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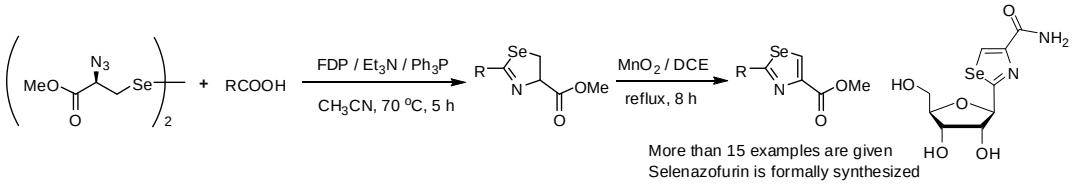

More than 15 examples are given
Selenazofurin is formally synthesized

Method to Build 2,4-Substituted Selenazole from β-Azido Diselenide and Carboxylic Acid: A Formal Synthesis of Selenazofurin

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ABSTRACT

A novel approach for the synthesis of 2,4-disubstituted selenazoles using carboxylic acids (or anhydrides) and β-azido diselenide was achieved via a one-pot cascade formation of selenazoline (Staudinger reduction / diselenide cleavage / selenocarbonylation / aza-Wittig reaction) and a following MnO_2-promoted oxidation. This method offers excellent substrate flexibility, and its valuable application is exemplified by an efficient total synthesis of Selenazofurin which exhibited good antitumor activities against K562 and A549 cells.

1. Introduction

The physiological roles of selenium are usually ascribed to selenium-containing proteins that have been identified in the human proteome and in other species.[1] Biological incorporation of selenium proceeds through selenocysteine which is considered as the twenty-first proteinogenic amino acid being coded by the UGA codon.[2] Studies also reveal that selenium deficiency is associated with Kashin-Beck disease, cardiovascular disease, thyroid disease, cognitive decline, male infertility and cancers.[3-5] There has been an increased attention on selenium-containing compounds due to their abilities to act as glutathione peroxidase (GPX) mimics, catalytic antioxidants, and cancer-chemopreventive compounds.[6] Accordingly, diverse selenium derivatives have been prepared and their anticancer activities were investigated both *in vivo* and *in vitro*, suggesting that their main action modes were carried out by inducing apoptosis of tumor cells and reducing cancerous metastasis in animals.[7-9] Other activities were also disclosed with antihypertensive, antiviral, antibacterial, and antifungal properties.[10]

In particular, 1,3-selenazoline and 1,3-selenzole, the interesting class of five-membered heterocycles containing selenium and nitrogen atoms at 1- and 3-positions respectively, have long been under investigations because of their

outstanding biological and medicinal potentials.[5, 11] For examples (Fig. 1), a ribose modified selenazole derivative (selenazofurin **1**) exhibited amazing anti-tumor properties, as well as a broad spectrum of antiviral activities in cell culture experiments.[12] Selenazole **2** showed potent antiproliferative activity against MCF-7 (Human breast cancer) cells.[13] More interestingly, when the thiazole structure of febuxostat was replaced by selenazole, the generated analog **3** provides an excellent inhibition activity on xanthine oxidase with IC_{50} at nM level, which is three times more potent than the marketed reference drug.[14]

Due to the importance of 1,3-selenazoles in drug development, many procedures have been reported to allow the synthesis of more diversified derivatives.[6] Among them, Hantzch condensation of α-haloketone with selenoureas or selenoamides remains the most widely explored method.[5] However, selenourea analogs are not convenient to use because of the high cost and low stability to the air and light.[15] Thus, an operational convenient and environmental benign method for the synthesis of 1,3-selenazole is still interesting. Herein, we would like to report our new method for the preparation of 1,3-selenazoles with *β*-azido diselenides and carboxylic acids or anhydrides, as well as the efficient synthesis of selenazofurin **1** using this protocol.

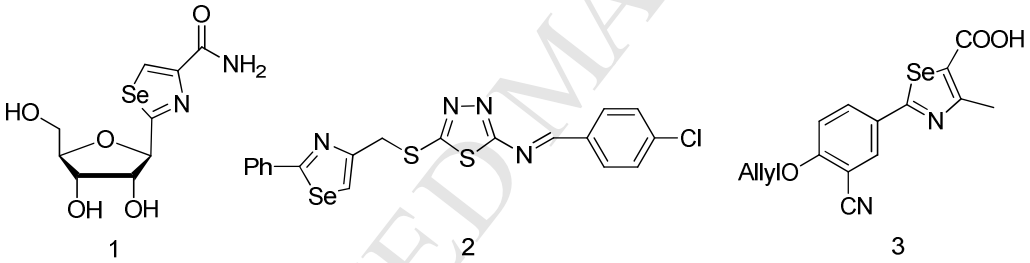
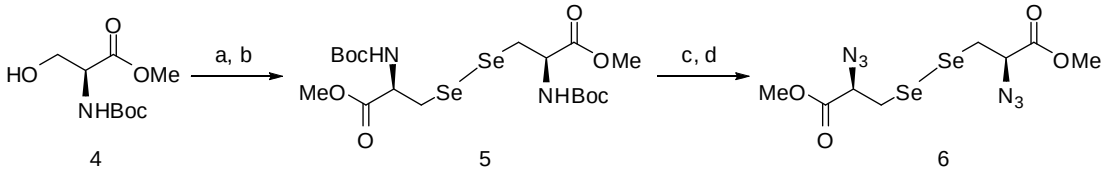


