RĪGAS TEHNISKĀ UNIVERSITĀTE

Sindija Lapčinska

CISTEĪNU UN SELĒNCISTĪNU SATUROŠO PEPTĪDU MODIFICĒŠANA ELEKTROFĪLĀS CIKLIZĀCIJAS UN REDZAMĀS GAISMAS INICIĒTĀS REAKCIJĀS

Promocijas darbs

MODIFICATION OF CYSTEINE AND SELENOCYSTINE-CONTAINING PEPTIDES BY ELECTROPHILIC CYCLIZATION AND VISIBLE LIGHT-INDUCED REACTIONS

Doctoral Thesis

RTU Izdevniecība
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Zinātniskais vadītājs / Scientific supervisor
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APSTIPRINĀJUMS

Apstiprinu, ka esmu izstrādājusi šo promocijas darbu, kas iesniegts izskatīšanai Rīgas Tehniskajā universitātē zinātnes doktora (*Ph. D.*) grāda iegūšanai. Promocijas darbs zinātniskā grāda iegūšanai nav iesniegts nevienā citā universitātē.

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Promocijas darbs ir tematiski vienota zinātnisko publikāciju kopa ar kopsavilkumu latviešu un angļu valodā. Tajā apkopoti seši zinātniskie oriģinālraksti. Publikācijas zinātniskajos žurnālos uzrakstītas angļu valodā, to kopējais apjoms, ieskaitot pielikumus, ir 820 lpp.

SATURS

SAĪSINĀJUMI

AEŠH	augstas efektivitātes šķidrumu hromatogrāfija
AIMS	augstas izšķirtspējas masspektrometrija
Arg	arginīns
Boc	*terc*-butoksikarbonil-
Bn	benzil-
t-Bu	*terc*-butil-
Cbz	benziloksikarbonil-
m-CPBA	*meta*-hlorperoksibenzoskābe
Cys	cisteīns
DCM	dihlormetāns
DDQ	2,3-dihlor-5,6-diciano-1,4-benzohinons
Dha	dehidroalanīns
EDC·HCl	1-etil-3-(3-dimetilaminopropil)karbodiimīda hidrogēnhlorīds
Gly	glicīns
GSH	glutations
His	histidīns
HOBt	1-hidroksibenzotriazols
it	istabas temperatūra
KMR	kodolu magnētiskā rezonanse
LED	gaismu emitējošas diodes
Lys	lizīns
Met	metionīns
NBS	*N*-bromsukcīnimīds
NCS	*N*-hlorsukcīnimīds
NIS	*N*-jodsukcīnimīds
NMM	*N*-metilmorfolīns
Ns	nozil-
PBS	fosfāta buferšķīdums
PLQY, Φ	fotoluminescences kvantu iznākums
RB	Bengālijas rozā
REN	relatīvā elektronegativitāte
Sec	selēncisteīns
ŠH-MS	šķidrumu hromatogrāfija-masspektrometrija
Trp	triptofāns
Ts	tozil-
Tyr	tirozīns
UV	ultravioletsts

DARBA VISPĀRĒJS RAKSTUROJUMS

Tēmas aktualitāte

Īsie peptīdi ir unikāla savienojumu klase ar daudzsološām īpašībām zāļvielu atklāšanā.[1] Kā daudzu fizioloģisko procesu endogēnas signālmolekulas peptīdi paver iespējas terapijām, kas līdzinās dabiskajiem metabolisma procesiem.[2] Peptīdu kā terapeitisko līdzekļu izmantošana ir ievērojami attīstījusies laika gaitā, turklāt ievērojams daudzums zāļvielu ir peptīdu saturoši līdzekļi.[1] Tomēr būtisks ierobežojums peptīdu izmantošanā ir to zemā perorālā biopieejamība un membrānu caurlaidība. Lai uzlabotu peptīdu biopieejamību, tiek veidoti peptīdu un mazu molekulu konjugāti. Konjugācija ir populārs mehānisms, kā uzlabot peptīdu īpašības. Apmēram trešdaļa no peptīdiem, kas iekļauti klīniskajos pētījumos pēdējo 10 gadu laikā, ir konjugāti, tādēļ jaunu peptīdu saturošu zāļvielu meklējumi turpinās.

Cisteīna (*Cys*), selēncisteīna (*Sec*), kā arī šo aminoskābju oksidēto formu iekļaušana peptīdu vai proteīnu molekulās paver iespēju tos selektīvi modificēt. Šos peptīdus var modificēt, ģenerējot halkogenilelektrofīlu, nukleofīlu vai radikāli.

Lai gan sērs un selēns abi ir VI A grupas elementi, to ķīmiskajām un fizikālajām īpašībām ir ievērojamas atšķirības. Selenoliem ir zemākas pKa vērtības nekā tioliem, piemēram, selēncisteīna pKa ir 5,2, savukārt cisteīna pKa – 8,3, kas nozīmē, ka fizioloģiskajos apstākļos (pH = 7,4) selēncisteīns ir deprotonēts. Selēns ir vairāk polarizēts, un tas atspoguļojas augstākā reaģētspējā.

Protams, literatūrā ir labi zināmas metodes sulfenil- un selenilelektrofīlu iegūšanai, tomēr šīs metodes ir lietojamas tikai vienkāršiem ariltioliem vai diarildisulfīdiem/diselenīdiem. Tipiski piemēri sulfenilelektrofīliem ir arilsulfenilhalīdi, ko iegūst no ariltioliem vai diarildisufīdiem reakcijā ar halogēniem,[3, 4] $SOCl_2$,[5] SO_2Cl_2,[3] katalītisku vai ekvimolāru daudzumu Luisa skābes (piemēram, dzelzs(III) sāļi,[6, 7, 8] $CuBr_2$[9]). Līdzīgā veidā, izmantojot halogēnus,[4] KI/*m-CPBA*,[10] oksidējošos aģentus (persulfātus,[11] oksonu[12]), Luisa skābes (CuI,[13] $FeCl_3$[6]) un hipervalentus joda savienojumus,[14] iespējams iegūt selenilelektrofīlu.

Peptīdu modificēšana ir ievērojami sarežģītāka sensitīvu funkcionālo grupu dēļ. Turklāt literatūrā zināmās metodes halkogenilpeptīdu modificēšanai parasti pamatojas uz tiola vai selenola nukleofilitāti, taču šo savienojumu elektrofīlās īpašības ir ļoti maz pētītas. Pretēji cisteīnam, selēncistīnu saturošie peptīdi daudz retāk tiek lietoti ķīmiskajā sintēzē.

Peptīdu funkcionalizēšana tiek veikta, lai uzlabotu šo savienojumu biopieejamību, membrānu caurlaidību un stabilitāti. Piemēram, peptīdu ciklizēšana uzlabo selektivitāti, metabolisko stabilitāti un saistīšanās afinitāti.[15] Vairākas zināmās antibiotikas (vankomicīns, daptomicīns, valinomicīns, gramicidīns S), imūnsupresanti (ciklosporīns), hormonu regulētāji (somatostatīns), pretvēža preparāti (aplidīns, daktinomicīns) ir peptīdu makrocikli.[16, 17] Laika posmā no 2015. līdz 2019. gadam *FDA* (*Food and drug administration*) apstiprināja 208 zāļvielas, no kurām 15 saturēja peptīdus.[18] Šie medikamenti tiek lietoti diabēta (*Tresiba*, *Lixisenatide*), osteoporozes (*Tymlos*), pazemināta asinsspiediena (*Giapreze*), neiroendokrīno audzēju (*Lutathera*) u. c. saslimšanu ārstēšanai. Savukārt 2021. gadā *FDA* apstiprināja

15 peptīdu zāļvielas (*Voxzogo, Korsuva, Besremi, Skytrofa, Nexviazyme, Jemperli, Evkeeza, Saphnelo, Rylaze, Aduhelm, Rybrevant, Empaveli, Zynlonta, Zegalogue, Lupkynis*).

Pētījuma mērķis un uzdevumi

Promocijas darba mērķis ir jaunu, efektīvu metožu izstrāde cisteīnu un selēncistīnu saturošo peptīdu modificēšanai, balstoties uz sulfenil- vai selenilelektrofīla ģenerēšanu, pievēršot uzmanību augstam iznākumam, atomekonomiskam un videi draudzīgam procesam.

Darba mērķa sasniegšanai definēti šādi uzdevumi:

1) izstrādāt ērtu protokolu sulfenilelektrofīla ģenerēšanai *in situ* no cisteīnu saturošiem peptīdiem;
2) izstrādāt jaunas metodes selenilelektrofīla ģenerēšanai, kas būtu piemērotas selēncistīnu saturošiem peptīdiem;
3) izstrādāt metodes sulfenil- un selenilelektrofīlu izmantošanai 5- un 6-*endo-dig* ciklizācijas reakcijās;
4) izstrādāt metodes selēncistīnu saturošu peptīdu modificēšanai redzamās gaismas iniciētās reakcijās.

Zinātniskā novitāte un galvenie rezultāti

Veikto pētījumu rezultātā ir izstrādātas vienkāršas un efektīvas metodes cisteīnu un selēncistīnu saturošu peptīdu funkcionalizēšanai. Cisteīna elektrofīls – cisteinilhlorīds – tika *in situ* iegūts, izmantojot *N*-hlorsukcīnimīdu, savukārt selēna elektrofīla iegūšanai tika izmantota Luisa skābe ($CuBr_2$) vai neorganisks oksidējošais aģents ($K_2S_2O_8$). Elektrofīlās daļiņas sekojoši tika izmantotas 5- un 6-*endo-dig* ciklizācijas reakcijās ar piemērotiem trīskāršo saiti saturošiem substrātiem, iegūstot cisteīnu un selēncisteīnu saturošus indolizīnija sāļus, indolus, benz[*b*]furānus, indēn[1,2-*c*]hromēnus, poliaromātiskus ogļūdeņražus, kumarīnus, izokumarīnus, hinolīn-2-onus un izohinolīn-2-onus. Lietotās metodes selenilelektrofīla ģenerēšanai uzrādīja augstu toleranci aminoskābēm ar "sensitīvām" grupām (piemēram, *Tyr*, *Glu*, *Lys*). Izstrādāta jauna, atomekonomiska metode aizsargātu un neaizsargātu selēncistīnu saturošo peptīdu modificēšanai, izmantojot redzamās gaismas iniciētu reakciju. Ar šo metodi veikta selēncisteīnu saturošu *N*-heterociklu sintēze ar augstiem iznākumiem. Turklāt, izmantojot iekšmolekulāru indolu selenilēšanu, iegūti selēncisteīnu saturoši makrocikli. Izmantojot redzamās gaismas iniciētu reakciju, iegūti arī Se–S saiti saturoši peptīdi. Metode balstās uz glutationa S-radikāļa ģenerēšanu organiskās krāsvielas klātienē.

Darba struktūra un apjoms

Promocijas darbs ir tematiski vienota zinātnisko publikāciju kopa, kas veltīta jaunu metožu izstrādei cisteīnu un selēncistīnu saturošu peptīdu modificēšanai elektrofīlās ciklizācijas un redzamās gaismas iniciētās reakcijās. Promocijas darbs ietver sešas oriģinālpublikācijas, kas indeksētas *Scopus* un *Web of Science* datubāzēs.

Darba aprobācija un publikācijas

Promocijas darba rezultāti publicēti sešos zinātniskajos oriģinālrakstos, pētījuma rezultāti prezentēti sešās zinātniskajās konferencēs.

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2. Peptide functionalization through the generation of selenocysteine electrophile. A. Ivanova, S. Lapcinska, P. Arsenyan. *14th International Conference on the Chemistry of Selenium and Tellurium (ICCST-14)*, June 3–7, 2019, Santa Margherita di Pula (Italy).
3. Formation of indole and benzofuran moieties attached to selenocysteine containing peptides. S. Lapcinska, P. Arsenyan. *14th International Conference on the Chemistry of Selenium and Tellurium (ICCST-14)*, June 3–7, 2019, Santa Margherita di Pula (Italy).

4. Performance of chalcogen-containing peptides in 5- and 6-*endo-dig* cyclization reactions. S. Lapcinska. *Paul Walden 11th Symposium on Organic Chemistry*, September 19–20, 2019, Riga (Latvia).
5. Visible light-mediated functionalization of selenocystine-containing peptides. S. Lapcinska, P. Arsenyan. *International Conference on Photochemistry – 30th edition (ICP2021)*, July 19-23, 2021, Geneva (Switzerland), online conference.
6. Photocatalytic macrocyclization of selenocystine-containing peptides. S. Lapcinska, P. Arsenyan. *International Symposium on Synthesis and Catalysis (IsySyCat2021)*, August 31–September 3, 2021, Evora (Portugal), online conference.
7. Light-driven modifications of Cys and Sec containing peptides. S. Lapcinska. *Paul Walden 12th Symposium on Organic Chemistry*, October 28–29, 2021, Riga (Latvia), online conference.

PROMOCIJAS DARBA GALVENIE REZULTĀTI

1. Sulfenilelektrofīla iegūšana un izmantošana 5- un 6-*endo-dig* ciklizācijas reakcijās

Cisteīns (*Cys*) ir samērā reti sastopams proteīnos (1–2 %), tāpēc tas ir populārs mērķis proteīnu reģioselektīvai modificēšanai. Iespējams, vissvarīgākais *Cys* saturošais peptīds ar mazu molekulāro masu, kas sastopams cilvēku organismā, ir glutations (*GSH*). *GSH* ir svarīga nozīme daudzos šūnā notiekošos procesos, tajā skaitā šūnu diferenciācijā, proliferācijā un apoptozē. *GSH* pasargā šūnas no bojājumiem, ko izraisa lipīdu peroksīdi, reaktīvās skābekļa un slāpekļa daļiņas un ksenobiotiķi. Samazināta *GSH* un *GSH*/glutationa disulfīda (*GSSG*) attiecība izraisa palielinātu uzņēmību pret oksidatīvo stresu, kas saistīts ar vēža attīstību, taču paaugstināts *GSH* daudzums izraisa rezistenci pret oksidatīvo stresu. Turklāt pēdējo gadu laikā tiolu saturoši peptīdi ir izmantoti lipofīlo zāļvielu piegādes sistēmās.

Zināmās metodes konjugātu sintēzei parasti balstās uz *Cys* nukleofilitāti. Plaši lietotas metodes cisteīna funkcionalizēšanai ir alkilēšana ar alkilhalogenīdiem, arilēšana un Maikla pievienošanās reakcija. Vērtīga metode ir cisteīna sašūšana (*stapling*) ar dažādiem arillinkeriem. Cisteīnu var viegli oksidēt par tā dimēru – cistīnu. S–S saites izveide proteīnos ir atkarīga no cisteīnu novietojuma, un tās ievietošana proteīnos atbild par to stabilitāti un izkārtojumu.

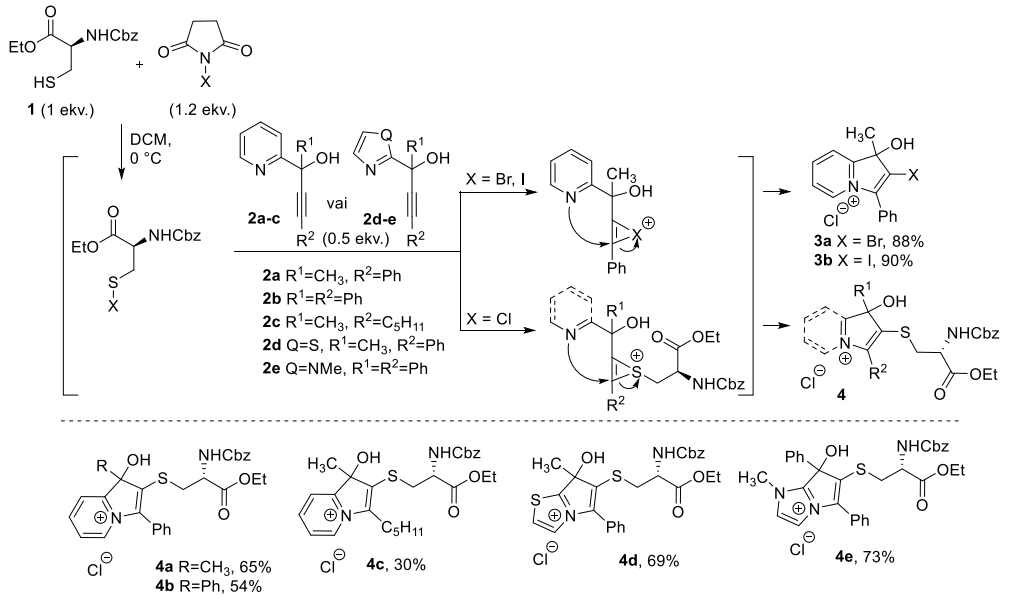
S–H saites homolītiskās dissociācijas enerģija ir neliela (86 kcal/mol), kas ļauj viegli ģenerēt attiecīgo cisteīna radikāli.[19] Pēdējo gadu laikā izstrādātas metodes cisteīna konjugācijai fotokatalizatoru klātienē, izmantojot redzamo vai *UV* gaismu.[20] Iespējams, ka plašāk lietotās radikāļu reakcijas cisteīna modificēšanai ir tiola-ēna/īna reakcijas. Savukārt *Cys* desulfenilēšanas rezultātā veidojas *Dha* atvasinājumi. Parasti reakciju veic fosfīnu klātienē.

Elektrofīlais centrs uz sēra atoma atrodas savienojumos ar S–O, S–N vai S–Cl saiti. Sulfēnskābes ir nestabilas, taču sulfēnamīdi parasti tiek sintezēti no sulfenilhalogenīdiem, tāpēc sulfenilhalogenīdi tiek plašāk lietoti kā sulfenilelektrofīli. Šos elektrofīlus var iegūt no tioliem vai disulfīdiem, izmantojot halogēnus, $SOCl_2$, SO_2Cl_2 vai *N*-halosukcīnimīdus. Cisteīnu iespējams modificēt, lietojot tā spēju saistīties ar Luisa skābēm. Tādas Luisa skābes kā $FeCl_3$, $CuBr_2$ un $AlCl_3$ izmanto, lai ģenerētu elektrofīlu no tioliem vai disulfīdiem. Elektrofīlu var iegūt, izmantojot (diacetoksijod)benzolu KI klātienē. Iepriekš pagatavoti *N*-tioalkil- vai arilftalimīdi un *N*-tiosukcīnimīdi arī spēj kalpot kā efektīvi elektrofīli.

Promocijas darba pamatā esošajos pētījumos tika izlemts ģenerēt sulfenilelektrofīlu ar *N*-halosukcīnimīdu palīdzību. *N-Cbz-Cys-OEt* (**1**) un 4-fenil-2-(piridīn-2-il)but-3-īn-2-ols (**2a**) tika izmantoti par modeļvielām optimālo reakcijas apstākļu meklējumos. Analizējot reakcijas maisījumus, noskaidrots, ka *N*-bromsukcīnimīda (NBS) un *N*-jodsukcīnimīda (NIS) gadījumā ar augstiem iznākumiem īsā reakcijas laikā (1 h) veidojās attiecīgi halo-indolizīnija sāļi **3a** un **3b**. Savukārt *N*-hlorsukcīnimīds (NCS) izrādījās piemērots, lai iegūtu *Cys* saturošo indolizīnija sāli **4a**, kas tika izolēts kā diastereomēru maisījums (1 : 1) ar 65 % iznākumu (1. shēma). NCS nespēja veicināt 5-*endo-dig* ciklizēšanu ar **2a**, bet reakcijā ar NBS un NIS trīs dienu laikā

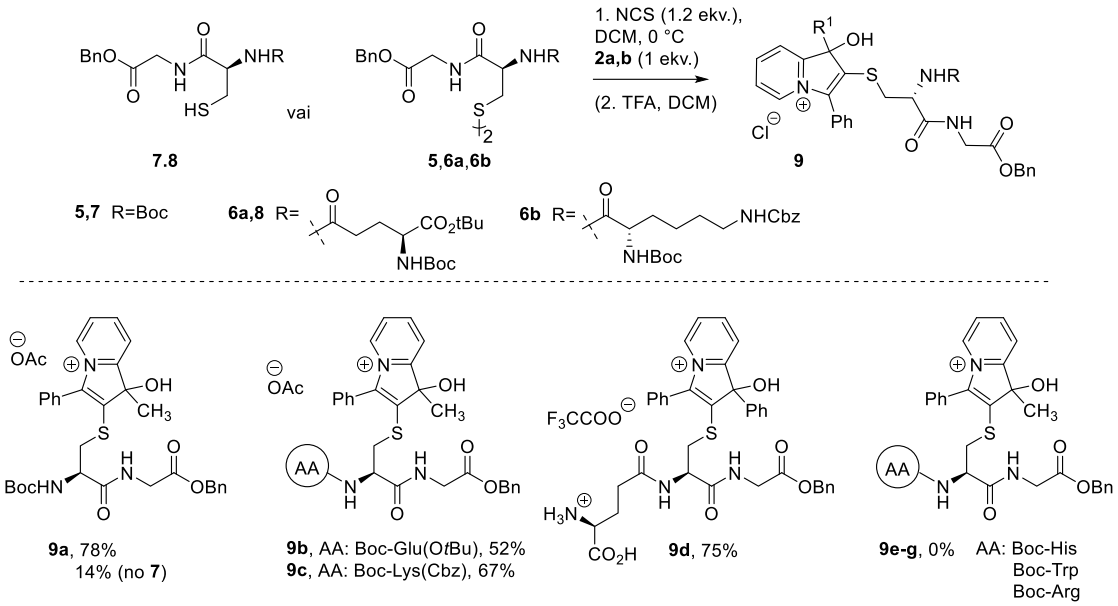
neselektīvi veidojās **3a** un **3b**. Tas apstiprināja hipotēzi par attiecīgo cisteinilhalogenīdu izveidi cisteīna reakcijā ar *N*-halosukcīnimīdiem. Iegūtie rezultāti liecina par to, ka elektrofīlais centrs cisteinilhlorīdā ir sēra atoms, savukārt cisteinilbromīda un cisteiniljodīda gadījumā elektrofīlais centrs ir halogēna atoms, jo šajos gadījumos ir mazāka relatīvās elektronegativitātes (REN) atšķirība starp konkrētajiem atomiem (REN S = 2,58, Cl = 3,16, Br = 2,96, I = 2,66).

Substrātu klāsta pārbaude parādīja, ka ne tikai propargilpiridīni **2a-c**, bet arī propargiltiazols **2d** un propargil-*N*-metilimidazols **2e** ir piemēroti substrāti ciklizācijas reakcijai un veido indolizīnija tipa sistēmas **4d**,**e** ar labiem iznākumiem (69–73 %).



1. shēma. *Cys* saturošu indolizīnija tipa sāļu sintēze.
Visi produkti tika attīrīti ar apgrieztās fāzes hromatogrāfijas palīdzību
(MeCN/H_2O, pH = 4, sālsskābe).

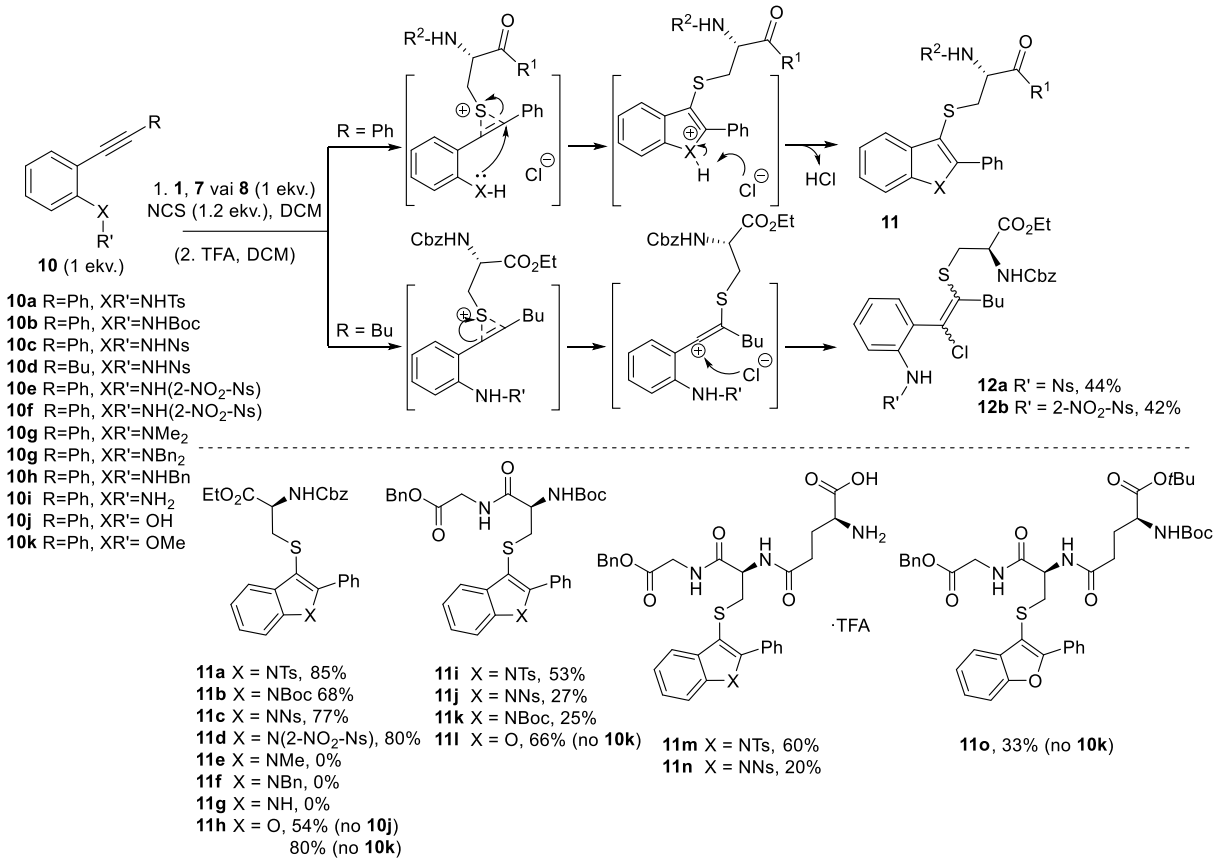
Izstrādātie reakcijas apstākļi nebija piemēroti savienojuma **9a** iegūšanai (2. shēma). Lai gan produkta veidošanās tika novērota, veidojās arī dažādi piemaisījumi. Reakciju veicot acetonitrilā, izdevās izolēt **9a** ar 14 % iznākumu. Taču izrādījās, ka ir iespējams izmantot S–S saiti saturošus substrātus, lai ģenerētu sulfenilelektrofīlu un izmantotu to 5-*endo-dig* ciklizācijā. Sekojoši (*Boc-Cys-Gly-OBn*)$_2$ **5** un (*Boc-Glu(OtBu)-Cys-Gly-OBn*)$_2$ **6a** izmantošana ļāva iegūt attiecīgos indolizīnija sāļus **9a-c** ar vidējiem līdz labiem iznākumiem. Peptīdi, kas saturēja aminoskābes ar "jutīgām" funkcionālajām grupām (*His*, *Trp*, *Arg*), nebija piemēroti reakcijas apstākļiem.



2. shēma. *Cys* peptīdus saturošu indolizīnija sāļu sintēze.
Produkti tika attīrīti ar apgrieztās fāzes hromatogrāfijas palīdzību
(**9a-c**: MeCN/H_2O, pH = 4, etiķskābe; **9d**: MeCN/H_2O, pH = 4, trifluoretiķskābe).

Daudzas dabasvielas, kā arī ievērojams skaits zāļvielu, satur indola un benz[*b*]furāna motīvus.[21, 22] Turklāt šos heterociklus uzskata par "priviliģētām struktūrām".[23] Tāpēc tika izlemts lietot izstrādātos reakcijas apstākļus citai 5-*endo-dig* ciklizācijai, kas rezultātā ļautu iegūt indolus un benz[*b*]furānus.

Reakcijā starp **1** un 2-(feniletinil)anilīnu NCS klātienē veidojās 3-*Cys*-indoli **11a-d** ar augstiem iznākumiem (3. shēma). Taču ne dimetilamino-, ne dibenzilamino-, kā arī neaizsargāti (feniletinil)anilīni nebija piemēroti substrāti ciklizācijas reakcijai.

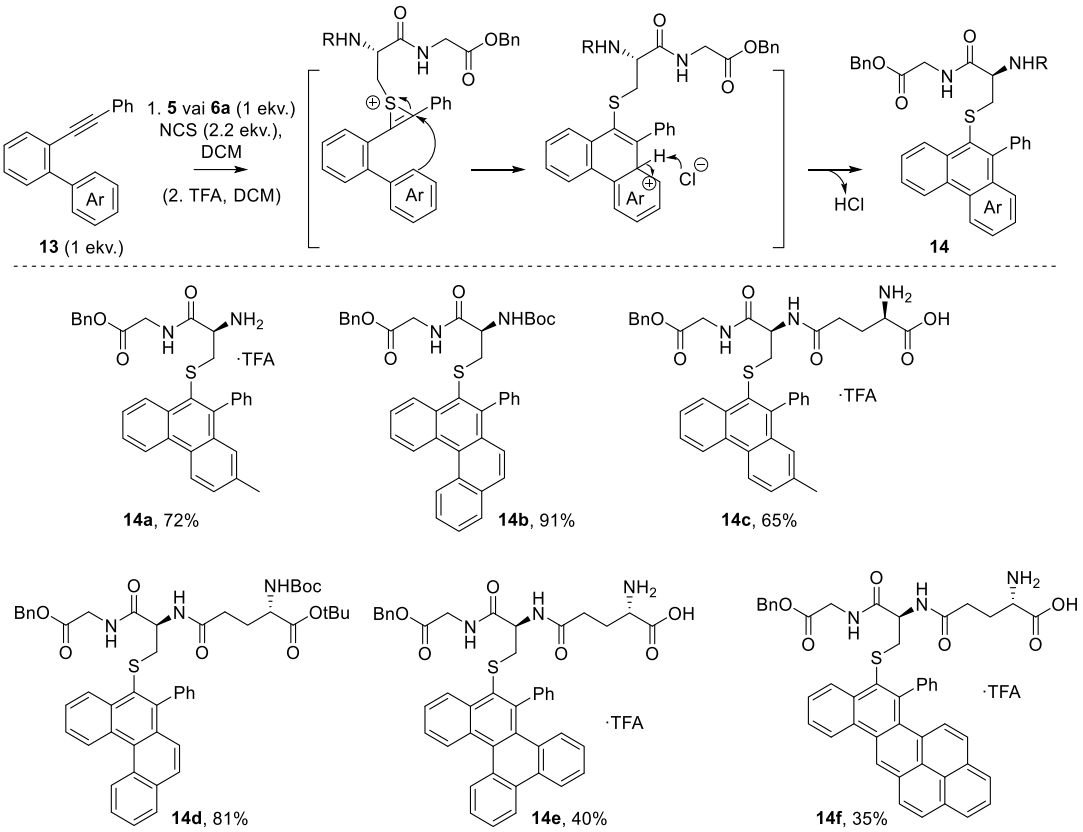


3. shēma. *Cys* peptīdu saturošu indolu un benz[*b*]furānu sintēze.

Līdzīgā veidā tika iegūts 3-*Cys*-benz[*b*]furāns **11h**. Svarīgi, ka reakcijas iznākumu izdevās ievērojami uzlabot, 2-(feniletinil)fenola **10j** vietā izmantojot 2-(feniletinil)anizolu **10k**. Peptīdu *Boc-Cys-Gly-OBn* (**7**) un *Boc-Glu(OtBu)-Cys-Gly-OBn* (**8**) lietošana ļāva izolēt attiecīgos indolus un benz[*b*]furānus, tomēr reakcijas iznākumi bija dažādi (20–66 %). S–S saiti saturošie peptīdi nebija piemēroti savienojumu **11** sintēzei. Jāpiemin, ka, izmantojot 2-heksīnilanilīnus, reakcijā veidojās tikai trīskāršās saites pievienošanās produkti **12a**-**b**, iespējams, tādēļ, ka izveidojās arilgrupas stabilizēts vinilkatjons, kas novērsa 5-*endo-dig* ciklizāciju.

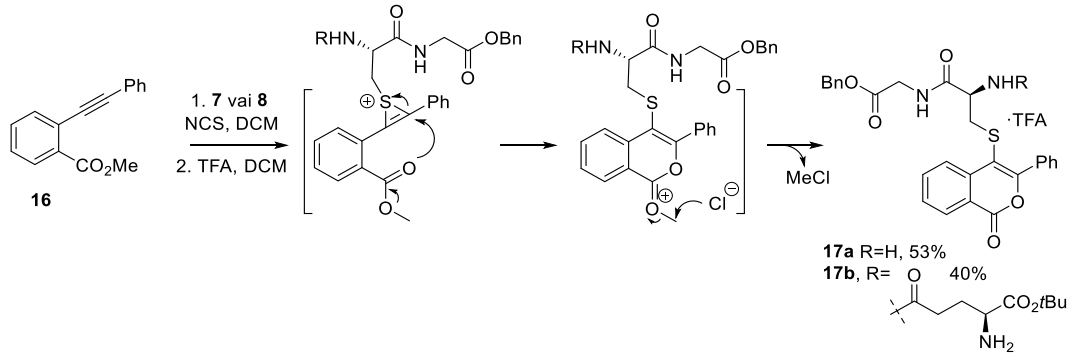
Tālāk tika nolemts izpētīt iespēju ģenerēt sulfenilelektrofīlu, lai izmantotu to 6-*endo-dig* ciklizācijā ar 2-(feniletinil)biarilsistēmu saturošiem savienojumiem. Policikliski aromātiskie ogļūdeņraži ir lietderīgi savienojumi, kas tiek izmantoti ne tikai kā izejvielas organiskajā sintēzē, bet arī kā fluorescenti marķieri.

Reakcija starp (*Boc-Cys-Gly-OBn*)$_2$ (**5**) un alkīnu **13a** NCS klātienē ļāva iegūt 6-*endo-dig* ciklizācijas produktu **14a** ar 72 % iznākumu (4. shēma). Trīskāršā saite koordinējas ar elektrofīlo sēra aromu, veidojot tiirēnija ciklu, kam uzbrūk tuvākais aromātiskais gredzens, veidojot 6 locekļu ciklu. Pēc deprotonēšanas ar hlorīda anjonu veidojas galaprodukts. Tā viegli tika konstruētas benz[*c*]fenantrēna, benz[*g*]krizēna un benz[*pqr*]picēna sistēmas. Lai arī izejvielu konversija bija augsta, produktu iznākumi ir atkarīgi no izdalīšanas un attīrīšanas procesiem.



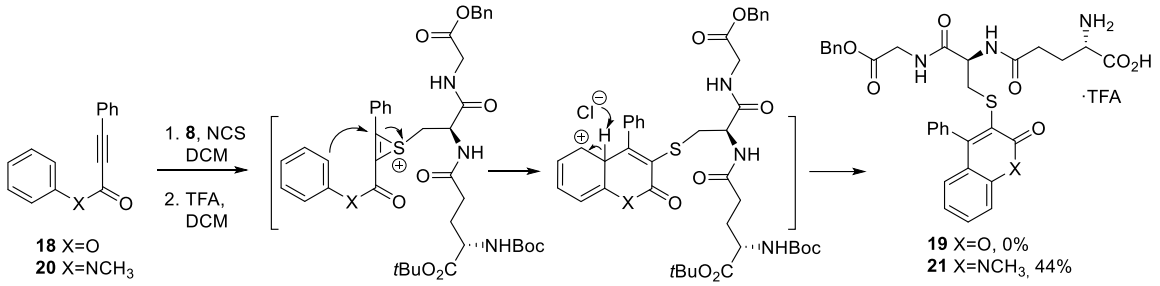
4. shēma. *Cys* peptīdu saturošu poliaromātisku ogļūdeņražu sintēze.

Novērtētas arī iespējas NCS metodi lietot glutationu saturošu izokumarīnu **17a** un **17b** sintēzei. Izokumarīnu motīvs bieži sastopams bioloģiski aktīvu dabasvielu struktūrās.[24] Šiem savienojumiem ir zināma antimikrobiāla, pretsēnīšu, citotoksiska un pretiekaisumu aktivitāte. Cerētos produktus izdevās izolēt ar viduvējiem iznākumiem, izmantojot S–H saiti saturošos peptīdus **7** un **8** (5. shēma).



5. shēma. *Cys* peptīdu saturošu izokumarīnu sintēze.

Veiksmīgi izdevās veikt citu 6-*endo-dig* ciklizāciju ar arilpropiolamīdu **20**, iegūstot 1-metil-3-sulfanilhinolīn-2-onu **21** (6. shēma). Zināms, ka hinolīn-2-oni ietilpst dažādu zāļvielu sastāvā, tajā skaitā plaši lietotu antibiotiku sastāvā (ciprofloksacīns, levofloksacīns).[25]



6. shēma. *Cys* peptīdu saturoša hinolīn-2-ona sintēze.

Oriģinālpublikācija par šajā apakšnodaļā aprakstītajiem pētījumiem – 3. pielikumā.

2. Selenilelektrofīla ģenerēšana un izmantošana 5- un 6-*endo-dig* ciklizācijas reakcijās

Pretēji tioliem, selenoli viegli oksidējas gaisā, tāpēc standartapstākļos tie pastāv kā diselenīdi. Selenoli ir nukleofilāki par tioliem, un tos parasti iegūst *in situ* reducējošo aģentu klātienē (piemēram, $NaBH_4$). Diselenīdi ir ļoti svarīgi daudzpusīgi savienojumi, un tos var izmantot ne tikai kā selēna nukleofīla avotu, bet arī lai ģenerētu selenilelektrofīlu vai radikāli. Tipiski selenilelektrofīli ir selenilhalogenīdi RSeX (X=Br, Cl, I), ko viegli iespējams iegūt, apstrādājot diselenīdus ar halogēniem vai SO_2Cl_2. Nesen literatūrā tika aprakstīta fenilselenilfluorīda *in situ* iegūšana, izmantojot *Selectfluor*.[26] Jāpiebilst, ka fenilselenilhlorīds un fenilselenilbromīds ir komerciāli pieejami.

Protams, ka halogenīda nukleofilitāte spēj izraisīt nevēlamas blakus reakcijas, tādēļ dažkārt priekšroka jādod sintēzes protokoliem, kuros netiek izmantots halogenīds. Selenilhalogenīdi reaģē ar dažādiem sudraba sāļiem, apmaiņas reakcijā veidojot seleniltriflātu, tozilātu, acetātu, heksafluorfosfātu. No iepriekš minētajiem savienojumiem visplašāk lietotais ir seleniltriflāts.

Alternatīva metode selenilelektrofīla ģenerēšanai ir diselenīdu oksidēšana. Piemēram, persulfātu sāļu klātienē var iegūt selenilsulfātu.[27] Pēdējos pāris gados oksons tiek lietots selenilelektrofīla ģenerēšanai un sekojošai reakcijai ar trīskāršo saiti saturošiem substrātiem, ciklizācijas reakcijā iegūstot selenilētus heterociklus (indolus, pirazolus). Difenildiselenīda reakcijā ar oksonu veidojas fenilselenīnskābe un fenilselenilsulfāts.[28] Selenilelektrofīla iegūšanai lietoti arī citi reaģenti: hipervalenti joda savienojumi, *m-CPBA*, $Ce(NH_4)_2(NO_3)_6$, KNO_3, *DDQ*. Difenildiselenīds reaģē ar 1,4-dicianonaftalīnu pēc SET mehānisma, veidojot selēna elektrofīlu. Turklāt selenilelektrofīlu var ģenerēt diselenīda reakcijā ar Luisa skābēm, piemēram, bieži tiek lietoti dzelzs(III), vara(I) un vara(II) sāļi.

Selenolu un diselenīdu oksidēšanā iespējams iegūt Se–O saiti saturošus savienojumus: selenēnskābi (RSeOH), selenīnskābi ($RSeO_2H$) vai selenoskābi ($RSeO_3H$). Selenēnskābes ir ļoti reaģētspējīgi savienojumi, tikai arilselenēnskābes ir detektētas. Turklāt selenēnskābe ar stēriski apjomīgām arilgrupām ir izolēta un raksturota. Selenīnskābes un selenoskābes ir stabilākas, un tās var izmantot dažādu organisko savienojumu oksidēšanai. Selenīnskābes var izmantot Se–S saiti saturošu savienojumu sintēzei,[29] turklāt fenilselenīnskābe[30] ir izmantota indolu un anilīnu selenilēšanai.

Literatūrā ir aprakstītas tikai dažas metodes selēncistīnu saturošo peptīdu modificēšanai. Ir zināma metode *Sec* peptīdu konjugēšanai ar mazām molekulām, kas balstās uz (5-nitropiridīniltio)-*Sec* peptīdu elektrofīlo raksturu.[31] Šie substrāti reaģē arī ar nukleofīlām arilborskābēm, veidojot arilētus *Sec* peptīdus.[32] Plašāk lietotās metodes selēncistīnu saturošo peptīdu modificēšanai balstās uz deselenilēšanu, veidojot alanīna peptīdus reducējošo aģentu[33] klātienē vai oksidējošā aģenta ierosinātu[34] dehidroalanīna izveidi.

Lai atrastu piemērotu substrātu selenilelektrofīla ģenerēšanai, tika pētīta reakcija starp Ph_2Se_2 (**22a**), **2a** un dažādām Luisa skābēm ($FeCl_3$, $FeBr_2$, $FeBr_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $CuBr_2$, $Cu(OAc)_2$, $RuCl_3$, $In(OTf)_3$, $Bi(OTf)_3$).

Vara(II) bromīds uzrādīja vislabākos rezultātus (1. tabula). Turklāt visi pārbaudītie šķīdinātāji ļāva iegūt produktu **23a** ar pieņemamu iznākumu, savukārt produkta veidošanās ar izcilu iznākumu noritēja *DCM*, MeCN un EtOH. Maksimāla iznākuma nodrošināšanai bija jāizmanto ekvimolārs $CuBr_2$ daudzums. Reakciju veicot bez difenildiselenīda, galvenais produkts bija 2-bromindolizīnija bromīds. Metodi varēja lietot arī telenilelektrofīla ģenerēšanai, taču ne sulfenilelektrofīla ģenerēšanai – reakcijā veidojās tikai 2-bromindolizīnija bromīds.

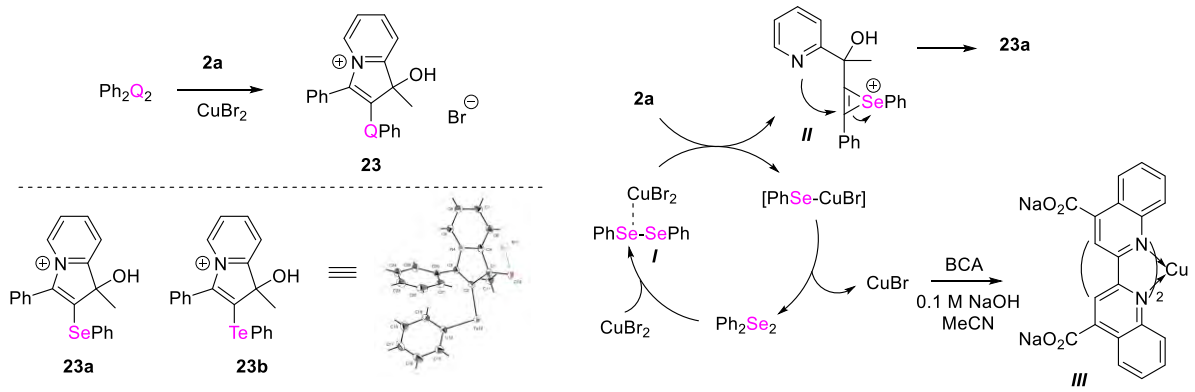
Lai noskaidrotu reakcijas mehānismu, tika uzņemts ekvimolāra daudzuma $CuBr_2$ un Ph_2Se_2 maisījuma ^{77}Se KMR spektrs (1. A att.). $Ph_2Se_2 \cdot CuBr_2$ maisījuma spektrā tika novērots viens plats signāls pie 450 ppm, kas ir līdzīgs Ph_2Se_2 signālam (462 ppm). Tas liecina par $Ph_2Se_2 \cdot CuBr_2$ izveidi, turklāt Se–Br un Se–Cu saites saturošie starpprodukti netika detektēti. Šo apgalvojumu apstiprina arī fakts, ka Se–Cu saiti saturoša savienojuma ($Cu(SePh)_2 \cdot$2-fenantrolīns) ķīmiskā nobīde KMR spektrā ir 274 ppm; savukārt PhSeBr signāls atrodas pie 867 ppm. Protams, teorētiski selēna spektrā vajadzētu parādīties diviem signāliem, taču starpprodukta dinamiskās dabas dēļ šie signāli ir saplūduši, turklāt signāls ir plats vara paramagnētisko īpašību dēļ.

Balstoties uz eksperimentālajiem datiem, ir piedāvāts iespējamais reakcijas mehānisms. Vispirms veidojas $Ph_2Se_2 \cdot CuBr_2$ pievienošanās produkts ***I***. Tad ***I*** ģenerē selēna elektrofīlu, kas koordinējas ar substrāta **2a** trīskāršo saiti, veidojot selēnirēnija katjonu ***II***. Slāpekļa nedalītais elektronu pāris uzbrūk divkāršajai saitei, veidojot 5 locekļu ciklu ar pozitīvi lādēto slāpekļa atomu. Reakcijas blakusprodukts PhSeCuBr sadalās, veidojot Ph_2Se_2 un CuBr.

CuBr veidošanās tika pierādīta eksperimentā ar bicinhonīnskābi (BCA), kas selektīvi veido helātu ar Cu(I) joniem, veidojot violeti krāsotu kompleksu ***III*** (absorbcijas maksimums pie 562 nm). Tika veikti absorbcijas mērījumi (1. B att.) trīs paraugiem: reakcijas maisījumam ar BCA, CuI un BCA, $CuBr_2$ un BCA. Absorbcijas vērtība reakcijas maisījumam ar BCA un CuI ar BCA bija līdzīgas vērtības (absorbcija attiecīgi 1,1503 pie 560 nm un 1,4749 pie 560 nm). Tādējādi tika apstiprināta nepieciešamība izmantot ekvimolāru daudzumu $CuBr_2$ – tas tiek izlietots, veidojot CuBr, kas nespēj iniciēt selēna elektrofīla veidošanos. Tas tika arī apstiprināts testa reakcijā, $CuBr_2$ vietā izmantojot CuBr vai CuI.

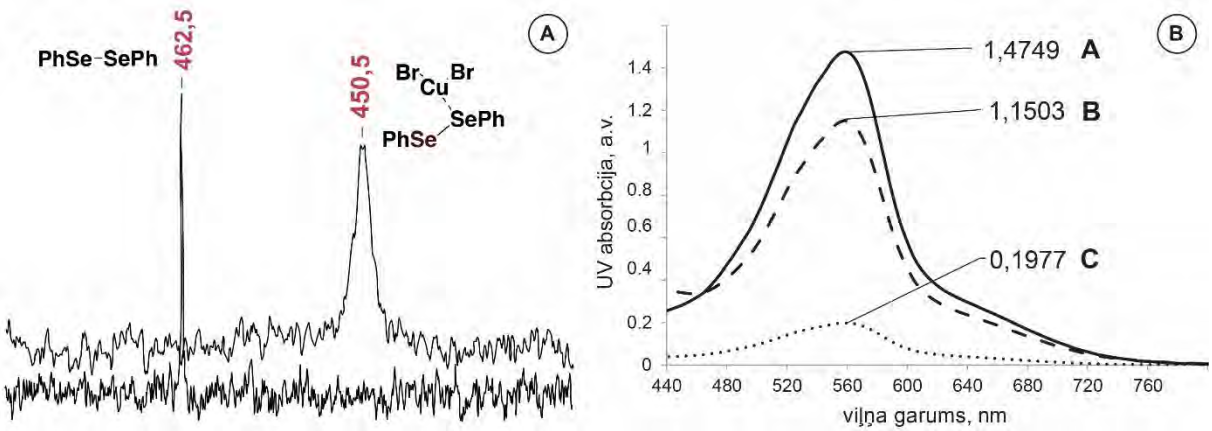
1. tabula

Reakcijas apstākļu optimizēšana savienojumu **23a** un **23b** iegūšanai



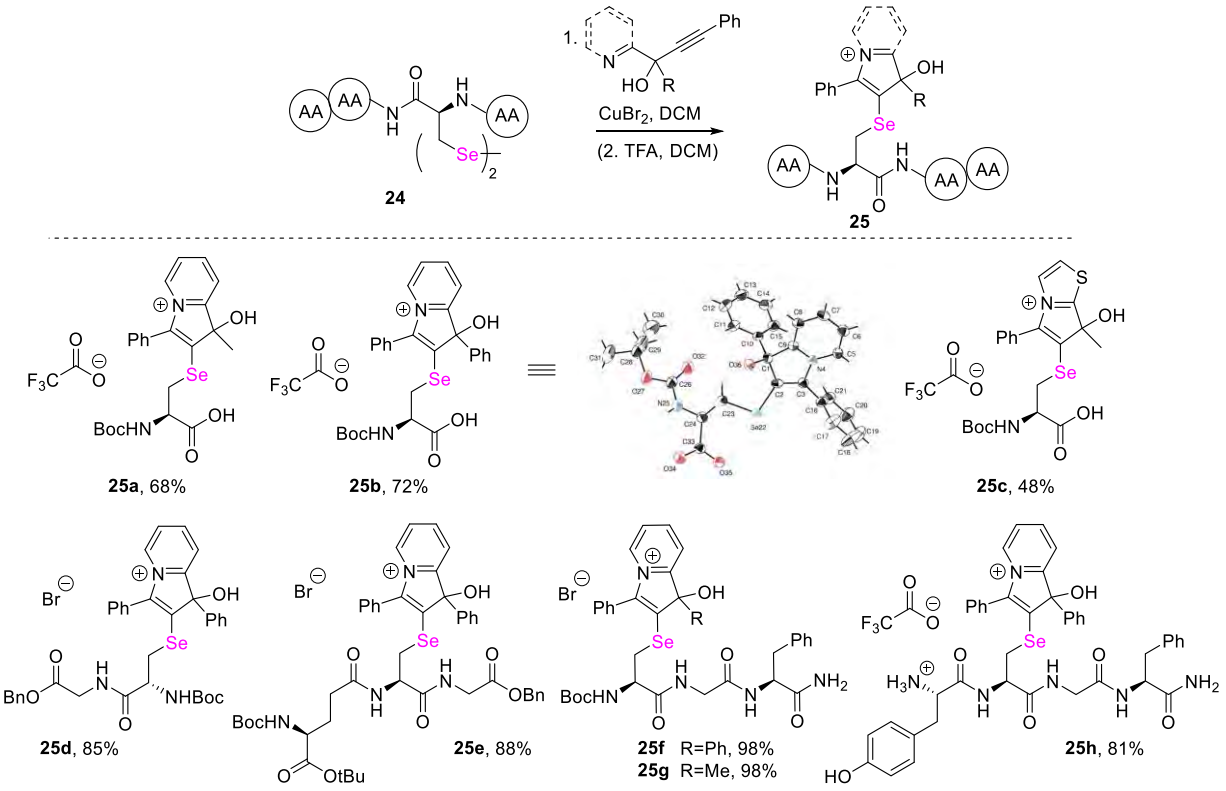
Nr. p. k.	**22**	Šķīdinātājs	Reaģentu attiecība **22** : $CuBr_2$: **2a**	Laiks, h	Iznākums, %
1.	Ph_2Se_2[a]	DCM	2 : 2: 1	4	-[c]
2.	Ph_2Se_2[b]	DCM	2 : 2: 1	4	-[c]
3.	Ph_2Se_2	DCM	2 : 2 : 1	4	86
4.	Ph_2Se_2	DCM	2 : 0.5 : 1	4	47
5.	Ph_2Se_2	DCM	1,2 : 1,2 : 1	4	94
6.	Ph_2Se_2	DCM	0,6 : 1,2 : 1	4	62
7.	Ph_2Se_2	DMSO	1,2 : 1,2 : 1	24	70[e]
8.	Ph_2Se_2	EtOAc	1,2 : 1,2 : 1	24	62[e]
9.	Ph_2Se_2	MeCN	1,2 : 1,2 : 1	0,5	90
10.	Ph_2Se_2	EtOH	1,2 : 1,2 : 1	12	96
11.	-	DCM	0 : 2 : 1	72	86[d]
12.	Ph_2Se_2	MeCN	1,2 : 1,2 : 1	1	32 + 58[f]
13.	Ph_2S_2	DCM	1,2 : 1,2 : 1	24	78[d]
14.	Ph_2S_2	MeCN	1,2 : 1,2 : 1	24	87[d]
15.	Ph_2Te_2	DCM	1,2 : 1,2 : 1	0,25	94
16.	Ph_2Te_2	MeCN	1,2 : 1,2 : 1	4	76

[a] MX_n – $FeCl_3$; [b] MX_n – $FeBr_3$; [c] produktu maisījums; [d] veidojās 2-bromindolizīnija bromīds; [e] nepilna izejvielu konversija; [f] vispirms pie **2a** pievieno $CuBr_2$ un pēc 20 min pievieno Ph_2Se_2. Veidojas 2-bromindolizīnija bromīds (32 %) un **23a** (58 %).



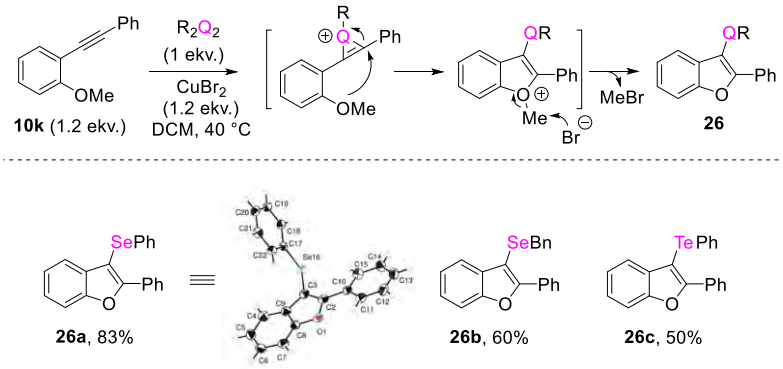
1. att. A: $Ph_2Se_2 \cdot CuBr_2$ un Ph_2Se_2 ^{77}Se KMR spektri. B: UV absorbcijas spektri: A: CuI + BCA; B: reakcijas maisījums + BCA; C: $CuBr_2$ + BCA.

Noskaidrots ka arī *Boc*-aizsargāts selēncistīns **24a** ir savietojams ar reakcijas apstākļiem. Reakcijā starp $(Boc\text{-}Sec)_2$ un **2a,b,d** vara(II) bromīda klātienē veidojās atbilstošie indolizīnija **25a**,**b** un pirolotiazolija **25c** sāļi ar labiem iznākumiem kā diastereomēru maisījumi, kas tika sadalīti ar AEŠH palīdzību (7. shēma). Turklāt iegūts ***(1R,S)*-25b** kristāls kā cviterjona sāls, apstiprinot tā struktūru. Reakcijā arī lietoti dažādi selēncistīnu saturoši peptīdi un izolēti indolizīnija sāļi **25d-h** ar augstiem iznākumiem.



7. shēma. *Sec*-indolizīnija sāļu sintēze. **24a** $(Boc\text{-}Sec)_2$; **24b** $(Boc\text{-}Sec\text{-}Gly\text{-}OBn)_2$; **24c** $(Boc\text{-}Glu(OtBu)\text{-}Sec\text{-}Gly\text{-}OBn)_2$; **24d** $(Boc\text{-}Sec\text{-}Gly\text{-}Phe\text{-}NH_2)_2$; **24e** $(Boc\text{-}Tyr\text{-}Sec\text{-}Gly\text{-}Phe\text{-}NH_2)_2$. Produkti tika attīrīti ar apgrieztās fāzes hromatogrāfijas palīdzību (**25a-c**: MeCN/H_2O, pH = 4, trifluoretiķskābe).

Cita veiksmīga 5-*endo-dig* ciklizācija tika veikta, izmantojot Ph_2Se_2 un 2-(feniletinil)anizolu (**10k**). Iepriekš izstrādātie reakcijas apstākļi bija piemēroti šai ciklizācijai, taču, lai sasniegtu pilnu izvejvielu konversiju 16 h laikā, bija jāpaaugstina temperatūra (40 °C). 3-Selenilbenz[*b*]furāns **26a** tika iegūts ar 83 % iznākumu, un tā struktūra tika viennozīmīgi apstiprināta ar retgenstruktūras analīzes datiem (8. shēma). Līdzīgā veidā tika izolēti 3-halkogenilbenz[*b*]furāni **26b** and **26c**. Turklāt reakcijas iznākumi bija līdzīgi vai augstāki nekā literatūrā aprakstītajai $FeCl_3$ iniciētajai 2-feniletinilanizola ciklizācijai (**26b** 64 %, **26c** 36 %).[6]



8. shēma. 3-halkogenilbenz[*b*]furānu veidošanās.

Attiecīgi, izmantojot peptīdus **24b**,**c** un anizolu $CuBr_2$ klātienē veidojās *Sec* saturošie benz[*b*]furāni **27a** un **27d** (9. shēma). Jāpiebilst, ka dzelzs(III) hlorīds nebija spējīgs iniciēt produktu **27** veidošanos. Līdzīgā veidā tos pašus savienojumus varēja iegūt no 2-(feniletinil)fenola (**10j**). Tomēr $CuBr_2$ iniciēta selenilelektrofīla ģenerēšana nebija piemērota, lai veiktu 2-alkil-3-selenilbenz[*b*]furānu sintēzi. Reakcijā veidojās vairāku produktu maisījums, iespējams, ka veidojās arilgrupas stabilizēts vinilkatjons, kas novērš 5-*endo-dig* ciklizāciju. Tāpēc bija jāatrod cita metode, lai iegūtu selenilelektrofīlu un veiktu ciklizācijas reakciju.

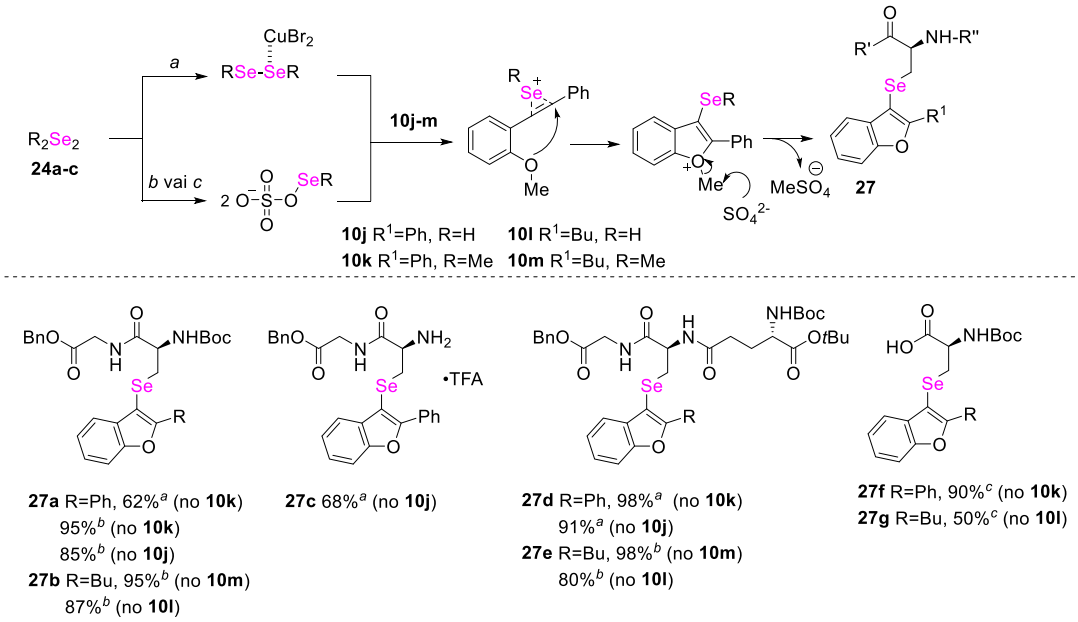
Piemērotu reaģentu izdevās atrast, pārbaudot dažādus oksidējošos aģentus. Zināms, ka reakcijā starp diselenīdiem un persulfātu sāļiem veidojas selenilsulfāts, kas ir stiprs elektrofīls. Lai gan reakcija starp **24b** un **10l** $K_2S_2O_8$ (5 ekv.) klātienē bija lēna (pilnīga izejvielu konversija tika sasniegta 3 dienu laikā), svarīgi, ka reakcija bija selektīva un benz[*b*]furāns veidojās ar augstu iznākumu. Citi pārbaudītie oksidējošie aģenti bija mazāk efektīvi (amonija persulfāts, kālija jodāts) vai arī notika neselektīva reakcija (oksons, *meta*-hlorperoksibenzoskābe, nātrija perjodāts, cērija amonija nitrāts, (diacetoksijod)benzols), jo ātra oksidēšana izraisīja deselenilēšanas reakcijas.

Savienojuma **27c** iznākumu varēja uzlabot, lietojot 2-(heks-1-īn-1-il)anizolu (**10m**). Jāpiebilst, ka $K_2S_2O_8$ iniciēta selenilelektrofīla ģenerēšana un sekojoša ciklizēšana ļāva iegūt arī **27a** ar augstāku iznākumu nekā $CuBr_2$ gadījumā.

Reakcijā starp (*Boc*-*Sec*)$_2$ un **10k** gan $CuBr_2$, gan $K_2S_2O_8$ (5 equiv.) klātienē veidojās tikai zīmes no cerētā benz[*b*]furāna. Taču, palielinot $K_2S_2O_8$ daudzumu līdz 50 ekv., izdevās panākt pilnīgu izejvielu konversiju 16 h laikā un izolēt savienojumu **27f** ar augstu iznākumu. Ņemot vērā to, ka $K_2S_2O_8$ šķīdība acetonitrilā ir ārkārtīgi zema, faktiskais oksidējošā aģenta daudzums reakcijas maisījumā ir zems. Tādējādi ir pilnīgi pieņemami izmantot lielāku daudzumu $K_2S_2O_8$,

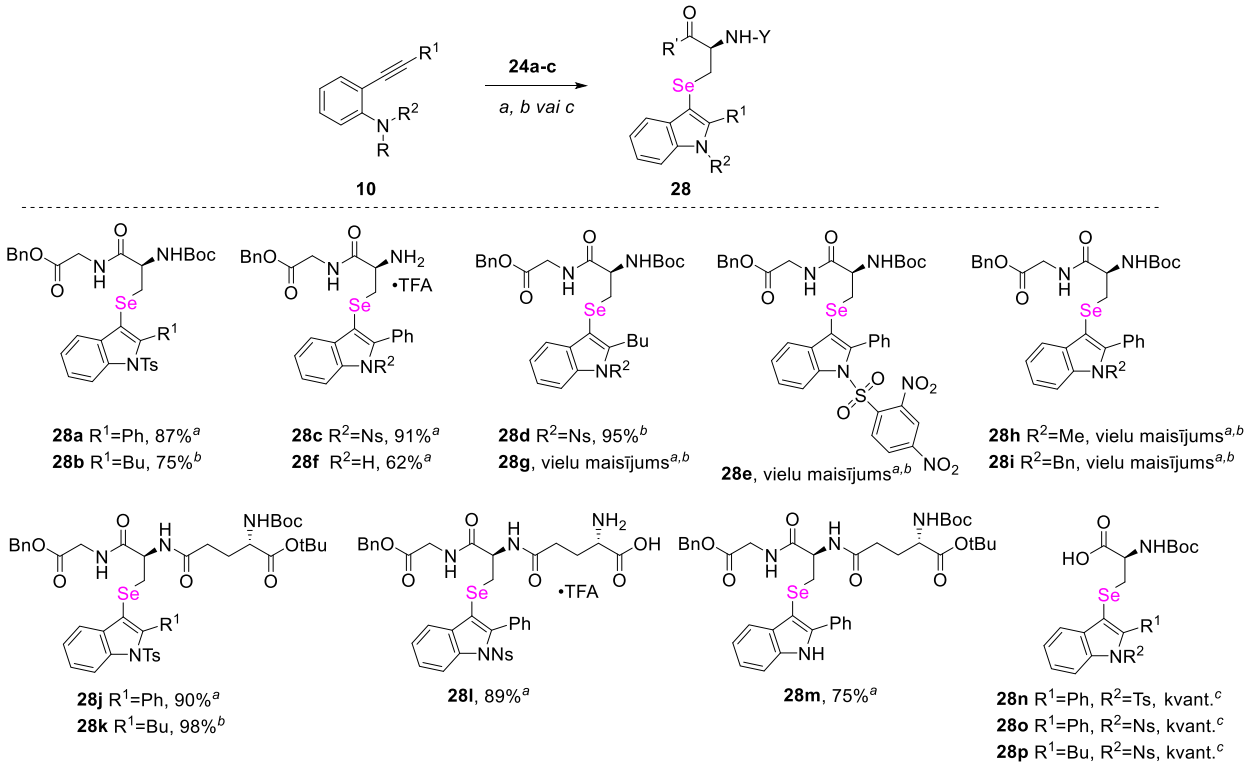
jo tas nebojā izejvielas, pēc reakcijas beigām to var vienkārši nofiltrēt, un jāuzsver, ka tas ir lēts, neorganisks reaģents.

Diemžēl neviena no iepriekš minētajām metodēm nebija piemērota sulfenilelektrofīla ģenerēšanai no **8** un sekojošai ciklizēšanai, veidojot glutationu saturošu benz[*b*]furānu.



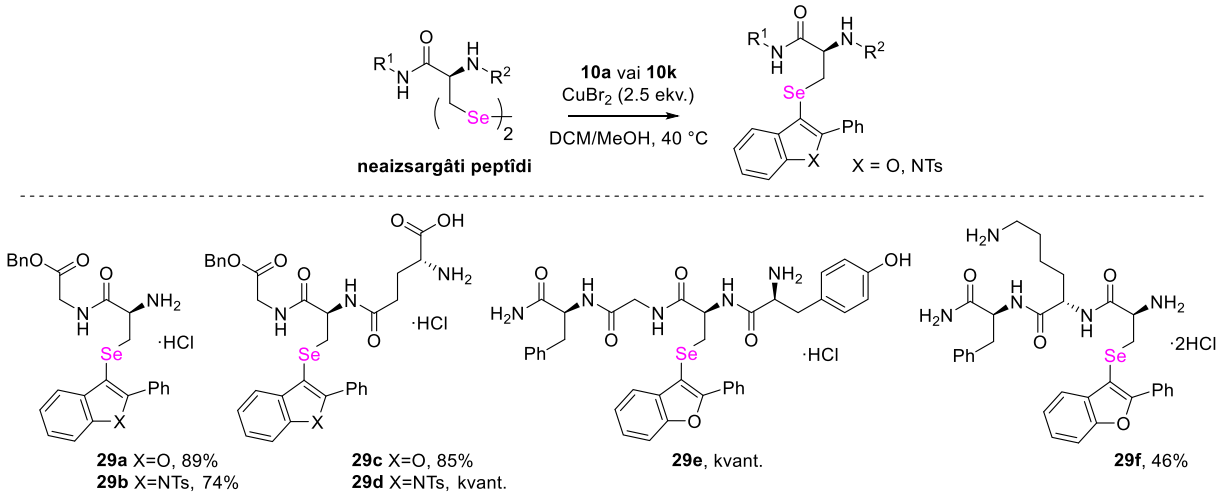
9. shēma. 3-selenilbenz[*b*]furānu sintēze. Reakcijas apstākļi: a) 1. $CuBr_2$ (1,5 ekv.), *DCM*, 40 °C; (2. *TFA*, *DCM*); b) $K_2S_2O_8$ (5 ekv.), MeCN, it; c) $K_2S_2O_8$ (50 ekv.), MeCN, it.

Analogā veidā $CuBr_2$ iniciēta selenilelektrofīla ģenerēšana un sekojoša 5-*endo-dig* ciklizācija tika lietota 2-aril-3-selenilindolu sintēzei, savukārt $K_2S_2O_8$ iniciēta selenilelektrofīla ģenerēšana bija piemērota 2-alkil-3-selenilindolu, kā arī *Boc-Sec* saturošu indolu sintēzei (10. shēma). Substrātu klāsta pārbaude parādīja, ka *Ts*, *Ns* un *Boc* aizsarggrupas ir tolerantas pret reakcijas apstākļiem, savukārt 2,4-dinitrofenilsulfonil, *N,N*-dimetil-, *N,N*-dibenzil, *N*-benzil-2-(feniletinil)anilīni, kā arī neaizsargāti 2-(feniletinil)anilīni nav piemēroti substrāti.



10. shēma. 3-selenilindolu sintēze. Reakcijas apstākļi: a) 1. $CuBr_2$ (1,5 ekv.), *DCM*, 40 °C; (2. *TFA*, *DCM*); b) $K_2S_2O_8$ (5 ekv.), MeCN, it; c) $K_2S_2O_8$ (50 ekv.), MeCN, it.

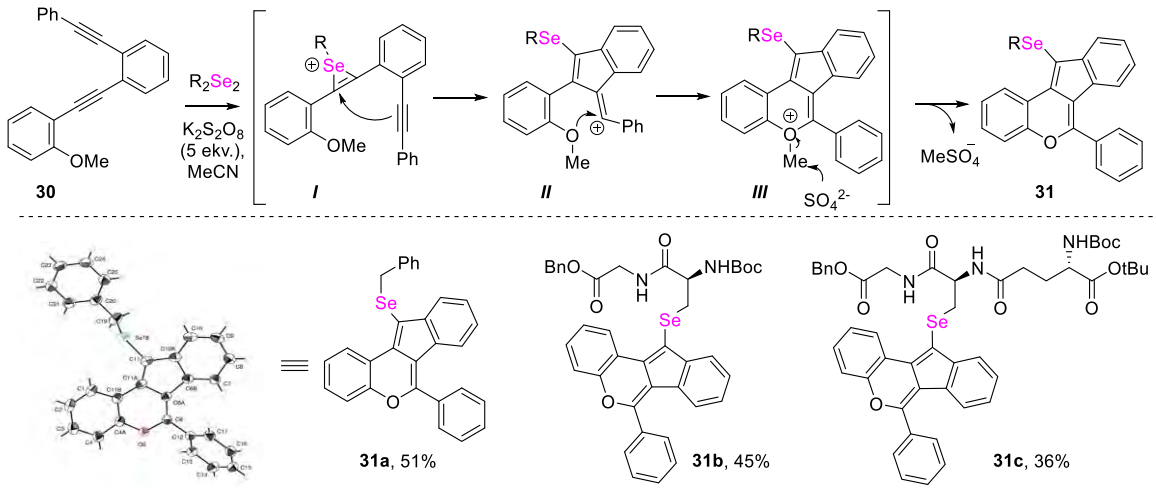
Svarīgi, ka $CuBr_2$ iniciētu selenilelektrofīla ģenerēšanu var lietot arī neaizsargātiem peptīdiem. Šis fakts ir nozīmīgs un ievērojami atvieglo sarežģītāku peptīdu lietošanu. Reakcija noritēja viegli, jo protonētās aminogrupas nespēj veidot kompleksu ar $CuBr_2$. Visi pārbaudītie selēncistīnu saturošie peptīdi uzrādīja izcilu reaģētspēju, un produkti veidojās ar augstiem iznākumiem (11. shēma).



11. shēma. Benz[*b*]furānu un indolu sintēze, izmantojot neaizsargātus peptīdus. Produkti tika attīrīti ar apgrieztās fāzes hromatogrāfijas palīdzību ($MeCN/H_2O$, pH = 4, sālsskābe).

Nākamais izaicinājums bija noskaidrot, vai ir iespējams ģenerēt selēna elektrofīlu un izmantot to kaskādes tipa reakcijā. Tika nolemts izmantot anizolu saturošu arildiīnu **30**, kas veiksmīgas reakcijas gadījumā (sekojoša 5- un 6-*endo-dig* ciklizācija) veidotu indēn[1,2-*c*]hromēnu. Hromēna cikls sastopams bioloģiski aktīvos dabas produktos, turklāt savienojumi, kas satur indēn[1,2-*c*]hromēna fragmentu, ir uzrādījuši augstu potenciālu lietojumam krāsvielu fotoelektriskajās šūnās. Turklāt šāda tipa savienojumu sintēzei ir zināmas tikai dažas metodes. Zināms, ka iespējams izmantot TfOH iniciētu kaskādes reakciju, lai no anizola **30** iegūtu 6-fenilindēn[1,2-*c*]hromēnu,[35] vai arī ciklizēšanu iespējams veikt halogēnu klātienē, iegūstot halogēna atomu saturošus 6-fenilindēn[1,2-*c*]hromēnus.[36]

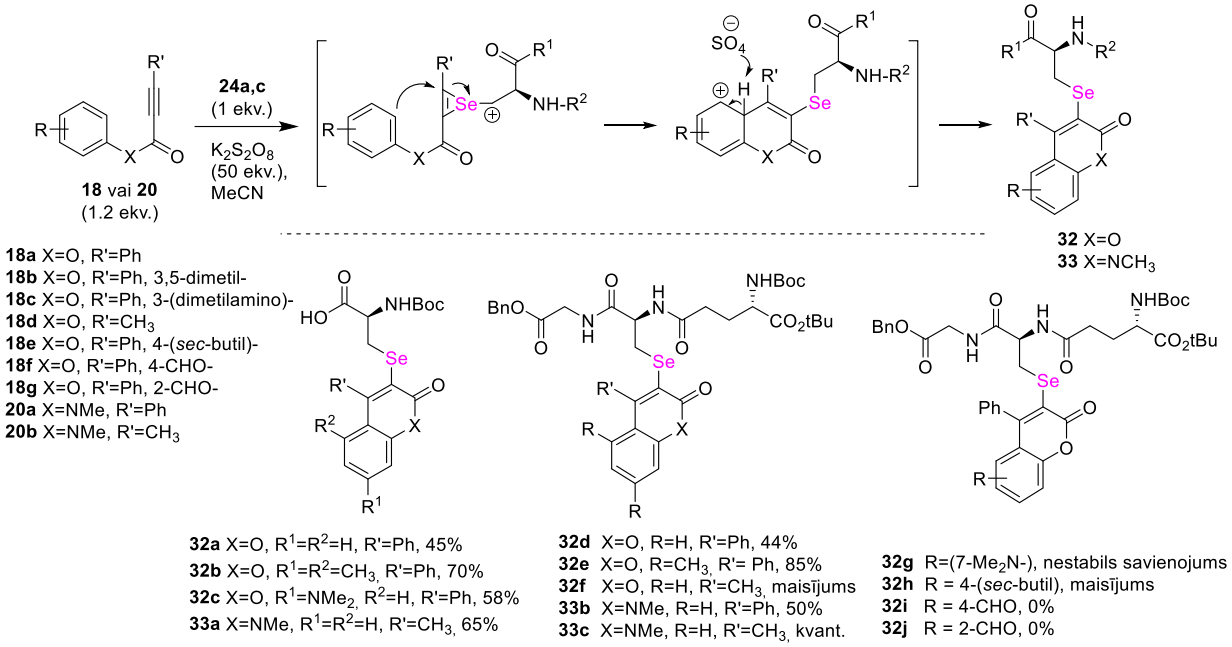
Vispirms tika pārbaudīts Ph_2Se_2 ar **30** $CuBr_2$ klātienē, taču tika novērota tikai 11-brom-6-fenilindēn[1,2-*c*]hromēna veidošanās. Cerētais produkts netika iegūts arī, izmantojot $K_2S_2O_8$. Savukārt $K_2S_2O_8$ bija spējīgs iniciēt selenilelektrofīla veidošanos no Bn_2Se_2 un selēncistīnu saturošiem peptīdiem, kas tālāk reaģēja ar elektroniem bagātāko trīskāršo saiti, veidojot selēnirēnija jonu ***I***. Sekojošs otras trīskāršās saites uzbrukums selēnirēnija katjonam veidoja indēna ciklu (starpprodukts ***II***). Metoksigrupas uzbrukums karbkatjonam veidoja ciklizēšanās starpproduktu ***III***, kas pēc demetilēšanas veidoja 11-(benzilselenil)-6-fenilindēn[1,2-*c*]hromēnu **31a** (12. shēma). Savienojuma **31a** struktūra tika viennozīmīgi apstiprināta ar rentgenstruktūras analīzes datiem. Līdzīgā veidā tika iegūti *Sec*-peptīdu saturošie 6-fenilindēn[1,2-*c*]hromēni, produktu iznākumi gan bija tikai viduvēji sarežģītās produktu attīrīšanas dēļ.



12. shēma. Piedāvātais kaskādes 5-*endo-dig*/6-*endo-dig* ciklizācijas mehānisms.

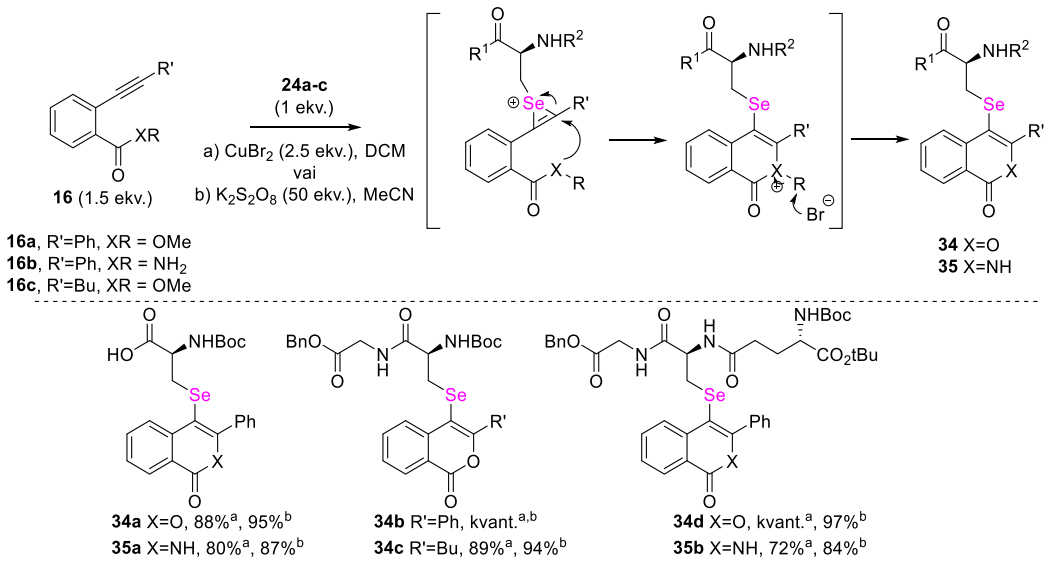
Tālāk tika nolemts pārbaudīt, vai iespējams lietot $CuBr_2$ vai $K_2S_2O_8$ metodi selenilelektrofīla ģenerēšanai un sekojošai 6-*endo-dig* ciklizācijai. Vispirms nolemts šādā veidā iegūt kumarīnus. Izrādījās, ka reakcija starp $(Boc\text{-}Sec)_2$ un fenil-3-fenilpropiolātu $K_2S_2O_8$ ļauj iegūt atbilstošo *Boc-Sec* saturošo kumarīnu **32a** (13. shēma). Substrātu klāsta pārbaude ļāva secināt, ka EDG aromātiskā gredzena 3. vai 3. un 5. pozīcijā veicināja ciklizāciju, savukārt EAG ievadīšana aromātiskajā gredzenā samazināja substrāta reaģētspēju. Aizsargāts selēnglutations arī bija piemērots substrāts ciklizācijas reakcijai, taču diemžēl neizdevās izolēt selēnglutationu saturošo 7-aminokumarīnu tā nestabilitātes dēļ. Tādos pašos apstākļos ar labiem

iznākumiem bija iespējams iegūt analogos slāpekli saturošos heterociklus – hinolīn-2-onus **33a-c**.



13. shēma. 3-*Sec*-kumarīnu un hinolīn-2-onu sintēze.

Svarīgi, ka citu 6-*endo*-*dig* ciklizāciju, kas ļāva iegūt *Sec* saturošus izokumarīnus un izohinolīn-2-onus (14. shēma), varēja viegli veikt, izmantojot gan $K_2S_2O_8$, gan $CuBr_2$ iniciētu selenilelektrofīla ģenerēšanu. Abos gadījumos produkti veidojās ar izciliem iznākumiem, vienīgā atšķirība bija reakcijas laiks – $CuBr_2$ klātienē pilnīga izejvielu konversija tika sasniegta 2 h, $K_2S_2O_8$ klātienē – 24 h.



14. shēma. 4-*Sec*-izokumarīnu un izohinolīn-2-onu sintēze.

Pirmais solis gan izokumarīnu izveidei, gan kumarīnu izveidei ir selenilelektrofīla ģenerēšana, kas pēc tam reaģē ar trīskāršo saiti, veidojot selēnirēnija jonu. Pretēji kumarīna cikla izveidei, kas balstās uz elektrofīlo aromātisko aizvietošanos, izokumarīna cikls veidojas nukleofīlā heteroatoma uzbrukuma selēnirēnija jonam rezultātā, tāpēc šī reakcija notiek daudz vieglāk.

Oriģinālpublikācijas par šajā apakšnodaļā aprakstītajiem pētījumiem – 1., 2. un 6. pielikumā.

3. Redzamās gaismas iniciēta selēncistīnu saturošo peptīdu funkcionalizēšana: indolu selenilēšana un makrociklizēšana

Fotokatalizētas reakcijas ir ērta pieeja sarežģītu savienojumu sintēzei maigos apstākļos. Šis reakciju tips tiek uzskatīts par videi draudzīgu, efektīvu un selektīvu, turklāt reakcijas var veikt arī biosavietojamos apstākļos. Parasti reakcijas efektīvai norisei nepieciešams fotokatalizators. Populārākie homogēnu katalizatoru piemēri ir pārejas metālu kompleksi un organiskās krāsvielas. Protams, ka pārejas metālu (Ru, Ir) kompleksi ir dārgi un tos nevar uzskatīt par videi draudzīgu izvēli. Tāpēc pievilcīga alternatīva ir organiskās krāsvielas. Iespējams izmantot arī heterogēnus katalizatorus (neorganiskus pusvadītājus (metālu oksīdus vai sulfīdus), grafīta tipa oglekļa nitrīdu polimērus, fotoaktīvus MOFus). Heterogēns katalizators sniedz iespēju katalizatora atkārtotai izmantošanai un atvieglo produkta attīrīšanu.

