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Efficient and Scalable Syntheses of 1,2-Thiaselenane-4-amine and 1,2-Thiaselenane-5-amine

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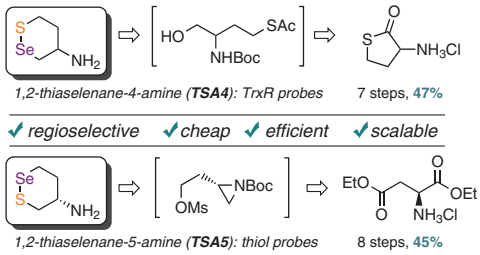

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Abstract The first regioselective syntheses of 1,2-thiaselenane-4-amine (TSA4) and 1,2-thiaselenane-5-amine (TSA5) are developed. Both are redox motifs with high value in chemical biology that until now were hindered by tedious synthesis. An aziridine intermediate and a kinetically controlled S-acylation were leveraged for regioselective chalcogen installations. Short, fast sequences were optimised with just one or two chromatographic steps that cheaply deliver these motifs on scale for high throughput inhibitor screening, and thus provide a robust methodology for assembling other selenenyl sulfides.

Key words redox, chemical probe, selenenyl sulfide, dichalcogenide, disulfide, fluorogenic probe, thioredoxin reductase, regioselective synthesis

Cyclic 6-membered selenenyl sulfides (1,2-thiaselenanes) have crucial roles as probes to study cellular redox biology, specifically of mammalian thioredoxin reductase (TrxR). TrxR is a vital oxidoreductase relying on a unique vicinal selenol/thiol redox-active centre, installed at great bioenergetic cost for its chemical functions, which include being the upstream reductant for DNA synthesis.[1] Iwaoka has used 1,2-thiaselenane-4/5-acetamides as functional mimics of TrxR, for example, studying their ability to catalyse the reduction of H_2O_2 by thiols in cells (Figure 1a).[2] As tractable model compounds, they provide insight into TrxR's active site chemistry, which is otherwise notoriously challenging to study by biochemical methods.[3–5]

We have instead exploited 1,2-thiaselenanes as the reducible 'triggers' of redox probes that function in live cells with remarkable regioselectivity profiles.[6,7] The 1,2-thiaselenane-4-amine (**TSA4**) probes are selectively activated by TrxR, without any turn-on by even 10 mM of monothiols over 6 hours, whereas their regioisomeric 1,2-thiaselenane-5-amine (**TSA5**) probes are strongly activated just by glutathione (GSH) and so are not TrxR reporters. This remarkable difference is entirely due to the position of the selenium atom. Chalcogenols attack both **TSA**s >10^3 faster at Se than at sulfur,[8] setting an 'at-Se' regioselectivity for their reduction.[6] This can be exploited with the **TSA4** regioisomer, where (unwanted) activation by GSH requires a prohibitive net trimolecular reaction, making for TrxR-selective probes. For example, the TrxR-selective **TSA4**-based probe **RX1** has enabled the first cellular high-throughput screen for TrxR

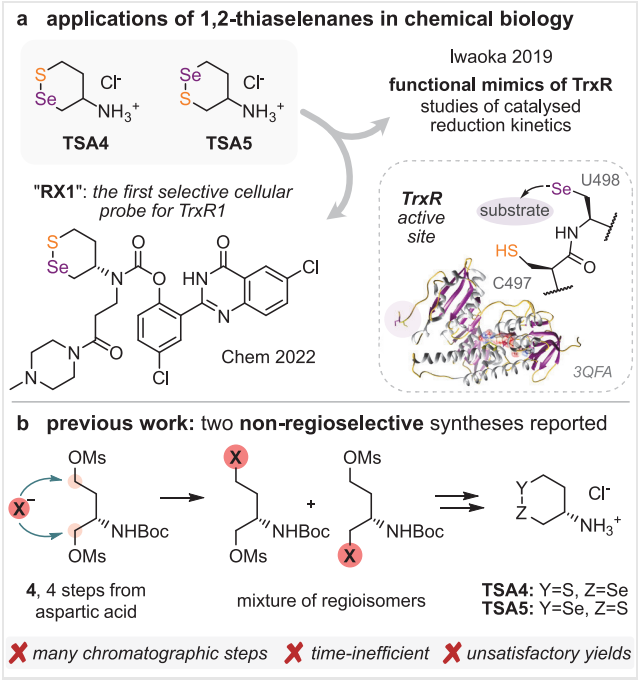


Figure 1 (**a**) 1,2-Thiaselenanes are important redox-active motifs for chemical biology; (**b**) Known routes to **TSA**s are not regioselective, are inefficient, and have high step count (X = RSe^-, RS^-).

inhibitors, and may guide future developments of TrxR-targeted drugs for cancer and inflammation.[9,10] Alternatively, at-Se selectivity can be subverted with **TSA5**, whose bimolecular reaction with one monothiol is enough for activation: giving fast, nonselective thiol probes (Figure S1).[6]

Redox probes therefore require strict regioisomeric purity of the **TSA4** or **TSA5** motifs they use, since this entirely determines their performance. Thus, **TSA** syntheses must differentiate the 4- and 5-regioisomers efficiently, all the while avoiding problems due to intramolecular reactions of the N, S, and Se nucleophiles and their protecting groups (see below).

The key to synthesising cyclic 1,2-selenenyl sulfides is installing the sulfur and selenium groups, often done by chalcogenide substitution of (pseudo)halides. After this, forming the Se–S bond is generally successful: for example, by *in situ* unmasking a thiol-selenol then oxidising with air[11,12] or I_2;[2,13] or by unmasking a thiol that can attack an electrophilic selenium (e.g., RSeCN).[6,14,15] Therefore, the challenge for accessing **TSAs** for biological uses is to obtain just one sulfur/selenium substitution pattern, with appropriate *N*-protection, by an efficient, regioisomerically pure synthesis that works on scale. This challenge remains unmet.