Figure 1. Structures of bioactive 1,3-selenazole compounds

2. Results and discussion

Based on our previous research on the phosphine triggered thiazole formation,[16] we envisaged that its structural analog selenazole could also be made through the sequential diselenide cleavage / selenocarbonylation / intramolecular Staudinger Reduction / Wittig reactions using *β*-azido diselenide and carboxylic compound in one-pot. To verify this hypothesis, the substrate *β*-azido diselenide **6** was prepared from commercial available N-Boc-L-serine methyl ester (**4**) in two steps (Scheme 1).

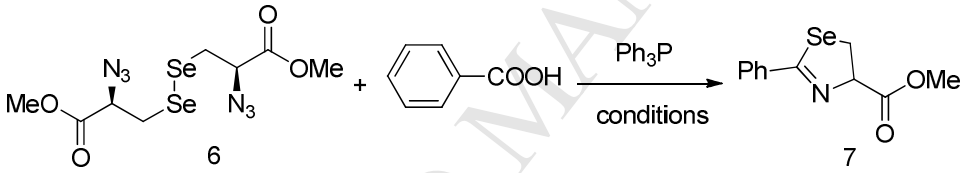


Scheme 1. Synthesis of the *β*-azido diselenide **6**.

Reagents and conditions: (a) compound **4** (1.0 equiv), Br_2 (2.0 equiv), Ph_3P (2.0 equiv), imidazole (2.0 equiv) in DCM, rt, 2h, 86%; (b) N_2H_4•H_2O/Se/NaOH/DMF, rt, 3h, 70%; (c) TFA/DCM, rt, 3h; (d) NaN_3/Tf_2O, $ZnSO_4$, Et_3N, CH_3OH/H_2O/DCM, 0 ºC, overnight, 44% over two steps.

Thus, compound **4** was treated with triphenylphosphine, bromine, and imidazole in dry dichloromethane (DCM) to convert hydroxyl group into the corresponding bromide in 86% yield. The bromide was added to a DMF solution containing a mixture of elemental selenium, hydrazine monohydrate, and NaOH. The reaction between selenium and hydrazine in the presence of NaOH gave Na_2Se_2, which condensed with the bromide to give the *N*-Boc-protected homoselenocystine **5**. It is worth to note that the molar ratio between selenium and NaOH had better be kept at 1:1 to ensure the predominant product diselenide **5**. Treatment of **5** with 10% TFA in DCM removed Boc group, followed by $ZnSO_4$ promoted azide transfer using freshly prepared TfN_3,[17] obtained the expected *β*-azido diselenide **6** in 44% overall yield.

Table 1. Screening of the reaction conditions for selenazoline synthesis [a]



Entry	Coupling reagent	Base	Time (h)	Temperature (°C)	Solvent	Yield (%)
1	EDCI	DIPEA	5	reflux	DCM	23
2	FDP	Et_3N	5	reflux	DCM	29
3	FDP	NMP	5	reflux	DCM	10
4	EDCI	DIPEA	5	40	CH_3CN	70
5	FDP	Et_3N	5	40	CH_3CN	73
6	FDP	Et_3N	5	70	CH_3CN	**84**
7	FDP	Et_3N	5	70	DMF	49
8	FDP	Et_3N	5	70	dioxane	30
9	FDP	Et_3N	5	70	toluene	40
10	FDP	Et_3N	3	70	CH_3CN	45

[a] Unless otherwise noted, **6** (1.0 equiv), acid (4.0 equiv), coupling reagent (4.0 equiv), base (8.0 equiv), Ph_3P (8.0 equiv).

With substrate **6** in hand, we next investigated the possibility of making selenazoline, the precursor of selenazole preparation. Following our previous synthesis of 2,4-disubstituted thiazoline,[16] *β*-azido diselenide **6** was condensed with benzoic acid in the presence of Ph_3P, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

hydrochloride (EDCI) and 1,1'-Dimethyltriethylamine (DIPEA) in DCM under reflux. However, the desired selenazoline product **7** was obtained in an unacceptable low yield (23%, Table 1, entry 1). A series of experimental results suggested that the variation of coupling agent and base did not improve the reaction outcome much (Table 1, entries 1-3), until we realized that the stability of the Staudinger intermediate during selenazoline formation might be crucial.[18] We found that the solvent had a great effect on the reaction in terms of the yield, and the relative polar solvent CH_3CN was found to be the best in selenazoline synthesis (entries 4-9). This is quite different from that of in thiazoline synthesis wherein the nonpolar solvent DCM was favorable to the target formation. The reaction time and temperature were also important factors impacting on the reaction (entries 5, 6, 10). Thus, an optimized typical process for selenazoline synthesis was exemplified as follows: condensation of *β*-azido diselenide **6** with benzoic acid in the presence of pentafluorophenyl diphenylphosphate (FDP), Ph_3P, and Et_3N in CH_3CN at 70 ^oC for 5h afforded methyl 2-pheny1-1,3-selenazoline-4-carboxylate (**7**) in good yield (84%). The transformation of **6** into **7** could be explained as shown in the following proposed pathway (Figure 2).