Kā populārākās organisko krāsvielu klases jāmin akridīni un akridīnija sāļi, fluoresceīns un tā atvasinājumi, benzofenoni, pirīlija sāļi, rodamīni un fentiazīni. Bengālijas rozā (RB) ir fluoresceīna struktūranalogs, un tas ir zināms kā efektīvs fotokatalizators ar plašu lietojumu.[37]

Absorbējot gaismu, RB tiek aktivēts un nonāk ierosinātā singleta stāvoklī (RB*) ($t^{1/2} = 10^{-6}$ līdz 10^{-9} s). Ierosinātais singleta stāvoklis var atgriezties pamata stāvoklī vai arī tas var pārvērsties par ilgāk dzīvojošo tripleta ierosināto stāvokli ($t^{1/2} = 10^{-3}$ s) caur starpsistēmu šķēršošanu (*intersystem crossing*).[38] Visbiežāk reakcijas ar RB notiek pēc viena elektrona pārneses (SET) mehānisma, bet iespējams arī enerģijas pārneses (EnT) mehānisms, it sevišķi singleta skābekļa veidošanās procesā. Šajā gadījumā veidojas ierosinātais RB*, kas pārnes enerģiju uz substrātu, veidojot reaģētspējīgu vielu.[37, 39]

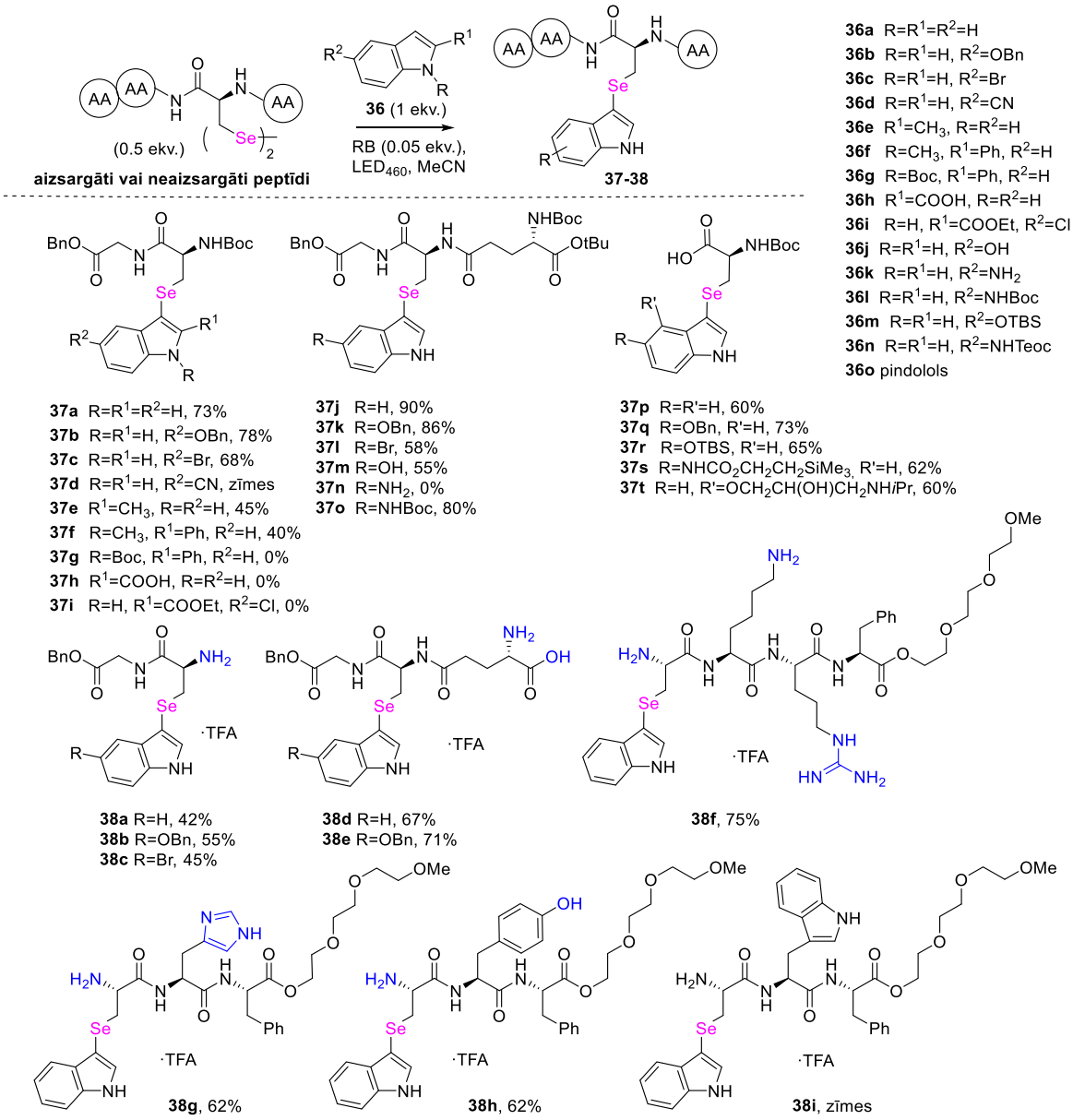
Ierosinātais RB* var būt gan oksidētājs, gan reducētājs. Reducējošās dzēšanas ciklā organiskā viela tiek oksidēta. Šajā gadījumā RB* pārvēršas par $RB^{\bullet -}$ pēc SET mehanisma, vienlaikus notiek substrāta pārveide par radikāļa katjonu. Tad, oksidējot $RB^{\bullet -}$ ar skābekli, veidojas pamata stāvokļa RB. Līdzīgi oksidējošās dzēšanas ciklā substrāts tiek reducēts, savukārt katalizators oksidējas.[39]

Lai gan ir veikti pētījumi par vienkāršu diarildiselenīdu izmantošanu redzamās gaismas iniciētās reakcijās,[40, 41] to pašu nevar apgalvot par Se–Se saiti saturošiem peptīdiem. Zināms, ka, apstarojot selēncistīnu saturošus peptīdus ar *UV* gaismu, iespējams iegūt selēnlantionīnus,[42] savukārt diselenīdu metatēzi starp vienkāršiem diorganildiselenīdiem[43] vai Se–Se saiti saturošiem peptīdiem[44] var veikt, izmantojot redzamo gaismu.

Tika nolemts sākt ar reakciju starp (*Boc*-*Sec*-*Gly*-*OBn*)$_2$ **24b** un 1*H*-indolu (**36a**), apstarojot šķīdumu ar zilo *LED* gaismu (maks. 460 nm, koši zila, $x = 0{,}1440$, $y = 0{,}0395$, > 50 000 lx) (15. shēma). Lai atrastu piemērotāko fotokatalizatoru, reakcija tika veikta acetonitrilā, izmantojot 0,5 ekv. **24b**, 1 ekv. **36a**, 2 mol% pārejas metālu kompleksu vai 5 mol% organisko krāsvielu. Pārsteidzoši, bet tikai divi no pārbaudītajiem fotokatalizatoriem bija spējīgi nodrošināt selektīvu 3-selenilindola **37a** sintēzi: Bengālijas rozā (RB) un tā analogs eritrozīns B. RB bija nedaudz efektīvāks, tādēļ tas izmantots turpmākajiem pētījumiem. Citi pārbaudītie fotokatalizatori (5-TAMRA, niķeļa tetrafenilporfirīns, 4-CzIPN, krezolsarkanais, hlorfenola sarkanais, bromkrezolzaļais, metiloranžs, Kongo sarkanais, tiešais sarkanais 81, tiešais dzeltenais 27, metilēnzilais, bāziskais fuksīns, indigokarmīns, alciānzilais, 2,4,6-trifenilpirīlija tetrafluorborāts, 9-mezitil-10-metilakridīnija tetrafluorborāts, akridīns un *N*-metilakridīnija jodīds) bija ievērojami mazāk efektīvi vai arī ierosināja neselektīvu reakciju. Acetonitrils izrādījās piemērotākais šķīdinātājs, jo citos pārbaudītajos protonos šķīdinātājos (MeOH, EtOH, EtOH/H_2O un *i*PrOH) notika neselektīva reakcija savienojuma **24b** ātras oksidēšanas un deselenilēšanas dēļ. Kontroles reakcijās bez RB, dienasgaismā vai tumsā neveidojās **37a**, tādējādi apstiprinot, ka reakcijas norisei ir nepieciešams gan RB, gan LED_{460}. Reakciju veicot degazētos apstākļos, izejvielas konversija bija ievērojami zemāka. Produkta veidošanās netika novērota, reakciju veicot 4-amino-*TEMPO* klātienē, kas liecināja par radikāļu mehānismu. Secinājām, ka optimālie reakcijas apstākļi 3-Sec-indolu sintēzei ir 5 mol% RB, MeCN, zilā *LED* gaisma, 90 min. Jāuzsver, ka šī ir atomekonomiska metode, jo tiek izmantotas abas diselenīda daļas.

Substrātu klāsta pārbaude atklāja, ka EDG indola C5 pozīcijā uzlabo reakcijas iznākumu, halogēna atomi īpaši neietekmēja reakciju, savukārt EAG samazināja indola reaģētspēju. EDG C2 pozīcijā samazināja produkta iznākumu, savukārt elektroniem nabadzīgi indoli (EAG N1 vai C2 pozīcijās) bija pilnīgi reaģētnespējīgi. Svarīgi, ka reakcija ar 5-hidroksiindolu noritēja veiksmīgi, savukārt 5-aminoindols nebija reaģētspējīgs, taču *terc*-butil-(1*H*-indol-5-il)karbamāta **2l** izmantošana ļāva sintezēt vēlamo produktu **3o**. Turklāt arī (*Boc*-*Sec*)$_2$ bija piemērots substrāts indolu selenilēšanai, tādējādi tika iegūti produkti **37p-t**, kas ir vērtīgi starpprodukti sarežģītāku vielu sintēzei.

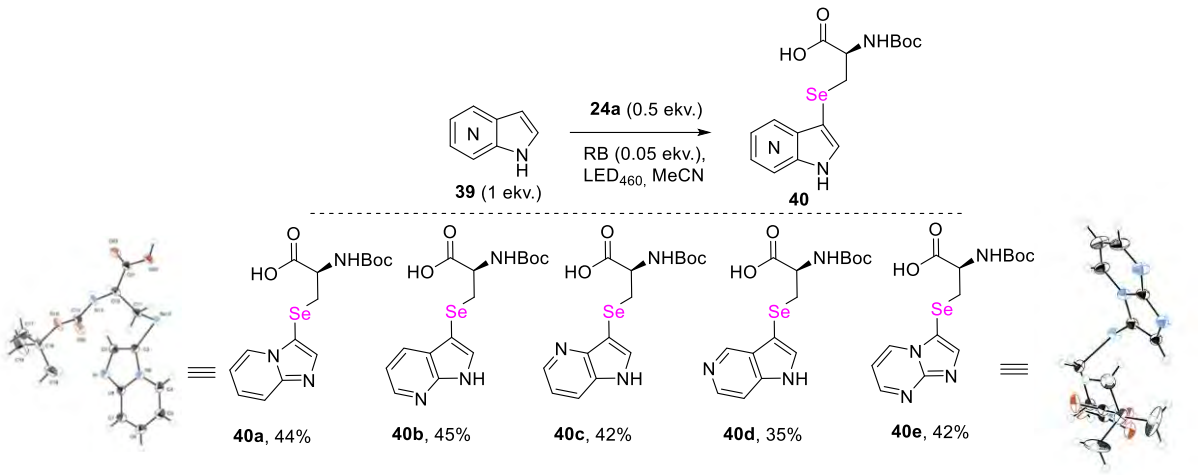
Svarīgi, ka neaizsargāti selēncistīnu saturošie peptīdi uzrādīja augstu toleranci reakcijas apstākļos, kas, protams, ievērojami paplašina metodes lietojumu. Pat sarežģītāki selēncistīnu saturošie peptīdi ar "sensitīvām" aminoskābēm (*Lys*, *Arg*, *His*, *Tyr*) veidoja attiecīgos *Sec*-indolus ar labiem iznākumiem. Vienīgais izņēmums bija *Trp* saturošais peptīds – šajā gadījumā notika neselektīva reakcija, visticamāk, tāpēc, ka *Trp* spēj veidot radikāli redzamās gaismas ietekmē.



15. shēma. *Sec*-indolu sintēze.

38a-i: produktu iegūšanai izmantoti neaizsargāti peptīdi TFA sāls formā.

Lai paplašinātu substrātu klāstu, tika iegūti arī *Boc-Sec*-azaindoli **40** (16. shēma). Taču tika atklāts, ka tikai protonēti azaindoli spēj reaģēt ar $(Boc\text{-}Sec)_2$, turklāt izejvielas **24a** daļējas sadalīšanās dēļ reakcijas iznākumi bija tikai viduvēji. Produktu **40a** un **40e** struktūras tika apstiprinātas ar rentgenstaru spektroskopijas analīzes datiem.

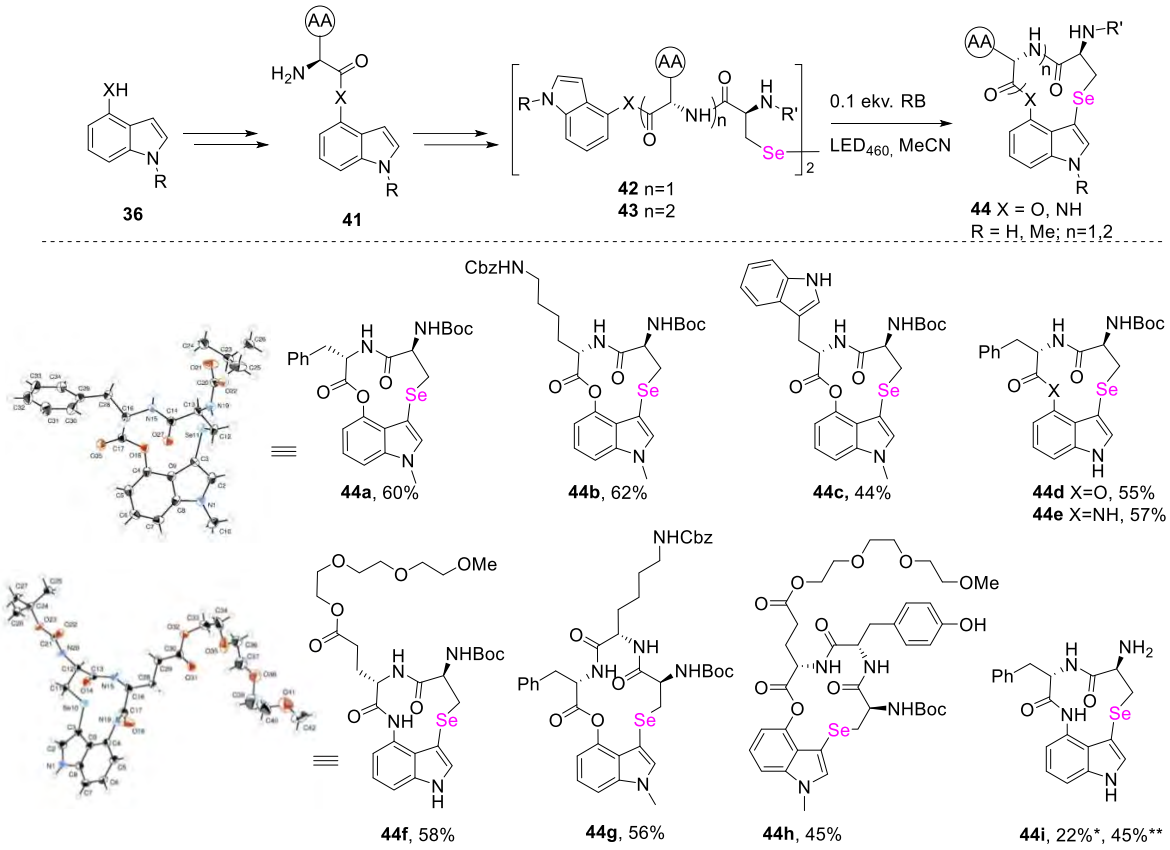


16. shēma. *Boc-Sec*-azaindolu sintēze.

Tika nolemts izmantot izstrādāto protokolu, lai pārbaudītu iespējas veikt iekšmolekulāru indolu selenilēšanu (A stratēģija). Šādā veidā veiksmīgas reakcijas rezultātā veidotos *Sec* saturoši indola makrocikli. Turklāt izstrādātas arī alternatīvas pieejas makrociklu sintēzei: redzamās gaismas iniciēta indola (kas jau satur aminoskābi vai īsu peptīdu C4 vai C5 pozīcijās) selenilēšana un sekojoša iekšmolekulāra amīda saites izveide (B stratēģija); redzamās gaismas iniciēta reakcija starp $(Boc\text{-}Sec)_2$ un aizsargātu 5-hidroksi- vai 5-aminoindolu, sekojoša reakcija ar nelielu peptīdu, aizsarggrupu nošķelšana un iekšmolekulāra amīda saites izveide (C stratēģija).

Redzamās gaismas iniciēta reakcija izrādījās piemērota iekšmolekulārai indolu selenilēšanai, kā rezultātā makrocikli **44a-h** tika izolēti ar labiem iznākumiem (17. shēma). Lai gan reakcija noritēja lēnāk nekā starpmolekulāra selenilēšana, svarīgi, ka tā bija selektīva. Turklāt makrociklu **44a** un **44f** struktūras tika viennozīmīgi apstiprinātas ar rentgenstruktūras analīzes datiem. Tika izdalīts arī neaizsargāts makrociklu **44i**. Produkts tika iegūts makrociklizācijas reakcijā, izmantojot *Fmoc* aizsargātu substrātu un pēc tam nošķeļot aizsarggrupu. Alternatīva metode savienojuma **44i** sintēzei ir vispirms nošķelt *Fmoc* aizsarggrupu un pēc tam veikt ciklizēšanas reakciju, taču šajā gadījumā reakcijas iznākums bija zemāks.

Diemžēl tika secināts, ka A stratēģija nav piemērota, lai ciklizētu indolus, kas peptīdu satur C5 pozīcijā, iespējams, konformācijas ierobežojumu dēļ, tādēļ nolemts lietot citu pieeju šo savienojumu sintēzei.

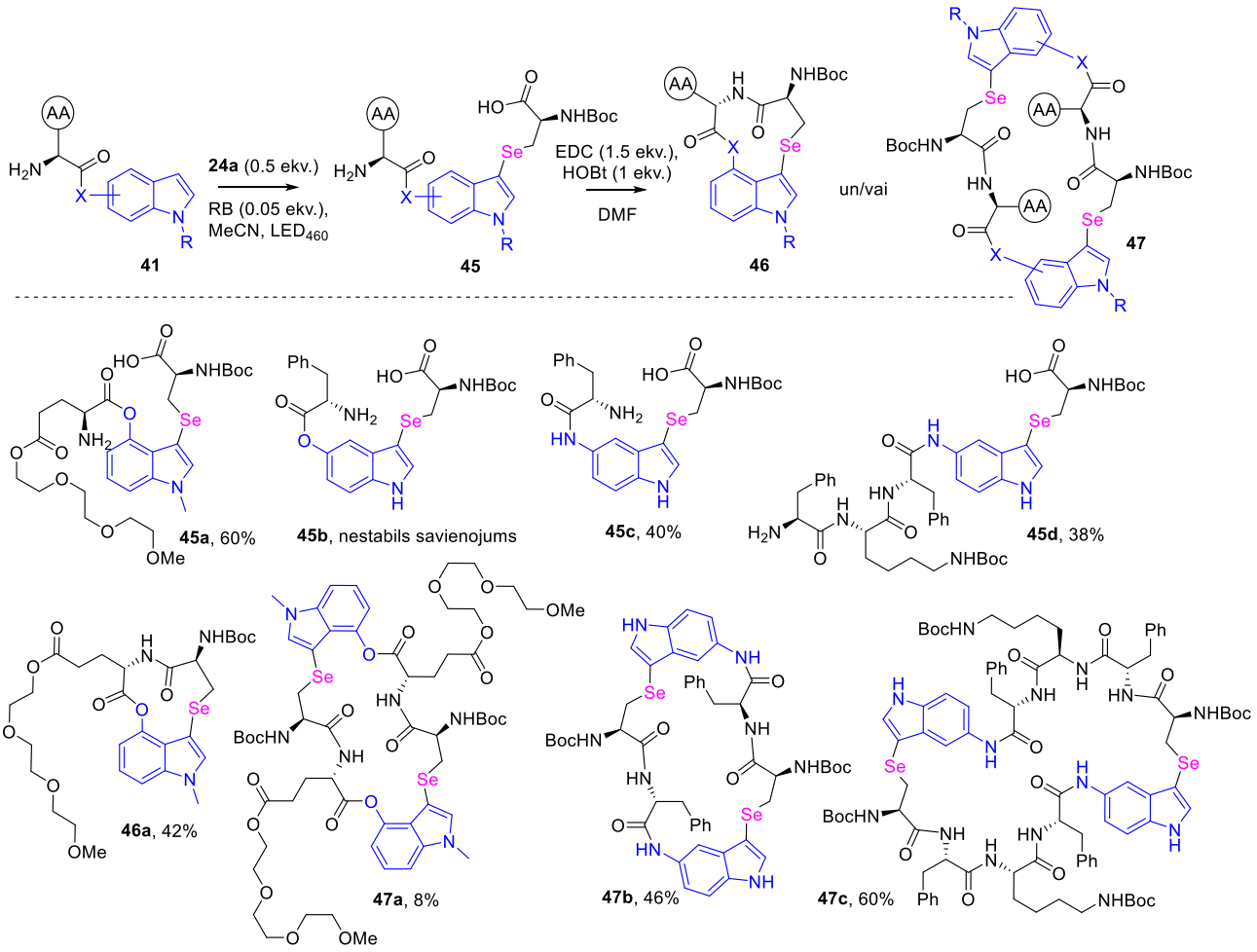


17. shēma. Makrociklu sintēze: A stratēģija.

**Fmoc* nošķelšana, ciklizēšana, **ciklizēšana, *Fmoc* nošķelšana.

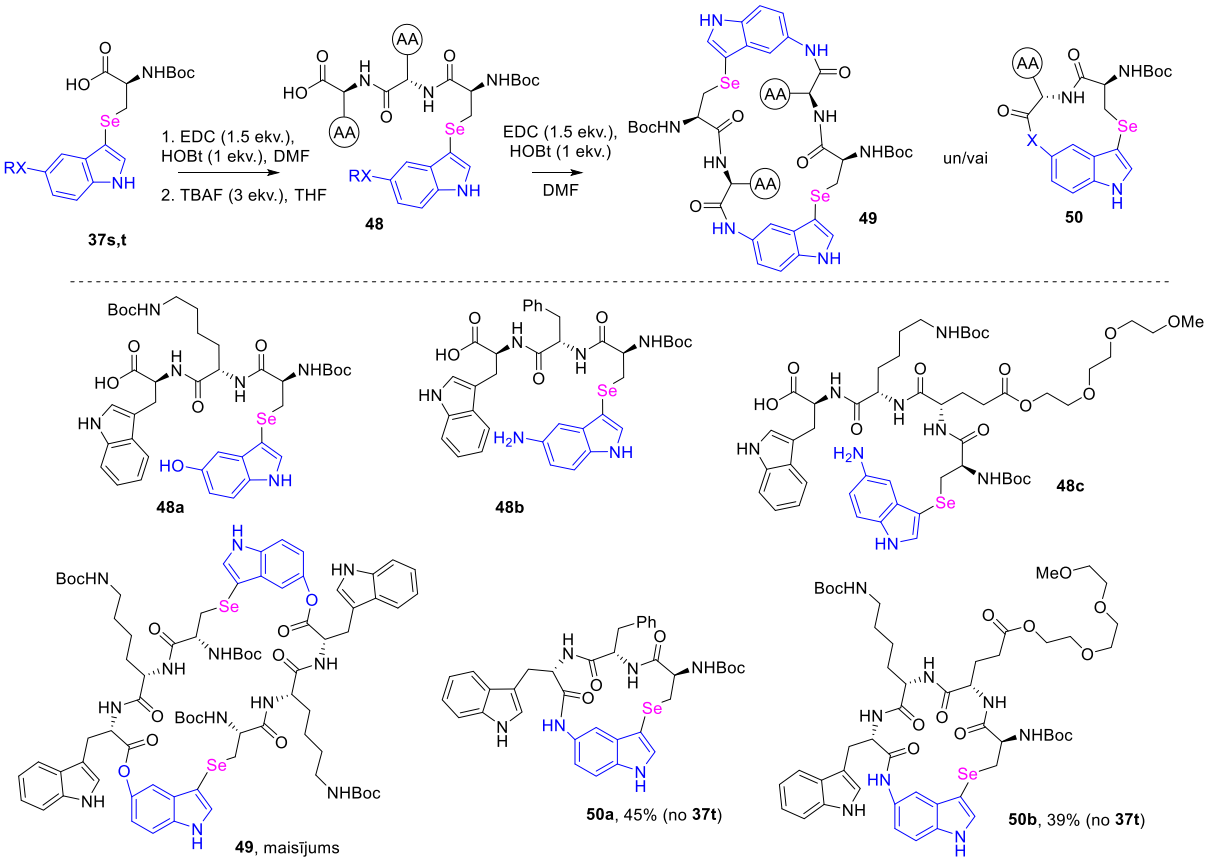
B stratēģija balstījās uz redzamās gaismas iniciētu indolu **41** selenilēšanu, kas jau saturēja aminoskābi vai īsu peptīdu C4 vai C5 pozīcijā. Sekojoša iekšmolekulāra amīda saites izveide ļāva iegūt makrociklus (18. shēma).

Redzamās gaismas iniciēta reakcija ļāva iegūt *Boc-Sec* saturošus indolus **45** ar viduvējiem līdz labiem iznākumiem. Iekšmolekulārai amīda saites izveidei tika izmantota EDC/HOBt metode. Rezultātā makrocikls **46a** tika izolēts, izmantojot 4-aizvietotu indolu **45a** kā izejvielu. Izdevās izolēt arī reakcijas minoro produktu – bis-makrociklizēšanas produktu **47a**. Savukārt indolu **45c** un **45d**, kas saturēja aizvietotāju C5 pozīcijā, izmantošana rezultējās ar bis-makrociklizēšanas produktu **47b** un **47c** izolēšanu.



18. shēma. Makrociklu sintēze: B stratēģija.

C stratēģija balstījās uz produktu **37s** un **37t** izmantošanu, kas tika iegūti redzamās gaismas iniciētā reakcijā, selenilējot aizsargātus 5-hidroksi- un 5-aminoindolus (19. shēma). Nākamā stadija bija starpmolekulāra amīda saites izveidei, tad pēc aizsarggrupu nošķelšanas tika veikta iekšmolekulāra amīda saites izveide. Bis-makrocikla **49** sintēze nenoritēja selektīvi, savukārt makrocikli **50a** and **50b** tika izolēti ar labiem iznākumiem, izmantojot savienojumus **48b** un **48c**.



19. shēma. Makrociklu sintēze: C stratēģija.

Nākamais uzdevums bija izpētīt redzamās gaismas iniciētu indolu selenilēšanas mehānismu. Pirmkārt, tika novērots, ka, apstarojot šķīdumu, kas satur **24b** un RB, veidojas nestabila selenīnskābe **51** un *Boc-Dha-Gly-OBn* **53** (20. shēma). Savienojuma **51** izolēšana nebija iespējama, taču tas tika detektēts ar ŠH-MS, AIMS ([M + Na] = 471,0633) un ^{77}Se KMR spektroskopiju ($RSeO_2H$ 1217,5 ppm) (2. A att.). Reakcijas maisījums KMR stobriņā pēc 24 h uzrādīja, ka savienojums **51** ir sadalījies, veidojot citu vielu – selēnpaskābi (H_2SeO_3 **52**) (1302,7 ppm).

Kontroles testi apstiprināja, ka ūdens un skābeklis šķīdinātājā ir nepieciešami reakcijas norisei. Produkts **53** netika iegūts, reakciju veicot *TEMPO* klātienē, kas apstiprināja, ka reakcija notiek pēc radikāļu mehānisma.

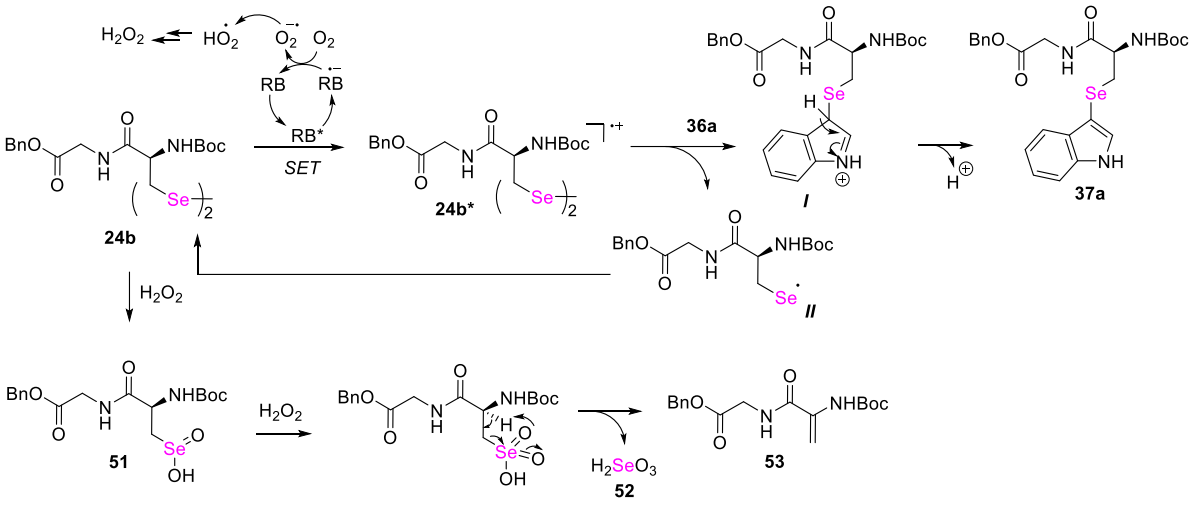
Redzamās gaismas ietekmē RB klātienē skābeklis veido superoksīda anjonradikāli ($O^{\bullet -}$), savukārt $RB^{\bullet -}$ atgriežas pamata stāvoklī (20. shēma). Reakcijā starp $O^{\bullet -}$ un ūdeni veidojas hidroperoksiradikāļi, kas veido ūdeņraža peroksīdu. Ūdeņraža peroksīds reaģē ar **24b**, notiek oksidēšanas reakcija un sekojoša deselenilēšana, veidojot dubultsaiti saturošo savienojumu.

Savienojumiem **24b**, **36a**, RB, etileozīnam, eritrozīnam B, FIrPic, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ un 4-CzIPN tika uzņemti *UV* spektri sausā acetonitrila šķīdumā (2. B un 2. C att.). Fotoķīmiskā reakcija starp **36a** un diselenīdu **24b** ir neefektīva bez katalizatora, jo indola absorbija ir 200 nm līdz 305 nm, savukārt **24b** piemīt absorbcija līdz 430 nm, taču ar zemu intensitāti. Svarīgi, ka absorbijas plecs pie 275–430 nm savienojuma **24b** *UV* spektrā ir raksturīgs Se–Se saitei, kas atvieglo selēna radikāļa izveidi fotokatalizatora klātienē.

Fotoluminescences dzēšanas eksperimenti RB, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ un FIrPic tika veikti, izmantojot **24b** vai **36a** degazētā acetonitrilā. Dzēšanas ātruma konstante RB eksperimentā ar **24b** bija $10{,}42 \times 10^{-3}$ l/mol (2. D att.). Savukārt **36a** nedzēš RB fluorescenci ievērojamā daudzumā.

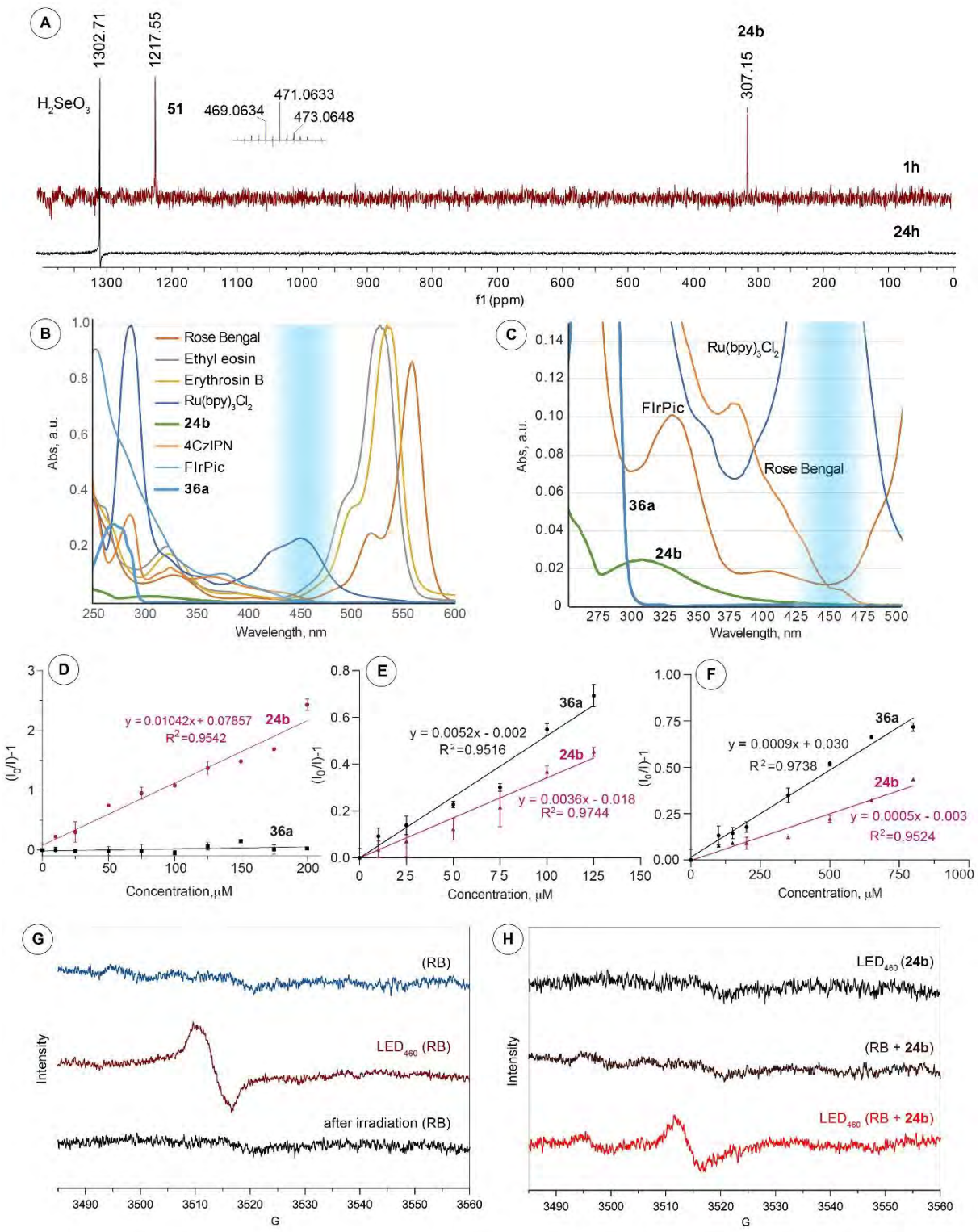
EPR eksperimenti apstiprināja radikāļu veidošanos RB šķīdumā, apstarojot to ar LED_{460} gaismu. Turklāt diselenīda **24b** klātiene samazināja RB* signāla intensitāti, ļaujot apstiprināt dinamiska līdzsvara izveidi LED_{460} apstākļos: RB* + **24b** ↔ (RB + **24b***).

Tādējādi, balstoties uz iegūtajiem rezultātiem, secināts, ka, ierosinot RB veidojas diselenīda katjonradikālis **24b***, kas reaģē ar indolu **36a**, veidojot starpproduktu ***I*** un selēna radikāli ***II***. Radikālis ***II*** var dimerizēties par diselenīdu **24b**, savukārt starpprodukts ***I*** pēc deprotonēšanas izveido produktu **37a** (20. shēma).



20. shēma. Piedāvātais mehānisms redzamās gaismas iniciētai indolu selenilēšanai.

Oriģinālpublikācija par šajā apakšnodaļā aprakstītajiem pētījumiem – 4. pielikumā.



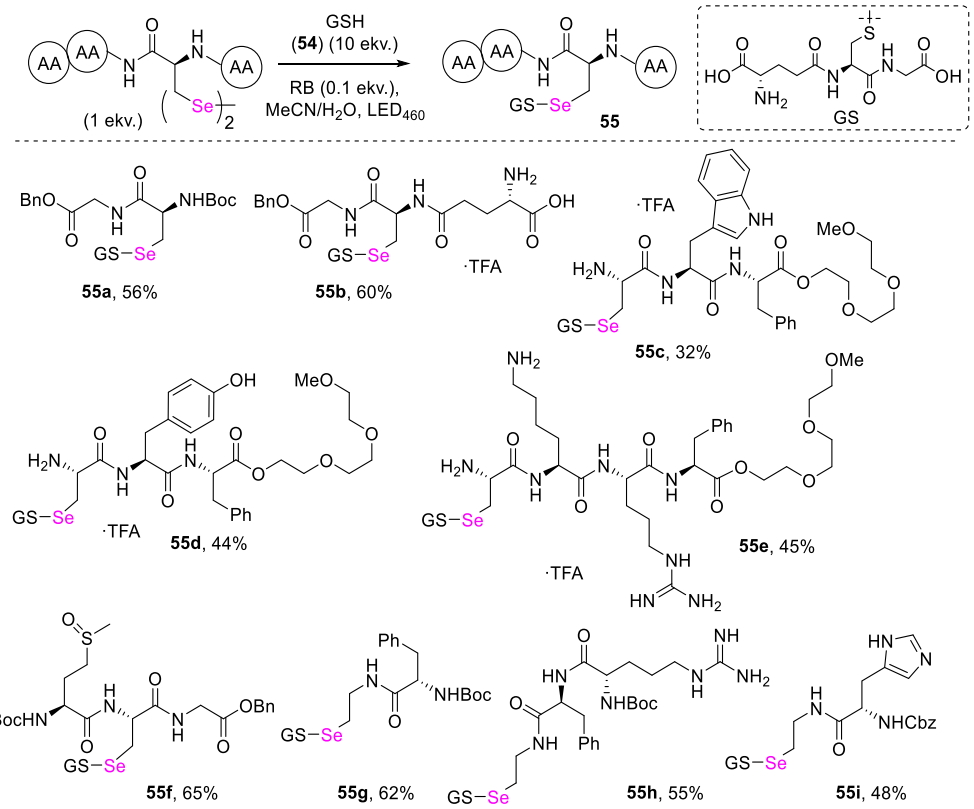
2. att. (A) ^{77}Se KMR spektri **24b** sadalīšanās produktiem pēc 1 h ilgas apstarošanas un pēc 24 h uzglabāšanas KMR stobriņā; (B) **24b**, **36a** un fotokatalizatoru *UV* spektri; (C) pietuvināts *UV* spektru 250-500 nm reģions; (D) RB fotoluminescences dzēšana ar **24b** un **36a**; (E) $Ru(bpy)_3Cl_2 \cdot 6H_2O$ fotoluminescences dzēšana ar **24b** un **36a**; (F) FIrPic fotoluminescences dzēšana ar **24b** un **36a**; (G) EPR spektri: RB un RB + **24b** ar un bez LED_{460} apstarošanas.

4. Redzamās gaismas iniciēta Se–S saiti saturošu peptīdu sintēze

Se–S saite ir sastopama tioredoksīna reduktāzes aktīvajā centrā, kas ir viena no svarīgākajām antioksidantu sistēmas sastāvdaļām cilvēka šūnās.[45, 46] Se–S saiti saturošs starpprodukts veidojas glutationa peroksidāzes katalītiskajā ciklā – selēnenzīms, kas ir atbildīgs par H_2O_2 un citu peroksīdu reducēšanu ar glutationu.[47] Mazas molekulārās masas savienojumi ar Se–S saiti ir izmantoti kā fluorescenti marķieri reaģētspējīgu sēra daļiņu detektēšanai[48, 49] un arī kā priekšzāles proteīna tirozīna fosfatāzes inhibēšanai.[50]

Savienojumi, kas satur Se–S saiti, tiek uzskatīti par nestabiliem, tāpēc to sintēze ir sarežģīta.[51] Teorētiski ir iespējama apmaiņas reakcija starp diselenīdu un tiolu, tomēr tā nav efektīva, jo selenolāta blakusprodukts ir stiprāks nukleofīls nekā tiols.[52] Tomēr piemērotos apstākļos reakciju ir iespējams veikt.[51] Difenildiselenīds reaģē ar $AgSCF_3$, veidojot attiecīgo Se–S saiti saturošo produktu, jo selenolāta blakusprodukts ir stabilizēts ar sudraba jonu.[53] Parasti Se–S saites izveidei izmanto reakciju starp tiolu un elektrofīliem selēna savienojumiem – selenilhalogenīdiem[54,55] vai organilselenīnskābēm.[56,29] Taču arī fenilselenols var reaģēt ar aril- vai alkiltioliem *t*BuOK klātienē, savukārt fenilselenola reakcija ar elektrofīlo *N*-fenil-trifluorometānsulfēnamīdu notiek skābā vidē.[38] Dažādi ogļhidrātu-selenilsulfīda atvasinājumi tika iegūti reakcijā starp diorganildiselenīdiem un glutationu fosfāta buferšķīdumā. Šī metode tika lietota arī proteīna (subtilizīns) modificēšanai.[57] Svarīgi, ka apmaiņas reakcijai starp diarildisulfīdu un dialkildiselenīdu ir izmantota *UV* gaisma. Turklāt attiecīgās publikācijas autori apgalvoja, ka Se–S saites izveide notiek *UV* gaismā, bet pie garākiem viļņiem (>410 nm, redzamā gaisma) notiek apgriezeniskā reakcija.[58]

Promocijas darba ietvaros tika izstrādāta jauna metode Se–S saiti saturošu peptīdu sintēzei, izmantojot redzamās gaismas iniciētu reakciju. Optimālie reakcijas apstākļi tika atrasti, pētot reakciju starp dipeptīda dimēru (*Boc-Sec-Gly-OBn*)$_2$ **24b** un glutationu (*GSH*) (**54**) MeCN/H_2O maisījumā, apstarojot to ar LED_{460} gaismu. Arī šajā gadījumā Bengālijas rozā (RB) izrādījās vispiemērotākais katalizators savienojuma **55a** sintēzei (21. shēma). Neviens no citiem pārbaudītajiem katalizatoriem (FIrPic, $Ru(bpy)_3Cl_2$, 4-CzIPN, 9-mezitil-10-metilakridīnija tetrafluorborāts, etileozīns, fluoresceīns, 5-TAMRA) neuzrādīja augstāku selektivitāti un spēju nodrošināt pilnu izejvielu konversiju 1 h laikā. Novērots, ka notiek arī konkurējošā reakcija – *GSH* oksidēšanās, veidojot GS–SG, tādēļ šo substrātu nepieciešams ņemt pārākumā – 10 ekv. Taču, ņemot vērā to, ka rakcijā tiek izmantotas abas diselenīda daļas, GSH attiecība pret vienu selēnu ir 5 : 1. Kontroltesti pierādīja, ka reakcijas norisei ir nepieciešami gan LED_{460}, gan RB. Radikāļu ķērāja *TEMPO* klātiene nenovērsa produkta **55a** veidošanos, jo, visticamāk, ka radikāļa reakcija ar *TEMPO* ir daudz lēnāka nekā glutationa ($GS^{\cdot}$) radikāļa ģenerēšana un sekojošā Se–S saites izveides reakcija vai glutationa dimerizēšanās reakcija.

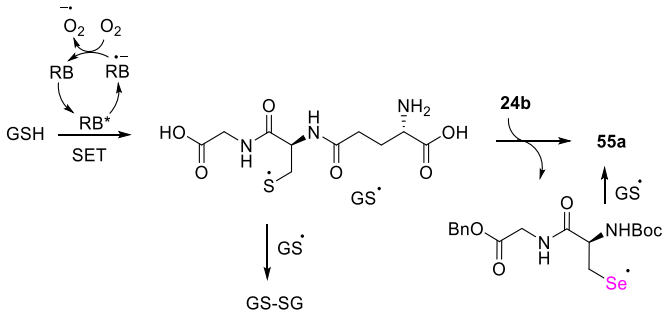


21. shēma. Se–S saiti saturošo peptīdu sintēze.

55b-e: produktu iegūšanai izmantoti neaizsargāti peptīdi TFA sāls formā.

Svarīgi, ka substrātu klāsta pārbaude uzrādīja, ka dažādi selēncistīna un selēncistamīna peptīdi ar "sensitīvām" aminoskābēm (*Arg*, *Glu*, *Lys*, *His*, *Tyr*) uzrādīja lielisku toleranci un veidoja gaidītos Se–S saiti saturošos produktus. Lai gan ir zināms, ka *Trp* ir ļoti jutīgs pret oksidēšanos ar radikāļiem un singleta skābekli, tomēr izdevās izolēt arī *Trp* saturošo produktu **55c**, taču iznākums bija zemāks blakusproduktu veidošanās dēļ. Savukārt *Met* saturošā peptīda gadījumā tika izolēts *Met* sulfoksīdu saturošais produkts **55f**.

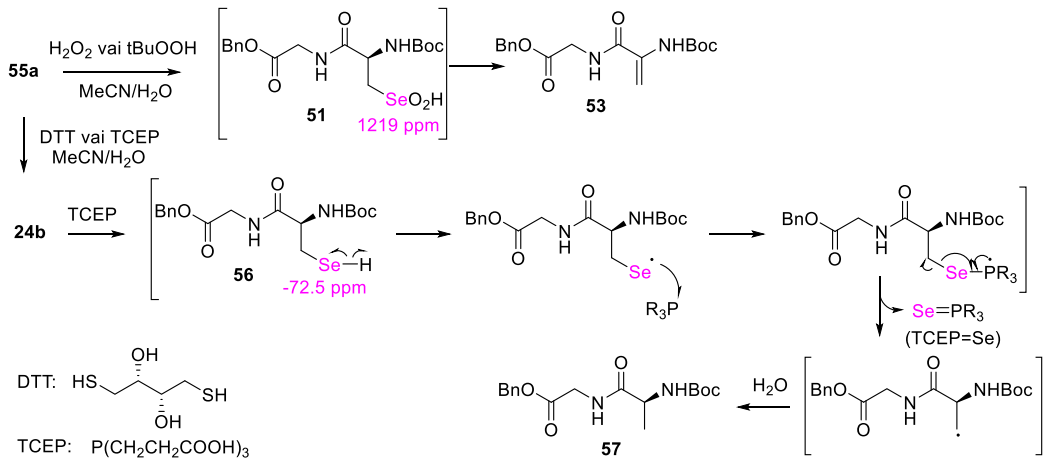
Sterna–Volmera eksperiments uzrādīja, ka RB fluorescences dzēšanas ātrums ar GSH ir ievērojami augstāks ($8{,}8 \times 10^{-3}$ l/mol) nekā ar **24b** ($0{,}12 \times 10^{-3}$ l/mol). Tāpēc var uzskatīt, ka S-centrēts radikālis ātri izveidojas redzamās gaismas ietekmē fotokatalizatora klātienē. Tad GS$^{\cdot}$ radikālis reaģē ar diselenīdu, veidojot Se–S saiti saturošo peptīdu **55a**, vai arī tas veido oksidētu glutationu (GS-SG) (22. shēma).



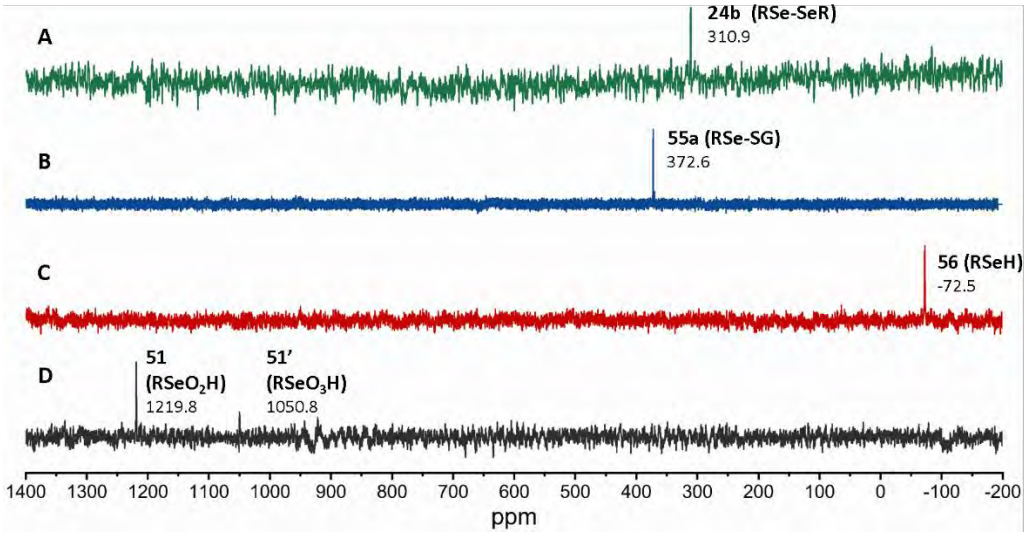
22. shēma. Piedāvātais mehānisms Se–S saiti saturošo peptīdu sintēzei.

Tika nolemts izpētīt Se–S saiti saturošā peptīda **55a** stabilitāti dažādos apstākļos: apstarojot to ar *LED*$_{460}$, oksidētāju un reducētāju klātienē (23. shēma). Ilgākas apstarošanas (*LED*$_{460}$ > 3 h) rezultātā RB klātienē MeCN/H_2O maisījumā veidojās attiecīgā selenīnskābe **51** un *Boc-Dha-Gly-OBn* **53**, jo, visticamāk, notika savienojuma **55a** reakcija ar H_2O_2, kas veidojas reakcijas apstākļos. Oksidējot **55a** ar H_2O_2 ar *t*BuOOH, veidojās attiecīgā selenīnskābe **51**, kas tika detektēta ar AIMS ([M + Na] = 471,0633) un ^{77}Se KMR spektroskopiju (δ 1219,8 ppm). Turklāt neliels selenoskābes **51′** signāls (δ 1050,8 ppm) arī tika detektēts ^{77}Se KMR spektrā (3. att.).

Reducējot **55a** ar 3 ekv. 1,4-ditiotreitola (DTT) vai tris(2-karboksietil)fosfīna hidrogēnhlorīdu (TCEP), veidojās **24b** un GSH īsā reakcijas laikā (30 min). Turklāt, palielinot TCEP daudzumu (10 ekv.), tika novērots, ka notiek deselenilēšanās un veidojas *Boc-Ala-Gly-OBn* **57** un TCEP=Se ([M-H] = 328,9704) (23. shēma). Reducēšanas reakcijas starpprodukts – selenols **56** – tika detektēts ar ŠH-MS ([M + Na] = 439,03) un ^{77}Se KMR spektroskopiju (δ –72,5 ppm). Deselenilēšanas mehānisms ir literatūrā zināms.[33]



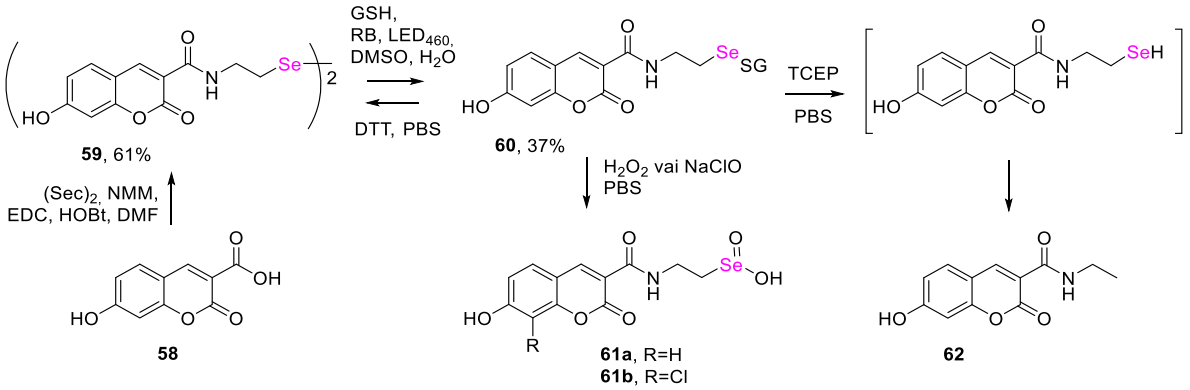
23. shēma. 55a stabilitāte reducētāju un oksidētāju klātienē.



3. att. ^{77}Se KMR spektri: **24b** (A), **55a** (B), **56** (C), **51** (D).

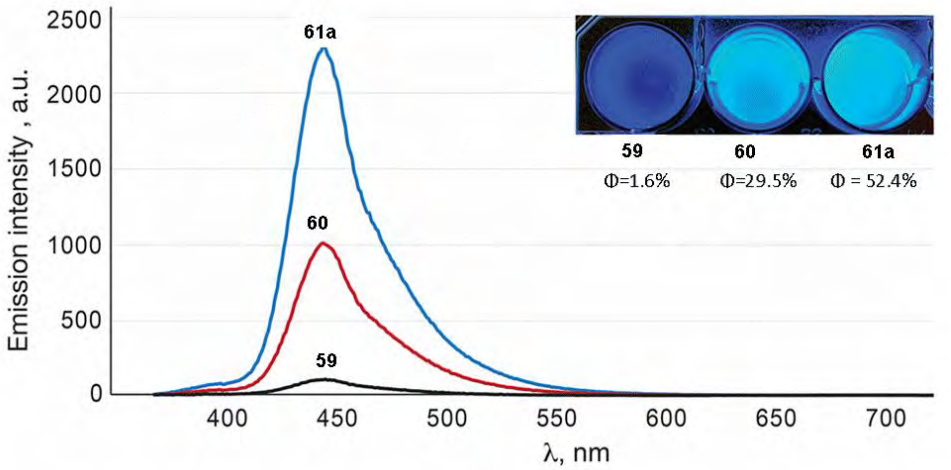
Lai novērtētu alkilselenilsulfīda izmantošanu kā viegli nošķeļamu linkeri fluorescenta marķiera pievienošanai pie tiola grupu saturoša peptīda, tika sintezēts diselenīds **59** no 7-hidroksi-2-okso-2*H*-hromēn-3-karbonskābes **58** (24. shēma). Redzamās gaismas iniciētā reakcijā starp GSH un diselenīdu **59** veidojās Se–S saiti saturošais produkts **60** ar 37 % iznākumu.

Tālāk tika pārbaudīta savienojuma **60** stabilitāte. H_2O_2 klātienē notika ātra oksidēšanās un veidojās attiecīgā selenīnskābe **61a**. NaClO klātienē veidojās hlorēta selenīnskābe **61b** ([M-H] = 377,9290). DTT (10 ekv.) izmantošanas rezultātā tika iegūts diselenīds **59**. Nesen literatūrā tika aprakstīts, ka reducējošos apstākļos (TCEP/DDT) ciklisks selenilsulfīds spontāni eliminē etilselenilgrupu, veidojot etilēnu, turklāt šī reakcija notika ļoti lēni (sešas dienas).[59] Līdzīga pārvērtība netika novērota savienojuma **60** gadījumā – reakcijā ar TCEP (10 ekv.) veidojās etilamīds **62** jau 30 min laikā.



24. shēma. Savienojuma **60** sintēze un stabilitāte.

Papildus tika noteikta savienojuma **60** stabilitāte fosfāta buferšķīdumā (*PBS*) pie dažādām pH vērtībām, atklājot, ka savienojumam ir stabils pH robežās 3,0–8,0 vismaz 24 h. Tika iegūti absorbcijas un emisijas spektri savienojumu **59**, **60** un **61a** *PBS* šķīdumiem (pH = 7,4, c = 10 µM), kā arī tika noteikti šo vielu fotoluminescences kvantu iznākumi (*PLQY*) (4. att.). Se–S saturošā savienojuma *PLQY* ir ievērojami augstāks (Φ = 29,5 %) nekā attiecīgajam diselenīdam **59** (Φ = 1,6 %). Taču visaugstākais kvantu iznākums tika novērots selenīnskābes **61a** (Φ = 52,4 %) gadījumā.



4. **att**. Savienojumu **59**, **60**, **61a** emisijas spektri (ierosinot pie 350 nm (*PBS*, pH = 7,4)).

Oriģinālpublikācija par šajā apakšnodaļā aprakstītajiem pētījumiem – 5. pielikumā.

SECINĀJUMI

1. Cisteīnu saturošus peptīdus var funkcionalizēt maigos apstākļos, izmantojot *N*-hlorsukcīnimīdu *in situ* cisteinilhlorīda iegūšanai.
2. $CuBr_2$ ir piemērota Luisa skābe halkogenilelektrofīla ģenerēšanai no diaril/dibenzil dihalkogenīdiem, kā arī no aizsargātiem un neaizsargātiem selēncistīnu saturošiem peptīdiem.
3. $K_2S_2O_8$ ir piemērots reaģents selenilelektrofīla ģenerēšanai no (*Boc*-*Sec*)$_2$ un selēncistīnu saturošiem peptīdiem.
4. Sulfenil- un selenilelektrofīlus var izmantot 5- un 6-*endo*-*dig* ciklizācijas reakcijās ar piemērotiem trīskāršo saiti saturošiem savienojumiem, veidojot cisteīnu un selēncisteīnu saturošas (hetero)cikliskas sistēmas (indolizīnija sāļus, indolus, benz[*b*]furānus, kumarīnus, izokumarīnus, hinolīn-2-onus, izohinolīn-2-onus, poliaromātiskus ogļūdeņražus, indēn[1,2-*c*]hromēnus).
5. Bengālijas rozā ir efektīvs fotokatalizators selenilelektrofīla ģenerēšanai no aizsargātiem un neaizsargātiem selēncistīnu saturošiem peptīdiem. Iegūtais elektrofīls reaģē ar elektroniem bagātiem *N*-heterocikliem. Izstrādātā atomekonomiskā metode uzrādīja izcilu toleranci arī neaizsargātiem selēncistīnu saturošiem peptīdiem ar "sensitīvām" aminoskābēm (*Lys*, *Arg*, *His*, *Glu*, *Tyr*). Reakcijas apstākļos iespējams veikt arī iekšmolekulāru indolu selenilēšanu, iegūstot selēncisteīnu saturošus makrociklus.
6. Bengālijas rozā ir piemērots fotokatalizators Se–S saiti saturošu peptīdu sintēzei.

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PATEICĪBAS

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Sindija Lapčinska

DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council on February 17, 2022 at 14:00 at the Faculty of Materials Science and Applied Chemistry of Riga Technical University, 3 Paula Valdena Street, Room 272.

OFFICIAL REVIEWERS

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis had not been submitted to any other university for the promotion to a scientific degree.

Sindija Lapčinska ……. (signature)
Date: ……

The Doctoral Thesis has been prepared as a collection of thematically related scientific publications complemented by summaries in both Latvian and English. The Doctoral Thesis includes six scientific publications. The scientific publications have been written in English, with the total volume of 820 pages, including supplementary data.

CONTENTS

Appendix I: Arsenyan, P.; Lapcinska, S.; Ivanova, A.; Vasiljeva, J. Peptide functionalization through the generation of selenocysteine electrophile. *Eur. J. Org. Chem*. **2019**, 4951–4961.

Appendix II: Lapcinska, S.; Arsenyan, P. Selenocystine peptides performance in 5-*endo-dig* reactions. *Eur. J. Org. Chem*. **2020**, 784–795.

Appendix III: Lapcinska, S.; Arsenyan, P. Straightforward functionalization of sulfur-containing peptides via 5- and 6-*endo-dig* cyclization reactions. *Synthesis* **2021**, *53*, 1805–1820.

Appendix IV: Lapcinska, S.; Dimitrijevs, P.; Lapcinskis, L.; Arsenyan, P. Visible light-mediated functionalization of selenocystine-containing peptides. *Adv. Synth. Cat.* **2021**, *363*, 3318–3328.

Appendix V: Lapcinska, S.; Dimitrijevs, P.; Arsenyan, P. Visible light-mediated synthesis of Se–S bond-containing peptides. *Adv. Synth. Cat.* **2021**, *363*, 3968–3972.

Appendix VI: Lapcinska, S.; Arsenyan, P. Selenocysteinyl electrophiles efficiently promote the formation of coumarin and quinolinone cores by 6-*endo-dig* cyclization. *New. J. Chem*. **2021**, *45*, 16625–16634.

ABBREVIATIONS

Arg	arginine
Boc	*tert*-butoxycarbonyl-
Bn	benzyl-
t-Bu	*tert*-butyl-
Cbz	benzyloxycarbonyl-
m-CPBA	*meta*-chloroperoxybenzoic acid
Cys	cysteine
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
Dha	dehydroalanine
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Gly	glycine
GSH	glutahione
His	histidine
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
LC-MS	liquid chromatography-mass spectrometry
LED	light-emitting diodes
Lys	lysine
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
Ns	nosyl
PAHs	polyaromatic hydrocarbons
PBS	phosphate-buffered saline
PLQY, Φ	photoluminescence quantum yield
RB	Rose Bengal
REN	relative electronegativity
rt	room temperature
Sec	selenocysteine
Trp	tryptophan
Ts	tosyl
Tyr	tyrosine
UV	ultraviolet

GENERAL OVERVIEW OF THE THESIS

Introduction

Short peptides are a unique class of molecules that have promising properties for drug discovery.[1] As intrinsic signaling molecules for many physiological functions, peptides present an opportunity for therapeutic intervention that closely mimics natural pathways.[2] The use of peptides as therapeutics has evolved over time, consequently, a significant number of marketed drugs already are peptide-based compounds.[1] With purpose to overcome some issues associated with peptides, e.g. oral bioavailability and membrane permeability, peptide and small molecule conjugates have been designed. Conjugation is an attractive mechanism for enhancing the properties of peptides. Notably, 30 % of peptides that have entered clinical development in the last decade are conjugates, and the search for novel peptide-based pharmaceuticals continues.

The introduction of cysteine (Cys), selenocysteine (Sec) or the oxidized forms of these amino acids into peptide or protein provides a handle for selective modification. Peptides with cysteine or selenocysteine residues can be modified through the generation of chalcogenyl electrophile, nucleophile or radical.

Although sulfur and selenium are close analogues, significant differences in chemical and physical properties exist. Selenols have lower pKa values than thiols, for example, pKa of selenocysteine is 5.2 while pKa of cysteine is 8.3, meaning that under physiological conditions (pH = 7.4) selenocysteine is deprotonated. Selenium is more polarizable that results in higher reactivity.

In general, methods for generation of sulfenyl and selenyl electrophiles exist, however, these protocols are usually limited with simple aryl thiols or diaryl disulfides/diselenides. Typical examples of sulfenyl electrophiles are aryl sulfenyl halides prepared from aryl thiols or diaryl disulfides using halogens,[3,4] $SOCl_2$,[5] SO_2Cl_2,[3] catalytic or equimolar amount of Lewis acids (e.g. iron(III) salts,[6,7,8] $CuBr_2$[9]). Similarly, selenyl electrophile can be generated using halogens,[4] KI/*m*-CPBA,[10] oxidants (persulfates,[11] oxone[12]), metal salts (CuI,[13] $FeCl_3$[6]) and hypervalent iodine compounds[14].

The modification of peptides obviously is more complicated due to the presence of 'sensitive' functional groups. Notably, the known methods usually rely on the high nucleophilicity of the thiol/selenol group while only few methods exist for generation of cysteine/selenocysteine electrophiles. Contrary to cysteine, selenocystine-containing peptides are rarely used for chemical synthesis.

Peptide functionalization is performed with the purpose to improve the bioavailability, cell permeability and stability. It is known that cyclization of peptides can improve their selectivity, metabolic stability and binding affinity.[15] Some of the most widely used antibiotics (vancomycin, daptomycin, valinomycin, gramicidin S), immunosuppressants (cyclosporine), anti-cancer drugs (aplidine, dactinomycin) are macrocyclic peptides.[16,17] From 2015 to 2019, FDA (*Food and Drug Administration*) approved 208 drugs, and 15 of them were peptide-based compounds that were used for treating diabetes (*Tresiba*, *Lixisenatide*), osteoporosis (*Tymlos*), hypotension (*Giapreze*), neuroendocrine tumors (*Lutathera*) and other diseases.[18] Additionally,

in 2021, FDA approved 15 peptide-based drugs (*Voxzogo, Korsuva, Besremi, Skytrofa, Nexviazyme, Jemperli, Evkeeza, Saphnelo, Rylaze, Aduhelm, Rybrevant, Empaveli, Zynlonta, Zegalogue, Lupkynis*).

Aims and objectives

The main goal of the Thesis is the development of new effective synthetic approaches for modification of cysteine and selenocystine-containing peptides through the generation of sulfenyl or selenyl electrophile, focusing on high yield, atom-economic and sustainable processes.

The following tasks were set:

1) to develop convenient protocols for the *in situ* generation of sulfenyl electrophile from cysteine containing peptides;
2) to establish novel methods for selenyl electrophile generation suitable for selenocystine-containing peptides;
3) to elaborate methods for utilization of sulfenyl and selenyl electrophiles in 5- and 6-*endo-dig* cyclization reactions;
4) to develop methods for modification of selenocystine-containing peptides by visible light induced reactions.

Scientific novelty and main results

As the result of the Thesis, simple and efficient methods for functionalization of cysteine and selenocystine-containing peptides were established. The electrophilic cysteinyl species – cysteinyl chloride – was *in situ* obtained employing *N*-chlorosuccinimide, while Lewis acid or oxidant induced selenyl electrophile generation was employed for the selenocystine-containing peptides. The electrophilic species were subsequently trapped with triple bond-containing substrates followed by 5- and 6-*endo-dig* cyclization reactions yielding indolizinium salts, indoles, benzo[*b*]furans, indeno[1,2-*c*]chromenes, polyaromatic hydrocarbons, isocoumarins, coumarins, isoquinolin-2-ones and quinolin-2-ones attached to cysteine or selenocysteine peptides. Methods employed for selenyl electrophile generation showed high tolerance for amino acids with sensitive groups (e.g. Tyr, Glu, Lys). A novel atom-economic method was established for modification of selenocystine-containing peptides under visible light irradiation. This method enabled the synthesis of selenocysteine-containing *N*-heterocycles in high yields. Furthermore, intramolecular selenylation was performed resulting in preparation of selenocysteine-containing macrocycles. Additionally, visible light-initiated reaction was developed for synthesis of Se–S bond-containing peptides, and it was based on the generation of thiyl radical of glutathione in the presence of organic dye.

Structure and volume of the Thesis

The Thesis is a collection of thematically related scientific publications devoted to the development of new methods for modification of cysteine and selenocystine-containing peptides by electrophilic cylization and visible light-initiated reactions. The Thesis compiles results from 6 original scientific papers indexed in Scopus and Web of Science.

Publications and approbation of the Thesis

The results of the Thesis have been published in 6 scientific papers. Additionally, the results have also been disseminated in 6 scientific conferences.

Scientific publications

1. Arsenyan, P.; Lapcinska, S.; Ivanova, A.; Vasiljeva, J. Peptide functionalization through the generation of selenocysteine electrophile. *Eur. J. Org. Chem*. **2019**, 4951–4961.
   [Lapcinska, S.; Ivanova, A.; Vasiljeva, J., Arsenyan, P. Synthesis of selenocysteine-based peptides. *Synfacts* **2019**, *15*(10), 1206.]
2. Lapcinska, S.; Arsenyan, P. Selenocystine peptides performance in 5-*endo-dig* reactions. *Eur. J. Org. Chem*. **2020**, 784–795.
   [Lapcinska, S.; Arsenyan, P. Site-selective modification of selenocystine peptides. *Synfacts* **2020**, *16*(05), 0606.]
3. Lapcinska, S.; Arsenyan, P. Straightforward functionalization of sulfur-containing peptides via 5- and 6-*endo-dig* cyclization reactions. *Synthesis* **2021**, *53*, 1805–1820.
4. Lapcinska, S.; Dimitrijevs, P.; Lapcinskis, L.; Arsenyan, P. Visible light-mediated functionalization of selenocystine-containing peptides. *Adv. Synth. Cat.* **2021**, *363*, 3318–3328.
5. Lapcinska, S.; Dimitrijevs, P.; Arsenyan, P. Visible light-mediated synthesis of Se–S bond- containing peptides. *Adv. Synth. Cat.* **2021**, *363*, 3968–3972.
   [Lapcinska, S.; Dimitrijevs, P.; Arsenyan, P. Visible light-mediated selenium–sulfur bond formation to afford selenium–sulfur bond-containing peptides. *Synfacts* **2021**, *17*(11), 1289.]
6. Lapcinska, S.; Arsenyan, P. Selenocysteinyl electrophiles efficiently promote the formation of coumarin and quinolinone cores by 6-*endo-dig* cyclization. *New. J. Chem*. **2021**, *45*, 16625–16634.

Scientific conferences

1. S. Lapčinska. Cysteinyl- and selenocysteinyl indoles and benzo[*b*]furanes. *University of Latvia 77th International Scientific Conference, Organic Chemistry section,* February 18, 2019, Riga (Latvia).
2. Peptide functionalization through the generation of selenocysteine electrophile. A. Ivanova, S. Lapcinska, P. Arsenyan. *14th International Conference on the Chemistry*

of Selenium and Tellurium (ICCST-14), June 3–7, 2019, Santa Margherita di Pula (Italy).

3. Formation of indole and benzofuran moieties attached to selenocysteine containing peptides. S. Lapcinska, P. Arsenyan. *14th International Conference on the Chemistry of Selenium and Tellurium (ICCST-14*), June 3–7, 2019, Santa Margherita di Pula (Italy).
4. Performance of chalcogen-containing peptides in 5- and 6-*endo-dig* cyclization reactions. S. Lapcinska. *Paul Walden 11th Symposium on Organic Chemistry*, September 19–20, 2019, Riga (Latvia).
5. Visible light-mediated functionalization of selenocystine-containing peptides. S. Lapcinska, P. Arsenyan. *International Conference on Photochemistry – 30th edition (ICP2021)*, July 19–23, 2021, Geneva (Switzerland), online conference.
6. Photocatalytic macrocyclization of selenocystine-containing peptides. S. Lapcinska, P. Arsenyan. *International Symposium on Synthesis and Catalysis (IsySyCat2021)*, August 31 – September 3, 2021, Evora (Portugal), online conference.
7. Light-driven modifications of Cys and Sec containing peptides. S. Lapcinska. *Paul Walden 12th Symposium on Organic Chemistry*, October 28–29, 2021, Riga (Latvia), online conference.

MAIN RESULTS OF THE THESIS

1. Sulfenyl electrophile generation and subsequent use in 5- and 6-*endo-dig* cyclization reactions

Cysteine (Cys) is a very popular target for regioselective modification of proteins due to its low natural abundance (1–2 % in proteins). Probably, the most important Cys containing intracellular low-molecular weight peptide present in mammalians is glutathione (GSH). GSH plays a critical role in many processes in the cells, including differentiation, proliferation and apoptosis. GSH protects the cells from damage caused by lipid peroxides, reactive oxygen and nitrogen species, and xenobiotics. Diminished levels of GSH and the GSH/glutathione disulfide (GSSG) ratio leads to an increased susceptibility to oxidative stress implicated in the progression of cancer, while elevated GSH levels leads to the resistance to oxidative stress. Notably, in the last few years thiol-containing peptides have been utilized in drug delivery of lipophilic drugs.

The current bioconjugation methods usually rely on the high nucleophilicity of Cys. Widely employed reactions of cysteine are: alkylation with haloalkyl reagents, arylation, and Michael addition reaction. A valuable modification of cysteine is stapling with different aryl linkers. Additionally, cysteine can be easily oxidized to its dimer – cystine. The disulfide bond in proteins is a prevalent approach for stabilization and protein folding. Obviously, the S–S bond formation in proteins depends on appropriate position of Cys residues.

The homolytic bond dissociation energy of the S–H bond in cysteine is low (86 kcal/mol), therefore the corresponding cysteinyl radical can be easily generated.[19] During the last few years, methods for cysteine conjugation under irradiation conditions have been developed using photocatalysts.[20] Probably, the most well-known radical-mediated reaction of Cys is the thiol-ene/yne reaction.

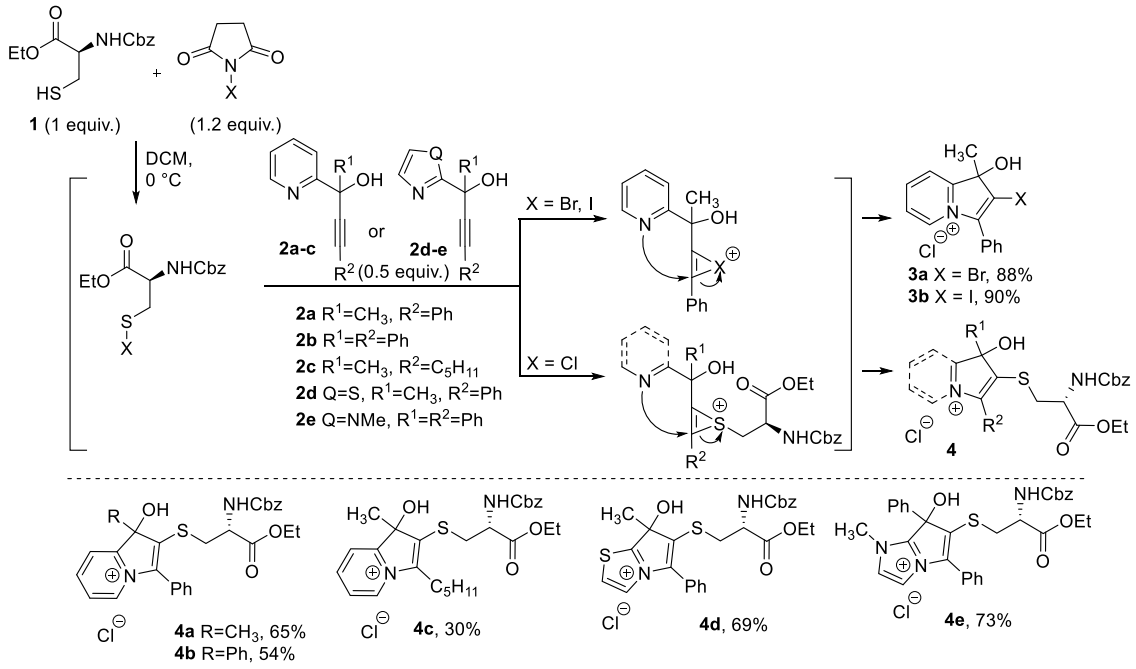
Another method for modification of cysteine is based on the formation of Dha peptide via desulfurization. Typically, the reaction is performed in the presence of phosphines.

Electrophilic center is located on the sulfur atom in compounds containing S–O, S–N or S–Cl bonds. Sulfenic acids are unstable but sulfenamides are usually synthesized from sulfenyl halides, thus the most common sulfenyl electrophiles are sulfenyl halides. These electrophilic species can be prepared from thiols or disulfides using halogens, $SOCl_2$, SO_2Cl_2 or *N*-halosuccinimides. The ability of Cys to bind with Lewis acids can result in efficient modifications. For example, Lewis acids such as $FeCl_3$, $CuBr_2$ and $AlCl_3$ have been used to induce electrophile formation from thiols or disulfides. Furthermore, phenyl iododiacetate induces the formation of electrophilic sulfur species in the presence of KI. Notably, pre-made *N*-thioalkyl or aryl phthalimides or *N*-thiosuccinimides can serve as efficient electrophiles as well.

The current use of reaction of Cys-derivatives and *N*-halosuccinimides is limited to the preparation of disulfides. Nevertheless, we envisioned to prepare the electrophilic sulfenyl species *in situ* using *N*-halosuccinimides. The optimal reaction conditions were found

investigating reaction of *N*-Cbz-Cys-OEt (**1**) and 4-phenyl-2-(pyridin-2-yl)but-3-yn-2-ol (**2a**). The examination of the reaction mixtures revelead that the employment of *N*-bromosuccinimide (NBS) and *N*-iodosuccinimide (NIS) led to the formation of the corresponding halo-indolizinium salts **3a** and **3b** in high yields (88 and 90 %, respectively) in short reaction time (1 h). However, *N*-chlorosuccinimide (NCS) proved to be the reagent of choice – utilization of NCS resulted in the synthesis of the desired Cys-containing indolizinium salt **4a** (65 % yield) as a mixture of diastereomers (1:1) (Scheme 1). NCS was not able to induce 5-*endo-dig* cyclization of **2a**, while the reactions with NBS and NIS led to unselective formation of **3a** and **3b** in 3 days. The results suggested that the electrophilic centre in cysteinyl chloride is the sulfur atom, whereas the electrophilic centres in cysteinyl bromide and iodide are halogen atoms due to smaller difference in relative electronegativity (REN S = 2.58, Cl = 3.16, Br = 2.96, I = 2.66).

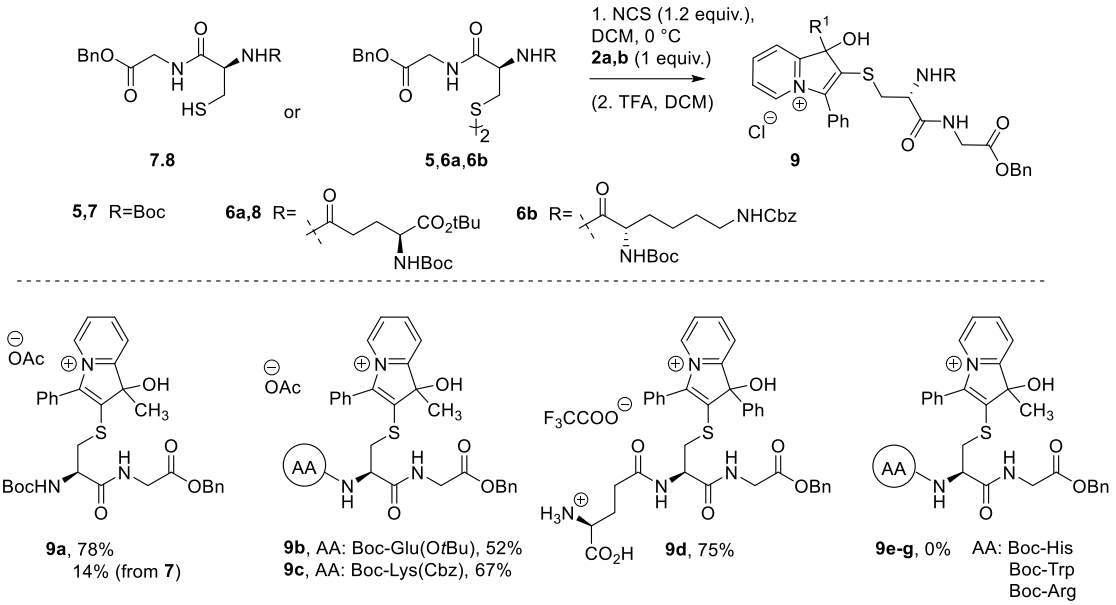
The substrate scope showed that not only propargyl pyridines **2a-c** were suitable substrates for the cyclization reaction, but also propargyl thiazole **2d** and *N*-methylimidazole **2e** effectively trapped the *in situ* prepared cysteinyl chloride and provided indolizinium type systems **4d, e** in even better yields (69–73 %).



Scheme 1. Synthesis of indolizinium type salts.
All products were purified by reverse phase chromatography
(MeCN/H_2O, pH = 4, hydrochloric acid).

Previously used conditions were not appropriate for the synthesis of **9a** (Scheme 2). Although the product was detected, various impurities were formed as well. Solvent change to acetonitrile yielded the desired product in 14 % yield. Nevertheless, we found out that S–S bond containing substrated can also be used for the generation of sulfenyl electrophile and subsequent 5-*endo-dig* cyclization. Employment of (Boc-Cys-Gly-OBn)$_2$ (**5**) and (Boc-Glu(O*t*Bu)-Cys-Gly-OBn)$_2$ (**6a**) provided the corresponding indolizinium salts **9a-c** in moderate to good yields.

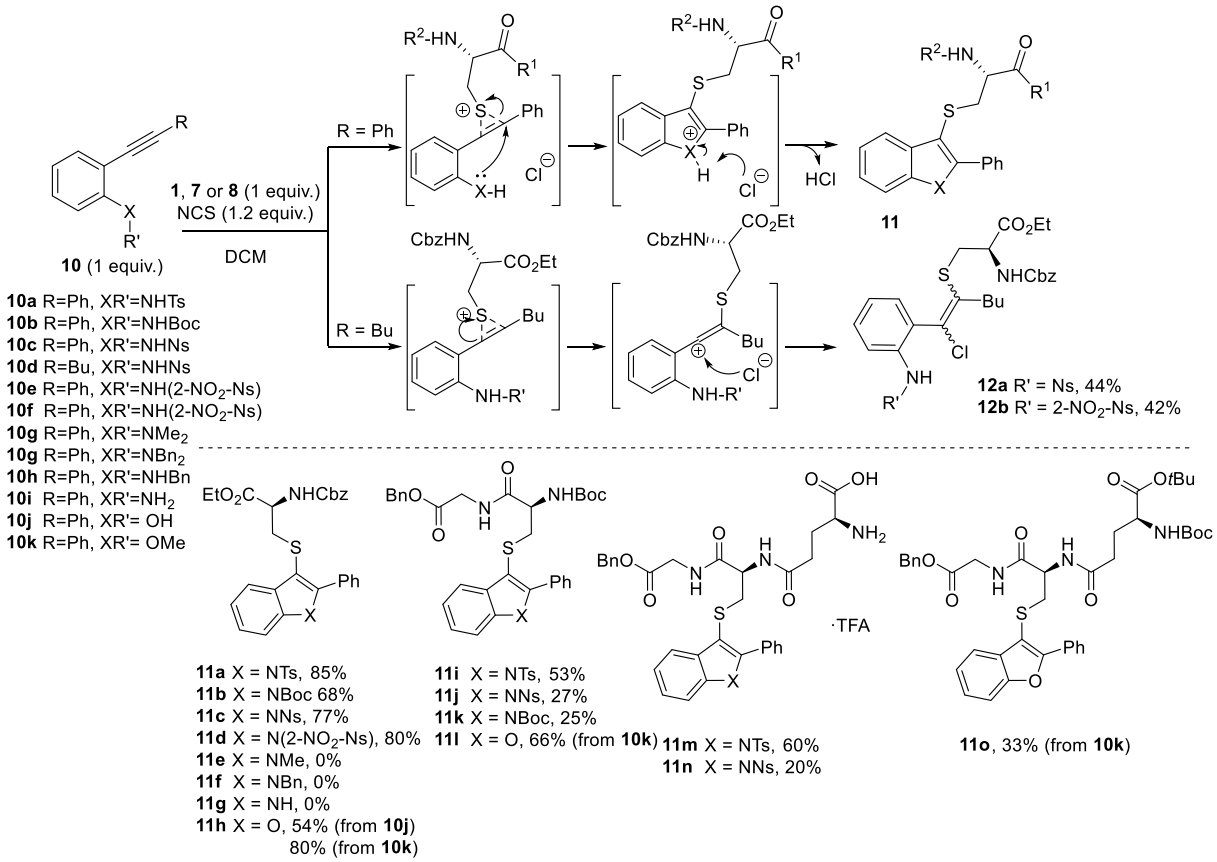
The substrate scope showed that peptides with ‘sensitive’ amino acids (His, Trp, Arg) were not suitable for the reaction.



Scheme 2. Synthesis of Cys-peptide containing indolizinium salts.
All products were purified by reverse phase chromatography
(**9a-c**: MeCN/H_2O, pH = 4, acetic acid; **9d**: MeCN/H_2O, pH = 4, trifluoroacetic acid).

Many natural products and a significant number of marketed drugs contain indole and benzo[*b*]furan moieties.[21,22] Furthermore, both of these heterocycles are considered as ‘privileged structures’.[23] Thus, we decided to apply the same reaction conditions for 5-*endo-dig* cyclization that would result in the formation of indole and benzo[*b*]furan rings.

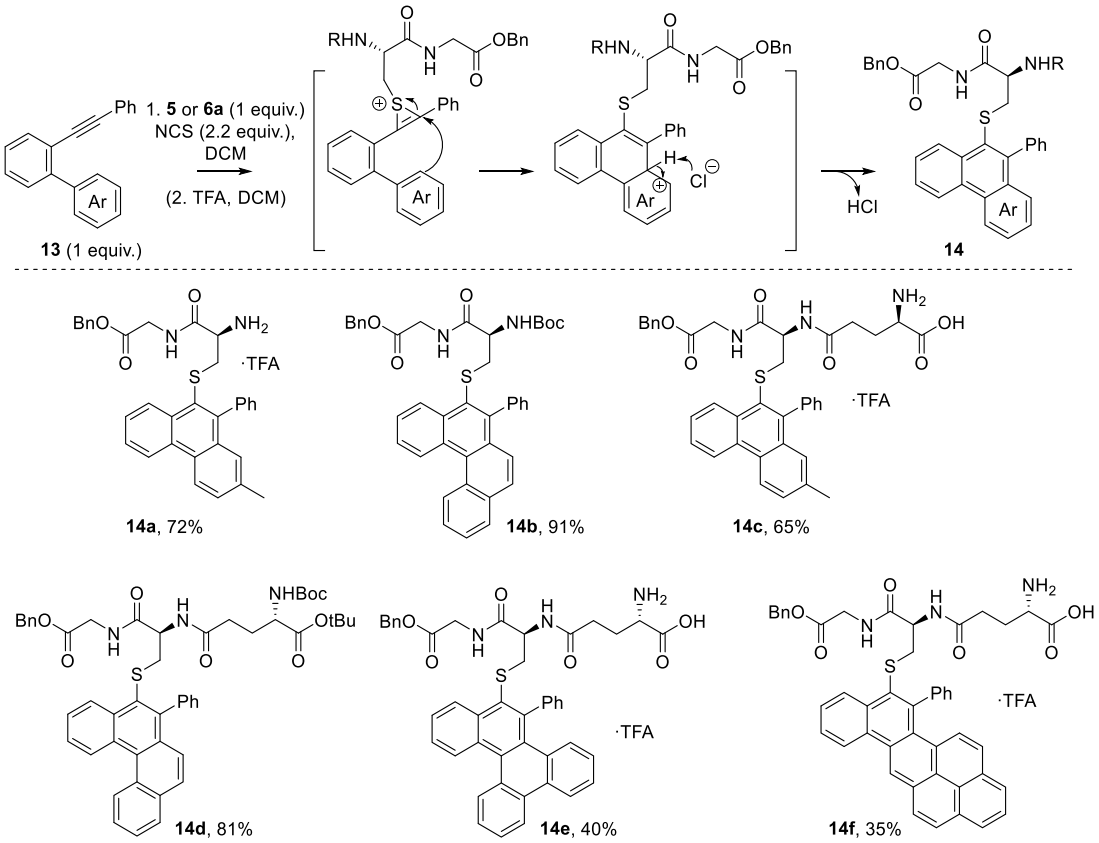
Reaction of **1** with 2-(phenylethynyl)anilines in the presence of NCS provided 3-Cys-indoles **11a-d** in high yields (Scheme 3). Neither dimethyl and dibenzylamino, nor unprotected (phenylethynyl)aniline were found as suitable substrates. Similarly, 3-Cys benzo[*b*]furan **11h** was easily prepared using 2-(phenylethynyl)phenol **10j**. Notably, reaction yield was significantly improved employing 2-(phenylethynyl)anisole **10k**. The use of peptides Boc-Cys-Gly-OBn (**7**) and Boc-Glu(O*t*Bu)-Cys-Gly-OBn (**8**) yielded the desired indoles as well; however, the reaction yield was inconsequent (20–66 %). S–S bond containing peptides were not suitable for the preparation of **11**. Unfortunately, 2-hexynylanilines under the same reaction conditions provided only triple bond addition products **12a-b** due to formation of aryl group stabilized vinyl cation that prevents 5-*endo-dig* cyclization.