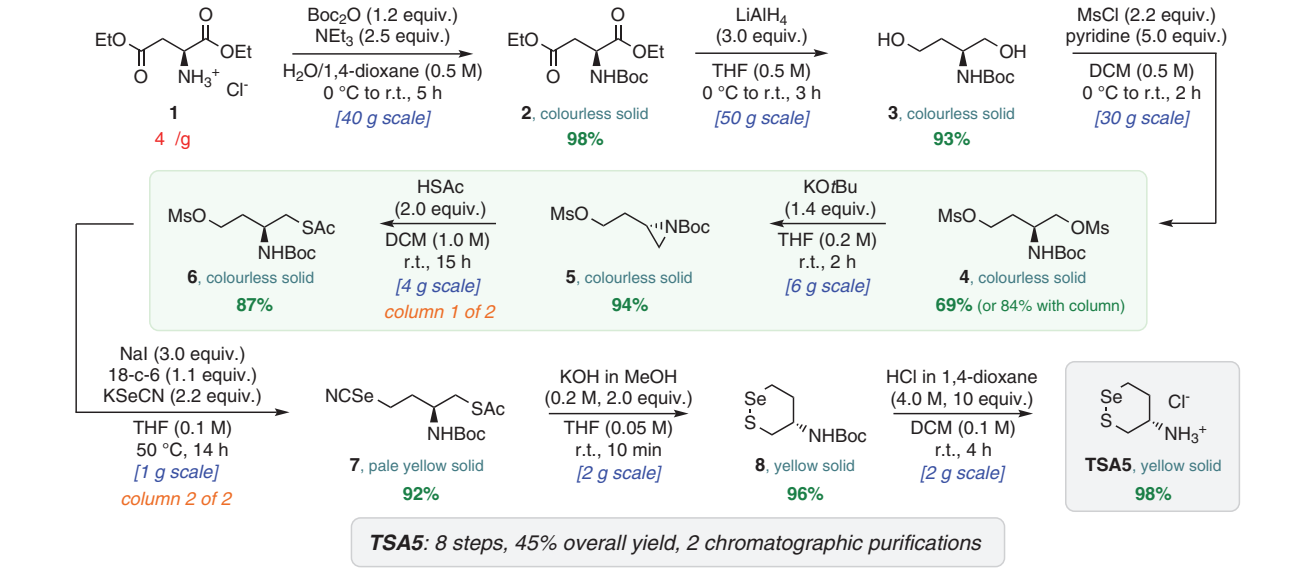
Iwaoka and Thorn-Seshold synthesised **TSAs** from the 1,2,4-trifunctional building block aspartic acid, converted to the convenient Boc-protected dimesylate **4** as a common intermediate in four steps (Figure 1b).[16] Iwaoka introduced sulfur by KSAc, selenium by *in situ* prepared PMBSeNa (non-commercial reagent), and finally obtained **TSA4** (21%) and **TSA5** (35%) in 9 steps after deprotection to the thiol-selenol then oxidation. Thorn-Seshold used KSAc then KSeCN as chalcogen sources, unmasked the thiolate to trigger cyclisation, and obtained **TSA4** (49%) and **TSA5** (28%) in 8 steps. The conceptual drawback of both sequences is that the mesylates of **4** have similar reactivity: so tedious, poorly scalable chromatographic separations of the mixed C1- and C4-substituted intermediates are needed to obtain pure fractions. Four to five additional chromatographic steps were also needed in the rest of the sequences (Scheme S1).

Instead to enable practical **TSA** synthesis on scale for screening applications, we aimed at regioselective and efficient syntheses of **TSA4** and **TSA5**. We focused on cost- and time-efficiency: using cheap commercially available reagents, minimising chromatographic purifications, and reducing the step count.[17,18] The straightforward syntheses we now provide address the growing need for functionalisable selenenyl sulfides in chemical biology and will also fill gaps in organochalcogen chemistry.

Aziridine Route to 1,2-Thiaselenane-5-amine (TSA5)

The major challenge here is to develop regioselective syntheses. Since their intermolecular reactivity was comparable (Figure 1b), we aimed to differentiate the C1- and C4-mesylates in **4** by their *intramolecular* reactivity: converting **4** to a C1-aziridine, for chemoselective opening later. Treating dimesylate **4** with KO*t*Bu efficiently gave Boc-aziridine **5**. As a test, we treated **5** with HSAc, regioselectively opening the aziridine to give the 1-*S*Ac-product **6** in excellent yield. We now optimised a regioselective route to **TSA5** around this key step.

Our route to **TSA5** began with aspartic acid diester **1**, which is cheap[19] or can be readily synthesised from aspartic acid.[20,21] Boc-protection on a multi-decagram scale gave **2** in 98% yield without further purification after aqueous workup (Scheme 1). The diester was reduced with excess



Scheme 1 Regioselective 8-step 2-column sequence to (*S*)-1,2-thiaselenane-5-amine **TSA5** (as hydrochloride salt), from diethyl L-aspartate

$LiAlH_4$. In contrast to Raines' established procedure,[16] we used lower grade THF, yet upon Fieser workup (see experimental), diol **3** was obtained in 93% yield and excellent purity without further purification. Small-scale conditions for activating **3** to the dimesylate **4** are well-established, but again we aimed for a procedure that is practical on a decagram scale. We found conditions to precipitate **4** directly from the organic extracts after aqueous workup, collecting a reproducible 69% yield of analytically pure solid **4** by simple filtration (though subsequent concentration of the filtrate and chromatography can supply an additional 15% yield if desired).

Treating dimesylate **4** with KO*t*Bu on scale gave the key Boc-aziridine **5** in 94% yield, again without purification (Scheme 1); a slight excess of KO*t*Bu can compensate for any hydrolysis of bench-stored reagent. Boc-aziridine **5** was reacted with HSAc in DCM using our key step conditions, giving the 1-*SAc*-product **6** in excellent yield (87%). We performed a first, straightforward chromatographic purification at this point, to ensure the chemical purity of the material used in the selenocyanation.

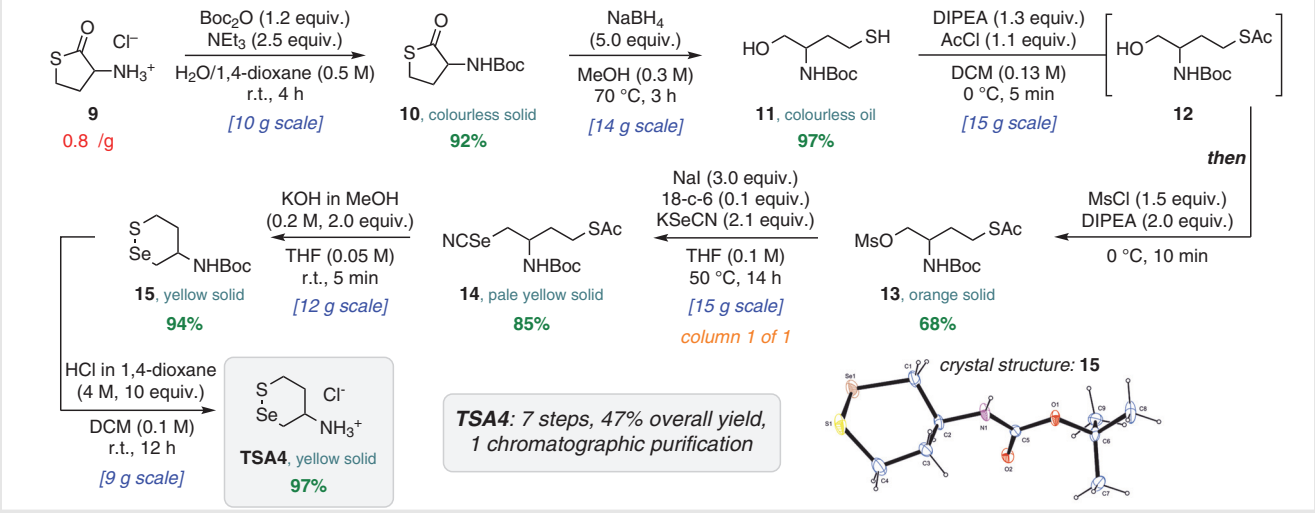
We completed the **TSA5** synthesis by our reported procedure:[6] introducing selenium using KSeCN (commercial at 10 €/g; can also be made *in situ* by Blacker's method[22]) to give **7**; and unmasking the thiol with base, for tandem cyclisation onto RSeCN giving selenenyl sulfide **8**. Boc-deprotection with HCl completed this cheap, concise, and regioselective route, giving **TSA5** on multigram scale, in 8 steps, with 45% overall yield, and just 2 chromatographic purifications (Scheme 1).