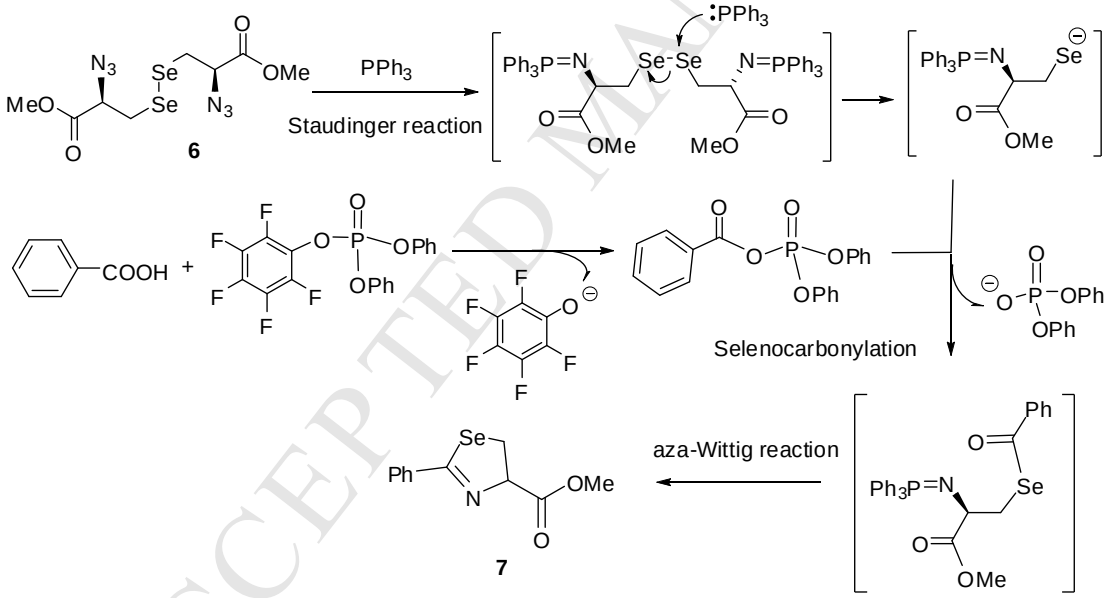


Figure 2. Proposed pathway for the transformation of compound **6** into **7**.

Table 2. Exploration of conditions to convert selenazoline to the corresponding selenazole

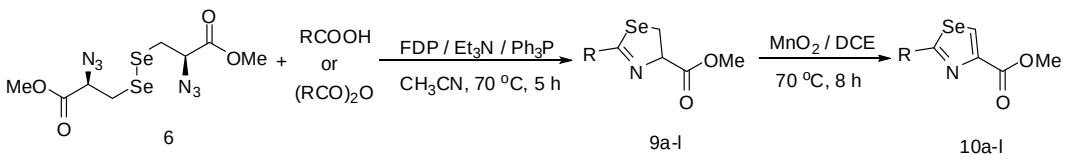
Entry	Oxidation reagent	Temperature	Time	Yield (%)

1	I_2 / DCM[a]	rt	24h	0
2	I_2 / THF[a]	rt or reflux	24h	0
3	IBX / DMSO[b]	45 °C	8h	0
4	DBU / $BrCCl_3$ / DCM[c]	rt	8h	45-71
5	Fresh MnO_2 / DCE[d]	70 °C	8h	94

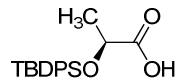
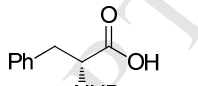
[a] selenazoline **7** (1.0 equiv), I_2 (2.0 equiv). [b] IBX (1.0 equiv). [c] $BrCCl_3$ (1.0-4.0 equiv), DBU (2.0-8.0 equiv). [d] MnO_2 (10 equiv)/DCE, 70 °C, 8h.