Scheme 3. Synthesis of Cys-indoles and benzo[*b*]furans.

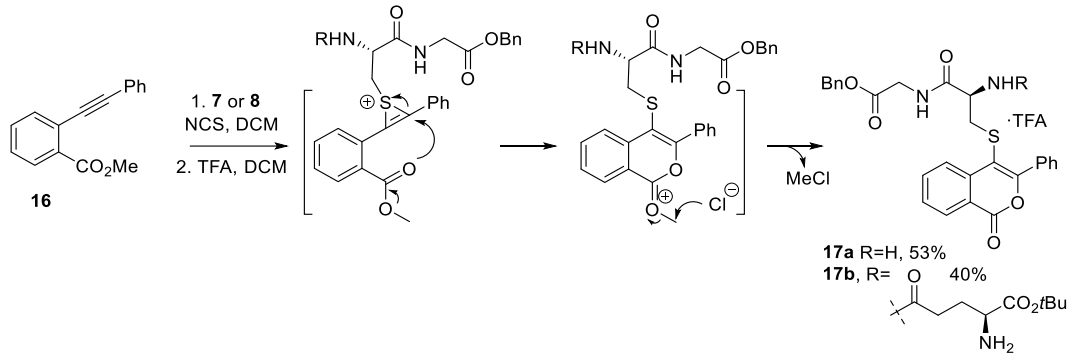
Next, we checked the possibility to generate sulfenyl electrophile and perform 6-*endo-dig* cyclization with 2-(phenylethynyl)biaryls. Polycyclic aromatic hydrocarbons (PAHs) are perspective compounds employed not only as starting materials in organic synthesis but also as fluorescent markers in bioimaging.

The reaction between **5** and alkyne **13a** in the presence of NCS provided 6-*endo-dig* cyclization product **14a** in 72 % yield (Scheme 4). We preferred to use the S–S (not S–H) bond containing substrate due to improved yields and diminished formation of side products. Triple bond coordinates with electrophilic sulfur atom forming thiirenium cycle, which is attacked by the closest aromatic ring producing 6-member cycle. Aromaticity is restored after deprotonation with chloride anion, providing the final product. Furthermore, benzo[*c*]phenantrene, benzo[*g*]chrysene and benzo[*pqr*]picene systems were easily constructed applying our methodology. The isolated yields were variable due to difficulties in purification process.



Scheme 4. Synthesis of Cys-peptide containing PAHs.

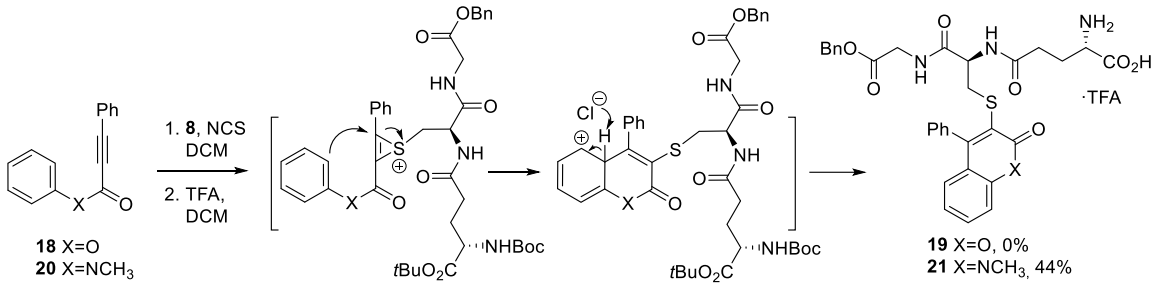
Next, evaluation of the use of NCS method was applied for synthesis of isocoumarins with glutathione moiety. Isocoumarin scaffold is present in many biologically active natural products.[24] Notably, compounds containing isocoumarin moiety exhibit antimicrobial, antifungal, cytotoxic, anti-inflammatory activities. The products were obtained in moderate yields employing peptides **7** and **8** (Scheme 5), whereas peptides with S–S bond (**5** and **6**) were not able to provide the products.



Scheme 5. Synthesis of Cys containing isocoumarins.

Then we moved on to another 6-*endo-dig* cyclization with the aim to prepare coumarins and quinolinones. Unfortunately, attempts to prepare coumarins failed. However, the reaction of glutathione **8** with NCS and aryl propiolamide **20** resulted in preparation of 1-methyl-3-

sulfenylquinolinone **21** (Scheme 6). Noteworthy, quinolinones are the core structure of many drugs, including some of the most widely used antibiotics (ciprofloxacin, levofloxacin).[25]



Scheme 6. Synthesis of Cys containing quinolinone.

The article about the studies described in this chapter can be found in Appendix III.

2. Selenyl electrophile generation and subsequent use in 5- and 6-*endo-dig* cyclization reactions

Contrary to thiols, the selenols are more prone to oxidation by air, thus they usually exist as diselenides under ambient conditions. The selenols are even more nucleophilic than thiols and can be prepared *in situ* by reducing agents (for example, $NaBH_4$). The diselenides are very important versatile compounds and can be used not only as a source of selenium nucleophile, but selenyl electrophile and radical species as well. Typical selenyl electrophiles are selenyl halides RSeX (X = Br, Cl, I) that can be easily obtained by the reaction of diselenides with halogens or SO_2Cl_2. Recently, the *in situ* preparation of phenylselenyl fluoride was reported using Selectfluor.[26] Furthermore, phenylselenyl chloride and phenylselenyl bromide are commercially available.

Obviously, the nucleophilicity of the halide can lead to undesired side reactions, thus synthetic protocols employing halide-free selenyl electrophiles are preferred. Selenyl halides react with various silver salts providing selenyl triflate, tosylate, acetate, hexafluorophosphate via exhange reaction. From the above-mentioned substrates, the selenyl triflate is the most commonly employed.

Alternatively, the selenyl electrophile can be obtained by the oxidation of the diselenide. For example, persulfate salts are known to induce the formation of strongly electrophilic selenyl sulfate.[27] In the last few years oxone has been frequently used for generation of selenyl electrophile, which further is trapped by triple bond-containing substrates, and the resulting cyclization yields selenylated heterocycles (indoles, pyrazoles). The reaction of oxone with diphenyl diselenide provides a mixture of phenyl selenenic acid and phenyl selenyl sulfate.[28] Other reagents, e.g. hypervalent iodine compounds, *m*-CPBA, $Ce(NH_4)_2(NO_3)_6$, KNO_3, DDQ, are known to produce selenyl electrophile as well. Furthermore, the reaction of diphenyl diselenide with 1,4-dicyanonaphthalene leads to the formation of electrophilic species through single electron transfer mechanism. Additionally, the formation of selenyl electrophile can be

induced by reaction of diselenide with Lewis acids, for example, iron(III), copper(I) and copper(II) salts have been frequently employed.

The oxidation of selenols or diselenides can also lead to formation of Se–O bond-containing intermediates: selenenic acid (RSeOH), seleninic acid ($RSeO_2H$) or selenonic acid ($RSeO_3H$). Selenenic acids are very reactive intermediates, only aryl selenenic acids have been detected. Notably, selenenic acid with bulky aryl groups have been isolated and characterized. Seleninic and selenonic acids are more stable and are useful for oxidation of organic compounds. Seleninic acids have been used for preparation of Se–S bond-containing compounds,[29] benzeneseleninic acid[30] has been used for selenylation of indoles and anilines.

Only few methods exist for modification of selenocystine-containing peptides in the literature. An efficient method has been reported for conjugation of Sec-peptides and small molecules based on the electrophilic character of (5-nitropyridylthio)-Sec peptides.[31] These substrates were also shown to react with nucleophilic aryl boronic acids providing arylated Sec peptides.[32] Other more common modifications of selenocystine-containing peptides with loss of selenium have also been demonstrated: deselenylation to alanine peptides by reducing agents[33] or oxidant-induced[34] formation of dehydroalanine derivatives.

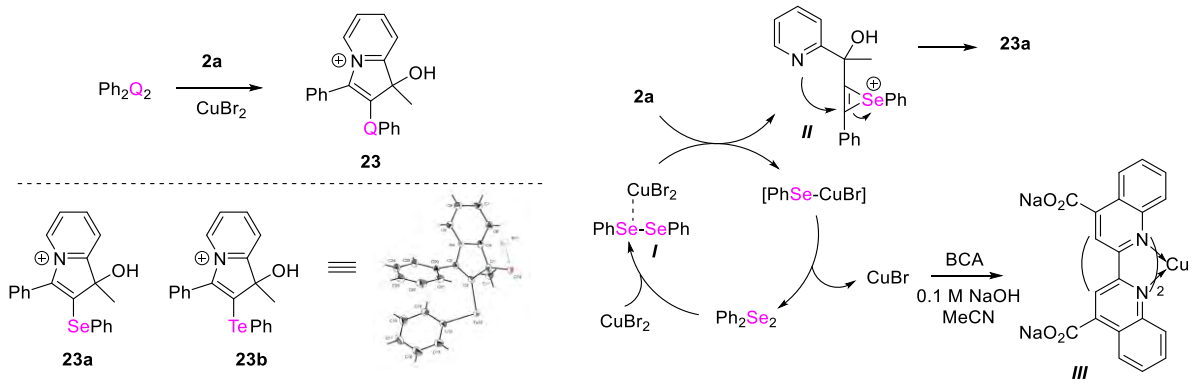
In order to find a suitable promoter for selenyl electrophile generation, the reaction between Ph_2Se_2 (**21**) and **2a** was examined in the presence of several Lewis acids ($FeCl_3$, $FeBr_2$, $FeBr_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $CuBr_2$, $Cu(OAc)_2$, $RuCl_3$, $In(OTf)_3$, $Bi(OTf)_3$) (Scheme 6).

Copper(II) bromide showed the best results (Table 1, entries 1–3). Importantly, all tested solvents provided acceptable yield, but DCM, MeCN and EtOH enabled excellent yield (Table 1, entries 5, 7–10). Significantly, an equimolar amount of $CuBr_2$ was required for maximizing the yield of the reaction. The reaction performed in an absence of diphenyl diselenide, yielded 2-bromoindolizinium bromide (Table 1, entry 11). The method was also suitable for tellanyl electrophile generation (Table 1, entries 15–16) but not for sulfenyl electrophile generation due to relatively low copper-philicity of sulfur; the main product of the reaction was 2-bromoindolizinium bromide (Table 1, entries 13–14).

To understand the product of interaction between $CuBr_2$ and Ph_2Se_2, ^{77}Se NMR spectra of a mixture of an equimolar amount of Ph_2Se_2 and $CuBr_2$ was acquired and compared to a spectrum of Ph_2Se_2. Overlay of these spectra is shown in Fig. 1A. A single signal at 450 ppm was observed in the $Ph_2Se_2 \cdot CuBr_2$ spectrum, meaning that the reaction was complete and the chemical shift was similar to that of Ph_2Se_2 (462 ppm). Thus, we concluded that neither Se–Br, nor Se–Cu species were formed but the reaction of Ph_2Se_2 with $CuBr_2$ produces coordinated adduct. This statement is also based on the fact that Se–Cu bond-containing compound $Cu(SePh)_2 \cdot$2-Phenanthroline has upfield-shifted signal (274 ppm); while PhSeBr signal is strongly downfield (867 ppm). Theoretically two signals in selenium spectra should be seen, however, due to dynamic nature of the intermediate, these signals were merged, and the signal is broad due to paramagnetic properties of copper.

Table 1

Optimization of reaction conditions for the preparation of **23a** and **23b**



Entry	**22**	Solvent	Stoichiometry, **22** : $CuBr_2$: **2a**	Time, h	Yield, %
1	Ph_2Se_2[a]	DCM	2 : 2: 1	4	-[c]
2	Ph_2Se_2[b]	DCM	2 : 2: 1	4	-[c]
3	Ph_2Se_2	DCM	2 : 2 : 1	4	86
4	Ph_2Se_2	DCM	2 : 0.5 : 1	4	47
5	Ph_2Se_2	DCM	1.2 : 1.2 : 1	4	94
6	Ph_2Se_2	DCM	0.6 : 1.2 : 1	4	62
7	Ph_2Se_2	DMSO	1.2 : 1.2 : 1	24	70 [e]
8	Ph_2Se_2	EtOAc	1.2 : 1.2 : 1	24	62[e]
9	Ph_2Se_2	MeCN	1.2 : 1.2 : 1	0.5	90
10	Ph_2Se_2	EtOH	1.2 : 1.2 : 1	12	96
11	-	DCM	0 : 2 : 1	72	86[d]
12	Ph_2Se_2	MeCN	1.2 : 1.2 : 1	1	32+58 [f]
13	Ph_2S_2	DCM	1.2 : 1.2 : 1	24	78[d]
14	Ph_2S_2	MeCN	1.2 : 1.2 : 1	24	87[d]
15	Ph_2Te_2	DCM	1.2 : 1.2 : 1	0.25	94
16	Ph_2Te_2	MeCN	1.2 : 1.2 : 1	4	76

[a] MX_n – $FeCl_3$; [b] MX_n – $FeBr_3$; [c] Mixture of products; [d] 2-bromoindolizinium bromide was formed; [e] Reaction was not completed; [f] $CuBr_2$ was added to **2a** first, and after 20 min Ph_2Se_2 was added. The formation of 2-bromoindolizinium bromide (32 %) and **23a** (58 %) was observed.

Based on the experimental results above, a plausible reaction mechanism is proposed. Firstly, the reaction of diphenyl diselenide with copper(II) bromide leads to the formation of coordinated $Ph_2Se_2 \cdot CuBr_2$ adduct ***I***. Then, adduct ***I*** generates selenium electrophile which coordinates to the triple bond of substrate **2a** forming selenirenium cation ***II***. Then, nitrogen's lone pair attacks the double bond, forming the five-membered cycle with positively charged nitrogen. As a co-product of this reaction PhSeCuBr intermediate that decomposes regenerating diphenyl diselenide and producing copper(I) bromide.

The formation of CuBr in the reaction mixture was confirmed in the experiment with bicinchoninic acid (BCA), which chelates with Cu(I) ion but not with Cu(II) ion, giving deep purple-colored complex ***III*** (absorbance maximum at 562 nm). The absorbance measurements (Fig. 1 B) of three samples were taken: the reaction mixture plus BCA, CuI plus BCA, and $CuBr_2$ plus BCA. The absorption values for the reaction mixture plus BCA and CuI plus BCA were similar (absorption 1.1503 at 560 nm and 1.4749 at 560 nm, respectively) due to formation of complex ***III***. Therefore, the use of equimolar amount of $CuBr_2$ is justified – it is consumed and forms copper(I) bromide that cannot induce the formation of selenyl electrophile. The last statement was also confirmed by the control tests using CuBr or CuI instead of $CuBr_2$.

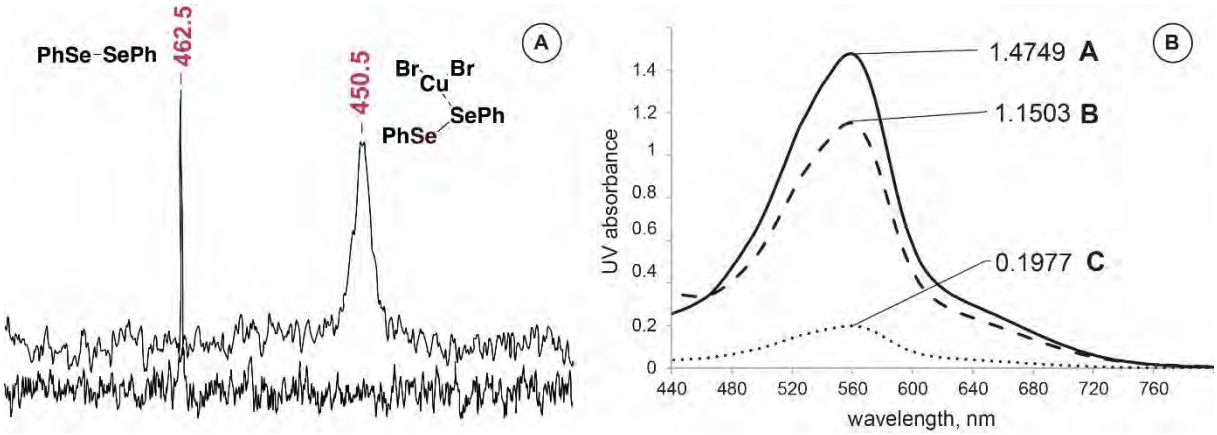
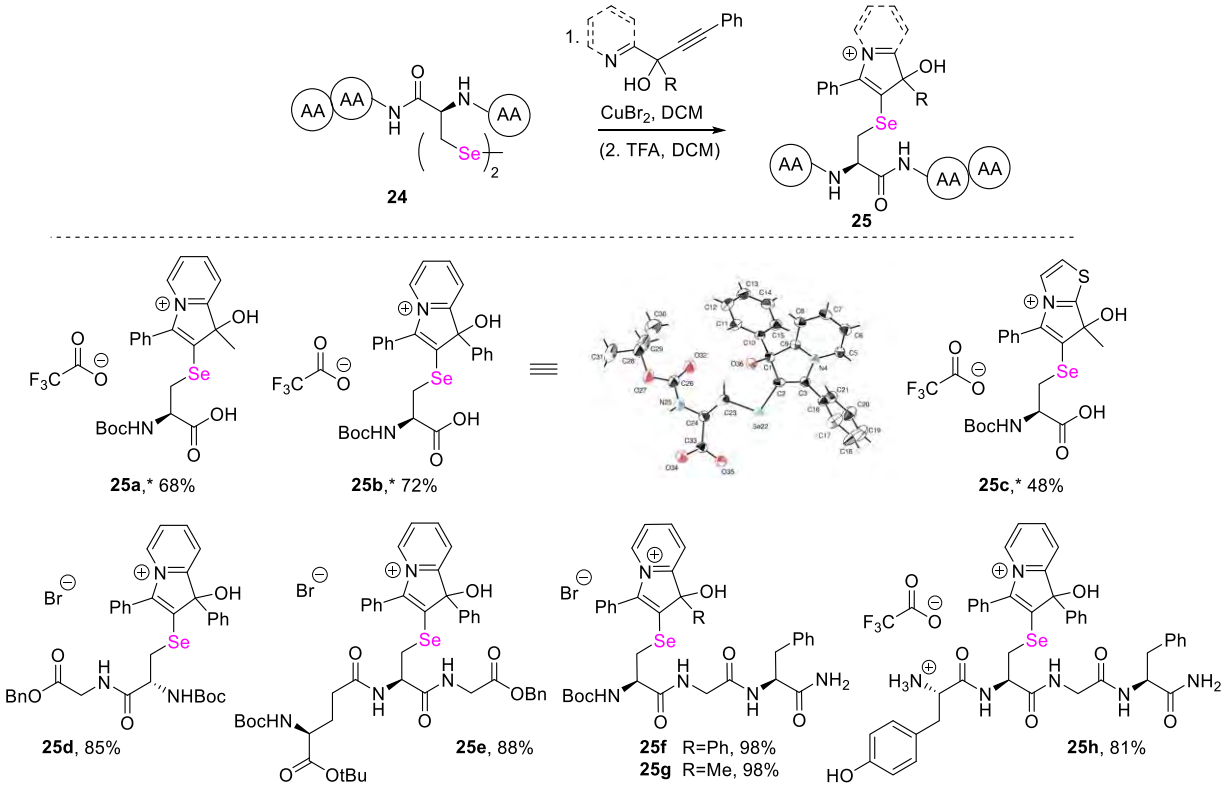


Fig. 1. A – overlay of ^{77}Se NMR spectra of $Ph_2Se_2 \cdot CuBr_2$ and Ph_2Se_2. B – UV absorbance spectrum: A – CuI + BCA; B – the reaction mixture + BCA; C – $CuBr_2$ + BCA.

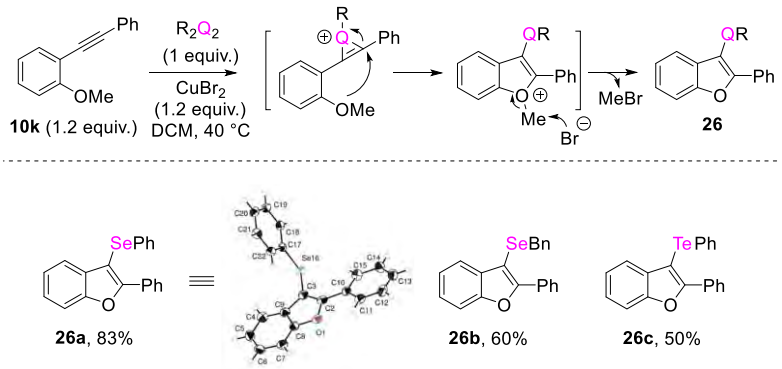
Next, we tested whether Boc-protected selenocystine **24a** is compatible with the developed conditions. The reaction between Boc-Sec and **2a, b, d** in the presence of copper(II) bromide provided corresponding indolizinium **25a**, **b** and pyrrolothiazolium **25c** salts in good yields as a mixture of diastereomers that were separated by HPLC (Scheme 7). We obtained single crystals of ***(1R,S)*-25b** as zwitterionic salt, unambiguously confirming the structure. Next, we extended 5-*endo-dig* reaction to selenocystine-containing peptides. Gratifyingly, all tested selenocystine-containing peptides readily reacted with **2b** in the presence of copper(II) bromide forming indolizinium salts **25d–h** in high yields.



Scheme 7. Synthesis of Sec-indolizinium salts.

24a (Boc-Sec)$_2$; **24b** (Boc-Sec-Gly-OBn)$_2$; **24c** (Boc-Glu(O*t*Bu)-Sec-Gly-OBn)$_2$; **24d** (Boc-Sec-Gly-Phe-NH$_2$)$_2$; **24e** (Boc-Tyr-Sec-Gly-Phe-NH$_2$)$_2$. All products were purified by reverse phase chromatography (**25a–c** MeCN/H$_2$O, pH = 4, trifluoroacetic acid).

Another successful 5-*endo-dig* cyclization was performed utilizing Ph_2Se_2 and 2-(phenylethynyl)anisole (**10k**). The elaborated reaction conditions were suitable for this cyclization reaction, except elevated temperature (40 °C) was required for full consumption of starting materials in 16 h. Thus the 3-selanylbenzo[*b*]furan **26a** was obtained in 83 % yield and the structure was unambiguously confirmed by X-ray analysis (Scheme 8). Analogously, 3-chalcogenyl benzo[*b*]furans **26b** and **26c** were synthesized with similar or better yield than described in literature by $FeCl_3$ promoted cyclization of 2-phenylethynyl anisole (**26b** 64 %, **26c** 36 %).[6]



Scheme 8. 3-Selanyl and 3-tellanyl benzo[*b*]furans formation.

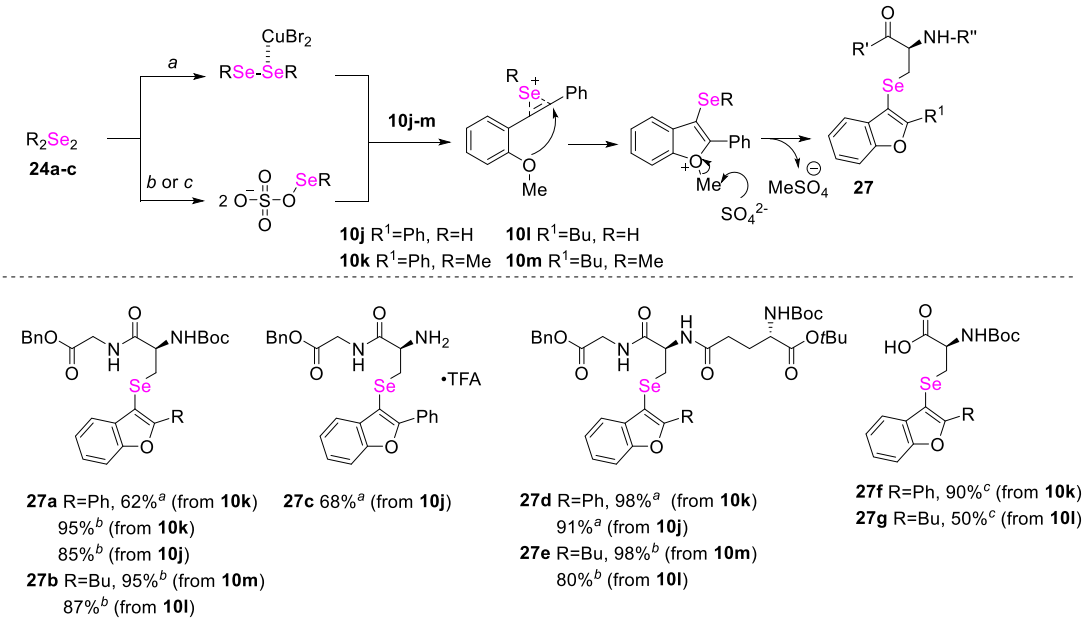
The reaction between peptides **24b**, **c** and anisole in the presence of $CuBr_2$ resulted in preparation of Sec-containing benzo[*b*]furans **27a** and **27d** (Scheme 9). Notably, another Lewis base – $FeCl_3$ – failed to initiate the formation of products **27**. Similarly, 2-(phenylethynyl)phenol (**10j**) provided the same products. Unfortunately, $CuBr_2$-induced selenyl electrophile generation was not suitable for preparation of 2-alkyl-3-selanylbenzo[*b*]furans. A complex mixture was formed utilizing **10l** probably due to the formation of aryl group stabilized vinyl cation that prevents 5-*endo-dig* cyclization. Accordingly, a different method was required for selenyl electrophile generation and subsequent 5-*endo-dig* cyclization.

A suitable promoter was found among oxidants. The formation of strongly electrophilic organoselenyl sulfate is known as a resulting interaction between diselenides and persulfates. Although the reaction between **24b** and **10l** in the presence of 5 equiv. of $K_2S_2O_8$ was slow (3 days were required for full conversion of the starting materials), more importantly, it was selective and provided the desired benzo[*b*]furan in high yield. Other tested oxidants were less efficient (ammonium persulfate, potassium iodate) or provided unselective reaction (oxone, *meta*-chloroperoxybenzoic acid, sodium periodate, cerium ammonium nitrate, (diacetoxyiodo)benzene) due to fast oxidation and deselenylation.

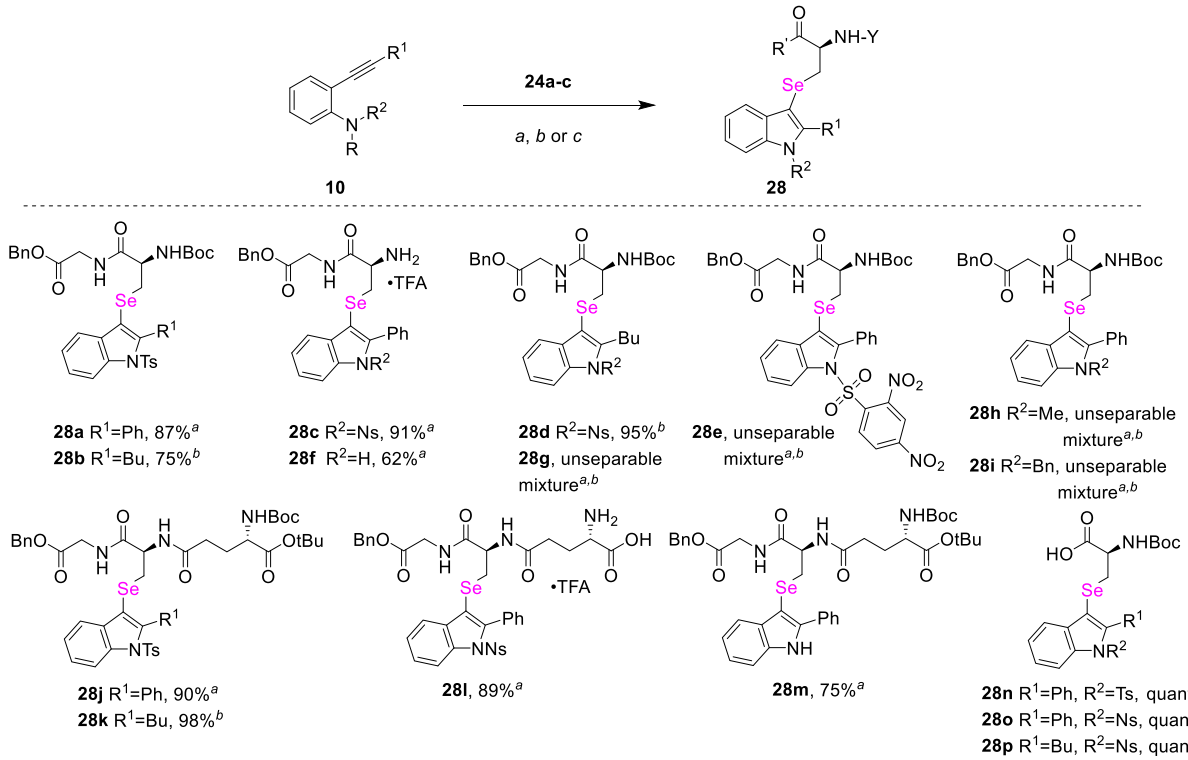
Notably, the yield of **27c** was improved employing 2-(hex-1-yn-1-yl)anisole (**10m**). It should be noted that $K_2S_2O_8$-induced selenium electrophile generation and subsequent cyclization provided **27a** in high yield as well. Furthermore, the yield was higher than in the case of $CuBr_2$-promoted cyclization.

The reaction between $(Boc\text{-}Sec)_2$ and **10k** in the presence of $CuBr_2$ or $K_2S_2O_8$ (5 equiv.) provided only traces of the respective benzo[*b*]furan. However, utilization of 50 equiv. of $K_2S_2O_8$ resulted in high yielding preparation of **27f** in 16 h without damaging starting materials. Due to the low solubility of $K_2S_2O_8$ in acetonitrile, only a small amount of oxidant is actually present in the reaction mixture. Therefore, it is completely acceptable to utilize a considerable excess of this cheap inorganic reagent.

Analogously, $CuBr_2$-induced selenyl electrophile generation and following 5-*endo-dig* cyclization was applied for the synthesis of 2-aryl-3-selanylindoles, while $K_2S_2O_8$-promoted selenyl electrophile generation was employed for synthesis of 2-alkyl-3-selanylindoles as well as for the preparation of Boc-Sec-indoles (Scheme 10). Substrate scope showed that Ts, Ns and Boc protecting groups were well tolerated, but 2,4-dinitrobenzenesulfonyl protection, *N,N*-dimethyl-, *N,N*-dibenzyl, *N*-benzyl- 2-(phenylethynyl)anilines and unprotected 2-(phenylethynyl)aniline were not suitable substrates.



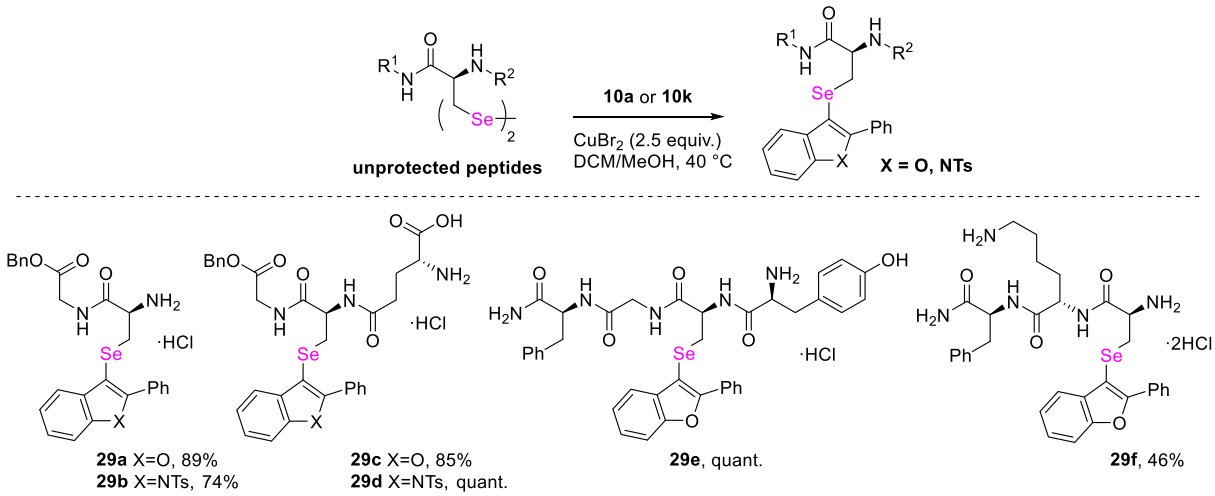
Scheme 9. 3-selanyl benzo[*b*]furan formation: scope and limitation studies. *Reaction conditions*: *a* – 1. $CuBr_2$ (1.5 equiv.), DCM, 40 °C; (2. TFA, DCM, 0 °C); *b* – $K_2S_2O_8$ (5 equiv.), MeCN, rt; *c* – $K_2S_2O_8$ (50 equiv.), MeCN, rt.



Scheme 10. Synthesis of 3-selanylindoles: scope and limitation studies. *Reaction conditions*: *a* – 1. $CuBr_2$ (1.5 equiv.), DCM, 40 °C; (2. TFA, DCM, 0 °C); *b* – $K_2S_2O_8$ (5 equiv.), MeCN, rt; *c* – $K_2S_2O_8$ (50 equiv.), MeCN, rt. **10n** R^1Bu, R^2=Ts, R=H; **10o** R^1=Bu, R^2=Boc, R=H

Unfortunately, both above-mentioned methods failed to promote the generation of sulfenyl electrophile from **8** with consequent formation of glutathione containing benzo[*b*]furans and indoles.

Significantly, $CuBr_2$-induced Sec electrophile generation was applicable to unprotected peptides as well, employing tosyl-aniline **10a** and anisole **10k** as the traps for the electrophile. This finding greatly improves the application scope for the method and allows the use of more sophisticated peptides. The reaction proceeded smoothly due to inability of protonated amino groups to form a complex with $CuBr_2$. Notably, the tested selenocystine-containing peptides showed excellent reactivity and the products were prepared with excellent yields (Scheme 11).



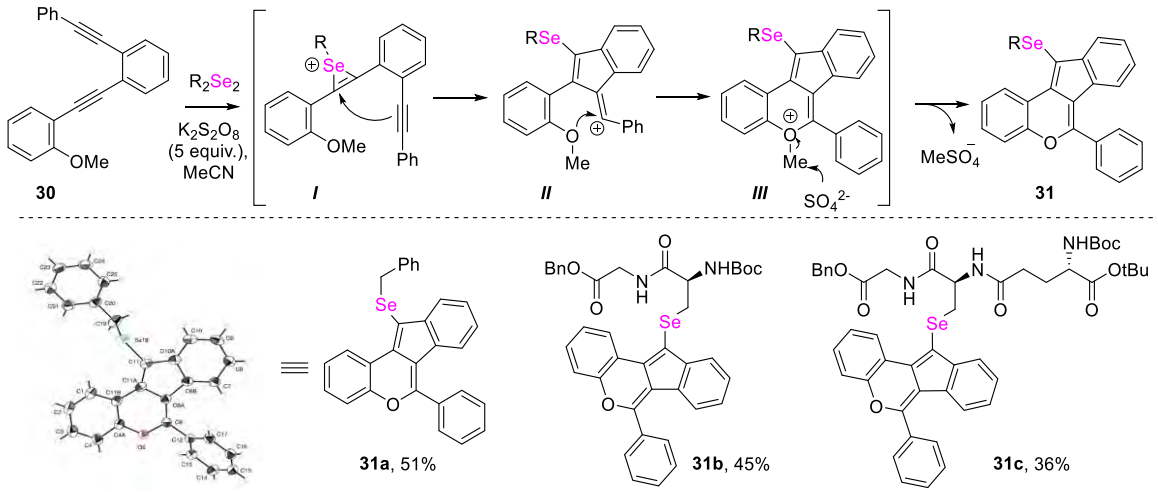
Scheme 11. Preparation of benzo[*b*]furans and indoles employing unprotected peptides. Products were purified by reverse phase chromatography ($MeCN/H_2O$, pH=4, hydrochloric acid).

The next challenge was to determine whether selenyl electrophile can be employed in the cascade reaction. We decided to use anisole-containing aryldiyne **30** that would provide indeno[1,2-*c*]chromene skeleton as a consequence of successful and sequential 5- and 6-*endo-dig* cyclization reactions. Chromene moiety is often found in biologically active natural products, furthermore, compounds containing indeno[1,2-*c*]chromene core show high potential for use in dye-sensitized photovoltaic cells. Notably, only few methods exist for the construction of indeno[1,2-*c*]chromene moiety. Previously, TfOH mediated cascade reaction of anisole **30** has been performed for the synthesis of 6-phenylindeno[1,2-*c*]chromene,[35] while halogen-mediated cascade reaction has been reported by Chen *et al.* for the synthesis of halogenated 6-phenylindeno[1,2-*c*]chromenes.[36]

Initially, we tested the reaction of Ph_2Se_2 with **30** in the presence of $CuBr_2$. However, only 11-bromo-6-phenylindeno[1,2-*c*]chromene was detected in the reaction mixture. Potassium persulfate induced electrophile generation did not lead to the desired product as well.

Next, we decided to test Bn_2Se_2. While the use of $CuBr_2$ resulted in formation of 11-bromo-6-phenylindeno[1,2-*c*]chromene, $K_2S_2O_8$ was capable to generate selenyl electrophile from Bn_2Se_2 that further was trapped with the more electron rich triple bond forming selenirenium cation ***I***. Next, the attack of the other triple bond to the selenirenium cation resulted in the

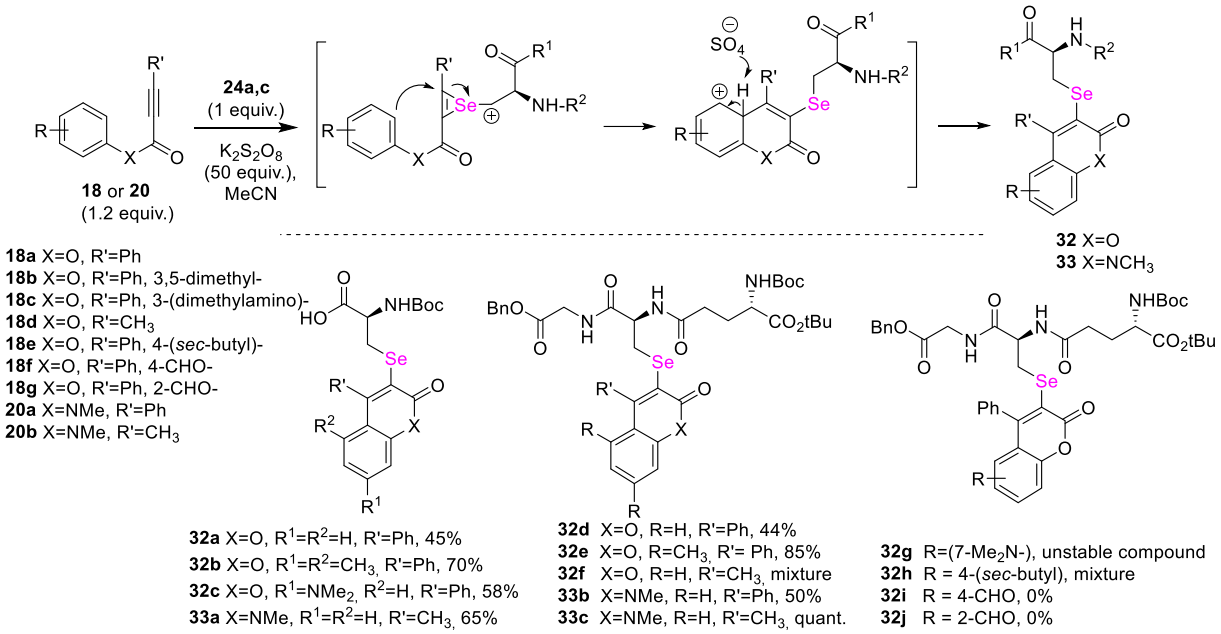
closure of indene cycle (intermediate ***II***). Following attack from the methoxy group to the carbocation provided cyclization intermediate ***III*** that after demethylation gave the product 11-(benzylselanyl)-6-phenylindeno[1,2-*c*]chromene **31a** (Scheme 12). The structure of **31a** was unambiguously confirmed by X-ray analysis. Then, 6-phenylindeno[1,2-*c*]chromenes attached to Sec-peptides were also prepared, unfortunately, the yields were only moderate due to complicated purification process.



Scheme 12. Plausible mechanism for cascade 5-*endo*/6-*endo*-*dig* cyclization.

Next, we moved on to see whether we can apply $CuBr_2$ or $K_2S_2O_8$ method for selenyl electrophile generation and subsequent 6-*endo*-*dig* cyclization. Reaction between $(Boc\text{-}Sec)_2$ and phenyl 3-phenylpropiolate in the presence of $K_2S_2O_8$ provided respective Boc-Sec-containing coumarin **32a** (Scheme 13). The examination of substrate scope revealed that the presence of EDG in the 3rd or 3rd and 5th positions of aryl ring promoted the reaction, whereas introduction of EWG diminished the reactivity. Protected selenoglutathione was also a suitable substrate for cyclization reaction. However, we were not able to isolate the selenoglutathione-containing 7-aminocoumarin due to instability of the product.

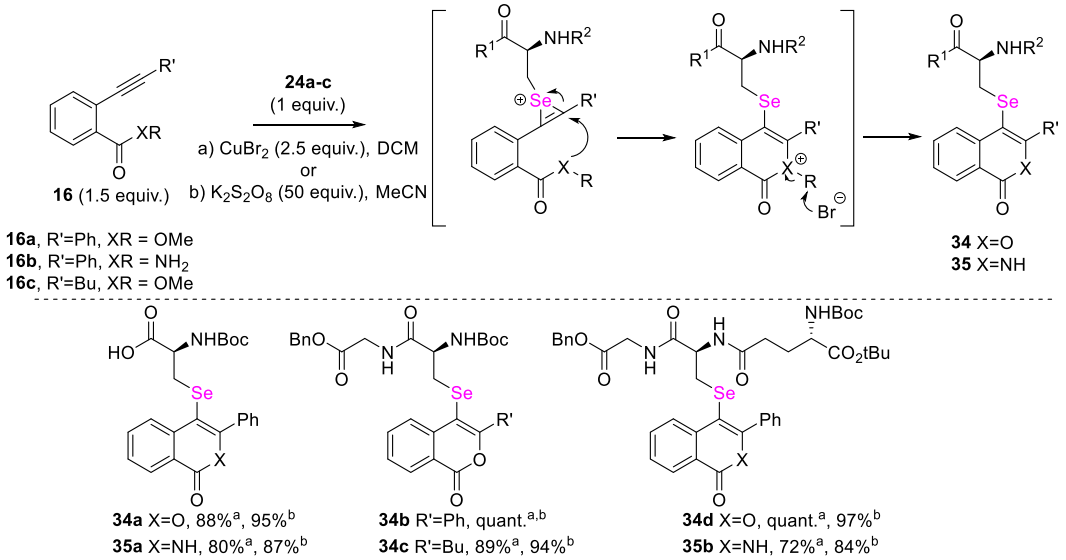
Under the same conditions the analogous nitrogen heterocycles – quinolin-2-ones **33a-c** were prepared in good yields.



Scheme 13. Synthesis of 3-Sec-coumarins and quinolin-2-ones.

Notably, another 6-*endo-dig* cyclization that provided Sec-containing isocoumarins and isoquinolin-2-ones (Scheme 14) was easily performed utilizing either $K_2S_2O_8$ or $CuBr_2$-induced selenyl electrophile generation. The product formation occured in excellent yields under both conditions, although it should be pointed out that the reaction time for full conversion of starting materials was 2 h for $CuBr_2$-induced selenyl electrophile generation and 24 h for $K_2S_2O_8$.

The first step for isocoumarin and coumarin formation is the generation of selenyl electrophile, which is then trapped by the triple bond providing selenirenium ion. Unlike the coumarin ring formation that relies on electrophilic aromatic substitution, the isocoumarin ring is closed by the attack of the nucleophilic heteroatom to the selenirenium atom. Therefore, the reaction occurs more easily.



Scheme 14. Synthesis of 4-Sec-isocoumarins and isoquinolin-2-ones.

The articles about the studies described in this chapter can be found in Appendices I, II, VI.

3. Visible light-mediated functionalization of selenocystine-containing peptides: selenylation of indoles and macrocyclization

Photocatalyzed reactions are a convenient way for synthesis of complex structures under mild conditions. This reaction type is considered as sustainable, ensuring efficient and selective synthesis that can be perfomed under biocompatible conditions as well. Usually the presence of photosensitizers is required for efficient transformation. Typical examples of homogenous catalysts are transition metal complexes and organic dyes. The use of transition metal (Ru, Ir) complexes is expensive, and they are not considered as an environmentally-friendly option. On the contrary, the use of organic dyes is an attractive alternative. Obviously, heterogenous catalysts can also be employed (inorganic semiconductors (metal oxides or sulfides), graphitic carbon nitride polymers, photoactive MOFs). A heterogenous catalyst offers the possibility to use the catalyst repeatedly and facilitates the purification of products.

Some of the most popular clases of organic dyes are: acridines and acridinium salts, fluorescein and its derivatives, benzophenones, pyrylium salts, rhodamines, and phenothiazines. Rose Bengal is a structural analogue of fluorescein, and it has emerged as an efficient photocatalyst with a wide application.[37]

Upon absorption of light, RB is activated to its excited singlet state (RB*) with $t^{1/2}$ ranging from 10^{-6} to 10^{-9} s. The singlet excited state can return to ground state or it is converted to a longer lived triplet excited state ($t^{1/2} = 10^{-3}$ s) via intersystem crossing.[38] Mostly reactions with RB proceed through single electron transfer (SET), but it can also work through energy transfer (EnT) pathway, particularly as a singlet oxygen sensitizer. In this process, excited state RB* is formed and then RB* transfers its energy to the substrate to generate a reactive substrate.[37,39]

Excited RB* can work as an oxidant or as a reductant. In the reductive quenching cycle, the organic reactant gets oxidised. For example, in reductive activation the excited state of RB* is converted to $RB^{\bullet -}$ radical anion via (SET), with the simultaneous conversion of a susbtrate to the radical cation intermediate. Then oxidation of $RB^{\bullet -}$ by oxygen leads to the formation of the ground state RB. Similarly, in the oxidative quenching cycle, the organic reactant initially gets reduced with the oxidation of the photoexcited species.[39]

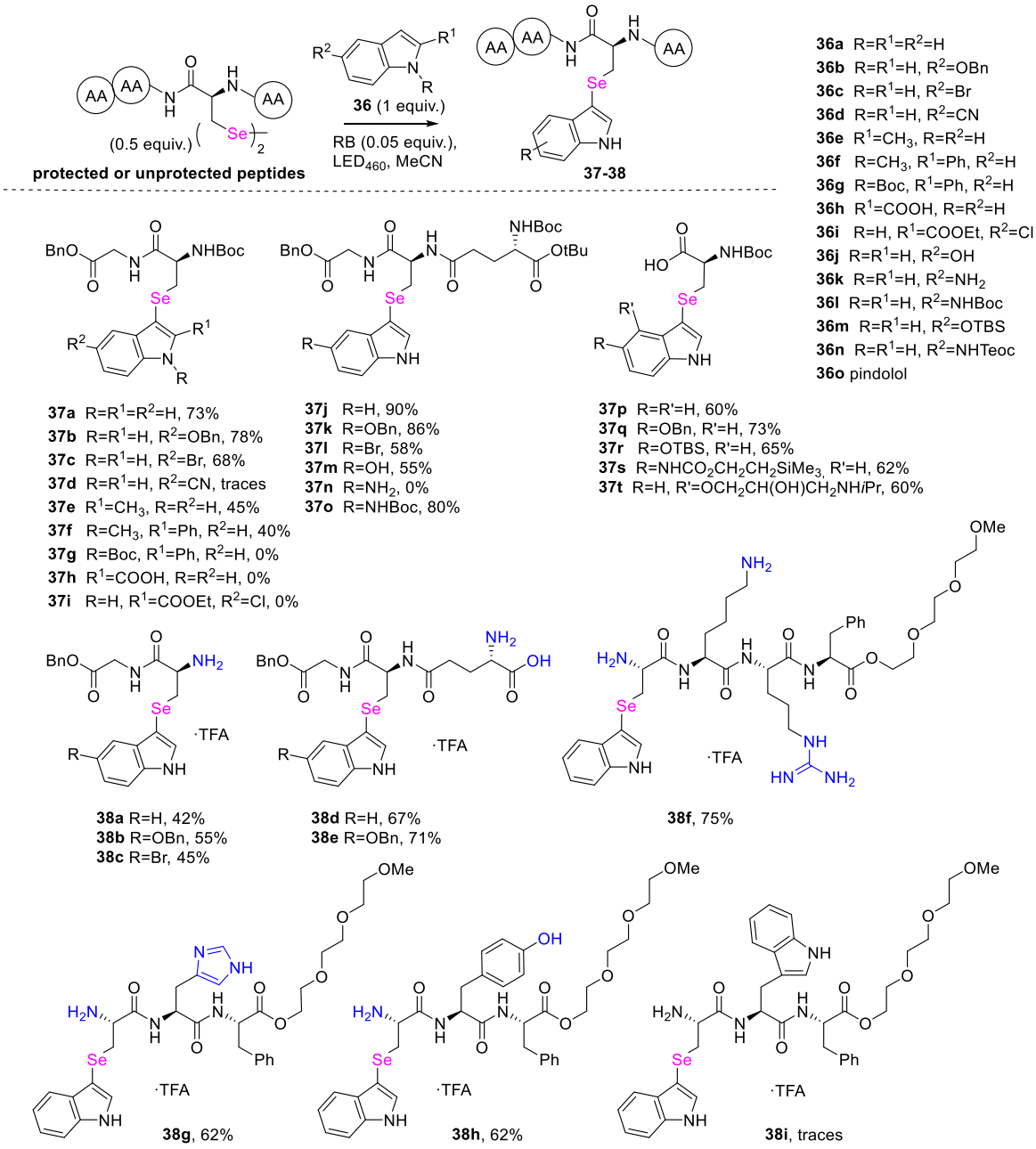
Although the use of simple diaryl diselenides in the visible light-mediated reacions has been investigated,[40-41] the same cannot be said abot Se–Se bond-containing peptides. Notably, selenocystine-containing peptides can be converted to selenolanthionines by UV irradiation,[42] while diselenide metathesis between simple diorganyl diselenides[43] or Se–Se bond-containing peptides[44] has been established under visible light irradiation.

Therefore, we set a goal to investigate the possibilites to functionalize selenocystine-containing peptides under visible light irradiation. We decided to examine the reaction between $(Boc\text{-}Sec\text{-}Gly\text{-}OBn)_2$ **24b** and 1*H*-indole (**36a**) under blue LED light (max 460 nm, bright blue, $x = 0.1440$, $y = 0.0395$, >50 000 lx) conditions (Scheme 15). The preliminary screening of photocatalysts (transition metal complexes, organic dyes >25) was performed in acetonitrile

using 0.5 equiv. **24b**, 1 equiv. **36a**, 2 mol% transition metal catalyst or 5 mol% organic dye. Surprisingly, only two photocatalysts were able to ensure selective synthesis of the desired 3-selanylindole **37a**: Rose Bengal (RB) and its analogue erythrosin B. RB was slightly more efficient, thus it was the catalyst of choice for further studies. Other tested photocatalysts (5-carboxytetramethylrhodamine (5-TAMRA), nickel tetraphenyl porphyrin, 4-CzIPN, cresol red, chlorophenol red, bromocresol green, methyl orange, congo red, direct red 81, direct yellow 27, methylene blue, basic fuchsine, indigo carmine, alcian blue, 2,4,6-triphenylpyrylium tetrafluoroborate, 9-mesityl-10-methylacridinium tetrafluoroborate, acridine and *N*-methylacridinium iodide) were either significantly less efficient or provided unselective reaction. Acetonitrile appeared to be the most suitable solvent because other tested greener protic solvents such as MeOH, EtOH, EtOH/H_2O, and *i*PrOH resulted in a nonselective reaction due to the fast oxidation and deselenylation of **24b**. Control reactions performed without RB, in the daylight or dark did not provide the formation of **37a**, therefore confirming the need of both – RB and LED_{460}. The reaction performed under Ar atmosphere resulted in lower conversion of the starting materials. The reaction performed in the presence of 4-amino-TEMPO did not yield **37a** as well, suggesting the radical mechanism. We concluded that the optimal conditions for the synthesis of 3-Sec-indoles are 5 mol% RB, MeCN and blue LED light for 90 min. Notably, both diselenide parts are utilized in the reaction, thus an atom economic method is demonstrated.

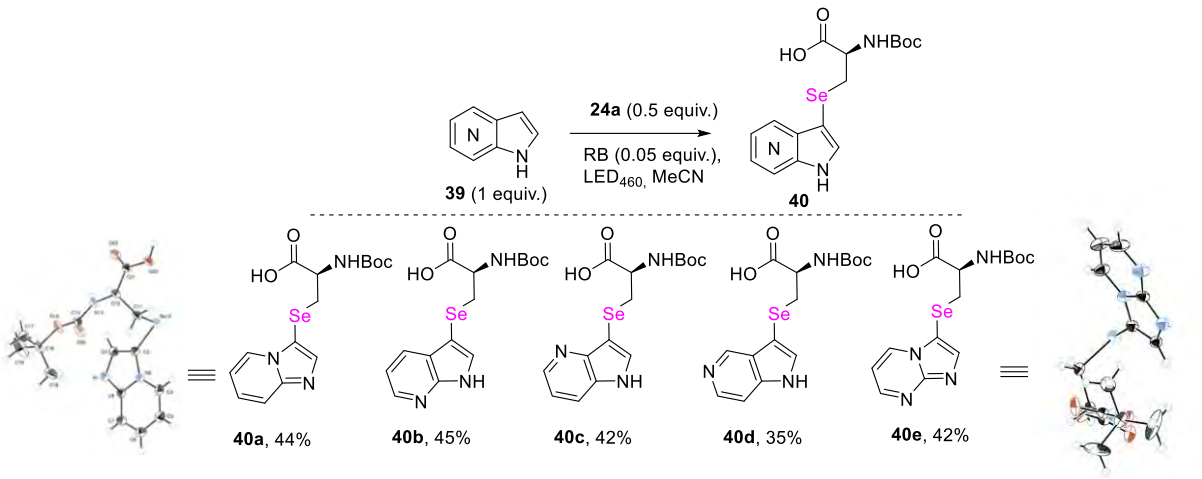
The examination of substrate scope revealed that the presence of an EDG at the C5 position of indole improved the reaction yield; halogen atoms did not significantly affect the process, but the presence of an EWG diminished the reactivity and provided only trace amounts of the products. An EDG at the C2 position provided the product in lower yield, but electron-deficient indoles (EWG at the N1 or C2 position) were completely unreactive. Importantly, a hydroxy group at the C5 position of indole was also tolerated under the reaction conditions, whereas the reaction with 5-aminoindole failed. However, the use of *tert*-butyl (1*H*-indol-5-yl)carbamate **36l** resulted in the formation of desired product **37o** in high yield. Furthermore, not only protected selenocystine-containing peptides, but also $(Boc\text{-}Sec)_2$ was a suitable substrate for selanylation of indoles leading to formation of useful building blocks **37p-t**.

Importantly, unprotected selenocystine-containing peptides were also well tolerated under the reaction conditions facilitating the developed protocol. Even more sophisticated selenocystine-containing peptides with ‘sensitive’ amino acid residues (Lys, Arg, His, Tyr) provided the expected Sec-indoles in good yields. The only exception was the Trp-containing peptide – nonselective reaction occurred, most likely due to ability of Trp to form radical under irradiation conditions.



Scheme 15. Sec-indole formation: scope and limitation studies.
38a-l: the products were obtained employing unprotected peptides as TFA salts.

Additionally, Boc-Sec-azaindoles were prepared employing visible light-initiated reaction (Scheme 16). However, we found out that only protonated azaindoles were able to react with $(Boc\text{-}Sec)_2$. The yields were moderate due to partial degradation of $(Boc\text{-}Sec)_2$. Notably, the structures of products **40d** and **40e** were confirmed by X-ray analysis.

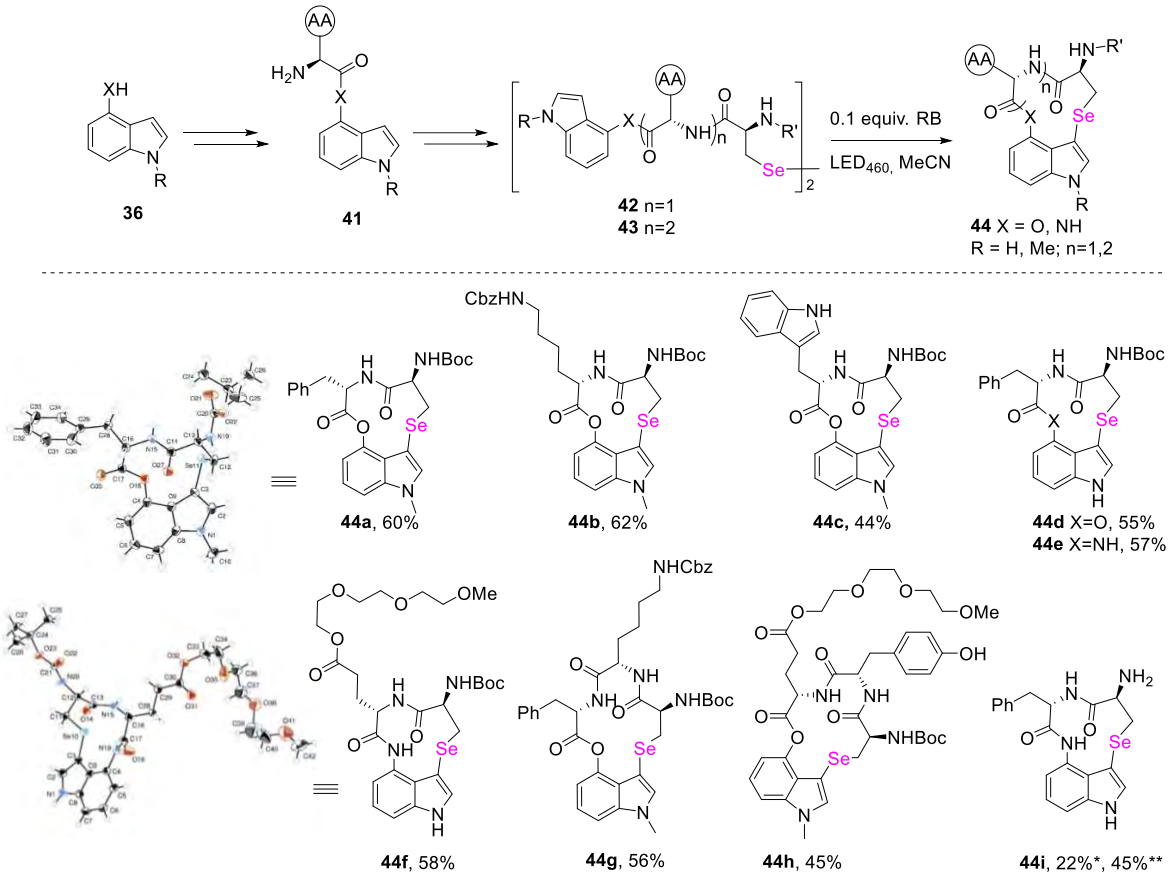


Scheme 16. Synthesis of Boc-Sec-azaindoles.

Next, we decided to establish whether the intramolecular indole selenylation (Approach A) can be performed under the same conditions. A successful reaction would afford Sec-containing indole-embedded macrocycles. Additionally, we envisioned alternative approaches towards synthesis of macrocycles: selenylation of an indole attached to a peptide and subsequent intramolecular amide bond formation (Approach B); and a visible light-initiated reaction between Boc-Sec and protected 5-hydroxy- or aminoindole, then coupling reaction with a small peptide, deprotection and intramolecular amide bond formation (Approach C).

Gratifyingly, visible light-mediated intramolecular selanylation proceeded smoothly providing macrocycles **44a-h** in good yields (Scheme 17). Although the reaction was slower than the intermolecular reaction, it was selective. Furthermore, the structures of macrocycles **44a** and **44f** were unambiguously confirmed by X-ray analysis. We also succeeded in isolation of unprotected macrocycle **44i**. The product was obtained by visible light-mediated macrocylization of Fmoc protected substrate and subsequent deprotection. Alternatively, **44i** was prepared by utilizing deprotected **42g** in the macrocyclization reaction. However, the yield was lower utilizing unprotected substrate.

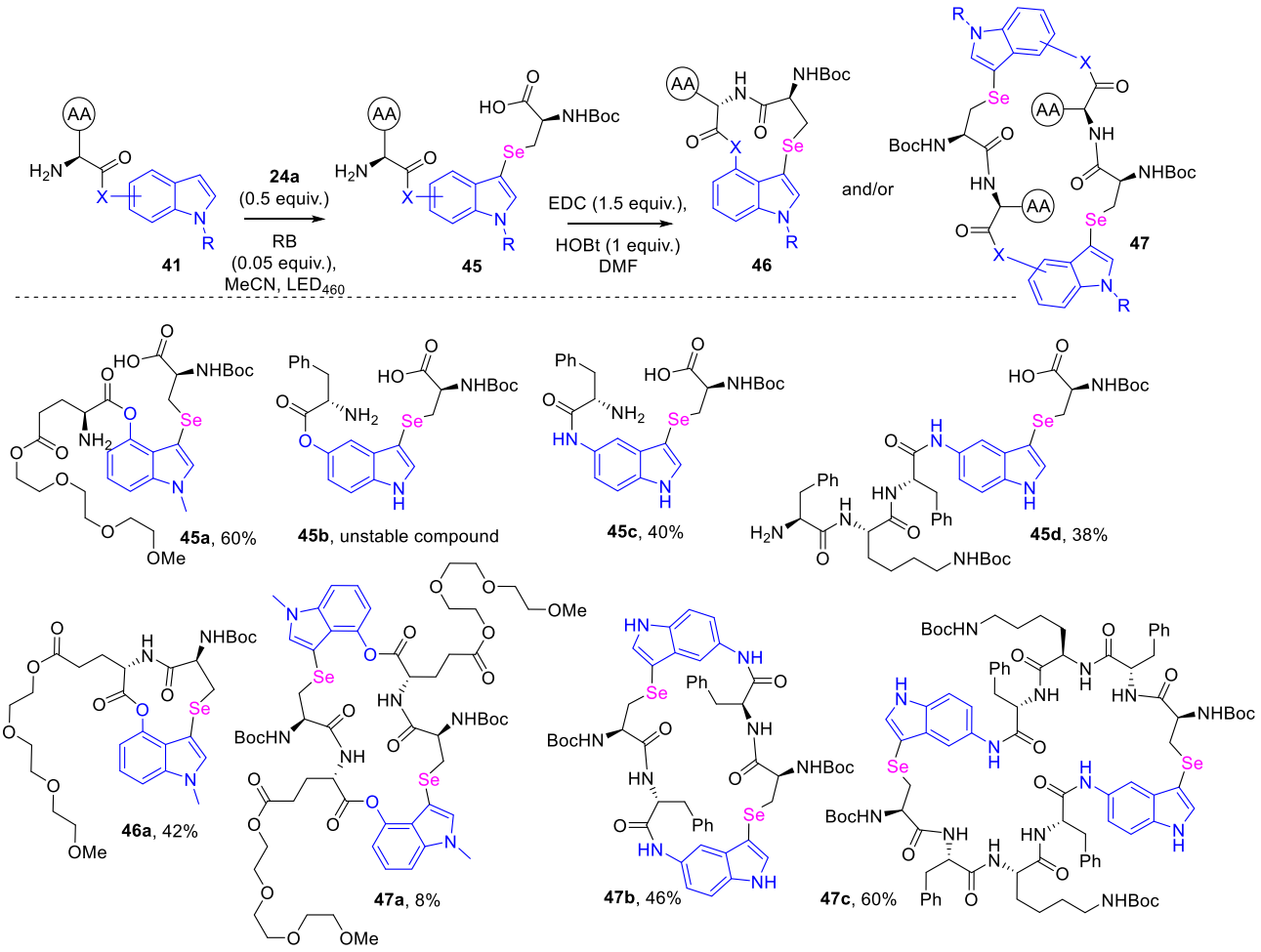
Unfortunately, we concluded that Approach A is not suitable for macrocycle formation starting from indoles with peptides attached to its C5 position, probably due to conformational restrictions. Therefore, we proposed alternative approaches for synthesis of these compounds.



Scheme 17. Synthesis of macrocycles: Approach A.
*Fmoc cleavage, cyclization, **cyclization, Fmoc cleavage.

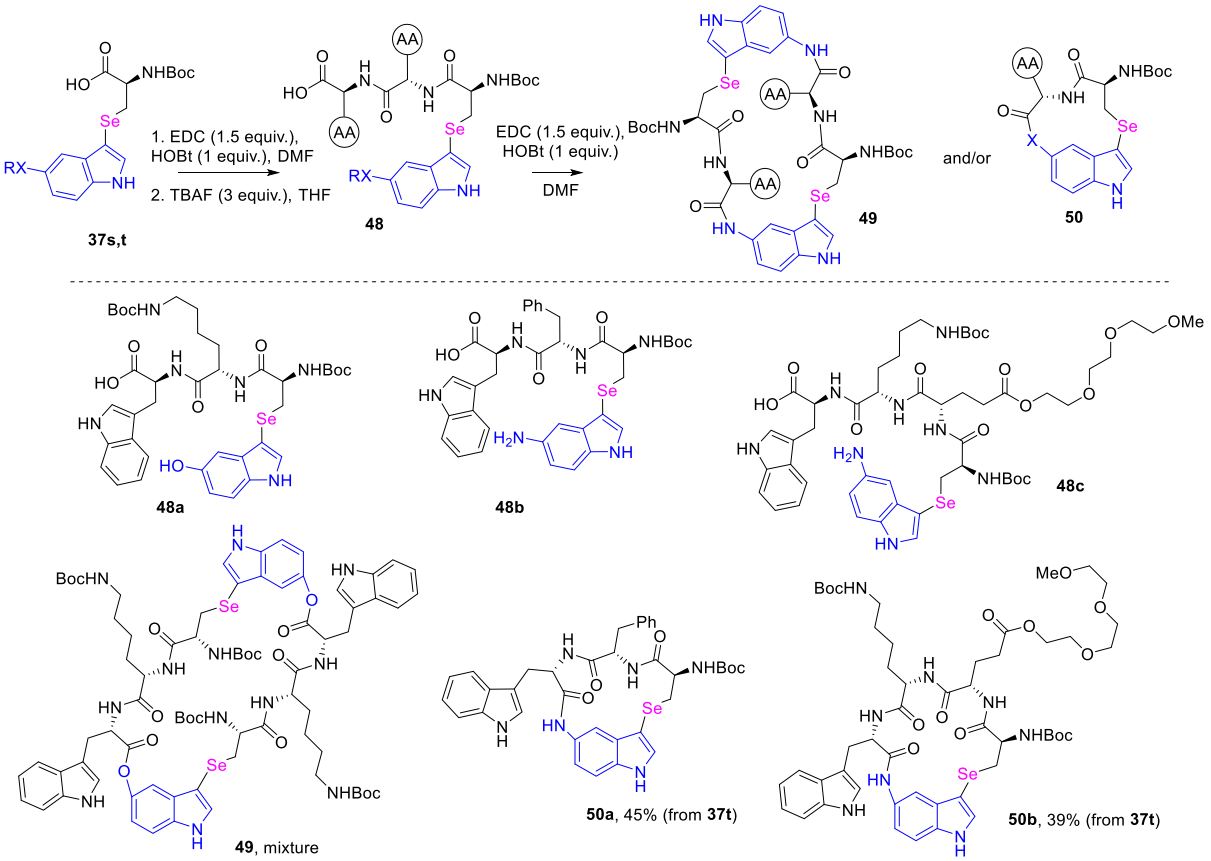
Approach B relied on visible light-mediated selenylation of indoles **41** that contained the amino acid or short peptide at the C4 or C5 position of the indole ring and subsequent intramolecular peptide bond formation reaction (Scheme 18).

Visible light-mediated reaction yielded Boc-Sec-indoles **45** in moderate to good yields. Then EDC/HOBt coupling was performed for intramolecular amide bond formation. As a result, macrocycle **46a** was isolated utilizing 4-substituted indole as the starting material. Notably, we also isolated the bis-macrocyclization product **47a** as the minor product. Surprisingly, indoles **45c**,**d** with substituent at the C5 position provided only bis-macrocyclization products **47b**,**c**.



Scheme 18. Synthesis of macrocycles: Approach B.

The third approach (Approach C) required the utilization of products **37s** and **37t** that were obtained by visible light-mediated selenylation of protected 5-hydroxy- and 5-aminoindoles (Scheme 19). The next step was intermolecular amide bond formation reaction, then after deprotection, an intramolecular amide bond formation reaction was performed. The formation of bis-macrocycle **49** was not selective, while stable macrocycles **50a** and **50b** were isolated.



Scheme 19. Synthesis of macrocycles: Approach C.

The next task was to explore the mechanism of visible light-mediated selanylation. First, we discovered that irradiation of the solution of **24b** and RB affords unstable seleninic acid **51** and Boc-Dha-Gly-OBn **53** (Scheme 20). We were not able to isolate **51** but it was detected by LC-MS, HRMS ([M+Na] = 471.0633) and ^{77}Se NMR spectroscopy ($RSeO_2H$ 1217.5 ppm) (Fig. 2 A). Storage of the reaction mixture in an NMR tube for 24 h led to the disappearance of the signal in the ^{77}Se NMR spectra attributed to **51**, however, a new signal was formed – seleninic acid (H_2SeO_3 **52**) (1302.7 ppm).

The control tests proved that the presence of water and oxygen in the solvent is necessary for the reaction to occur. The reaction performed in the presence of TEMPO did not lead to the formation of **53**, thus confirming radical pathway.

Under irradiation conditions, oxygen is converted to superoxide radical anion ($O^{\bullet -}$), while $RB^{\bullet -}$ returns to the ground state. Reaction between $O^{\bullet -}$ and water generates hydroperoxyl radical that produces hydrogen peroxide, which is trapped by **24b**, resulting in oxidation and deselenylation with the formation of a double bond.

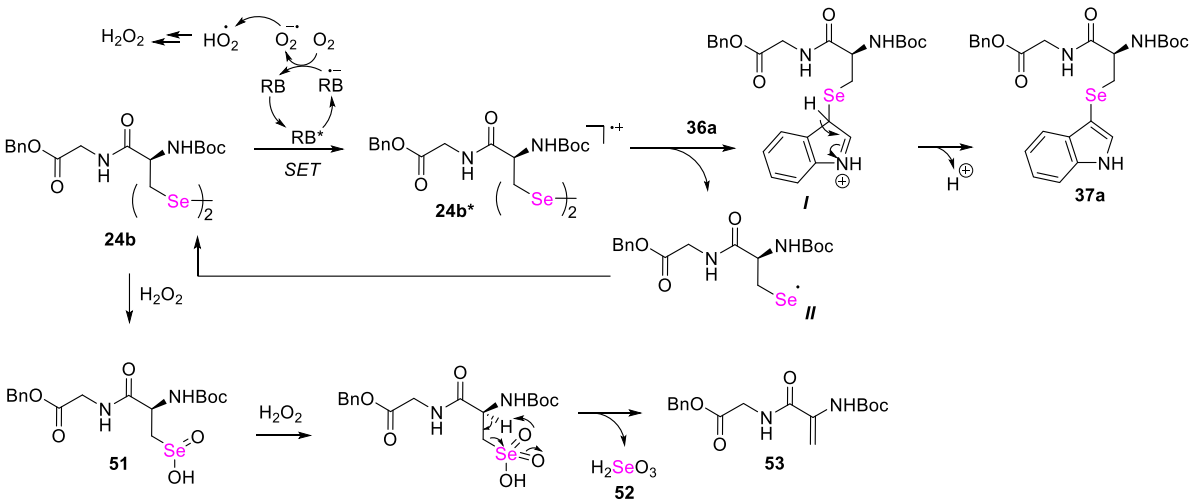
UV spectra were recorded for **24b**, **36a**, RB, ethyl eosin, erythrosin B, FIrPic, $Ru(bpy)_3Cl_2 \cdot 6H_2O$, and 4-CzIPN in dry acetonitrile solutions (Fig. 2 B and 2 C). The photochemical reaction between **36a** and diselenide **24b** was not effective in the absence of a catalyst under LED_{460} light because indole has an absorption band from 200 to 305 nm and **24b** exhibits absorption until 430 nm, albeit with low intensity.

Notably, the absorption shoulder at 275–430 nm in the **24b** UV spectrum is characteristic of Se–Se bond, which facilitates the formation of selenyl radicals in the presence of a photocatalyst.

The photoluminescence quenching of RB, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and FIrPic was performed using **24b** or **36a** in degassed acetonitrile. The quenching rate constant of RB in the experiment with **24b** was determined to be 10.42×10^{-3} l/mol (Fig. 2D). In contrast, **36a** does not quench the fluorescence of RB to any significant extent.

EPR studies confirmed radical formation of RB in solution under irradiation conditions. Furthermore, the presence of diselenide **24b** during irradiation, reduced the intensity of RB* signal twice, allowing to confirm the establishment of dynamic equilibrium under LED_{460} light: RB* + **24b** ↔ (RB + **24b***).

Thus, based on the obtained results, it can be concluded that upon excitation of RB, radical cation **24b*** is produced, which reacts with indole **36a**, forming intermediate ***I*** and selenyl radical ***II***. The radical ***II*** can provide diselenide **24b** via dimerization while intermediate ***I*** delivers the product **37a** after deprotonation (Scheme 20).



Scheme 20. Proposed mechanism of visible light mediated reaction.

The article about the studies described in this chapter can be found in Appendix IV.

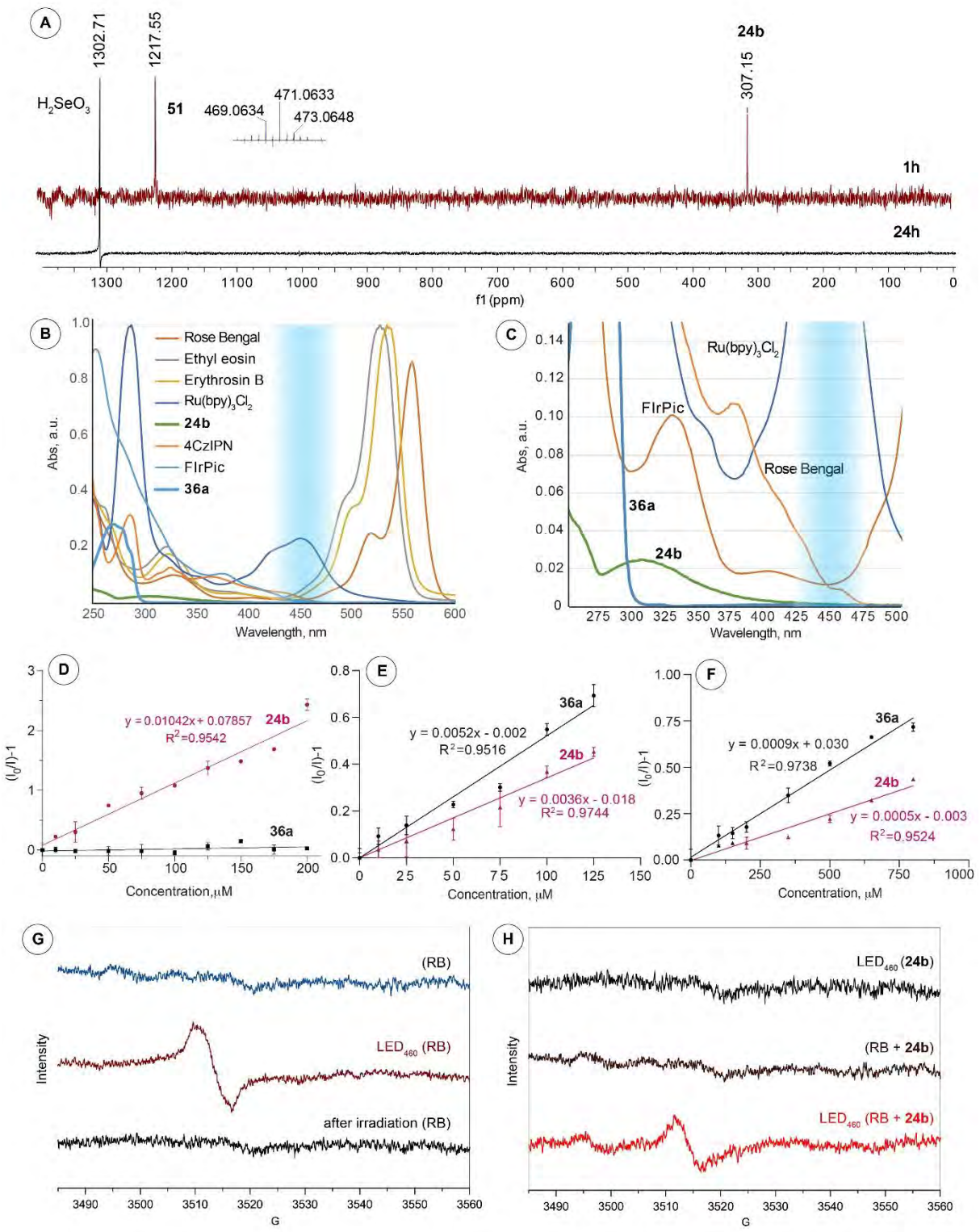


Fig 2. A – ^{77}Se NMR spectrum of **24b** degradation products after 1 h of irradiation and after storage of the reaction mixture in NMR tube for 24 h; B – UV spectra of **24b**, **36a** and photocatalysts; C – magnified UV spectra of 250–500 nm region; D – photoluminescence quenching of RB with **24b** and **36a**; E – photoluminescence quenching of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ with **24b** and **36a**; F – photoluminescence quenching of FIrPic with **24b** and **36a**; G – EPR spectra of RB and RB + **24b** with and without LED_{460} irradiation.

4. Visible light-mediated synthesis of Se–S bond-containing peptides

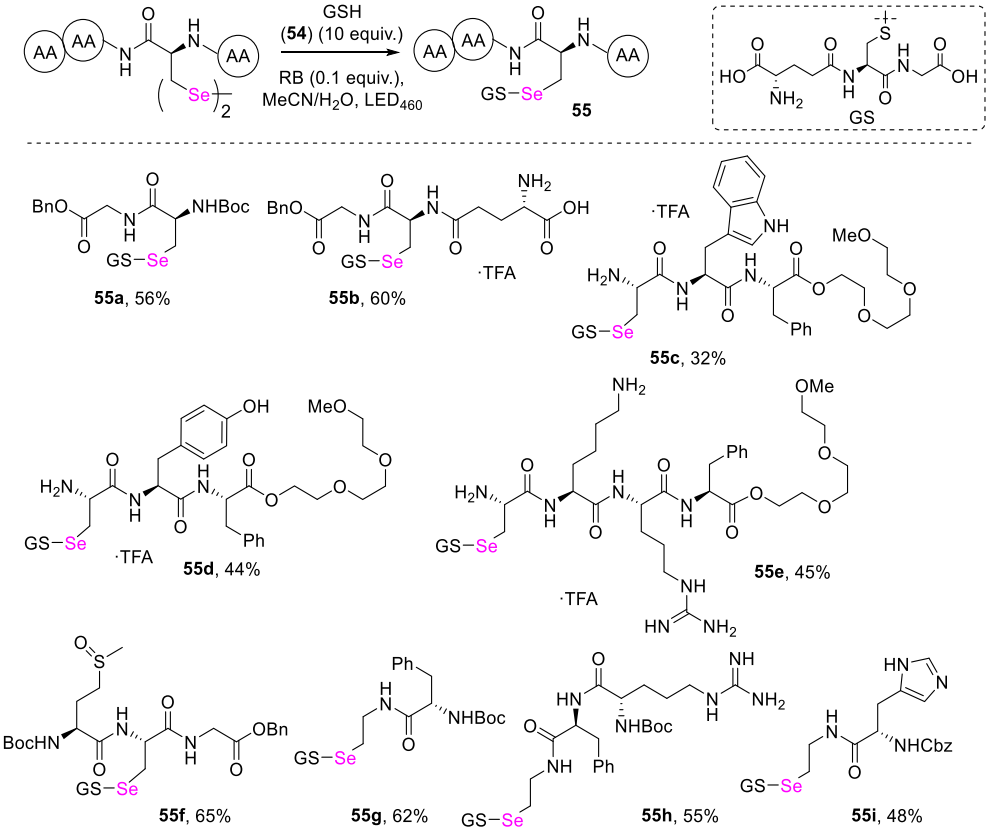
Se–S bond is found in the active center of thioredoxin reductase – one of the major components of the antioxidant system in mammalian cells.[45,46] Se–S bond-containing intermediate is formed in the catalytic cycle of the glutathione peroxidase – selenoenzyme that is responsible for reduction of H_2O_2 and other peroxides by glutathione.[47] Low-molecular weight compounds with Se–S bond have been used as fluorescent probes for detection of reactive sulfur species[48,49] and as prodrugs for inhibition of protein tyrosine phosphatases.[50]

Compounds with Se–S bond are considered unstable, thus the synthesis can be challenging.[51] The exchange reaction between diselenide and thiol, although theoretically possible, is unfavorable because the selenolate byproduct is a stronger nucleophile than thiol.[52] However, the reaction can be performed under suitable conditions.[51] Diphenyl diselenide reacts with $AgSCF_3$ providing respective Se–S bond-containing substrate while the selenolate by-product is stabilized with the silver ion.[53] Typically, Se–S bond is prepared by reaction between thiol and electrophilic selenyl species – selenyl halides[54,55] or organyl seleninic acids.[56,29] Nevertheless, benzeneselenol can also react with aryl or alkyl thiols in the presence of a catalytic amount of *t*BuOK, while the reaction of benzeneselenol with electrophilic *N*-phenyl-trifluoromethanesulfenamide occurs in acidic conditions.[38] Notably, various sugar-selenyl sulfides have been synthesized directly from sugar diselenides and glutathione in a phosphate buffer. Moreover, this efficient method has been extended from using glutathione as a thiol group containing substrate to a protein – a single-cysteine mutant of subtilisin.[57] Significantly, UV light has been employed for the exchange reaction between diaryl disulfide and dialkyl diselenide. The authors have also stated that Se–S bond is formed under UV light, while longer wavelength (>410 nm, visible light) reverses the reaction.[58]

We developed a novel method for the synthesis of Se–S bond-containing peptides via visible light-initiated reaction. The optimal reaction conditions were found examining the reaction of dipeptide dimer $(Boc\text{-}Sec\text{-}Gly\text{-}OBn)_2$ **24b** and glutathione (GSH) (**54**) in the mixture of $MeCN/H_2O$ under visible light irradiation. Rose Bengal (RB) again turned out to be the best photocatalyst for the reaction. None of the other tested catalysts (FIrPic, $Ru(bpy)_3Cl_2$, 4-CzIPN, 9-mesityl-10-methylacridinium tetrafluoroborate, ethyl eosin, fluorescein, 5-TAMRA) showed superior selectivity and ability to ensure higher conversion of the starting materials in 1 h. Due to the competing reaction, homocoupling of GSH forming GS-SG, this substrate should be employed in an excess – 10 equiv. However, both parts of the diselenide are consumed; therefore, the ratio of GSH versus one selenium is 5:1. Control tests proved that both LED_{460} and RB are required for the reaction. Surprisingly, the presence of radical scavenger (TEMPO) did not prevent the formation of **55a**. Most likely, radical quenching with TEMPO is slower than the generation of glutathionyl ($GS^{\cdot}$) radical under visible light irradiation, followed by the formation of Se–S bond or homocoupling reaction.