Protection and Route to TSA4 from Homocysteine

However, when attempting similar use of the aziridine route for **TSA4** (using HSeAc generated *in situ*[23] instead of HSAc to open the Boc-aziridine **5** and give the 2-amino-1-selenol precursor (Scheme S4)), it failed: the selenoester mesylate **S3** (visible by LCMS) decomposed during purification. We tried instead opening the aziridine with AcOH, giving the differentiated C1-acetate/C4-mesylate **S4**, which could have been reacted at C4 with KSAc, deprotected, activated, and reacted at C1 with KSeCN to give **TSA4** (Scheme S4), but at a high step-count. A related plan to use oxazolidinones as masked 2-amino-1-alcohols, allowing C4 thiol installation before Se functionalisation, also failed (Scheme S6).

For **TSA4**, we therefore re-routed to use racemic homocysteine thiolactone hydrochloride (**9**) as a cheap, commercially available building block with the required 1-functionalised 2-aminobutan-4-thiol framework (Scheme 2). Conceptually, reduction of the thiolactone at C1 yields a synthetic equivalent of 1-hydroxy-2-aminobutan-4-thiol, which with a suitable protecting group strategy could be selenised and cyclised to give **TSA4**. In practice, **9** undergoes intermolecular amide formation unless *N*-protected, which prevented us from using the nitrogen to control reactivity; and the thiol also re-cyclises readily to the thiolactone. These restrict the synthetic options from **9** (e.g., with or without *N*-protection, we failed to convert it to the cyclic 6-membered thiocarbamate that could permit selective C1 selenisation; Scheme S5). Therefore, we first Boc-protected the nitrogen, giving crude **10** in 92% yield and excellent purity on a decagram scale (Scheme 2), then examined protecting group strategies to mask the thiol, before attempting C1-selenisation.

We first tested intermolecular disulfides as 'zero-weight' thiol protecting groups. Boc-disulfide **S12** was easily reduced to C1-alcohol **S13** and mesylated to **S14**. Unfortunately, KSeCN reacted with this dimesylate-disulfide to give complex mixtures with only traces of the target **S15** (Scheme S7).



Scheme 2 Regioselective 7-step 1-column route to *rac*-1,2-thiaselenane-4-amine (**TSA4**, as hydrochloride salt), starting from *rac*-homocysteine thiolactone. The crystal structure of **15** confirms the desired regiochemistry (thermal ellipsoids of non-hydrogen atoms are set to the 50% probability level).

We therefore chose thioesters as *S*-protecting groups that are well-behaved with KSeCN (see **6**→**7**). Compound **10** was refluxed in MeOH with excess $NaBH_4$ to give mercaptobutanol **11** (Scheme 2); heating was essential for full conversion of the thiolactol intermediate. The borate salts were removed by filtration, and aqueous workup gave crude **11** in 97% yield and excellent purity. We next devised a procedure to *S*-mask **11** as the thioester before mesylating its alcohol, seeking controlled conditions to favour the *S*-ester kinetic product over the *O*-ester thermodynamic product (that can be formed directly, or after intra/intermolecular trans-acylation). At 0 °C, we screened equimolar amounts of acid chlorides for *S*-masking. Bulky BzCl and PivCl, which give kinetically stable thioesters, allowed rapid chemoselective *S*-masking; alternatively, lower temperatures, controlled stoichiometry, and short reaction times, allowed unhindered AcCl to reach similar efficiency. One-pot mesylation of the alcohol was smooth, with aqueous workup giving excellently pure *S*-acetate mesylate **13** in 68% yield, ready for selective C1-selenisation (Scheme 2).

To reach **TSA4** from **13**, selenium was first introduced by our known protocol using KSeCN. Despite good purity of crude **14**, chromatography was used to remove atrocious-smelling minor side products from Se-introduction. Basic thioester deprotection for the tandem Se–S bond formation step (→**15**) proceeded faster for the *S*-acetate **14** (<5 min) than for bulkier *S*-Bz/Piv analogues (e.g., **S2**, ~15 min).[6] We retained *S*-acetyl as the thioester of choice for the full scale preparation of **15**, since its shorter reaction time blocks parasitic deprotection of the selenocyanate [which gives a copolar 3-(*N*-Boc-amino)tetrahydroselenophene impurity that is tedious to remove]. Compound **15** was obtained in 94% yield on scale, and was confirmed as the desired regioisomer by X-ray crystallography (Scheme 2). Concluding the sequence, Boc-deprotection of **15** with HCl gave **TSA4** hydrochloride as a yellow solid that precipitated from the reaction mixture and was simply washed with diethyl ether to remove residual apolar impurities, obtaining the target in excellent purity and 97% yield. This completed the regioselective synthesis of selenenyl sulfide **TSA4** on decagram scale, in overall yield of 47% over 7 steps, with only one chromatographic purification (Scheme 2).

rac-TSA4 Enables a High-Performance Probe for TrxR

In contrast to aspartate-derived enantiopure **TSA5** (Scheme 1), the racemic thiolactone starting material **9** gives racemic **TSA4**. If enantiopure **TSA4** were needed, (*S*)-homocysteine (a more expensive starting material) could be used, or racemic **9** could be enantiomerically resolved.[24] However, for applications in redox biology probes, we expected that this cheap racemic **TSA4** would have the same performance as enantiopure **TSA4**, because: (i) the selenolthiol active site of the *target* oxidoreductase TrxR is only one residue away from the protein C-terminus, on an unstructured tail at the enzyme surface, and thus it has such low steric/geometric constraints for reacting with small molecules[25] that even *regio*isomeric probes using **TSA4** and **TSA5** have identical TrxR reaction kinetics; similarly, (ii) the major off-targets that probes have to resist are monothiols such as GSH, which impose even lower steric constraints.[6] Therefore, we expected both on-target and off-target behaviour to be independent of C2 stereochemistry. To test this, we assembled **RX1b** (Figure 2a), which is the racemic version of the enantiopure TrxR1 probe **RX1**,[6] and compared their cell-free and cellular performance side-by-side. They indeed had essentially identical performance with target TrxR1 (fast processing) and off-target GSH (full resistance) in cell-free conditions, plus identical cellular performance (Figure 2b,c and Figure S2).