There are very few literatures[19,20] about the direct oxidation of selenazoline to selenazole. Our exploration indicated that the activated MnO_2 in 1,2-dichlorethane (DCE) was promising in case of the substrate **7** (Table 2), and the followed scope research results are summarized in Table 3. The current method is applicable to a wide variety of carboxylic substrates (acids and anhydrides, entries 1-12), except for the relatively strong organic acids which might cause intermediate decomposition (entries 3 and 7). Curious about the chiral stability at selenazoline C-4, compound **9b** was subjected to Et_3N in CD_3OD at 60 °C for 4 h. A clear hydrogen–deuterium exchange was observed at C-4 in proton NMR experiment, presumably through base-catalyzed ester-enol epimerization (see ESI Fig. S1). Attempts to prepare the selenazole *via* one-pot five-step reaction following an established procedure[16] gave low yields of product, presumably due to the easy decomposition of α-bromoester selenazoline intermediate under $BrCCl_3$ oxidation. We thus prepared the desired selenazole in two steps, i.e., to prepare selenazoline *via* four-reaction in one-pot and followed by a further MnO_2 oxidation. It was reported that racemization on the α-stereogenic position of thiazole was encountered with Hantzsch method.[21,22] To check this, the optically pure carboxylic acid **11** and Boc-D-Phenylalanine **12** were subjected to the selenazole formation under our reaction conditions (entries 14, 15), respectively. The experiments turned out that both compounds were good substrates for this reaction and no detectable racemization was observed in the corresponding selenazoles (see ESI Fig. S2-S5). This finding certainly enlarges the potential applications of our method comparing to the reported methods.[19, 23]

Table 3. Synthesis of 1,3-selenazolines and selenazoles[a, b]

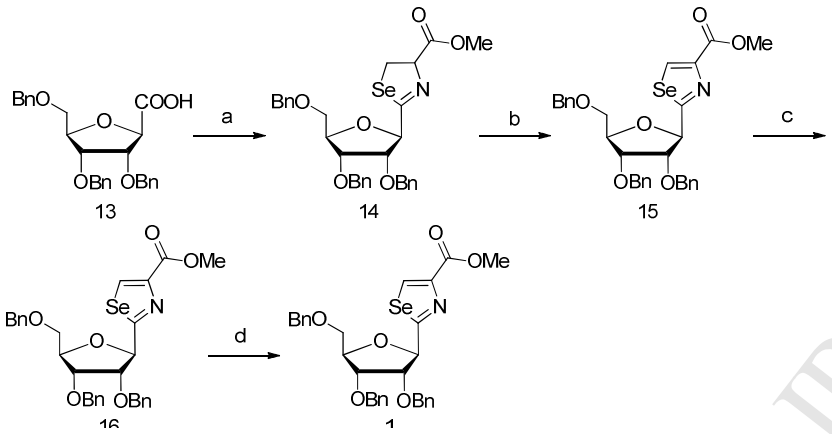


Entry	Substrate	Product	Yield (%)	Product	Yield (%)

1	2-I-PhCOOH	**9a**	60	**10a**	89
2	4-Cl-PhCOOH	**9b**	65	**10b**	92
3	4-NO_2-PhCOOH	**9c**	0	**10c**	/
4	3-Br-PhCOOH	**9d**	75	**10d**	90
5	2-OAc-PhCOOH	**9e**	65	**10e**	93
6	3-CH_3-PhCOOH	**9f**	71	**10f**	92
7	CF_3COOH	**9g**	0	**10g**	/
8	*n*-BuCOOH	**9h**	60	**10h**	95
9	*n*-$C_5H_{11}COOH$	**9i**	71	**10i**	93
10	*n*-$C_7H_{15}COOH$	**9j**	65	**10j**	90
11	*n*-butyric anhydride	**9h**	54[c]	**10h**	95
12	*n*-caproic anhydride	**9i**	66[c]	**10i**	93
13	benzoic anhydride	**7**	76[c]	**8**	94
14	 (S)-2-((tert-butyldiphenyl-silyl)oxy)propanoic acid (**11**)	**9k**	77	**10k**	90
15	 Boc-D-phenylalanine (**12**)	**9l**	62	**10l**	91

[a] Unless otherwise noted, *β*-azido diselenide **6** (1.0 equiv), acid (4.0 equiv), FDP (4.0 equiv), Et_3N (8.0 equiv), Ph_3P (8.0 equiv), 70 ºC in CH_3CN, 5h. [b] MnO_2 (10 equiv)/DCE, 70 ºC, 8h. [c] coupling reagent FDP was not added.

As the methodology has been established, we then focused on the application of our method to a formal synthesis of 2-*β*-D-ribofuranosylselenazole-4-carboxamide (selenazofurin **1**). Being an anti-tumor drug candidate under clinical evaluation,[24] selenazofurin is 10-fold more dose potent than its sulfur counterpart[25] in several *in vitro* and *in vivo* antitumor screenings acting as an inhibitor of inosine monophosphate dehydrogenase (IMPD) *via* selenazofurin adenosine dinucleotide (SAD).