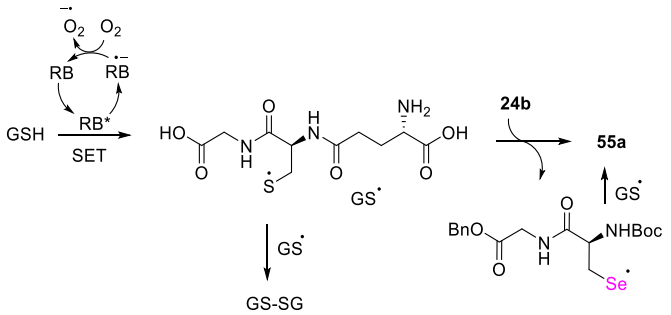
Importantly, the substrate scope evaluation showed that various selenocystine and selenocystamine peptides with sensitive amino acids (Arg, Glu, Lys, His, Tyr) showed excellent tolerance and provided the desired Se–S bond-containing products (Scheme 21). Although Trp is known to be prone to oxidation by radicals and singlet oxygen, the Trp-containing product

55c was also isolated; however, the yield was lower due to formation of side products. Met-containing peptide yielded the Met sulfoxide-containing product **55f**.



Scheme 21. Scope and limitation studies for the preparation of Se–S bond containing compounds. **55b–e**: the products were obtained employing unprotected peptides as TFA salts.

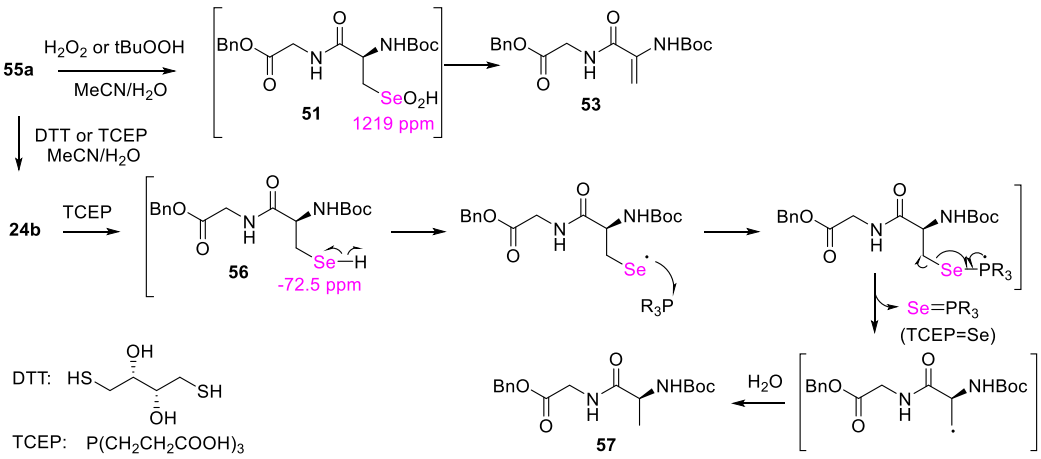
The Stern-Volmer analysis disclosed that the quenching rate constant of RB with GSH was significantly higher (8.8×10^{-3} l/mol) than with **24b** (0.12×10^{-3} l/mol). Therefore, it can be inferred that S-centered radical is rapidly formed under LED_{460} irradiation. Next, the GS radical reacts with the diselenide forming the desired Se–S bond-containing peptide **55a** or it undergoes the homocoupling reaction forming oxidized glutahione (GS-SG), thus an excess of **54** is required for full consumption of **24b** (Scheme 22).



Scheme 22. Proposed mechanism for the formation of Se–S bond-containing substrates.

Next, we examined the stability of Se–S bond-containing peptide **55a** under visible light irradiation, as well as in the presence of oxidants and reducing agents (Scheme 23). Prolonged irradiation (>3 h) of **55a** and RB in MeCN/water with LED_{460} provided alkyl seleninic acid **6** and Boc-Dha-Gly-OBn **53** due to the reaction with H_2O_2 that is formed under irradiation conditions. The oxidation of **55a** with H_2O_2 or *t*BuOOH afforded alkyl seleninic acid. Intermediate **51** was detected by HRMS ([M+Na] = 471.0633) and ^{77}Se NMR (δ 1219.8 ppm) spectroscopy. Besides, a small signal of selenonic acid **51'** (δ 1050.8 ppm) was also detected in the ^{77}Se NMR spectra (Fig. 3).

The reduction of **55a** with 3 equiv. 1,4-dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) delivered diselenide **24b** and GSH in a short reaction time (30 min). Furthermore, the use of increased amount of TCEP (10 equiv.) led to deselenylation reaction yielding Boc-Ala-Gly-OBn **57** and TCEP=Se ([M-H] = 328.9704) (Scheme 23). The selenol **56** was detected by LC-MS ([M+Na] = 439.03) and ^{77}Se NMR spectroscopy (δ –72.5 ppm) as an intermediate of the reduction. The mechanism of deselenylation is known in the literature.[33]



Scheme 23. Stability of **55a** in the presence of reducing agents and oxidants.

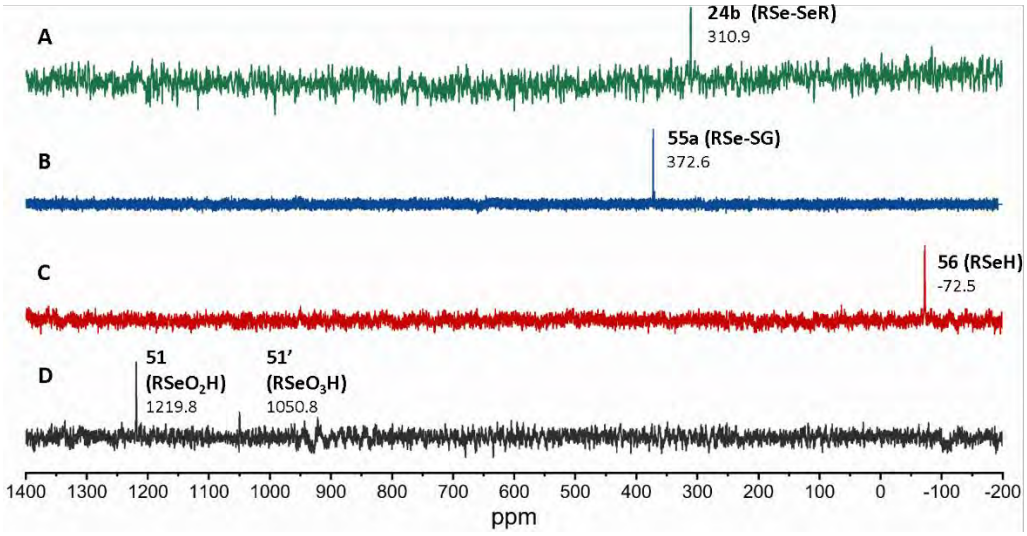
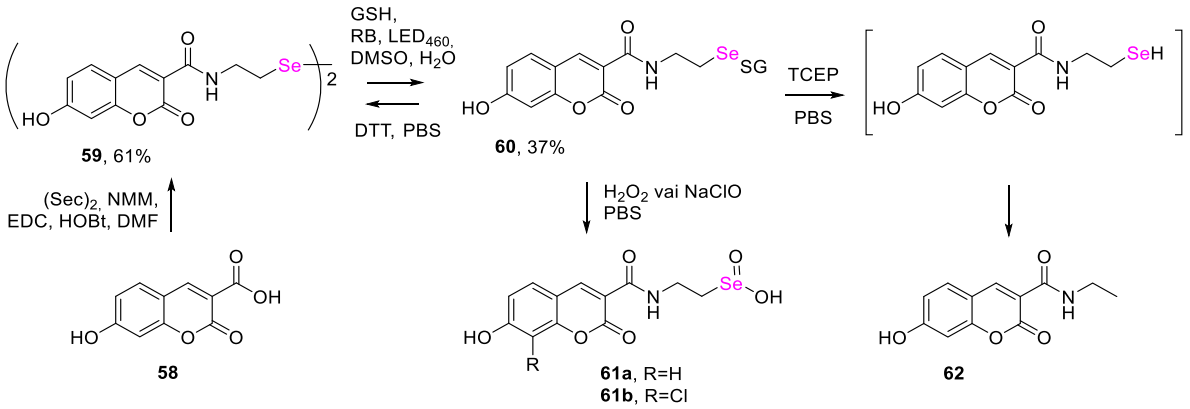


Fig. 3. ^{77}Se NMR spectra of **24b** (A), **55a** (B), **56** (C), **51** (D)

Next, aiming to evaluate the possibility to utilize alkylselenylsulfide moiety as a cleavable linker for the introduction of fluorescent probe to thiol group-containing peptide, diselenide **59** was prepared starting with 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid **58** (Scheme 24). The visible light-initiated reaction between GSH and diselenide **59** provided the desired Se–S bond-containing product **60** in 37 % yield.

Next, the stability of **60** was established. A quick oxidation and formation of the respective seleninic acid **61a** was observed in the presence of H_2O_2. Another oxidant – NaClO provided the respective chlorinated seleninic acid **61b** ([M-H] = 377.9290). The use of DTT (10 equiv.) led to formation of diselenide **59**. Recently, it was demonstrated that a cyclic selenosulfide subjected to reductive conditions (TCEP or DDT) spontaneously eliminates selenoethyl moiety releasing ethylene molecule, besides, all reactions were very slow (up to 6 days).[59] We did not observe such transformation in the case of **60** – interaction with TCEP (10 equiv.) was very efficient providing ethyl amide **62** in 30 min.



Scheme 24. Synthesis and stability of **60**.

Additionally, the stability of **60** in phosphate-buffered saline (PBS) was established under various pH revealing that the compound is stable in pH range 3.0–8.0 for 24h. Absorption and emission spectra were collected for the solutions of **59**, **60** and **61a** in PBS (pH = 7.4, c = 10 μM), as well as the photoluminescence quantum yield (PLQY) of the studied compounds were determined (Fig. 4). The PLQY of the Se–S bond-containing substrate is considerably higher (Φ = 29.5 %) than the PLQY of diselenide **59** (Φ = 1.6 %). However, the highest emission quantum yield was observed for the seleninic acid **61a** (Φ = 52.4 %).

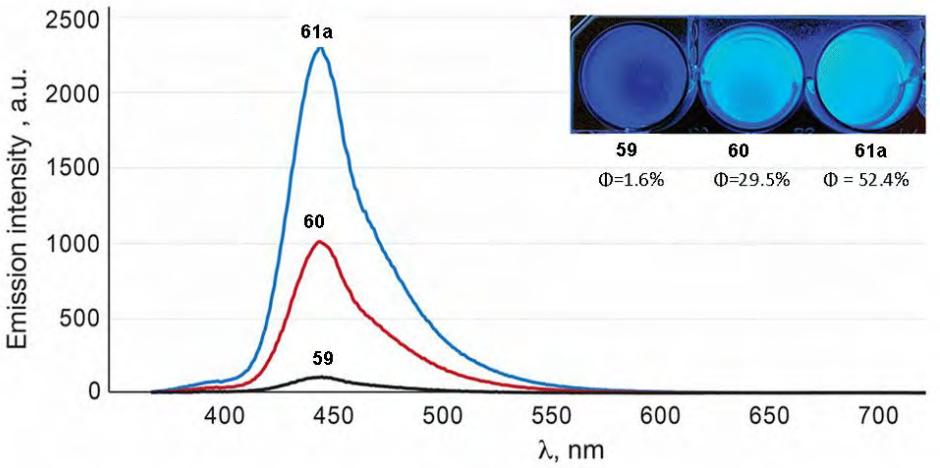


Fig. 4. Emission spectra of **59**, **60**, **61a** upon excitation at 350 nm (PBS, pH = 7.4).

The article about the studies described in this chapter can be found in Appendix V.

CONCLUSIONS

1. Peptides with cysteine residue can be functionalized under mild conditions through the *in situ* preparation of cysteinyl chloride using *N*-chlorosuccinimide.
2. $CuBr_2$ is an efficient promoter for chalkogenyl electrophile generation from diaryl/dibenzyl dichalcogenides, protected and unprotected selenocystine-containing peptides.
3. $K_2S_2O_8$ is an efficient promoter for generation of selenyl electrophile from $(Boc\text{-}Sec)_2$ and protected selenocystine-containing peptides.
4. The sulfenyl and selenyl electrophiles can be trapped by suitable triple bond-containing substrates and sequential 5- or 6-*endo-dig* cyclization reactions provides (hetero)cyclic systems attached to cysteine or selenocysteine (indolizinium salts, indoles, benzo[*b*]furans, coumarins, isocoumarins, quinolin-2-ones, isoquinolin-2-ones, polyaromatic hydrocarbons, indeno[1,2-*c*]chromenes).
5. Rose Bengal is an efficient photocatalyst for selenyl electrophile generation from protected and unprotected selenocystine-containing peptides. The electrophile can be trapped by electron-rich *N*-heterocycles. The elaborated atom-economic protocol shows excellent tolerance for selenocystine-containing peptides with 'sensitive' amino acids (Lys, Arg, His, Glu, Tyr). Visible light-mediated intramolecular indole selenylation can be performed under the same conditions affording Sec-macrocycles.
6. Rose Bengal is a suitable photocatalyst to induce formation of Se–S bond-containing peptides.

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Sindija Lapčinska

PIELIKUMI/APPENDICES

1. pielikums/ Appendix I

Arsenyan, P.; Lapcinska, S.; Ivanova, A.; Vasiljeva, J.

Peptide functionalization through the generation of selenocysteine electrophile.

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Full Paper

Selenocysteine Electrophile

Peptide Functionalization Through the Generation of Selenocysteine Electrophile

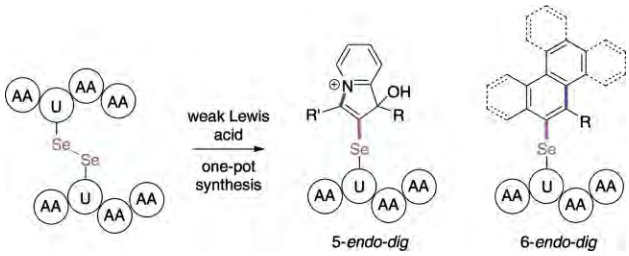
Pavel Arsenyan,*[a] Sindija Lapcinska,[a] Anna Ivanova,[a] and Jelena Vasiljeva[a]

In memoriam of Professor Edvards Liepinsh (1944–2019).

Abstract: Herein we report the first example of a strategy for peptide functionalization through the generation of selenocysteine electrophile in 5- and 6-*endo-dig* cyclization reactions. This simple approach allows bio-conjugation of selenocystine-based peptides. The developed protocol is based on copper(II) bromide mediated reactions of selenocystine with either 2-propargyl *N*-heterocycles through 5-*endo-dig* closure or with 2-ethynylbiaryls through 6-*endo-dig* closure. It allows construction of indolizinium moiety on selenocysteine residue as well as formation of polyaromatic fragment bonded to selenium in a simple one-pot process under mild reaction conditions.

Introduction

Alkyl and aryl(hetaryl) diselenides are extremely convenient and widely used reagents in synthetic organic chemistry.[1a,1b] Depending on reaction conditions Se–Se can be a source of selenium electrophile, nucleophile, or radical. Unfortunately, probably the most interesting diselenide – selenocystine – was generally utilized as a nucleophilic agent. According to published reports, aryl(hetaryl)selenium electrophile can be easily generated using halogens,[2a] KI/*m*-CPBA,[2b] persulfates,[2c,2d] copper(I) salts,[2e,2f] and silver triflate.[2g] However, none of the above-mentioned methods were applied to generate electrophilic selenocysteine (Sec), leaving this part of Sec properties almost unexplored. It must be noted that interest in Sec-containing peptides' functionalization is a hot topic.[3] Different scientific groups have put efforts in this field.[4] Whedon's and Chatterjee's teams reported an elegant procedure for the functionalization of Sec-containing enzymes through the formation of *exo* double bond by deselenation of Sec residue.[5a] Buchwald's and Pentelute's labs reported operationally simple approach for oxidized Sec (Se–S bond) to react with a nucleophilic arylboronic acids to provide arylated selenocysteines.[5b] Moreover, the same team just published a method for late-stage functionalization of unprotected Sec-containing peptides by electrophilic reactions.[5c] Thus, herein we would like to report a novel synthetic procedure for generating Sec electrophile and utilizing it in 5- and 6-*endo-dig* cyclization reactions.



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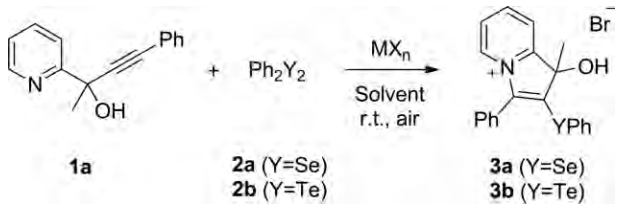
Results and Discussion

Initially, we devoted our efforts to find a suitable promoter for an efficient Se–Se bond activation, compatible with peptide chemistry. 4-Phenyl-2-(pyridin-2-yl)but-3-yn-2-ol (**1a**) and diphenyl diselenide (**2a**) were chosen for *5-endo-dig* cyclization reaction (Scheme 1). It is known that transition metal salts, especially halides, may form complexes with diselenides.[6] Therefore, several Lewis acids [such as $FeCl_3$, $FeBr_2$, $FeBr_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $CuBr_2$, $Cu(OAc)_2$, $RuCl_3$, $In(OTf)_3$, and $Bi(OTf)_3$] were evaluated for their ability to activate Se–Se bond and promote the cyclization. Iron and copper reagents were preferred as most suitable and affordable compounds, and, to our delight, copper(II) bromide showed the best results (Table 1, entries 1–3). It is important to mention that the use of diphenyl diselenide and copper(II) bromide in equimolar quantities is essential for maximizing yield of the reaction. The insufficiency of copper(II) bromide or diphenyl diselenide led to unsatisfactory yields, e.g. treatment with 0.5 equivalent of $CuBr_2$ led to halved yield (Table 1, entries 3–6). Then, we have tested different solvents to assess their influence on the reaction. However, all tested solvents provided acceptable yield, dichloromethane (DCM), MeCN and EtOH enabled excellent yield (Table 1, entries 5, 7–10). An experiment, which has been carried out in an absence of diphenyl diselenide, yielded 2-bromoindolizinium bromide as product (Table 1, entry 11). Likewise, 2-bromoindolizinium bromide was observed as co-product when $CuBr_2$ was added

to **1** first, and after 20 min, Ph_2Se_2 was added (Table 1, entry 12). The method was expanded on diphenyl disulfide and ditelluride. However, only ditelluride provided the expected product in good yield (Table 1, entries 15–16). Molecular structure of **3b** was unambiguously confirmed by X-ray analysis (Figure 1). In case of sulfur, this method presumed to be inefficient due to relatively low copper-philicity of sulfur; the main reaction's product was 2-bromoindolizinium bromide (Table 1, entries 13–14).



Scheme 1. 5-*endo-dig* cyclization of **1a** with diphenyl diselenide and ditelluride.

Table 1. Optimization of reaction conditions for the preparation of **3a** and **3b**.

Entry	**2**	Solvent	Stoichiometry **1a**/$CuBr_2$/**2**	Yield, % (time [h])
1	Ph_2Se_2[a]	DCM	2:2: 1	– (4)[c]
2	Ph_2Se_2[b]	DCM	2:2: 1	– (4)[c]
3	Ph_2Se_2	DCM	2:2:1	86 (4)
4	Ph_2Se_2	DCM	2:0.5:1	47 (4)
5	Ph_2Se_2	DCM	1.2:1.2:1	94 (4)
6	Ph_2Se_2	DCM	0.6:1.2:1	62 (4)
7	Ph_2Se_2	DMSO	1.2:1.2:1	70 (24)[e]
8	Ph_2Se_2	EtOAc	1.2:1.2:1	62 (24)[e]
9	Ph_2Se_2	MeCN	1.2:1.2:1	90 (0.5)
10	Ph_2Se_2	EtOH	1.2:1.2:1	96 (12)
11	–	DCM	0:2:1	86 (72)[d]
12	Ph_2Se_2	MeCN	1.2:1.2:1	32 + 58 (1)[f]
13	Ph_2S_2	DCM	1.2:1.2:1	78 (24)[d]
14	Ph_2S_2	MeCN	1.2:1.2:1	87 (24)[d]
15	Ph_2Te_2	DCM	1.2:1.2:1	94 (0.25)
16	Ph_2Te_2	MeCN	1.2:1.2:1	76 (4)

[a] MX_n – $FeCl_3$. [b] MX_n – $FeBr_3$. [c] Mixture of products. [d] 2-Bromoindolizinium bromide was formed. [e] Reaction was not completed. [f] $CuBr_2$ was added to **1a** first and after 20 min Ph_2Se_2 was added. The formation of 2-bromoindolizinium bromide (32 %) and **3a** (58 %) was observed.

Discussing possible pathways for mechanism of this reaction the behavior of $CuBr_2$ remained unclear. The problem was to determine the product of interaction between $CuBr_2$ and Ph_2Se_2. Thus, several ^{77}Se NMR experiments were performed. A spectrum of equimolar mixture of Ph_2Se_2 with $CuBr_2$ and a spectrum of single Ph_2Se_2 were acquired. Overlay of these spectra is shown in Figure 2. The only signal in the Ph_2Se_2•$CuBr_2$ spectrum means that the reaction was complete and a single product was formed. The chemical shift of Ph_2Se_2•$CuBr_2$ (^{77}Se δ at 450 ppm) is similar to that of Ph_2Se_2 (^{77}Se δ at 462 ppm), which led us to thinking that neither Se–Br, nor Se–Cu bond was formed. This statement is based on the following facts: Se–Cu bond-containing compound Cu(SePh)$_2$•2-Phen has ^{77}Se upfield-shifted signal (274 ppm); Se–Br bond-containing PhSeBr ^{77}Se signal is strongly downfield (867 ppm).[7] In theory two signals in selenium spectra should be seen, however, due to

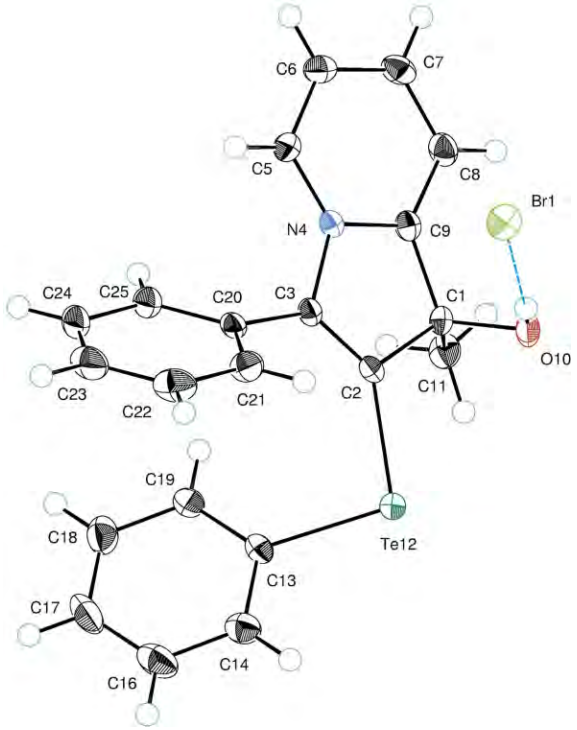


Figure 1. ORTEP molecular structure of **3b**.

dynamic nature of intermediate these signals were merged and the signal is broad due to paramagnetic properties of copper. Under these circumstances, the reaction of Ph_2Se_2 with $CuBr_2$ produces coordinated adduct, as Se–Se bond of diselenide is not cleaved retaining the chemical shift. Based on the experimental results above and previous reports,[8a,8b] a plausible reaction mechanism is proposed in Scheme 2. Firstly, the reaction of diphenyl diselenide with copper(II) bromide leads to the formation of coordinated Ph_2Se_2•$CuBr_2$ adduct **B**. Then adduct **B** can generate selenium electrophile which subsequently coordinates to the triple bond of substrate **A** forming selenirenium cation, intermediate **C**. Then nitrogen's lone pair attacks the double bond, restoring neutral charge of selenium. As a result, the five-membered cycle with positively charged nitrogen closes to yield the product **D**. As a co-product of this reaction PhSeCuBr moiety **E** is formed to regenerate diphenyl diselenide and to produce copper(I) bromide. To prove the formation of CuBr in the reaction mixture we made the following experiment. It is known that bicinchoninic acid (BCA) chelates with Cu^{I} ion, rather than with Cu^{II} ion, giving deep purple-colored complex **F** (absorbance maximum at 562 nm).[9] Therefore, these copper ions can be distinguished using the absorbance measurements. Three samples were analyzed: the reaction mixture plus BCA, CuI plus BCA, and $CuBr_2$ plus BCA. All samples contained equimolar quantities of BCA and copper reagents. For this purpose, 50 nM solution of BCA in 0.1 M solution of NaOH was used. The presence of complex **F** was detected as a result of interactions, both BCA with copper(I) (absorption 1.4749 at 560 nm) and BCA with the reaction mixture (absorption 1.1503 at 560 nm). These samples have similar absorption values, different from lower absorption of BCA plus copper(II)

(absorption 0.1977 at 560 nm). Therefore, copper(II) bromide does not act as a catalyst in this reaction. It is consumed and converted to copper(I) bromide which is not able to promote the formation of selenium electrophile.

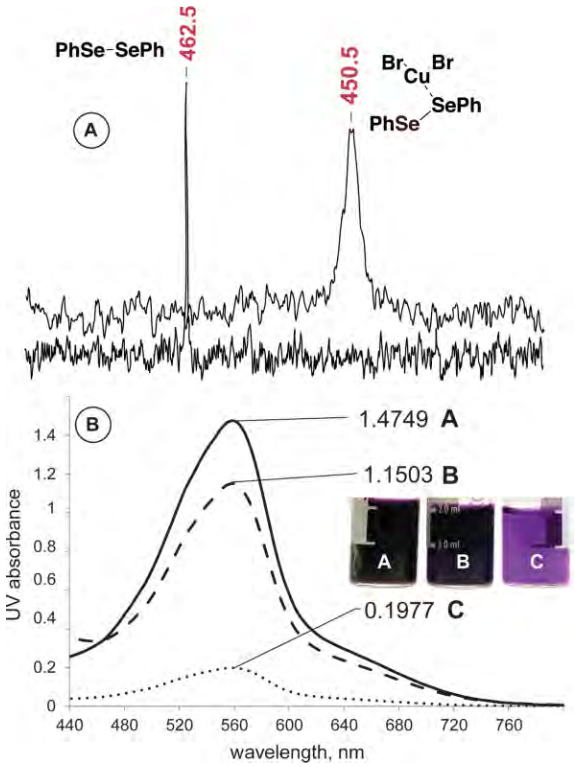
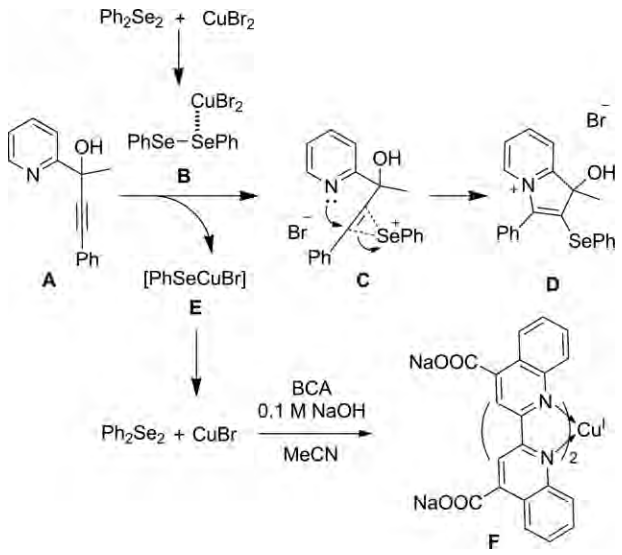


Figure 2. A: overlay of ^{77}Se NMR spectra of Ph_2Se_2•$CuBr_2$ and Ph_2Se_2. B:UV absorbance spectrum of three samples: A: CuI + BCA; B: the reaction mixture + BCA; and C – $CuBr_2$ + BCA.

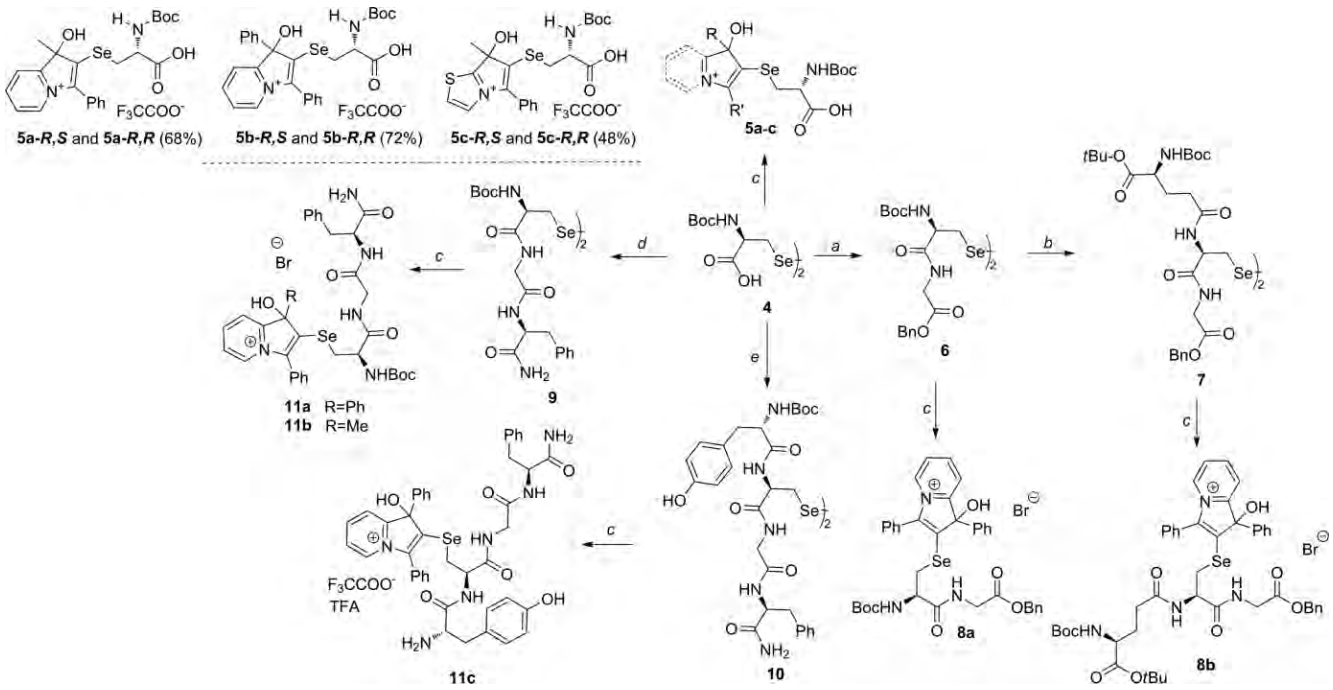


Scheme 2. A plausible mechanism for $CuBr_2$-mediated *5-endo-dig* cyclization.

Having optimized method in hands we moved forward to use peptides with Se–Se bonds as sources of selenium electrophile. As it was expected, unprotected selenocystine was not able to serve as reagent due to low solubility. However, Boc-protected selenocystine **4** readily reacted with **1a**–**1c** in presence of copper(II) bromide to yield the corresponding indolizinium **5a**,**b** and pyrrolothiazolium **5c** salts in good yields (Scheme 3). In each case a mixture of *R*,*S*- and *R*,*R*-diastereomers was formed. Every diastereomeric pair was separated by HPLC. The structures of all products were confirmed by ^{1}H, ^{13}C, ^{77}Se and HRMS spectra. Pyrrolothiazolium **5c** derivatives of selenocysteine were unstable when stored for more than a day, however, indolizinium salts **5a**,**b** were stable enough and, luckily, we obtained single crystals of **5b-*R*,*S*** as zwitterionic salt (Figure 3), unambiguously confirming the structure. Notably, using this method there is no need to protect carboxylic acid moiety In continuation of our investigation, we expanded 5-*endo-dig* reaction to di-, tri- and tetrapeptides containing the Se–Se bond (Scheme 3). Peptides **6**–**10** were prepared starting with di(Boc)-selenocystine **4** by regular methodology in peptide chemistry yielding desired di-, tri- and tetrapeptides in good yields. First target tripeptide chosen for the further modification was selenoglutathione **7**. Both (Boc)Sec-Gly-OBn **6** and **7** readily reacted with **1b** in the presence of copper(II) bromide forming indolizinium moiety in 85–88 % yield (**8a**,**b**). For two diastereomers of **8b** NMR signals in ^{77}Se spectra were found at 213.9 and 207.2 ppm. Next, (Boc)Tyr-Sec-Gly-Phe-NH_2 tetrapeptide **10** was synthesized with the aim to mimic the active center of glutathione peroxidase 4 (GPx-4) enzyme [Tyr(48)-Sec(49)-Gly(50)]. Phospholipid hydroperoxide GPx (GPx-4) plays a unique and vital antioxidant role; it is the only enzyme that can directly catalyze the reduction of membrane phospholipid hydroperoxides.[10] Indolizinium derivatives **11a**–**c** were isolated in high yields by treatment of peptides **9** and **10** with propargyl pyridines **1a**,**b** in presence of $CuBr_2$. It should be noted that isolation of conjugates was very simple. Reaction mixture was evaporated till dryness and product was isolated by reverse-phase chromatography using acetonitrile/water (1 % AcOH) system as eluent.

The next challenge for copper(II) mediated selenium electrophile generation was to promote *6-endo-dig* cyclization. Under the same reaction conditions selenocystine-based peptides are able to react with 2-(phenylethynyl)biphenyl type compounds, producing polyaromatic systems fused with Sec-peptides (Scheme 4). Unsubstituted 2-(phenylethynyl)biphenyl **12a** undergoes 6-*endo-dig* cyclization with dipeptide **6** in 85 % yield. In this reaction, aromatic system acts as nucleophile, so the impact of EDG and EWG on aromatics' activity was estimated. Introduction of EDG at phenyl ring **B** increased reaction's yield (**13b**). On the opposite, introduction of EWG, such as cyano group, lead to deactivation of aromatic system and formation of the product **13c** was not observed.

Elaborated method is tolerant to both aryl and alkyl R^1 substituents at ethynyl group. Moreover, the yield was not affected by the extension of **B** to naphthyl or phenanthrene. Polyaromatic systems such as benzo[*c*]phenanthrene (**13e**) and benzo[*g*]chrysene (**13f**) were formed in high yields and with low expenses. Benzene ring **A** can be swapped for thiophene moiety without diminishing the reactions' yield (**13g**, **13h**). Notably, benzo[*c*]phenanthrene cycle was formed in very high



Scheme 3. Synthesis of Sec-peptides and 5-*endo-dig* cyclization a) Gly-OBn (5 equiv.), EDC•HCl (6 equiv.), HOBt (1 equiv.), NMM (6 equiv.), DMF, r.t.; b) TFA, DCM, then (Boc)Glu-*O*-*t*Bu(4 equiv.),EDC•HCl (6 equiv.), HOBt (1 equiv.), NMM (6 equiv.), DMF, r.t.; c) propargyl pyridine (2 equiv.) $CuBr_2$, solvent, r.t., 4–24 h; d) Gly-Phe-NH_2(4 equiv.), EDC•HCl (6 equiv.), HOBt (1 equiv.), NMM (6 equiv.), DMF, r.t.; e) TFA, DCM, then (Boc)Tyr-OH(4 equiv.), EDC•HCl (6 equiv.), HOBt (1 equiv.), NMM (6 equiv.), DMF, r.t.

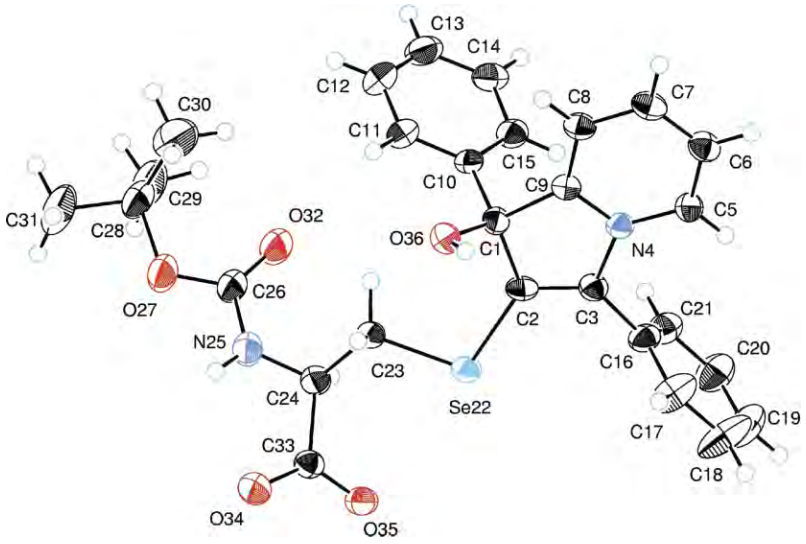
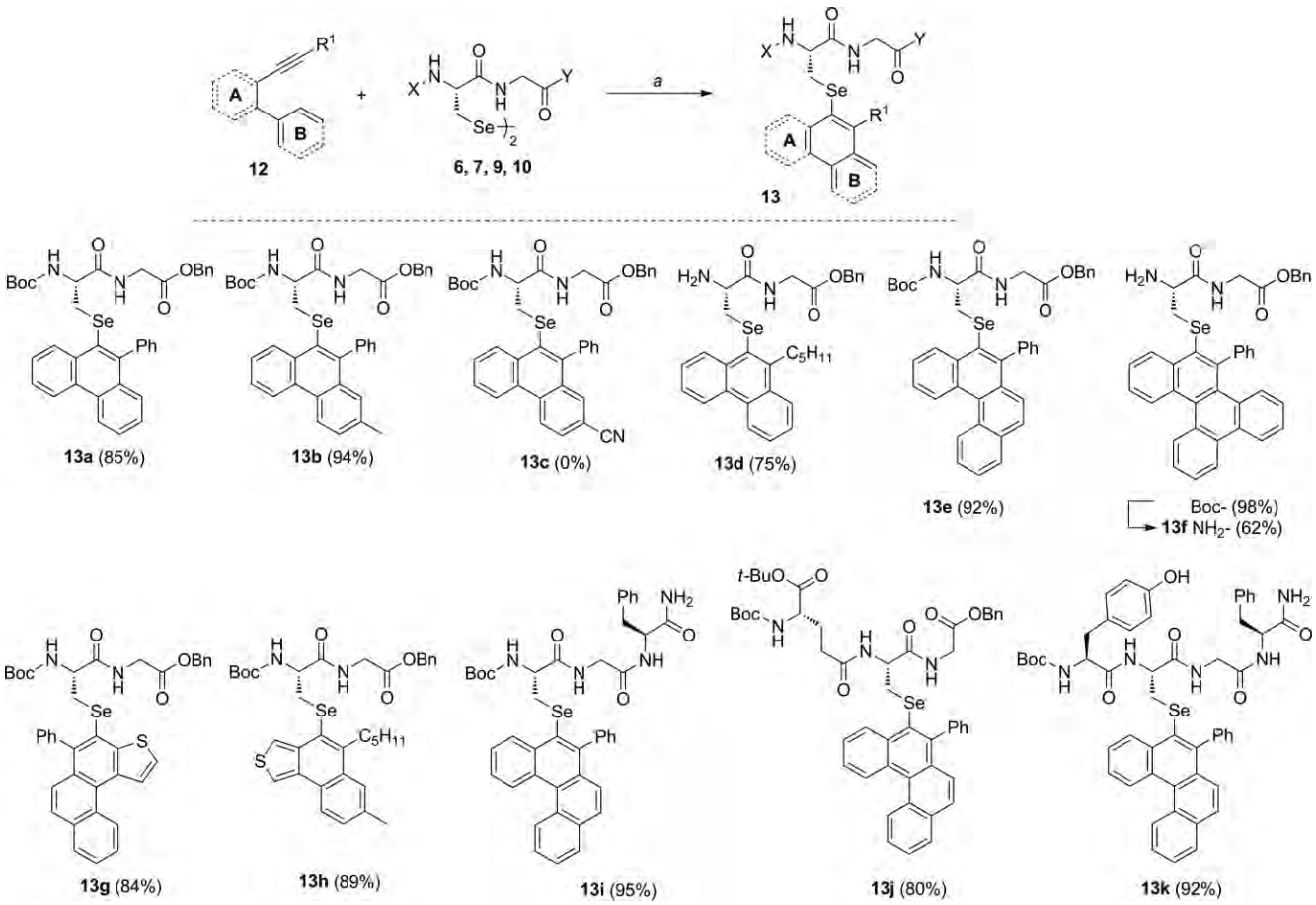


Figure 3. ORTEP molecular structure of **5b-*R,S***.

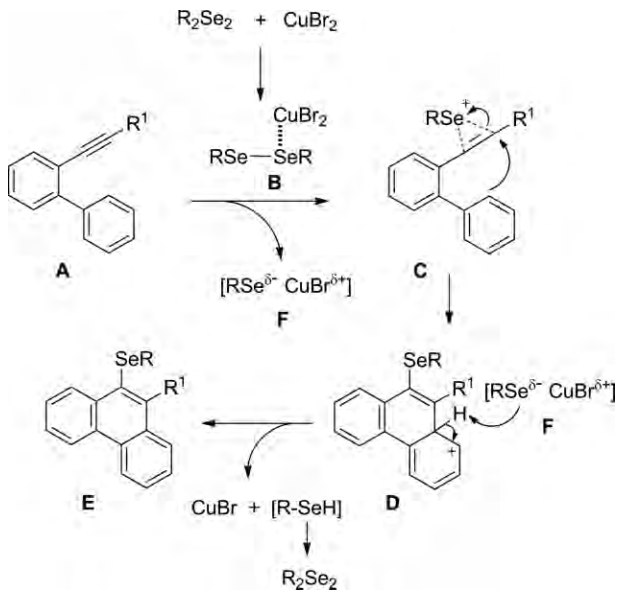
yields (80–95 %) using selenoglutathione **7** and tripeptide **9** as well as (Boc)Tyr-Sec-Gly-Phe-NH_2 tetrapeptide **10** as substrates (**13i**–**k**). Besides, selenium-modified benzo[*g*]chrysene (**13f**) formation was achieved in mild, simple and selective reaction. Synthesis of polycyclic aromatic hydrocarbons is a pertinent topic due to its physical and chemical properties.[11]

Possible mechanism for $FeCl_3$ promoted *6-endo-dig* cyclization was proposed by Zeni et al.[12] Despite iron(III) chloride mediated cyclization, utilizing copper(II) bromide, we were able to confirm intermediate **B** and copper(I) bromide formation experimentally. Based on received data a plausible mechanism presented herein (Scheme 5) has a different interpretation. Thus, adduct **B**, generated from diselenide and $CuBr_2$ coordinates to substrate's **A** triple bond, producing **C** and **F**. Then selenirenium cycle is intramolecularly attacked by the nearest aromatics, enclosing six-member ring and restoring neutral Se charge. Through intermediate **D** aromaticity is restored by deprotonation with **F**. As a result, the final product **E** and a corre-



Scheme 4. 6-*endo-dig* cyclization reaction. a) $CuBr_2$, CH_2Cl_2, r.t., 4–24 h.

sponding selenol are formed; selenols are unstable and recombine to diselenides. We must acknowledge that late-stage formation of polyaromatic hydrocarbons on peptides yields potential biomarkers.



Scheme 5. Plausible mechanism for $CuBr_2$ promoted *6-endo-dig* cyclization.

Conclusions

A convenient and simple $CuBr_2$ mediated functionalization of Sec-containing peptides by 5-*endo*- and 6-*endo-dig* protocols opens a possibility to be successfully transferred to polypeptides. The formation of indolizinium systems and polyaromatics using Sec electrophilic properties is achieved due to their possible further utilization, both in development of new medicines, biomarkers, and materials as well as usage as intermediates in the synthesis of more sophisticated peptides. We hope that the results of our research will be useful and increase the interest in modification of selenocysteine containing peptides.

Experimental Section

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was performed using MERCK Silica gel 60 F254 plates and visualized by UV (254 nm) fluorescence. ZEOCHEM silica gel (ZEOprep 60/35–70 microns – SI23501) was used for column chromatography. ^{1}H, ^{13}C, ^{77}Se, ^{125}Te NMR spectra were recorded on a Bruker Avance Neo spectrometer at 400, 101, 76, and 126 MHz correspondingly at 298 K in CD_3OD or $CDCl_3$. Infrared (IR) spectra were recorded with a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). GC–MS was recorded on Agilent 7690 GC with MSD. HRMS were recorded on Waters Synapt GII Q-ToF UPLC/MS system. Single crystals of **3b** and **5b-*R,S*** were investigated on a Rigaku

XtaLAB Synergy, Dualflex, HyPix diffractometer. The crystal was kept at 140.0(1) K during data collection. Using Olex2,[13a] the structure was solved with the olex2.solve.[13b] structure solution program using Charge Flipping and refined with the ShelXL[14] refinement package using Least Squares minimisation. Optical density was studied on a Tecan Infinite1000 microplate reader.

Propargylpyridines **1a**,[15] **1b**,[16] Boc-L-selenocystine **4**,[17] arene-alkynes **12a**–**c**,[18] **12d**[19] are known compounds which were prepared according to literature procedures.

4-Phenyl-2-(thiazol-2-yl)but-3-yn-2-ol (1c): To the solution of phenylacetylene (2.73 g, 26.77 mmol) and 18-crown-6 (0.83 g, 3.15 mmol) in dry toluene freshly dried CsF (0.5 g, 3.15 mmol) and trimethylsilylacetylene (2.62 g, 26.77 mmol) were added. Then reaction mixture was stirred at 50 °C for 3 hours. After cooling to room temperature solution of 2-acetylthiazole (2 g, 15.75 mmol) in dry toluene was added and the mixture was stirred overnight. Toluene was then evaporated and to the crude residue methanol (100 mL) and an excess of K_2CO_3 were added. After stirring for 12 hours, solvent was evaporated and crude residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/EtOAc, 3:1) to yield the product as white solid (g, 44 %). M.p. 114–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.77 (d, J = 3.4 Hz, 1H), 7.50–7.40 (m, 2H), 7.30 (d, J = 3.4 Hz, 1H), 7.35–7.26 (m, 3H), 4.02 (d, J = 0.9 Hz, 1H), 2.05 (d, J = 0.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.1, 142.6, 132.0, 128.9, 128.4, 122.1, 120.0, 90.7, 85.2, 69.1, 31.9.

General Method for the Preparation of 3a,b: A vial charged with diphenyl diselenide **2a** or diphenyl ditelluride **2b** (1 mmol), copper(II) bromide (0.27 g, 1.2 mmol) and 25 mL of acetonitrile was stirred for 30 min. Then solution of 4-phenyl-2-(pyridin-2-yl)but-3-yn-2-ol (**1a**) in 10 mL of acetonitrile was added in one portion. Resulting mixture continued stirring for 30 min. Then 1 mL of water was added and volatiles were evaporated under reduced pressure. Crude product was purified by flash chromatography yielding **3a** or **3b** in 90 % and 76 %, correspondingly. It should be noted that this reaction could be performed in ethanol and dichloromethane as well.

1-Hydroxy-1-methyl-3-phenyl-2-(phenylselanyl)-1*H*-indolizin-4-ium Bromide (3a): Yellowish solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.57 (td, J = 7.8, 1.2 Hz, 1H), 8.40 (dt, J = 6.2, 1.1 Hz, 1H), 8.36 (dt, J = 7.8, 1.1 Hz, 1H), 7.96 (ddd, J = 7.6, 6.2, 1.2 Hz, 1H), 7.53–7.48 (m, 1H), 7.47–7.36 (m, 7H), 7.26–7.19 (m, 1H), 7.15–7.08 (m, 2H), 1.86 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 161.9, 145.8, 142.8, 141.0, 136.3, 136.0, 132.2, 131.9, 130.6, 130.4, 129.9, 128.5, 126.3, 126.0, 123.9, 83.6, 25.1. ^{77}Se NMR (76 MHz, CD_3OD) δ = 303.78. HRMS (ESI/Q-TOF) m/z: $[M]^+$ calcd. for $[C_{21}H_{18}NOSe]^+$ 380.0548, found 380.0562.

1-Hydroxy-1-methyl-3-phenyl-2-(phenyltellanyl)-1*H*-indolizin-4-ium Bromide (3b): Reddish solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.53 (td, J = 7.6, 1.2 Hz, 1H), 8.41–8.34 (m, 2H), 7.92 (ddd, J = 7.6, 6.2, 1.2 Hz, 1H), 7.64–7.59 (m, 2H), 7.54 (tt, J = 7.6, 1.4 Hz, 1H), 7.30–7.24 (m, 1H), 7.12–7.04 (m, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 148.3, 141.7, 131.9, 129.0, 128.7, 128.7, 128.5, 126.9, 125.9, 123.6, 122.3, 87.8 (d, $J_{(Te\text{-}C)}$ = 21.2 Hz), 70.2, 34.1. ^{125}Te NMR (126 MHz, CD_3OD) δ = 502.3. HRMS (ESI/Q-TOF) m/z: $[M]^+$ calcd. for $[C_{21}H_{18}NOTe^+]^+$ 430.0445, found 430.0457. CCDC 1901311. Single crystals of $C_{21}H_{18}BrNOTe$ [**3b**] were investigated on a Rigaku XtaLAB Synergy, Dualflex, HyPix diffractometer. The crystal was kept at 130.0(1) K during data collection. Crystal Data for $C_{21}H_{18}BrNOTe$ (M =507.89 g/mol): triclinic, space group $P\bar{1}$ (no. 2), a = 7.3244(2) Å, b = 8.05570(10) Å, c = 17.7657(3) Å, α = 86.1550(10)°, β = 89.988(2)°, γ = 64.242(2)°, V = 941.46(4) Å^3, Z = 2, T = 130.0(1) K, μ(Mo-K_α) = 3.710 mm^{-1}, $Dcalc$ = 1.7915 g/cm^3, 15841 reflections measured ($2\Theta \leq 63.5°$), 5236 unique (R_{int} = 0.0252, R_{sigma} = 0.0248) which were used in all calculations. The final R_1 was 0.0212 [$I \geq 2u(I)$] and wR_2 was 0.0508 (all data).

CCDC 1901311 (for **3b**), 1889565 (for **5b-*R,S***) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

General Method for the Preparation of 5a–c: Compound **4** (0.5 mmol, 269 mg) and copper(II) bromide (1 mmol, 224 mg) were dissolved in acetonitrile and stirred for 30 minutes. Then propargyl *N*-heterocycle (**1a**–**c**) (1.2 mmol) in acetonitrile was added and the stirring was continued for 4–16 hours. After consumption of the initial compound volatiles were removed and crude product was purified by reverse phase chromatography [MeCN/water (1 % AcOH)] giving a mixture of the corresponding diastereomers **5a**–**c**. Separation of diastereomers was performed on preparative HPLC Shimadzu prominence apparatus, equipped with XBridge® peptide BEH C18 OBD™ prep column, 130Å, 10 μm, 19 mm × 250 mm as eluents were used MeCN:H_2O (28:72 to 100:0 %) at flow rate 4mL/min.

(*S*)-2-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Trifluoroacetate (5a-*S,R*): Foam (53 mg, 35 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.55 (td, J = 7.8, 1.0 Hz, 1H), 8.50 (d, J = 6.1 Hz, 1H), 8.36 (d, J = 7.8 Hz, 1H), 7.95 (td, J = 6.9, 6.3, 1.1 Hz, 1H), 7.67 (m, 5H), 4.12 (t, J = 5.2 Hz, 1H), 3.39 (dd, J = 12.0, 5.6 Hz, 1H), 3.12 (dd, J = 11.7, 5.9 Hz, 1H), 1.83 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 176.4, 162.7, 140.9, 135.8, 132.6, 132.1, 130.9, 128.3, 126.7, 123.8, 84.2, 49.0, 30.4, 28.7, 25.4. HRMS (ESI/Q-TOF) m/z: $[M]^+$ calcd. for $[C_{23}H_{27}N_2O_5Se]^+$ 491.1080, found 491.1107.

(*R*)-2-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Trifluoroacetate (5a-*R,R*): Foam (50 mg, 33 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.54 (td, J = 7.8, 1.2 Hz, 1H), 8.49–8.43 (m, 1H), 8.37–8.30 (m, 1H), 7.94 (ddd, J = 7.6, 6.2, 1.3 Hz, 1H), 7.73 (s, 5H), 4.23 (t, J = 4.2 Hz, 1H), 3.93 (dd, J = 12.7, 4.3 Hz, 1H), 3.16 (dd, J = 12.6, 4.6 Hz, 1H), 1.84 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.4, 163.1, 145.3, 140.8, 140.8, 135.8, 132.5, 132.2, 130.7, 128.1, 126.8, 123.7, 84.6, 49.0, 48.8, 29.4, 28.7, 26.2. HRMS (ESI/Q-TOF) m/z: $[M]^+$ calcd. for $[C_{23}H_{27}N_2O_5Se]^+$ 491.1080, found 491.1103.

(*S*)-2-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Trifluoroacetate (5b-*S,R*): Foam. (63 mg, 38 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.57 (d, J = 6.1 Hz, 1H), 8.44 (td, J = 7.8, 1.0 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.98–7.91 (m, 1H), 7.80–7.74 (m, 2H), 7.73–7.67 (m, 3H), 7.65–7.59 (m, 2H), 7.50–7.34 (m, 3H), 4.13 (s, 1H), 3.19 (s, 1H), 2.81 (d, J = 47.4 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 162.6, 145.6, 142.8, 140.7, 137.5, 136.0, 132.8, 132.0, 131.0, 130.5, 130.4, 128.4, 126.6, 126.4, 124.8, 87.4, 49.6, 30.0, 28.7. HRMS (ESI/Q-TOF) m/z: $[M]^+$ calcd. for $[C_{28}H_{29}N_2O_5Se]^+$ 553.1236, found 553.1257. CCDC 1889565. Single crystals of $C_{84}H_{79}N_6O_{17}Se_3$ [**5b-*R,S***] were were investigated on a Rigaku XtaLAB Synergy, Dualflex, HyPix diffractometer. Crystal Data for $C_{84}H_{79}N_6O_{17}Se_3$ (M =1681.41 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 17.8073(2) Å, b = 12.2429(1) Å, c = 19.8681(1) Å, β = 99.527(1)°, V = 4271.76(6) Å^3, Z = 2, T = 130.0(1) K, μ(Cu-K_α) = 2.121 mm^{-1}, $Dcalc$ = 1.307 g/cm^3, 61372 reflections measured ($7.294° \leq 2\Theta \leq 154.506°$), 16681 unique ($R_{int}$ = 0.0705, R_{sigma} = 0.0386) which were used in all calculations. The final R_1 was 0.0544 [$I > 2\sigma(I)$] and wR_2 was 0.1446 (all data).

(*R*)-2-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Trifluoroacetate (5b-*R*,*R*): Foam (56 mg, 34 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.54 (d, *J* = 6.1 Hz, 1H), 8.44 (t, *J* = 7.6 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.93 (t, *J* = 6.6 Hz, 1H), 7.81–7.69 (m, 5H), 7.60 (d, *J* = 7.0 Hz, 2H), 7.48–7.38 (m, 3H), 4.21–4.05 (m, 1H), 3.80–3.71 (m, 1H), 2.92–2.84 (m, 1H), 1.39 (s, 8H). ^{13}C NMR (101 MHz, CD_3OD) δ = 145.60, 141.97, 141.12, 138.12, 136.05, 132.68, 132.09, 130.92, 130.37, 128.33, 126.60, 126.35, 124.66, 87.94, 80.52, 28.68. HRMS (ESI/Q-TOF) *m*/*z*: $[M]^+$ calcd. for $[C_{28}H_{29}N_2O_5Se]^+$ 553.1236, found 553.1257.

(*S*)-6-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-7-hydroxy-7-methyl-5-phenyl-7*H*-pyrrolo[2,1-*b*]thiazol-4-ium Trifluoroacetate (5c-*R*,*S*): Foam (42 mg, 28 %). Compound is not stable. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.34 (d, *J* = 3.6 Hz, 1H), 8.17 (d, *J* = 3.6 Hz, 1H), 7.75 (m, 2H), 7.63 (m, 3H), 4.16 (d, *J* = 6.8 Hz, 1H), 3.42 (dd, *J* = 12.3, 5.2 Hz, 1H), 3.05 (dd, *J* = 12.3, 7.0 Hz, 1H), 1.90 (s, 3H), 1.41 (s, 10H). HRMS (ESI/Q-TOF) *m*/*z*: $[M]^+$ calcd. for $[C_{21}H_{25}N_2O_5SSe]^+$ 497.0644, found 497.0648.

(*R*)-6-({(*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-carboxyethyl}-selanyl)-7-hydroxy-7-methyl-5-phenyl-7*H*-pyrrolo[2,1-*b*]thiazol-4-ium Trifluoroacetate (5c-*R*,*R*): Foam (39 mg, 25 %). Compound is not stable. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.31 (d, *J* = 3.7 Hz, 1H), 8.14 (d, *J* = 3.7 Hz, 1H), 7.75–7.72 (m, 2H), 7.63–7.61 (m, 3H), 4.27 (m, 2H), 3.77 (dd, *J* = 12.8, 4.7 Hz, 1H), 3.15 (dd, *J* = 12.8, 4.7 Hz, 1H), 1.91 (s, 3H), 1.41 (s, 9H). HRMS (ESI/Q-TOF) *m*/*z*: $[M]^+$ calcd. for $[C_{21}H_{25}N_2O_5SSe]^+$ 497.0644, found 497.0650.

Benzyl (6*R*,11*R*)-6-{[2-(Benzyloxy)-2-oxoethyl]carbamoyl}-11-[(*tert*-butoxycarbonyl)amino]-2,2-dimethyl-4,12-dioxo-3-oxa-8,9-diselena-5,13-diazapentadecan-15-oate (6): To a solution of benzyl glycinate (3.17 g, 15.72 mmol, 5 equiv.) in DMF (30 mL) NMM (2.07 mL, 18.87 mmol, 6 equiv.) was added at 0 °C and the mixture was stirred for 5 minutes. Then HOBt (0.48 g, 3.14 mmol, 1 equiv.) was added to the reaction mixture followed by the addition of **4** (1.68 g, 3.14 mmol, 1 equiv.) in DMF (8 + 2 mL) and EDC (3.62 g, 18.87 mmol, 6 equiv.). The reaction mixture was stirred at 0 °C for 10 minutes and then at r.t. for 2 h then it was evaporated and the residue was purified by reverse phase chromatography (MeCN/H_2O) to yield **6** as yellow solid (2.1 g, 80 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.40–7.27 (m, 10H), 5.17 (s, 4H), 4.54–4.46 (m, 2H), 4.08–3.93 (m, 4H), 3.40–3.33 (m, 1H), 3.16–3.07 (m, 2H), 1.43 (s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.9, 170.8, 157.7, 137.1, 129.6, 129.3, 80.9, 67.9, 56.6, 42.2, 28.7. ^{77}Se NMR (76 MHz, CD_3OD) δ = 310.9. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ calcd. for $[C_{34}H_{47}N_4O_{10}Se_2]^+$ 831.1623, found 831.1608.

Dibenzyl 11,11′-[Diselanediylbis(methylene)](6*S*,6′*S*,11*R*,11′*R*)-bis[6-(*tert*-butoxycarbonyl)-2,2-dimethyl-4,9,12-trioxo-3-oxa-5,10,13-triazapentadecan-15-oate] (7): To a solution of **6** (1 g, 1.21 mmol, 1 equiv.) in CH_2Cl_2 (18 mL) TFA (12 mL) was added at 0 °C and the reaction mixture was stirred for 2 h at r.t. Then it was evaporated and dissolved in DMF (30 mL) and NMM (0.80 mL, 7.24 mmol, 6 equiv.) was added at 0 °C and the mixture was stirred for 5 minutes. Then HOBt (0.37 g, 2.41 mmol, 1 equiv.), a solution of compound (Boc)Glu-O*t*Bu (1.55 g, 4.83 mmol, 4 equiv.) in DMF (8 + 2 mL) and EDC (1.39 g, 7.24 mmol, 6 equiv.) was added and the reaction mixture was stirred at 0 °C for 10 minutes and then at r.t. for 2 hours. The reaction mixture was evaporated and the residue was purified by reverse phase chromatography (MeCN/H_2O) to give the title compound as yellow solid (1.2 g, 80 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.40–7.26 (m, 10H), 5.16 (s, 4H), 4.94–4.86 (m, 2H), 4.09–3.94 (m, 6H), 3.38–3.32 (m, 1H), 3.19–09 (m, 2H), 2.36 (t, *J* = 7.6 Hz, 4H), 2.17–2.01 (m, 3H), 1.95–1.79 (m, 3H), 1.46 (s, 18H), 1.43 (s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 173.3, 173.2, 170.9, 158.1, 137.1, 129.6, 129.4, 82.8, 80.5, 68.0, 55.4, 55.1, 42.3, 33.1, 33.0, 28.8, 28.5, 28.3. ^{77}Se NMR (76 MHz, CD_3OD) δ = 312.6. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ calcd. for $[C_{52}H_{76}N_6O_{16}Se_2]^+$ 1201.3724, found 1201.3789.

2-[((*R*)-3-{[2-(Benzyloxy)-2-oxoethyl]amino}-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl)selanyl]-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Bromide (8a): To a solution of **6** (100 mg, 0.12 mmol, 1 equiv.) in CH_2Cl_2 (5 mL) was added $CuBr_2$ (67 mg, 0.3 mmol, 2.5 equiv.) at r.t. and the mixture was stirred for 30 min. Then a solution of **1b** (103 mg, 0.36 mmol, 3 equiv.) in CH_2Cl_2 (3 + 1 mL) was added. Reaction mixture was stirred for 16 h, then it was evaporated and purified by reverse phase chromatography (MeCN/H_2O + AcOH) to give **8a** as yellow oil (80 mg, 85 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.61–8.54 (m, 1H), 8.45 (t, *J* = 7.8 Hz, 1H), 8.02–7.93 (m, 2H), 7.83–7.73 (m, 2H), 7.74–7.63 (m, 5H), 7.51–7.39 (m, 3H), 7.38–7.28 (m, 5H), 5.15–5.11 (m, 2H), 4.22–4.19 (m, 0.5H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.2, 173.0, 170.7, 161.9, 157.4, 145.8, 145.7, 142.1, 141.8, 140.2, 140.1, 137.3, 137.1, 136.1, 132.9, 131.8, 131.1, 131.1, 130.7, 130.6, 129.6, 129.4, 129.3, 129.2, 128.7, 126.4, 126.4, 124.8, 124.7, 87.5, 87.4, 81.0, 67.8, 55.9, 55.6, 42.1, 42.1, 40.1, 28.7, 27.2, 27.1. ^{77}Se NMR (76 MHz, CD_3OD) δ = 215.0, 206.2. HRMS (ESI/Q-TOF) *m*/*z*: $[M]^+$ calcd. for $[C_{37}H_{37}N_3O_6Se]^+$ 700.1920, found 700.1924.

2-[((*R*)-3-{[2-(Benzyloxy)-2-oxoethyl]amino}-2-{(*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanamido}-3-oxopropyl)selanyl]-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Bromide (8b): To a solution of **7** (100 mg, 0.083 mmol, 1 equiv.) in CH_2Cl_2 (5 mL) was added $CuBr_2$ (47 mg, 0.208 mmol, 2.5 equiv.) at r.t. and the mixture was stirred for 30 min. Then a solution of **1b** (71 mg, 0.25 mmol, 3 equiv.) in CH_2Cl_2 (3 + 1 mL) was added. Reaction mixture was stirred for 16 h and then it was evaporated and purified by reverse phase chromatography (MeCN/H_2O + AcOH) to give product as yellow oil (70 mg, 88 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.57 (t, *J* = 5.0 Hz, 1H), 8.45 (t, *J* = 7.7 Hz, 1H), 8.04–7.92 (m, 2H), 7.79–7.69 (m, 4H), 7.68–7.62 (m, 2H), 7.50–7.41 (m, 3H), 7.38–7.27 (m, 5H), 5.14 and 5.13 (2 s, 2H), 4.44 (dd, *J* = 8.5, 5.8 Hz, 0.5H), 4.34 (t, *J* = 7.1 Hz, 0.5H), 4.00–3.86 (m, 3H), 3.52–3.42 (m, 1H), 3.09–3.01 (m, 1H), 2.76–2.65 (m, 1H), 2.32–2.19 (m, 2H), 2.07–1.97 (m, 1H), 1.87–1.75 (m, 2H), 1.47 and 1.43 (2 s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 173.2, 172.7, 172.52, 170.8, 162.1, 158.1, 145.8, 137.5, 137.14, 136.2, 132.90, 131.8, 131.1, 130.7, 130.6, 129.6, 129.3, 129.2, 128.6, 128.0, 126.4, 124.8, 87.6, 87.4, 82.8, 80.5, 67.9, 55.4, 54.7, 54.3, 42.2, 32.9, 28.8, 28.3, 26.5. ^{77}Se NMR (76 MHz, CD_3OD) δ = 213.9, 207.2. HRMS (ESI/Q-TOF) *m*/*z*: $[M]^+$ calcd. for $[C_{46}H_{52}N_4O_9Se]^+$ 885.2972, found 885.2972.

Di-*tert*-butyl [(2*S*,8*R*,13*R*,19*S*)-1,20-Diamino-2,19-dibenzyl-1,4,7,14,17,20-hexaoxo-10,11-diselena-3,6,15,18-tetraazaicosane-8,13-diyl]dicarbamate (9): To a solution of (*S*)-2-(2-aminoacetamido)-3-phenylpropanamide (245 mg, 0.730 mmol, 3 equiv.) in DMF (5 mL) at 0 °C was added NMM (0.11 mL, 0.973 mmol, 4 equiv.) and the mixture was stirred for 5 minutes. Then to the reaction mixture was added HOBt (37 mg, 0.243 mmol, 1 equiv.), a solution of compound **4** (130 mg, 0.243 mmol, 1 equiv.) in DMF (3 + 1 mL) and EDC (187 mg, 0.973 mmol, 4 equiv.). The reaction mixture was stirred at 0 °C for 10 minutes and at r.t. for 3 hours, then it was evaporated and the residue was purified by reverse phase chromatography (MeCN/H_2O) to give the product as light yellow solid (101 mg, 44 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.30–7.16 (m, 10H), 4.65–4.57 (m, 2H), 4.42–4.35 (m, 2H), 3.88 (d, *J* = 16.7 Hz, 2H), 3.74 (d, *J* = 16.7 Hz, 2H), 3.38 (dd, *J* = 12.7, 5.3, 2H), 3.23–3.08 (m, 4H),

2.92 (dd, *J* = 13.9, 9.1 Hz, 2H), 1.44 (s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.9, 173.9, 171.2, 157.8, 138.3, 129.5, 127.7, 81.0, 56.8, 55.9, 43.7, 38.7, 32.7, 28.8. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{38}H_{55}N_8O_{10}Se_2]^+$ 943.2372, found 943.2379.

***tert*-Butyl {(6*R*,9*R*,14*R*,17*R*)-9,14-Bis[(2-{[(*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)carbamoyl]-6-(4-hydroxybenzyl)-18-(4-hydroxyphenyl)-2,2-dimethyl-4,7,16-trioxo-3-oxa-11,12-diselena-5,8,15-triazaoctadecan-17-yl}carbamate (10):** To a solution of compound **9** (270 mg, 0.287 mmol, 1 equiv.) in CH_2Cl_2 (5 mL) was added TFA (2 mL) at 0 °C and reaction mixture was stirred for 2 hours at room temperature, then it was evaporated, dissolved in DMF (5 mL) and to this was added NMM (0.19 mL, 1.72 mmol, 6 equiv.) at 0 °C and the mixture was stirred for 5 minutes. Then HOBt was added (88 mg, 0.574 mmol, 2 equiv.) and a solution of Boc-L-tyrosine (323 mg, 1.15 mmol, 4 equiv.) in DMF (3 + 1 mL) was added. Then EDC (330 mg, 1.72 mmol, 6 equiv.) was added and the reaction mixture was stirred at 0 °C for 5 minutes and then at r.t. for 3 hours. The reaction mixture was evaporated and the residue was purified by reverse phase chromatography (MeCN/H_2O) to give product as white solid (185 mg, 51 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.30–7.11 (m, 10H), 7.04 (d, *J* = 8.6 Hz, 4H), 6.69 (d, *J* = 8.5 Hz, 4H), 4.69–4.57 (m, 2H), 4.31–4.23 (m, 2H), 3.87 (d, *J* = 16.7 Hz, 2H), 3.74 (d, *J* = 16.7 Hz, 2H), 3.41–3.34 (m, 2H), 3.24–3.11 (m, 4H), 3.03–2.92 (m, 4H), 2.84–2.75 (m, 2H), 1.36 (s, 9H). ^{13}C NMR spectra was not recorded due to lack of solubility. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{56}H_{73}N_{10}O_{14}Se_2]^+$ 1269.3638, found 1269.3722.

2-({(*R*)-3-[(2-{[(*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)amino]-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}selanyl)-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Bromide (11a): To a solution of **9** (100 mg, 0.11 mmol, 1 equiv.) in CH_2Cl_2 (3 mL) and MeOH (1 mL) was added $CuBr_2$ (59 mg, 0.27 mmol, 2.5 equiv.) at r.t. and the mixture was stirred for 30 min. Then a solution of **1b** (91 mg, 0.32 mmol, 3 equiv.) in CH_2Cl_2 (3 + 1 mL) was added. Reaction mixture was stirred for 16 h, then it was evaporated and the product was purified by reverse phase chromatography (MeCN/H_2O) to give product in 98 % yield. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.57 (t, *J* = 6.5 Hz, 1H), 8.46 (t, *J* = 7.8 Hz, 1H), 8.03–7.94 (m, 2H), 7.79–7.64 (m, 7H), 7.49–7.40 (m, 3H), 7.28–7.15 (m, 5H), 4.56–4.45 (m, 1H), 4.12 (t, *J* = 7 Hz, 0.5H), 4.03 (t, *J* = 6.8 Hz, 0.5H), 3.86–3.75 (m, 1H), 3.62 (d, *J* = 16.1 Hz, 1H), 3.40 (dd, *J* = 12.6, 5.9 Hz, 0.5), 3.22–3.11 (m, 1H), 3.01 (dd, *J* =12.4, 6.1 Hz, 0.5H), 2.88 (ddd, *J* = 13.5, 9.2, 3.6 Hz, 1H), 2.70 (dd, *J* = 12.4, 8.2 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.88, 173.11, 172.93, 171.14, 171.03, 162.11, 162.03, 157.50, 145.73, 142.19, 142.01, 140.19, 138.52, 137.36, 137.26, 136.14, 132.89, 131.82, 131.11, 130.70, 130.56, 130.24, 129.47, 128.68, 127.74, 126.43, 126.38, 124.76, 87.51, 81.18, 55.98, 55.74, 43.51, 38.70, 38.62, 28.72, 27.00, 26.87. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 212.3, 206.1. HRMS (ESI/Q-TOF) *m/z*: $[M]^+$ calcd. for $[C_{39}H_{41}N_5O_6Se]^+$ 756.2295, found 756.2291.

2-({(*R*)-3-[(2-{[(*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)amino]-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-selanyl)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Bromide (11b): To a solution of compound **9** (100 mg, 0.11 mmol, 1 equiv.) in CH_2Cl_2 (4 mL) and MeOH (1 mL) was added $CuBr_2$ (59 mg, 0.27 mmol, 2.5 equiv.) at r.t. and the mixture was stirred for 30 min. Then a solution of alkyne (71 mg, 0.32 mmol, 3 equiv.) in CH_2Cl_2 (2 + 1 mL) was added. Reaction mixture was stirred for 16 h, then it was evaporated and the product was purified by reverse phase chromatography (MeCN/H_2O) to give product as yellow oil in 98 % yield. ^{1}H NMR (400 MHz, CD_3OD): 8.58 (t, *J* = 7.7 Hz, 1H), 8.50 (t, *J* = 7.0 Hz, 1H), 8.41–8.35 (m, 1H), 7.98 (t, *J* = 7Hz, 1H), 7.70–7.62 (m, 5H), 7.29–7.16 (m, 5H), 4.53 (dd, *J* = 9.4, 5 Hz, 0.5H), 4.45 (dd, *J* = 9.3, 5 Hz, 0.5H), 4.34 (t, *J* = 6.6 Hz, 0.5H), 4.20 (dd, *J* = 9.0, 5.3 Hz, 0.5H), 3.89–3.78 (m, 1H), 3.74–3.63 (m, 1H), 3.55 (dd, *J* = 12.6, 5.2 Hz, 0.5), 3.42–3.34 (m, 0.5H), 3.26 (dd, *J* = 12.5, 5.9 Hz, 0.5H), 3.17 (ddd, *J* = 14.0, 11.1, 5.0 Hz, 1H), 2.99–2.84 (m, 1.5H), 1.89 un 1.88 (2 signals, s, 3H), 1.42 and 1.41 (2 signals, s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.9, 175.9, 173.4, 173.1, 171.2, 171.1, 162.2, 157.7, 145.7, 141.2, 140.8, 140.1, 138.6, 135.9, 132.7, 131.9, 130.9, 130.2, 129.5, 128.5, 127.7, 126.5, 126.5, 123.9, 84.3, 81.3, 56.3, 56.0, 43.5, 38.7, 38.6, 28.7, 27.7, 25.4. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 184.1, 177.8. HRMS (ESI/Q-TOF) *m/z*: $[M]^+$ calcd. for $[C_{34}H_{39}N_5O_6Se]^+$ 694.2138, found 694.2132.

2-({(*R*)-3-[(2-{[(*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)amino]-2-[(*S*)-2-amino-3-(4-hydroxyphenyl)propanamido]-3-oxopropyl}selanyl)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Trifluoroacetate (11c): To a solution of compound **10** (84 mg, 0.07 mmol, 1 equiv.) in CH_2Cl_2 (10 mL) and MeOH (4 mL) was added $CuBr_2$ (37 mg, 0.17 mmol, 2.5 equiv.) at r.t. and the mixture was stirred for 30 min. Then a solution of alkyne (57 mg, 0.20 mmol, 3 equiv.) in CH_2Cl_2 (3 + 1 mL) was added. Reaction mixture was stirred for 16 h and then it was evaporated. The product was dissolved in CH_2Cl_2 (3 mL) and TFA (3 mL) was added at 0 °C. The reaction mixture was stirred for 2 hours, then it was evaporated and purified by reverse phase chromatography (MeCN/H_2O + HCl) to give product as yellow oil (50 mg, 81 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.56 (t, *J* = 6.4 Hz, 1H), 8.45 (t, *J* = 7.8 Hz, 1H), 8.04–7.93 (m, 2H), 7.81–7.74 (m, 2H), 7.72–7.63 (m, 6H), 7.51–7.40 (m, 3H), 7.28–7.21 (m, 5H), 7.21–7.15 (m, 1H), 7.13–7.07 (m, 2H), 6.77 (d, *J* = 8.3 Hz, 2H), 4.55 (dd, *J* = 9.4, 5.3 Hz, 0.5H), 4.49 (dd, *J* = 9.4, 5.2 Hz, 0.5H), 4.23 (t, *J* = 7.0 Hz, 0.5H), 4.13–4.06 (m, 1.5H), 3.95–3.83 (m, 1H), 3.63–3.53 (m, 1H), 3.22–3.07 (m, 3H), 2.99–2.79 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 176.0, 171.4, 171.0, 170.4, 162.0, 158.3, 145.7, 141.9, 140.2, 138.4, 137.3, 136.1, 132.9, 131.8, 131.7, 131.2, 130.7, 130.6, 130.4, 129.5, 128.6, 127.7, 126.5, 126.4, 125.8, 124.8, 116.9, 87.5, 55.9, 55.5, 43.4, 39.0, 37.7, 25.4. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 216.2, 211.0. HRMS (ESI/Q-TOF) *m/z*: $[M]^+$ calcd. for $[C_{43}H_{42}N_6O_6Se]^+$ 819.2404, found 819.2396.

General Procedure for the Synthesis of Arene-alkynes 12e–h: 1-Bromo-2-substituted (ethynyl)benzene (1 equiv.), arylboronic acid (2 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), Na_2CO_3 (6 equiv.), and mixture of solvents (60 mL, EtOH/H_2O/toluene = 1:1:4) were added subsequently into a high pressure vial. The resulting mixture was stirred at 70–80 °C for 8 h. After that the mixture was cooled to r.t. and extracted with ethyl acetate (40 mL × 3 times). The combined organic layers were dried with $MgSO_4$. Then it was filtered, volatiles were evaporated under reduced pressure, and the residue was purified by flash column chromatography on silica gel (eluent: petroleum ether/ethyl acetate) to afford **12e**–**h** majorly as an oils.

1-[2-(Phenylethynyl)phenyl]naphthalene (12e): Yellowish oil which solidified in a fridge (0.8 g, 84 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 (t, *J* = 8.1 Hz, 2H), 7.74–7.68 (m, 2H), 7.60–7.54 (m, 1H), 7.53–7.39 (m, 6H), 7.18–7.08 (m, 3H), 6.82–6.77 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.3, 139.0, 133.7, 132.1, 132.0, 131.3, 130.8, 128.2, 128.1, 128.1, 128.0, 127.6, 127.5, 126.6, 126.0, 125.8, 125.3, 123.8, 123.2, 93.1, 89.2. GC–MS (*m/z*): 303.1.1(100) $[M]^{+\cdot}$, 151.1(11).

9-[2-(Phenylethynyl)phenyl]phenanthrene (12f): White solid (0.62 g, 31 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.85–8.80 (m, 4H), 7.93 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.87 (s, 2H), 7.74 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 2H), 7.70–7.63 (m, 4H), 7.51 (ddd, *J* = 8.2, 1.5, 0.7 Hz, 2H), 7.38 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.3, 132.3,

131.8, 130.5, 128.9, 128.6, 127.7, 127.1, 126.9, 126.8, 126.7, 123.0, 122.8. GC–MS (*m/z*): 353.1(100) $[M - 1]^{+\cdot}$, 175.0(40).

2-(Naphthalen-1-yl)-3-(phenylethynyl)thiophene (12g): Yellowish solid (0.84 g, 77 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.89–7.85 (m, 1H), 7.81 (ddt, *J* = 10.6, 8.1, 1.5 Hz, 2H), 7.56 (dd, *J* = 7.1, 1.5 Hz, 1H), 7.46 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.43–7.35 (m, 2H), 7.29 (d, *J* = 5.1 Hz, 1H), 7.14–7.08 (m, 3H), 7.08–7.02 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.8, 134.0, 133.6, 131.8, 131.3, 130.3, 128.4, 128.4, 128.3, 128.1, 126.4, 126.1, 126.0, 125.9, 125.4, 123.1, 120.8, 95.4, 83.0. GC–MS (*m/z*): 309.1(100) $[M]^{+\cdot}$, 175.0(34).

4-(Hept-1-yn-1-yl)-3-(*p*-tolyl)thiophene (12h): Yellowish oil (0.4 g, 79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61–7.57 (m, 2H), 7.43 (d, *J* = 3.3 Hz, 1H), 7.24–7.19 (m, 2H), 7.20 (s, 1H), 2.39 (s, 3H), 2.36 (t, *J* = 7.0 Hz, 2H), 1.62–1.50 (m, 2H), 1.44–1.24 (m, 4H), 0.90 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.4, 137.2, 132.9, 129.2, 129.0, 128.2, 122.6, 121.5, 92.2, 75.9, 31.2, 28.4, 22.4, 21.4, 19.6, 14.1. GC–MS (*m/z*): 268.0(80) $[M]^{+\cdot}$, 210.9(100).

General Procedure for the Synthesis of Aryl Fused Peptides via 6-*endo*-*dig* Cyclization: A vial loaded with selenocystine-based peptide (1 equiv.) and $CuBr_2$ (1 equiv.) dissolved in dry CH_2Cl_2 (2 mL) was stirred for 4 h at r.t. (*Note*: if the mixture is not transparent, a few drops of acetonitrile can be added). Then dry CH_2Cl_2 (1 mL) solution of 2-ethynyl biphenyl (2 equiv.) was added dropwise, and the mixture was stirred at r.t. overnight. After this period, the solvents were evaporated, and the products (**13a**–**h**) was isolated by reverse-phase flash chromatography on silica gel C-18 using the mixture of water/acetonitrile as eluent.

Benzyl (*R*)-{2-[(*tert*-Butoxycarbonyl)amino]-3-[(10-phenylphenanthren-9-yl)selanyl]propanoyl}glycinate (13a): Foam (51 mg, 85 %). Synthesized from 2-(phenylethynyl)-1,1′-biphenyl (**12a**) (0.181 mmol, 0.046 g) and **6** (0.09 mmol, 0.075 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.89–8.81 (m, 3H), 7.76–7.65 (m, 3H), 7.55–7.42 (m, 4H), 7.38 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.35–7.21 (m, 7H), 5.11 (s, 2H), 4.13–4.06 (m, 1H), 3.89–3.75 (m, 2H), 2.97 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.92–2.83 (m, 1H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7, 169.3, 142.2, 135.3, 132.5, 131.9, 130.9, 130.3, 129.5, 129.1, 128.8, 128.7, 128.7, 128.5, 128.5, 128.2, 128.1, 127.8, 127.7, 127.6, 127.4, 127.2, 126.9, 123.2, 122.7, 80.5, 67.3, 41.3, 31.0, 28.3. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 167.6. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{37}H_{36}N_2O_5Se]^+$ 669.1868, found 669.1866.

Benzyl (*R*)-{2-[(*tert*-Butoxycarbonyl)amino]-3-[(2-methyl-10-phenylphenanthren-9-yl)selanyl]propanoyl}glycinate (13b): Foam (38 mg, 94 %). Synthesized from 4′-methyl-2-(phenylethynyl)-1,1′-biphenyl (**12b**) (0.121 mmol, 0.032 g) and **6** (0.06 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.82–8.74 (m, 2H), 8.69 (d, *J* = 8.5 Hz, 1H), 7.72–7.62 (m, 2H), 7.54–7.42 (m, 4H), 7.33–7.20 (m, 7H), 7.15 (s, 1H), 5.09 (s, 2H), 4.09 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.87–3.74 (m, 2H), 2.95 (dd, *J* = 12.5, 4.8 Hz, 1H), 2.86 (dd, *J* = 12.5, 8.3 Hz, 1H), 2.34 (s, 3H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.7, 170.7, 157.1, 147.2, 143.4, 137.7, 137.1, 133.6, 133.4, 132.0, 131.5, 131.4, 131.3, 130.1, 130.1, 129.5, 129.3, 129.3, 129.2, 129.0, 128.4, 128.2, 128.1, 123.9, 123.7, 80.8, 67.9, 56.2, 42.1, 31.8, 28.5, 21.6. ^{77}Se NMR (76 MHz, CD_3OD) δ = 178.3. IR $\tilde{\nu}_{max}$ (film): 3327, 3061, 3026, 2977, 2932, 1751, 1685, 1497, 1367, 1246, 1171, 1029, 758, 701. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{38}H_{38}N_2O_5Se]^+$ 683.2024, found 683.2007.

Benzyl (*R*)-{2-Amino-3-[(10-pentylphenanthren-9-yl)selanyl]-propanoyl}glycinate Trifluoroacetate (13d): Foam (40 mg, 94 %). Synthesized from 2-(hept-1-yn-1-yl)-1,1′-biphenyl (**12d**) (0.121 mmol, 0.032 g) and **6** (0.060 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.46–7.40 (m, 2H), 7.40–7.26 (m, 11H), 5.24–5.16 (m, 2H), 4.04 (s, 1H), 3.98 (d, *J* = 2.1 Hz, 1H), 3.88 (q, *J* = 5.9, 5.3 Hz, 1H), 3.02–2.73 (m, 3H), 2.38–2.26 (m, 1H), 1.39–1.23 (m, 5H), 1.20–1.11 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 170.5, 143.0, 142.9, 141.9, 141.1, 137.1, 132.1, 131.3, 130.9, 130.4, 130.2, 129.6, 129.5, 129.4, 129.0, 128.6, 128.5, 121.8, 68.2, 42.2, 38.3, 38.0, 32.1, 28.1, 23.6, 14.3. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 270.8. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{31}H_{35}N_2O_3Se]^+$ 563.1813, found 563.1803.

Benzyl (*R*)-{2-[(*tert*-Butoxycarbonyl)amino]-3-[(6-phenylbenzo[*c*]phenanthren-5-yl)selanyl]propanoyl}glycinate (13e): Foam (39 mg, 92 %). Synthesized from compounds **12e** (0.121 mmol, 0.037 g) and **6** (0.06 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 9.03–8.96 (m, 2H), 8.91–8.84 (m, 1H), 7.97 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.74–7.59 (m, 5H), 7.53–7.43 (m, 3H), 7.34–7.19 (m, 8H), 5.06 (s, 2H), 4.08 (dd, *J* = 7.6, 4.8 Hz, 1H), 3.82–3.69 (m, 2H), 3.03–2.88 (m, 2H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.7, 170.6, 157.1, 146.6, 143.2, 137.1, 135.1, 134.9, 131.8, 131.7, 131.6, 131.2, 130.9, 130.7, 129.8, 129.7, 129.7, 129.5, 129.4, 129.3, 129.3, 128.6, 128.2, 128.2, 127.6, 127.4, 127.3, 126.3, 80.8, 67.8, 56.2, 54.8, 42.0, 31.9, 28.5. ^{77}Se NMR (76 MHz, CD_3OD) δ = 178.0. IR $\tilde{\nu}_{max}$ (film): 3396, 3332, 3061, 2977, 2932, 1747, 1691, 1508, 1367, 1237, 1168, 1024, 826, 753, 699. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for [for $C_{41}H_{39}N_2O_5Se]^+$ 719.2024, found 719.2001.