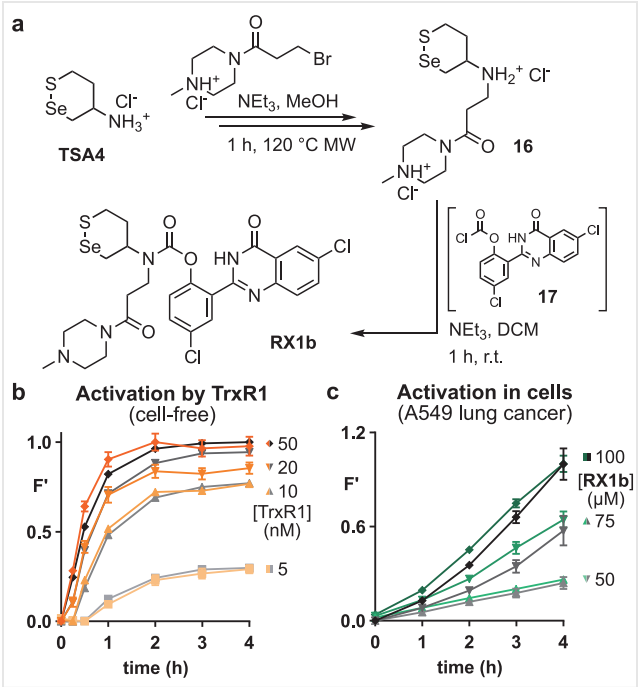


Figure 2 (**a**) Overview of the synthesis of the racemic **TSA-4**-based fluorogenic reporter probe **RX1b** (see Supporting Information for details). This probe integrates turnover by TrxR1 since selenenyl sulfide reduction leads to 5-*exo*-*trig* cyclisation and expulsion of the fluorogenic hydroxyphenylquinazolinone cargo; (**b**,**c**) **RX1b** (coloured traces) performs identically to enantiopure **RX1** (greyscale traces) as a cell-active redox probe for TrxR1 activity.

In summary, these new routes provide regioisomerically pure access to the biologically important selenenyl sulfides **TSA4** and **TSA5**, in short and streamlined synthetic sequences, and on multigram scale. The routes rely on easy-to-perform transformations, inexpensive reagents and starting materials, and largely avoid chromatographic purifications. The time economy of both sequences allows rapid access to both thiaselenanes on scale in ~3 days. Notably, these practical routes ensure chemically pure supply of TrxR-selective probes such as **RX1b** for large-scale (high-

throughput) cellular screening, for example, in cancer therapy and inflammation research.[9,10] More broadly, the simplicity, efficiency, and regioselectivity of the chalcogen introductions, and the insights into protecting group chemistries and selenenyl sulfide formation methods that are compatible with these densely functionalised scaffolds, may support further research into new chemical probes for redox biology.

Unless stated otherwise, all reactions were performed without precautions in regard of potential air- and moisture-sensitivity and were stirred with Teflon-coated magnetic stir bars. Reactions were monitored by TLC and visualised by UV irradiation and/or $KMnO_4$ stain (3.0 g $KMnO_4$, 20 g K_2CO_3, 0.30 g KOH, 0.30 L H_2O). The term 'column chromatography' refers to either (a) manual flash column chromatography conducted under positive N_2 pressure or (b) purification on a Biotage Selekt system using prepacked silica cartridges purchased from Biotage. All eluent and solvent mixtures are given as volume ratios, unless otherwise specified. Yields do not necessarily refer to chromatographically isolated and spectroscopically pure materials – sufficiently pure synthetic intermediates were often directly used for further transformations to increase cost and time efficiency. All chemicals were used as received and without purification. TLC control, extractions and column chromatography were conducted using distilled, technical grade solvents. The term *hexanes* refers to distilled, technical-grade crude isohexane.

Caution: KSeCN (toxic) and all Se-containing organics were treated with great care during reaction setup, workup, and purification. The selenocyanation reactions towards **7** and **14** produce species with an atrocious smell; we advise to wear a lab coat, use two layers of gloves, and work under a well-ventilated hood. We also recommend the use of a 'selenium-only' liquid and solid waste to dispose of contaminated towels or filter papers. Se-contaminated glassware can be cleaned using 2% aqueous NaOCl. Early fractions during column chromatography contain species with a highly unpleasant, garlicky smell.

Diethyl *N*-Boc-L-aspartate (2)

Diethyl L-aspartate hydrochloride (**1**; 40 g, 0.18 mol, 1.0 equiv.) was dissolved in a 1:1 mixture of H_2O and 1,4-dioxane (0.5 M, 0.20 + 0.20 L), cooled to 0 °C and charged with NEt_3 (61 mL, 0.44 mol, 2.5 equiv.) and Boc_2O (46 g, 0.21 mol, 1.2 equiv.). The reaction mixture was stirred for 5 h, then acidified to pH 2 using aq HCl (1 M). The aqueous layer was extracted with DCM (3 × 0.40 L) and the combined organic layers were washed with brine (0.40 L), dried ($MgSO_4$), filtered and concentrated. Boc-protected diester **2** was obtained as a colourless solid (50 g, 0.17 mol, 98%) and used without further purification; R_f = 0.39 (hexanes/EtOAc 3:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.47 (d, *J* = 8.6 Hz, 1 H), 4.54 (dd, *J* = 9.0, 4.8 Hz, 1 H), 4.21 (qd, *J* = 7.1, 2.2 Hz, 2 H), 4.14 (q, *J* = 7.1 Hz, 2 H), 2.98 (dd, *J* = 16.8, 4.8 Hz, 1 H), 2.79 (dd, *J* = 16.8, 4.8 Hz, 1 H), 1.45 (s, 11 H), 1.42 (s, 9 H), 1.26 (td, *J* = 7.1, 5.5 Hz, 6 H).

LRMS (ESI+): *m*/*z* calcd for $C_{13}H_{23}NO_6Na$ [M + Na]$^+$: 312.1; found: 312.2.