Scheme 2. Formal synthesis of Selenazofurin **1.**

Reagents and conditions: (a) *β*-azido diselenide **6** (1.0 equiv), **13** (4.0 equiv), FDP (4.0 equiv), Et_3N (8.0 equiv), Ph_3P (8.0 equiv), 70 ºC in CH_3CN, 5h, 70%; (b) MnO_2 (10 equiv)/DCE, 70 ºC, 8h, 85%; (c) MsOH/CH_3Cl, rt, 8h, 90%; (d) CH_3OH/NH_3, rt, 5h, in quantitative.

Our strategy towards the total synthesis of selenazofurin is depicted in Scheme 2. The precursor, 2,3,5-tri-*O*-benzyl-*β*-D-ribofuranosyl formic acid (**13**), was synthesized from commercially available D-Ribose following a combination of two literature procedures.[23, 26] Treatment of **13** with *β*-azido diselenide **6** in the presence of FDP, Ph_3P, and Et_3N in CH_3CN afforded ribofuranosyl selenazoline **14** (Scheme 2) in 70% yield as an enantiomeric mixture at C-4 of selenazoline (*R*/*S* = 1.00:1.38, ESI Fig. S6). The crude mixture was further oxidized with active MnO_2 in DCE affording the expected ribosyl selenazole **15** in 85% isolated yield. Coupling constant of *J* Hz at 2.8 ppm in ^{1}H NMR spectrum clearly indicated that ribosyl selenazole **15** was in *β* configuration.[26] No *α* product was observed in our studies. This was quite encouraging as the anomeric mixture of ribofuranosyl residue was obtained from other reported method.[25] Hydrogenolysis of **15** with various Pd catalysts on carbon did not give a complete removal of benzyl ethers, possibly due to the poisoning of Pd catalyst by selenium in the substrate.[27] To our delight, the clean cleavage of benzyl ethers from **15** was carried out smoothly with methanesulfonic acid in $CHCl_3$ to afford the desired **16** in a yield of 90%. Finally, treatment of **16** with NH_3 in methanol furnished selenazofurin **1** quantitatively. The physical data of the synthetic selenazofurin **1** is in consistent with that of the reported previously.[28]

The antitumor activity of the synthetic **1** was evaluated against three tumor cell lines (K562, A549 and HCT-116) according to a literature procedure,[29] and the results are outlined in Table 4. Compound **1** exhibited good antitumor activities against K562 and A549 cells with IC_{50} at 0.29 and 0.85 μM, respectively.

Table 4. *In Vitro* antitumor activity of the synthetic compound **1**

Compound	IC_{50} (μM)		
	K562	A549	HCT-116
1	0.29	0.85	>10
Taxol	0.0026	0.0026	0.0025

3. Conclusions

We have successfully demonstrated a facile transformation of dialkyl diselenides into selenazoles in good yields *via* a cascade Staudinger reduction / diselenide cleavage / selenocarbonylation / aza-Wittig reaction and a following oxidation. The method provides a broad substrate applicability and its potentials are also exemplified by an efficient total synthesis of Selenazofurin which exhibited good antitumor activities against K562 and A549 cells. Further applications of this strategy to the design and synthesis of other related selenazole-containing bioactive compounds are currently under investigation in our laboratory.

4. Experimental section

4.1 General Experimental

All solvents were dried according to the established procedures before use. All reagents were purchased from commercial corporations. Flash chromatography (FC) was performed using silica gel (200−300 meshes) according to the protocol of Still, Kahn, and Mitra.[30] All reactions under standard conditions were monitored by thin-layer chromatography (TLC) on gel F254 plates. Optical rotations were measured using a polarimeter. High-resolution mass spectrometry data (HRMS) were acquired using a Q-TOF analyzer. ^{1}H NMR and ^{13}C NMR were measured on 400 MHz and 100 MHz spectrometers (NMR in $CDCl_3$ with TMS as an internal standard). Chemical shifts (δ) are given in parts per million and coupling constants (J) in Hertz.

4.2. Experimental Procedures

Synthesis of Boc-L-selenocystine methyl ester **(5)**

To a pre-cooled mixture of triphenylphosphine (4.40 g, 16.8 mmol) in dry DCM (50 mL) was dropwisely added a solution of Br_2 (0.86 mL, 16.8 mmol) in DCM (20 mL) at 0 °C under nitrogen protection. After 20 min, a mixture of N-Boc-L-serine methyl ester (1.84 g, 8.4 mmol) and imidazole (1.14 g, 16.8 mmol) in DCM (50 mL) was slowly added to the above pale yellow solution and the mixture was stirred for another two hours at 0 °C. Subsequently, the solids were filtered off and the solvent was evaporated. The resulting solid was re-dissolved in DCM (10 mL) and purified by column chromatography (hexanes /EtOAc, 6:1) to give the bromide intermediate in 86% yield (2.03 g, 7.2 mmol).