Benzyl (*R*)-{2-Amino-3-[(5-phenylbenzo[*g*]chrysen-6-yl)selanyl]-propanoyl}glycinate (13f): Foam (47 mg, 62 %). Synthesized from compounds **12f** (0.121 mmol, 0.041 g) and **6** (0.06 mmol, 0.05 g) followed by removing of Boc protecting group with TFA (0.5 mL) in DCM (1 mL), at r.t. for 3 h. The product was isolated by reverse-phase flash chromatography. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.79–8.67 (m, 4H), 8.63 (dt, *J* = 8.2, 0.8 Hz, 1H), 7.79–7.67 (m, 3H), 7.62 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.51–7.38 (m, 6H), 7.35–7.25 (m, 6H), 7.02 (ddd, *J* = 8.4, 7.0, 1.3 Hz, 1H), 5.03 (d, *J* = 2.3 Hz, 2H), 3.64 (dd, *J* = 6.8, 6.0 Hz, 1H), 3.64–3.40 (m, 3H), 3.00 (dd, *J* = 6.4, 3.8 Hz, 2H), 1.29 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.6, 144.7, 142.7, 135.3, 133.4, 132.5, 131.7, 131.5, 131.0, 130.9, 130.2, 130.0, 129.8, 129.5, 129.4, 129.2, 129.1, 128.7, 128.6, 128.5, 128.3, 127.8, 127.5, 127.4, 126.8, 126.4, 125.7, 123.6, 123.2, 67.1, 41.0, 35.3. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 163.9. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{40}H_{33}N_2O_3Se]^+$ 669.1656, found 669.1645.

Benzyl (R)-{2-[(*tert*-Butoxycarbonyl)amino]-3-[(5-phenylphenanthro[3,4-*b*]thiophen-4-yl)selanyl]propanoyl}glycinate (13g): Foam (24 mg, 84 %). Synthesized from compounds **12g** (0.121 mmol, 0.037 g) and **6** (0.06 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.95 (d, *J* = 8.2 Hz, 1H), 7.93–7.88 (m, 1H), 7.84 (t, 1H), 7.67–7.61 (m, 1H), 7.59–7.53 (m, 1H), 7.52–7.39 (m, 3H), 7.35–7.14 (m, 9H), 6.95–6.90 (m, 1H), 6.88–6.81 (m, 1H), 6.79 (d, *J* = 5.2 Hz, 1H), 6.69 (t, *J* = 7.6 Hz, 1H), 6.38 (d, *J* = 7.0 Hz, 1H), 5.11 (d, *J* = 9.6 Hz, 2H), 3.97–3.81 (m, 3H), 2.39–2.27 (m, 1H), 2.27–2.16 (m, 1H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.4, 170.6, 137.1, 137.1, 135.4, 135.3, 134.9, 131.4, 130.8, 130.1, 129.9, 129.6, 129.5, 129.4, 129.3, 129.3, 129.0, 128.9, 128.9, 128.8, 128.3, 127.7, 127.4, 127.2, 127.0, 126.9, 126.6, 126.3, 80.9, 67.9, 55.5, 42.1, 28.7. ^{77}Se NMR (76 MHz, CD_3OD) δ = 392.1. IR $\tilde{\nu}_{max}$ (film): 3410, 3340, 2978, 2932, 1748, 1682, 1514, 1367, 1170, 1046, 1024, 756, 699. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{39}H_{37}N_2O_5SSe]^+$ 725.1588, found 725.1580.

Benzyl (*R*)-{2-[(*tert*-Butoxycarbonyl)amino]-3-[(7-methyl-5-pentylnaphtho[1,2-*c*]thiophen-4-yl)selanyl]propanoyl}glycinate (13h): Foam (36 mg, 89 %). Synthesized from compounds **12h** (0.121 mmol, 0.032 g) and **6** (0.06 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.38–7.30 (m, 7H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.16–7.11

(m, 2H), 5.15 (s, 2H), 4.14 (broad s, 1H), 4.01–3.88 (m, 2H), 3.03–2.43 (m, 4H), 2.33 (s, 3H), 1.61–1.23 (m, 15H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.7, 170.8, 157.4, 143.1, 142.1, 138.1, 137.1, 135.5, 134.4, 129.9, 129.6, 129.4, 129.3, 129.3, 127.6, 123.4, 81.0, 67.9, 56.1, 49.6, 42.2, 37.8, 32.3, 28.7, 28.4, 23.6, 21.3, 14.4. IR $\tilde{\nu}_{max}$ (film): 3316, 2955, 2929, 2858, 1752, 1674, 1507, 1456, 1367, 1249, 1188, 1046, 1023, 755, 698. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd. for $[C_{35}H_{43}N_2O_5SSe]^+$ 683.2058, found 683.2047.

***tert*-Butyl {(*R*)-1-[(2-{[(*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)amino]-1-oxo-3-[(6-phenylbenzo[*c*]phenanthren-5-yl)selanyl] Propan-2-yl}carbamate (13i):** Foam (39 mg, 95 %). Synthesized from compounds **12e** (0.106 mmol, 0.032 g) and **9** (0.053 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 9.06–8.97 (m, 2H), 8.94–8.86 (m, 1H), 7.98 (dd, J = 7.8, 1.6 Hz, 1H), 7.77–7.59 (m, 5H), 7.56–7.45 (m, 3H), 7.35–7.28 (m, 3H), 7.23–7.13 (m, 4H), 7.15–7.06 (m, 1H), 4.53 (dd, J = 8.9, 5.2 Hz, 1H), 3.98 (dd, J = 8.9, 4.8 Hz, 1H), 3.71 (d, J = 16.7 Hz, 1H), 3.51 (d, J = 16.7 Hz, 1H), 3.14 (dd, J = 13.3, 5.2 Hz, 1H), 3.03 (dd, J = 13.3, 4.8 Hz, 1H), 2.98–2.80 (m, 2H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 172.4, 169.6, 156.0, 145.3, 141.9, 137.0, 133.6, 133.5, 130.4, 130.3, 130.2, 129.8, 129.5, 129.3, 128.8, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.9, 127.2, 126.8, 126.8, 126.3, 126.3, 126.0, 124.9, 79.6, 55.2, 54.4, 42.2, 37.3, 30.2, 27.2. ^{77}Se NMR (76 MHz, CD_3OD) δ = 178.2. IR $\tilde{\nu}_{max}$ (film): 3302, 1640, 1527, 1428, 1251, 1167, 1047, 1030, 826, 754, 699. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd. for $[C_{43}H_{43}N_4O_5Se]^+$ 775.2399, found 775.2390.

***tert*-Butyl N^5-((*R*)-1-{[2-(Benzyloxy)-2-oxoethyl]amino}-1-oxo-3-[(6-phenylbenzo[*c*]phenanthren-5-yl)selanyl]propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (13j):** Foam (28 mg, 80 %). Synthesized from compounds **12e** (0.085 mmol, 0.026 g) and **7** (0.043 mmol, 0.05 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 9.04–8.98 (m, 2H), 8.93–8.88 (m, 1H), 7.98 (dd, J = 7.7, 1.7 Hz, 1H), 7.76–7.61 (m, 5H), 7.54–7.44 (m, 3H), 7.34–7.20 (m, 8H), 5.06 (s, 2H), 4.27 (dd, J = 9.0, 5.0 Hz, 1H), 3.91 (dd, J = 9.0, 5.0 Hz, 1H), 3.76 (s, 2H), 3.06 (dd, J = 12.7, 5.0 Hz, 1H), 2.89–2.81 (m, 1H), 2.20–2.02 (m, 2H), 1.99–1.88 (m, 1H), 1.79–1.67 (m, 1H), 1.44 (s, 9H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 173.2, 173.2, 170.6, 158.0, 146.9, 143.2, 137.0, 135.2, 134.9, 131.8, 131.5, 131.3, 130.9, 130.7, 129.7, 129.6, 129.5, 129.3, 129.3, 129.2, 129.1, 128.6, 128.2, 128.0, 127.7, 127.4, 127.4, 126.4, 82.7, 80.5, 67.8, 55.4, 54.9, 42.0, 32.9, 31.3, 28.7, 28.3. ^{77}Se NMR (76 MHz, CD_3OD) δ = 179.8. IR $\tilde{\nu}_{max}$ (film): 3311, 3061, 2978, 2932, 1742, 1652, 1524, 1367, 1154, 1055, 1030, 826, 754, 701. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd. for $[C_{54}H_{54}N_3O_8Se]^+$ 904.3076, found 904.3055.

***tert*-Butyl [(*S*)-1-({(*R*)-1-[(2-{[(*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]amino}-2-oxoethyl)amino]-1-oxo-3-[(6-phenylbenzo[*c*]-phenanthren-5-yl)selanyl]propan-2-yl}amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]carbamate (13k):** Foam (23 mg, 92 %). Synthesized from compounds **12e** (0.055 mmol, 0.017 g) and **10** (0.028 mmol, 0.035 g). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.97–8.88 (m, 2H), 8.81 (dd, J = 7.9, 1.5 Hz, 1H), 7.89 (dd, J = 7.9, 1.5 Hz, 1H), 7.69–7.50 (m, 5H), 7.45–7.31 (m, 3H), 7.26–7.16 (m, 3H), 7.14–7.05 (m, 4H), 7.06–6.96 (m, 1H), 6.78 (d, J = 8.2 Hz, 2H), 6.51 (d, J = 8.2 Hz, 2H), 4.42 (dd, J =8.7, 5.6 Hz, 1H), 4.07 (dd, J = 8.2, 5.6 Hz, 1H), 3.96 (dd, J = 8.7, 5.6 Hz, 1H), 3.62 (d, J = 16.9 Hz, 1H), 3.34 (d, J = 16.9 Hz, 1H), 3.06–2.95 (m, 2H), 2.87–2.69 (m, 3H), 2.48 (dd, J = 14.0, 8.7 Hz, 1H), 1.23 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 176.0, 174.5, 172.8, 171.0, 157.8, 157.3, 146.8, 143.2, 138.5, 135.1, 134.9, 131.8, 131.8, 131.6, 131.4, 131.3, 130.9, 130.7, 130.3, 129.8, 129.7, 129.4, 129.4, 129.3, 129.3, 128.6, 128.3, 128.2, 127.7, 127.4, 126.4, 116.2, 81.0, 57.6, 56.0, 55.7, 43.6, 38.7, 30.8, 28.7. ^{77}Se NMR (76 MHz, CD_3OD) δ = 180.9. IR $\tilde{\nu}_{max}$ (film): 3230, 1682, 1638, 1516, 1436, 1247, 1167, 825, 754, 699. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd. for $[C_{52}H_{52}N_5O_7Se]^+$ 938.3032, found 938.2994.

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Selenocysteine Electrophile

Selenocystine Peptides Performance in 5-*endo-dig* Reactions

Sindija Lapcinska[a] and Pavel Arsenyan*[a]

Abstract: Herein, we present methods for the generation of selenocysteinyl electrophile by weak Lewis acids or oxidants. The electrophilic selenium species were further utilized in 5-*endo-dig* cyclization reactions with 2-ethynyl phenols, anisoles, and anilines, yielding substituted benzo[*b*]furans and indoles bearing short selenocysteine-containing peptides. Copper(II) bromide promoted 5-*endo-dig* cyclization can be successfully applied for protected and unprotected peptides in high yields. Elaborated protocol allows the construction of phenylindeno[1,2-*c*]chromene moiety in 5-*endo-dig*/6-*endo-dig* cascade reactions.

Introduction

Benzo[*b*]furan[1] and indole[2] are among the most important heterocycles found in many natural products. Due to the biological activity of natural and synthetic derivatives of these heterocycles they are relevant scaffolds for pharmaceuticals.[3,4] Furthermore, both – benzo[*b*]furan and indole – are considered as "privileged structures" thanks to their ability to act as ligands for various receptors.[5]

The most popular approaches for introduction of selenium atom in benzo[*b*]furan and indole moieties is either direct selenylation[6–10] of these heterocycles or electrophilic cyclization of 2-(1-alkynyl)anisoles/phenols and 2-(1-alkynyl)anilines in the presence of selenium electrophile.[11] The electrophilic species usually employed are arylselanyl chloride[12,13] or arylselanyl iodide generated in situ from diselenides using I_2,[14] Fe/I_2,[15] KI/*m*-CPBA system[6] or copper(I) iodide.[16] Copper(I) iodide has also been used as catalyst for the generation of selenium electrophile.[17] Lewis acid ($FeCl_3$) mediated generation of selenium electrophile has been used for preparation of 3-selanyl benzo[*b*]furans[18] and indoles[19] as well. Very recently, Perin et al. reported the synthesis of 2,3-dichalcogenyl-substituted benzo[*b*]chalcogenophenes employing oxone induced generation of selenium electrophile.[20] Another sulfur-containing oxidant (persulfates) induced generation of selenium electrophile has been used for the direct selenylation of indole[8] and arenofurans.[9] It is noteworthy that previous research has only focused on the use of simple diaryl diselenides except for Cohen et al. who reported a sophisticated method[21,22] for the generation of selenocysteine[23,24] (Sec, U) electrophile. This approach is based on the electrophilic character of (5-nitropyridylthio)-Sec peptides and was used in Sec-peptide and small molecule (including some indoles) late stage conjugation. Recently, our group demonstrated novel copper(II) bromide mediated method[25] for the generation of selenocysteinyl electrophile from selenocystine containing peptides. The electrophilic species were used in 5-*endo-dig* cyclization with 2-propargyl *N*-pyridines forming corresponding indolizinium salts whereas 6-*endo-dig* cyclization with 2-ethynylbiaryls led to formation of polyaromatic systems containing Sec peptides. In continuation of our research we would like to present the formation of selenocysteine containing benzo[*b*]furans and indoles via 5-*endo-dig* cyclization utilizing copper(II) bromide or oxidant induced generation of Sec electrophile. Notably, methods are tolerant to both unprotected and protected selenocystine containing peptides. Moreover, developed protocol allows the formation of phenylindeno[1,2-*c*]chromene moiety in 5-*endo-dig*/6-*endo-dig* cascade reactions.

Results and Discussion

Knowing that copper(II) bromide is a suitable promoter for the Sec electrophile generation, we continued our research in purpose to expand the application scope of this method in 5-*endo-dig* cyclization reactions. Initially we chose 2-(phenylethynyl)anisole (**1a**) and diphenyl diselenide as model substrates employing previously established optimal reaction conditions (Scheme 1). Copper(II) bromide (1.2 equiv.) was added to a solution of Ph_2Se_2 (1.0 equiv.) in CH_2Cl_2 followed by addition of anisole **1a** (1.2 equiv.) after 30 min of stirring. After 24 hours we were delighted to observe the formation of product **2a**, however, the diselenide still remained in the reaction mixture

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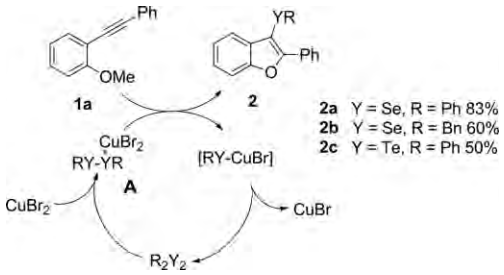
therefore we elevated the reaction temperature to 40 °C, which resulted in full consumption of the diselenide in 16 hours and the product **2a** was obtained in 83 % yield. Furthermore, the structure of **2a** was unambiguously confirmed by X-ray analysis (Figure 1, CCDC 1943954). Interestingly, that Kazmierczak et al., who developed method for synthesis of 3-selanyl benzo[*b*]furans via 5-*endo-dig* cyclization of 2-alkynyl phenols using diaryl diselenide and copper(I) iodide,[17] stated that the reaction of diphenyl diselenide and 2-(phenylethynyl)phenol in the presence of $CuBr_2$ in DMSO provides the product **2a** in only 9 % yield. They hypothesized that the halogen atom plays a crucial role. Probably, less polar solvent elongates the existence of diphenyl diselenide – copper(II) bromide adduct in the reaction mixture providing an increase of a product yield up to 83 %. Analogously, 3-chalcogenyl benzo[*b*]furans **2b** and **2c** were synthesized employing dibenzyl diselenide and diphenyl ditelluride. Previously **2b** and **2c** have been obtained only by Zeni et al. using $FeCl_3$ promoted cyclization of 2-phenylethynyl anisole.[18] Inspecting both methods, it could be concluded that in case of **2b** the yields are similar (60 % - $CuBr_2$ method, 64 % - $FeCl_3$). However, $CuBr_2$ promoted cyclization led to the formation of 2-phenyl-3-(phenyltellanyl)benzo[*b*]furan (**2c**) in 50 % yield which is a considerable improvement comparing to $FeCl_3$ promoted phenyltellanyl electrophile generation (36 %). Based on previous studies[25] we believe that electrophilic selenium species are formed via $CuBr_2$-diselenide adduct **A**.



Scheme 1. 3-Selanyl and 3-tellanyl benzo[*b*]furans formation: feasibility studies. *Reaction conditions*: **1a** (1.2 equiv.), R_2Y_2 (1.0 equiv.), $CuBr_2$ (1.2 equiv.), CH_2Cl_2, 40 °C.

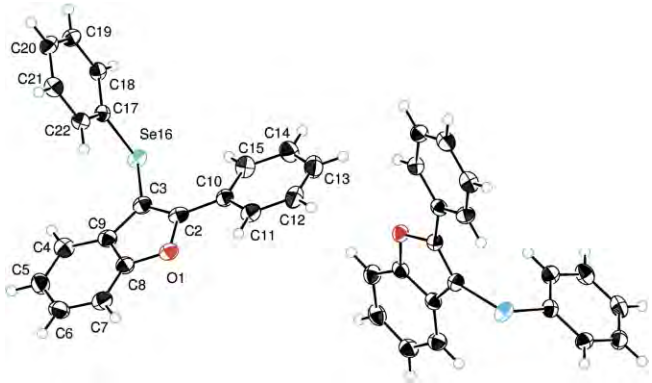
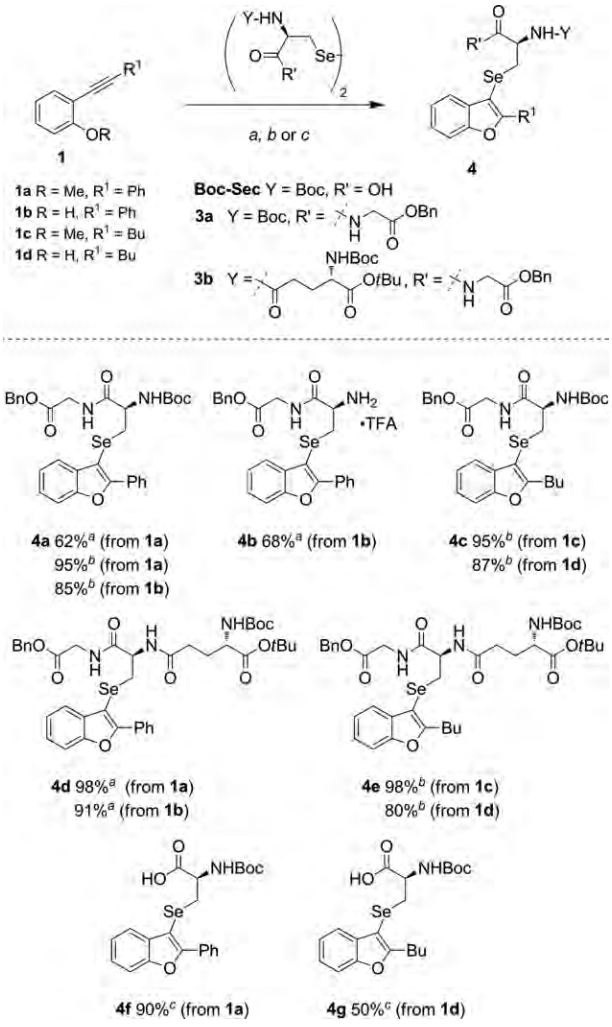


Figure 1. ORTEP molecular structure of **2a**.

Satisfied with the results, we turned our attention to preparation of Sec containing benzo[*b*]furans. Gratifyingly, generation of selenium electrophile from bis-dipeptide (Boc)Sec-Gly-OBn **3a** with $CuBr_2$ and reaction with anisole **1a** finalized with the isolation of **4a** in 62 % yield (Scheme 2). Similarly, 2-(phenylethynyl)phenol (**1b**) provided product **4b** in 68 % yield. Since dipeptide **3a** was convenient for the benzo[*b*]furan formation process, we decided to test more complicated system – *bis*-tripeptide selenoglutathione **3b** – a seleno-analogue of natural glutathione.[26] Under the same reaction conditions benzo[*b*]furan **4d** with selenoglutathione moiety was isolated in almost quantitative yield (98 % employing **1a**, 91 % - **1b**). Obviously, the complexity of the diselenide does not interfere with the reaction yield. However, treatment of 2-(hex-1-yn-1-yl)phenol (**1d**) with dipeptide **3a** in the presence of $CuBr_2$ led to the formation of a mixture of unidentified compounds probably due to the formation of aryl group stabilized vinyl cation thus preventing 5-*endo-dig* cyclization. The desired 2-butyl-3-selanylbenzo[*b*]furan **4c** formed in low yield forcing us to search for a different method for the generation of Sec electrophile. It is known that selenium electrophile can be generated from diselenides using inorganic and organic oxidants. The choice of reagent depends on the substrate and its functional groups.[27]



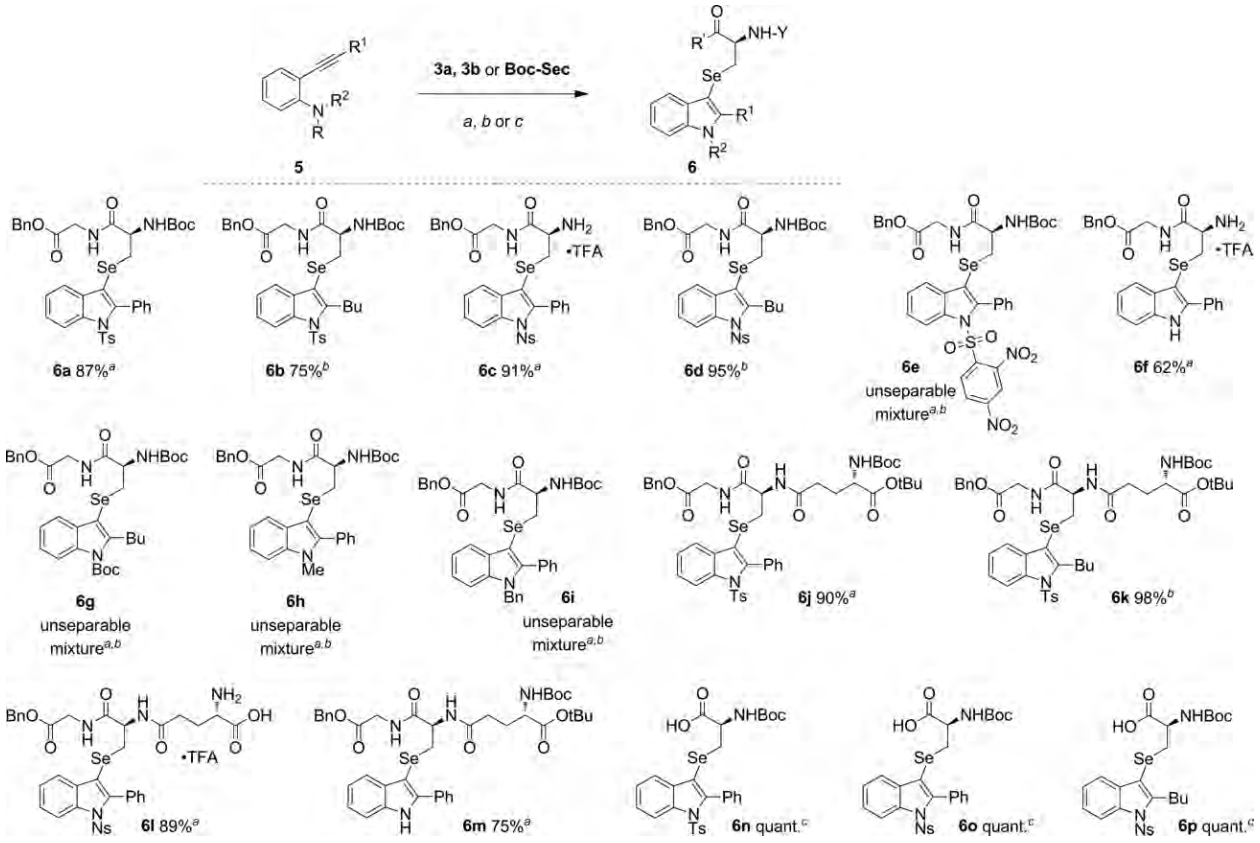
Scheme 2. 3-selanyl benzo[*b*]furan formation: scope and limitation studies. *Reaction conditions*: *a)* 1. $CuBr_2$ (1.5 equiv.), CH_2Cl_2, 40 °C; (2. TFA, CH_2Cl_2, 0 °C); *b)* $K_2S_2O_8$ (5 equiv.), MeCN, r.t.; *c*) $K_2S_2O_8$ (50 equiv.), MeCN, r.t.

We tested several of the most popular oxidation agents used for diselenide oxidation to see whether it is possible to employ oxidant for generation of Sec electrophile from Sec containing peptide and use it further in 5-*endo-dig* cyclization for benzo[*b*]furan ring formation. Among the most popular oxidants for selenium electrophile generation are persulfates producing strongly electrophilic alkyl or aryl selanyl sulfate which has been proposed by Tiecco et al.[28] and proven by Kumar et al.[29] based on ^{77}Se NMR data.[9,28–31] Interestingly, Kumar et al. emphasized that TFA is crucial for phenylselanyl electrophile generation in the presence of $K_2S_2O_8$. Probably, it is related with the fact that diphenyl diselenide reaction with persulfate is very slow, furthermore, the solubility of $K_2S_2O_8$ in organic solvents is low as well. Tiecco et al. also reported[31] that addition of TfOH to a mixture of Ph_2Se_2 and ammonium persulfate in acetonitrile resulted in completion of reaction in just several minutes. Although most likely that under these conditions the selanylating agent was a mixture of phenylselanyl sulfate and phenylselanyl triflate.[31] Unfortunately, the addition of TFA was prohibited since it would remove protecting groups from peptide. Furthermore, Hajra et al. in 2017 reported direct selanylation of arenofurans using $Na_2S_2O_8$ mediated oxidation of diaryl diselenide[9] without addition of any acid. Utilization of only 1.2 equivalents of oxidant was enough for reaction completion. Treating mixture of dipeptide **3a** and 2-(hexynyl)phenol (**1d**) in MeCN with 5 equiv. of $K_2S_2O_8$ resulted in selective, but slow formation of the product **4c**. Ammonium persulfate provided similar results, yet it was slightly less effective and required more time for full conversion of **3a**. In case of potassium iodate, the reaction was even slower than persulfates whereas oxone provided complex mixture of unidentified compounds and only traces of product. Unsatisfactory results were also obtained employing *m*-CPBA, $NaIO_4$, cerium ammonium nitrate and (diacetoxyiodo)benzene. Therefore, $K_2S_2O_8$ is the most suitable oxidant for 2-alkyl-3-selanylbenzo[*b*]furan formation. Next, the necessary quantity of oxidant for the reaction completion was studied (1, 2, 3 and 5 equiv. of $K_2S_2O_8$). According to experimental results, 5 equivalents of oxidant were required for full conversion of starting material in the shortest period (3 days). It should be noted that 5 equivalent excess of oxidant is acceptable for the further studies only because potassium persulfate is a very cheap inorganic reagent. The solvent change from MeCN to CH_2Cl_2 and temperature increase to 40 °C did not give any improvement, so we settled reaction conditions on more environmentally friendly solvent at room temperature. Next, we were focused on the scope and limitations studies. Reaction of **3a** with 2-(hex-1-yn-1-yl)phenol (**1d**) proceeded smoothly to yield 2-butyl-3-selanylbenzo[*b*]furan **4c** in very good yield (87 %). The use of 2-(hex-1-yn-1-yl)anisole (**1c**) resulted in even higher yield of **4c** (95 %). We also prepared previously obtained compound **4a** to compare the impact of alkyl and aryl substituents attached to the triple bond. Both substituents were equally suitable for the preparation of 2-substituted benzo[*b*]furans. It is worth mentioning that product **4a** was prepared in significantly higher yield than employing $CuBr_2$ mediated cyclization.

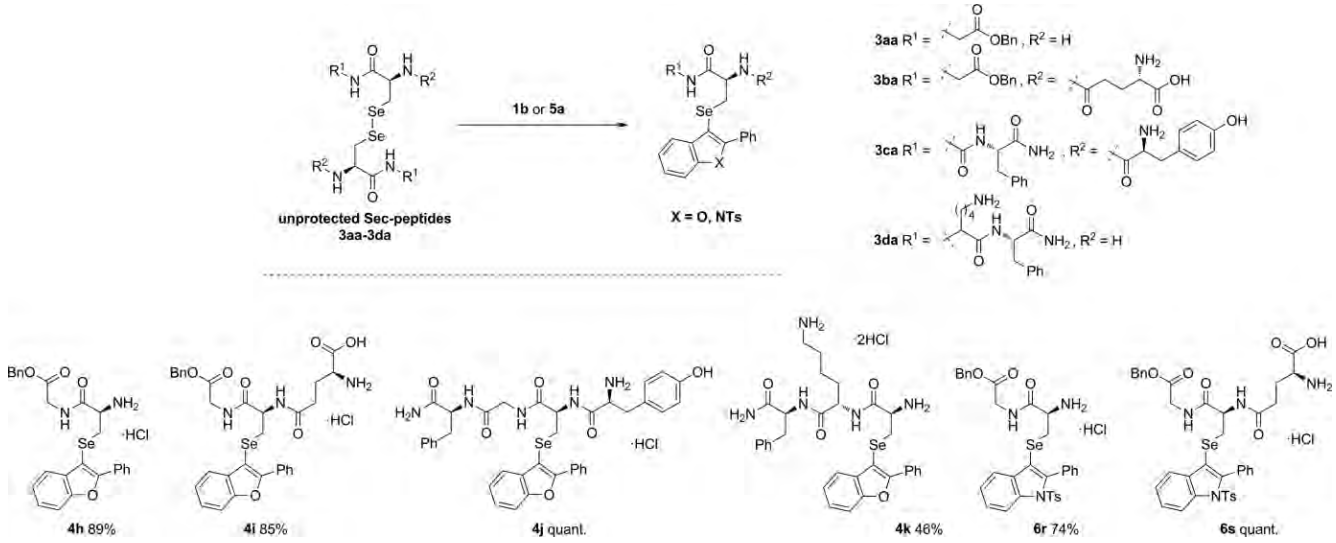
Notably, selenoglutathione-benzo[*b*]furan conjugate **4e** was obtained from phenol **1d** in 80 % yield whereas the use of anisole **1c** resulted in preparation of **4e** in almost quantitative yield (98 %). Unfortunately, neither $CuBr_2$, nor $K_2S_2O_8$ was suitable for the generation of respective sulfenyl electrophile from sulfur analogue of **3b** with consequent formation of glutathione containing benzo[*b*]furan. Treatment of Boc-Sec with **1a** in the presence of $CuBr_2$ or $K_2S_2O_8$ (5 equiv.) led only to traces of the desired product **4f** even after several days. However, increasing the amount of oxidant up to 50 equiv. led to selective formation of **4f** only in 16 hours providing the product in high yield. Product **4g** was obtained analogously, but in lower yield.

Elaborated reaction conditions using 2-ethynyl-substituted anilines were tested in purpose to obtain Sec-containing indoles. Previously, Zeni's group reported an elegant $FeCl_3$ promoted cyclization[19] of *o*-alkynyl anilines with diphenyl diselenide, unfortunately, this method led to a very low conversion of peptide **3a**. Similarly to benzo[*b*]furan formation, the treatment of (Boc)Sec-Gly-OBn **3a** with *N*-tosyl-2-(phenylethynyl)aniline **5a** in the presence of $CuBr_2$ in CH_2Cl_2 at room temperature was slow. However, elevation of the temperature to 40 °C led to full consumption of diselenide within 16 hours yielding **6a** in 87 % yield (Scheme 3). Under the optimal reaction conditions, substrate scope of alkynyl anilines was examined. Nosyl protected 2-(phenylethynyl)aniline **5c** showed even better result compared to tosyl aniline. Corresponding *N*-nosylindole **6c** was isolated in 91 % yield after treatment with TFA. Notably, 2,4-dinitro-benzenesulfonyl protection (**5e**) resulted in loss of reactivity, the reaction mixture showed only traces of product **6e**. Pleasingly, Boc protected 2-(phenylethynyl)aniline **5f** produced indole **6f** in 62 % yield after treatment with TFA. However, the developed method does not allow the use of unprotected 2-(phenylethynyl)aniline as a starting material: only a mixture of unidentified compounds was obtained. Additionally, *N*,*N*-dimethyl- and *N*,*N*-dibenzyl 2-(phenylethynyl)anilines **5h** and **5i** were tested with the aim to improve the substrate scope. In both cases complex mixture of unidentified compounds was obtained. The same was observed employing *N*-benzyl-2-(phenylethynyl)aniline **5j**. Under the optimal reaction conditions, we prepared selenoglutathione-indole conjugates **6j**, **6l**, **6m**. Again, we observed that the use of selenoglutathione **3b** showed superior yield (75–90 %) compared to (Boc)Sec-Gly-OBn **3a** probably due to steric hindrance of the Boc protecting group in **3a**. Treatment of peptide **3a** with 2-(hexynyl)aniline **5b** in the presence of $CuBr_2$ led to formation of a mixture of unidentified compounds showing only traces of product. Thus, an oxidant-promoted selenium electrophile generation was utilized for preparation of 2-alkyl-3-selanylindoles. Tosyl and nosyl anilines were well tolerated under the chosen reaction conditions providing corresponding 3-selanyl indoles in good yields (75–98 %). However, (2,4-dinitrophenylsulfonyl), Boc and Bn protected anilines were not suitable substrates, as well as dimethyl and dibenzyl anilines. Remarkably, selenocysteinyl indoles **6n–p** were obtained in quantitative yield employing 50 equiv. of $K_2S_2O_8$. Boc-Sec moiety containing benzo[*b*]furans and indoles are without doubt important building blocks that can be easily used for synthesis of more sophisticated structures.

Although both methods for Sec electrophile generation provided products in good yields and the substrate scope was



Scheme 3. Scope and limitation studies in synthesis of 3-selanylindoles. *Reaction conditions*: *a*) 1. $CuBr_2$ (1.5 equiv.), CH_2Cl_2, 40 °C; (2. TFA, CH_2Cl_2, 0 °C); *b*) $K_2S_2O_8$ (5 equiv.), MeCN, r.t.; *c*) $K_2S_2O_8$ (50 equiv.), MeCN, r.t. **5a** R^1 = Ph, R^2 = Ts, R = H; **5b** R^1 = Bu, R^2 = Ts, R = H; **5c** R^1 = Ph, R^2 = Ns, R = H; **5d** R^1 = Bu, R^2 = Ns, R = H; **5e** R^1 = Ph, R^2 = (2,4-dinitrophenylsulfonyl), R = H; **5f** R^1 = Ph, R^2 = Boc, R = H; **5g** R^1 = Bu, R^2 = Boc, R = H; **5h** R^1 = Ph, R^2 = R= Me; **5i** R^1 = Ph, R^2 = R = Bn; **5j** R^1 = Ph, R^2 = Bn, R = H.



Scheme 4. Preparation of benzo[*b*]furans and indoles employing unprotected Sec peptides. *Reaction conditions*: $CuBr_2$ (2.5 equiv.), CH_2Cl_2/MeCN, 40 °C.

quite broad, obviously the main limitation of this reaction to be used for more sophisticated peptides is the employment of protecting groups. Consequently, we decided to test the reaction between Ts-aniline **5a** and unprotected dipeptide Sec-Gly-OBn **3aa**, although we did not have high expectations. However, the reaction in the presence of $CuBr_2$ led to selective formation of corresponding 3-Sec-indole due to protonation of amino group that prevented the formation of complex with copper salt. Increase of temperature to 40 °C provided the product **6r** in very good yield (74 %) (Scheme 4). The product was

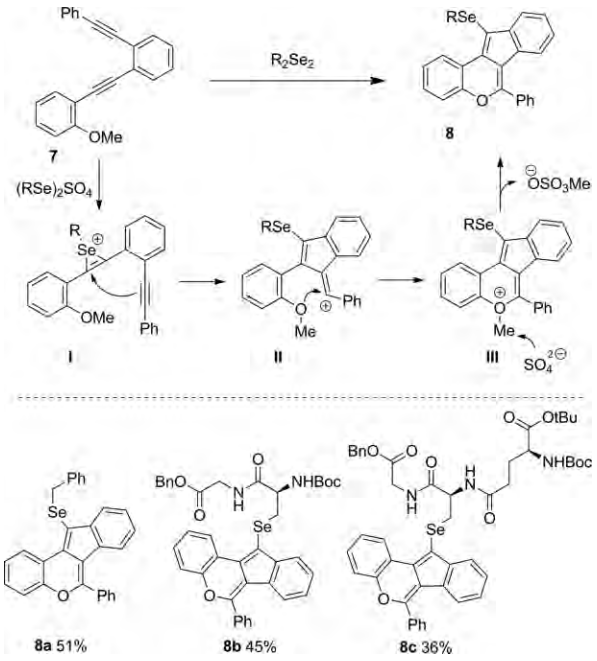
also formed employing $K_2S_2O_8$, however, with considerably lower yield and formation of side products.

Next, the substrate scope was determined, and we found out that only Ts-aniline **5a** and anisole **1a** were suitable substrates for 5-*endo-dig* cyclization. In both cases, the use of unprotected dipeptide **3aa** resulted in excellent yields, furthermore, they exceeded the ones obtained employing protected dipeptide **3a**. This observation encouraged us to test more complex Sec peptides. Unprotected selenoglutathione Glu-Sec-Gly-OBn **3ba** provided benzo[*b*]furan **4i** and indole **6s** in excellent yields (85 % and quantitative yield, correspondingly). Tetrapeptide dimer Tyr-Sec-Gly-Phe-NH_2 **3ca** that mimics the active site of glutathione peroxidase[32] (GPx-4) gave corresponding benzo[*b*]furan **4j** in quantitative yield. Tripeptide Sec-Lys-Phe-NH_2 **3da** provided benzo[*b*]furan **4k** in lower yield due to complications in purification process.

Next, we were interested to see whether it is possible to generate selenium electrophile and perform cascade reaction due to the attractive nature of such transformation. This route allows the formation of multiple bonds in a single step providing polycyclic structures. The main advantages of cascade reaction are atom economy, short reaction time and less waste supporting the basic principles of green chemistry. We chose to utilize anisole-containing aryldiyne **7** for the construction of indeno[1,2-*c*]chromene skeleton. Chromene moiety is often found in biologically active natural products, furthermore, compounds containing indeno[1,2-*c*]chromene core show high potential for use in dye-sensitized photovoltaic cells.[33,34] Notably, only few methods exist for the construction of indeno[1,2-*c*]chromene moiety. Previously, TfOH mediated cascade reaction of anisole **7** has been performed for the synthesis of 6-phenylindeno[1,2-*c*]chromene,[34] while halogen-mediated cascade reaction has been reported by Chen et al. for the synthesis of halogenated 6-phenylindeno[1,2-*c*]chromenes.[35]

Initially, we tested the reaction of Ph_2Se_2 with **7** in the presence of $CuBr_2$. However, only 11-bromo-6-phenylindeno[1,2-*c*]chromene was detected in the reaction mixture. Potassium persulfate induced electrophile generation lead only to the traces of the desired product even employing 50 equiv. of oxidant. Next, we decided to test Bn_2Se_2. Fortunately, the reaction of Bn_2Se_2 with anisole **7** in the presence of $K_2S_2O_8$ provided 11-(benzylselanyl)-6-phenylindeno[1,2-*c*]chromene (**8a**) (Scheme 5).

The structure of **8a** was unambiguously confirmed by X-ray analysis (Figure 2, CCDC 1949753). Similarly, the use of peptides **3a** and **3b** led to the formation of Sec containing 6-phenylindeno[1,2-*c*]chromenes as the major product. However, complicated purification of products **8b** and **8c** was responsible for the low yield of products. Unfortunately, Boc-Sec was not suitable substrate for this reaction. The plausible mechanism for this transformation includes following steps: selanyl electrophile coordinates to the more electron rich double bond forming selenirenium cation **I**. Next, the other triple bond attacks the selenirenium cation with the closure of indene cycle (intermediate **II**). Methoxy group then attacks the carbocation giving cyclization intermediate **III** that after demethylation provides the product **8**. Chen et al.[35] performed DFT studies to confirm



Scheme 5. Plausible mechanism for cascade 5-*endo*/6-*endo-dig* cyclization. *Reaction conditions*: $K_2S_2O_8$ (5 equiv.), MeCN, r.t.

the cyclization path for *o*-methoxy aryldiynes and stated that the pathway for *o*-amino, thio and carboxy substituents would be different due to the nucleophilic attack of heteroatom to the coordinated triple bond.

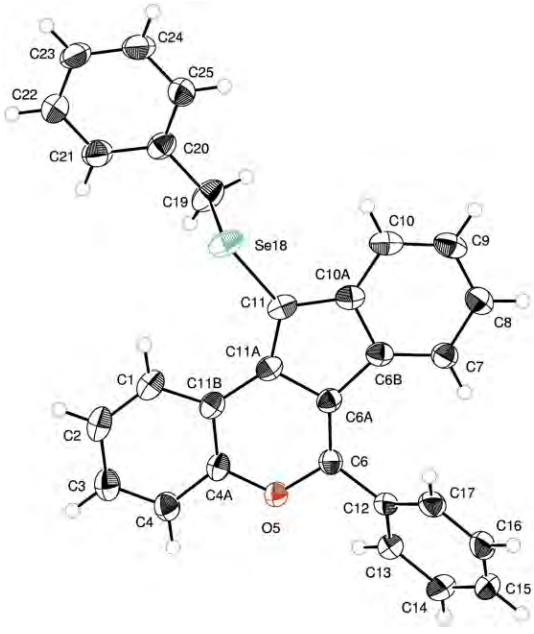


Figure 2. ORTEP molecular structure of **8a**.

Conclusions

Preparation of 2-aryl- as well as 2-alkyl-3-selanylbenzo[*b*]furans and indoles by ring closure via 5-*endo-dig* cyclization employing $CuBr_2$ and $K_2S_2O_8$ mediated generation of Sec electrophile

was presented. Copper(II) bromide is effective promoter for the generation of Sec electrophile from protected and unprotected peptides for the formation of 2-aryl-3-Sec-benzo[*b*]furans and indoles up to quantitative yields. However, utilization of $CuBr_2$ is not suitable for preparation of 2-alkyl-3-Sec-benzo[*b*]furans and indoles. Out of all tested oxidants, potassium persulfate is the top choice for the Sec electrophile generation to provide both 2-alkyl- and 2-aryl-3-Sec benzo[*b*]furans and indoles as well. Although the reaction is rather slow and more than equimolar amount of oxidant is required, the yields are excellent and superior to the ones obtained by $CuBr_2$ promoted reaction. Oxidant induced Sec electrophile generation tolerated anilines with Ts and Ns protecting groups whereas $CuBr_2$ promoted cyclization tolerated also Boc aniline. Moreover, based on optimized conditions construction of indeno[1,2-*c*]chromene skeleton is possible. As a result, the use of selenocystine containing peptides results in the formation of Sec containing 6-phenylindeno[1,2-*c*]chromenes. To sum up, the elaborated methods are efficient for the synthesis of 3-Sec-benzo[*b*]furans, indoles and indeno[1,2-*c*]chromenes employing mild conditions, tolerating broad substrate scope and providing products in good to excellent yields.

Experimental Section

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was performed using MERCK Silica gel 60 F254 plates and visualized by UV (254 nm) fluorescence. ZEOCHEM silica gel (ZEOprep 60/35–70 microns – SI23501) was used for column chromatography. ^{1}H, ^{13}C and ^{77}Se NMR[36] spectra were recorded on a Bruker Avance Neo spectrometer at 400, 101 and 76 MHz correspondingly at 298 K in CD_3OD or $CDCl_3$. Dimethyl selenide was used as a standard. Infrared (IR) spectra were recorded with a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). HRMS were recorded on Waters Synapt GII Q-ToF UPLC/MS system. Single crystals of **2a** and **8a** were investigated on a Rigaku XtaLAB Synergy, Dualflex, HyPix diffractometer. The crystal was kept at 140.0(1) K during data collection. Using Olex2,[36] the structure was solved with the olex2.solve[37] structure solution program using Charge Flipping and refined with the ShelXL[38] refinement package using Least Squares minimization.

Boc-L-selenocystine,[39] peptides **3a**,**b**,**c**,[25] 2-(1-alkynyl)anisoles **1a**,[40] **b**,[41] 2-(1-alkynyl)phenols **1c**,**d**,[42] 2-(1-alkynyl)anilines **5a–c**,[43] **f**,[44] **g**,[45] **h**,**i**,[46] **j**,[47] and **7**[34] were prepared according to literature procedures.

***N*-(2-(Hex-1-yn-1-yl)phenyl)-4-nitrobenzenesulfonamide (5d):** To a solution of 2-(hex-1-yn-1-yl)aniline (0.3 g, 1.73 mmol, 1 equiv.) in CH_2Cl_2 (7 mL) pyridine (0.28 mL, 3.46 mmol, 2 equiv.) and *p*-nitrobenzenesulfonyl chloride (0.48 g, 2.08 mmol, 1.2 equiv.) were added at 0 °C. The reaction mixture was stirred for 16 h at room temperature, then it was poured into ice water and extracted with CH_2Cl_2, washed with 2 M HCl and brine, dried with Na_2SO_4. After filtration and evaporation, the residue was purified by flash chromatography (PE/EtOAc, 10:1–3:1) to give the title compound **5d** (0.43 g, 69 %) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.24 (d, *J* = 8.9 Hz, 2H), 7.91 (d, *J* = 9.0 Hz, 2H), 7.60 (d, *J* = 7.9, 1H), 7.30–7.21 (m, 3H), 7.07 (td, *J* = 7.6, 1.2 Hz, 1H), 2.38 (t, *J* = 7.0 Hz, 2H), 1.60–1.51 (m, 2H), 1.49–1.37 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 150.4, 144.8, 136.4, 132.4, 129.2, 128.6, 125.6, 124.2, 120.9, 116.1, 98.4, 75.2, 30.7, 22.2, 19.3, 13.7. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{18}H_{19}N_2O_4S]^+$ 359.1066, found 359.1071.

2,4-Dinitro-*N*-(2-(phenylethynyl)phenyl)benzenesulfonamide (5e): To a solution of 2-(phenylethynyl)aniline (0.3 g, 1.55 mmol, 1 equiv.) in CH_2Cl_2 (7 mL) pyridine (0.25 mL, 3.10 mmol, 2 equiv.) and 2,4-dinitrobenzenesulfonyl chloride (0.51 g, 1.86 mmol, 1.2 equiv.) were added at 0 °C. The reaction mixture was stirred for 16 h at room temperature, then it was poured into ice water and extracted with CH_2Cl_2, washed with 2 M HCl and brine, dried with Na_2SO_4. After filtration and evaporation, the residue was purified by flash chromatography (PE/EtOAc, 10:1–3:1) to give the title compound **5e** (0.34 g, 52 %) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.30 (dd, *J* = 8.6, 2.2 Hz, 1H), 8.18 (d, *J* = 2.2 Hz, 1H), 8.05 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.62 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.38–7.24 (m, 6H), 7.18–7.12 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 149.9, 148.1, 138.9, 135.8, 133.0, 132.5, 131.5, 130.0, 129.6, 128.8, 127.0, 126.8, 124.2, 121.6, 121.0 117.2, 96.1, 83.6, 77.5, 77.2, 76.8. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{20}H_{14}N_3O_6S]^+$ 424.0603, found 424.0592.

Preparation of Sec-Peptide 3d

Benzyl *tert*-Butyl ((*S*)-6-(((*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (9): To a solution of (*S*)-2-amino-3-phenylpropanamide hydrochloride (0.79 g, 3.94 mmol, 1.5 equiv.) in DMF (5 mL) at 0 °C was added NMM (0.58 mL, 5.26 mmol, 2 equiv.) and the mixture was stirred for 5 minutes. Then to the reaction mixture was added a solution of N^6-((benzyloxy)carbonyl)-N^2-(*tert*-butoxycarbonyl)-L-lysine (1 g, 2.63 mmol, 1 equiv.) in DMF (5 mL), HOBt (0.402 g, 2.63 mmol, 1 equiv.) and EDC·HCl (1 g, 5.26 mmol, 2 equiv.). The reaction mixture was stirred at 0 °C for 10 minutes and at r.t. for 2 hours. After evaporation, the residue was purified by reverse phase chromatography (C-18, MeCN/H_2O + AcOH 10–85 %) to give the title compound **9** (1.2 g, 87 %) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.39–7.15 (m, 10H), 5.07 (s, 2H), 4.63 (dd, *J* = 8.9, 5.4 Hz, 1H), 3.84 (dd, *J* = 8.1, 5.8 Hz, 1H), 3.20 (dd, *J* = 13.9, 5.4 Hz, 1H), 3.06 (t, *J* = 6.9 Hz, 2H), 2.95 (dd, *J* = 13.8, 9.0 Hz, 1H), 1.58–1.47 (m, 2H), 1.46–1.34 (m, 11H), 1.30–1.12 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.9, 175.0, 158.9, 158.2, 138.5, 138.4, 130.4, 129.5, 128.9, 128.8, 127.8, 80.9, 67.3, 56.8, 55.2, 41.3, 38.4, 32.7, 32.4, 30.5, 28.7, 23.8, 23.7, 14.4. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{28}H_{39}N_4O_6]^+$ 527.2870, found 527.2868.

***tert*-Butyl (2-(Trimethylsilyl)ethyl) ((*S*)-6-(((*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (10):** To a solution of **9** (0.54 g, 1.02 mmol) in MeOH with few drops of AcOH was added Pd/C (0.11 g, 0.09 mmol) and H_2 was bubbled through the mixture for 1 hour. The product was filtered, evaporated and dissolved in a mixture of THF (2 mL) and saturated $NaHCO_3$ (2 mL) and Teoc-OSu (0.396 g, 1.53 mmol, 1.5 equiv.) was added. The resulting reaction mixture was stirred for 2 hours, followed by evaporation. Residue was extracted with EtOAc, and washed with brine yielding the title compound (0.4 g, 74 %) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.36–7.12 (m, 5H), 4.64 (dd, *J* = 8.8, 5.4 Hz, 1H), 4.18–4.07 (m, 2H), 3.86 (dd, *J* = 8.2, 5.7 Hz, 1H), 3.20 (dd, *J* = 13.9, 5.4 Hz, 1H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.96 (dd, *J* = 13.9, 8.9 Hz, 1H), 1.59–1.47 (m, 2H), 1.46–1.35 (m, 11H), 1.30–1.13 (m, 2H), 1.03–0.93 (m, 2H), 0.05 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.9, 175.0, 159.3, 158.2, 138.5, 130.4, 129.5, 127.8, 80.9, 63.7, 56.8, 55.2, 41.2, 38.4, 32.5, 30.6, 28.7, 23.9, 18.7, –1.4. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{26}H_{45}N_4O_6Si]^+$ 537.3108, found 537.3109.

(12*S*,15*S*)-16-Amino-15-benzyl-2,2-dimethyl-6,13,16-trioxo-5-oxa-7,14-diaza-2-silahexadecan-12-aminium 4-Methylbenzenesulfonate (11): To a solution of **10** (0.4 g, 0.75 mmol, 1 equiv.) in Et_2O (7 mL) *p*TsOH (0.156 g, 0.82 mmol, 1.1 equiv.) was added and the mixture was stirred until full dissolution and then it was evaporated and held at 40 °C for 4 hours. The residue was purified by reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) to give the title compound (220 mg, 51 %) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.72 (d, *J* = 8.2 Hz, 2H), 7.32–7.16 (m, 7H), 4.64 (dd, *J* = 8.8, 6.0 Hz, 1H), 4.12 (t, *J* = 8.4 Hz, 2H), 3.82 (t, *J* = 6.3 Hz, 1H), 3.14 (dd, *J* = 13.9, 6.0 Hz, 1H), 3.08 (t, *J* = 6.9 Hz, 2H), 2.97 (dd, *J* = 13.9, 9.0 Hz, 1H), 2.37 (s, 3H), 1.90–1.76 (m, 2H), 1.54–1.44 (m, 2H), 1.44–1.31 (m, 2H), 1.02–0.93 (m, 2H), 0.05 (s, 8H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.5, 170.1, 159.4, 143.5, 141.7, 138.3, 130.3, 129.8, 129.5, 127.9, 127.0, 63.8, 56.1, 54.2, 41.1, 38.8, 32.3, 30.5, 22.9, 21.3, 18.7, –1.5. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{21}H_{37}N_4O_4Si]^+$ 437.2584, found 437.2586.

Di-*tert*-Butyl (2-(Trimethylsilyl)ethyl) ((12*S*,15*R*,20*R*,23*S*)-23-(((*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl)carbamoyl)-12-(((*S*)-1-amino-1-oxo-3-phenylpropan-2-yl)carbamoyl)-2,2-dimethyl-6,14,21-trioxo-5-oxa-17,18-diselena-7,13,22-triaza-2-silaheptacosane-15,20,27-triyl)tricarbamate (3d): To a solution of **10** (200 mg, 0.33 mmol, 3 equiv.) in DMF (5 mL) NMM (0.05 mL, 0.45 mmol, 4 equiv.) was added and the mixture was stirred for 5 minutes at 0 °C. Then HOBt (34 mg, 0.22 mmol, 2 equiv.) was added followed by the addition of solution of Boc-L-selenocystine (60 mg, 0.11 mmol, 1 equiv.) in DMF (3 mL) and EDC•HCl (87 mg, 0.45 mmol, 4 equiv.). The reaction mixture was stirred for 10 minutes at 0 °C and additionally for 1 hour at r.t., then it was evaporated and the residue was purified by reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) to give the title compound (110 mg, 71 %) as light yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.33–7.14 (m, 5H), 4.67 (t, *J* = 7.1 Hz, 1H), 4.48–4.43 (m, 2H), 4.11 (t, *J* = 8.3 Hz, 2H), 3.30–3.24 (m, 1H), 3.13 (dd, *J* = 14.0, 6.5 Hz, 1H), 3.04 (t, *J* = 6.9 Hz, 2H), 2.96 (dd, *J* = 13.7, 8.3 Hz, 1H), 1.77–1.51 (m, 2H), 1.50–1.37 (m, 11H), 1.39–1.22 (m, 2H), 1.03–0.92 (m, 2H), 0.04 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.8, 173.8, 173.3, 159.2, 157.6, 138.3, 130.4, 129.5, 127.8, 80.9, 63.7, 56.2, 55.7, 54.9, 41.4, 39.1, 33.5, 33.0, 30.5, 28.8, 23.9, 18.7, –1.4. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{58}H_{97}N_{10}O_{14}Se_2Si_2]^+$ 1373.5055, found 1373.5100.

General Procedure for $CuBr_2$ Promoted Cyclization of Diorganyl Dichalcogenide and 2-(Phenylethynyl)anisole (1a): $CuBr_2$ (1.2 equiv.) was added to a solution of diorganyl dichalcogenide (1 equiv.) in CH_2Cl_2 and the mixture was stirred for 30 min at r.t. Then a solution of **1a** (1.2 equiv.) in CH_2Cl_2 was added. Reaction mixture was stirred for 16 h at 40 °C, and then it was evaporated and purified by flash chromatography (petroleum ether/ethyl acetate, 10:0–4:1) to give **2a**–**c**.

2-Phenyl-3-(phenylselanyl)benzofuran (2a):[18] Colorless crystals (104 mg, 83 %). Prepared from Ph_2Se_2 (112 mg, 0.36 mmol), $CuBr_2$ (97 mg, 0.43 mmol), and **1a** (90 mg, 0.43 mmol). Crystallized from petroleum ether/ethyl acetate. Melting point: 86–87 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.22–8.16 (m, 2H), 7.56–7.10 (m, 12H).

3-(Benzylselanyl)-2-phenylbenzofuran (2b):[18] Yellow oil (64 mg, 60 %). Prepared from Bn_2Se_2 (123 mg, 0.36 mmol), $CuBr_2$ (97 mg, 0.43 mmol), and **1a** (90 mg, 0.43 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.11–8.02 (m, 2H), 7.60–7.56 (m, 1H), 7.52 (m, 1H), 7.44–7.24 (m, 5H), 7.16–7.02 (m, 5H), 3.98 (s, 2H).

2-Phenyl-3-(phenyltellanyl)benzofuran (2c):[18] Yellow oil (72 mg, 50 %). Prepared from Ph_2Te_2 (148 mg, 0.36 mmol), $CuBr_2$ (97 mg, 0.43 mmol), and **1a** (90 mg, 0.43 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.15–8.10 (m, 2H), 7.57–7.52 (m, 2H), 7.50–7.42 (m, 5H), 7.38–7.30 (m, 2H), 7.18–7.07 (m, 3H).

General Procedure for Preparation of 3-Selanyl Benzo[*b*]furans 4a–e and Indoles 6a–m

Method A: To a solution of Sec-peptide **3** (1 equiv.) in CH_2Cl_2 $CuBr_2$ (1.5 equiv.) was added and the mixture was stirred for 30 min at r.t. Then a solution of 2-(1-alkynyl)anisole/phenol/aniline (2 equiv.) in CH_2Cl_2 was added. Reaction mixture was stirred for 16 h at 40 °C, and then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) to give the product.

Method B: To a solution of peptide **3a** or **3b** (1 equiv.) and 2-(1-alkynyl)aniline/phenol/anisole (2 equiv.) in MeCN $K_2S_2O_8$ (5 equiv.) was added and the mixture was stirred for 3 days at r.t. After evaporation the mixture was purified by reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) to give the product.

Boc and *t*Bu Cleavage*:* To a solution of protected peptide in CH_2Cl_2 TFA at 0 °C was added. Reaction mixture was stirred until disappearance of starting material (1–3 hours). After evaporation the mixture was purified by reverse phase chromatography (C-18, MeCN/H_2O 10–80 %).

Benzyl (*R*)-(2-((*tert*-Butoxycarbonyl)amino)-3-((2-phenylbenzofuran-3-yl)selanyl)propanoyl)glycinate (4a): White solid. Prepared by method A in 62 % yield from **3a** (100 mg, 0.12 mmol), $CuBr_2$ (40 mg, 0.18 mmol), alkyne **1a** (50 mg, 0.24 mmol), CH_2Cl_2 (5 mL). Prepared by method B from **3a** (100 mg, 0.12 mmol), $K_2S_2O_8$ (165 mg, 0.61 mmol), alkyne **1a** 50 mg, 0.24 mmol) or alkyne **1b** (47 mg, 0.24 mmol, MeCN (4 mL) in 95 % (70 mg) or 85 % (63 mg) yield, correspondingly. NMR spectra presented in SI are from product obtained by method B using alkyne **1a**. $[\alpha]_D^{20}$ –9.0 (c 0.96, $CHCl_3$). IR ν_{max} (film): $\tilde{\nu}$ = 3064, 2978, 2933, 1751, 1686, 1517, 1253, 1178. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.21–8.10 (m, 2H), 7.61–7.52 (m, 1H), 7.45–7.32 (m, 3H), 7.32–7.13 (m, 8H), 6.64 (s, 1H), 5.14 (d, *J* = 8.2 Hz, 1H), 5.04 (s, 2H), 4.25 (s, 1H), 3.77 (qt, *J* = 18.3, 2.7 Hz, 2H), 3.12 (m, 1H), 2.99 (dd, *J* = 12.6, 5.7 Hz, 1H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7, 169.3, 156.1, 155.4, 153.9, 135.2, 132.0, 130.2, 129.3, 128.7, 128.6, 128.4, 127.8, 125.3, 123.5, 120.8, 111.3, 100.0, 80.5, 67.2, 54.3, 41.4, 29.7, 28.2. ^{77}Se (76 MHz, $CDCl_3$) δ = 68.1. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{31}H_{33}N_2O_6Se]^+$ 609.1504, found 609.1501.

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenylbenzo-furan-3-yl)selanyl)propan-2-aminium Trifluoroacetate (4b): Yellow solid (50 mg, 68 %). Prepared by method A from **3a** (100 mg, 0.12 mmol), $CuBr_2$ (40 mg, 0.18 mmol), alkyne **1b** (71 mg, 0.24 mmol), CH_2Cl_2 (5 mL). Product was isolated after Boc deprotection. $[\alpha]_D^{20}$ +5.2 (c 1.2, MeOH). IR ν_{max} (film): $\tilde{\nu}$ = 3064, 3031, 2934, 1748, 1674, 1668, 1662, 1512, 1192, 1189. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.30–8.22 (m, 2H), 7.75–7.68 (m, 1H), 7.60–7.48 (m, 2H), 7.46–7.37 (m, 1H), 7.37–7.28 (m, 7H), 5.12 (s, 2H), 3.76 (s, 2H), 3.41 (dd, *J* = 7.3, 5.2 Hz, 1H), 3.12 (dd, *J* = 12.3, 5.2 Hz, 1H), 2.98 (dd, *J* = 12.3, 7.3 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.8, 170.9, 157.5, 155.3, 137.1, 133.4, 131.6, 130.3, 129.6, 129.5, 129.4, 128.8, 126.5, 124.6, 122.0, 112.1, 101.0, 67.9, 55.9, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 41.9, 34.3. ^{77}Se NMR (76 MHz, CD_3OD) δ = 59.5. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{26}H_{25}N_2O_4Se]^+$ 509.0980, found 509.0989.

Benzyl (*R*)-(2-Amino-3-((2-butylbenzofuran-3-yl)selanyl)propanoyl)glycinate (4c): White solid. Prepared by method B from **3a** (100 mg, 0.12 mmol), $K_2S_2O_8$ (165 mg, 0.61 mmol), alkyne **1c** (45 mg, 0.24 mmol) or alkyne **1d** (42 mg, 0.24 mmol, MeCN (4 mL) in 95 % (67 mg) and 87 % (62 mg) yield, correspondingly. $[\alpha]_D^{20}$ –23.0 (c 1.1, $CHCl_3$). IR ν_{max} (film): $\tilde{\nu}$ = 3322, 2958, 2930, 1752, 1685,

1523, 1452, 1367, 1251, 1171. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.58–7.53 (m, 1H), 7.46–7.40 (m, 1H), 7.37–7.18 (m, 7H), 5.13 (s, 2H), 4.14 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.89 (d, *J* = 5.7 Hz, 2H), 3.11 (dd, *J* = 12.8, 4.5 Hz, 1H), 2.99 (td, *J* = 7.4, 5.4 Hz, 2H), 2.90–2.77 (m, 1H), 1.72 (q, *J* = 7.6 Hz, 2H), 1.43 (s, 8H), 1.40–1.26 (m, 4H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.9, 170.8, 164.3, 157.5, 155.8, 137.1, 132.2, 129.5, 129.3, 125.2, 124.2, 121.1, 111.8, 100.8, 80.9, 67.9, 56.1, 42.1, 31.6, 30.3, 28.7, 27.9, 23.3, 14.2. ^{77}Se (76 MHz, CD_3OD) δ = 58.2. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{29}H_{37}N_2O_6Se]^+$ 589.1817, found 589.1813.

***tert*-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenylbenzofuran-3-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (4d):** Yellow solid. Prepared by method A from **3b** (100 mg, 0.083 mmol), $CuBr_2$ (28 mg, 0.12 mmol), alkyne **1c** (35 mg, 0.167 mmol) or alkyne **1d** (49 mg, 0.167 mmol), CH_2Cl_2 (5 mL) in 98 % (65 mg) or 91 % (60 mg) yield, correspondingly. IR ν_{max} (film): $\tilde{\nu}$ = 3295, 3069, 2978, 2933, 1734, 1653, 1646, 1517, 1455, 1368, 1253, 1154. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.29–8.22 (m, 2H), 7.71–7.65 (m, 1H), 7.57–7.24 (m, 12H), 5.10 (s, 2H), 4.43 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.92 (dd, *J* = 9.4, 4.7 Hz, 1H), 3.81 (d, *J* = 2.4 Hz, 2H), 3.25 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.97 (dd, *J* = 12.6, 9.1 Hz, 1H), 2.24–2.11 (m, 1H), 2.08–1.87 (m, 2H), 1.81–1.67 (m, 1H), 1.54–1.37 (m, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.6, 173.3, 173.0, 170.7, 158.1, 157.5, 155.3, 137.1, 133.4, 131.6, 130.3, 129.6, 129.5, 129.3, 128.9, 126.4, 124.6, 122.0, 112.1, 100.8, 82.8, 80.5, 67.9, 55.4, 54.8, 49.4, 42.1, 32.8, 29.8, 28.8, 28.3. ^{77}Se NMR (76 MHz, CD_3OD) δ = 82.1. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{40}H_{49}N_3O_9Se]^+$ 794.2556, found 794.2559.

***tert*-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((2-butylbenzofuran-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (4e):** Yellow oil. Prepared by method A from **3b** (100 mg, 0.083 mmol), $CuBr_2$ (28 mg, 0.12 mmol), alkyne **1c** (32 mg, 0.17 mmol) or alkyne **1d** (29 mg, 0.17 mmol), CH_2Cl_2 (5 mL) in 98 % (63 mg) or 80 % (51 mg) yield, correspondingly. IR ν_{max} (film): $\tilde{\nu}$ = 3295, 2977, 2932, 1718, 1646, 1529, 1452, 1367, 1250, 1155. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.56–7.51 (m, 1H), 7.44–7.40 (m, 1H), 7.36–7.21 (m, 7H), 5.12 (s, 2H), 4.42 (dd, *J* = 9.4, 4.8 Hz, 1H), 3.98 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.87 (d, *J* = 1.2 Hz, 2H), 3.13 (dd, *J* = 12.7, 4.8 Hz, 1H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.86 (dd, *J* = 12.7, 9.5 Hz, 1H), 2.35–2.20 (m, 2H), 2.10–2.00 (m, 1H), 1.88–1.78 (m, 1H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.51–1.34 (m, 22H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 173.3, 170.8, 164.3, 155.8, 137.1, 132.2, 129.6, 129.34, 129.31, 128.3, 128.0, 125.3, 124.2, 121.2, 111.8, 82.8, 80.6, 67.9, 65.2, 55.4, 55.0, 42.1, 41.8, 33.1, 31.6, 28.8, 28.5, 28.3, 27.9, 23.4, 14.2. ^{77}Se (76 MHz, CD_3OD) δ = 62.9. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd. for $[C_{38}H_{51}N_3O_9SeNa]^+$ 796.2688, found 796.2698.

Benzyl (*R*)-(2-((*tert*-Butoxycarbonyl)amino)-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)selanyl)propanoyl)glycinate (6a): Yellow solid (40 mg, 87 %). Prepared by method A from **3a** (50 mg, 0.06 mmol), $CuBr_2$ (20.2 mg, 0.09 mmol), alkyne **5a** (41.9 mg, 0.12 mmol), CH_2Cl_2 (5 mL). $[\alpha]_D^{20}$ –13.1 (c 1.1, MeOH). IR ν_{max} (film): $\tilde{\nu}$ = 2982, 2931, 1669, 1448, 1369, 1177, 1091. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.28 (d, *J* = 8.3 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.49–7.24 (m, 13H), 7.15 (d, *J* = 8.2 Hz, 2H), 5.10 (s, 2H), 4.05–3.95 (m, 1H), 3.76 (s, 2H), 2.83 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.69 (dd, *J* = 12.5, 8.2 Hz, 1H), 2.27 (s, 3H), 1.42–22 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.4, 170.6, 157.1, 146.8, 145.3, 138.4, 137.1, 136.4, 133.6, 133.2, 132.6, 130.7, 130.1, 129.5, 129.32, 129.29, 128.3, 127.9, 126.6, 125.6, 122.2, 117.0, 111.1, 80.9, 67.9, 55.9, 42.0, 30.0, 28.7, 21.5. ^{77}Se NMR (76 MHz, CD_3OD) δ = 86.3. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{38}H_{40}N_3O_7SSe]^+$ 762.1752, found 762.1741.

Benzyl (*R*)-(2-((*tert*-Butoxycarbonyl)amino)-3-((2-butyl-1-tosyl-1*H*-indol-3-yl)selanyl)propanoyl)glycinate (6b): Yellow oil (67 mg, 75 %). Prepared by method B from **3a** (100 mg, 0.12 mmol), $K_2S_2O_8$ (165 mg, 0.61 mmol), alkyne **5b** (80 mg, 0.24 mmol), MeCN (5 mL). $[\alpha]_D^{20}$ –10.2 (c 1.12, $CHCl_3$). IR ν_{max} (film): $\tilde{\nu}$ = 2959, 2931, 1750, 1686, 1455, 1368, 1173. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.09–8.03 (m, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.58–7.53 (m, 1H), 7.35–7.19 (m, 9H), 5.11 (s, 2H), 4.00 (dd, *J* = 10.0, 4.4 Hz, 1H), 3.86 (s, 2H), 3.36–3.32 (m, 2H), 3.08 (dd, *J* = 12.6, 4.5 Hz, 1H), 2.76 (dd, *J* = 12.6, 9.9 Hz, 1H), 2.24 (s, 3H), 1.75–1.55 (m, 2H), 1.51–1.28 (m, 12H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.9, 170.7, 157.5, 148.0, 146.6, 138.1 137.0, 136.7, 133.4, 131.1, 129.5, 129.31, 129.26, 127.4, 125.7, 125.2, 121.5, 116.1, 108.8, 80.9, 67.9, 55.7, 42.1, 34.8, 30.3, 29.3, 28.8, 23.7, 21.5, 14.3. ^{77}Se (76 MHz, CD_3OD) δ = 68.6. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd. for $[C_{36}H_{43}N_3O_7SeSNa]^+$ 764.1885, found 764.1893.

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((1-((4-nitrophenyl)-sulfonyl)-2-phenyl-1*H*-indol-3-yl)selanyl)-1-oxopropan-2-aminium 2,2,2-Trifluoroacetate (6c): Yellow oil (87 mg, 78 %). Prepared by method A from **3a** (80 mg, 0.1 mmol), $CuBr_2$ (32.3 mg, 0.16 mmol), alkyne **5c** (73 mg, 0.19 mmol), CH_2Cl_2 (5 mL). Product was isolated after Boc deprotection. $[\alpha]_D^{20}$ –4.2 (c 1.2, MeOH). IR ν_{max} (film): $\tilde{\nu}$ = 3109, 3028, 1748, 1683, 1532, 1349, 1184, 1088. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.29 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 8.9 Hz, 2H), 7.73–7.63 (m, 3H), 7.54–7.37 (m, 7H), 7.32 (s, 5H), 5.12 (s, 2H), 3.70 (d, *J* = 2.6 Hz, 2H), 3.20–3.02 (m, 1H), 2.88 (dd, *J* = 12.2, 4.5 Hz, 1H), 2.73–2.57 (m, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 170.8, 152.3, 145.3, 143.8, 138.3, 137.1, 133.8, 133.2, 132.3, 130.4, 129.6, 129.39, 129.36, 128.6, 127.2, 126.3, 125.4, 122.5, 117.0, 112.3, 67.9, 41.9. ^{77}Se NMR (76 MHz, $CDCl_3$) δ = 332.5. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{32}H_{29}N_4O_7SSe]^+$ 693.0922, found 693.0934.

Benzyl (*R*)-(2-((*tert*-Butoxycarbonyl)amino)-3-((2-butyl-1-((4-nitrophenyl)sulfonyl)-1*H*-indol-3-yl)selanyl)propanoyl)glycinate (6d): Yellow oil (90 mg, 95 %). Prepared by method B from **3a** (100 mg, 0.12 mmol), $K_2S_2O_8$ (165 mg, 0.61 mmol), alkyne **5d** (80 mg, 0.24 mmol), MeCN (5 mL). $[\alpha]_D^{20}$ +5.1 (c 1.06, $CHCl_3$). IR ν_{max} (film): $\tilde{\nu}$ = 2960, 2931, 1749, 1684, 1539, 1179. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.21 (d, *J* = 8.8 Hz, 2H), 8.07 (d, *J* = 7.9 Hz, 1H), 7.96–7.91 (m, 2H), 7.54 (d, *J* = 7.1 Hz, 1H), 7.34–7.22 (m, 8H), 5.11 (s, 2H), 3.85 (s, 2H), 3.75 (dd, *J* = 10.4, 4.2 Hz, 1H), 3.38–3.32 (m, 2H), 3.06 (dd, *J* = 12.8, 4.2 Hz, 1H), 2.68 (dd, *J* = 12.8, 10.4 Hz, 1H), 1.82–1.70 (m, 1H), 1.67–1.58 (m, 1H), 1.51–1.33 (m, 12H), 1.33–1.25 (m, 1H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.7, 170.7, 157.6, 152.2, 148.2, 143.9, 138.1, 137.0, 133.5, 129.5, 129.27, 129.26, 129.0, 126.3, 126.0, 125.8, 121.9, 116.3, 110.3, 80.9, 67.9, 55.5, 42.1, 34.7, 29.9, 29.5, 28.8, 23.6, 14.3. ^{77}Se (76 MHz, CD_3OD) δ = 74.9. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd. for $[C_{35}H_{40}N_4O_9SeSNa]^+$ 795.1579, found 795.1579.

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1*H*-indol-3-yl)selanyl)propan-2-aminium 2,2,2-Trifluoroacetate (6f): Yellow oil (45 mg, 62 %). Prepared by method A from **3a** (100 mg, 0.12 mmol), $CuBr_2$ (40.4 mg, 0.18 mmol), alkyne **5f** (71 mg, 0.24 mmol), CH_2Cl_2 (5 mL). Product was isolated after Boc deprotection. $[\alpha]_D^{20}$ +6.3 (c 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.31–8.23 (m, 2H), 7.75–7.65 (m, 1H), 7.59–7.27 (m, 11H), 5.12 (s, 2H), 3.76 (s, 2H), 3.45–3.36 (m, 1H), 3.12 (dd, *J* = 12.2, 5.0 Hz, 1H), 2.98 (dd, *J* = 12.3, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 170.9, 157.4, 155.3, 137.1, 133.4, 131.6, 130.3, 129.6, 129.5, 129.3, 128.8, 126.4, 124.6, 122.0, 112.1, 101.0, 67.9, 42.0. ^{77}Se NMR (76 MHz, CD_3OD) δ = 59.5. ESI-MS *m/z*: 508.20 $[M + H]^+$. Elemental analysis calculated for $C_{26}H_{25}N_3O_3Se$•TFA•1.3H_2O: C, 52.30; H, 4.48; N, 6.53; found C, 52.39; H, 4.57; N, 6.38.

***tert*-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (6j):** Yellow oil (76 mg, 90 %). Prepared by method A from **3b** (100 mg, 0.082 mmol), $CuBr_2$ (27.8 mg, 0.13 mmol), alkyne **5a** (87 mg, 0.24 mmol), CH_2Cl_2 (5 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3306, 2 978, 2933, 1739, 1654, 1517, 1368, 1178, 1090. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.28 (d, *J* = 8.3 Hz, 1H), 7.64 (d, *J* = 7.7, 1H), 7.50–7.27 (m, 16H), 7.16 (d, *J* = 8.1 Hz, 2H), 5.11 (s, 2H), 4.23–4.11 (m, 1H), 3.98–3.90 (m, 1H), 3.76 (d, *J* = 3.8 Hz, 2H), 2.91 (dd, *J* = 12.5, 5.4 Hz, 1H), 2.65 (dd, *J* = 12.5, 8.8 Hz, 1H), 2.29 (s, 3H), 2.20–2.09 (m, 1H), 2.09–1.98 (m, 1H), 1.98–1.91 (m, 1H), 1.80–1.70 (m, 1H), 1.46 (m, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 173.3, 172.9, 170.7, 158.1, 146.8, 145.6, 138.5, 137.1, 136.3, 133.6, 133.3, 132.6, 130.7, 130.1, 129.6, 129.34, 129.31, 128.3, 127.9, 126.7, 125.7, 122.2, 117.0, 110.9, 82.8, 80.5, 67.9, 55.4, 54.6, 49.4, 42.0, 32.8, 29.2, 28.8, 28.3, 21.5. ^{77}Se (76 MHz, CD_3OD) δ = 89.4. HRMS (ESI/Q-TOF) *m*/*z*: [M + H]$^+$ calcd. for $[C_{47}H_{55}N_4O_{10}SSe]^+$ 947.2804, found 947.2789.

***tert*-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((2-butyl-1-tosyl-1*H*-indol-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (6k):** Yellow oil (37 mg, 98 %). Prepared by method B from **3b** (50 mg, 0.04 mmol), $K_2S_2O_8$ (56 mg, 0.21 mmol), alkyne **5b** (27 mg, 0.08 mmol), MeCN (4 mL). IR ν_{max} (film): $\tilde{\nu}$ = 2971, 2931, 1662, 1457, 1369, 1172. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.09–8.05 (m, 1H), 7.67–7.61 (m, 2H), 7.59–7.53 (m, 1H), 7.37–7.23 (m, 10H), 5.12 (s, 2H), 4.37–4.28 (m, 1H), 4.04–3.98 (m, 1H), 3.87 (s, 2H), 3.10 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.80 (dd, *J* = 12.6, 9.7 Hz, 1H), 2.34–2.23 (m, 5H), 2.13–2.02 (m, 1H), 1.96–1.84 (m, 1H), 1.75–1.57 (m, 2H), 1.51–1.37 (m, 20H), 1.32–1.25 (m, 1H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 173.3, 173.1, 170.7, 158.1, 147.9, 146.7, 138.1, 137.1, 136.8, 133.4, 131.1, 129.6, 129.34, 129.29, 127.5, 125.8, 125.3, 121.5, 116.2, 108.9, 82.8, 80.6, 67.9, 55.4, 54.5, 42.1, 34.7, 33.1, 29.4, 28.8, 28.3, 23.7, 21.5, 14.3. ^{77}Se (76 MHz, CD_3OD) δ = 71.4. HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd. for $[C_{45}H_{58}N_4O_{10}SeSNa]^+$ 949.2937, found 949.2926.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((1-((4-nitrophenyl)sulfonyl)-2-phenyl-1H-indol-3-yl)selanyl)-1-oxopropan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium Trifluoroacetate (6l): Yellow solid (30 mg, 89 %). Prepared by method A from **3b** (50 mg, 0.042 mmol), $CuBr_2$ (14.0 mg, 0.06 mmol), alkyne **5c** (39.4 mg, 0.10 mmol), CH_2Cl_2 (5 mL). Product was isolated after Boc deprotection. IR ν_{max} (film): $\tilde{\nu}$ = 3062, 3028, 2932, 1744, 1653, 1532, 1349, 1184. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.32 (d, *J* = 8.1 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 2H), 7.67 (dd, *J* = 8.0, 3.7 Hz, 3H), 7.59–7.25 (m, 12H), 5.14 (s, 2H), 4.06 (d, *J* = 9.3 Hz, 1H), 3.79 (s, 2H), 3.55 (s, 1H), 3.03 (dd, *J* = 12.6, 4.4 Hz, 1H), 2.72–2.60 (m, 1H), 2.35 (s, 2H). Due to low solubility of **6l** only ^{1}H NMR spectra was acquired. HRMS (ESI/Q-TOF) *m*/*z*: [M + H]$^+$ calcd. for $[C_{37}H_{36}N_5O_{10}SSe]^+$ 822.1348, found 822.1346.

***tert*-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1*H*-indol-3-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-l-glutaminate (6m):** Yellow oil (49 mg, 75 %). Prepared by method A from **1c** (100 mg, 0.083 mmol), $CuBr_2$ (28.4 mg, 0.124 mmol), alkyne **5f** (49 mg, 0.167 mmol), CH_2Cl_2 (5 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3295, 2977, 2934, 1743, 1645, 1534, 1455, 1367, 1253, 1175, 1172, 1154. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.30–8.21 (m, 2H), 7.67 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.57–7.46 (m, 3H), 7.45–7.39 (m, 1H), 7.39–7.26 (m, 7H), 5.10 (s, 2H), 4.43 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.93 (d, *J* = 4.7 Hz, 1H), 3.80 (d, *J* = 2.5 Hz, 2H), 3.24 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.97 (dd, *J* = 12.6, 9.1 Hz, 1H), 2.24–2.13 (m, 1H), 2.08–1.89 (m, 2H), 1.80–1.67 (m, 1H), 1.45 (d, *J* = 8.0 Hz, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.6, 173.3, 173.0, 170.7, 158.1, 157.5, 155.3, 137.1, 133.4, 131.6, 130.3, 129.6, 129.5, 129.33, 129.30, 128.9, 126.4, 124.6, 122.0, 112.1, 100.8, 82.8, 80.5, 67.9, 55.4, 54.8, 42.4, 32.8, 29.8, 28.8, 28.3. ^{77}Se NMR (76 MHz, CD_3OD) δ = 82.0. HRMS (ESI/Q-TOF) *m*/*z*: [M + H]$^+$ calcd. for $[C_{40}H_{49}N_4O_8Se]^+$ 793.2716, found 794.2537.