Analytics match literature reports.[26]

N-Boc-(*S*)-2-aminobutane-1,4-diol (3)

Under N_2 atmosphere, $LiAlH_4$ (20 g, 0.52 mol, 3.0 equiv.) was suspended in THF (1 M, 0.50 L) and cooled to 0 °C. A solution of **2** (50 g, 0.17 mol, 1.0 equiv.) in THF (1 M, 0.17 L) was added carefully (gas formation!), and the resulting grey suspension was allowed to warm to r.t. and vigorously stirred for 3 h. Once TLC control indicated full conversion of the starting material, the reaction was cooled to 0 °C, and excessive $LiAlH_4$ was quenched with H_2O (20 mL) and aq NaOH (2 M, 20 mL), giving a thick colourless slurry. The precipitates were filtered off and washed with EtOAc (3 × 0.20 L). The combined filtrates were dried (Na_2SO_4), filtered, and concentrated to give diol **3** (33 g, 0.16 mol, 93%) as a colourless oil, which crystallised under high vacuum and was used directly for the following step; R_f = 0.30 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.99 (s, 1 H), 3.91–3.82 (m, 1 H), 3.78–3.62 (m, 4 H), 2.24 (s, 2 H), 1.81 (ddt, *J* = 14.1, 9.3, 4.6 Hz, 1 H), 1.63 (tt, *J* = 10.1, 4.2 Hz, 1 H), 1.45 (s, 9 H).

HRMS (EI+): *m*/*z* calcd for $C_8H_{16}NO_3$ [M – CH_3O]$^{+\bullet}$: 174.1125; found: 174.1124.

NMR data for **3** match literature reports.[16]

N-Boc-(*S*)-2-aminobutane-1,4-diyl Dimethanesulfonate (4)

To a solution of diol **3** (33 g, 0.16 mol, 1.0 equiv.) in DCM (0.5 M, 0.32 L) were added pyridine (65 mL, 0.80 mmol, 5.0 equiv.) and MsCl (27 mL, 0.35 mmol, 2.2 equiv.) at 0 °C. Addition of MsCl resulted in an immediate colour change from colourless to orange. The reaction mixture was allowed to warm to r.t. and stirred for 2 h. Once LCMS-control indicated full conversion of the starting material, the reaction was quenched with sat. aq NH_4Cl (0.20 L) and the aqueous phase was extracted with DCM (3 × 0.20 L). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to about 1/10 of their volume. Toluene (0.15 L) was added, and upon removal of remaining DCM, colourless solids precipitated from the solvent mixture. The solids were collected by filtration, washed with toluene (3 × 50 mL), then hexanes (2 × 0.10 L) and were left to dry on air overnight giving **4** as a colourless powder; yield: 40 g (0.11 mol, 69%); R_f = 0.37 (hexanes/EtOAc 1:2).

Note: Additional product can be recovered from the mother liquors and washings of the precipitates with toluene, which can increase the yield by 15%. To obtain pure material, purification by flash column chromatography (SiO_2, hexanes/EtOAc 1:1 → EtOAc) is recommended.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.78 (d, *J* = 8.7 Hz, 1 H), 4.39–4.23 (m, 4 H), 4.10–4.02 (m, 1 H), 3.05 (s, 3 H), 3.04 (s, 3 H), 2.11–2.04 (m, 1 H), 2.02–1.93 (m, 1 H), 1.45 (s, 9 H).

HRMS (EI+): *m*/*z* calcd for $C_{11}H_{23}NO_8S_2$ [M]$^{+\bullet}$: 361.0860; found: 361.0859.

Analytical data match those of Raines and co-workers.[16]

N-Boc-(*S*)-2-(aziridin-2-yl)ethyl Methanesulfonate (5)

Dimesylate **4** (6.0 g, 17 mmol, 1.0 equiv.) was dissolved in THF (0.2 M, 0.10 L) at r.t., and KO*t*Bu (2.1 g, 18 mmol, 1.1 equiv.) was added in small batches. The reaction mixture soon turned from colourless to orange. The reaction progress was closely monitored by TLC, and additional KO*t*Bu (0.60 g, 5.0 mmol, 0.3 equiv.) was carefully added in portions until full conversion of the starting material was observed. After 2 h, the reaction was quenched with H_2O (50 mL), the organic phase was separated, and the aqueous layer was extracted with Et_2O (2 × 0.1 L). The combined organic layers were dried ($MgSO_4$), filtered

and concentrated, giving crude aziridine **5** as a colourless solid (4.1 g, 16 mmol, 94%) with good purity, which was used directly for the following transformation; R_f = 0.71 (hexanes/EtOAc 1:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.39 (t, *J* = 6.1 Hz, 2 H), 3.04 (s, 3 H), 2.55–2.46 (m, 1 H), 2.33 (d, *J* = 6.2 Hz, 1 H), 2.12–2.01 (m, 1 H), 1.99 (d, *J* = 3.7 Hz, 1 H), 1.77–1.68 (m, 1 H), 1.44 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.2, 81.6, 67.9, 37.5, 34.4, 32.3, 31.8, 28.0.

LRMS (ESI+): *m*/*z* calcd for $C_{10}H_{19}NO_5SNa$ [M + Na]$^+$: 288.1; found: 288.1.

N-Boc-(*S*)-(2-amino-4-(methanesulfonyl)oxybutyl) Ethanethioate (6)

To a solution of crude aziridine **5** (4.0 g, 15 mmol, 1.0 equiv.) in DCM (1.0 M, 15 mL) was added thioacetic acid (2.1 mL, 30 mmol, 2.0 equiv.) at r.t. The reaction mixture was stirred overnight, upon which the orange solution turned almost colourless and LCMS analysis indicated full conversion of the starting material. The reaction was quenched with sat. aq $NaHCO_3$ (50 mL) and the aqueous layer was extracted with DCM (3 × 80 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated giving a yellow crude solid. Purification by flash column chromatography (SiO_2, hexanes/EtOAc 3:1 → 1:1) gave thioacetate **6** as a colourless solid; yield: 4.3 g (13 mmol, 87%); R_f = 0.42 (hexanes/EtOAc 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.59 (d, *J* = 8.9 Hz, 1 H), 4.33–4.25 (m, 2 H), 3.90 (s, 1 H), 3.08 (dd, *J* = 6.2, 2.9 Hz, 2 H), 3.04 (s, 3 H), 2.37 (s, 3 H), 2.07–1.99 (m, 1 H), 1.90–1.80 (m, 1 H), 1.43 (s, 9 H).

NMR data match reported values.[6]

N-Boc-(*S*)-(2-amino-4-selenocyanatobutyl) Ethanethioate (7)

In analogy to a reported procedure,[6] mesylate **6** (0.55 g, 1.6 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 12 mL), and NaI (0.60 g, 3.8 mmol, 3.0 equiv.), 18-crown-6 (0.48 g, 1.8 mmol, 1.1 equiv.) and KSeCN (0.52 g, 3.6 mmol, 2.2 equiv.) were added sequentially. The hetereogeneous, colourless mixture was heated to 50 °C and was stirred for 16 h. Once LCMS-control indicated full conversion of the starting material, the reaction was cooled to r.t. and diluted with brine (50 mL) and DCM (50 mL). The layers were separated, the aqueous phase was extracted with DCM (3 × 50 mL), and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The remaining orange oil was purified by flash column chromatography (SiO_2, hexanes/EtOAc 5:1 → 3:1) giving the target compound as a pale-yellow solid; yield: 0.53 g (1.5 mmol, 92%); R_f = 0.55 (hexanes/ EtOAc 1:1).