To a suspension of Se (3.945 g, 47.6 mmol) and pulverized NaOH (1.998 g, 47.6

mmol) in DMF was added $N_2H_4 \cdot H_2O$ (0.4 mL). The mixture was stirred under argon for 3h at 60 °C, then the above bromide (1.9 g, 6.8 mmol) was added dropwise and stirred for 3h further. TLC indicated complete disappearance of the starting material, and the mixture was poured into aqueous HCl (1 M, 50 mL) and extracted with DCM (3 x 50 mL). The combined organic solvent was dried over Na_2SO_4 and evaporated *in vacuo*. The crude mixture was purified by FC (silica gel, hexanes/EtOAc 2:1) to give compound **5** in 70% yield as a yellow solid (1.33 g). m.p. 36-37 ºC; $[\alpha]_D^{25}$ 68.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, MeOD), δ (ppm) 5.39 (d, J = 7.6 Hz, 1H, NH), 4.61-4.62 (m, 1H, CH), 3.75 (s, 3H, CH_3), 3.32-3.43 (m, 2H, CH_2); ^{13}C NMR (100 MHz, MeOD), δ (ppm) 28.5, 32.5, 52.8, 53.8, 80.5, 155.2, 171.4; HRMS (ESI): calcd for $C_{18}H_{32}N_2O_8Se_2$, $[M+Na]^+$ 587.0387; found: 587.0369.

Synthesis of (2R, 2'R)-dimethyl 3,3'-diselanediylbis(2-azidopropanoate) (6)

In flask one, selenocystine methyl ester **5** (562 mg, 1 mmol) was dissolved in TFA (1.5 mL) in dry DCM (15 mL) and the mixture was stirred for 3 h. When TLC indicated complete reaction, the mixture was evaporated under reduced pressure to give L-selenocystine methyl ester trifluoroacetic acid salt quantitatively as a yellow solid. m.p. 148-149 ºC; $[\alpha]_D^{25}$ -39.0 (c 1.0, CH3OH); 1H NMR (400 MHz, MeOD), δ (ppm) 3.44-3.49 (m, 2H, CH_2), 3.56-3.60 (m, 2H, CH_2), 3.88 (s, 6H, OCH_3), 4.45 (m, 2H, CH); 13C NMR (100 MHz, MeOD), δ (ppm) 28.4, 54.1, 54.4, 169.7; HRMS (ESI): calcd for $C_8H_{16}N_2O_4Se_2$, $[M+H]^+$ 364.9519; found: 364.9500.

In flask two, a solution of NaN_3 (390 mg, 6 mmol) in H_2O (1 mL) and CH_2Cl_2 (1 mL) was added Tf_2O (846 mg, 3 mmol) and stirred vigorously for 2 h at 0 °C. The biphasic mixture was neutralized with saturated aqueous $NaHCO_3$ (1 mL), extracted with CH_2Cl_2 (2 x 1 mL), and the combined organic phase was washed with saturated aqueous $NaHCO_3$ solution (2 x 1 mL) again. The resulting TfN_3 solution in CH_2Cl_2 was used directly without further purification.

To a pre-mixed mixture of L-selenocystine methyl ester TFA salt (662 mg, 1 mmol), $ZnSO_4$ (4 mg, 0.02 mmol) and Et_3N (253 mg, 2.5 mmol) in MeOH (6.7 mL) and water (1.3 mL) was added dropwise the freshly prepared TfN_3 solution at 0 °C. The homogeneous mixture was stirred at 0 °C until the reaction completed (TLC monitoring), then quenched by addition of phosphate buffer (pH 3). The pH of the aqueous layer was carefully adjusted to pH 2 by addition of dilute aqueous HCl. The water phase was separated and extracted with CH_2Cl_2 (3 x 5 mL), and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by FC (silica gel, hexanes/EtOAc 3:1) to give compound 6 as an orangered oil (0.85 g, 44% for two steps). $[\alpha]_D^{25}$ -35.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.22 (dd, J = 8.0 Hz, J = 12.8 Hz, 1H); 3.37 (dd, J = 5.6 Hz, J = 12.8 Hz, 1H); 3.84 (s, 3H); 4.24-4.27 (m, 1H); ^{13}C NMR (100 MHz, CDCl3),

δ (ppm) 30.2, 53.2, 62.6, 169.6; HRMS (ESI): calcd for $C_8H_{12}N_6O_4Se_2$, $[M+Na]^+$ 438.9148; found: 438.9137.

General Procedure for Selenazoline Formation.

Typical procedure for the synthesis of methyl-2-phenylselenazoline-carboxylate **7**. To a solution of benzoic acid (122 mg, 1 mmol) in anhydrous acetonitrile (10 mL) were added triethylamine (Et_3N) (202 mg, 2 mmol) and pentafluorophenyl diphenylphosphate (FDP) (416 mg, 1 mmol) at room temperature. After stirring for 5 min, dialkyl *β*-azido diselenide **6** (104 mg, 0.25 mmol) and Ph_3P (524 mg, 2 mmol) were added, and the mixture was stirred at 70 ℃ for another 5h, then concentrated under reduced pressure. The residue was purified by FC (silica gel, hexanes/EtOAc 10:1) to give compound **7** as yellow oil (113 mg).