General Procedure for Preparation of Boc-Sec Containing Benzo[*b*]furans 4f,g and Indoles 6n–p: To a solution of Boc-Sec (100 mg, 0.187 mmol, 1 equiv.) and 2-(1-alkynyl)aniline/phenol/anisole (0.28 mmol, 1.5 equiv.) in MeCN (10 mL) $K_2S_2O_8$ (2.53 g, 9.36 mmol, 50 equiv.) was added and the mixture was stirred for 16 hours at r.t. After filtration and evaporation, the mixture was purified by reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) to give the product.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((2-phenylbenzofuran-3-yl)selanyl)propanoic Acid (4f): White solid (76 mg, 88 %). Prepared from alkyne **1a** (58 mg). $[\alpha]_D^{20}$ +24.2 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.31–8.25 (m, 2H), 7.70–7.65 (m, 1H), 7.54–7.44 (m, 3H), 7.43–7.37 (m, 1H), 7.37–7.28 (m, 2H), 4.25 (dd, *J* = 8.0, 4.4 Hz, 1H), 3.34–3.26 (m, 1H, overlaps with CD_3OD signal), 3.08 (dd, *J* = 12.5, 8.0 Hz, 1H), 1.37–1.17 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.0, 157.3, 157.2, 155.3, 133.4, 131.6, 130.2, 129.5, 128.7, 126.3, 124.5, 121.9, 112.1, 100.8, 80.6, 55.3, 30.1, 28.6. ^{77}Se (76 MHz, CD_3OD) δ = 83.9. HRMS (ESI/Q-TOF) *m*/*z*: [M – H]$^-$ calcd. for $[C_{22}H_{22}NO_5Se]^-$ 460.0663, found 460.0677.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((2-butylbenzofuran-3-yl)-selanyl)propanoic Acid (4g): Light yellow oil (40 mg, 50 %). Prepared from alkyne **1d** (46 mg, 0.24 mmol). $[\alpha]_D^{20}$ +14.1 (c 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.59–7.53 (m, 1H), 7.45–7.39 (m, 1H), 7.29–7.22 (m, 2H), 4.19 (dd, *J* = 8.4, 4.4 Hz, 1H), 3.21 (dd, *J* = 12.6, 4.4 Hz, 1H), 3.03–2.88 (m, 3H), 1.73 (p, *J* = 7.4 Hz, 2H), 1.49–1.25 (m, 11H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.1, 164.2, 157.5, 155.8, 132.1, 125.2, 124.2, 121.1, 111.8, 100.7, 80.6, 55.3, 31.6, 29.6, 28.6, 27.9, 23.3, 22.1, 14.2. ^{77}Se (76 MHz, CD_3OD) δ = 59.0. HRMS (ESI/Q-TOF) *m*/*z*: [M – H]$^-$ calcd. for $[C_{20}H_{26}NO_5Se]^-$ 440.0976, found 440.0982.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)selanyl)propanoic Acid (6n): Light yellow solid (115 mg, quant.). Prepared from alkyne **5a** (97 mg). $[\alpha]_D^{20}$ +12.7 (c 0.93, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.28 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.51–7.26 (m, 9H), 7.15 (d, *J* = 8.1 Hz, 2H), 4.09–4.01 (m, 1H), 2.95 (dd, *J* = 12.6, 4.5 Hz, 1H), 2.76 (dd, *J* = 12.6, 7.6 Hz, 1H), 2.29 (s, 3H), 1.39–1.20 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.7, 157.1, 146.7, 145.3, 138.6, 136.3, 133.6, 133.1, 132.6, 130.6, 130.0, 128.3, 127.8, 126.6, 125.7, 122.1, 117.1, 111.2, 80.7, 55.1, 29.4, 28.6, 21.5. ^{77}Se (76 MHz, CD_3OD) δ = 91.3. HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd. for $[C_{29}H_{30}N_2O_6SSeNa]^+$ 637.0887, found 637.0909.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-((4-nitrophenyl)sulfonyl)-2-phenyl-1*H*-indol-3-yl)selanyl)propanoic Acid (6o): Yellow solid (120 mg, quant.). Prepared from alkyne **5c** (106 mg). $[\alpha]_D^{20}$ +12.6 (c 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3CN) δ = 8.25 (dt, *J* = 8.4, 0.9 Hz, 1H), 8.21–8.10 (m, 2H), 7.73–7.60 (m, 3H), 7.60–7.39 (m, 7H), 5.25 (d, *J* = 7.9 Hz, 1H), 4.09–3.96 (m, 1H), 2.99 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.83 (dd, *J* = 12.8, 7.3 Hz, 1H), 1.39–1.15 (m, 9H). ^{13}C NMR (101 MHz, CD_3CN) δ = 172.1, 155.9, 152.0, 144.7, 143.2, 137.8, 133.2, 132.8, 132.0, 130.3, 129.1, 128.5, 127.2, 126.2, 125.5, 122.2, 116.9, 112.1, 80.3, 54.5, 29.1, 28.4. ^{77}Se (76 MHz, CD_3CN) δ = 91.3. HRMS (ESI/Q-TOF) *m*/*z*: [M – H]$^-$ calcd. for $[C_{28}H_{26}N_3O_8SSe]^-$ 644.0606, found 644.0645.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((2-butyl-1-((4-nitrophenyl)sulfonyl)-1*H*-indol-3-yl)selanyl)propanoic Acid (6p): Yellow oil (117 mg, quant.). Prepared from alkyne **5d** (100 mg). $[\alpha]_D^{20}$

28.8 (c 0.97, $CHCl_3$). ^1H NMR (400 MHz, CD_3OD) δ = 8.31–8.25 (m, 2H), 8.12–8.05 (m, 1H), 8.01–7.93 (m, 2H), 7.60–7.55 (m, 1H), 7.36–7.25 (m, 2H), 3.78 (dd, *J* = 10.0, 4.1 Hz, 1H), 3.36 (t, *J* = 7.9 Hz, 2H), 3.09 (dd, *J* = 12.7, 4.1 Hz, 1H), 2.75 (dd, *J* = 12.7, 10.0 Hz, 1H), 1.83–1.72 (m, 1H), 1.69–1.58 (m, 1H), 1.50–1.36 (m, 10H), 1.27–1.20 (m, 1H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.4, 157.6, 152.2, 148.4, 144.0, 138.1, 133.6, 129.0, 126.4, 126.0, 125.8, 121.8, 116.4, 110.2, 80.5, 54.4, 34.7, 29.4, 29.3, 28.8, 23.6, 14.2. ^{77}Se (76 MHz, CD_3OD) δ = 78.5. HRMS (ESI/Q-TOF) *m/z*: $[M - H]^-$ calcd. for $[C_{26}H_{30}N_3O_8SSe]^-$ 624.0919, found 624.0943.

General Procedure for Preparation of 3-Selanyl Benzo[*b*]furans 4h–k and Indoles 6r,s: To a solution of Sec-peptide **3a-d** in CH_2Cl_2 TFA was added at 0 °C. Reaction mixture was stirred until disappearance of starting material. After evaporation the peptides **3aa**–**3da** were used further without additional purification. To a solution of unprotected Sec-peptide (1 equiv.) in CH_2Cl_2/MeCN (and MeOH in the case of peptides **3ca** and **3da**) $CuBr_2$ (2.5 equiv.) was added and the mixture was stirred for 30 min at r.t. followed by addition of a solution of **1a** or **5a** (1.5 equiv.) in CH_2Cl_2. Reaction mixture was stirred for 16 h at 40 °C, and then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O+HCl 10–85 %) to give the desired product.

Benzyl (*R*)-(2-Amino-3-((2-phenylbenzofuran-3-yl)selanyl)propanoyl)glycinate (4h): White solid (53 mg, 89 %). Prepared from **3a** (90 mg, 0.11 mmol), $CuBr_2$ (61 mg, 0.27 mmol), alkyne **1a** (34 mg, 0.16 mmol), CH_2Cl_2 (4 mL), MeCN (1 mL). ^1H NMR (400 MHz, CD_3OD) δ = 8.26–8.19 (m, 2H), 7.77–7.69 (m, 1H), 7.60–7.26 (m, 11H), 5.11 (s, 2H), 4.04 (t, *J* = 6.2 Hz, 1H), 3.73 (d, *J* = 17.8 Hz, 1H), 3.51 (d, *J* = 17.8 Hz, 1H), 3.28–3.21 (m, 2H).

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenylbenzofuran-3-yl)selanyl)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium Chloride (4i): White solid (47 mg, 85 %). Prepared from **3b** (100 mg, 0.083 mmol), $CuBr_2$ (47 mg, 0.21 mmol), alkyne **1a** (26 mg, 0.12 mmol), CH_2Cl_2 (4 mL), MeCN (1 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3280, 3064, 2936, 1743, 1653, 1539, 1200. ^1H NMR (400 MHz, CD_3OD) δ = 8.28–8.22 (m, 2H), 7.69–7.64 (m, 1H), 7.56–7.27 (m, 11H), 5.10 (s, 2H), 4.39 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.93 (t, *J* = 6.4 Hz, 1H), 3.80 (s, 2H), 3.25 (dd, *J* = 12.7, 5.1 Hz, 1H), 2.96 (dd, *J* = 12.7, 9.3 Hz, 1H), 2.46–2.34 (m, 1H), 2.31–2.19 (m, 1H), 2.12–1.94 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.1, 173.0, 171.4, 170.8, 157.6, 155.3, 137.0, 133.4, 131.6, 130.4, 129.6, 129.59, 129.55, 129.4, 129.32, 129.31, 128.9, 126.5, 124.6, 122.0, 112.2, 100.6, 67.9, 55.0, 53.5, 42.0, 32.3, 29.5, 26.9. ^{77}Se (76 MHz, CD_3OD) δ = 83.4. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{31}H_{32}N_3O_7Se]^+$ 638.1405, found 638.1409.

(*S*)-1-(((*R*)-1-((2-(((*S*)-1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-3-((2-phenylbenzofuran-3-yl)selanyl)propan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-aminium chloride (4j): White solid (50 mg, 100 %). Prepared from (Boc)Tyr-Sec-Gly-Phe-NH_2 (83 mg, 0.066 mmol), $CuBr_2$ (44 mg, 0.19 mmol), alkyne **1a** (20 mg, 0.098 mmol), CH_2Cl_2 (2 mL), MeCN (1 mL), MeOH (2 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3269, 3032, 1652, 1506, 1214. ^1H NMR (400 MHz, CD_3OD) δ = 8.25–8.19 (m, 2H), 7.73–7.67 (m, 1H), 7.58–7.53 (m, 1H), 7.51–7.45 (m, 2H), 7.43–7.29 (m, 3H), 7.27–7.21 (m, 4H), 7.19–7.13 (m, 1H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 2H), 4.59 (dd, *J* = 9.0, 5.5 Hz, 1H), 4.39 (t, *J* = 7.2 Hz, 1H), 3.89 (dd, *J* = 8.4, 5.6 Hz, 1H), 3.79 (d, *J* = 16.9 Hz, 1H), 3.38 (d, *J* = 16.9 Hz, 1H), 3.29–3.24 (m, 1H), 3.18–3.02 (m, 3H), 2.91 (dd, *J* = 13.9, 9.1 Hz, 1H), 2.82 (dd, *J* = 14.4, 8.7 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 176.1, 171.7, 170.7, 169.9, 158.3, 157.4, 155.4, 138.4, 133.3, 131.6, 131.5, 130.4, 130.3, 129.6, 129.5, 128.9, 127.8, 126.5, 125.8, 124.6, 122.0, 116.9, 112.2, 101.1, 55.84, 55.78, 55.6, 43.4, 39.0, 37.6, 29.1. ^{77}Se (76 MHz, CD_3OD) δ = 83.8. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{37}H_{38}N_5O_6Se]^+$ 728.1987, found 728.1984.

(*S*)-6-(((*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl)amino)-5-((*R*)-2-ammonio-3-((2-phenylbenzofuran-3-yl)selanyl)propanamido)-6-oxohexan-1-aminium Dichloride (4k): White solid (25 mg, 46 %). Prepared from **3d** (100 mg, 0.076 mmol), $CuBr_2$ (42 mg, 0.19 mmol), alkyne **1a** (24 mg, 0.11 mmol), CH_2Cl_2 (2 mL), MeCN (1 mL), MeOH (2 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3441, 1646, 1521, 1180, 1142. ^1H NMR (400 MHz, CD_3OD) δ = 8.29–8.20 (m, 2H), 7.81–7.72 (m, 1H), 7.61–7.50 (m, 3H), 7.49–7.43 (m, 1H), 7.42–7.34 (m, 2H), 7.24–7.19 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 2H), 7.08–7.01 (m, 1H), 4.60 (dd, *J* = 8.7, 5.8 Hz, 1H), 4.31 (t, *J* = 6.9 Hz, 1H), 4.02 (dd, *J* = 7.8, 5.5 Hz, 1H), 3.24–3.05 (m, 3H), 3.00–2.84 (m, 3H), 1.80–1.69 (m, 1H), 1.67–1.58 (m, 3H), 1.44–1.30 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ = 175.8, 172.7, 168.2, 158.1, 155.4, 138.2, 133.0, 131.3, 130.7, 130.3, 129.8, 129.4, 129.0, 127.7, 126.7, 124.9, 121.9, 112.3, 100.2, 55.7, 54.7, 54.2, 40.4, 39.0, 32.5, 29.1, 28.0, 23.3. ^{77}Se (76 MHz, CD_3OD) δ = 67.9. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{32}H_{38}N_5O_4Se]^+$ 636.2089, found 636.2079.

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)selanyl)propan-2-aminium Chloride (6r): White solid (56 mg, 74 %). Prepared from **3a** (90 mg, 0.11 mmol), $CuBr_2$ (61 mg, 0.27 mmol), alkyne **1a** (57 mg, 0.16 mmol), CH_2Cl_2 (4 mL), MeCN (1 mL).). $[\alpha]_D^{20}$ +23.5 (c 1, $CHCl_3$). IR ν_{max} (film): $\tilde{\nu}$ = 1738, 1669, 1369, 1175. ^1H NMR (400 MHz, CD_3OD) δ = 8.31 (d, *J* = 8.3 Hz, 1H), 7.75–7.67 (m, 1H), 7.52–7.31 (m, 15H), 7.18 (d, *J* = 8.2 Hz, 2H), 5.13 (d, *J* = 2.9 Hz, 2H), 3.76 (t, *J* = 6.4 Hz, 1H), 3.67 (d, *J* = 17.8 Hz, 1H), 3.48 (d, *J* = 17.8 Hz, 1H), 2.90 (dd, *J* = 6.4, 1.5 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 170.3, 168.4, 147.0, 145.8, 138.3, 137.0, 136.3, 133.3, 133.1, 132.3, 130.8, 130.4, 129.6, 129.45, 129.42, 128.6, 127.9, 126.9, 125.8, 122.1, 116.9, 109.8, 68.1, 54.0, 41.9, 28.1, 21.5. ^{77}Se (76 MHz, CD_3OD) δ = 74.8. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{33}H_{32}N_3O_5SSe]^+$ 662.1228, found 662.1223.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)selanyl)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium Chloride (6s): White solid (69 mg, 100 %). Prepared from **3b** (100 mg, 0.083 mmol), $CuBr_2$ (47 mg, 0.28 mmol), alkyne **1a** (26 mg, 0.12 mmol), CH_2Cl_2 (4 mL), MeCN (1 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3376, 3050, 1652, 1517, 1176. ^1H NMR (400 MHz, CD_3OD) δ = 8.29 (dt, *J* = 8.4, 0.9 Hz, 1H), 7.63 (ddd, *J* = 7.7, 1.4, 0.7 Hz, 1H), 7.49–7.27 (m, 14H), 7.20–7.14 (m, 2H), 5.11 (s, 2H), 4.10 (dd, *J* = 9.1, 5.3 Hz, 1H), 3.95 (t, *J* = 6.4 Hz, 1H), 3.75 (s, 2H), 2.92 (dd, *J* = 12.7, 5.3 Hz, 1H), 2.63 (dd, *J* = 12.7, 9.1 Hz, 1H), 2.41–2.33 (m, 1H), 2.30 (s, 3H), 2.29–2.21 (m, 1H), 2.08 (p, *J* = 7.5, 6.9 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.0, 172.9, 171.5, 170.7, 146.9, 145.7, 138.5, 137.1, 136.3, 133.5, 133.31, 132.6, 130.7, 130.1, 129.6, 129.4, 129.3, 128.3, 127.9, 126.7, 125.7, 122.1, 117.1, 110.6, 67.9, 54.8, 42.0, 32.2, 28.9, 26.9, 21.5.^{77}Se (76 MHz, CD_3OD) δ = 90.7. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{31}H_{32}N_3O_7Se]^+$ 638.1405, found 638.1409.

General Procedure for Preparation of 11-Selanyl-6-phenylindeno[1,2-*c*]chromenes: To a solution of diorganyl diselenide (1 equiv.) and alkyne (1 equiv.) in MeCN $K_2S_2O_8$ (5 equiv.) was added and the mixture was stirred for 3 days at r.t. After volatiles evaporation residue was purified by flash chromatography on silica gel (PE/EtOAc, 10:1–6:1) (**8a**) or reverse phase chromatography (C-18, MeCN/H_2O 10–85 %) (**8b,c**) to give the product.

11-(Benzylselanyl)-6-phenylindeno[1,2-*c*]chromene (8a): Orange crystals (57 mg, 51 %). Prepared from dibenzyl diselenide (83 mg, 0.24 mmol), $K_2S_2O_8$ (326 mg, 1.21 mmol), alkyne **7** (75 mg,

0.24 mmol), MeCN (5 mL). Crystallized from *i*PrOH. Melting point: 84–85 °C. ^{1}H NMR (400 MHz, CD_3OD) δ = 9.48 (dd, J = 8.0, 1.4 Hz, 1H), 7.90–7.83 (m, 3H), 7.67–7.60 (m, 3H), 7.53–7.41 (m, 4H), 7.37 (ddd, J = 8.0, 7.0, 1.4 Hz, 1H), 7.19–7.05 (m, 6H), 4.05 (s, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ = 152.9, 150.4, 145.5, 139.1, 133.7, 131.6, 130.9, 129.8, 129.0, 128.9, 128.5, 128.4, 127.1, 126.8, 125.8, 124.6, 123.2, 121.4, 120.6, 120.5, 118.0, 117.7, 110.6, 32.4.^{77}Se NMR (76 MHz, CD_3OD) δ = 191.5. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{29}H_{21}OSe]^+$ 465.0758, found 465.0729.

(*R*)-Benzyl 2-(2-((*tert*-Butoxycarbonyl)amino)-3-((6-phenylindeno[1,2-*c*]chromen-11-yl)selanyl)propanamido)acetate (8b): Orange oil (77 mg, 45 %). Prepared from **3a** (200 mg, 0.24 mmol), $K_2S_2O_8$ (326 mg, 1.21 mmol), alkyne **7** (75 mg, 0.24 mmol), MeCN (7 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3341, 2926, 1748, 1684, 1653, 1456, 1214, 755. $[\alpha]_D^{20}$ –7.26 (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ = 9.56 (dd, J = 7.6, 2.0 Hz, 1H), 7.85–7.74 (m, 3H), 7.72–7.62 (m, 3H), 7.56–7.43 (m, 3H), 7.39 (ddd, J = 7.9, 7.2, 1.1 Hz, 1H), 7.33–7.26 (m, 6H), 7.05 (ddd, J = 8.1, 7.3, 1.1 Hz, 1H), 5.10 (s, 2H), 4.27–4.17 (m, 1H), 3.81 (s, 2H), 3.23 (dd, J = 12.7, 4.7 Hz, 1H), 3.14–3.05 (m, 1H), 1.29 (s, 10H). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.8, 170.7, 154.2, 151.8, 146.3, 137.1, 134.8, 132.3, 132.0, 131.0, 130.6, 130.0, 129.9, 129.5, 129.3, 128.2, 126.8, 126.0, 124.2, 122.4, 121.43, 121.38, 118.9, 118.8, 116.8, 80.8, 67.9, 56.5, 42.1, 28.6. ^{77}Se NMR (76 MHz, CD_3OD) δ = 91.7. HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd. for $[C_{39}H_{37}N_2O_6Se]^+$ 709.1817, found 709.1807.

(6*S*,11*R*)-Benzyl 6-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4,9,12-trioxo-11-(((6-phenylindeno[1,2-*c*]chromen-11-yl)selanyl)methyl)-3-oxa-5,10,13-triazapentadecan-15-oate (8c): Orange oil (52 mg, 36 %). Prepared from **3b** (194 mg, 0.16 mmol), $K_2S_2O_8$ (219 mg, 0.81 mmol), alkyne **7** (50 mg, 0.16 mmol), MeCN (7 mL). IR ν_{max} (film): $\tilde{\nu}$ = 3307, 2978, 1701, 1636, 1455, 1152. ^{1}H NMR (400 MHz, CD_3OD) δ = 9.51 (dd, J = 7.9, 1.7 Hz, 1H), 7.80 (m, 3H), 7.76–7.62 (m, 3H), 7.62–7.23 (m, 11H), 7.05 (m, 1H), 5.09 (s, 2H), 4.45 (dd, J = 9.0, 4.8 Hz, 1H), 3.89 (dd, J = 9.4, 4.9 Hz, 1H), 3.78 (d, J = 2.4 Hz, 2H), 3.28 (m, 1H), 3.05 (dd, J = 12.7, 9.2 Hz, 1H), 2.18–2.05 (m, 1H), 2.05–1.82 (m, 2H), 1.76–1.64 (m, = 1H), 1.43 and 1.42 (2 s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 173.2, 170.7, 158.0, 154.3, 151.7, 146.3, 137.1, 134.8, 132.4, 132.0, 131.0, 130.7, 130.0, 129.9, 129.5, 129.31, 129.29, 128.2, 126.8, 125.9, 124.3, 122.4, 121.5, 121.3, 118.9, 110.0, 82.7, 80.5, 67.9, 55.4, 55.3, 42.1, 32.9, 30.8, 29.9, 28.8, 28.3, 28.2. ^{77}Se NMR (76 MHz, CD_3OD) δ = 96.2. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd. for $[C_{48}H_{51}N_3O_9SeNa]^+$ 916.2688, found 916.2653.

CCDC 1943954 (for **2a**), and 1949753 (for **8a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

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Keywords: Diselenide · Peptides · Selenocysteine · 5-*endo*-dig · 6-*endo*-dig

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Straightforward functionalization of sulfur-containing peptides via 5- and 6-*endo-dig* cyclization reactions.

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Synthesis S. Lapcinska, P. Arsenyan Paper

Straightforward Functionalization of Sulfur-Containing Peptides via 5- and 6-*endo-dig* Cyclization Reactions

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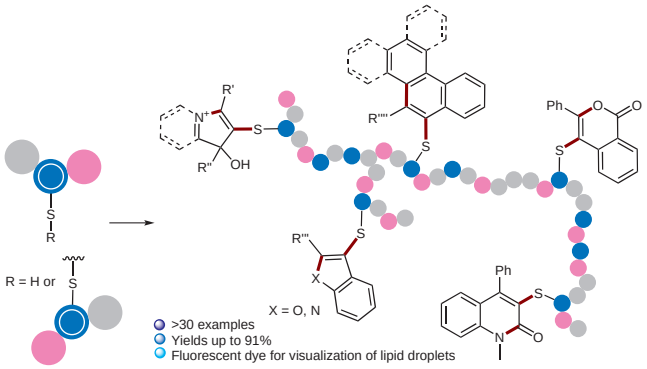

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Abstract We present a simple and convenient method for the generation of sulfenyl electrophiles from peptides containing S–S or S–H bonds by employing *N*-chlorosuccinimide. The corresponding sulfenyl electrophiles are further utilized in 5- and 6-*endo-dig* cyclization reactions yielding indolizinium salts, indoles, benzo[*b*]furans, polyaromatic hydrocarbons (PAHs) and isocoumarins, as well as quinolinones bearing a glutathione moiety. PAH derivatives can be used as selective fluorescent dyes for the visualization of lipid droplets in living cells.

Key words benzofurans, cysteine, 5-*endo-dig*, 6-*endo-dig*, indoles, lipid droplets, peptides, polyaromatic hydrocarbons

Short peptides[1–3] are a unique class of molecules that have promising properties for drug discovery; furthermore, a significant number of marketed drugs are already peptide-based compounds.[4–6] As intrinsic signaling molecules for many physiological functions, peptides present an opportunity for therapeutic intervention that closely mimics natural pathways.[7,8] Peptides containing sulfur atoms exhibit unique properties,[9] whilst the existence of a disulfide bond in peptides can improve their pharmacological properties, stability, activity and selectivity.[9,10] With the aim of overcoming some problems associated with peptides, e.g., low solubility, oral bioavailability and membrane permeability,[10] peptide and small molecule conjugates have been designed.[6,11,12] Conjugation is an attractive mechanism for enhancing the properties of peptides.[8] Notably, 30% of peptides that have entered clinical development in the last decade are conjugates, and the search for novel peptide-based pharmaceuticals continues.[8] Essentially, the conjugation protocol requires high tolerance of different functional groups, compatibility with various solvents, including water, and the reaction should provide products in short reaction times and in high yields.

A list of methods exist for the modification of cysteine motifs in proteins.[13,14] The sulfhydryl group in cysteine can be easily alkylated,[14] arylated[15,16] and used in Michael addition reactions.[13] Methods for cysteine stapling with different aryl linkers have been demonstrated[17–21] as well as thiol-ene/yne reactions that are usually radical-mediated.[22–24] Another widespread modification of sulfhydryl groups is oxidation reactions forming a disulfide bond.[13,14] Respectively, two thiol groups within a peptide can form an intramolecular disulfide bridge.[13] However, the above-mentioned methods mostly rely on the high nucleophilicity of the sulfhydryl group, although a sulfur atom can be converted into an electrophilic species as well. Very often sulfenyl halides serve as electrophilic sulfenyl species that can be easily prepared from thiols or disulfides using halogens,[25,26] $SOCl_2$,[27] SO_2Cl_2[28] or *N*-halosuccinimides.[29–31] Metal salts can also be used to generate sulfenyl electrophiles: $FeCl_3$ has been used to induce electrophile formation from thiols[32] or disulfides;[33,34] another iron salt, FeF_3,[35] has been employed in the presence of a catalytic amount of iodine; $CuBr_2$[36] and $AlCl_3$[37] can be used as well. Furthermore, phenyl iododiacetate induces the formation of electrophilic sulfur species in the presence of KI.[38] Pre-made *N*-thioalkyl or aryl phthalimides[39] or *N*-thiosuccinimides[40] can also serve as effective electrophiles. Recently we have reported methods[41,42] for the generation of selenium electrophiles from selenocystine-containing peptides by employing a mild Lewis acid and an inorganic oxidant. Herein, we report a

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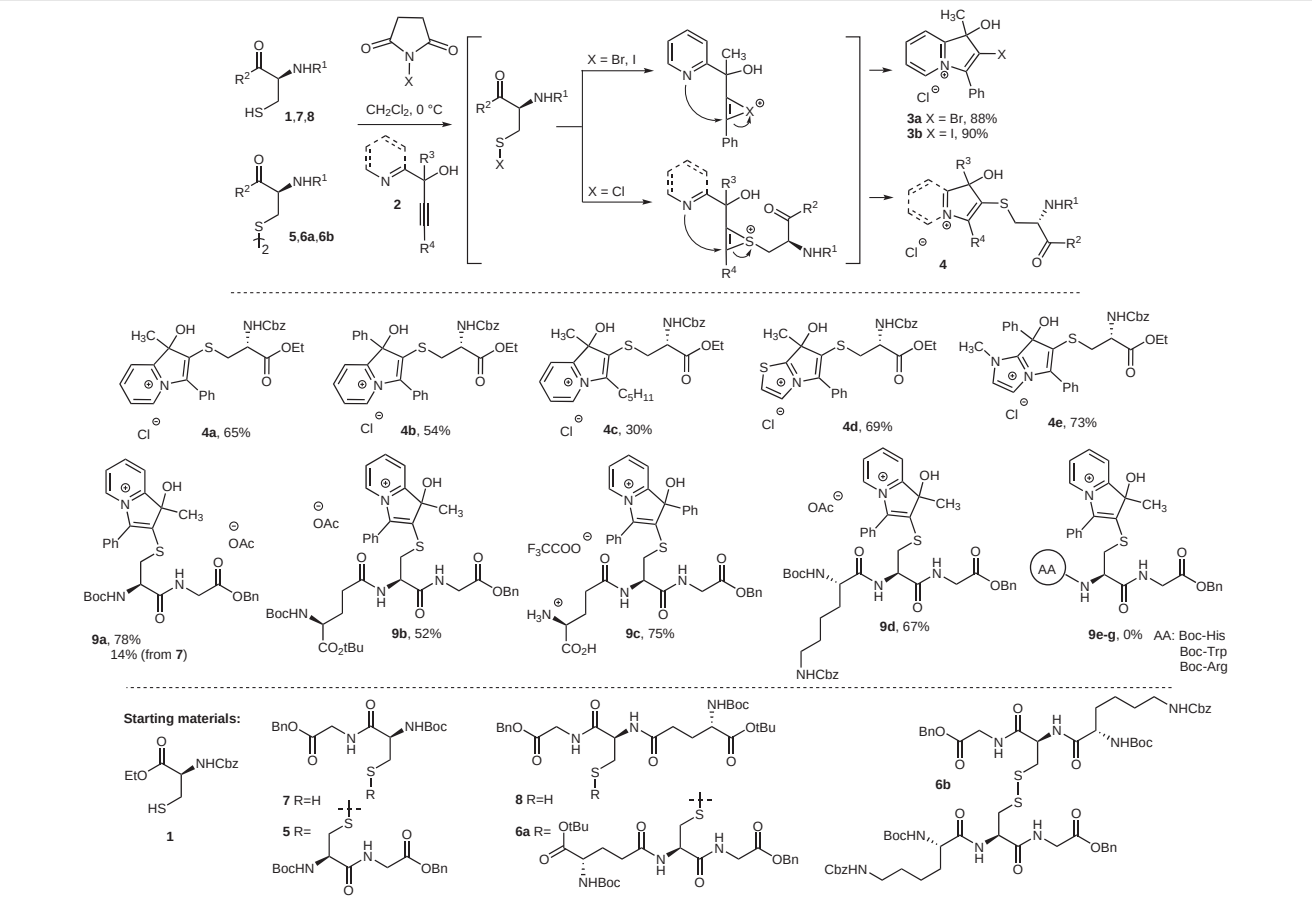
method for the generation of sulfenyl electrophiles from analogous sulfur-containing peptides.

Due to the presence of a heterocyclic moiety in a vast array of biologically active compounds, an important part of research in heterocyclic chemistry is devoted towards developing novel strategies for ring formation.[43] Many naturally occurring alkaloids such as swainsonine, monomorine, gephyrotoxin and lamellarins contain indolizine as the core structure.[44] Indolizine-ring-containing molecules have drawn attention because of their pharmacological properties, e.g., antibacterial, anti-inflammatory, antiviral, antitumor and antioxidant.[45]

Due to the necessity of mild reaction conditions while working with peptides, we decided to prepare electrophilic sulfenyl species in situ using *N*-halosuccinimides. *N*-Cbz-cysteine ethyl ester **1** was chosen as a model compound. A slight excess (1.2 equiv) of the *N*-halosuccinimide was added to a solution of **1** (1 equiv) in dichloromethane at 0 °C. After stirring the reaction mixture for 1 hour, 4-phenyl-2-(pyridin-2-yl)but-3-yn-2-ol (**2a**) (0.5 equiv) was added. Examination of the reaction mixture led us to conclude that the employment of *N*-bromosuccinimide (NBS) and *N*-iodosuccinimide (NIS) led to the formation of the corresponding halo-indolizinium salts **3a** and **3b** in high yields (88 and 90%, respectively) in a short reaction time (1 h). In contrast, the utilization of *N*-chlorosuccinimide (NCS) resulted in the desired Cys-containing indolizinium salt **4a** (65% yield) as a mixture of diastereomers (1:1) (Scheme 1). The results clearly showed that the electrophilic center in cysteinyl chloride is the sulfur atom, unlike in cysteinyl bromide and iodide where the electrophilic centers are halogen atoms due to the smaller difference in the relative electronegativities (RENs) (S = 2.58, Cl = 3.16, Br = 2.96, I = 2.66). Accordingly, the triple bond coordinates with the electrophilic sulfur atom forming a thiirenium cation, which upon nitrogen atom attack forms a 5-membered ring.

Control reactions of alkyne **2a** with *N*-halosuccinimides revealed that NCS does not react with alkyne **2a**, whereas NBS and NIS provided the respective halo-indolizinium salts in 3 days, together with unidentified side products confirming that a sulfenyl halide indeed is formed and rapidly reacts with propargyl pyridines providing indolizinium salts via 5-*endo*-*dig* cyclization. Notably, cyanuric chloride was less convenient than NCS. A reaction with 0.33 equiva-



Scheme 1 Synthesis of indolizinium-type salts. *Reagents and conditions*: Cbz-Cys-OEt or S–S peptide (1 equiv), NCS (1.2 equiv), alkyne **2** (0.5 equiv for Cbz-Cys-OEt, 1 equiv for S–S peptides), CH_2Cl_2, 0 °C to rt. **2a**, R^3 = Me, R^4 = Ph; **2b**, R^3,R^4 = Ph; **2c**, R^3 = Me, R^4 = C_5H_{11}; **2d**, R^3 = Me, R^4 = Ph; **2e**, R^3,R^4 = Ph.

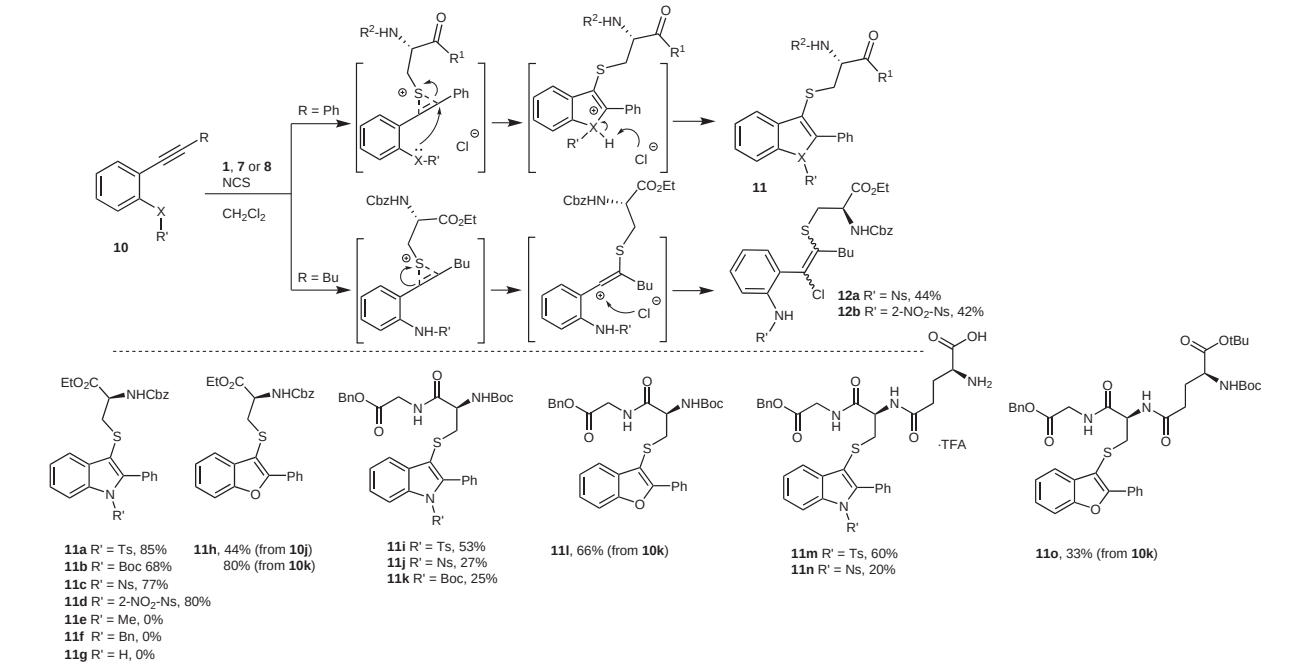
lents of cyanuric chloride with **1** in the presence of **2a** did not lead to full conversion of the starting material in 16 hours, whereas the employment of 1 equivalent of cyanuric chloride led to a non-selective reaction with many side products. Since the initially chosen reaction conditions proved to be suitable for 5-*endo*-*dig* cyclization, we moved on to examine the substrate scope of the cyclization reaction. The introduction of a phenyl group at position R^3 resulted in a slightly lower yield (**4b**, 54%), whereas changing R^2 from an aryl to an alkyl group resulted in a significantly lower yield (**4c**, 30%).

Notably, not only were propargyl pyridines **2a–c** suitable substrates, propargyl thiazole **2d** and *N*-methylimidazole **2e** also effectively trapped the in situ prepared cysteinyl chloride and provided indolizinium-type systems **4d**,**e** in higher yields (69–73%). However, in the case of imidazole derivative **2e**, the reaction was slower: 24 hours were required for full conversion of the starting materials (the reaction time for propargyl pyridines and thiazole was 3 h).

Next, we moved on to the preparation of cysteine-containing peptides **7** and **8** by employing conventional methodology[46] used in peptide chemistry. Unfortunately, previously used conditions were not appropriate for the synthesis of **9a**. Even after 48 hours, starting materials (**7** and **2a**) were observed in the reaction mixture with only traces of the desired product. Changing the solvent to acetonitrile resulted in isolation of the desired product in only 14% yield, which encouraged us to seek another route to prepare the sulfenyl chloride. Lei et al. recently demonstrated that NCS can be used in reactions with disulfides in the presence of TEMPO, thus obtaining a sulfenyl chloride and *N*-sulfenyl succinimide in situ.[47]

Encouraged by these results, we tested the reaction between peptide dimer **5**, alkyne **2a** and NCS in dichloromethane, both with and without TEMPO. Luckily, the reaction without TEMPO resulted in the isolation of product **9a** in 76% yield, whereas the addition of TEMPO did not improve the yield, thereby indicating that the reaction did not proceed through a radical pathway. Electrophilic sulfenyl chloride is formed that reacts with the alkyne. The employment of dipeptide **5** and glutathione **6a** provided the corresponding indolizinium salts **9a–c** in moderate to good yields. Next, we were interested in determining the tolerance of different amino acids under the chosen reaction conditions. Unfortunately, the desired product was obtained only with protected lysine-containing tripeptide dimer **6b**, whereas histidine (His)-, tryptophan (Trp)- and arginine (Arg)-containing peptides were not suitable for this reaction.

The indole ring is one of the most important heterocycles and is widely found in biologically active natural products.[48] One of the proteinogenic α-amino acids contains an indole ring, i.e., tryptophan, which is also the biogenetic precursor for all indole alkaloids.[49] Notably, a significant



Scheme 2 Synthesis of Cys-indoles and benzo[*b*]furans. *Reagents and conditions*: Cbz-Cys-OEt or S–H peptide (1 equiv), NCS (1.2 equiv), alkyne **2** (0.5 equiv for Cbz-Cys-OEt, 1 equiv for S–H peptides), CH_2Cl_2, 0 °C to rt. **10a**, X = NTs, R′ = H, R = Ph; **10b**, X = NBoc, R′ = H, R = Ph; **10c**, X = NNs, R′ = H, R = Ph; **10d**, X = NNs, R′ = H, R = Bu; **10e**, X = *N*-(2,4-dinitrophenylsulfonyl), R′ = H, R = Ph; **10f**, X = *N*-(2,4-dinitrophenylsulfonyl), R′ = H, R = Bu; **10g**, X = NMe, R′ = Me, R = Ph; **10h**, X = NBn, R′ = Bn, R = Ph; **10i**, X = NBn, R′ = H, R = Ph; **10j**, X = O, R′ = H, R = Ph; **10k**, X = O, R′ = Me, R = Ph.

number of marketed drugs contain an indole scaffold, e.g., indomethacin (an anti-inflammatory drug), vinblastine and vincristine (anticancer agents) and delavirdine (an anti-HIV drug).[50,51] Furthermore, indole and benzo[*b*]furan are considered as 'privileged structures'.[52] Benzofuran derivatives are present in both natural product structures and synthetic drugs.[53]

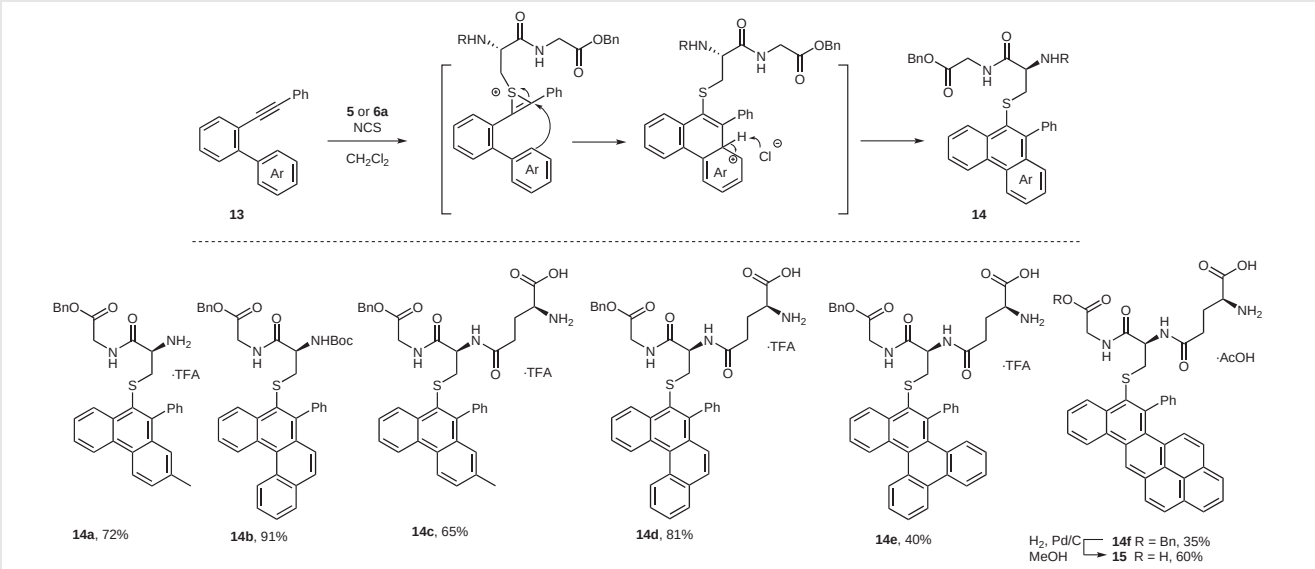
We decided to use the same method for the generation of sulfenyl electrophiles and to perform the 5-*endo*-*dig* cyclization with 2-(phenylethynyl)anilines, phenol and anisole that could result in the formation of 3-Cys-containing indoles and benzo[*b*]furans. The reaction of **1** with protected 2-(phenylethynyl)anilines in the presence of NCS provided 3-Cys indoles **11a–d** in high yields (Scheme 2). Neither dimethyl and dibenzyl amino, nor unprotected (phenylethynyl)aniline were suitable substrates. Obviously, the properties of the methyl and benzyl substituents as leaving groups are too weak to facilitate ring closure under the given conditions. Tosyl, nosyl, 2,4-dinitrophenylsulfonyl and Boc protecting groups were well tolerated under the current reaction conditions.

Unfortunately, 2-hexynylanilines, under the same reaction conditions, provided only triple bond addition products **12a**,**b** due to formation of an aryl-group-stabilized vinyl cation that prevents 5-*endo*-*dig* cyclization. Similarly, 3-Cys-benzo[*b*]furan **11h** was easily prepared using 2-(phenylethynyl)phenol **10j**. Notably, the reaction yield was significantly improved by employing 2-(phenylethynyl)anisole **10k**. Next, peptides **7** and **8** were used as substrates; however, the reaction yields were inconsistent. Products **11i–o** were obtained in 20–66% yield.

Polycyclic aromatic hydrocarbons (PAHs) are compounds employed not only as starting materials in organic synthesis, but also as fluorescent markers in bio-imaging, for example, naphthalene,[54] pyrene,[55] carbazole[56] and rhodamine[57] derivatives have been commonly used for this purpose. We therefore turned our attention to exploring whether it was possible to generate sulfenyl electrophiles using the same methodology and to perform 6-*endo*-*dig* cyclizations with 2-(phenylethynyl)biaryls.[58,59]

Gratifyingly, the reaction between **5** and alkyne **13a** in the presence of NCS provided 6-*endo*-*dig* cyclization product **14a** in 72% yield (Scheme 3). We preferred to use substrates containing S–S (not S–H) bonds due to the improved yields and diminished formation of side products. The triple bond coordinates with the electrophilic sulfur atom forming a thiirenium ring, which is attacked by the closest aromatic ring to produce a 6-membered cycle. Aromaticity is restored after deprotonation with a chloride anion, providing the final product. Furthermore, benzo[*c*]phenanthrene system **14b** was easily constructed in almost quantitative yield by applying our methodology. The use of glutathione **7** resulted in the isolation of glutathione-containing polyaromatic systems **14c**,**d** in good yields as well. Benzo[*g*]chrysene and benzo[*pqr*]picene systems with a glutathione moiety, **14e** and **14f**, were successfully synthesized, however, the isolated yields were moderate due to difficulties in the purification process.

The development of selective fluorescent dyes for imaging of living cells is a very popular research direction due to the beneficial visualization of organelles and *in vitro* processes in different cells. Polyaromatic hydrocarbons are known for their fluorescent properties; however, bioavailability was



Scheme 3 Synthesis of S-peptide-containing PAHs. *Reagents and conditions*: S–S peptide (1 equiv), NCS (2.2 equiv), alkyne (1 equiv), CH_2Cl_2, 0 °C to rt.

always under question because of low solubility. Luckily, the functionalization of PAHs with peptide moieties increases their solubility in physiological media, thereby allowing the utilization of such derivatives as fluorescent dyes. Derivative **15** was characterized by UV/Vis and fluorescence spectroscopy; the excitation and emission spectra in 20 mM HEPES buffer solution (pH 7.4) are presented in Figure 1. The absorption and emission maxima were determined at 322 nm and 489 nm (CIE coordinates: X: 0.050031497, Y: 0.274001803, turquoise, sky blue), respectively (PLQY: 3.8%). To evaluate cell permeability and the fluorescent properties, peptide derivative **15** was incubated for 17 hours with rat embryo myoblast cell line H9C2 (2-1) and internalization was visualized using fluorescence microscopy (Figure 2). According to the obtained data, derivative **15** crosses the cell membrane and accumulates in organelles (Figure 2A); notably, extracellular media does not have an emission, confirming full accumulation into the cell.

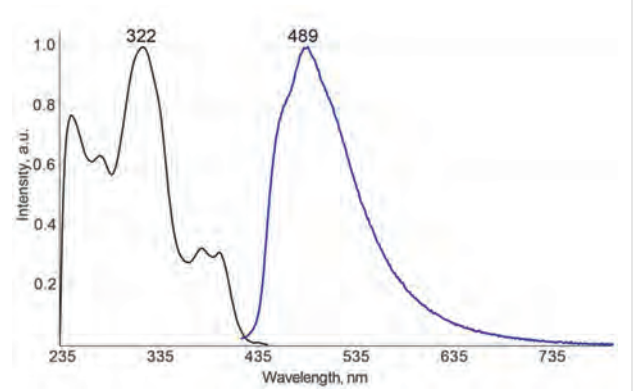


Figure 1 Absorption (black line) and emission (blue line) spectra of **15** in 20 mM HEPES buffer solution (pH 7.4)

With the purpose of specifying the nature of the organelle, the same cells were treated with Nile Red, which is a well-known and selective fluorescent dye for the determination of lipid droplets (Figure 2, B). Lipid droplets (LDs) are cytoplasmic lipid-enriched organelles and are of extremely high interest due to the correlation of the amount of LDs in cells with cardio diseases and cancer.[60–65] The overlap of both Figures 2A and 2B (Figure 2C) allowed us to unambiguously conclude that derivative **15** selectively accumulated in cell lipid droplets and emitted in the blue region. In other words, this type of PAH-substituted glutathione **15** can serve as a blue fluorescent dye in *in vitro* cell media, however, further improvement of the dye structure in terms of increasing the quantum yield will be necessary.

The isocoumarin scaffold is present in many biologically active natural products.[66,67] Notably, compounds containing an isocoumarin moiety exhibit antimicrobial,[68] antifungal,[69] cytotoxic[70] and anti-inflammatory[70] activities. An attractive way to construct an isocoumarin ring is via 6-*endo*-

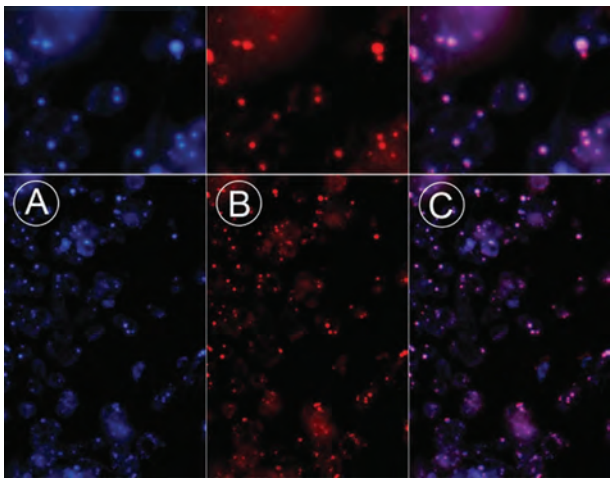
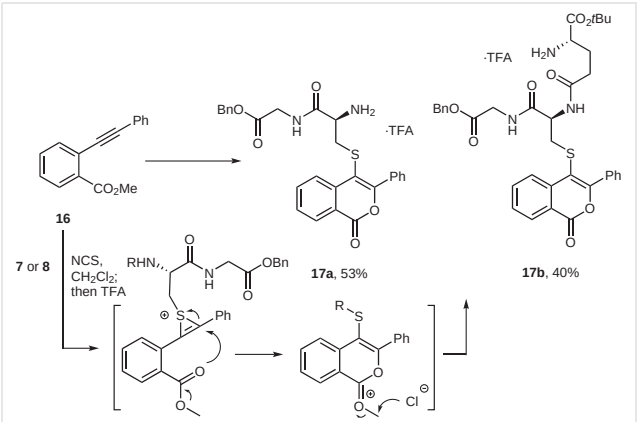


Figure 2 A: Visualization of LDs with **15**. B: Visualization of LDs with Nile Red. C: Overlap of Figures 2A and 2B.

dig cyclization of methyl 2-(phenylethynyl)benzoate (**16**) in the presence of electrophilic species.[71] For the synthesis of 4-sulfenyl isocoumarins, sulfenyl chloride, which can be prepared in situ from a disulfide and $PhICl_2$, was used as the source of electrophilic sulfur.[72] A recent report on the synthesis of 4-(methylthio)isocoumarins demonstrated that methyl sulfenyl chloride can be formed in situ by employing DMSO and $SOCl_2$.[73] Such cyclization has also been performed using diorganyl disulfide and $FeCl_3$.[34] For the introduction of $-SCF_3$ or $-SCF_2$-moieties, bismuth(III) chloride can be employed alone[74] or in combination with $BF_3{\cdot}Et_2O$.[75]

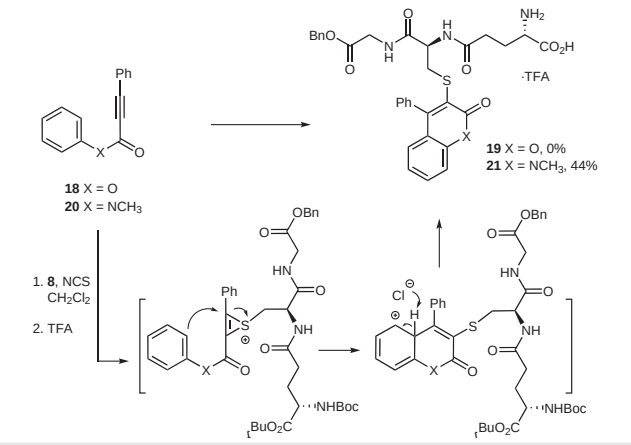
An evaluation of the NCS method was applied for synthesis of isocoumarins with a glutathione moiety. Luckily, the desired products **17a**,**b** were obtained in moderate yields by employing peptides with S–H bonds (**7** and **8**) (Scheme 4), whereas peptides with S–S bonds (**5** and **6**) were not able to provide the products.



Scheme 4 Synthesis of S-peptide-containing isocoumarins. *Reagents and conditions*: peptide (1 equiv), NCS (1.2 equiv), alkyne (1 equiv), CH_2Cl_2, 0 °C to rt.

We next moved on to another 6-*endo-dig* cyclization with the aim to prepare coumarins and quinolinones. Unfortunately, attempts to prepare coumarins, an important class of heterocycle, failed.

Peptides containing S–H or S–S bonds were unable to provide the desired compounds. However, the reaction of glutathione **8** with NCS and aryl propiolamide **20** resulted in the formation of 1-methyl-3-sulfenyl-quinolinone **21** (Scheme 5). It is noteworthy that quinolinones are the core structures of many drugs, including some of the most widely used antibiotics (e.g., ciprofloxacin and levofloxacin).[76–78] Furthermore, a literature search for methods for the preparation of 3-sulfenyl quinolinones indicated that there is clearly room for novel methods.



Scheme 5 Synthesis of S-peptide-containing quinolinones. *Reagents and conditions*: peptide (1 equiv), NCS (1.2 equiv), alkyne (1 equiv), CH_2Cl_2, 0 °C to rt.

In conclusion, a simple method has been demonstrated for the in situ preparation of cysteinyl chloride using *N*-chlorosuccinimide. As a result, series of indolizinium salts, indoles and benzo[*b*]furans have been obtained by a 5-*endo-dig* mechanism. The yields of the products depend on the nature of the substituent and on the source of sulfenyl chloride. Notably, the developed methodology was also suitable for 6-*endo-dig* cyclization, providing glutathione-containing polyaromatic systems, isocoumarins and quinolinones in good yields. Importantly, a glutathione-containing PAH was found to selectively accumulate in cell lipid droplets, thus serving as a blue fluorescent dye.

Unless otherwise stated, all reagents were purchased from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed using MERCK Silica gel 60 F254 plates, which were visualized by UV (254 nm) fluorescence. ZEOCHEM silica gel (ZEOprep 60/35–70 microns, SI23501) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance Neo spectrometer at 400 or 300 MHz (product **3b**) and 101 MHz, respectively, at 298 K in CD_3OD or $CDCl_3$. HRMS were recorded on a Waters Synapt GII Q-ToF UPLC/MS system. Infrared (IR) spectra were recorded with a Prestige-21 FTIR spectrophotometer (Shimadzu, Kyoto, Japan). *N*-Cbz-cysteine ethyl ester **1**,[79] propargyl pyridines **2a**[80] and **2b**,[81] thiazole **2d**,[41] imidazole **2e**,[82] phenyl ethynyl anilines **10a**,[83] **10b**,[84] **10c**,[85] **10d**,**e**,[42] **10g**,**h**,[86] and **10i**,[87] phenol **10j**,[88] arene-alkynes **13a–c**[41] and alkynes **16**,[89] **18**[90] and **20**[91] were prepared according to literature procedures.

2-(Pyridin-2-yl)non-3-yn-2-ol (2c)

To a solution of 1-heptyne (0.72 mL, 5.46 mmol, 2 equiv) in THF (20 mL) was added BuLi (1.90 mL, 2.3 M in cyclohexane, 4.37 mmol, 1.6 equiv) at –20 °C. The reaction mixture was stirred for 20 min, cooled to –78 °C and a solution of benzoyl pyridine (0.5 g, 2.73 mmol, 1 equiv) in THF (20 mL) was added. The mixture was stirred for 1 h, then it was warmed to rt and stirred for 16 h. Saturated aqueous NH_4-Cl was added and the mixture was extracted with EtOAc. The combined extracts were dried, filtered and evaporated. The residue was purified by flash chromatography (PE/EtOAc) to give the title compound as a dark oil (0.46 g, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1 H), 7.72 (td, *J* = 7.9, 7.4, 1.7 Hz, 1 H), 7.60 (dt, *J* = 8.0, 1.1 Hz, 1 H), 7.22 (ddd, *J* = 7.4, 4.9, 1.1 Hz, 1 H), 5.38 (s, 1 H), 2.20 (t, *J* = 7.2 Hz, 2 H), 1.73 (s, 3 H), 1.50 (quin, *J* = 7.2 Hz, 2 H), 1.40–1.22 (m, 4 H), 0.87 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.7, 147.4, 137.3, 122.7, 120.1, 84.8, 83.4, 68.8, 32.7, 31.2, 28.4, 22.3, 18.9, 14.1.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{14}H_{20}NO]^+$: 218.1539; found: 218.1547.

Indolizinium Salts 3a,b and 4a–e; General Procedure

To a solution of *N*-halosuccinimide (1.2 equiv) in CH_2Cl_2 (3 mL) was added a solution of *N*-Cbz-cysteine ethyl ester (1 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 1 h, then a solution of alkyne (0.5 equiv) in CH_2Cl_2 (3 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated and the residue purified by reverse-phase chromatography (MeCN/H_2O + HCl, 10–85%) to afford the indolizinium salts.

2-Bromo-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Chloride (3a)

Prepared from **1** (100 mg, 0.35 mmol), NBS (75 mg, 0.43 mmol) and **2a** (39 mg, 0.18 mmol).

Yield: 53 mg (88%); yellow solid.

IR (film): 3060, 1627, 1488, 1448, 1126 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.74–8.63 (m, 2 H), 8.45 (d, *J* = 7.5 Hz, 1 H), 8.05 (t, *J* = 6.6 Hz, 1 H), 7.78–7.64 (m, 5 H), 1.83 (s, 3 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 160.7, 146.9, 140.6, 137.6, 133.1, 132.9, 131.7, 131.0, 128.9, 125.1, 124.5, 81.8, 23.5.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{15}H_{13}NOBr]^+$: 302.0175; found: 302.0194.

1-Hydroxy-2-iodo-1-methyl-3-phenyl-1*H*-indolizin-4-ium Chloride (3b)[92]

Prepared from **1** (100 mg, 0.35 mmol), NIS (96 mg, 0.43 mmol) and **2a** (39 mg, 0.18 mmol).

Yield: 61 mg (90%); yellow solid.

^{1}H NMR (300 MHz, CD_3OD): δ = 8.67–8.56 (m, 2 H), 8.48–8.41 (m, 1 H), 7.99 (ddd, *J* = 7.7, 6.2, 1.4 Hz, 1 H), 7.76–7.59 (m, 5 H), 1.75 (s, 3 H).

2-(((*R*)-2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)-thio)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Chloride (4a)

Prepared from **1** (381 mg, 1.34 mmol), NCS (215 mg, 1.61 mmol) and **2a** (150 mg, 0.67 mmol).

Yield: 0.237 g (65%); yellow solid.

IR (film): 3054, 2471, 1742, 1635, 1520, 1455, 1245, 1046, 760 cm^{-1}.

^1H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.63–8.54 (m, 2 H), 8.51 (dd, *J* = 11.6, 6.2 Hz, 2 H), 8.35 (d, *J* = 7.8 Hz, 2 H), 8.03–7.94 (m, 2 H), 7.71–7.60 (m, 10 H), 7.37–7.24 (m, 10 H), 5.11–5.01 (m, 4 H), 4.38–4.25 (m, 2 H), 4.17–4.02 (m, 4 H), 3.68 (dd, *J* = 13.9, 4.1 Hz, 1 H), 3.29–3.16 (m, 2 H), 2.95–2.85 (m, 1 H), 1.88 (s, 3 H), 1.85 (s, 3 H), 1.25–1.09 (m, 6 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 171.6, 171.4, 161.3, 161.2, 158.6, 158.2, 145.8, 145.7, 143.6, 143.0, 138.7, 138.6, 138.1, 138.0, 136.0, 135.9, 132.9, 132.8, 132.1, 131.00, 130.98, 129.5, 129.12, 129.09, 128.85, 128.80, 128.60, 128.56, 125.9, 123.73, 123.70, 83.5, 83.4, 67.83, 67.78, 62.80, 62.77, 55.5, 55.0, 33.5, 33.2, 25.2, 25.1, 14.4.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{28}H_{29}N_2O_5S]^+$: 505.1792; found: 505.1800.

2-(((*R*)-2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)-thio)-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium Chloride (4b)

Prepared from **1** (398 mg, 1.05 mmol), NCS (169 mg, 1.26 mmol) and **2b** (150 mg, 0.53 mmol).

Yield: 0.170 g (54%); yellow solid.

IR (film): 3064, 2958, 2778, 1734, 1512, 1455, 1319, 1272, 1217, 1057, 748 cm^{-1}.

^1H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.58 (d, *J* = 6.0 Hz, 2 H), 8.52–8.41 (m, 2 H), 8.05–7.93 (m, 4 H), 7.81–7.64 (m, 14 H), 7.55–7.39 (m, 6 H), 7.39–7.23 (m, 10 H), 5.11–4.99 (m, 4 H), 4.32 (dd, *J* = 9.2, 4.5 Hz, 1 H), 4.11 (q, *J* = 7.0 Hz, 2 H), 4.04 (q, *J* = 7.0 Hz, 2 H), 3.93 (dd, *J* = 9.2, 5.3 Hz, 1 H), 3.60 (dd, *J* = 13.7, 4.5 Hz, 1 H), 3.25 (dd, *J* = 13.7, 5.3 Hz, 1 H), 3.07 (dd, *J* = 13.7, 9.2 Hz, 1 H), 2.72–2.61 (m, 1 H), 1.21 (t, *J* = 7.1 Hz, 3 H), 1.16 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 171.4, 171.2, 160.9, 145.9, 145.8, 138.0, 137.2, 137.1, 136.2, 132.9, 132.0, 131.12, 131.07, 130.81, 130.77, 130.7, 130.6, 129.5, 129.0, 128.8, 128.73, 128.71, 126.42, 126.36, 125.79, 125.75, 124.6, 86.8, 86.7, 67.7, 62.74, 62.68, 55.5, 55.2, 32.6, 32.3, 17.7, 14.44, 14.42.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{33}H_{31}N_2O_5S]^+$: 567.1948; found: 567.1957.

2-(((*R*)-2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)-thio)-1-hydroxy-1-methyl-3-pentyl-1*H*-indolizin-4-ium Chloride (4c)

Prepared from **1** (782 mg, 2.76 mmol), NCS (442 mg, 3.31 mmol) and **2c** (300 mg, 1.38 mmol).

Yield: 0.217 g (30%); yellow oil.

IR (film): 3091, 2956, 2930, 2872, 1718, 1716, 1534, 1327, 1215, 1052, 748 cm^{-1}.

^1H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 9.12–9.02 (m, 2 H), 8.65–8.54 (m, 2 H), 8.29 (d, *J* = 7.8 Hz, 2 H), 8.15–8.03 (m, 2 H), 7.43–7.23 (m, 10 H), 5.22–5.01 (m, 4 H), 4.57–4.49 (m, 1 H), 4.36 (dd, *J* = 10.9, 3.7 Hz, 1 H), 4.26–4.13 (m, 3 H), 4.06 (dd, *J* = 13.8, 3.7 Hz, 1 H), 3.58–3.48 (m, 2 H), 3.08–3.00 (m, 1 H), 2.99–2.91 (m, 3 H), 2.93–2.81 (m, 2 H), 1.78 and 1.77 (2 s, 6 H), 1.73–1.57 (m, 2 H), 1.62–1.47 (m, 2 H), 1.48–1.30 (m, 8 H), 1.33–1.17 (m, 6 H), 0.99–0.87 (m, 6 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 171.9, 171.7, 162.0, 161.9, 158.7, 158.3, 146.2, 146.1, 144.5, 144.2, 139.8, 139.1, 138.2, 136.6, 136.5, 129.5, 129.1, 128.8, 128.73, 128.67, 128.6, 123.6, 83.3, 83.2, 67.8, 67.7, 62.9, 62.8, 56.4, 55.5, 34.2, 34.0, 32.4, 27.4, 25.12, 25.05, 24.8, 23.3, 14.5, 14.4, 14.3.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{27}H_{35}N_2O_5S]^+$: 499.2261; found: 499.2281.

6-(((*R*)-2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)-thio)-7-hydroxy-7-methyl-5-phenyl-7*H*-pyrrolo[2,1-*b*]thiazol-4-ium Chloride (4d)

Prepared from **1** (371 mg, 1.31 mmol), NCS (210 mg, 1.57 mmol) and **2d** (150 mg, 0.66 mmol).

Yield: 0.247 g (69%); yellow solid.

IR (film): 3086, 2804, 1718, 1529, 1261, 1213, 1049, 748 cm^{-1}.

^1H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.35 (t, *J* = 3.8 Hz, 2 H), 8.20 (dd, *J* = 9.8, 3.7 Hz, 2 H), 7.74–7.65 (m, 4 H), 7.65–7.54 (m, 6 H), 7.40–7.25 (m, 10 H), 5.13–4.98 (m, 4 H), 4.39 (t, *J* = 7.1 Hz, 1 H), 4.29 (dd, *J* = 10.8, 3.9 Hz, 1 H), 4.16–4.04 (m, 4 H), 3.73 (dd, *J* = 13.9, 4.0 Hz, 1 H), 3.30–3.20 (m, 2 H), 2.90 (dd, *J* = 13.9, 10.8 Hz, 1 H), 1.94 and 1.92 (2 s, 6 H), 1.25–1.11 (m, 6 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 178.0, 177.9, 171.7, 171.5, 158.5, 158.2, 141.6, 140.9, 139.3, 139.2, 138.04, 137.99, 132.7, 132.6, 131.1, 130.60, 130.55, 129.96, 129.90, 129.5, 129.1, 128.83, 128.81, 128.77, 128.72, 126.7, 84.0, 83.8, 67.8, 67.8, 62.8, 62.7, 55.7, 55.0, 34.5, 33.8, 26.2, 26.0, 14.42, 14.41.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{26}H_{27}N_2O_5S_2]^+$: 511.1356; found: 511.1370.

6-(((*R*)-2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)-thio)-7-hydroxy-1-methyl-5,7-diphenyl-1,7-dihydropyrrolo[1,2-*a*]imidazol-4-ium Chloride (4e)

Prepared from **1** (590 mg, 2.08 mmol), NCS (334 mg, 2.5 mmol) and **2f** (150 mg, 0.52 mmol).

Yield: 0.23 g (73%); yellow solid.

IR (film): 2974, 2496, 1733, 1616, 1587, 1515, 1302, 1265, 1214, 1049, 756 cm^{-1}.

^1H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 7.75–7.71 (m, 4 H), 7.66–7.54 (m, 12 H), 7.49–7.26 (m, 18 H), 5.08–4.99 (m, 4 H), 4.09 (s, 3 H), 4.06 (s, 3 H), 4.05–3.91 (m, 5 H), 3.88 (dd, *J* = 8.7, 4.7 Hz, 1 H), 2.91–2.78 (m, 2 H), 2.68–2.57 (m, 2 H), 1.20–1.08 (m, 6 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 171.5, 171.4, 158.1, 158.0, 149.22, 149.15, 140.4, 140.3, 138.6, 138.5, 138.43, 138.35, 138.02, 137.97, 137.0, 136.9, 131.5, 131.4, 130.8, 130.7, 129.93, 129.90, 129.8, 129.5, 129.1, 128.89, 128.86, 128.5, 128.4, 126.8, 119.5, 79.2, 79.1, 67.7, 62.7, 62.6, 54.9, 54.8, 38.7, 38.4, 37.2, 14.42, 14.39.

HRMS (ESI/Q-TOF): *m*/*z* [M – Cl]$^+$ calcd for $[C_{32}H_{32}N_3O_5S]^+$: 570.2057; found: 570.2049.

Benzyl (6*R*,11*R*)-6-((2-(Benzyloxy)-2-oxoethyl)carbamoyl)-11-((*tert*-butoxycarbonyl)amino)-2,2-dimethyl-4,12-dioxo-3-oxa-8,9-dithia-5,13-diazapentadecan-15-oate (5)

To a solution of glycine benzyl ester hydrochloride (1.37 g, 6.81 mmol, 3 equiv) in DMF (5 mL) was added NMM (1 mL, 9.08 mmol, 4 equiv) at 0 °C and the mixture was stirred for 5 min. Next, a solution of Boc-L-

cystine (1 g, 2.27 mmol, 1 equiv) in DMF (5 mL) was added to the reaction mixture followed by the addition of HOBt (0.697 g, 4.54 mmol, 2 equiv) and EDC·HCl (1.74 g, 9.08 mmol, 4 equiv). The reaction mixture was stirred at 0 °C for 10 min and then at rt for 1 h. The reaction mixture was evaporated and the residue was purified by reverse-phase chromatography (MeCN/H_2O, 10–85%) to give the product as white solid in 95% yield.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.41–7.26 (m, 10 H), 5.16 (s, 4 H), 4.49 (d, *J* = 18.0 Hz, 2 H), 4.08–3.92 (ABm, 4 H), 3.23–3.09 (m, 2 H), 2.86 (dd, *J* = 13.8, 9.5 Hz, 2 H), 1.44 (s, 18 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.8, 170.8, 157.8, 137.1, 129.6, 129.3, 80.9, 67.9, 55.2, 42.3, 42.2, 28.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{34}H_{46}N_4O_{10}S_2Na]^+$: 757.2553; found: 757.2548.

Peptides 6a–d; General Procedure

To a solution of **5** (1 equiv) in CH_2Cl_2 (4 mL) was added TFA (2 mL) at 0 °C and the reaction mixture was stirred at rt for 1 h and then evaporated. The residue was dissolved in DMF (5 mL), NMM (3 equiv) was added at 0 °C and the mixture was stirred for 5 min. Next, a solution of protected amino acid (3 equiv) in DMF (5 mL) was added followed by HOBt (1 equiv) and EDC·HCl (3 equiv). The resulting mixture was stirred at 0 °C for 10 min and then at rt for 2 h. The mixture was evaporated and the residue was purified by reverse-phase chromatography (MeCN/H_2O, 5–85%) to provide the product.

Dibenzyl 11,11′-(Disulfanediylbis(methylene))(6*S*,6′*S*,11*R*,11′*R*)-bis(6-(*tert*-butoxycarbonyl)-2,2-dimethyl-4,9,12-trioxo-3-oxa-5,10,13-triazapentadecan-15-oate) (6a)

Prepared from **5** (1.5 g, 2 mmol), Boc-Glu-O^{t}Bu (1.97 g, 6.12 mmol), NMM (0.67 mL, 6.12 mmol), HOBt (0.306 g, 2 mmol) and EDC·HCl (1.18 g, 6.12 mmol).

Yield: 1.8 g (80%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.39–7.27 (m, 10 H), 5.16 (s, 4 H), 4.84–4.81 (m, 2 H), 4.07–3.96 (m, 6 H), 3.17 (dd, *J* = 14.0, 4.9 Hz, 2 H), 2.91 (dd, *J* = 14.0, 9.5 Hz, 2 H), 2.36 (t, *J* = 7.6 Hz, 4 H), 2.18–2.05 (m, 2 H), 1.95–1.83 (m, 2 H), 1.46 (s, 18 H), 1.44 (s, 18 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 175.0, 173.3, 173.1, 170.9, 158.1, 137.1, 129.6, 129.36, 129.35, 82.8, 80.5, 68.0, 55.4, 53.8, 42.3, 41.9, 33.0, 28.9, 28.3.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{52}H_{77}N_6O_{16}S_2]^+$: 1105.4837; found: 1105.4818.

Benzyl ((9*S*,12*R*,17*R*)-12-((2-(Benzyloxy)-2-oxoethyl)carbamoyl)-17-((*S*)-6-(((benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)-amino)hexanamido)-9-((*tert*-butoxycarbonyl)amino)-3,10-dioxo-1-phenyl-2-oxa-14,15-dithia-4,11-diazaoctadecan-18-oyl)glycinate (6b)

Prepared from **5** (150 mg, 0.2 mmol), Boc-Lys(Cbz) (233 mg, 0.61 mmol), NMM (0.07 mL, 0.61 mmol), HOBt (30 mg, 0.2 mmol) and EDC·HCl (117 mg, 0.61 mmol).

Yield: 190 mg (73%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.37–7.25 (m, 20 H), 5.14 (s, 4 H), 5.04 (s, 4 H), 4.07–3.94 (m, 6 H), 3.18 (dd, *J* = 14.0, 4.7 Hz, 2 H), 3.10 (t, *J* = 6.7 Hz, 4 H), 2.95 (dd, *J* = 14.0, 9.4 Hz, 2 H), 1.83–1.70 (m, 2 H), 1.70–1.58 (m, 2 H), 1.55–1.41 (m, 8 H), 1.41 (s, 18 H), 1.40–1.35 (m, 2 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 175.4, 172.8, 170.9, 158.9, 158.2, 138.4, 137.1, 129.6, 129.5, 129.36, 129.35, 128.9, 128.8, 116.1, 80.9, 68.0, 67.3, 56.4, 53.6, 42.3, 41.6, 41.4, 32.8, 30.5, 28.8, 24.0.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{62}H_{82}N_8O_{16}S_2Na]^+$: 1281.5188; found: 1281.5208.

Dibenzyl 9,9′-(Disulfanediylbis(methylene))(6*S*,6′*S*,9*R*,9′*R*)-bis(6-((1*H*-indol-3-yl)methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate) (6c)

Prepared from **5** (150 mg, 0.2 mmol), Boc-Trp (186 mg, 0.61 mmol), NMM (0.07 mL, 0.61 mmol), HOBt (30 mg, 0.2 mmol) and EDC·HCl (117 mg, 0.61 mmol).

Yield: 150 mg (73%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.58 (d, *J* = 7.9 Hz, 2 H), 7.36–7.25 (m, 12 H), 7.11 (s, 2 H), 7.06 (t, *J* = 7.2 Hz, 2 H), 6.98 (t, *J* = 7.3 Hz, 2 H), 5.11 (s, 4 H), 4.84–4.79 (m, 2 H), 4.43 (dd, *J* = 7.7, 6.0 Hz, 2 H), 3.97–3.76 (m, 4 H), 3.27 (dd, *J* = 14.1, 6.1 Hz, 2 H), 3.14–2.96 (m, 4 H), 2.86 (dd, *J* = 14.1, 8.8 Hz, 2 H), 1.34 (s, 18 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.9, 172.5, 170.7, 157.9, 137.9, 137.0, 129.6, 129.37, 129.35, 128.8, 124.9, 122.5, 119.9, 119.5, 112.3, 110.8, 80.9, 68.0, 57.2, 53.8, 42.2, 41.6, 29.1, 28.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{56}H_{66}N_8O_{12}S_2Na]^+$: 1129.4139; found: 1129.4172.

Dibenzyl 9,9′-(Disulfanediylbis(methylene))(6*S*,6′*S*,9*R*,9′*R*)-bis(6-((1*H*-imidazol-4-yl)methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate) (6d)

Prepared from **5** (150 mg, 0.2 mmol), Boc-His (156 mg, 0.61 mmol), NMM (0.07 mL, 0.61 mmol), HOBt (30 mg, 0.2 mmol) and EDC·HCl (117 mg, 0.61 mmol).

Yield: 130 mg (65%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.60 (s, 2 H), 7.39–7.26 (m, 10 H), 6.89 (s, 2 H), 5.16 (s, 4 H), 4.85–4.77 (m, 2 H), 4.37–4.29 (m, 2 H), 4.08–3.96 (ABm, 4 H), 3.19 (dd, *J* = 14.0, 4.7 Hz, 2 H), 3.07 (dd, *J* = 14.8, 5.6 Hz, 2 H), 2.94 (dd, *J* = 14.1, 9.1 Hz, 4 H), 1.39 (s, 18 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.4, 172.9, 170.9, 157.8, 137.1, 136.3, 134.4, 129.6, 129.4, 118.4, 81.0, 68.0, 56.3, 53.8, 42.3, 41.4, 30.6, 28.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{46}H_{61}N_{10}O_{12}S_2]^+$: 1009.3912; found: 1009.3925.

Peptides 7 and 8

To a solution of S–S-bond-containing peptide (1 equiv) in MeOH (5 mL) and H_2O (0.5 mL) was added *n*Bu_3P (1.3 equiv), and after stirring for 30 min, the reaction mixture was evaporated and purified by flash chromatography (EtOAc/PE, 2:1 to 5:0)

Benzyl (*tert*-Butoxycarbonyl)-L-cysteinylglycinate (7)

Prepared from **5** (200 mg, 0.27 mmol, 1 equiv) and *n*Bu_3P (0.088 mL, 0.37 mmol).

Yield: 144 mg (72%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.45–7.22 (m, 5 H), 5.17 (s, 2 H), 4.29–4.19 (m, 1 H), 4.09–3.92 (ABm, 2 H), 2.88–2.73 (m, 2 H), 1.45 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.6, 170.9, 157.7, 137.1, 129.6, 129.4, 81.0, 68.0, 58.0, 42.2, 28.7, 27.5.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{17}H_{24}N_2O_5SNa]^+$: 391.1304; found: 391.1299.

tert-Butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-mercapto-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (8)

Prepared from **6a** (200 mg, 0.18 mmol, 1 equiv) and nBu$_3$P (0.059 mL, 0.23 mmol).

Yield: 0.59 mg (75%); white solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.40–7.25 (m, 5 H), 5.16 (s, 2 H), 4.84–4.80 (m, 1 H), 4.08–3.95 (m, 3 H), 3.17 (dd, J = 14.0, 4.9 Hz, 1 H), 2.91 (dd, J = 14.0, 9.4 Hz, 1 H), 2.36 (t, J = 7.6 Hz, 2 H), 2.18–2.05 (m, 1 H), 1.94–1.82 (m, 1 H), 1.46 (s, 9 H), 1.44 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 175.0, 173.3, 173.1, 170.9, 158.1, 137.1, 129.6, 129.36, 129.35, 82.8, 80.5, 68.0, 55.4, 53.8, 42.3, 41.9, 33.0, 28.8, 28.3.

HRMS (ESI/Q-TOF): m/z [M + Na]$^+$ calcd for [$C_{26}H_{39}N_3O_8SNa$]$^+$: 576.2356; found: 576.2346.

Indolizinium Salts 9a–f; General Procedure

To a solution of *N*-halosuccinimide (2.2 equiv) in CH_2Cl_2 (3 mL) was added a solution of S–S-bond-containing peptide (1 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 1 h, then a solution of the corresponding alkyne (1 equiv) in CH_2Cl_2 (3 mL) was added. The resulting mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by reverse-phase chromatography (MeCN/H_2O + AcOH, 10–85%) to afford the indolizinium salt.

2-(((*R*)-3-((2-(Benzyloxy)-2-oxoethyl)amino)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)thio)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Acetate (9a)

Prepared from **5** (100 mg, 0.14 mmol), NCS (41 mg, 0.31 mmol) and alkyne **2a** (46 mg, 0.20 mmol).

Yield: 69 mg (78%); white solid.

IR (film): 3290, 2980, 1682, 1534, 1188 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.62–8.53 (m, 2 H), 8.52 (d, J = 6.1 Hz, 2 H), 8.36 (d, J = 7.9 Hz, 2 H), 8.02–7.94 (m, 2 H), 7.72–7.60 (m, 10 H), 7.40–7.28 (m, 10 H), 5.17–5.09 (m, 4 H), 4.34–4.28 (m, 1 H), 4.22 (dd, J = 9.9, 4.5 Hz, 1 H), 3.98–3.92 (m, 4 H), 3.63 (dd, J = 13.8, 4.5 Hz, 1 H), 3.31–3.24 (m, 2 H), 2.88 (dd, J = 13.8, 9.9 Hz, 1 H), 1.89 (s, 6 H), 1.46–1.36 (m, 18 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.1, 172.8, 170.9, 170.8, 161.5, 157.8, 157.6, 145.7, 145.6, 143.3, 137.2, 135.9, 132.80, 132.78, 132.0, 131.0, 129.6, 129.3, 129.24, 129.19, 128.50, 128.47, 128.0, 125.91, 125.89, 123.72, 123.69, 83.5, 81.2, 81.1, 67.90, 67.88, 65.2, 55.7, 55.6, 42.2, 33.8, 28.71, 28.65, 25.2, 23.9.

HRMS (ESI/Q-TOF): m/z [M – OAc]$^+$ calcd for [$C_{32}H_{36}N_3O_6S$]$^+$: 590.2319; found: 590.2325.

2-(((*R*)-3-((2-(Benzyloxy)-2-oxoethyl)amino)-2-((*S*)-5-(*tert*-butoxy)-4-((*tert*-butoxycarbonyl)amino)-5-oxopentanamido)-3-oxopropyl)thio)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Acetate (9b)

Prepared from **6a** (100 mg, 0.09 mmol), NCS (27 mg, 0.20 mmol) and alkyne **2a** (30 mg, 0.13 mmol).

Yield: 40 mg (52%); colorless oil.

IR (film): 3246, 2978, 1670, 1518, 1246, 1153 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.62–8.52 (m, 2 H), 8.48 (t, J = 7.1 Hz, 2 H), 8.38 (t, J = 8.4 Hz, 2 H), 8.03–7.93 (m, 2 H), 7.69–7.60 (m, 10 H), 7.39–7.27 (m, 10 H), 5.14–5.08 (m, 4 H), 4.59–4.49 (m, 2 H), 3.96 (s, 3 H), 3.93 (s, 3 H), 3.77 (dd, J = 14.0, 4.5 Hz, 1 H), 3.39–3.32 (m, 1 H), 3.36–3.20 (m, 2 H), 2.90 (dd, J = 14.0, 10.3 Hz, 1 H), 2.36–2.14 (m, 4 H), 2.13–1.95 (m, 2 H), 1.91–1.78 (m, 8 H), 1.50–1.42 (m, 36 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 175.0, 173.3, 172.7, 172.4, 170.83, 170.80, 161.4, 161.3, 158.1, 145.9, 145.8, 143.3, 142.5, 139.9, 138.8, 137.17, 137.16, 136.0, 135.9, 132.7, 132.2, 130.91, 130.86, 129.6, 129.4, 129.33, 129.28, 129.22, 129.17, 128.5, 128.4, 126.0, 125.9, 123.88, 123.86, 83.6, 83.4, 82.72, 82.65, 80.51, 80.47, 67.81, 67.78, 55.5, 54.5, 54.3, 42.23, 42.19, 33.6, 33.2, 33.0, 28.8, 28.3, 28.2, 25.3.

HRMS (ESI/Q-TOF): m/z [M – OAc]$^+$ calcd for [$C_{41}H_{51}N_4O_9S$]$^+$: 775.3371; found: 775.3395.

2-(((*R*)-2-((*S*)-4-Ammonio-4-carboxybutanamido)-3-((2-(benzyloxy)-2-oxoethyl)amino)-3-oxopropyl)thio)-1-hydroxy-1,3-diphenyl-1*H*-indolizin-4-ium 2,2,2-Trifluoroacetate (9c)

Prepared from **6a** (100 mg, 0.09 mmol), NCS (27 mg, 0.20 mmol) and alkyne **2b** (40 mg, 0.14 mmol). The product was isolated after treatment with TFA.

Yield: 61 mg (75%); colorless oil.

^{1}H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.60–8.53 (m, 2 H), 8.51–8.40 (m, 2 H), 8.04–7.93 (m, 4 H), 7.81–7.63 (m, 14 H), 7.52–7.41 (m, 6 H), 7.39–7.26 (m, 10 H), 5.20–5.11 (m, 4 H), 4.51 (dd, J = 9.3, 4.9 Hz, 1 H), 4.36 (dd, J = 8.2, 5.8 Hz, 1 H), 4.05–3.97 (m, 2 H), 3.97–3.86 (m, 6 H), 3.61 (dd, J = 13.8, 4.9 Hz, 1 H), 3.25–3.15 (m, 1 H), 3.16–3.07 (m, 1 H), 2.74–2.65 (m, 1 H), 2.55–2.35 (m, 4 H), 2.25–2.08 (m, 4 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.2, 172.6, 172.3, 172.0, 171.4, 170.80, 170.77, 160.9, 146.0, 145.9, 144.6, 144.0, 142.7, 139.3, 138.4, 137.18, 137.13, 137.12, 136.4, 136.3, 132.9, 131.11, 131.05, 130.78, 130.73, 130.65, 130.60, 129.6, 129.4, 129.34, 129.30, 129.23, 129.21, 128.7, 128.3, 128.0, 126.5, 126.4, 125.8, 125.74, 124.66, 86.9, 86.7, 67.92, 67.90, 65.2, 54.6, 54.1, 53.5, 42.3, 42.12, 42.05, 41.8, 32.7, 32.4, 32.3, 27.0.

HRMS (ESI/Q-TOF): m/z [M – CF_3COO]$^+$ calcd for [$C_{37}H_{37}N_4O_7S$]$^+$: 681.2377; found: 681.2376.

2-(((*R*)-3-((2-(Benzyloxy)-2-oxoethyl)amino)-2-((*S*)-6-(((benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)hexanamido)-3-oxopropyl)thio)-1-hydroxy-1-methyl-3-phenyl-1*H*-indolizin-4-ium Acetate (9d)

Prepared from **6b** (120 mg, 0.092 mmol), NCS (27 mg, 0.20 mmol) and alkyne **2a** (31 mg, 0.139 mmol).

Yield: 59 mg (67%); colorless oil.

IR (film): 3305, 2935, 1704, 1533, 1249, 1176 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) (1:1 mixture of diastereomers): δ = 8.59–8.50 (m, 2 H), 8.48 (dd, J = 6.2, 2.8 Hz, 2 H), 8.34 (d, J = 7.8 Hz, 2 H), 7.99–7.92 (m, 2 H), 7.69–7.56 (m, 10 H), 7.38–7.25 (m, 20 H), 5.14–5.10 (m, 4 H), 5.05 (s, 4 H), 4.67–4.51 (m, 2 H), 4.02–3.88 (m, 5 H), 3.89–3.79 (m, 2 H), 3.39 (dd, J = 13.8, 7.9 Hz, 1 H), 3.30–3.24 (m, 1 H), 3.13–3.04 (m, 4 H), 2.98 (dd, J = 13.9, 9.9 Hz, 1 H), 1.91–1.86 (m, 6 H), 1.75–1.60 (m, 2 H), 1.60–1.50 (m, 2 H), 1.47–1.29 (m, 28 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 175.4, 172.1, 171.9, 170.8, 170.7, 161.4, 161.2, 158.9, 158.2, 157.9, 145.73, 145.65, 143.1, 142.9, 139.1, 138.4, 137.2, 137.1, 136.2, 135.9, 132.7, 132.1, 132.0, 131.00, 130.96, 129.6, 129.5, 129.33, 129.25, 129.2, 129.0, 128.8, 128.5, 128.4, 125.9, 125.8, 123.7, 83.6, 83.5, 80.9, 80.6, 67.9, 67.3, 56.5, 56.1, 54.6, 54.1, 42.2, 42.1, 41.4, 33.1, 32.7, 30.5, 30.4, 28.8, 25.3, 25.2, 23.9, 22.5.

HRMS (ESI/Q-TOF): *m*/*z* [M – OAc]$^+$ calcd for [$C_{46}H_{54}N_5O_9S$]$^+$: 852.3637; found: 852.3687.