Note: Scale-up (>4 g starting material) of the reaction proceeded smoothly, although with compromised yield (82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.60 (d, *J* = 9.0 Hz, 1 H), 3.91 (s, 1 H), 3.19 (ddd, *J* = 12.6, 7.8, 5.1 Hz, 1 H), 3.07 (d, *J* = 6.2 Hz, 2 H), 2.99 (dt, *J* = 12.3, 7.9 Hz, 1 H), 2.38 (s, 3 H), 2.18–2.07 (m, 1 H), 2.06–1.97 (m, 1 H), 1.43 (s, 9 H).

NMR data match our previous report.[6]

N-Boc-(*S*)-1,2-thiaselenan-5-amine (8)

N-Boc-(*S*)-(2-amino-4-selenocyanatobutyl) ethanethioate (**7**; 2.4 g, 6.8 mmol, 1.0 equiv.) was dissolved in MeOH (0.2 M, 35 mL) and a methanolic solution of KOH (0.2 M, 41 mL, 8.2 mmol, 1.2 equiv.) was slowly added at r.t. Within seconds, the reaction mixture turned from colourless to yellow, indicating thiaselenane formation. Full conversion was monitored by TLC and was achieved within 10 min. The reaction was concentrated to 10% of its volume and was diluted with brine (0.10 L) and DCM (0.10 L). The layers were separated, the aqueous phase was extracted with DCM (2 × 0.10 L) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The title compound was received as a pale-yellow solid (1.8 g, 6.5 mmol, 96%) and used without further purification; R_f = 0.64 (hexanes/EtOAc 3:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.02 (s, 1 H), 3.96–3.83 (m, 1 H), 3.30–3.13 (m, 2 H), 2.97 (s, 1 H), 2.80 (dd, *J* = 13.5, 7.7 Hz, 1 H), 2.25 (t, *J* = 11.8 Hz, 1 H), 2.04 (s, 1 H), 1.45 (s, 9 H).

LRMS (ESI+): *m*/*z* calcd for $C_9H_{17}NO_2SSe$ [M]$^{+\bullet}$: 283.0140; found: 283.0133.

NMR data match our previous report.[6]

(*S*)-1,2-Thiaselenan-5-amine Hydrochloride (TSA5)

N-Boc-(*S*)-1,2-thiaselenan-5-amine (**8**; 1.8 g, 6.5 mmol, 1.0 equiv.) was dissolved in DCM (0.1 M, 65 mL) and HCl (4 M in 1,4-dioxane, 16 mL, 65 mmol, 10 equiv.) was added at r.t. in a dropwise manner. Within minutes, pale yellow solids preciptitated from the reaction mixture. The suspension was stirred for 4 h, upon which TLC control indicated full deprotection of the starting material. The stir bar was removed, and the solids were allowed to settle to the bottom of the flask. The liquid phase was decanted, and the solids were washed with Et_2O (3 × 20 mL) and then dried under high vacuum giving the target amine hydrochloride as a pale-yellow solid; yield: 1.4 g (6.4 mmol, 98%).

^{1}H NMR (400 MHz, D_2O): δ = 3.57–3.44 (m, 1 H), 3.16 (dd, *J* = 14.6, 7.5 Hz, 3 H), 2.97 (dd, *J* = 13.8, 9.6 Hz, 1 H), 2.42 (dq, *J* = 13.5, 4.3 Hz, 1 H), 2.05 (td, *J* = 14.3, 6.5 Hz, 1 H).

LRMS (ESI+): *m*/*z* calcd for $C_4H_{10}NSSe$ [M + H]$^+$: 184.0; found: 184.0.

Analytics match our previous results.[6]

N-Boc-homocysteine Thiolactone (10)

DL-Homocysteinethiolactone hydrochloride (**9**; 10 g, 65 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of H_2O and 1,4-dioxane (0.5 M, 65 + 65 mL) and cooled to 0 °C. NEt_3 (23 mL, 0.16 mol, 2.5 equiv.) and Boc_2O (17 g, 78 mmol, 1.2 equiv.) were added sequentially, and the reaction mixture was allowed to warm to r.t. and stirred for 4 h. Upon completion, the thick, colourless precipitates that had formed during the reaction were redissolved by addition of EtOAc (0.30 L), and the pH was adjusted to 2 by aq HCl (1 M). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 0.30 L). The combined organic layers were dried, filtered, and concentrated, giving **10** as a colourless solid; yield: 13 g (60 mmol, 92%); R_f = 0.77 (hexanes/EtOAc 1:1).

As NMR analysis of the crude solids showed excellent purity, **10** was used without further purification.

Note: Compound **10** can be recrystallised from EtOAc. In our hands, two rounds of crystallisation gave **10** as crystalline, colourless solid and in barely compromised yield (88%, 10 g scale).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.98 (s, 1 H), 4.42–4.18 (m, 1 H), 3.38–3.19 (m, 2 H), 2.87 (d, J = 10.8 Hz, 1 H), 1.98 (td, *J* = 12.4, 7.1 Hz, 1 H), 1.45 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 205.5, 155.7, 80.6, 60.7, 32.3, 28.4, 27.6.

HRMS (ESI+): *m*/*z* calcd for $C_9H_{15}NO_3SNa$ [M + Na]$^+$: 240.06649; found: 240.60874.

N-Boc-2-amino-4-mercaptobutan-1-ol (11)

Thiolactone **10** (14 g, 65 mmol, 1.0 equiv.) was dissolved in MeOH (0.3 M, 0.20 L) and $NaBH_4$ (12 g, 0.33 mol, 5.0 equiv.) was carefully added in small batches at r.t. The reaction mixture was heated to 70 °C for 3 h, and once TLC-control indicated full conversion of the starting material, concentrated under reduced pressure. The residue was taken up in Et_2O (0.20 L), and the resulting mixture was filtered. The filter cake was washed with Et_2O (3 × 80 mL) and the combined filtrates were concentrated to half of its volume. The crude ether solution was washed with aq NH_4Cl (0.30 L) and brine (0.20 L), dried ($MgSO_4$), filtered and concentrated. Product **11** was obtained as a colourless oil (14 g, 63 mmol, 97%), which was used without further purification.

Notes: (1) Addition of $NaBH_4$ results in vigorous gas formation and should be conducted with great care – especially when working on scale. (2) Even though theoretically prone to oxidation on air, isolated **11** proved to be surprisingly stable. No significant disulfide formation was observed even after >1 week of storage under ambient conditions. (3) An analytically pure sample of **11** was prepared by purification by column chromatography (SiO_2, hexanes/EtOAc 2:1 → 1:1); R_f = 0.33 (hexanes/EtOAc 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.76 (s, 1 H), 3.79 (m, 1 H), 3.68 (dd, *J* = 11.1, 3.9 Hz, 1 H), 3.57 (dd, *J* = 11.0, 5.2 Hz, 1 H), 2.59 (q, *J* = 7.5 Hz, 2 H), 2.41–2.22 (m, 1 H), 1.79 (q, *J* = 7.2 Hz, 2 H), 1.60–1.50 (m, 1 H), 1.44 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.4, 80.0, 65.5, 51.4, 36.1, 28.5, 21.5.