General Procedure to Oxide Selenazoline to Selenazole

Typical procedure for the formation of methyl-2-phenylselenazole-carboxylate **8**. To a solution of methyl-2-phenylselenazoline-carboxylate **7** (177 mg, 1 mmol) in 1,2-dichloroethane (10 mL) was added activated MnO_2 (860 mg, 10 mmol). The mixture was then heated at 70 ^oC for 8 h under a nitrogen atmosphere. After filtration, the mixture was evaporated *in vacuo*. The residue was chromatographed on silica gel (hexanes/EtOAc 8:1) to give compound **8** (105 mg).

Methyl 2-(2-iodophenyl)selenazoline-4-carboxylate (7)

The title compound was obtained in 84% yield as pale yellow oil. ^1H NMR (400 MHz, $CDCl_3$), *δ* (ppm) 3.71-3.78 (m, 2H), 3.81 (d, *J* = 4.0 Hz, 3H), 5.22 (t, *J* = 8.8 Hz, 1H), 7.37−7.40 (m, 2H), 7.44−7.47 (m, 1H), 7.78 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$), *δ* (ppm) 31.5, 52.9, 81.0, 128.7, 129.3. 131.8, 135.1, 169.7, 171.4; HRMS (ESI): calcd for $C_{11}H_{11}NO_2Se$, $[M+Na]^+$ 291.9853; found: 291.9860.

Methyl 2-phenylselenazole-4-carboxylate (8)

The title compound was obtained in 94% yield according to the typical procedure as white solid. m.p. 108-110 ºC; ^1H NMR (400 MHz, $CDCl_3$), *δ* (ppm) 3.96 (d, *J* = 1.6 Hz, 3H), 7.41−7.49 (m, 3H), 7.93-7.96 (m, 2H), 8.88 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), *δ* (ppm) 52.7,127.6, 129.2, 131.0, 134.6, 135.7, 149.0, 162.4, 175.4; HRMS (ESI): calcd for $C_{11}H_9NO_2Se$, $[M+Na]^+$ 289.9696; found: 289.9702.

Methyl 2-(2-iodophenyl)selenazoline-4-carboxylate (9a)

The title compound was obtained in 60% yield as pale yellow oil. ^1H NMR (400 MHz, $CDCl_3$), *δ* (ppm) 3.82-3.95 (m, 2H), 3.84 (s, 3H), 5.25 (t, *J* = 9.0 Hz, 1H), 7.04-7.09 (m, 1H), 7.35 (td, J_1 = 0.8 Hz, J_2 = 7.6 Hz, 1H), 7.45 (dd, J_1 = 1.2 Hz, J_2 = 7.6 Hz, 1H), 7.88 (dd, J_1 = 0.8 Hz, J_2 = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), *δ* (ppm) 33.3, 53.0, 80.7, 94.4, 128.2, 129.5, 131.3, 140.3, 140.4, 170.7, 170.9; HRMS (ESI): calcd for $C_{11}H_{10}INO_2Se$, $[M+Na]^+$ 417.8814; found: 417.8822.

Methyl 2-(2-iodophenyl)selenazole-4-carboxylate (10a)

The title compound was obtained in 89% yield as a yellowish oil (89%): ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.96 (s, 3H), 7.11 (td, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 7.40-7.44 (m, 1H), 7.82 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 9.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 52.7, 96.9, 128.5, 131.3, 131.3, 137.0, 140.1, 140.7, 147.8, 162.4, 174.5; HRMS (ESI): calcd for $C_{11}H_8INO_2Se$, $[M+Na]^+$ 415.8663; found: 415.8664.

Methyl 2-(4-chlorophenyl)selenazoline-4-carboxylate (9b)

The title compound was obtained in 65% yield as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.75-3.81 (m, 2H), 3.96 (s, 3H), 5.21 (t, J = 9.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 31.9, 53.0, 80.9, 129.0, 130.5, 133.6, 138.0, 168.6, 171.2; HRMS (ESI): calcd for $C_{11}H_{10}ClNO_2Se$, $[M+Na]^+$ 325.9463; found: 325.9439.

Methyl 2-(4-chlorophenyl)selenazole-4-carboxylate (10b)

The title compound was obtained in 92% yield as white solid. m.p. 136-138 ºC; ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.96 (s, 3H), 7.41 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 8.89 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 52.7, 128.7(2), 129.5(2), 134.2, 134.8, 137.1, 149.1, 162.2, 173.9; HRMS (ESI): calcd for $C_{11}H_8ClNO_2Se$, $[M+Na]^+$ 323.9298; found: 323.9292, $[M+K]^+$ 339.9038; found: 339.9077.

Methyl 2-(3-bromophenyl)selenazoline-4-carboxylate (9d)

The title compound was obtained in 75% yield as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.75-3.82 (m, 2H), 3.84 (s, 3H), 5.23 (t, J = 8.8 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.58-7.60 (m, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.98 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 33.2, 54.3, 82.2, 124.2, 129.5, 131.6, 133.0, 136.0, 138.3, 169.6, 172.4; HRMS (ESI): calcd for $C_{11}H_{11}NO_2Se$, $[M+Na]^+$ 369.8949; found: 369.8973.