N-(2-(Hex-1-yn-1-yl)phenyl)-2,4-dinitrobenzenesulfonamide (10f)

To a solution of 2-(hex-1-yn-1-yl)aniline (0.3 g, 1.73 mmol, 1 equiv) in CH_2Cl_2 (7 mL) were added pyridine (0.28 mL, 3.46 mmol, 2 equiv) and 2,4-dinitrobenzenesulfonyl chloride (0.57 g, 2.08 mmol, 1.2 equiv) at 0 °C. The reaction mixture was stirred for 16 h at room temperature and then poured into ice water and extracted with CH_2Cl_2. The combined organic layer was washed with 2 M HCl and brine and then dried over Na_2SO_4. After filtration and evaporation, the residue was purified by flash chromatography (PE/EtOAc, 10:1 to 3:1) to give the title compound (0.42 g, 61%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.68 (d, *J* = 2.2 Hz, 1 H), 8.42 (dd, *J* = 8.6, 2.2 Hz, 1 H), 8.17–8.12 (m, 2 H), 7.70 (dd, *J* = 8.6, 1.2 Hz, 1 H), 7.34–7.26 (m, 2 H), 7.09 (td, *J* = 7.6, 1.2 Hz, 1 H), 2.38 (t, *J* = 7.1 Hz, 2 H), 1.57–1.49 (m, 2 H), 1.48–1.34 (m, 2 H), 0.93 (t, *J* = 7.3 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.2, 148.3, 138.4, 136.0, 133.0, 132.7, 129.1, 127.0, 125.8, 120.9, 120.7, 116.3, 99.2, 74.8, 30.6, 22.2, 19.4, 13.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{18}H_{18}N_3O_6S$]$^+$: 404.0916; found: 404.0906.

Indoles and Benzo[*b*]furans 11a–h; General Procedure

To a solution of NCS (1.2 equiv) in CH_2Cl_2 was added a solution of *N*-Cbz-cysteine ethyl ester **1** in CH_2Cl_2 at 0 °C. The mixture was stirred for 1 h, then a solution of the corresponding alkyne (0.5 equiv) in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by flash chromatography (PE/EtOAc, 10–100%) to give the product.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(2-phenyl-1-tosyl-1*H*-indol-3-yl)-L-cysteinate (11a)

Prepared from **1** (245 mg, 0.86 mmol), NCS (138 mg, 1.04 mmol), alkyne **10a** (150 mg, 0.43 mmol) and CH_2Cl_2 (10 mL).

Yield: 230 mg (85%); yellow oil; $[\alpha]_D^{20}$ +46.5 (*c* 1.0, $CHCl_3$).

IR (film): 3405, 3032, 1746, 1511, 1377, 1178 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (d, *J* = 8.2 Hz, 1 H), 7.65 (d, *J* = 7.2 Hz, 1 H), 7.45–7.24 (m, 14 H), 7.06 (d, *J* = 8.1 Hz, 2 H), 5.05 (d, *J* = 7.8 Hz, 1 H), 4.99 (d, *J* = 12.2 Hz, 1 H), 4.89 (d, *J* = 12.2 Hz, 1 H), 4.35–4.23 (m, 1 H), 3.88–3.73 (m, 1 H), 3.55–3.42 (m, 1 H), 3.18 (dd, *J* = 14.0, 4.3 Hz, 1 H), 2.97 (dd, *J* = 14.0, 4.9 Hz, 1 H), 2.30 (s, 3 H), 0.97 (t, *J* = 7.0 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.8, 155.4, 145.1, 143.9, 137.0, 136.3, 135.1, 132.0, 131.1, 130.2, 129.6, 129.4, 128.6, 128.3, 128.1, 127.5, 127.0, 125.8, 124.6, 119.9, 116.3, 114.0, 67.0, 61.7, 53.5, 35.8, 21.7, 13.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{34}H_{33}N_2O_6S_2$]$^+$: 629.1780; found: 629.1766.

tert-Butyl (*R*)-3-((2-(((Benzyloxy)carbonyl)amino)-3-ethoxy-3-oxopropyl)thio)-2-phenyl-1*H*-indole-1-carboxylate (11b)

Prepared from **1** (289 mg, 1.02 mmol), NCS (164 mg, 1.23 mmol), alkyne **10b** (150 mg, 0.51 mmol) and CH_2Cl_2 (10 mL).

Yield: 199 mg (68%); light yellow oil; $[\alpha]_D^{20}$ +33.8 (*c* 1.0, $CHCl_3$).

IR (film): 3404, 2981, 1733, 1516, 1318, 1058 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 7.5 Hz, 1 H), 7.76–7.69 (m, 1 H), 7.47–7.27 (m, 12 H), 5.12–5.06 (m, 1 H), 5.00 (d, *J* = 12.2 Hz, 1 H), 4.93 (d, *J* = 12.2 Hz, 1 H), 4.37–4.28 (m, 1 H), 3.88–3.79 (m, 1 H), 3.58–3.44 (m, 1 H), 3.27 (dd, *J* = 13.9, 4.5 Hz, 1 H), 3.01 (dd, *J* = 13.9, 5.0 Hz, 1 H), 1.21 (s, 9 H), 1.00 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.0, 155.5, 149.7, 143.2, 136.6, 133.2, 130.2, 130.1, 128.6, 128.4, 128.2, 128.1, 127.9, 125.4, 123.6, 119.6, 115.5, 110.5, 84.0, 66.9, 61.6, 53.4, 36.1, 27.5, 13.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{32}H_{35}N_2O_6S$]$^+$: 575.2216; found: 575.2219.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(1-((4-nitrophenyl)sulfonyl)-2-phenyl-1*H*-indol-3-yl)-L-cysteinate (11c)

Prepared from **1** (225 mg, 0.79 mmol), NCS (127 mg, 0.95 mmol), alkyne **10c** (150 mg, 0.40 mmol) and CH_2Cl_2 (10 mL).

Yield: 203 mg (77%); yellow solid; $[\alpha]_D^{20}$ +49.1 (*c* 1.0, $CHCl_3$).

IR (film): 3406, 2983, 1729, 1533, 1348, 1185 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.28 (d, *J* = 8.2 Hz, 1 H), 8.11–8.03 (m, 2 H), 7.63 (d, *J* = 8.0 Hz, 1 H), 7.55–7.47 (m, 2 H), 7.47–7.22 (m, 11 H), 5.01 (d, *J* = 7.8 Hz, 1 H), 4.94 (d, *J* = 12.2 Hz, 1 H), 4.84 (d, *J* = 12.2 Hz, 1 H), 4.30–4.19 (m, 1 H), 3.85–3.73 (m, 1 H), 3.59–3.43 (m, 1 H), 3.13 (dd, *J* = 14.0, 4.3 Hz, 1 H), 2.97 (dd, *J* = 14.0, 4.9 Hz, 1 H), 0.96 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7, 155.3, 150.7, 143.3, 142.9, 136.8, 136.2, 131.9, 131.2, 129.8, 129.6, 128.6, 128.30, 128.25, 128.1, 127.7, 126.4, 125.4, 124.1, 120.4, 116.2, 115.7, 77.5, 77.2, 76.8, 67.0, 61.7, 53.5, 35.6, 13.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{33}H_{30}N_3O_8S_2$]$^+$: 660.1474; found: 660.1455.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(1-((2,4-dinitrophenyl)sulfonyl)-2-phenyl-1*H*-indol-3-yl)-L-cysteinate (11d)

Prepared from **1** (200 mg, 0.71 mmol), NCS (114 mg, 0.85 mmol), alkyne **10e** (150 mg, 0.35 mmol) and CH_2Cl_2 (10 mL).

Yield: 200 mg (80%); yellow solid; $[\alpha]_D^{20}$ +35.5 (*c* 1.0, $CHCl_3$).

IR (film): 3404, 2928, 1723, 1554, 1347, 1181 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 2.3 Hz, 1 H), 8.11–8.04 (m, 2 H), 7.76 (dd, *J* = 6.1, 3.0 Hz, 1 H), 7.46–7.41 (m, 2 H), 7.39–7.30 (m, 5 H), 7.28–7.20 (m, 6 H), 5.15 (d, *J* = 7.8 Hz, 1 H), 4.98 (d, *J* = 12.2 Hz, 1 H), 4.88 (d, *J* = 12.2 Hz, 1 H), 4.39–4.30 (m, 1 H), 3.91–3.79 (m, 1 H), 3.60–3.47 (m, 1 H), 3.25 (dd, *J* = 14.0, 4.2 Hz, 1 H), 3.03 (dd, *J* = 14.0, 5.2 Hz, 1 H), 1.00 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9, 155.4, 150.1, 147.8, 141.9, 137.6, 137.3, 136.2, 132.6, 132.3, 130.04 129.96, 129.0, 128.6, 128.3, 128.1, 128.0, 126.6, 126.2, 125.2, 120.4, 120.3, 116.0, 115.1, 77.5, 77.2, 76.8, 67.1, 61.9, 53.5, 35.9, 13.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{33}H_{29}N_4O_{10}S_2$]$^+$: 705.1325; found: 705.1318.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(2-phenylbenzofuran-3-yl)-L-cysteinate (11h)

Prepared from **1** (438 mg, 1.55 mmol), NCS (248 mg, 1.85 mmol), alkyne **10j** (150 mg, 0.77 mmol) and CH_2Cl_2 (10 mL).

Yield: 160 mg (44%); white solid; $[\alpha]_D^{20}$ +42.8 (c 1.0, $CHCl_3$).

IR (film): 3337, 2981, 1736, 1501, 1202, 1067 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 7.1 Hz, 2 H), 7.73–7.64 (m, 1 H), 7.54–7.29 (m, 11 H), 5.42 (d, *J* = 7.7 Hz, 1 H), 5.00 (d, *J* = 12.2 Hz, 1 H), 4.91 (d, *J* = 12.2 Hz, 1 H), 4.55–4.45 (m, 1 H), 3.99–3.86 (m, 1 H), 3.77–3.63 (m, 1 H), 3.44–3.32 (m, 2 H), 1.02 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.0, 156.4, 155.5, 153.7, 136.3, 131.0, 129.9, 129.4, 128.7, 128.6, 128.3, 128.1, 127.5, 125.4, 123.6, 120.1, 111.5, 106.3, 67.0, 61.9, 53.9, 36.8, 13.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{27}H_{26}NO_5S]^+$: 476.1532; found: 476.1536.

Indoles and Benzo[*b*]furans 11i–o; General Procedure

To a solution of NCS (1.2 equiv) in CH_2Cl_2 was added a solution of S–H-bond-containing peptide (1 equiv) in CH_2Cl_2 at 0 °C. The mixture was stirred for 1 h, then a solution of the corresponding alkyne (1 equiv) in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by reverse-flash chromatography (MeCN/H_2O, 30–85%) to give the product.

Benzyl *N*-(*tert*-Butoxycarbonyl)-*R*-(2-phenyl-1-tosyl-1*H*-indol-3-yl)-L-cysteinylglycinate (11i)

Prepared from **7** (100 mg, 0.27 mmol), NCS (43 mg, 0.33 mmol), alkyne **10a** (94 mg, 0.27 mmol) and CH_2Cl_2 (10 mL).

Yield: 103 mg (53%); white solid; $[\alpha]_D^{20}$ +23.0 (*c* 1.0, $CHCl_3$).

IR (film): 3340, 2978, 1747, 1680, 1522, 1449, 1178 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.29 (d, *J* = 8.3 Hz, 1 H), 7.68 (ddd, *J* = 7.7, 1.3, 0.8 Hz, 1 H), 7.49–7.25 (m, 14 H), 7.17 (d, *J* = 7.9 Hz, 2 H), 5.11 (s, 2 H), 3.89 (dd, *J* = 8.2, 5.0 Hz, 1 H), 3.76 (s, 2 H), 2.87 (dd, *J* = 13.5, 5.0 Hz, 1 H), 2.71 (dd, *J* = 13.5, 8.2 Hz, 1 H), 2.30 (s, 3 H), 1.38 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.2, 170.6, 157.2, 146.9, 145.3, 138.3, 137.1, 136.3, 133.1, 132.7, 131.9, 130.7, 130.1, 129.5, 129.3, 128.4, 127.9, 126.7, 125.7, 121.1, 117.1, 116.2, 80.9, 67.9, 55.6, 42.1, 37.5, 28.7, 21.5.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{38}H_{39}N_3O_7S_2Na]^+$: 736.2127; found: 736.2094.

Benzyl *N*-(*tert*-Butoxycarbonyl)-*R*-(1-((4-nitrophenyl)sulfonyl)-2-phenyl-1*H*-indol-3-yl)-L-cysteinylglycinate (11j)

Prepared from **7** (100 mg, 0.27 mmol), NCS (44 mg, 0.33 mmol), alkyne **10c** (103 mg, 0.27 mmol) and CH_2Cl_2 (10 mL).

Yield: 54 mg (27%); yellow solid; $[\alpha]_D^{20}$ +17.4 (*c* 1.0, $CHCl_3$).

IR (film): 3335, 2927, 1695, 1533, 1183, 758 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.33 (d, *J* = 8.3 Hz, 1 H), 8.31–8.20 (m, 2 H), 7.76–7.64 (m, 3 H), 7.55–7.38 (m, 7 H), 7.38–7.27 (m, 5 H), 5.14 (s, 2 H), 3.88 (dd, *J* = 8.2, 4.9 Hz, 1 H), 3.78 (s, 2 H), 2.94 (dd, *J* = 13.7, 4.9 Hz, 1 H), 2.74 (dd, *J* = 13.7, 8.2 Hz, 1 H), 1.40 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.0, 169.9, 169.1, 150.8, 143.3, 142.9, 136.9, 135.3, 132.0, 131.3, 130.5, 129.9, 129.8, 128.80, 128.76, 128.6, 128.2, 127.9, 126.5, 125.5, 124.3, 120.4, 116.4, 116.2, 80.7, 77.5, 77.2, 76.8, 67.4, 41.4, 41.3, 28.3.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{37}H_{36}N_4O_9S_2Na]^+$: 767.1816; found: 767.1827.

tert-Butyl (*R*)-3-((3-((2-(Benzyloxy)-2-oxoethyl)amino)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)thio)-2-phenyl-1*H*-indole-1-carboxylate (11k)

Prepared from **7** (200 mg, 0.54 mmol), NCS (87 mg, 0.65 mmol), alkyne **10b** (158 mg, 0.54 mmol) and CH_2Cl_2 (10 mL).

Yield: 89 mg (25%); colorless oil; $[\alpha]_D^{20}$ +9.3 (*c* 1.0, $CHCl_3$).

IR (film): 3331, 2979, 2481, 1733, 1456, 1340, 1178 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (d, *J* = 8.2 Hz, 1 H), 7.75–7.72 (m, 1 H), 7.48–7.26 (m, 12 H), 5.12 (s, 2 H), 3.99–3.93 (m, 1 H), 3.83–3.75 (ABm, 2 H), 2.95 (dd, *J* = 13.6, 4.7 Hz, 1 H), 2.77 (dd, *J* = 13.6, 8.3 Hz, 1 H), 1.38 (s, 9 H), 1.21 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.5, 170.7, 157.3, 150.9, 144.4, 137.8, 137.1, 134.9, 131.9, 131.4, 129.5, 129.31, 129.25, 129.0, 126.2, 124.5, 120.6, 116.2, 112.5, 84.9, 80.9, 67.9, 55.8, 42.1, 38.0, 28.7, 27.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{36}H_{41}N_3O_7SNa]^+$: 682.2563; found: 682.2556.

Benzyl *N*-(*tert*-Butoxycarbonyl)-*R*-(2-phenylbenzofuran-3-yl)-L-cysteinylglycinate (11l)

Prepared from **7** (200 mg, 0.54 mmol), NCS (87 mg, 0.65 mmol), alkyne **10k** (113 mg, 0.54 mmol) and CH_2Cl_2 (10 mL).

Yield: 200 mg (66%); white solid; $[\alpha]_D^{20}$ –2.4 (*c* 1.0, $CHCl_3$).

IR (film): 3315, 2977, 1749, 1684, 1528, 1169 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31–8.19 (m, 2 H), 7.74–7.64 (m, 1 H), 7.61–7.43 (m, 3 H), 7.45–7.28 (m, 8 H), 5.15 (s, 2 H), 4.19 (s, 1 H), 3.94–3.71 (m, 2 H), 3.35–3.20 (m, 1 H), 3.18 (dd, *J* = 13.7, 5.7 Hz, 1 H), 1.33 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.34, 170.28, 169.27, 169.24, 153.8, 135.2, 131.0, 130.0, 129.4, 128.8, 128.7, 128.5, 127.6, 125.5, 123.7, 120.0, 111.6, 106.6, 80.7, 67.3, 54.4, 41.5, 41.3, 28.2.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{31}H_{32}N_2O_6SNa]^+$: 583.1879; found: 583.1876.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenyl-1-tosyl-1*H*-indol-3-yl)thio)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (11m)

Prepared from **8** (150 mg, 0.27 mmol), NCS (43 mg, 0.33 mmol), alkyne **10a** (94 mg, 0.27 mmol) and CH_2Cl_2 (10 mL). The product was isolated after treatment with TFA.

Yield: 120 mg (60%); colorless oil.

IR (film): 3063, 1740, 1653, 1375, 1178 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.29 (d, *J* = 8.2 Hz, 1 H), 7.67–7.63 (m, 1 H), 7.48–7.26 (m, 13 H), 7.15 (d, *J* = 8.8 Hz, 2 H), 5.14–5.05 (m, 2 H), 3.98 (dd, *J* = 9.9, 4.5 Hz, 1 H), 3.76 (s, 2 H), 3.54 (t, *J* = 6.5 Hz, 1 H), 3.02 (dd, *J* = 13.8, 4.5 Hz, 1 H), 2.64 (dd, *J* = 13.8, 9.9 Hz, 1 H), 2.39–2.30 (m, 1 H), 2.28 (s, 3 H), 2.25 (s, 1 H), 2.15–1.99 (m, 1 H), 2.01–1.86 (m, 1 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.8, 173.1, 170.8, 146.8, 145.7, 138.4, 137.0, 136.1, 133.2, 132.6, 131.7, 130.7, 130.2, 129.5, 129.34, 129.29, 128.4, 127.9, 126.8, 125.8, 121.1, 117.2, 115.6, 67.9, 55.2, 54.6, 42.0, 36.8, 32.6, 27.6, 21.5.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{38}H_{39}N_4O_8S_2]^+$: 743.2204; found: 743.2214.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((1-((4-nitrophenyl)sulfonyl)-2-phenyl-1*H*-indol-3-yl)thio)-1-oxopropan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (11n)

Prepared from **8** (140 mg, 0.25 mmol), NCS (41 mg, 0.3 mmol), alkyne **10c** (95 mg, 0.25 mmol) and CH_2Cl_2 (10 mL). The product was isolated after treatment with TFA.

Yield: 53 mg (20%); yellow solid.

IR (film): 3283, 1743, 1653, 1533, 1183, 752 cm^{-1}.

^1H NMR (400 MHz, CD_3OD): δ = 8.30 (d, *J* = 8.3 Hz, 1 H), 8.24–8.16 (m, 2 H), 7.70–7.58 (m, 3 H), 7.53–7.35 (m, 7 H), 7.33–7.25 (m, 5 H), 5.12–5.08 (ABm, 2 H), 3.95 (dd, *J* = 10.0, 4.4 Hz, 1 H), 3.76 (s, 2 H), 3.54 (dd, *J* = 7.3, 6.0 Hz, 1 H), 3.06 (dd, *J* = 13.8, 4.4 Hz, 1 H), 2.67 (dd, *J* = 13.8, 10.0 Hz, 1 H), 2.42–2.30 (m, 1 H), 2.29–2.20 (m, 1 H), 2.14–2.01 (m, 1 H), 1.97–1.86 (m, 1 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.9, 173.9, 173.0, 170.8, 152.3, 145.5, 143.7, 138.3, 137.1, 133.1, 132.8, 131.3, 130.5, 129.5, 129.34, 129.31, 128.6, 127.3, 126.4, 125.5, 121.4, 117.2, 117.0, 67.9, 55.2, 54.6, 42.0, 36.7, 32.6, 27.6.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{37}H_{36}N_5O_{10}S_2]^+$: 774.1898; found: 774.1904.

***tert*-butyl N^5-((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-phenylbenzofuran-3-yl)thio)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (11o)**

Prepared from **8** (140 mg, 0.25 mmol), NCS (41 mg, 0.3 mmol), alkyne **10k** (53 mg, 0.25 mmol) and CH_2Cl_2 (10 mL).

Yield: 62 mg (33%); white solid.

IR (film): 3289, 2977, 1719, 1700, 1653, 1507, 1155, 748 cm^{-1}.

^1H NMR (400 MHz, CD_3OD): δ = 8.32–8.22 (m, 2 H), 7.77–7.66 (m, 1 H), 7.62–7.23 (m, 11 H), 5.10 (s, 2 H), 4.34 (dd, *J* = 9.4, 4.8 Hz, 1 H), 3.96–3.87 (m, 1 H), 3.86–3.76 (ABm, 2 H), 3.38–3.32 (m, 1 H), 3.01 (dd, *J* = 13.7, 9.4 Hz, 1 H), 2.25–2.09 (m, 1 H), 2.05–1.82 (m, 2 H), 1.82–1.60 (m, 1 H), 1.46 (s, 9 H), 1.44 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.4, 171.5, 170.1, 169.3, 156.4, 155.9, 153.8, 135.3, 131.0, 130.0, 129.5, 128.8, 128.7, 128.6, 128.5, 127.7, 125.5, 123.7, 120.1, 111.6, 106.7, 82.4, 80.1, 67.2, 53.4, 53.0, 41.4, 41.3, 36.0, 32.0, 28.4, 28.1.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{40}H_{47}N_3O_9SNa]^+$: 768.2925; found: 768.2933.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(1-chloro-1-(2-((4-nitrophenyl)sulfonamido)phenyl)hex-1-en-2-yl)-L-cysteinate (12a)

To a solution of NCS (85 mg, 0.63 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL) was added a solution of *N*-Cbz-cysteine ethyl ester **1** (150 mg, 0.53 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 1 h, then a solution of alkyne **10d** (100 mg, 0.26 mmol, 0.5 equiv) in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by flash chromatography (PE/EtOAc, 10–70%) to give the product as a yellow solid (78 mg, 44%).

^1H NMR (400 MHz, $CDCl_3$): δ = 8.25–8.13 (m, 2 H), 8.00–7.86 (m, 2 H), 7.67–7.60 (m, 1 H), 7.41–7.28 (m, 6 H), 7.20–7.04 (m, 2 H), 5.22–5.09 (m, 2 H), 4.51–4.39 (m, 1 H), 4.32–4.09 (m, 2 H), 3.23–3.08 (m, 1 H), 3.00–2.82 (m, 1 H), 2.68–2.53 (m, 1 H), 2.49–2.27 (m, 1 H), 1.62–1.46 (m, 2 H), 1.45–1.34 (m, 2 H), 1.32–1.20 (m, 3 H), 1.03–0.92 (m, 3 H).

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{31}H_{35}N_3O_8S_2Cl]^+$: 676.1549; found: 676.1575.

Ethyl *N*-((Benzyloxy)carbonyl)-*R*-(1-chloro-1-(2-((2,4-dinitrophenyl)sulfonamido)phenyl)hex-1-en-2-yl)-L-cysteinate (12b)

To a solution of NCS (75 mg, 0.57 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL) was added a solution of *N*-Cbz-cysteine ethyl ester **1** (134 mg, 0.47 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 1 h, then a solution of alkyne **10f** (100 mg, 0.24 mmol, 0.5 equiv) in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by flash chromatography (PE/EtOAc, 10–70%) to give the product as a yellow oil (71 mg, 42%).

^1H NMR (400 MHz, $CDCl_3$): δ = 8.66–8.62 (m, 1 H), 8.35 (dd, *J* = 8.6, 2.3 Hz, 1 H), 8.03 (dd, *J* = 8.6, 3.7 Hz, 1 H), 7.67–7.62 (m, 1 H), 7.40–7.32 (m, 6 H), 7.26–7.19 (m, 1 H), 7.16–7.10 (m, 1 H), 5.17–5.07 (m, 2 H), 4.53–4.42 (m, 1 H), 4.30–4.12 (m, 2 H), 3.20–3.06 (m, 2 H), 2.89 (dd, *J* = 13.7, 6.0 Hz, 1 H), 2.61–2.49 (m, 1 H), 2.40–2.27 (m, 1 H), 1.54–1.46 (m, 2 H), 1.41–1.33 (m, 2 H), 1.29–1.20 (m, 3 H), 0.94 (t, *J* = 7.2 Hz, 3 H).

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{31}H_{34}N_4O_{10}S_2Cl]^+$: 721.1399; found: 721.1407.

1-(2-(Phenylethynyl)phenyl)pyrene (13d)

1-Bromo-2-(phenylethynyl)benzene (0.6 g, 2.33 mmol, 1 equiv), 1-pyrenylboronic acid (0.86 g, 3.5 mmol, 1.5 equiv), $Pd(PPh_3)_4$ (0.135 g, 0.167 mmol, 0.05 equiv), Na_2CO_3 (0.49 g, 4.67 mmol, 2 equiv) and a mixture of solvents [EtOH (6 mL), H_2O (6 mL), toluene (12 mL)] were added sequentially into a high-pressure vial. The resulting mixture was stirred at 70 °C for 8 h. Next, the mixture was cooled to rt and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (PE/EtOAc, 2–20%) to afford the product as a yellow solid (0.64 g, 72%).

^1H NMR (400 MHz, $CDCl_3$): δ = 8.28 (d, *J* = 7.8 Hz, 1 H), 8.23 (dd, *J* = 7.8, 1.2 Hz, 1 H), 8.18 (dd, *J* = 7.8, 1.2 Hz, 1 H), 8.14 (d, *J* = 1.8 Hz, 2 H), 8.11 (d, *J* = 7.8 Hz, 1 H), 8.07–7.97 (m, 3 H), 7.84–7.76 (m, 1 H), 7.63–7.56 (m, 1 H), 7.59–7.45 (m, 2 H), 7.11–7.02 (m, 1 H), 7.01–6.91 (m, 2 H), 6.79–6.71 (m, 2 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.5, 136.4, 132.3, 131.4, 131.3, 131.1, 131.0, 130.9, 129.1, 128.18, 128.15, 127.93, 127.87, 127.52, 127.49, 127.3, 126.0, 125.8, 125.1, 125.0, 124.9, 124.8, 124.3, 123.9, 123.0, 93.0, 89.2.

HRMS (ESI/Q-TOF): *m*/*z* [M]$^+$ calcd for $[C_{30}H_{18}]$: 378.1409; found: 378.1403.

S-Peptide-Containing Polyaromatic Hydrocarbons 14a–f; General Procedure

To a solution of *N*-chlorosuccinimide (2.2 equiv) in CH_2Cl_2 (3 mL) was added a solution of S–S- bond-containing peptide (1 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 1 h, then a solution of the corresponding alkyne (1 equiv) in CH_2Cl_2 (3 mL) was added. The reaction mixture was stirred until the starting material had disappeared and was then evaporated. The residue was purified by flash chromatography (PE/EtOAc, 10–85%) (**14b** and **14d**). Products **14a**, **14c** and **14e**,**f** were isolated after treatment with TFA and purified by reverse-phase chromatography (MeOH/H_2O, 10–85%).

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((2-methyl-10-phenylphenanthren-9-yl)thio)-1-oxopropan-2-aminium 2,2,2-Trifluoroacetate (14a)

Prepared from **5** (200 mg, 0.27 mmol), NCS (79 mg, 0.59 mmol) and alkyne **13a** (73 mg, 0.33 mmol).

Yield: 127 mg (72%); colorless oil; $[\alpha]_D^{20}$ +41.2 (*c* 1.0, MeOH).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.87–8.80 (m, 1 H), 8.82–8.75 (m, 1 H), 8.74 (d, *J* = 8.5 Hz, 1 H), 7.79–7.70 (m, 2 H), 7.61–7.47 (m, 4 H), 7.42–7.22 (m, 7 H), 7.20–7.15 (m, 1 H), 5.17–5.05 (ABm, 2 H), 3.86–3.73 (m, 3 H), 3.10 (dd, *J* = 13.8, 5.8 Hz, 1 H), 3.00 (dd, *J* = 13.8, 7.3 Hz, 1 H), 2.37 (s, 3 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 170.4, 168.6, 147.5, 141.6, 138.1, 136.9, 133.3, 132.7, 132.4, 131.7, 131.6, 130.7, 130.3, 129.58, 129.55, 129.43, 129.39, 128.93, 128.85, 128.81, 128.50, 128.48, 128.3, 124.2, 123.9, 68.1, 53.9, 42.1, 38.4, 21.6.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{33}H_{31}N_2O_3S]^+$: 535.2050; found: 535.2071.

Benzyl *N*-(*tert*-Butoxycarbonyl)-*S*-(6-phenylbenzo[*c*]phenanthren-5-yl)-L-cysteinylglycinate (14b)

Prepared from **5** (241 mg, 0.33 mmol), NCS (96 mg, 0.72 mmol) and alkyne **13b** (120 mg, 0.33 mmol).

Yield: 200 mg (91%); light yellow solid; $[\alpha]_D^{20}$ +26.7 (*c* 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, CD_3OD): δ = 9.01 (t, *J* = 8.8 Hz, 2 H), 8.95–8.89 (m, 1 H), 8.00–7.93 (m, 1 H), 7.77–7.58 (m, 5 H), 7.55–7.43 (m, 3 H), 7.38–7.17 (m, 8 H), 5.06 (s, 2 H), 4.03–3.95 (m, 1 H), 3.82–3.70 (ABm, 2 H), 2.99 (dd, *J* = 13.6, 4.6 Hz, 1 H), 2.88 (dd, *J* = 13.6, 8.4 Hz, 1 H), 1.27 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.5, 170.6, 157.2, 146.3, 141.6, 137.0, 134.9, 134.8, 131.9, 131.8, 131.7, 131.4, 130.7, 129.72, 129.66, 129.5, 129.4, 129.33, 129.26, 128.6, 128.3, 128.1, 127.7, 127.41, 127.37, 126.1, 80.8, 67.8, 55.9, 42.1, 39.2, 28.6.

HRMS (ESI/Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $[C_{41}H_{38}N_2O_5SNa]^+$: 693.2399; found: 693.2397.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((2-methyl-10-phenylphenanthren-9-yl)thio)-1-oxopropan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (14c)

Prepared from **6a** (200 mg, 0.18 mmol), NCS (53 mg, 0.40 mmol) and alkyne **13a** (48 mg, 0.18 mmol).

Yield: 91 mg (65%); colorless oil.

^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$): δ = 8.82–8.71 (m, 2 H), 8.66 (d, *J* = 8.5 Hz, 1 H), 7.74–7.62 (m, 2 H), 7.55–7.41 (m, 4 H), 7.38–7.20 (m, 7 H), 7.15 (s, 1 H), 5.13–5.02 (ABm, 2 H), 4.14 (dd, *J* = 9.6, 4.5 Hz, 1 H), 3.80 (s, 2 H), 3.52 (t, *J* = 6.5 Hz, 1 H), 3.05 (dd, *J* = 13.5, 4.5 Hz, 1 H), 2.80 (dd, *J* = 13.5, 9.6 Hz, 1 H), 2.42–2.33 (m, 4 H), 2.35–2.21 (m, 2 H), 2.11–1.98 (m, 1 H), 2.00–1.87 (m, 1 H).

^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$): δ = 174.5, 172.8, 170.4, 146.8, 141.3, 137.4, 136.4, 133.0, 132.8, 131.8, 131.31, 131.25, 130.0, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.6, 128.3, 128.1, 127.83, 127.81, 123.6, 123.4, 67.7, 54.8, 54.6, 41.8, 38.4, 32.5, 27.1, 21.7.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{38}H_{38}N_3O_6S]^+$: 664.2476; found: 664.2502.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((6-phenylbenzo[*c*]phenanthren-5-yl)thio)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (14d)

Prepared from **6a** (200 mg, 0.18 mmol), NCS (53 mg, 0.40 mmol) and alkyne **13b** (55 mg, 0.18 mmol).

Yield: 102 mg (81%); light yellow solid.

^{1}H NMR (400 MHz, CD_3OD): δ = 9.12–8.99 (m, 2 H), 8.99–8.89 (m, 1 H), 8.01 (d, *J* = 7.4 Hz, 1 H), 7.83–7.62 (m, 5 H), 7.59–7.45 (m, 3 H), 7.44–7.20 (m, 8 H), 5.08 (s, 2 H), 4.16 (dd, *J* = 9.3, 4.6 Hz, 1 H), 3.93 (t, *J* = 6.4 Hz, 1 H), 3.84–3.68 (m, 2 H), 3.09 (dd, *J* = 13.8, 4.6 Hz, 1 H), 2.86 (dd, *J* = 13.8, 9.3 Hz, 1 H), 2.48–2.27 (m, 2 H), 2.14–1.97 (m, 2 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.1, 173.0, 171.4, 170.7, 146.6, 141.6, 137.0, 135.0, 134.7, 132.0, 131.9, 131.8, 131.4, 130.7, 130.2, 129.84, 129.82, 129.6, 129.5, 129.4, 129.33, 129.26, 128.6, 128.3, 128.24, 128.18, 128.0, 127.8, 127.51, 127.48, 126.1, 67.9, 65.2, 54.8, 53.4, 42.0, 41.7, 38.6, 32.4, 26.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{41}H_{38}N_3O_6S]^+$: 700.2476; found: 700.2479.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((5-phenylbenzo[*g*]chrysen-6-yl)thio)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (14e)

Prepared from **6a** (105 mg, 0.095 mmol), NCS (28 mg, 0.21 mmol) and alkyne **13c** (34 mg, 0.095 mmol).

Yield: 33 mg (40%); light yellow oil.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.80 (dd, *J* = 8.4, 1.4 Hz, 1 H), 8.78–8.68 (m, 3 H), 8.60 (d, *J* = 8.2 Hz, 1 H), 7.78–7.59 (m, 4 H), 7.49–7.34 (m, 6 H), 7.34–7.21 (m, 6 H), 7.03–6.94 (m, 1 H), 5.10–4.96 (ABm, 2 H), 3.74 (dd, *J* = 9.5, 4.5 Hz, 1 H), 3.65 (s, 2 H), 3.42 (t, *J* = 6.6 Hz, 1 H), 2.99 (dd, *J* = 13.9, 4.5 Hz, 1 H), 2.74 (dd, *J* = 13.9, 9.5 Hz, 1 H), 2.28–2.16 (m, 1 H), 2.16–2.05 (m, 1 H), 1.99–1.88 (m, 1 H), 1.86–1.76 (m, 1 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.6, 173.1, 170.7, 144.1, 137.1, 134.3, 133.7, 133.4, 132.8, 132.1, 132.0, 131.3, 131.2, 131.04, 130.96, 130.4, 130.17, 130.13, 129.5, 129.4, 129.3, 129.3, 129.0, 128.7, 128.6, 128.4, 128.3, 127.8, 127.4, 126.5, 124.7, 124.2, 67.8, 55.2, 54.7, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 41.9, 38.2, 32.7, 27.5.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for $[C_{45}H_{40}N_3O_6S]^+$: 750.2632; found: 750.2643.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((12-phenylbenzo[*pqr*]picen-11-yl)thio)propan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium Acetate (14f)

Prepared from **6a** (200 mg, 0.18 mmol), NCS (53 mg, 0.40 mmol) and alkyne **13d** (110 mg, 0.18 mmol).

Yield: 50 mg (36%); yellow oil.

^{1}H NMR (400 MHz, CD_3OD): δ = 9.15 (d, *J* = 9.3 Hz, 1 H), 8.97 (d, *J* = 8.1 Hz, 1 H), 8.91 (d, *J* = 8.1 Hz, 1 H), 8.22 (d, *J* = 8.7 Hz, 2 H), 8.11 (d, *J* = 7.5 Hz, 1 H), 8.00 (t, *J* = 7.6 Hz, 1 H), 7.93–7.80 (m, 2 H), 7.82–7.62 (m, 3 H), 7.62–7.50 (m, 3 H), 7.42–7.26 (m, 2 H), 7.28–7.15 (m, 5 H), 5.03 (s, 2 H), 4.17 (dd, *J* = 9.3, 4.5 Hz, 1 H), 3.91 (t, *J* = 6.3 Hz, 1 H), 3.80–3.67 (ABm, 2 H), 3.12 (dd, *J* = 13.8, 4.5 Hz, 1 H), 2.90 (dd, *J* = 13.8, 9.3 Hz, 1 H), 2.47–2.27 (m, 2 H), 2.12–1.97 (m, 2 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.1, 172.9, 171.5, 170.6, 146.8, 141.6, 137.0, 133.9, 132.9, 132.3, 132.2, 132.1, 131.7, 131.0, 130.8, 130.7, 130.2, 129.5, 129.40, 129.35, 129.3, 129.2, 129.1, 128.8, 128.7, 128.4, 128.2, 128.0, 127.72, 127.66, 127.4, 126.9, 126.4, 126.2, 125.7, 124.7, 67.9, 54.8, 53.5, 42.0, 38.5, 32.4, 26.9, 22.1.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{47}H_{40}N_3O_6S$]$^+$: 774.2632; found: 774.2645.

(*S*)-1-Carboxy-4-(((*R*)-1-((carboxymethyl)amino)-1-oxo-3-((7-phenyldibenzo[*m*,*pqr*]tetraphen-8-yl)thio)propan-2-yl)amino)-4-oxobutan-1-aminium Acetate (15)

To a solution of **14f** (30 mg) in MeOH (2 mL) and THF (1 mL) was added Pd/C and then H_2 was bubbled through the reaction mixture for 1 h. After filtration and evaporation, product **15** was obtained as a yellow solid (16 mg, 60%).

^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$): δ = 9.19 (d, *J* = 9.3 Hz, 1 H), 9.09–9.02 (m, 1 H), 8.91–8.82 (m, 1 H), 8.20 (dd, *J* = 12.7, 8.5 Hz, 2 H), 8.08 (d, *J* = 7.0 Hz, 1 H), 8.02–7.93 (m, 2 H), 7.90 (d, *J* = 9.0 Hz, 1 H), 7.79 (d, *J* = 9.0 Hz, 1 H), 7.75–7.66 (m, 2 H), 7.56–7.47 (m, 3 H), 7.42–7.36 (m, 1 H), 7.34–7.28 (m, 1 H), 4.17–4.09 (m, 2 H), 3.78–3.62 (m, 2 H), 3.11 (dd, *J* = 13.5, 5.1 Hz, 1 H), 2.92 (dd, *J* = 13.5, 8.8 Hz, 1 H), 2.41–2.17 (m, 2 H), 2.09–1.99 (m, 2 H).

^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$): δ = 173.3, 172.0, 171.3, 145.7, 140.5, 132.7, 131.8, 131.09, 131.05, 130.8, 129.9, 129.8, 129.7, 129.0, 128.4, 128.1, 127.8, 127.39, 127.36, 127.2, 127.1, 126.8, 126.6, 126.4, 126.0, 125.4, 125.2, 124.9, 124.8, 124.0, 53.8, 37.2, 31.7, 25.6, 1.6.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{40}H_{34}N_3O_6S$]$^+$: 684.2163; found: 684.2170.

(*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((1-oxo-3-phenyl-1*H*-isochromen-4-yl)thio)propan-2-aminium 2,2,2-Trifluoroacetate (17a)

To a solution of NCS (43 mg, 0.33 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL) at 0 °C was added a solution of **7** (100 mg, 0.27 mmol, 1 equiv) in CH_2Cl_2 (4 mL). The mixture was stirred for 30 min, then a solution of alkyne **16** (64 mg, 0.27 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added and the mixture was stirred for 1 h. TFA (1 mL) was added and the mixture was stirred for 1 h. After evaporation, the mixture was purified by reverse-phase chromatography (C-18, MeCN/H_2O, 15–85%) to provide the product as a colorless oil (84 mg, 53%).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.33–8.25 (m, 2 H), 8.00–7.92 (m, 1 H), 7.85–7.78 (m, 2 H), 7.71–7.62 (m, 1 H), 7.57–7.48 (m, 3 H), 7.38–7.24 (m, 5 H), 5.19–5.07 (ABm, 2 H), 3.84 (t, *J* = 6.3 Hz, 1 H), 3.78 (s, 2 H), 3.06–2.95 (m, 2 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 170.3, 168.3, 162.8, 160.6, 139.0, 137.0, 136.9, 134.2, 131.6, 131.3, 130.7, 130.2, 129.6, 129.5, 129.44, 129.42, 126.8, 122.0, 109.4, 68.1, 53.5, 42.0, 37.4.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{27}H_{25}N_2O_5S$]$^+$: 489.1479; found: 489.1494.

(7*R*,12*S*)-15,15-Dimethyl-3,6,9,13-tetraoxo-7-(((1-oxo-3-phenyl-1*H*-isochromen-4-yl)thio)methyl)-1-phenyl-2,14-dioxa-5,8-diazahexadecan-12-aminium 2,2,2-Trifluoroacetate (17b)

To a solution of NCS (29 mg, 0.22 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL) at 0 °C was added a solution of **8** (100 mg, 0.18 mmol, 1 equiv) in CH_2Cl_2 (4 mL). The mixture was stirred for 30 min, then a solution of alkyne **16** (42 mg, 0.18 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added and the mixture was stirred for 1 h. TFA (1 mL) was added and the mixture was stirred for 1 h. After evaporation, the mixture was purified by reverse-phase chromatography (C-18, MeCN/H_2O, 15–85%) to provide the product as a colorless oil (57 mg, 40%).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.32–8.28 (m, 1 H), 8.25 (d, *J* = 8.0 Hz, 1 H), 7.95–7.89 (m, 1 H), 7.80–7.75 (m, 2 H), 7.67–7.61 (m, 1 H), 7.52–7.42 (m, 3 H), 7.36–7.26 (m, 5 H), 5.15–5.04 (m, 2 H), 4.15 (dd, *J* = 9.3, 5.0 Hz, 1 H), 3.87 (t, *J* = 6.4 Hz, 1 H), 3.78 (s, 2 H), 2.95 (dd, *J* = 13.8, 5.0 Hz, 1 H), 2.71 (dd, *J* = 13.8, 9.3 Hz, 1 H), 2.39–2.25 (m, 1 H), 2.23–2.13 (m, 1 H), 2.09–1.98 (m, 2 H), 1.52 (s, 9 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 173.8, 172.8, 170.6, 169.3, 163.1, 160.5, 139.4, 137.0, 136.7, 134.4, 131.5, 131.2, 130.7, 130.0, 129.6, 129.4, 129.3, 129.1, 126.9, 122.0, 109.7, 85.5, 67.9, 53.99, 53.95, 42.0, 37.5, 31.9, 28.2, 26.9.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{36}H_{40}N_3O_8S$]$^+$: 674.2531; found: 674.2543.

(*S*)-4-(((*R*)-1-((2-(Benzyloxy)-2-oxoethyl)amino)-3-((1-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)thio)-1-oxopropan-2-yl)amino)-1-carboxy-4-oxobutan-1-aminium 2,2,2-Trifluoroacetate (21)

To a solution of NCS (29 mg, 0.22 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL) at 0 °C was added a solution of **8** (100 mg, 0.18 mmol, 1 equiv) in CH_2Cl_2 (4 mL). The mixture was stirred for 30 min, then a solution of alkyne **20** (42 mg, 0.18 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added and the mixture was stirred for 1 h. TFA (1 mL) was added and the mixture was stirred for 1 h. After evaporation, the mixture was purified by reverse-phase chromatography (C-18, MeCN/H_2O, 15–85%) to provide the product as a colorless oil (45 mg, 44%).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.69–7.63 (m, 2 H), 7.54–7.47 (m, 3 H), 7.36–7.29 (m, 4 H), 7.26–7.22 (m, 2 H), 7.21–7.17 (m, 2 H), 7.13–7.09 (m, 1 H), 5.11 (s, 2 H), 4.30 (dd, *J* = 9.2, 4.5 Hz, 1 H), 4.04 (t, *J* = 6.4 Hz, 1 H), 3.92–3.89 (m, 2 H), 3.87 (s, 3 H), 3.30–3.25 (m, 1 H), 2.97 (dd, *J* = 13.8, 9.2 Hz, 1 H), 2.58–2.46 (m, 2 H), 2.27–2.10 (m, 2 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 174.3, 173.2, 171.5, 170.7, 162.7, 157.1, 140.7, 138.3, 137.1, 132.6, 130.3, 129.8, 129.7, 129.52, 129.50, 129.4, 129.3, 126.1, 123.8, 122.7, 116.1, 67.9, 54.9, 53.5, 42.1, 36.1, 32.5, 31.4, 27.0.

HRMS (ESI/Q-TOF): *m*/*z* [M + H]$^+$ calcd for [$C_{33}H_{35}N_4O_7S$]$^+$: 631.2221; found: 631.2242.

Evaluation of Cell Permeability and Fluorescence

Rat embryo myoblast cells lines H9C2 (2-1) were purchased from ATCC (ATCC® CRL-1446™, Rockville, MD) and maintained in DMEM supplemented with 10% FBS at 37 °C in humidified air containing 5% CO_2. All the media and serums were purchased from Sigma-Aldrich. During log-phase growth, the cells were collected and transferred to a 4 well on lumox® slide (Sarstedt) at a concentration of 50000 cells/mL and incubated for 72 h at 37 °C in humidified air containing 5% CO_2. The medium was then exchanged with a medium containing compound **15** at a concentration of 10 μM and incubated for 17 h. The medium was removed and washed once with fresh medium. Aspirate culture media and an equal volume of 10 μM Nile Red Staining Solution were added followed by incubation at 37 °C/5% CO_2 for 30 min. The Nile Red Staining Solution was removed and the wells were washed with PBS. Cells were photographed using a Nikon Eclipse TE300 inverted fluorescence microscope with a Nikon G-2A filter (EX 510-560, DM 575, BA 590) for Nile Red and a Nikon UV-2A filter (EX 330-380, DM 400, BA 420) for **15** (magnification: ×100).

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Supporting Information

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Visible Light-Mediated Functionalization of Selenocystine-Containing Peptides

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Abstract: A straightforward and atom-economic method for the functionalization of short selenocystine-containing peptides is presented. This method is shown to be tolerant to unprotected peptides. The detailed protocol is based on the generation of a selenium radical via visible light-initiated reaction in the presence of transition metal-free photocatalyst. The selenium radical is further oxidized to an electrophile and trapped by *N*-heterocycles. The mechanism is confirmed by NMR, HRMS, UV, EPR and cyclic voltammetry (CV) experiments and photocatalyst emission quenching studies. A visible light-initiated reaction is employed for the synthesis of selenocysteine-containing indole-based macrocycles via intramolecular Se–C bond formation.

Keywords: Macrocycle; *N*-heterocycle; peptide; photocatalysis; selenocysteine

Introduction

Visible light photocatalysis is a rapidly emerging field with attractive advantages such as efficiency, sustainability, atom economy, and selectivity.[1–3] Most organic molecules do not absorb visible light; therefore, the presence of photosensitizers such as transition metal complexes,[4–6] organic dyes[7] or semiconductors[8] facilitates the reaction.

A vast array of methods[9] for synthesis of 3-selenylindoles have been demonstrated employing metal salts (e.g. $FeCl_3$,[10] CuI[11]), bases[12,13] or oxidants.[14–16] More sustainable methods have been developed based on electrochemically-induced process.[17,18]

In the last few years, visible light-mediated C–H functionalization of (hetero)arenes with simple, mainly diaryl diselenides has been reported. Two catalysts outperform others in terms of efficiency of selenylation of indoles and other (hetero)arenes using blue LED light: FIrPic[19] and Rose Bengal[20,21] (RB). Notably, photocatalyst-free selenylation of indole can be performed by prolonged irradiation in ethanol[22] or in methanol under flow conditions.[23] Another green method for selenylation of allenes[24] and (hetero) arenes[25] has been established using LiCl as an additive and a household white LED lamp ($\lambda = 430–730$ nm) as the light source. The abovementioned methods were limited solely to the use of diorganyl diselenides, whereas photocatalyst- and additive-free methods for the synthesis of 3-selenyl-, 3-tellanyl-, 3-sulfenyl- and 3-thiocyanoindoles were achieved by employing 26 W CFL bulbs or sunlight, resulting in similar reaction yields.[26] Alternatively, 3-sulfenyl and 3-selenylindoles can be obtained by visible light-induced chalcogenylation of indolines in the presence of graphene oxide.[27]

An indole ring was formed via 5-*endo-dig* cyclization starting from alkynylanilines and diaryl diselenides or disulfides in the presence of H_2O_2 and blue LED light.[28] A simple method for the construction of various heterocycles, e.g., oxazoline, isoxazoline, pyrrolidine, and lactone, was established using diorganyl diselenides and alkene-containing substrates and employing 4-CzIPN and blue LED light to initiate the reaction.[29] Catalyst-free synthesis of arylselanyl- and arylsulfenyl-3,3-difluoro-γ-lactams was demonstrated in the presence of a base (KH_2PO_4).[30] Notably, functionalization of styrenes was demonstrated using diaryl diselenides in the presence of RB by irradiating the mixture with blue LEDs.[31] Visible light-initiated

generation of selenyl radicals with subsequent addition to terminal[32,33] or internal[34] alkynes has also been investigated. Significantly, catalyst-free conditions can be used to realize both visible light-induced metathesis reactions between diselenides and ditellurides[35] and diselenide metathesis between simple diorganyl diselenides[36] or Se–Se bond-containing peptides.[37] Strikingly, there has been no profound study on selenocysteine (Sec)-containing peptide modification under visible light conditions.

Late-stage modification of peptides is not an easy task; however, photocatalysis can provide a route to achieve chemoselective bioconjugation under mild and often biocompatible conditions.[38–41] However, other methods of Sec-containing peptide functionalization have been successfully employed, and common modifications of Sec in selenoproteins have been recently reviewed.[42,43] UV light (254 nm) irradiation of selenocystine-based peptides converts Se–Se bridges to selenolanthionine fragments.[44] Deselenylation of Sec-containing peptides can be effectively achieved by reduction of Sec to alanine with TCEP/DTT[45] or oxidation of Sec to dehydroalanine derivatives with hydrogen peroxide.[46] Notably, an elegant method has been established for Sec-containing peptides and small molecule conjugation based on the electrophilic character of (5-nitropyridylthio)-Sec-containing peptides.[47] Recently, Sec-containing peptide modification through the generation of selenyl electrophiles using weak Lewis acids or oxidants has been demonstrated.[48,49]

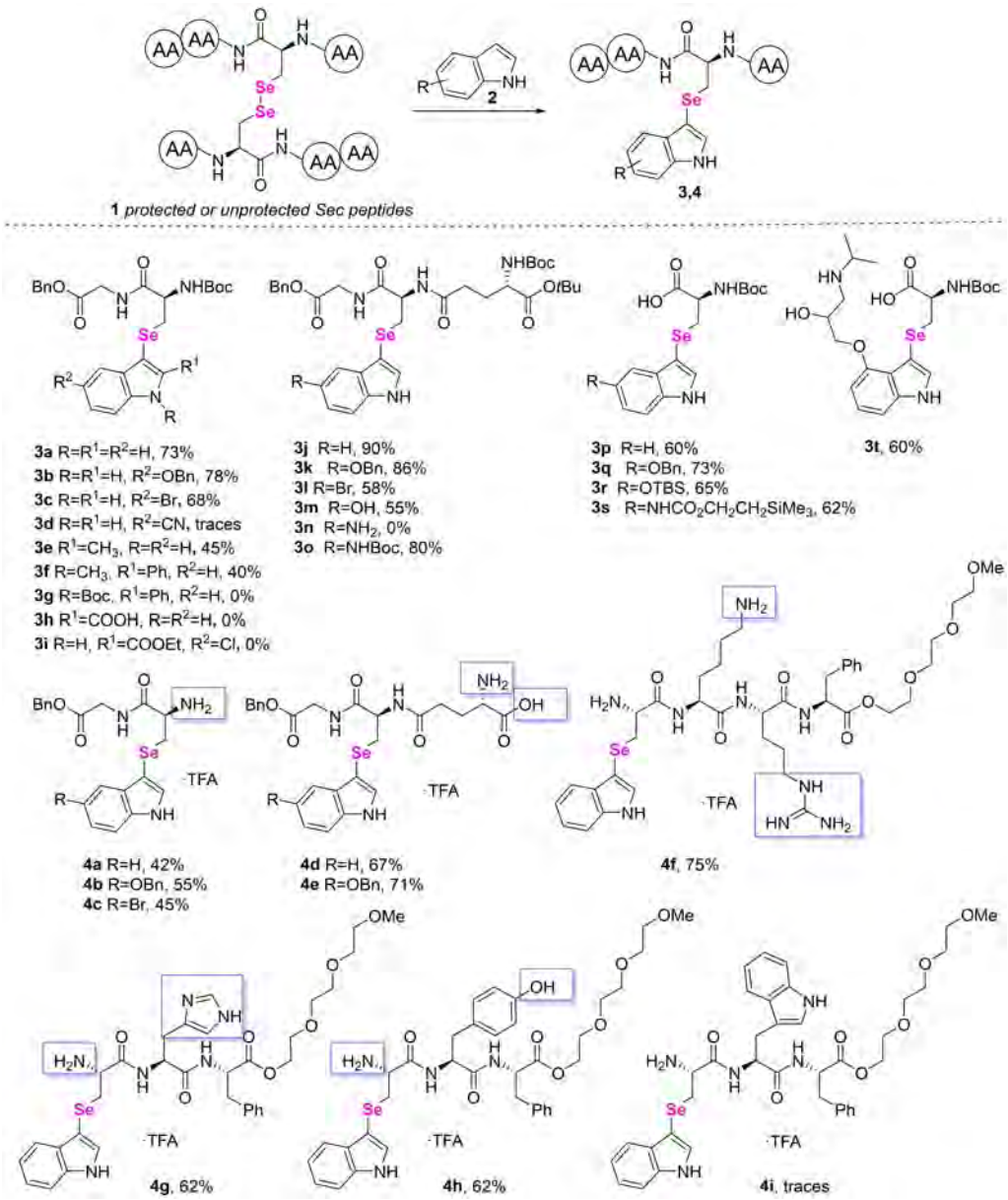
Here, we report our findings regarding Se–Se bond-containing peptide modification using visible light-initiated reactions, scope and limitation studies, formation of macrocyclic Sec-containing peptides, and mechanistic studies.

Results and Discussion

Dipeptide dimers Boc-Sec-Gly-OBn **1 a** and 1*H*-indole (**2 a**) were chosen as model substrates to optimize the reaction conditions. Preliminary screening of a photocatalyst panel (more than 25, ESI Figure S1) was performed in acetonitrile using 0.5 equiv. **1 a**, 1 equiv. **2 a**, 2 mol% transition metal catalyst or 5 mol% organic dye and irradiating the reaction mixture with blue LED light (max 461 nm, bright blue, x = 01440, y = 0.0395, > 50 000 lx) for 90 min. The absence of a photocatalyst resulted in no reaction and only a trace amount of desired product **3 a** (Scheme 1). Evaluation of the obtained data led to the conclusion that the popular transition metal catalysts FIrPic and Ru(bpy)$_3$Cl$_2$ were nonselective and provided a mixture of products, including **3 a**. However, a series of fluorescein derivatives, which are well-known organic dyes, were more promising. Fluorescein itself has very low solubility in MeCN; thus, DMF was added. Unfortunately, the formation of the desired product was not observed, although ethyl eosin was capable of inducing the formation of **3 a** (22%). Gratifyingly, RB and erythrosin B induced a full conversion of the starting materials and selective synthesis of 3-selanyl indole **3 a** in just 90 min.

The following catalysts were found to be unsuitable: 5-carboxytetramethylrhodamine (5-TAMRA), nickel tetraphenyl porphyrin, 4-CzIPN, cresol red, chlorophenol red, bromocresol green, methyl orange, congo red, direct red 81, direct yellow 27, methylene blue, basic fuchsine, indigo carmine, alcian blue, 2,4,6-triphenylpyrylium tetrafluoroborate, 9-mesityl-10-methyl acridinium tetrafluoroborate, acridine, and *N*-methyl-acridinium iodide. In most cases, the formation of **3 a** was observed; however, the reaction was nonselective, and the conversion of starting materials was less than 50%. Thus, RB was selected as the most suitable catalyst. The reduction in the catalyst load to 2 mol% resulted in a prolonged reaction time and decrease in yield. Unfortunately, the use of greener protic solvents such as MeOH, EtOH, EtOH/H_2O, and *i*PrOH resulted in a nonselective reaction due to the fast oxidation and deselenylation of **1 a**. Changing the light source to a CFL bulb resulted in a significantly slower reaction, whereas a red LED was incapable of initiating the formation of **3 a**. Control tests showed that the reaction did not occur under daylight or dark conditions; thus, the necessity of the LED$_{460}$ light was confirmed. The reaction under an Ar atmosphere did not differ from the reaction performed in an open flask, whereas the reaction in the presence of 4-amino-TEMPO did not result in the formation of **3 a**, confirming the radical mechanism for this process. In summary, the optimized conditions for the synthesis of 3-Sec-indoles were found to be 5 mol% RB, MeCN and blue LED light for 90 min.

To determine how the substituents in the indole ring affect the reaction, we tested the reaction of **1 a** with various indoles. Notably, the presence of an electron-donating group (EDG) at the C5 position of indole improved the reaction yield; halogen atoms did not significantly affect the process, whereas the presence of an electron-withdrawing group (EWG) diminished the reactivity and provided only trace amounts of the products. An EDG at the C2 position provided the product in lower yield, but electron-deficient indoles (EWG at the N1 or C2 position) were completely unreactive. Tripeptide dimer **1 b** showed an even better ability to react with indoles than **1 a**, and the corresponding products **3 k**–**n** and **3 p** were obtained in excellent yields. Importantly, a hydroxy group at the C5 position of indole was also tolerated under the reaction conditions, although the reaction yield was lower because prolonged irradiation was required for full conversion of the starting materials, whereas the reaction with 5-aminoindole failed, probably due to enamine-imino tautomerism. However, the use of *tert*-



Scheme 1. Sec-indole formation: scope and limitation studies. Reaction conditions: indole **2** (1 equiv.), **1** (0.5 equiv.), RB (0.05 equiv.), blue LED_{460}, MeCN. **1a** (Boc-Sec-Gly-OBn)$_2$, **1b** (Boc-Glu(O*t*Bu)-Sec-Gly-OBn)$_2$, **1c** (Boc-Sec)$_2$, **1d** (H_2N-Sec-Gly-OBn·TFA)$_2$, **1e** (H_2N-Glu(OH)-Sec-Gly-OBn·TFA)$_2$, **1f** (H_2N-Sec-Lys-Arg-Phe-OPEG^{3}OMe·TFA)$_2$, **1g** (H_2N-Sec-His-Phe-OPEG^{3}OMe·TFA)$_2$, **1h** (H_2N-Sec-Tyr-Phe-OPEG^{3}OMe·TFA)$_2$, **1i** (H_2N-Sec-Trp-Phe-OPEG^{3}OMe·TFA)$_2$; **2a** 1*H*-indole, **2b** 5-(benzyloxy)indole, **2c** 5-bromoindole, **2d** 5-cyanoindole, **2e** 2-methylindole, **2f** 1-methyl-2-phenylindole, **2g** 1-Boc-2-phenyl-indole, **2h** 1*H*-indole-2-carboxylic acid, **2i** ethyl 5-chloro-1*H*-indole-2-carboxylate, **2j** 5-hydroxyindole, **2k** 5-aminoindole, **2l** *tert*-butyl (1*H*-indol-5-yl)carbamate, **2m** 5-((*tert*-butyldimethylsilyl)oxy)-1*H*-indole, **2n** 2-(trimethylsilyl)ethyl (1*H*-indol-5-yl) carbamate, **2o** pindolol.

butyl (1*H*-indol-5-yl)carbamate **2l** resulted in the formation of desired product **3o** in high yield. Remarkably, we discovered that Boc-selenocystine **1c** can also be employed in visible light-mediated selenylation, leading to a selenium analog of tryptophan **3p** as well as products **3q**–**s**, which can serve as valuable building blocks. Notably, selenylation of pindolol – a nonselective β-adrenergic antagonist[50] – was successful.

Next, unprotected Sec-containing peptides were applied as starting materials to clearly facilitate the use of the developed protocol. Consequently, utilization of unprotected dipeptide dimer **1d** and tripeptide dimer **1e** led to the formation of desired products **4a**–**e**. Encouraged by these results, we decided to test more

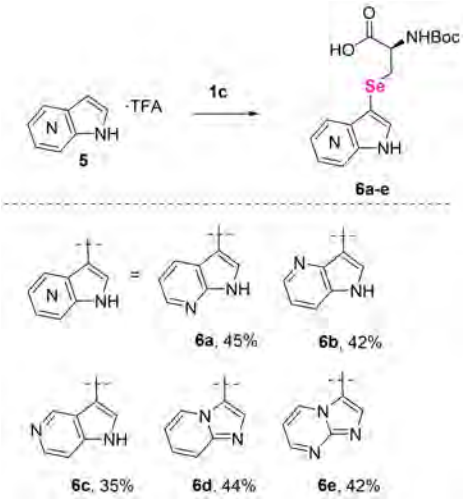
sophisticated Sec-containing peptides with "sensitive" amino acid residues to determine their tolerance under the developed reaction conditions. Thus, pegylated tetra- and tripeptide dimers **1f–i** were prepared and employed in visible light-mediated reactions.

We successfully obtained products **4f–h**, confirming that the Lys, Arg, His and Tyr moieties are well tolerated, whereas the reaction with Trp-containing peptide **1i** was nonselective.

To extend the method's scope, the possibilities for selenylation of indole ring bioisosteres – azaindoles **5** – were evaluated. These substrates proved to be significantly less reactive than 1*H*-indole. The formation of product **6a** (8%), along with side products, was observed when 7-azaindole was used; only trace amounts of product **6b** were formed when 4-azaindole was used, whereas 5-azaindole did not lead to product **6c**. Azaindoles[51] are electron-deficient heterocycles and are thus less reactive than indoles; moreover, these substrates might become deactivated due to excited-state tautomerization.[52]

However, protonation of azaindoles changes their electronic properties and promotes the formation of 3-Sec-azaindoles **6a–c** (Scheme 2). We observed significant differences in the reaction rate for different isomers: 1.5 h was needed for 7-azaindole and 24 h was needed for 5-azaindole to achieve 100% conversion.

These results correlate with the pKa of azaindoles: 7-azaindole has the highest acidity, whereas 5-azaindole has the highest basicity.[53] Notably, protonated imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyrimidine were also successfully employed in visible light-induced selenylation, and the structures of products **6d** (CCDC 2054758) and **6e** (CCDC 2054757) were unambiguously confirmed by X-ray analysis (Figure 1A and 1B).



Scheme 2. Synthesis of Sec-azaindoles. Reaction conditions: azaindole **5** (1 equiv.), **1c** (0.5 equiv.), RB (0.05 equiv.), LED_{460}, MeCN.

Next, we were interested in whether the developed protocol can be applied for intramolecular indole selenylation to form Sec-containing peptides with indole-embedded macrocycles. Furthermore, three strategies (Figure 2) were proposed for the formation of macrocycles: (A) visible light-mediated Se–C formation or, in other words, intramolecular indole selenylation; (B) selenylation of an indole attached to the peptide and subsequent intramolecular amide bond formation; and (C) a reaction between Boc-Sec and protected 5-hydroxy- or aminoindole, coupling with a small peptide, deprotection and intramolecular amide bond formation.

First, we intended to prepare Boc-Sec-containing dipeptide and tripeptide dimers (**8**,**9**) attached to the C4 or C5 position of indole through an ester or amide bond. Successive use of **8** and **9** for visible light-mediated intramolecular Se–C bond formation (Approach A) could result in the synthesis of macrocyclic structures **10** (Scheme 3). 1-Methyl-1*H*-indol-4-ol (**2p**) was coupled with Boc-Phe and then deprotected, affording **7a**, which was next coupled with Boc-Sec, yielding **8a**. Gratifyingly, substrate **8a** in the presence of RB under visible light irradiation provided macrocycle **10a** in 60% yield. The intramolecular selenylation proceeded slightly slower than the intermolecular

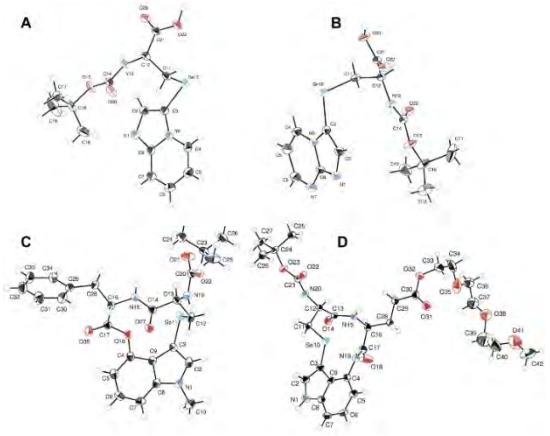


Figure 1. ORTEP molecular structures of **6d** (A), **6e** (B), **10a** (C), **10f** (D).

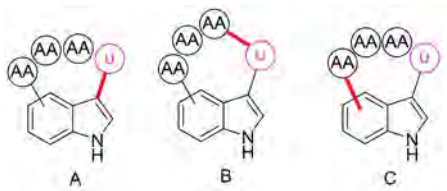
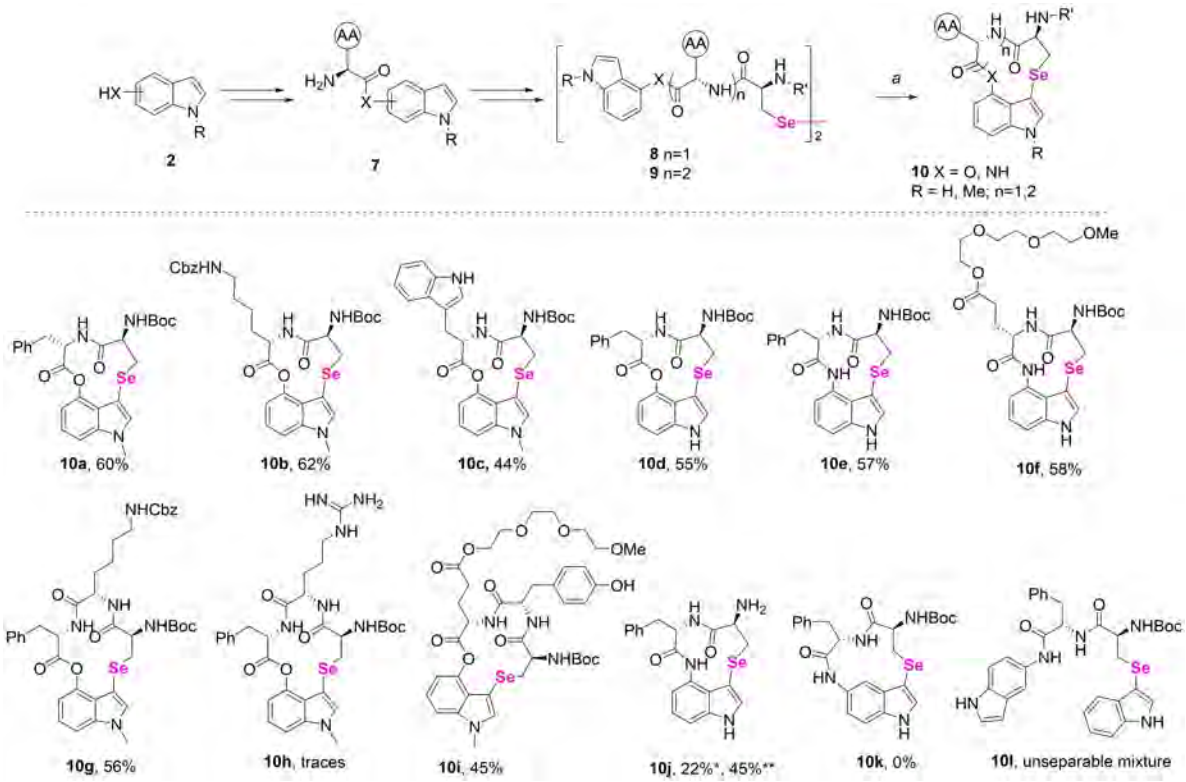


Figure 2. Proposed strategies for Sec-macrocycle formation.



Scheme 3. Synthesis of macrocycles: approach A. Reaction conditions: (a) **8** or **9** (1 equiv.), RB (0.1 equiv.), LED_{460}, MeCN. *Fmoc cleavage, cyclization, **cyclization, Fmoc cleavage.

reaction, but it was selective. Furthermore, the structure of **10a** (CCDC 2054762) was unambiguously confirmed by X-ray analysis (Figure 1C). Macrocycle with *N*(ε)-protected lysine **10b** was successfully synthesized, as was the tryptophan moiety containing macrocycle **10c**, although the yield was slightly lower due to the formation of side products. Next, we tested whether a Sec-containing peptide derivative with 1*H*-indole **8d** can be used to prepare macrocycles. Fortunately, the desired products were obtained starting from 4-hydroxyindole (**2q**) and 4-amino-1*H*-indole (**2r**). Compounds **10a–e** exhibited relatively low solubility; therefore, PEGylated glutamic acid was employed to resolve this issue. The molecular structure of **10f** (CCDC 2054761) was also confirmed by X-ray analysis (Figure 1D). Next, we investigated the preparation of macrocycles that contained tripeptides attached to the indole. Product **10g** containing a Boc-Sec-Lys-Phe moiety was successfully obtained, whereas Arg-containing substrate **9b** was insoluble in MeCN; consequently, the reaction did not occur, but the addition of DMF resulted in a nonselective reaction that yielded only trace amounts of the product **10h**. Fortunately, the introduction of tyrosine (substrate **9c**) was well tolerated under the reaction conditions, resulting in the successful isolation of Tyr-containing macrocycle **10i**. Unfortunately, the Boc protection strategy was unsuitable for the synthesis of unprotected macrocycles due to the instability of indoles under acidic conditions; after protonation, they formed dimers and trimers. However, the preparation of unprotected macrocycle **10j** was accomplished by employing a Fmoc protection strategy. Irradiation of **8g** with LED_{460} light in the presence of RB resulted in the formation of the corresponding macrocycle, and subsequent cleavage of the Fmoc group yielded unprotected macrocycle **10j**. The same product was also obtained by deprotection of **8g** followed by a macrocyclization reaction.

In this case, **10j** was obtained in lower yield; therefore, the advisable sequence for the preparation of unprotected Sec macrocycles is macrocyclization and then deprotection.

Next, the synthesis of Sec macrocycles with a short peptide at the C5 of indole was evaluated. For this purpose, substrate **8h** was irradiated with LED_{460} light. Unfortunately, the formation of product **10k** was not observed. However, we confirmed that intermolecular selenylation can be performed using **8h**. The addition of 1*H*-indole to the reaction mixture of **8h** and RB resulted in the formation of indole derivative **10l**, along with many side products. The results led us to conclusion that strategy A is not suitable for macrocycle formation starting from indoles with peptides

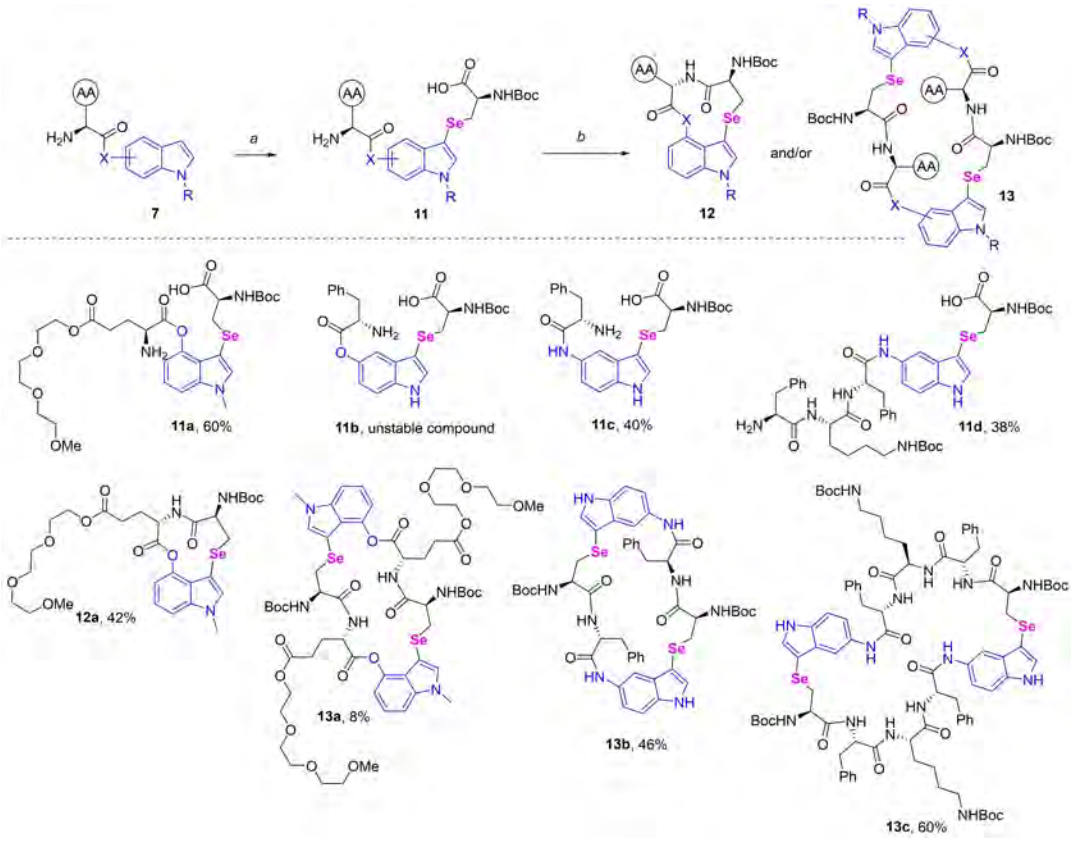
attached to its C5 position, probably due to conformational restrictions.

Approach B to prepare Sec-containing macrocycles relied on visible light-mediated selenylation of indoles **7** that contained the amino acid at the C4 or C5 position of the indole ring and subsequent intramolecular peptide bond formation using standard coupling conditions (Scheme 4). We were keen to investigate whether this synthesis pathway could lead to the formation of macrocycles in which amino acids are attached to the C5 position of indole due to the failed attempt to prepare these macrocycles through approach A.

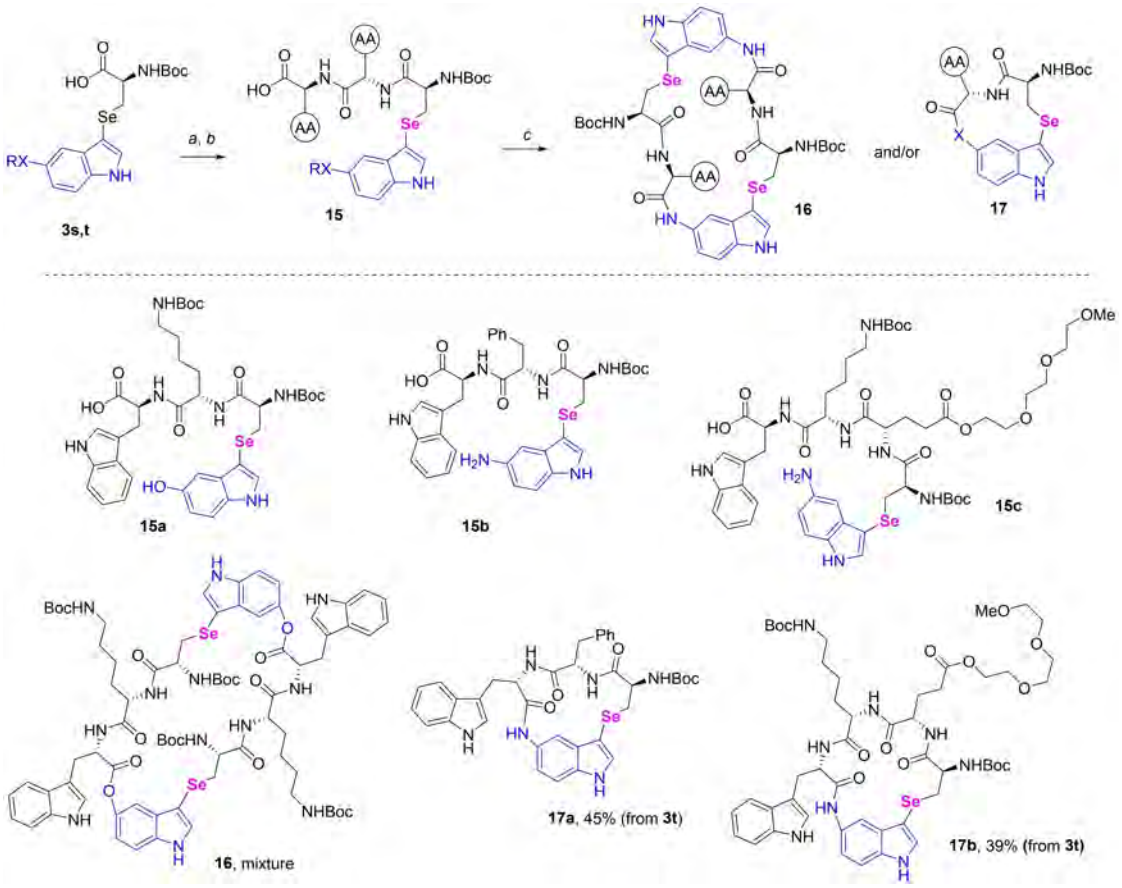
First, a mixture of Boc-Sec and indole **7h** with PEGylated Glu at the C4 position was irradiated in the presence of RB. The synthesis resulted in isolation of Boc-Sec-containing indole **11a** in good yield. Next, a routine EDC/HOBt method was used for intramolecular amide bond formation. Macrocycle **12a** was isolated in moderate yield along with bis-macrocyclization product **13a** as the minor product. Then, we moved on to selenylation of indoles that contained Phe at the C5 position of indole. We observed that the attachment of Phe to the C5 position of indole through ester bonds and the subsequent reaction with **1c** led to unstable product **11b**. However, indole **7j** was successfully selenylated, and product **11c** was further used for intramolecular amide bond formation. Surprisingly, only bis-macrocyclization product **13b** was formed in the reaction.

We conceded that if a small peptide instead of a single amino acid residue was attached to the C5 position of indole, it would allow the formation of a macrocycle rather than a bis-macrocyclization product. Thus, selenylation of indole **7l** with a Phe-Lys-Phe moiety resulted in formation of **11d**, and subsequent intramolecular amide bond formation provided bis-macrocyclization product **13c** as a single product.

The third approach (Approach C) involved the use of products **3s** and **3t** that were obtained by visible light-mediated selenylation of protected 5-hydroxy- and 5-aminoindoles and proved to be excellent building blocks for macrocycle formation (Scheme 5). Product **3s** was coupled with dipeptide **14a** and treated with TBAF to obtain the corresponding indole-containing peptide **15a**. Subsequent intramolecular amide bond formation resulted in a mixture of compounds, including bis-macrocyclization product **16**. Apparently, this product was also unstable, similar to the other indoles that contained amino acids attached at the C5



Scheme 4. Synthesis of macrocycles: approach B. (a) indole **7** (1 equiv.), **1c** (0.5 equiv.), RB (0.05 equiv.), LED_{460}, MeCN; (b) **11** (1 equiv.), HOBt (1 equiv.), EDC (1.5 equiv.), DMF.



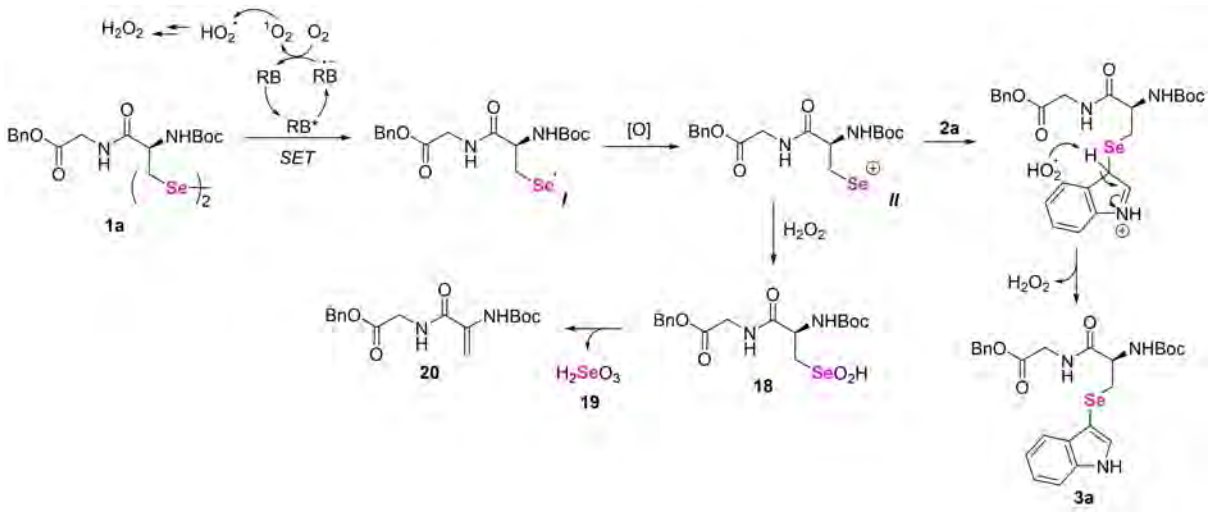
Scheme 5. Synthesis of macrocycles: approach C. Reaction conditions: (a) **3s** or **3t** (1 equiv.), **14** (1 equiv.), HOBt (0.5 equiv.), EDC (1.5 equiv.), DMF; (b) TBAF (3 equiv.), THF; (c) **15** (1 equiv.), HOBt (1 equiv.), EDC (1.5 equiv.), DMF.

position of the indole through an ester bond. Fortunately, stable macrocycles **17a** and **17b** were isolated by employing compounds **15b** and **15c** for intramolecular amide bond formation.

Mechanistic studies. To gain insight into visible light-mediated selenylation, we first examined the possible degradation of Sec-containing peptide **1a** under irradiation. Preliminary tests were performed in acetonitrile, and a solution of **1a** was irradiated with LED_{460} light for 90 min with and without a photocatalyst. No changes were observed in the absence of the photocatalyst, whereas the formation of two products was detected in the presence of RB.

Analysis of the LC/MS data showed that the products were seleninic acid **18** and a dehydroalanine (Dha) derivative **20**, respectively (Scheme 6). On the basis of ^{77}Se NMR spectroscopy data[54] (Figure 3A), alkyl seleninic acid **18** ($RSeO_2H$ 1217.5 ppm, [M+Na]=471.0633) was formed after 1 h of irradiation. Our attempts to isolate oxidized form of **1a** failed due to product lability. Furthermore, storage of an NMR tube for 24 h led to the disappearance of the **18** signal, confirming the formation of seleninic acid (H_2SeO_3 **19**) (1302.7 ppm) and Dha-peptide **20**. The use of Ru-$(bpy)_3Cl_2$ also resulted in the formation of **20**. Other catalysts, namely, FIrPic, ethyl eosin, and 4-CzIPN, induced the formation of **18** and **20** as well but were not able to achieve full consumption of the starting material. 2,4,6 Triphenyl-pyrylium tetrafluoroborate and 9-mesityl-10-methyl-acridinium tetrafluoroborate provided a less selective reaction, whereas DDQ and TAMRA were not efficient. Reactions performed in MeOH, EtOH or DMF did not reach full conversion of **1a** in the given time.

The addition of water did not interfere with the formation of **20**; however, the reaction performed in dry and degassed MeCN under an Ar atmosphere provided a considerably lower conversion of **1a**, thus confirming that the presence of water and oxygen in the solvent is necessary for the reaction to occur. Under irradiation conditions, oxygen is converted to short-living $^1O_2$, returning $RB^{\bullet-}$ to ground state by the energy transfer.[55–58] Singlet oxygen reacts with water generating hydroperoxyl radical that produces hydrogen peroxide,[22] which is trapped by **1a**, resulting in oxidation and deselenylation with the formation of a double bond. The control reaction with TEMPO did not lead to the formation of **20**, thus verifying the



Scheme 6. Proposed mechanism of visible light mediated reaction.

initial radical pathway. On the basis of previously published reports,[19–22,25,26,59] due to differences in reaction conditions, it was problematic to unambiguously specify whether the selenyl radical ***I*** attacks the indole at C3 position, or the indolyl radical is formed first or both selenyl and indolyl radicals are formed simultaneously. To answer this question, the following experiments were performed.

Optical absorption properties. UV spectra were recorded for **1 a**, **2 a**, RB, ethyl eosin, erythrosin B, FIrPic, $Ru(bpy)_3Cl_2 \cdot 6H_2O$, and 4-CzIPN in dry acetonitrile solutions (Figure 3B and 3 C). The photochemical reaction between **2 a** and diselenide **1 a** was not effective in the absence of a catalyst under LED_{460} light because indole has an absorption band from 200 to 305 nm and **1 a** exhibits absorption until 430 nm, albeit with low intensity. Notably, the absorption shoulder at 275–430 nm in the **1 a** UV spectrum is characteristic of Se–Se bonds, which facilitate the formation of selenyl radicals in the presence of a photocatalyst.