HRMS (ESI+): *m*/*z* calcd for $C_9H_{20}NO_3S$ [M + H]$^+$: 222.11584; found: 222.11572.

S-(3-((*tert*-Butoxycarbonyl)amino)-4-((methylsulfonyl)oxy)butyl) Ethanethioate (13)

N-Boc-2-amino-4-mercaptobutan-1-ol **11** (15 g, 68 mmol, 1.0 equiv.) was dissolved in DCM (0.20 M, 0.35 L) and the solution was cooled to 0 °C. AcCl (5.0 mL, 70 mmol, 1.03 equiv.) and DIPEA (18 mL, 0.10 mol, 1.5 equiv.) were added to the colourless solution and the reaction was monitored via TLC. Within 5 min, full conversion of the starting material was achieved. Then, MsCl (7.9 mL, 0.10 mmol, 1.5 equiv.) and DIPEA (24 mL, 0.14 mol, 2.0 equiv.) were added sequentially. Once more, TLC control indicated completion within 10 min, and the reaction was quenched using sat. aq NH_4Cl (400 mL). The layers were separated, the aqueous phase was extracted with DCM (2 × 300 mL) and the combined organic layers were dried, filtered and concentrated. **13** was isolated as a yellow oil that solidified over time (15 g, 46 mmol, 68%), and was used in the next step without further purification; R_f = 0.45 (hexanes/EtOAc 1:1).

Notes: (1) Double-functionalisation of **11** on a large scale does not proceed as smoothly with AcCl/MsCl (**13**) as for BzCl/MsCl (**S1**); TLC control indicates the formation of several side products for both steps. Yet, since the NMR spectrum of crude **13** showed good purity, we continued without chromatographic purification. (2) Purification of crude **13** by column chromatography (SiO_2, hexanes/EtOAc 3:1 → 1:1) gave analytically pure **13** as a colourless solid. (3) Compound **13** proved to be unstable when stored under ambient conditions or in $CDCl_3$ solution. It is advisable to rapidly acquire analytics and conduct follow-up transformations.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.77 (d, *J* = 8.7 Hz, 1 H), 4.24 (qd, *J* = 10.2, 3.9 Hz, 2 H), 3.90 (dp, *J* = 10.5, 5.7, 4.9 Hz, 1 H), 3.04 (s, 3 H), 2.99 (td, *J* = 8.2, 4.2 Hz, 1 H), 2.84 (dt, *J* = 13.8, 7.8 Hz, 1 H), 2.34 (s, 3 H), 1.93–1.76 (m, 2 H), 1.44 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 195.9, 155.4, 80.2, 71.0, 49.1, 37.5, 31.5, 30.7, 28.4, 25.6.

HRMS (ESI+): *m*/*z* calcd for $C_7H_{16}NO_4S_2$ [M – $C_5H_7O_2$]$^+$: 242.05153; found: 242.05146.

N-Boc-(3-amino-4-selenocyanatobutyl) Ethanethioate (14)

Mesylate **13** (15 g, 44 mmol, 1.0 equiv.) was dissolved in THF (0.15 M, 0.30 L), and NaI (20 g, 0.13 mol, 3.0 equiv.), 18-crown-6 (1.2 g, 4.4 mmol, 0.1 equiv.) and KSeCN (14 g, 94 mmol, 2.1 equiv.) were added sequentially. The heterogeneous, pale yellow reaction mixture was heated to 50 °C and stirred for 14 h. The reaction mixture was cooled to r.t., concentrated to about one fifth of its volume, and diluted with H_2O (0.30 L) and DCM (0.30 L). The organic phase was separated, and the aqueous layer was extracted with DCM (300 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated, giving a dark orange crude oil. Purification by column chromatography (SiO_2, hexanes/EtOAc 20:1 → 4:1) yielded selenocyanate **14** as a pale-yellow solid; yield: 13 g (37 mmol, 85%); R_f = 0.56 (hexanes/EtOAc 2:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.85 (s, 1 H), 3.94–3.82 (m, 1 H), 3.39–3.36 (m, 1 H), 3.31–3.21 (m, 1 H), 2.99 (dq, *J* = 12.3, 6.2, 5.5 Hz, 1 H), 2.84 (dt, *J* = 13.9, 7.7 Hz, 1 H), 2.34 (s, 3 H), 1.94–1.82 (m, 2 H), 1.44 (s, 9 H).

HRMS (ESI+): *m*/*z* calcd for $C_{12}H_{20}N_2O_3SSeNa$ [M + Na]$^+$: 375.02575; found: 375.02519.

Characterisation by NMR match our previously reported data.[6]

N-Boc-1,2-thiaselenan-4-amine (15)

Compound **14** (12 g, 33 mmol, 1.0 equiv.) was dissolved in THF (0.05 M, 0.60 L) and a methanolic solution of KOH (0.2 M, 0.33 L, 66 mmol, 2.0 equiv.) was added at r.t. The reaction was stirred for 5 min, upon which TLC control indicated full conversion of the starting material. The pale-yellow solution was then quenched with a 1:1 mixture of sat. aq $NaHCO_3$ (0.20 L) and H_2O (0.20 L) and the mixture was transferred into a separating funnel. The aqueous phase was extracted with Et_2O (2 × 0.40 L) and the combined organic layers were washed with brine (0.20 L), dried ($MgSO_4$) and filtered. Concentration *in vacuo* provided **15** as a bright yellow solid (8.9 g, 31 mmol, 94%), which was used without further purification; R_f = 0.75 (hexanes/EtOAc 3:1).

Notes: (1) Basic thioester cleavage and subsequent S_N2 at Se generates stoichiometric amounts of toxic CN^-. For disposal of the aqueous phase, especially when working on scale, it is advised to keep the liquid waste at high pH to avoid generation of gaseous HCN. (2) Selenenyl sulfide formation was conducted for three different thioesters, whose reactivity (Ac > Bz > Piv) was reflected in the time the reaction took for completion (<5, 15, 120 min, respectively). (3) If needed, an analytically pure sample can be obtained via column chromatography (hexane/EtOAc 9:1). For our purposes, we used **15** without further purification as the ensuing deprotection protocol is designed to remove all apolar impurities at this final step.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.98 (s, 1 H), 3.92 (t, *J* = 9.1 Hz, 1 H), 3.23 (ddd, *J* = 14.7, 7.9, 2.9 Hz, 2 H), 2.99 (t, *J* = 11.5 Hz, 1 H), 2.84 (dd, *J* = 12.1, 8.7 Hz, 1 H), 2.21–2.05 (m, 1 H), 1.81 (d, *J* = 33.6 Hz, 2 H), 1.44 (s, 11 H).