Photocatalyst emission quenching. The photoluminescence quenching of RB, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and FIrPic was performed using **1 a** or **2 a** in degassed acetonitrile. The quenching rate constant of RB in the experiment with **1 a** was determined to be 10.42×10^{-3} l/mol (Figure 3D). In contrast, **2 a** does not quench the fluorescence of RB to any significant extent. Notably, quenching experiments with $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and FIrPic showed the opposite pattern: the **2 a**-quenched fluorescence of both catalysts had a higher Stern-Volmer constant than **1 a** (**2 a**: 5.2×10^{-3} l/mol, **1 a**: 3.6×10^{-3} l/mol of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (Figure 3E); **2 a**: 0.9×10^{-3} l/mol, **1 a**: 0.5×10^{-3} l/mol of FIrPic) (Figure 3F). Based on the obtained results, it can be concluded that upon excitation of RB, only selenyl radical is formed. Then, selenyl radical ***I*** is oxidized with oxygen to selenyl electrophile ***II***,[19] which is further trapped by **2 a**, resulting in the formation of **3 a** (Scheme 6). Utilization of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and FIrPic leads to the formation of both selenyl and indolyl radicals, and consequently, an unspecific reaction with the formation of undesired byproducts occurs.

EPR studies. To confirm directly the generation of selenyl radical, EPR studies were conducted. We examined three solutions – RB (50 μM), **1 a** (50 μM), mixture of RB and **1 a** (1:5, 50 μM) in acetonitrile/water (9:1) under LED_{460} light irradiation. Thus, in the absence of light source, no radical formation was detected in the case of RB, **1 a** and the mixture of both (Figure 3G). However, radical formation was confirmed during irradiation of RB in solution ($g = 2.008$, $\Delta H_{pp} = 6G$). The amount of RB* is constant in time. Notably, switching off the light led to disappearance of RB radical. Received data are in agreement with already published research.[58] RB* is not stable and recombines to $RB^* + RB \rightarrow RB^{\bullet+} + RB^{\bullet-}$. Irradiation of **1 a** without photocatalyst did not produce a selenyl radical (Figure 3H). However, the presence of diselenide **1 a** during irradiation, reduced the intensity of RB* signal twice allowing to confirm the establishment of dynamic equilibrium under LED_{460} light: RB* + **1 a** ↔ (RB + **1 a***).

Cyclic voltammetry (CV) studies. To investigate the redox behavior of **1 a** and *N*-heterocycles, as well as the photocatalysts (RB, FIrPic, $Ru(bpy)_3Cl_2 \cdot 6H_2O$), CV studies were performed in dry degassed acetonitrile (details are presented in Table S1 and Figure S2).

The onset oxidation potentials ($E^{ox.vsFc/Fc*}{}_{onset}$) versus Fc were measured by CV from the first redox cycle. The CV of **2 a** shows $E^{ox.vsFc/Fc*}{}_{onset} = 0.43$ V, that of dipeptide **1 a** shows $E^{ox.vsFc/Fc*}{}_{onset} = 0.76$ V, that of RB

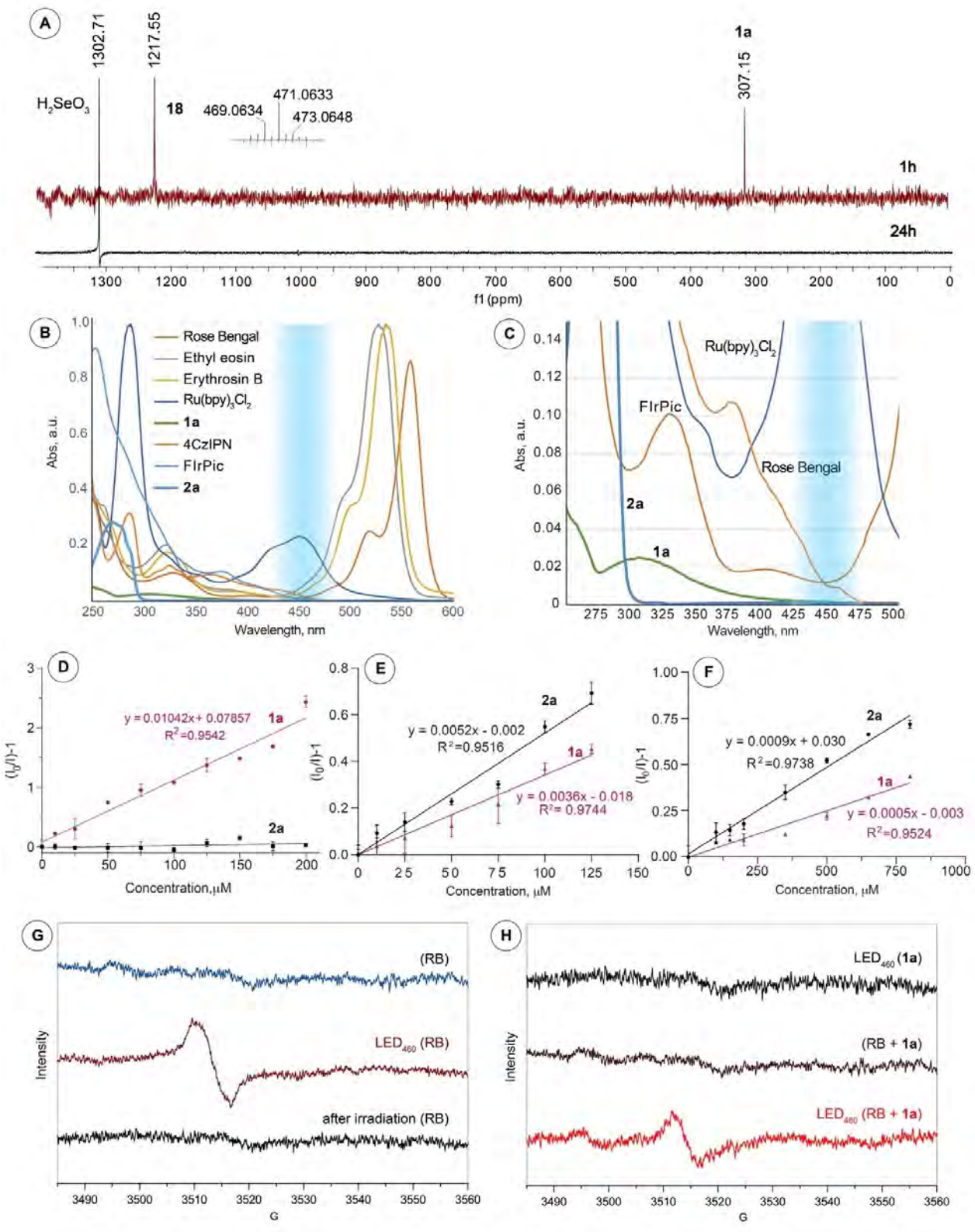


Figure 3. (A) ^{77}Se NMR spectrum of **1a** degradation products after 1 h of irradiation and after storage of the reaction mixture in NMR tube for 24 h; (B) UV spectra of **1a**, **2a** and photocatalysts; (C) Magnified UV spectra of 250–500 nm region (D) Photoluminescence quenching of RB with **1a** and **2a**; (E) Photoluminescence quenching of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ with **1a** and **2a**; (F) Photoluminescence quenching of FIrPic with **1a** and **2a**; (G, H) EPR spectra of RB and RB + **1a** with and without LED_{460} irradiation.

shows $E^{ox.vsFc/Fc*}_{onset} = -0.20$ V and $E^{red.vsFc/Fc*}_{onset} = -1.39$ V, that of FIrPic shows $E^{ox.vsFc/Fc*}_{onset} = 0.73$ V and $E^{red.vsFc/Fc*}_{onset} = 0.94$ V, and that of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ shows $E^{ox.vsFc/Fc*}_{onset} = 0.75$ V and $E^{red.vsFc/Fc*}_{onset} = -1.42$ V. Our experimental data are similar to those in a previously published report,[19] indicating that the photoreaction is mainly initiated by the interaction between the excited photocatalyst and diselenide **1a**. The oxidation potential of **1a** is much higher than the reduction potential of excited RB and $Ru(bpy)_3Cl_2 \cdot 6H_2O$; however, in contrast to that of diphenyl diselenide,[19] the oxidation potential of **1a** matches the reduction potential of FIrPic.[60,61] This result helps explain the low efficiency of FIrPic utilization in light-induced reactions with Sec-containing peptides under the developed reaction conditions. In addition, the redox potentials measured for the studied indoles did not provide clear insight into their reactivity, providing additional evidence that *N*-heterocycles do not involve an electron transfer step from exited RB* and participate solely in the reaction with selenyl electrophile ***II***, yielding Sec-containing peptides.

Conclusion

A straightforward, atom-economic method for the modification of selenocystine peptides was developed. The mechanism of the visible light-mediated reaction was confirmed by NMR, HRMS, UV, EPR and CV experiments and photocatalyst emission quenching studies. The novel method is based on a visible light-initiated reaction for the generation of selenium radical, which is then converted to selenium electrophile that is trapped by electron-rich *N*-heterocyles, thus providing Sec-containing indoles in good yields. Notably, because of initial homolytic cleavage of Se–Se bond the current method allows to utilize both parts of diselenide. Both protected and unprotected Sec-containing peptides can be successfully employed with excellent tolerance for sensitive amino acids (Lys, Arg, His, Glu, Tyr). Furthermore, three approaches were established for the synthesis of Sec-containing indole-based macrocycles. The utilization of visible light provides easy access to simple and sophisticated functionalized Sec-containing peptides, opening the way to a broad application for various types of reactions.

Experimental Section

Representative procedure for visible light-mediated indole selenylation: To a solution of Sec peptide **1a** (100 mg, 0.12 mmol, 0.5 equiv.) and indole **2** (0.24 mmol, 1 equiv.) in MeCN (5 ml) Rose Bengal (12 mg, 0.012 mmol, 0.05 equiv.) was added and the reaction mixture was irradiated by 36 W blue LEDs for 90 minutes. After evaporation the residue was purified by reverse phase flash chromatography (C-18, MeCN/H_2O, 10–85%) to give the product **3**.

CCDC 2054758 (**6a**), CCDC 2054757 (**6e**) CCDC 2054762 (**10a**), CCDC 2054761 (**10f**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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5. pielikums/ Appendix V

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Visible light-mediated synthesis of Se–S bond containing peptides.

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Visible Light-Mediated Synthesis of Se–S Bond-Containing Peptides

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Abstract: A visible light-initiated method has been developed for preparation of Se–S bond-containing peptides. The method is based on generation of sulfur-centered radical employing organic dye. The protocol is tolerant to unprotected peptides with "sensitive" amino acids. The stability of Se–S bond is evaluated in buffers at different pH (3.0–10.0) and also in the presence of oxidants and reducing agents. Additionally, the ability of Se–S bond to serve as an oxidation sensitive linker in biocompatible materials has been confirmed.

Keywords: glutathione; peptide; photocatalysis; selenocysteine

The biological importance of Se–S bond is based on the fact that this bond is found in the active center of redox regulating enzyme, namely, thioredoxin reductase – one of the major components of the antioxidant system in mammalian cells.[1,2] Se–S bond- containing intermediate is formed in the catalytic cycle of the glutathione peroxidase–selenoenzyme that is responsible for reduction of H_2O_2 and other peroxides by glutathione.[3] Recently, low-molecular weight compounds with Se–S bond have been used as fluorescent probes for detection of reactive sulfur species (RSS) – H_2S and H_2S_2.[4,5] Selenosulfides have also been used as prodrugs for inhibition of protein tyrosine phosphatases.[6]

Compounds with Se–S bond are considered unstable thus the synthesis can be challenging.[7] The exchange reaction between diselenide and thiol, although theoretically possible, is unfavorable because the selenolate byproduct is a stronger nucleophile than thiol.[8] However, the reaction can be performed under suitable conditions.[7] For example, the Se–S bond-containing compound has been obtained by reacting diphenyl diselenide with silver trifluoromethylsulfide. This exchange reaction is favored due to the selenolate stabilization with silver atom.[9] Typically, Se–S bond is prepared by reaction between thiol and electrophilic selenyl species – selenyl halides[10,11] and organyl seleninic acids.[12–14] Benzeneselenol can also be utilized for the synthesis of related phenylselenyl sulfide by employing aryl or alkyl thiols and a catalytic amount of *t*BuOK,[15] whereas reaction of benzeneselenol with electrophilic *N*-phenyl-trifluoromethanesulfenamide[16] occurs in acidic conditions. Notably, various sugar-selenyl sulfides have been synthesized directly from sugar diselenides and glutathione in a phosphate buffer. Moreover, this efficient method has been extended from using glutathione as a thiol group-containing substrate to a protein – a single-cysteine mutant of subtilisin.[17] Another example of synthesis of Se–S bond-containing substrate obtained by direct reaction between diselenide (selenocystine) and thiol (penicillamine) occurs in the presence of Et_3N.[18] Se–S bond-containing cyclic dipeptides are obtained by treating –Se-benzhydryl and –S-trityl dipeptide with iodine.[19] Significantly, UV light has been employed for the exchange reaction between diaryl disulfide and dialkyl diselenide. The authors have also stated that Se–S bond is formed under UV light, while longer wavelength (>410 nm, visible light) reverses the reaction.[20]

Recently, we have reported an efficient method for the functionalization of Se–Se bond-containing peptides, based on the generation of a selenium radical via visible light-initiated reaction.[21] As a continuation of our research related to development of methods for modification of selenocysteine[22–23] (Sec) and cysteine[24] peptides, here we report a simple protocol for preparation of Se–S bond-containing peptides using visible light-initiated reaction.

The optimization of reaction conditions was performed using dipeptide dimer Boc-Sec-Gly-OBn **1a** and glutathione (GSH) (**2**) as model substrates. The

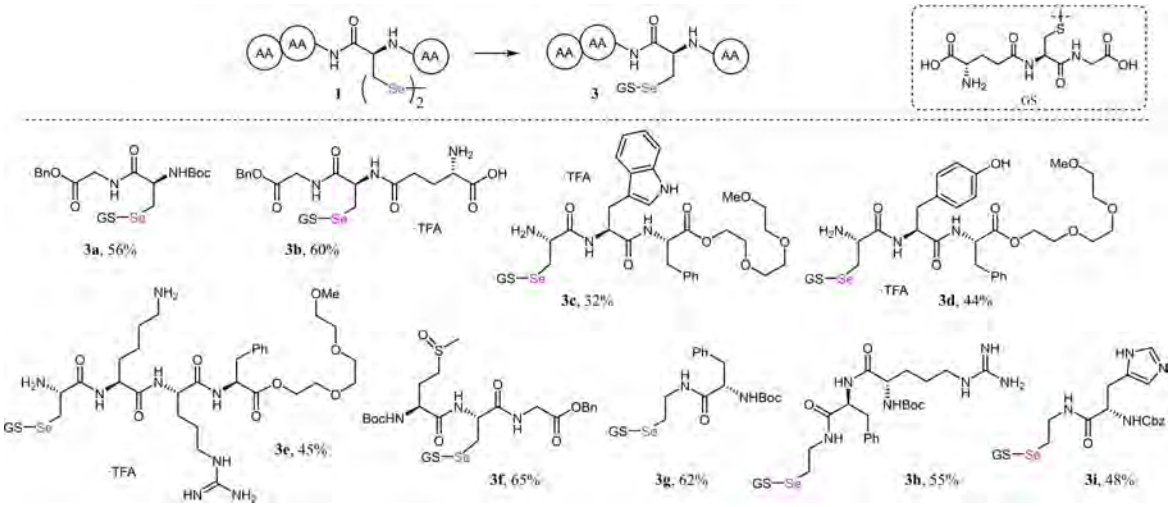
search for the most suitable photocatalyst was performed using 1 equiv. **1a**, 10 equiv. **2**, 0.02 equiv. transition metal catalyst or 0.05 equiv. organic dye in the mixture of acetonitrile and water (1:1), while irradiating the reaction mixture with blue LED light (max 460 nm, bright blue, x = 01440, y = 0.0395, > 50 000 lx) for 1 hour. The reaction performed without the catalyst showed only traces of the product **3a**. The highest selectivity and conversion of the starting materials was achieved employing Rose Bengal (RB). Other catalysts were: (i) less effective (bis[2-(4,6-difluorophenyl)pyridinato-C^2,*N*](picolinato)iridium(III) (FIrPic)); (ii) induced formation of the respective alkyl seleninic acid which subsequently led to deselenylation by elimination of H_2SeO_3 providing dehydroalanine (Dha) peptide[21] ($Ru(bpy)_3Cl_2$, 2,4,5,6-tetrakis(9*H*-carbazol-9-yl) isophthalonitrile (4-CzIPN), 9-mesityl-10-methylacridinium tetrafluoroborate, ethyl eosin, fluorescein), or (iii) failed to initiate the reaction (5-carboxytetramethylrhodamine). Performing the reaction in methanol and ethanol resulted in lower conversion of **1a** in the given time. The reaction performed in the day-light or in the dark did not lead to formation of **3a**, thus, confirming the necessity of LED_{460}. Other tested light sources (compact fluorescent lamp (CFL) – warm white (2291 K, x = 0.4915, y = 0.4105), red LED (max 660 nm, deep red, x = 0.5939, y = 0.2488)) were less efficient. The decrease of GSH amount (5 equiv.) did not result in full conversion of **1a**.

The presence of TEMPO did not prevent the formation of **3a**. Presumably, the generation of glutathionyl (GS•) radical under visible light irradiation, followed by the formation of Se–S bond or homocoupling reaction, proceeds much faster, than radical quenching with TEMPO.

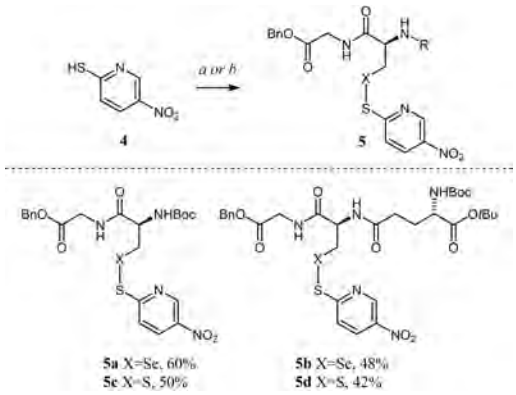
Next, the tolerance of amino acids with "sensitive" groups (Arg, Glu, Lys, His, Trp, Tyr, Met) was established (Scheme 1). Gratifyingly, selenocysteine and selenocystamine derivatives with Glu, Tyr, Arg, His, Lys showed excellent tolerance and provided the desired Se–S bond-containing products. Even Trp-containing product **3c** was isolated, albeit the yield was lower due to formation of side products that arise from Trp oxidation. A rapid oxidation of methionine has been reported under visible light irradiation in the presence of RB.[25] Thus, not surprisingly, Met sulfoxide-containing product **3f** was selectively obtained in good yield starting from Met-containing substrate.

Obviously, selenocystamine fragment can serve as a convenient linker. Furthermore, it has been demonstrated that in certain structures the selenoethyl moiety can be eliminated under reductive conditions.[26]

Notably, 5-nitropyridine-2-thiol (**4**) was also successfully employed in the visible light-mediated reaction providing the respective Se–S bond-containing products **5a**,**b** in good yields (Scheme 2). Therefore, a convenient and straightforward method is demonstrated for the preparation of 5-nitropyridin-2-yl)thio-Sec peptides as an alternative to the existing method.[27] This type of compounds has been reported to be used as electrophilic Se sources.[28] Furthermore, analogous S–S bond-containing products **5c**,**d** were synthesized employing Boc–Cys–Gly–OBn and Boc–Glu (O*t*Bu)–Cys–Gly–OBn. Although the preparation of asymmetrical disulfide may not be an easy task due to the formation of a mixture of symmetrical disulfides, the products **5c**,**d** were obtained in moderate yields and only negligible amounts of symmetrical disulfides were detected.



Scheme 1. Scope and limitation studies for the preparation of Se–S bond-containing compounds. Reaction conditions: diselenide (1 equiv.), GSH (10 equiv.), RB (0.1 equiv.), MeCN/H_2O, LED_{460}.



Scheme 2. Synthesis of (5-nitropyridin-2-yl)thio-Sec and Cys peptides. Reaction conditions: a) **1a** or **1b** (1 equiv.), **4** (5 equiv.), RB (0.1 equiv.), MeCN, LED_{460}; b) Cys peptide (1 equiv.), **4** (1 equiv.), RB (0.05 equiv.), MeCN, LED_{460}.

With the purpose to determine whether Se- or S-centered radical is formed via visible light-initiated reaction, Stern-Volmer analysis was performed (Figure 1). The photoluminescence quenching of RB was performed in a degassed mixture of acetonitrile/water (1:1). The quenching rate constant of RB with GSH was significantly higher ($8.8 \cdot 10^{-3}$ l/mol) than with **1a** ($0.12 \cdot 10^{-3}$ mol/l). Therefore, it allows the assumption that S-centered radical is rapidly formed under LED_{460} irradiation. Next, the GS radical reacts with the diselenide forming the desired Se–S bond-containing peptide **3a** or it undergoes the homocoupling reaction forming oxidized glutahione (GS-SG), therefore an excess of **2** is required for full consumption of **1a** (Scheme 3). Notably, both parts of the diselenide are utilized in the reaction.

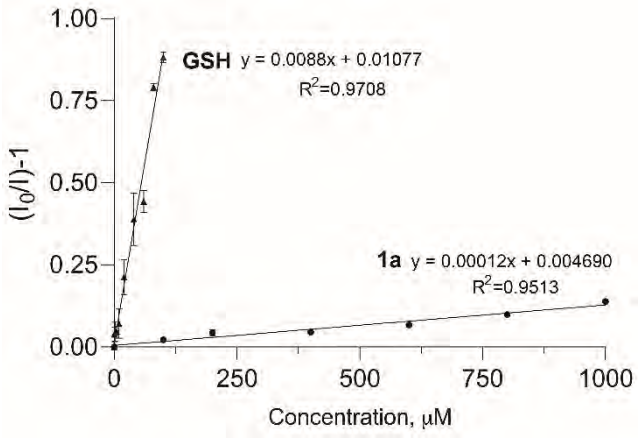
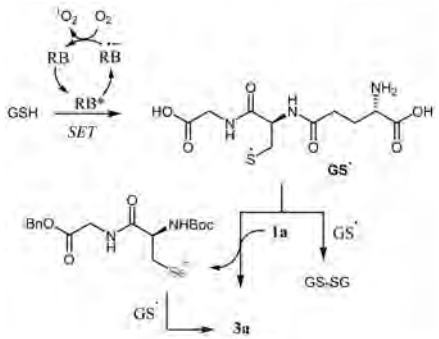


Figure 1. Photoluminescence quenching of RB with **1a** and GSH.

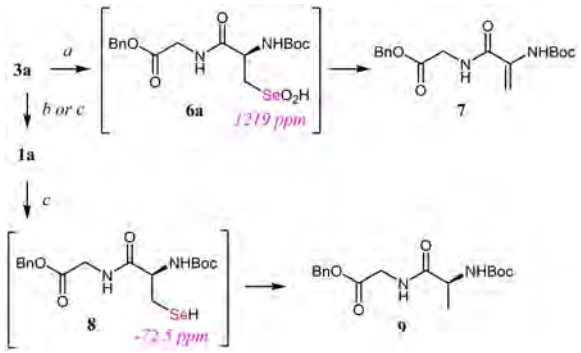
Next, the stability of Se–S bond-containing glutathione derivatives was evaluated. First, we examined the stability of **3a** under visible light irradiation. Prolonged irradiation (> 3 h) of **3a** and RB solution in MeCN/water with LED_{460} resulted in homolytic cleavage of Se–S bond, forming respective selenyl radical and GS radical. Selenyl radical is quickly oxidized to selenyl electrophile that reacts with H_2O_2 forming alkyl seleninic acid **6** and Boc–Dha–Gly–OBn **7** (Scheme 4).[21] H_2O_2 is produced from singlet oxygen reaction with water.

The reaction of **3a** with H_2O_2 led to fast oxidation to alkyl seleninic acid. Intermediate **6a** was detected by HRMS ([M+Na]=471.0633) and ^{77}Se NMR (δ 1219.8 ppm) spectroscopy. Besides, a small signal of selenonic acid **6b** (δ 1050.8 ppm) was also detected in the ^{77}Se NMR spectra (Figure S1). The alkyl seleninic acid **6a** is too unstable to be isolable, it delivers **7** via deselenylation.[21] The use of *t*BuOOH led to the formation of **7** in 2 h as well.

Reduction of **3a** with 3 equiv. 1,4-dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) provided diselenide **1a** and GSH in a short reaction time (30 min). Furthermore, increasing the amount of TCEP to 10 equiv. led to deselenylation and provided Boc-Ala-Gly-OBn **9** (Scheme 4). The reduction proceeds via formation of the respective selenol **8**



Scheme 3. Proposed mechanism for the formation of Se–S bond-containing substrates.



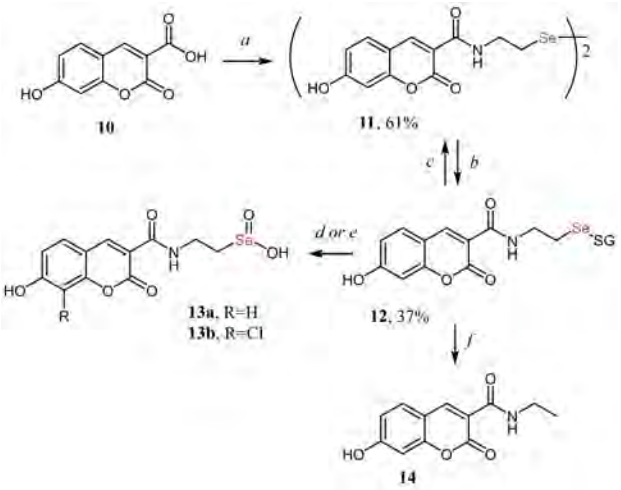
Scheme 4. Stability of **3a**. Reaction conditions: a) H_2O_2 or *t*BuOOH, MeCN/H_2O; b) DTT, MeCN/H_2O; c) TCEP, MeCN/H_2O.

that was detected by LC-MS ([M + Na] = 439.03) and ^{77}Se NMR spectroscopy (δ -72.5 ppm). The deselenylation proceeded smoothly yielding **9** and TCEP=Se ([M–H] = 328.9704).[29,30]

Further, derivative **12** was prepared starting with 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid **10** (Scheme 5) to evaluate the possibility to utilize alkylselenylsulfide moiety as a cleavable linker for the introduction of fluorescent probe to thiol group-containing peptide,. Diselenide **11** was successfully employed in the visible light-initiated reaction with glutathione, thus, a mixture of DMSO/H_2O was used to ensure that the starting materials are fully dissolved. Luckily, the desired Se–S bond-containing product **12** was easily obtained in 37% yield. Next, the stability of **12** was established. A quick oxidation and formation of the respective seleninic acid **13 a** was observed in the presence of H_2O_2. Another oxidant – NaClO provided the respective chlorinated seleninic acid **13 b** ([M–H] = 377.9290) The use of DTT (10 equiv.) led to formation of diselenide **11**. Recently, it was demonstrated that a cyclic selenosulfide subjected to reductive conditions (TCEP or DDT), spontaneously eliminates selenoethyl moiety releasing ethylene molecule, besides, all reactions were very slow (up to 6 days).[26] We did not observe such transformation in the case of **12** – interaction with TCEP (10 equiv.) was very efficient providing ethyl amide **14** in 30 min.

Considering a possible utilization of Se–S bond as a sensitive linker in biocompatible materials, the stability of **12** in phosphate-buffered saline (PBS, 20 mM) was established under various pH. The compound was stable in pH range 3.0–8.0 for 24 h, whereas a considerable amount of diselenide **11** was formed at pH 10.0 after 24 h (Table S1). Inspired by the good stability of a linker at physiological pH, absorption and emission spectra were collected for the solutions of **11**, **12** and **13 a** in PBS (pH = 7.4, c = 10 μM) (Figures S2–S4). The studied compounds showed absorbance maximum at 396–404 nm that is typical for the coumarin ring. Upon excitation at 350 nm the compounds' solutions emit light in blue region (Figure 2). Diselenide's **11** photoluminescence quantum yield is low (Φ = 1.6%), however, it is considerably higher in the case of **12** (Se–S, Φ = 29.5%). The highest emission quantum yield was observed after oxidation of **12** with hydrogen peroxide – the seleninic acid's **13 a** Φ is equal to 52.4%. The emission was characterized by CIE coordinates of pure blue light (x:0.1585, y:0.0160).

In conclusion, a simple protocol was developed for the synthesis of Se–S bond-containing peptides. A visible light-initiated reaction was employed for generation of sulfur-centered radical from unprotected glutathione that further reacted with protected and unprotected selenocysteine or selenocystamine peptides. Amino acids with sensitive groups (Arg, Lys, Trp, Tyr, His, Glu) showed tolerance under reaction conditions, although the products were obtained only in moderate yields. The use of Met-containing substrate provided the respective Met-sulfoxide. Notably, asymmetrical disulfides can be synthesized from 5-nitropyridine-2-thiol and Cys peptides. Fluorescent coumarin-selenocystamine conjugate with Se–S bond is stable under physiological conditions allowing to propose the Se–S bond as a valuable linker in biocompatible materials under oxidative stress conditions.



Scheme 5. Synthesis and stability of **12**. Reaction conditions: a) selenocystamine × 2HCl (1 equiv.), **10** (2.5 equiv.), *N*-methylmorpholine (NMM) (3 equiv.), 1-hydroxybenzotriazole (HOBt) (1 equiv.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (3 equiv.), DMF; b) GSH (10 equiv.), RB (0.1 equiv.), DMSO/H_2O, LED_{460}; c) DTT, PBS; d) H_2O_2; e) NaClO, PBS; f) TCEP, PBS.

Experimental Section

Representative procedure for visible light-mediated Se-S bond formation. Rose Bengal (0.1 equiv.) was added to a solution of Sec-peptide **1** (1 equiv.) and L-glutathione **2** (10 equiv.) in

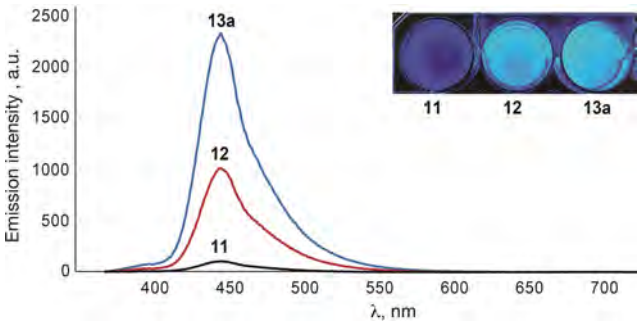


Figure 2. Emission spectra of **11**, **12**, **13 a** upon excitation at 350 nm (PBS, pH = 7.4).

mixture of MeCN/H_2O (1:1) and the mixture was irradiated by 36 W blue LEDs for 1 hour. After evaporation the residue was purified by reverse phase flash chromatography (C-18, MeCN/H_2O, 10–85%) to give the product **3**.

Acknowledgements

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6. pielikums/ Appendix VI

Lapcinska, S.; Arsenyan, P.

Selenocysteinyl electrophiles efficiently promote the formation of coumarin and quinolinone cores by 6-*endo-dig* cyclization.

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Selenocysteinyl electrophiles efficiently promote the formation of coumarin and quinolinone cores by 6-*endo-dig* cyclization†

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Efficient methods have been disclosed for the construction of nitrogen and oxygen containing heterocyclic systems attached to selenocysteine or selenoglutathione. An inorganic oxidant ($K_2S_2O_8$) or a mild Lewis acid ($CuBr_2$) was employed for the generation of selanyl electrophiles that were further trapped with phenyl propiolates, methyl 2-(phenylethynyl)benzoate, *N*-phenyl propiolamides and 2-(phenylethynyl)benzamide thus providing the respective coumarins, isocoumarins, quinolin-2-ones and isoquinolin-2-ones *via* 6-*endo-dig* cyclization. The use of visible light resulted in divergent reactivity providing triple bond addition products or spirocyclic compounds bearing selenocysteine moieties.

Introduction

2*H*-Chromen-2-one (coumarin) derivatives are widely present in nature possessing a broad spectrum of biological activity. Both natural and synthetic coumarins can act as pharmacologically active scaffolds in the treatment of various diseases, *e.g.*, cancer,[1–5] neurodegeneration,[6] and diseases induced by fungi,[7] viruses,[8,9] and pathological bacteria.[10] Polyhydroxycoumarins exhibit antioxidant activity.[11–14] Some examples of well-known coumarin-based drugs include warfarin and acenocoumarol (anticoagulants), and novobiocin and chlorobiocin (antibiotics).

Furthermore, not only medicinal chemists are interested in coumarins, but this scaffold is also widely used in fluorescent dye discovery[15] due to the strong photoluminescence properties displayed by 7-hydroxy and 7-amino substituted coumarins. Significantly, coumarins are often used as laser dyes[16] and as active components of optical brighteners,[17] solar cells[18] and organic light-emitting diodes.[19]

Remarkably, only a few examples have been reported for the preparation of 3-selanyl coumarins. Recently, Song *et al.* published an efficient method for direct selanylation of coumarins using diaryl or dialkyl diselenide and bis(trifluoroacetoxy)iodobenzene.[20] It should be pointed out that it is the first example of the direct selanylation of an unsubstituted coumarin ring. Previous methods were limited to the use of coumarins containing directing groups. A method for selanylation of 4-hydroxycoumarin and 4-hydroxy-1-methyl-2(1*H*)-quinolinone was developed employing elemental selenium, aryl iodide, a base, and a nickel ferrite nanocatalyst.[21] Jana *et al.* demonstrated that 4-hydroxycoumarin can be selenylated with benzeneselenol using the I_2/DMSO system as well.[22] A more sustainable protocol for the synthesis of 3-selanyl-4-(arylamino)coumarin was demonstrated by employing $(NH_4)_2S_2O_8$ under blue LED light irradiation.[23] An alternative approach for the synthesis of chalcogenated coumarins is 6-*endo-dig* cyclization of aryl propiolates in the presence of chalcogenyl electrophiles. A successful cyclization was established using propargylic aryl ethers, diaryl diselenides, *N*-iodosuccinimide and *tert*-butyl hydroperoxide.[24] Recently, an environmentally friendly and elegant method was reported for electrochemical oxidative cyclization of alkynoates and alkynamides using diorganyl diselenides for the synthesis of selanyl coumarins and quinolinones.[25] Zeni *et al.* showed that iron(III) chloride can be used for the generation of selanyl electrophiles with subsequent 6-*endo-dig* cyclization for the preparation of 3-selanyl coumarins and quinolinones.[26] Sahoo *et al.* examined the reaction between diaryl diselenides and *N*-aryl alkynamides in the presence of $K_2S_2O_8$.[27] They stated that the reaction proceeds *via* a radical mechanism under the following reaction conditions: 1 equiv. *N*-aryl alkynamide; 1.5 equiv. Ar_2Se_2; 1.5 equiv. $K_2S_2O_8$; 80 °C; dichloroethane.

Notably, a quinolinone scaffold is found in natural products[28] and biologically active compounds,[29,30] and its derivatives constitute an important class of many drugs, especially antibiotics and antituberculosis drugs,[31] thus the development of novel methods for the synthesis of this biologically active compound may be of interest for drug discovery.

Another important class of heterocycles that can be synthesized by 6-*endo-dig* cyclization is isocoumarins. Substituted isocoumarins are naturally occurring compounds, commonly exhibiting significant biological acitivities.[32–34] Recently, a sustainable method was established for the synthesis of 4-(phenylselanyl)isocoumarins using a continuous electrochemical

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1nj02633j

microreactor.[35] Alternatively, 6-*endo-dig* cyclization can be performed by employing $FeCl_3$[36] or *in situ* generated PhSeCl.[37,38] Jin *et al.* achieved the synthesis of selenylated isocoumarins using methyl 2-(phenylethynyl)benzoate, selenium powder, phenyl boronic acid, silver nitrate and $K_2S_2O_8$.[39]

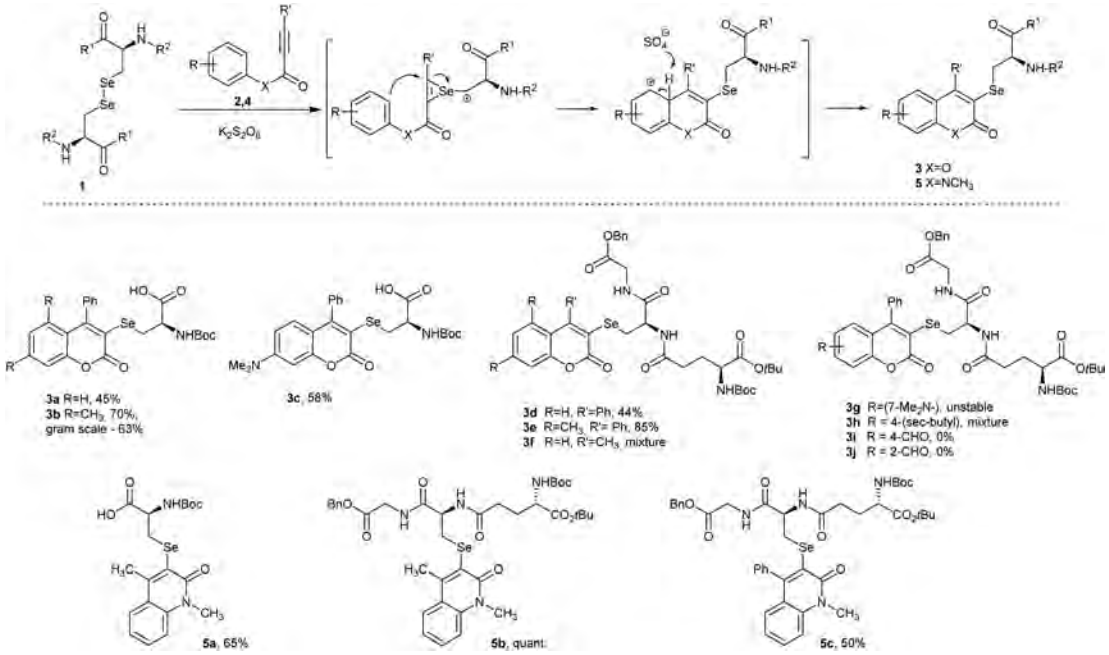
However, the aforementioned summary indicates that methodologies for the preparation of selenylated heterocycles need to be expanded, particularly, to aliphatic diselenides.

Herein we report our findings in the formation of coumarin and quinolin-2-one cores attached to selenocysteine moieties *via* 6-*endo-dig* cyclization of aryl propiolates and aryl propiolamides in the presence of selanyl electrophiles. Our previous results[40,41] that highlighted the use of a mild Lewis acid ($CuBr_2$) or an oxidant ($K_2S_2O_8$) for selenium electrophile generation from selenocystine containing peptides have laid a strong foundation for the exploration of broader application scope of these methods.

Results and discussion

We envisioned to find the optimal reaction conditions for the preparation of Sec-containing coumarins by selecting Boc-selenocysteine **1a** and phenyl 3-phenylpropiolate (**2a**) as model compounds. Initially, the reaction was tested by employing copper(II) bromide for selenium electrophile generation using the optimal reaction conditions reported[40,41] previously (**1a** – 1 equiv., **2a** – 1.5 equiv., and $CuBr_2$ – 2.5 equiv.). Unfortunately, cyclization was not observed, even on increasing the reaction temperature up to 40 °C. Another Lewis base reported in the literature ($FeCl_3$) capable of generating selanyl electrophiles with subsequent use in 6-*endo-dig* cyclization also failed to deliver the desired product. Gratifyingly, the use of an oxidant (5 equiv. of potassium persulfate) was the right choice to initiate the formation of selenocysteine containing coumarin rings. Test reactions with other oxidants such as *m*-CPBA, KIO_3, cerium ammonium nitrate and (diacetoxyiodo)benzene did not lead to the formation of even traces of **3a**. Due to the slow reaction rate with $K_2S_2O_8$ at ambient temperature, the increase of the temperature up to 40 °C resulted in the consumption of starting materials and formation of **3a** in 3 days. The utilization of 50 equiv. of an oxidant[41] accelerated the reaction without damaging the starting materials or products resulting in the formation of Sec-containing coumarin **3a** in 45% yield in 24 hours (Scheme 1). Therefore, we concluded that the optimal reaction conditions for the synthesis of 3-selanylcoumarins are 1 equiv. **1**, 1.2 equiv. **2** and 50 equiv. $K_2S_2O_8$. An excess amount of $K_2S_2O_8$ is required due to the extremely poor solubility of the oxidant in MeCN, thus the actual amount of the oxidant in the reaction mixture is very small. The decrease of the oxidant amount will result in a longer reaction time. The reaction yield was significantly improved by employing alkynes with EDGs in the 3rd or 3rd and 5th positions of the aryl ring (products **3b,c**). Additionally, a gram-scale reaction was performed for the synthesis of coumarin **3b** and the product was obtained in comparable yield.

Similarly, selenoglutathione moieties containing coumarins **3d** and **3e** were also successfully synthesized. The change of the



Scheme 1 Synthesis of 3-Sec-coumarins and quinolin-2-ones. Reaction conditions: diselenide **1** (1 equiv.), alkyne **2** or **4** (1.2 equiv.), $K_2S_2O_8$ (50 equiv.), MeCN, rt. **1a** $(Boc–Sec)_2$, **1b** (Boc–Glu(O*t*Bu)–Sec–Gly–OBn)$_2$, **2a** phenyl 3-phenylpropiolate, **2b** 3,5-dimethylphenyl 3-phenylpropiolate, **2c** 3-(dimethylamino)phenyl 3-phenylpropiolate, **2d** phenyl but-2-ynoate, **2e** 4-(*sec*-butyl)phenyl 3-phenylpropiolate, **2f** 4-formylphenyl 3-phenylpropiolate, **2g** 2-formylphenyl 3-phenylpropiolate, **4a** *N*-methyl-*N*-phenylbut-2-ynamide, **4b** *N*-methyl-*N*,3-diphenylpropiolamide.

phenyl substituent to the methyl group next to the triple bond resulted in an unselective reaction (**3f**). Unfortunately, the isolation of selenoglutathione containing 7-aminocoumarin **3g** failed. The reaction between **1b** and 3-(dimethylamino)phenyl propiolate was slower compared to that with Boc-selenocystine, and the appearance of side products was observed before the reaction reached 50% conversion. Although a large amount of oxidant was required for full conversion of **1** and **2**, the amino-substituted substances (the starting material and product) appeared to be unstable under the reaction conditions.

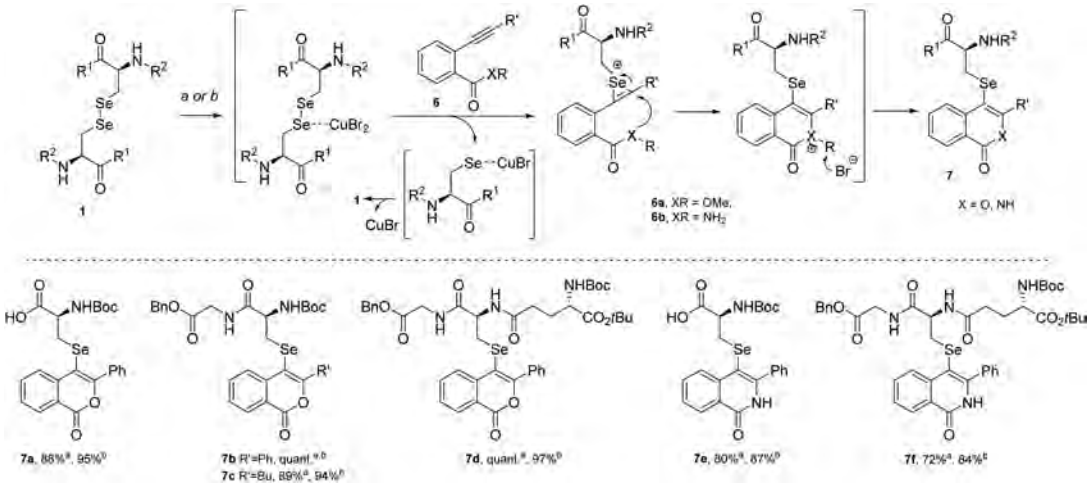
We believe that the mechanism of this reaction is as follows: the first step is the formation of selenium electrophiles which are trapped by the triple bond forming selenirenium ions (Scheme 1). Then electrophilic aromatic substitution occurs and after deprotonation, coumarin rings are formed. The results obtained let us conclude that the nature of the substituents strongly affected the coumarin ring closure. As in classical electrophilic aromatic substitution, the EDG increased the electron density in the π system thus making it more nucleophilic and promoted the reaction, whereas an EWG deactivated the aromatic ring by decreasing the electron density in the π system.

Satisfied with the results, we turned our attention to the preparation of analogous nitrogen heterocycles – quinolin-2-ones. Consequently, under the same reaction conditions, the respective selenocysteine and selenoglutathione containing quinolin-2-ones **5a–c** were obtained in good yields starting from *N*-methyl alkynyl amides (**4a**, **4b**).

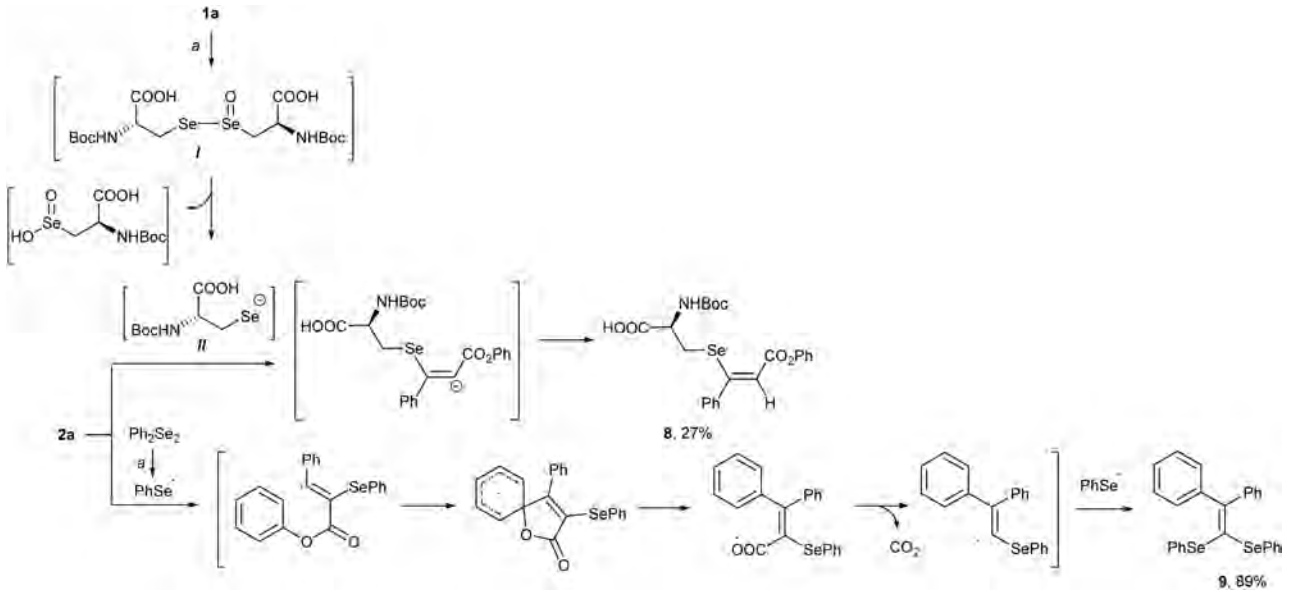
Next, we employed the same reaction conditions for the synthesis of isocoumarins and isoquinolin-2-ones. Luckily, alkyne **6a** easily reacted with selenium electrophiles that were generated using $K_2S_2O_8$ and provided Sec-containing isocoumarin **7a** in excellent yield (Scheme 2). Moreover, not only $K_2S_2O_8$ was suitable for the generation of selanyl electrophiles but also $CuBr_2$ (2.5 equiv.), thus expanding the application scope of the previously reported copper(II) bromide promoted selanyl electrophile generation.[40,41] Under both conditions, the products **7a–d** were obtained in equally high yields. The only difference was the reaction time – 24 hours for $K_2S_2O_8$, and 2 hours for $CuBr_2$-induced selanyl electrophile generation. Both aryl and alkyl substituents next to the triple bond were suitable for heterocycle formation. Besides, the synthesis of Sec-containing isoquinolin-2-ones was performed employing the same conditions. Also in this case, both methods were suitable for the generation of selenium electrophiles and resulted in the preparation of products **7e,f** in high yields. Alkynes reacted with selenium electrophiles forming selenirenium ions which are then attacked by the nucleophilic heteroatoms closing the cycle and after demethylation or deprotonation, the product is formed. Obviously, this ring closure occurs more easily compared to the coumarin ring formation because it is based on the attack of nucleophilic heteroatoms on the selenirenium ions, and not on the electrophilic aromatic substitution.

Unfortunately, none of the methods employed for selanyl electrophile generation and subsequent 6-*endo-dig* cyclization were suitable for sulfenyl electrophile generation from analogous S–S bonds containing $(Boc\text{-}Glu(O\mathit{t}Bu)\text{-}Cys\text{-}Gly\text{-}OBn)_2$. Obviously, the S–S bond is stronger than the Se–Se bond, therefore it is less reactive. However, recently we reported[42] a simple method for the preparation of glutathione containing quinolin-2-ones and isocoumarins. This protocol was based on *in situ* preparation of the respective sulfenyl chloride employing *N*-chlorosuccinimide. In this case, the symmetric disulfide was formed as a side product, thus the yields of glutathione containing heterocycles were only moderate. In contrast, the use of diselenide avoids undesired side-reactions; therefore, the products were synthesized in high yields.

Recently, we developed an efficient method[43] for the functionalization of Sec-peptides based on the visible light-initiated



Scheme 2 Synthesis of 4-Sec-isocoumarins and isoquinolin-2-ones. Reaction conditions: (a) diselenide **1** (1 equiv.), alkyne (1.5 equiv.), $CuBr_2$ (2.5 equiv.), CH_2Cl_2, rt; (b) diselenide **1** (1 equiv.), alkyne (1.5 equiv.), $K_2S_2O_8$ (50 equiv.), MeCN, rt. **1c** (Boc–Sec–Gly–OBn)$_2$, **6a** methyl 2-(phenylethynyl)-benzoate, **6b** methyl 2-(hex-1-yn-1-yl)benzoate, **6c** 2-(phenylethynyl)benzamide.

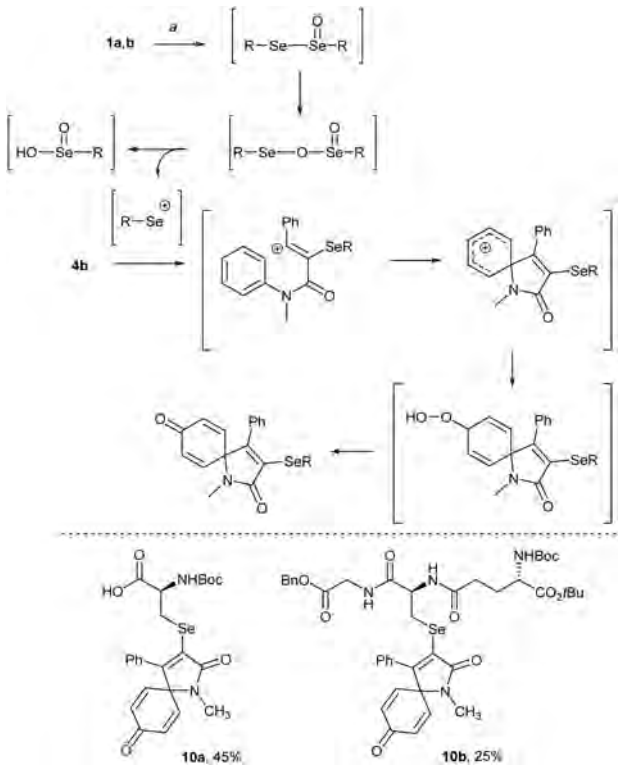


Scheme 3 Reaction between **2a** and **1a** or Ph_2Se_2 under visible light irradiation. Reaction conditions: (a) **1a** or Ph_2Se_2 (1 equiv.), **2a** (1 equiv.), MeCN, and LED_{460}.

reaction. Consequently, we envisioned to obtain **3a** *via* a visible light-induced reaction. It is noteworthy that this is an attractive way for the synthesis of selenylated heterocycles[44] due to being environmentally friendly and atom-economical because both diselenide parts are used for performing the reaction under mild conditions. The reaction between **1a** and **2a** under optimized reaction conditions[43] (5 mol% Rose Bengal, MeCN, LED_{460}) did not lead to the formation of **3a**, and only deselenylation was observed. Other tested photocatalysts, namely, FIrPic, $Ru(bpy)_3Cl_2$, fluorescein, acridine, and eosin K, did not enhance the formation of the product. However, photocatalyst-free conditions provided tri-substituted alkene **8** (Scheme 3). Alkene **8** was formed as a single isomer in MeCN or MeOH in 56 h utilizing 1 equiv. **1a** and 1 equiv. **2a**. Other tested solvents (EtOH, DMF, CH_2Cl_2, DMSO, EtOAc, and THF) were less effective. The reaction is very slow, because aliphatic diselenides do not form the corresponding radicals under LED-light irradiation without a photocatalyst. Mono-selenoxide intermediate ***I*** is formed in the reaction with hydrogen peroxide which is produced from water and oxygen during irradiation.[43,45] This intermediate decomposes forming respective seleninic acid and selenolate ***II*** which reacts with aryl propiolate *via* a Michael type reaction forming *Z*-alkene.[46,47] The formation of H_2O_2 under photocatalyst-free conditions is inefficient and it explains the low rate of the process and the low yield of **8** (27%).

In contrast, under the same conditions, the reaction between **2a** and Ph_2Se_2 provided tetrasubstituted alkene **9** in a short reaction time (1 h). This type of compound previously has been synthesized using Ar_2Se_2, **2a**, di-*tert*-butyl peroxide, 120 °C, PhCl, and N_2.[48] Therefore, an efficient and mild method is demonstrated as an alternative for the synthesis of 1,1-dichalcogenide olefins. The different reactivities of **1a** and Ph_2Se_2 are due to the facile formation of phenyl selanyl radicals[45] under visible light irradiation without the photocatalyst providing **9** in 89% yield.

The use of alkyne **4b** in the visible light-initiated reaction resulted in the synthesis of spirocyclic products **10** (Scheme 4).



Scheme 4 Synthesis of spirocyclic products **10**. Reaction conditions: (a) diselenide (1 equiv.), **4b** (1 equiv.), MeCN, LED_{460}.

Typically, aryl alkynes with *p*-OMe or *p*-F substituents are employed for spirocyclization reactions.[49–51] In this case, the keto group was introduced with the help of H_2O_2 that was produced by visible light irradiation.[43] The spirocycle formation was not observed employing **4a**. The selenium electrophile that is produced by the reaction between the diselenide and H_2O_2 adds to the triple bond providing vinyl cations which undergo *ipso*-cyclization. Sequential oxidation leads to product **10**. The change of solvent to methanol enriched with oxygen did not lead to significant improvement of conversion, because the limitating step is the *in situ* production of H_2O_2 in reaction media. Notably, the introduction of extra amount of hydrogen peroxide even at a low concentration resulted in side-reactions and decreased the yield of **10**.

Conclusions

Simple methods for the preparation of selenocysteine and selenoglutathione containing coumarins, quinolin-2-ones, isocoumarins and isoquinolin-2-ones are presented. The scaffolds of heterocycles are synthesized by the ring closure of the corresponding alkynes *via* 6-*endo-dig* cyclization.

The choice of exact reaction conditions strongly depends on the electronic behaviour of the initial compounds. For the synthesis of coumarins and quinolin-2-ones, $K_2S_2O_8$ is required for the generation of selenium electrophiles and ring closure whereas for the preparation of isocoumarins and isoquinolin-2-ones, either an oxidant or $CuBr_2$ can be used as an effective promoter for the generation of Sec electrophiles and heterocycle formation.

The change of reaction conditions to visible light irradiation led to different reactivities of **2a** or **4a** with aromatic and aliphatic diselenides based on the formation of arylselanyl radicals, selenocysteinyl nucleophiles or electrophiles.

Experimental section

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was performed using MERCK Silica gel 60 F254 plates and visualized by UV (254 nm) fluorescence. ZEOCHEM silica gel (ZEOprep 60/35-70 microns – SI23501) was used for column chromatography. ^{1}H, ^{13}C and ^{77}Se NMR spectra were recorded on a Bruker Avance Neo spectrometer at 400, 101 and 76 MHz correspondingly at 298 K in CD_3OD or $CDCl_3$. Infrared (IR) spectra were recorded with a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). HRMS were recorded using a Waters Synapt GII Q-ToF UPLC/MS system.

Boc-Sec[52] and Sec-peptides[40] **1b,c** were prepared according to literature procedures, as well as alkynes **2a-c**,[53] **2d**,[54] **2e**,[53] **2f**,[55] **2g**,[44] **4a,b**,[56] **6a**,[57] **6b**,[58] **6c**.[59]

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((2-oxo-4-phenyl-2*H*-chromen-3-yl)selanyl)propanoic acid (3a)

To a solution of Boc-Sec (100 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **2a** (50 mg, 0.22 mmol, 1.2 equiv.) and $K_2S_2O_8$ (2.53 g, 9.4 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **3a** as a yellow solid (42 mg, 45%). $[\alpha]_D^{20}$ +24.0 (c 1.0, MeOH). IR ν_{max} (film): 3436, 2980, 1718, 1539, 1167 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 7.61–7.49 (m, 4H), 7.40 (dd, J = 8.3, 1.1 Hz, 1H), 7.35–7.30 (m, 1H), 7.30–7.26 (m, 1H), 7.21 (ddd, J = 8.3, 7.1, 1.1 Hz, 1H), 7.01 (dd, J = 8.0, 1.5 Hz, 1H), 4.32 (t, J = 7.4, 4.6 Hz, 1H), 3.42 (dd, J = 13.0, 4.6 Hz, 1H), 3.30–3.26 (m, 1H, overlap with CD_3OD signal), 1.31 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.9, 161.2, 159.3, 157.3, 154.5, 137.8, 133.0, 130.1, 129.81, 129.79, 129.6, 129.4, 128.7, 125.6, 121.8, 119.7, 117.6, 80.7, 55.8, 28.7, 28.6. ^{77}Se NMR (76 MHz, CD_3OD) δ 244.9. HRMS (ESI/Q-TOF) m/z: [M + Na]$^+$ calcd for $[C_{23}H_{23}NO_6SeNa]^+$ 512.0588; found 512.0601.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((5,7-dimethyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)selanyl)propanoic acid (3b)

To a solution of Boc-Sec (100 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **2b** (56 mg, 0.22 mmol, 1.2 equiv.) and $K_2S_2O_8$ (2.53 g, 9.4 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **3b** as a white solid (70 mg, 70%). For the gram scale reaction Boc-Sec (0.92 g, 1.72 mmol, 1 equiv.), **2b** (0.52 g, 2.07 mmol, 1.2 equiv.) and $K_2S_2O_8$ (23.3 g, 86.1 mmol, 50 equiv.) were utilized to provide **3b** (0.56 g, 63%). $[\alpha]_D^{20}$ +23.3 (c 0.87, MeOH). IR ν_{max} (film): 3328, 2977, 2930, 1717, 1616, 1490 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 7.54–7.46 (m, 3H), 7.32–7.19 (m, 2H), 7.12 (s, 1H), 6.90 (s, 1H), 4.34–4.26 (m, 1H), 3.41 (dd, J = 13.1, 4.6 Hz, 1H), 2.38 (s, 3H), 1.68 (s, 3H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.0, 160.9, 159.8, 157.4, 155.5, 143.8, 142.0, 138.4, 131.1, 129.9, 129.8, 129.3, 129.1, 119.9, 117.5, 116.5, 80.7, 55.8, 28.7, 28.6, 23.4, 21.3. ^{77}Se NMR (76 MHz, CD_3OD) δ 246.7. HRMS (ESI/Q-TOF) m/z: [M + Na]$^+$ calcd for $[C_{25}H_{27}NO_6SeNa]^+$ 540.0901; found 540.0902.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((7-(dimethylamino)-2-oxo-4-phenyl-2*H*-chromen-3-yl)selanyl)propanoic acid (3c)

To a solution of **1a** (100 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **2c** (56 mg, 0.22 mmol, 1.4 equiv.) and $K_2S_2O_8$ (2.53 g, 9.4 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O + AcOH 20–85%) to provide **3c** as a brown solid (57 mg, 58%). $[\alpha]_D^{20}$ −36.5 (c 0.77, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 7.67–7.61 (m, 2H), 7.57–7.49 (m, 2H), 7.48–7.41 (m, 2H), 6.62 (dd, J = 8.7, 2.8 Hz, 1H), 6.55 (d, J = 2.7 Hz, 1H), 4.34–4.26 (m, 1H), 3.20 (dd, J = 12.6, 4.4 Hz, 1H), 3.06–2.98 (m, 1H), 2.96 (s, 6H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.3, 157.5, 154.1, 153.8, 153.6, 139.2, 134.2, 132.3, 130.0, 120. 5, 112.3, 107.6, 107.0, 89.5, 81.2, 80.6, 40.4, 31.0, 28.7. ^{77}Se NMR (76 MHz, CD_3OD) δ 189.3. HRMS (ESI/Q-TOF) m/z: [M + Na]$^+$ calcd for $[C_{25}H_{28}N_2O_6SeNa]^+$ 555.1010; found 555.1008.

***Tert*-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((2-oxo-4-phenyl-2*H*-chromen-3-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (3d)**

To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **2a** (22 mg, 0.1 mmol, 1.2 equiv.) and $K_2S_2O_8$ (1.12 g, 4.17 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **3d** as a white solid (30 mg, 44%). IR ν_{max} (film): 3369, 2979, 2930, 2479, 1722, 1662, 1528 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.63–7.48 (m, 4H), 7.41 (dd, J = 8.4, 1.0 Hz, 1H), 7.38–7.19 (m, 8H), 7.02 (dd, J = 8.1, 1.5 Hz, 1H), 5.12 (s, 2H), 4.48 (dd, J = 8.8, 4.9 Hz, 1H), 3.99–3.94 (m, 1H), 3.91 (s, 2H), 3.41 (dd, J = 12.9, 5.0 Hz, 1H), 3.03 (dd, J = 12.9, 8.9 Hz, 1H), 2.35–2.18 (m, 2H), 2.07–1.97 (m, 1H), 1.88–1.77 (m, 1H), 1.47 (s, 9H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 176.4, 174.7, 173.2, 170.7, 161.4, 159.9, 158.0, 154.5, 137.8, 137.1, 133.1, 130.2, 129.84, 129.77, 129.7, 129.59, 129.55, 129.4, 129.31, 129.29, 128.8, 125.7, 121.8, 119.5, 117.6, 82.8, 80.5, 67.9, 55.4, 54.8, 42.2, 33.0, 29.0, 28.8, 28.3, 22.1. ^{77}Se NMR (76 MHz, CD_3OD) δ 240.6. HRMS (ESI/Q-TOF) m/z: $[M + Na]^+$ calcd for $[C_{41}H_{47}N_3O_{10}SeNa]^+$ 844.2324; found 302.0194.

***Tert*-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-((5,7-dimethyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (3e)**

To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **2b** (25 mg, 0.1 mmol, 1.2 equiv.) and $K_2S_2O_8$ (1.12 g, 4.17 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **3e** as a white solid (60 mg, 85%). IR ν_{max} (film): 3515, 3378, 2983, 2934, 2507, 1749, 1689, 1636 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.51–7.45 (m, 3H), 7.36–7.32 (m, 4H), 7.31–7.25 (m, 1H), 7.25–7.21 (m, 1H), 7.20–7.16 (m, 1H), 7.13–7.11 (m, 1H), 6.91–6.88 (m, 1H), 5.12 (s, 2H), 4.46 (dd, J = 8.9, 4.7 Hz, 1H), 4.01–3.94 (m, 1H), 3.92 (s, 2H), 3.42 (dd, J = 13.0, 4.9 Hz, 1H), 3.04 (dd, J = 13.0, 8.9 Hz, 1H), 2.37 (s, 3H), 2.35–2.19 (m, 2H), 2.14–1.99 (m, 1H), 1.90–1.78 (m, 1H), 1.67 (s, 3H), 1.47 and 1.44 (2 s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.8, 173.3, 173.2, 170.7, 161.2, 160.4, 158.0, 155.5, 144.1, 142.0, 138.5, 137.2, 131.2, 129.9, 129.83, 129.81, 129. 6, 129.33, 129.31, 129.29, 129.1, 119.7, 117.6, 116.5, 82.8, 80.5, 67.9, 55.4, 55.0, 42.2, 33.1, 29.1, 28.8, 28.3, 23.4, 21.3. ^{77}Se NMR (76 MHz, CD_3OD) δ 241.7. HRMS (ESI/Q-TOF) m/z: $[M + Na]^+$ calcd for $[C_{43}H_{51}N_3O_{10}SeNa]^+$ 872.2637; found 872.2640.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((1,4-dimethyl-2-oxo-1,2-dihydroquinolin-3-yl)selanyl)propanoic acid (5a)

To a solution of **1a** (140 mg, 0.26 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **4a** (54 mg, 0.31 mmol, 1.2 equiv.) and $K_2S_2O_8$ (3.54 g, 13.1 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **5a** as a yellow solid (75 mg, 65%). $[\alpha]_D^{20}$ +9.4 (c 1, $CHCl_3$). IR ν_{max} (film): 3368, 2980, 2925, 1748, 1692, 1602, 1517 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.89 (d, J = 8.1 Hz, 1H), 7.67–7.59 (m, 1H), 7.57–7.51 (m, 1H), 7.36–7.27 (m, 1H), 4.29 (dd, J = 7.6, 4.4 Hz, 1H), 3.75 (s, 3H), 3.43 (dd, J = 12.9, 4.6 Hz, 1H), 3.29–3.21 (m, 1H), 2.85 (s, 3H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.2, 162.3, 157.4, 153.4, 140.4, 132.2, 127.5, 124.6, 123.8, 122.3, 116.0, 80.5, 55.7, 31.3, 29.1, 28.8, 28.6, 21.3. ^{77}Se NMR (76 MHz, CD_3OD) δ 192.4. HRMS (ESI/Q-TOF) m/z: $[M + Na]^+$ calcd for $[C_{19}H_{24}N_2O_5SeNa]^+$ 463.0748; found 463.0741.

***Tert*-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-((1,4-dimethyl-2-oxo-1,2-dihydroquinolin-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (5b)**

To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **4b** (18 mg, 0.10 mmol, 1.2 equiv.) and $K_2S_2O_8$ (1.12 g, 4.17 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **5b** as a yellow oil (65 mg, quant.). IR ν_{max} (film): 3293, 2978, 2933, 1653, 1539, 1507, 1361 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.88 (dd, J = 8.2, 1.4 Hz, 1H), 7.64 (ddd, J = 8.6, 7.1, 1.4 Hz, 1H), 7.54 (dd, J = 8.6, 1.1 Hz, 1H), 7.35–7.21 (m, 6H), 5.10 (s, 2H), 4.52 (dd, J = 8.2, 4.8 Hz, 1H), 3.97 (dd, J = 9.2, 5.1 Hz, 1H), 3.90 (s, 2H), 3.76 (s, 3H), 3.29–3.24 (m, 1H), 3.18 (dd, J = 12.8, 8.3 Hz, 1H), 2.82 (s, 3H), 2.40–2.32 (m, 2H), 2.13–2.03 (m, 1H), 1.92–1.80 (m, 1H), 1.45 and 1.42 (2 s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.9, 173.4, 173.3, 170.7, 162.4, 158.0, 153.6, 140.3, 137.1, 132.3, 129.5, 129.29, 129.25, 127.5, 124.7, 123.9, 122.2, 116.1, 82.7, 80.5, 67.9, 55.4, 55.0, 42.2, 33.1, 31.4, 29.2, 28.7, 28.3, 21.4. ^{77}Se NMR (76 MHz, CD_3OD) δ 187.7. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $[C_{37}H_{49}N_4O_9Se]^+$ 773.2659; found 773.2665.

***Tert*-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-((1-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (5c)**

To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **4c** (23 mg, 0.10 mmol, 1.2 equiv.) and $K_2S_2O_8$ (1.12 g, 4.17 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **5c** as a light-yellow solid (35 mg, 50%). IR ν_{max} (film): 3311, 2978, 2933, 1743, 1713, 1670, 1542 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.65–7.62 (m, 2H), 7.52–7.45 (m, 3H), 7.34–7.30 (m, 3H), 7.30–7.27 (m, 1H), 7.26–7.13 (m, 4H), 7.12–7.07 (m, 1H), 5.11 (s, 2H), 4.42 (dd, J = 8.8, 4.6 Hz, 1H), 4.03–3.95 (m, 1H), 3.92 (s, 2H), 3.86 (s, 3H), 3.25 (dd, J = 12.8, 4.7 Hz, 1H), 3.04 (dd, J = 12.8, 8.9 Hz, 1H), 2.38–2.27 (m, 2H), 2.12–2.01 (m, 1H), 1.91–1.80 (m, 1H), 1.45 and 1.42 (2 s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.9, 173.5, 173.2, 170.7, 162.6, 158.0, 156.8, 140.8, 139.7, 137.1, 132.3, 130.0, 129.8, 129.64, 129.57, 129.5, 129.30, 129.25, 124.4, 123.7, 122.7, 116.0, 82.7, 80.5, 67.8, 55.5, 55.1, 42.2, 33.1, 31.5, 28.8, 28.3. ^{77}Se NMR (76 MHz, CD_3OD) δ 132.9. HRMS

(ESI/Q-TOF) *m*/*z*: $[M + H]^+$ calcd for $[C_{42}H_{51}N_4O_9Se]^+$ 835.2816; found 835.2811.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((1-oxo-3-phenyl-1*H*-isochromen-4-yl)selanyl)propanoic acid (7a)

Method A. To a solution of Boc-Sec (100 mg, 0.187 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (100 mg, 0.47 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **5** (66 mg, 0.28 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7a** as a colorless oil (78 mg, 88%.). *Method B.* To a solution of Boc-Sec (100 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **5** (66 mg, 0.28 mmol, 1.5 equiv.) and $K_2S_2O_8$ (2.53 g, 9.36 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7a** (87 mg, 95%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (ddd, J = 8.0, 4.6, 1.2 Hz, 2H), 7.89 (ddd, J = 8.4, 7.3, 1.4 Hz, 1H), 7.74–7.66 (m, 2H), 7.66–7.58 (m, 1H), 7.53–7.44 (m, 3H), 4.21–4.10 (m, 1H), 2.98 (dd, J = 12.4, 4.1 Hz, 1H), 2.89 (dd, J = 12.4, 7.0 Hz, 1H), 1.40–1.24 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 176.4, 163.5, 159.5, 157.2, 140.0, 136.7, 135.6, 131.3, 131.1, 130.5, 129.9, 129.3, 129.0, 121.7, 106.2, 80.8, 30.4, 28.6, 22.1. ^{77}Se NMR (76 MHz, CD_3OD) δ 147.0. HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $[C_{23}H_{23}NO_6SeNa]^+$ 512.0588; found 512.0595.

Benzyl (*R*)-(2-((*tert*-butoxycarbonyl)amino)-3-((1-oxo-3-phenyl-1*H*-isochromen-4-yl)selanyl)propanoyl)glycinate (7b)

Method A. To a solution of **1c** (100 mg, 0.12 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (67 mg, 0.31 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **6a** (43 mg, 0.18 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7b** as a yellow solid (83 mg, quant.). *Method B.* To a solution of **1c** (100 mg, 0.12 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **6a** (40 mg, 0.17 mmol, 1.4 equiv.) and $K_2S_2O_8$ (1.6 g, 6.03 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7b** (83 mg, quant.). $[\alpha]_D^{20}$ −1.23 (c 0.88, $CHCl_3$). IR ν_{max} (film): 3321, 2978, 1739, 1529 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (dd, J = 8.0, 1.2 Hz, 1H), 8.20 (dd, J = 8.0, 1.2 Hz, 1H), 7.85–7.79 (m, 1H), 7.73–7.68 (m, 2H), 7.58–7.52 (m, 1H), 7.47–7.41 (m, 3H), 7.37–7.31 (m, 5H), 6.42 (s, 1H), 5.15 (s, 2H), 4.88 (d, J = 8.3 Hz, 1H), 4.24–4.16 (m, 1H), 3.88–3.81 (m, 2H), 3.04–2.96 (m, 1H), 2.75 (dd, J = 12.6, 6.2 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 169.2, 161.7, 158.2, 155.2, 138.5, 135.5, 135.2, 134.1, 130.4, 130.2, 130.0, 128.83, 128.77, 128.7, 128.5, 128.1, 128.0, 121.0, 105.3, 80.8, 67.3, 53.9, 41.4, 30.3, 28.3. ^{77}Se NMR (76 MHz, $CDCl_3$) δ 134.8. HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $[C_{32}H_{32}N_2O_7SeNa]^+$ 659.1272; found 659.1290.

Benzyl (*R*)-(2-((*tert*-butoxycarbonyl)amino)-3-((3-butyl-1-oxo-1*H*-isochromen-4-yl)selanyl)propanoyl)glycinate (7c)

Method A. To a solution of **1c** (100 mg, 0.12 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (67 mg, 0.31 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **6b** (29 mg, 0.18 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7c** as a yellow solid (66 mg, 89%). *Method B.* To a solution of **1a** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **6b** (36 mg, 0.17 mmol, 1.4 equiv.) and $K_2S_2O_8$ (1.6 g, 6.03 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7c** (70 mg, 94%). $[\alpha]_D^{20}$ −32.0 (c 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (dd, J = 8.0, 1.4 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.86–7.80 (m, 1H), 7.57–7.52 (m, 1H), 7.37–7.22 (m, 5H), 5.12 (s, 2H), 4.20 (dd, J = 9.2, 4.4 Hz, 1H), 4.01–3.82 (m, 2H), 3.20–2.95 (m, 3H), 2.88 (dd, J = 12.5, 9.6 Hz, 1H), 1.77–1.61 (m, 2H), 1.50–1.31 (m, 11H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.8, 170.8, 163.9, 157.5, 140.0, 137.1, 136.7, 130.5, 129.5, 129.33, 129.31, 129.2, 128.7, 128.0, 121.5, 105.3, 81.0, 67.9, 56.0, 42.1, 35.3, 31.4, 30.9, 28.7, 23.4, 14.2. ^{77}Se NMR (76 MHz, CD_3OD) δ 131.1. HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $[C_{30}H_{36}N_2O_7SeNa]^+$ 639.1585; found 639.1586.

Tert-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((1-oxo-3-phenyl-1*H*-isochromen-4-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (7d)

Method A. To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (47 mg, 0.208 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **6a** (30 mg, 0.125 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7d** as a white solid (66 mg, quant.). *Method B.* To a solution of **1a** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **6a** (26 mg, 0.117 mmol, 1.4 equiv.) and $K_2S_2O_8$ (1.13 g, 4.17 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7d** (64 mg, 97%). IR ν_{max} (film): 3295, 2917, 2849, 1734, 1635 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38–8.31 (m, 1H), 8.24–8.18 (m, 1H), 7.82 (ddd, J = 8.3, 7.3, 1.4 Hz, 1H), 7.76–7.67 (m, 2H), 7.59–7.52 (m, 1H), 7.49–7.41 (m, 3H), 7.38–7.28 (m, 5H), 5.17–5.09 (m, 2H), 4.37–4.30 (m, 1H), 4.15–4.05 (m, 1H), 3.93–3.73 (m, 2H), 3.01 (dd, J = 12.5, 6.3 Hz, 1H), 2.84 (dd, J = 12.4, 6.4 Hz, 1H), 2.10–1.96 (m, 3H), 1.78–1.71 (m, 1H), 1.47–1.41 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.3, 170.1, 169.1, 161.6, 158.2,

155.8, 138.5, 135.4, 135.1, 134.1, 130.4, 130.1, 129.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 120.8, 105.2, 82.4, 80.0, 67.1, 53.2, 52.3, 41.2, 41.1, 31.9, 28.3, 28.0. ^{77}Se NMR (76 MHz, $CDCl_3$) δ 132.9. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $[C_{41}H_{47}N_3O_{10}SeNa]^+$ 844.2324; found 844.2319.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((1-oxo-3-phenyl-1,2-dihydroisoquinolin-4-yl)selanyl)propanoic acid (7e)

Method A. To a solution of **1a** (100 mg, 0.187 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (100 mg, 0.47 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **6c** (62 mg, 0.28 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7e** as a yellow solid (73 mg, 80%.). *Method B.* To a solution of **1a** (100 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **6c** (58 mg, 0.26 mmol, 1.4 equiv.) and $K_2S_2O_8$ (2.53 g, 9.36 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7e** (79 mg, 87%). IR ν_{max} (film): 2979, 2933, 1734, 1718, 1507 cm^{-1}. ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$) δ 8.34–8.26 (m, 2H), 7.97–7.88 (m, 1H), 7.75–7.67 (m, 2H), 7.69–7.59 (m, 1H), 7.53–7.45 (m, 3H), 4.19–4.12 (m, 1H), 2.99 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.90 (dd, *J* = 12.6, 7.2 Hz, 1H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$) δ 163.4, 158.9, 156.5, 139.7, 136.5, 135.0, 130.9, 130.8, 130.3, 129.5, 129.0, 128.6, 121.3, 106.0, 80.6, 30.4, 28.5. ^{77}Se NMR (76 MHz, $CD_3OD/CDCl_3$) δ 147.1. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $[C_{23}H_{25}N_2O_5SeNa]^+$ 512.0821; found 512.0586.

Tert-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-1-oxo-3-((1-oxo-3-phenyl-1,2-dihydroisoquinolin-4-yl)selanyl)propan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (7f)

Method A. To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added $CuBr_2$ (47 mg, 0.208 mmol, 2.5 equiv.). The reaction mixture was stirred for 30 minutes and then alkyne **6c** (28 mg, 0.125 mmol, 1.5 equiv.) was added and the mixture was stirred until the disappearance of starting materials. Then it was evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7f** as a yellow solid (49 mg, 72%). *Method B.* To a solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was added alkyne **6c** (26 mg, 0.117 mmol, 1.4 equiv.) and $K_2S_2O_8$ (1.13 g, 4.16 mmol, 50 equiv.). The reaction mixture was stirred until the disappearance of starting materials, and then filtered, evaporated and purified by reverse phase chromatography (C-18, MeCN/H_2O 30–85%) to provide **7f** (57 mg, 84%). IR ν_{max} (film): 3295, 2980, 2933, 1734, 1688, 16136, 1523 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38–8.31 (m, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 7.87–7.78 (m, 1H), 7.75–7.68 (m, 2H), 7.60–7.51 (m, 1H), 7.49–7.43 (m, 3H), 7.39–7.29 (m, 5H), 6.63 (s, 1H), 6.26–6.15 (m, 1H), 5.23–5.15 (m, 1H), 5.13 (s, 2H), 4.36–4.27 (m, 1H), 4.16–4.05 (m, 1H), 3.94–3.71 (m, 2H), 3.02 (dd, *J* = 12.5, 6.2 Hz, 1H), 2.90–2.79 (m, 1H), 2.09–1.95 (m, 3H), 1.79–1.70 (m, 1H), 1.44 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 171.5, 170.2, 170.1, 169.2, 161.7, 158.4, 155.9, 138.6, 135.6, 135.3, 134.3, 130.6, 130.2, 130.1, 128.9, 128.8, 128.7, 128.6, 128.1, 121.0, 105.3, 82.5, 80.2, 67.3, 53.3, 52.5, 41.4, 41.3, 32.1, 29.7, 28.9, 28.5, 28.1. ^{77}Se NMR (76 MHz, $CDCl_3$) δ 132.5. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $[C_{41}H_{49}N_4O_9SeNa]^+$ 844.2557; found 844.2328.

(*R*,*Z*)-2-((*Tert*-butoxycarbonyl)amino)-3-((3-oxo-3-phenoxy-1-phenylprop-1-en-1-yl)selanyl)propanoic acid (8)

A solution of **1a** (100 mg, 0.187 mmol, 1 equiv.) and alkyne **2a** (42 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was irradiated by 36 W blue LEDs for 56 hours. The reaction mixture was filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **8** as a colorless oil (25 mg, 27%). $[\alpha]_D^{20}$ +17.2 (c 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 7.41–7.19 (m, 10H), 4.35 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.25 (dd, *J* = 13.0, 4.3 Hz, 1H), 2.98 (dd, *J* = 13.0, 9.5 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.3, 157.8, 147.7, 142.6, 142.5, 130.5, 130.0, 129.2, 129.12, 129.10, 123.7, 80.8, 55.9, 29.0, 28.8. ^{77}Se NMR (76 MHz, CD_3OD) δ 292.5. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $[C_{23}H_{25}NO_6SeNa]^+$ 514.0745; found 514.0754.

(2,2-Diphenylethene-1,1-diyl)bis(phenylselane) (9)[45]

A solution of Ph_2Se_2 (100 mg, 0.32 mmol, 1 equiv.) and alkyne **2a** (71 mg, 0.32 mmol, 1 equiv.) in MeCN (5 ml) was irradiated by 36 W blue LEDs for 1 hour. The reaction mixture was evaporated and purified by flash chromatography (hexane/EtOAc 1–4%) to provide **9** as a yellow oil (140 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 –7.15 (m, 16H), 7.14 –7.09(m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.45, 143.25, 133.64, 132.48, 129.43, 128.62, 128.13, 127.81, 127.34, 122.51. ^{77}Se NMR (76 MHz, $CDCl_3$) δ 468.9. GC-MS *m/z*: 489.9.

(*R*)-2-((*Tert*-butoxycarbonyl)amino)-3-((1-methyl-2,8-dioxo-4-phenyl-1-azaspiro[4.5]deca-3,6,9-trien-3-yl)selanyl)propanoic acid (10a)

A solution of **1a** (100 mg, 0.187 mmol, 1 equiv.) and alkyne **4b** (44 mg, 0.187 mmol, 1 equiv.) in MeCN (5 ml) was irradiated by 36 W blue LEDs for 56 hours. The reaction mixture was filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **10a** as a colorless oil (30 mg, 45%). $[\alpha]_D^{20}$ −4.2 (c 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 7.43–7.26 (m, 5H), 6.82–6.70 (m, 2H), 6.46–6.38 (m, 2H), 4.28 (dd, *J* = 8.4, 5.0 Hz, 1H), 3.51 (dd, *J* = 12.8, 5.0 Hz, 1H), 3.17 (dd, *J* = 12.8, 8.4 Hz, 1H), 2.92 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 185.9, 174.0, 171.1, 157.7, 156.0, 147.1, 133.9, 133.2, 130.7, 129.6, 129.4, 128.4, 80.7, 70.7, 55.1, 28.7, 27.0, 26.8. ^{77}Se NMR (76 MHz, CD_3OD) δ 182.3. HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $[C_{24}H_{26}N_2O_6SeNa]^+$ 541.0854; found 541.0872.

Tert-butyl N^5-((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-((1-methyl-2,8-dioxo-4-phenyl-1-azaspiro[4.5]deca-3,6,9-trien-3-yl)selanyl)-1-oxopropan-2-yl)-N^2-(*tert*-butoxycarbonyl)-L-glutaminate (10b)

A solution of **1b** (100 mg, 0.083 mmol, 1 equiv.) and alkyne **4b** (20 mg, 0.083 mmol, 1 equiv.) in MeCN (5 ml) was irradiated by

36 W blue LEDs for 56 hours. The reaction mixture was filtered, evaporated, and purified by reverse phase chromatography (C-18, MeCN/H_2O 20–85%) to provide **10b** as a colourless oil (18 mg, 25%). ^{1}H NMR (400 MHz, CD_3OD) 7.46–7.24 (m, 10H), 6.85–6.68 (m, 2H), 6.49–6.37 (m, 2H), 5.16 (s, 2H), 4.60–4.49 (m, 1H), 4.03–3.90 (m, 3H), 3.47 (dd, J = 12.8, 5.0 Hz, 1H), 3.03 (dd, J = 12.8, 9.0 Hz, 1H), 2.93 (s, 3H), 2.41–2.22 (m, 2H), 2.16–1.96 (m, 1H), 1.93–1.78 (m, 1H), 1.47 (s, 9H), 1.44 (s, 9H). ^{1}H NMR (400 MHz, CD_3OD) 185.8, 174.9, 173.3, 173.1, 171.3, 170.8, 158.1, 156.7, 146.93, 146.91, 137.2, 134.0, 133.9, 133.1, 130.8, 129.6, 129.5, 129.4, 129.34, 129.31, 128.5, 82.8, 80.6, 70.7, 67.9, 55.5, 54.5, 42.2, 33.1, 28.8, 28.4, 28.3, 27.4, 26.9. ^{77}Se NMR (76 MHz, CD_3OD) δ 180.7. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $[C_{42}H_{51}N_4O_{10}Se]^+$ 851.2765; found 851.2767.

Author contributions

P. A. was responsible for conceptualization, data curation, supervision, and visualization; S. L. developed the methodology and carried out the synthetic work. The manuscript was written by P. A. along with S. L.

Conflicts of interest

There are no conflicts to declare.

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