HRMS (ESI–): *m*/*z* calcd for $C_9H_{16}NO_2SSe$ [M – H]$^-$: 282.00724; found: 282.00711.

Analytical data match those of our previous report.[6]

1,2-Thiaselenan-4-amine Hydrochloride (TSA4)

Thiaselenane **15** (8.9 g, 31 mmol, 1.0 equiv.) was dissolved in DCM (0.1 M, 0.30 L) and reacted with HCl (4 M in 1,4-dioxane, 79 mL, 0.31 mol, 10 equiv.) at r.t. Within 10 min, a pale yellow precipitate started

to form, and after stirring for 4 h, a thick heterogenous reaction mixture was obtained. The solids were collected by filtration and were washed with DCM (3 × 80 mL), followed by Et_2O (2 × 80 mL) and dried under high vacuum overnight. 1,2-Thiaselenan-4-amine hydrochloride was obtained as a yellow solid; yield: 6.6 g (30 mmol, 97%).

^{1}H NMR (400 MHz, D_2O): δ = 3.64 (tt, *J* = 10.8, 3.3 Hz, 1 H), 3.26 (ddd, *J* = 14.4, 5.9, 3.1 Hz, 1 H), 3.16–3.04 (m, 3 H), 2.31 (ddt, *J* = 13.9, 5.5, 2.8 Hz, 1 H), 1.80 (dtd, *J* = 13.8, 10.8, 3.0 Hz, 1 H).

HRMS (ESI+): *m*/*z* calcd for C_4H_7SSe [M – NH_2]$^+$: 166.94282; found: 166.94275.

NMR data of **TSA4** match those of its reported, enantiopure counterpart.[6]

RX1b (= *rac*-RX1)

Step 1: **TSA4** (0.48 g, 2.2 mmol, 1.4 equiv.), NEt_3 (0.29 mL, 2.1 mmol, 1.3 equiv.), and 3-bromo-1-(4-methylpiperazin-1-yl)propan-1-one hydrochloride (0.44 g, 1.6 mmol, 1.0 equiv.) were added to a 10 mL microwave vial and dissolved in MeOH (6.0 mL, 0.4 M). The yellow solution was heated in a sealed tube using a laboratory microwave (120 °C, 25 W, 60 min), then transferred into a round-bottomed flask and concentrated under reduced pressure to yield a yellow crude oil. Though this crude was conceptually ready for carbamate formation, we found it advantageous to purify it first, by chromatography, before coupling. We use transient Boc protection to make the chromatography easier.[6]

Step 2: The yellow crude oil was redissolved in DCM (10 mL, 0.2 M). NEt_3 (0.67 mL, 4.8 mmol, 3.0 equiv.) and Boc_2O (0.53 g, 2.4 mmol, 1.5 equiv.) were added sequentially and the reaction was stirred at r.t. for 12 h. The organic solution was washed with a 1:1 mixture of H_2O:brine (3 ×), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude solid was purified by column chromatography (DCM → DCM/MeOH 9:1), giving *N*-Boc-3-((1,2-thiaselenan-4-yl)amino)-1-(4-methylpiperazin-1-yl)propan-1-one[6] as a yellow solid; yield: 0.40 g (0.92 mmol, 57% over 2 steps).

Step 3: The Boc-protected product of step 2 (0.76 g, 1.7 mmol, 1.0 equiv.) was dissolved in DCM (15 mL, 0.1 M) and HCl (4 M in 1,4-dioxane, 4.4 mL, 10 equiv.) was added. Within minutes, yellow solids precipitated from the reaction mixture. Once TLC control (hexanes/EtOAc 6:1) indicated full conversion of the starting material, all volatiles were removed *in vacuo*, giving the target dihydrochloride **16**[6] as a yellow solid; yield: 0.70 g (1.7 mmol, 98%).

Step 4: Amine **16** (0.25 g, 0.60 mmol, 1.0 equiv.) was reacted with chloroformate **17**[6] (PQ-OCOCl; 0.04 M in DCM, 26 mL, 1.3 mmol, 2.2 equiv.). Purification by flash column chromatography (DCM → DCM/MeOH 9:1) gave **RX1b** as a colourless solid. Further purification by preparative HPLC (MeCN/H_2O, 0.1% formic acid) yielded **RX1b** as a colourless solid; yield: 94 mg (0.14 mmol, 23%); R_f = 0.44 (DCM/MeOH 9:1). Light blue solid-state fluorescence was observed under UV-light, indicating no contamination by the violently green-fluorescent PQ-OH. NMR spectroscopy indicated two rotameric species for which individual signals could not be clearly identified at 298 K.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (t, *J* = 2.5 Hz, 1 H), 7.86 (dt, *J* = 8.9, 2.9 Hz, 1 H), 7.82 (d, *J* = 2.6 Hz, 1 H), 7.74 (dd, *J* = 8.8, 4.0 Hz, 1 H), 7.63 (dt, *J* = 9.0, 2.9 Hz, 1 H), 7.37 (dd, *J* = 8.8, 4.3 Hz, 1 H), 4.46 (s, 0.5 H), 3.88 (t, *J* = 11.7 Hz, 0.5 H), 3.72–3.47 (m, 4 H), 3.42–3.34 (m, 2 H), 3.29–3.23 (m, 2 H), 3.19–3.06 (m, 1 H), 2.88 (d, *J* = 11.7 Hz, 0.5 H), 2.77 (td, *J* = 7.2, 2.4 Hz, 1 H), 2.67–2.49 (m, 4.5 H), 2.44 (d, *J* = 4.9 Hz, 3 H), 2.34 (q, *J* = 7.2 Hz, 1 H), 2.18 (d, *J* = 13.3 Hz, 0.6 H), 2.00 (qd, *J* = 11.8, 3.7 Hz, 1 H), 1.86 (d, *J* = 13.6 Hz, 0.4 H).

^{13}C NMR (101 MHz, CD_3OD): δ = 171.4, 171.0, 136.5, 134.1, 133.0, 132.5, 131.0, 130.4, 130.3, 126.6, 126.5, 126.1, 126.0, 123.6, 60.5, 57.5, 55.4, 55.0, 45.4, 43.7, 41.7, 37.2, 35.8, 34.6, 34.4, 33.5, 28.3, 27.3.

HRMS (ESI+): *m*/*z* calcd for $C_{27}H_{30}Cl_2N_5O_4SSe$: [M + H]$^+$: 670.05553; found: 670.05600.

Conflict of Interest

L.Z. and O.T.-S. are inventors on patent application PCT/EP2022/059280 filed by the LMU Munich, covering RX1. The authors declare no competing financial interest.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-2022-1398.

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