Methyl 2-(3-bromophenyl)selenazole-4-carboxylate (10d)

The title compound was obtained in 90% yield as white solid. m.p. 122-123 ºC; ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.97 (s, 3H), 7.31 (t, J = 8.0 Hz, 1H), 7.60 (dt, J_1 = 0.8 Hz, J_2 = 8.0 Hz, 1H), 7.84-7.85 (m, 1H), 8.13 (m, 1H), 8.92 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 52.8, 123.4, 126.3, 130.1, 130.7, 133.8, 135.2, 137.3, 149.1, 162.2, 173.4; HRMS (ESI): calcd for $C_{11}H_8INO_2Se$, $[M+Na]^+$ 367.8792; found: 367.8795.

Methyl 2-(2-acetoxyphenyl)selenazoline-4-carboxylate (9e)

The title compound was obtained in 65% yield as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 2.36 (s, 3H), 3.70 (d, J = 8.4 Hz, 2H), 3.83 (s, 3H), 5.16 (t, J = 9.2 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.27-7.31 (m, 1H), 7.47 (t, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 21.6, 31.1, 52.9, 80.6, 123.7,

126.3, 127.8, 131.6, 132.2, 148.3, 164.3, 169.5, 171.2; HRMS (ESI): calcd for $C_{11}H_{11}NO_2Se$, $[M+Na]^+$ 349.9907; found: 349.9932.

Methyl 2-(2-acetoxyphenyl)selenazole-4-carboxylate (10e)

The title compound was obtained in 93% yield as white solid. m.p. 155-156 ºC; ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 2.49 (s, 3H), 3.97 (s, 3H), 7.24-7.26 (m, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 8.28 (t, J = 7.6 Hz, 1H), 8.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 22.0, 52.6, 123.7, 126.6, 127.6, 129.8, 131.4, 135.5, 147.8, 148.2, 162.4, 167.9, 168.9; HRMS (ESI): calcd for $C_{11}H_{11}NO_2Se$, $[M+Na]^+$ 347.9751; found: 347.9779.

Methyl 2-(4-methylhenyl)selenazoline-4-carboxylate (9f)

The title compound was obtained in 71% yield as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 2.36 (s, 3H), 3.70-3.78 (m, 2H), 3.82 (s, 3H), 5.20 (t, J = 8.8 Hz, 1H), 7.19 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 21.6, 31.3, 52.9, 80.8, 129.2(2), 129.4(2), 132.3, 142.3, 169.5, 171.5; HRMS (ESI): calcd for $C_{12}H_{13}NO_2Se$, $[M+Na]^+$ 306.0009; found: 306.0026.

Methyl 2-(4-methylphenyl)selenazole-4-carboxylate (10f)

The title compound was obtained in 92% yield as white solid. m.p. 115-117 ºC; ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 2.35 (s, 3H), 3.94 (s, 3H), 7.21 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 7.6 Hz, 2H), 8.83 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 21.6, 52.6, 127.4, 129.8, 132.9, 134.1, 141.4, 148.7, 162.3, 175.5; HRMS (ESI): calcd for $C_{12}H_{11}NO_2Se$, $[M+H]^+$ 282.0033; found: 282.0038.

Methyl 2-propylselenazoline-4-carboxylate (9h)

The title compound was obtained in 60% yield as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.96 (t, J = 7.4 Hz, 3H), 1.60−1.70 (m, 2H), 2.59 (m, 2H), 3.64 (dd, J_1 = 8.8 Hz, J_2 = 1.2 Hz, 2H), 3.79 (s, 3H), 4.98 (t, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 13.7, 21.7, 31.4, 39.6, 52.9, 80.1, 171.4, 173.7; HRMS (ESI): calcd for $C_8H_{13}NO_2Se$, $[M+Na]^+$ 258.0009; found: 257.9987.

Methyl 2-propylselenazole-4-carboxylate (10h)

The title compound was obtained in 95% yield as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 1.02 (t, J = 7.4 Hz, 3H), 1.77−1.86 (m, 2H), 3.03 (t, J = 7.8 Hz, 2H), 3.91 (s, 3H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 13.7, 24.4, 39.1, 52.6, 134.6, 147.2, 162.3, 179.7; HRMS (ESI): calcd for $C_8H_{11}NO_2Se$, $[M+Na]^+$ 255.9853; found: 255.9835.

Methyl 2-pentylselenazoline-4-carboxylate (9i)

The title compound was obtained in 71% yield as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.89 (t, J = 6.8 Hz, 3H), 1.31−1.35 (m, 4H), 1.59-1.65 (m, 2H), 2.61 (t, J = 7.9 Hz, 2H), 3.60-3.67 (m, 2H), 3.80 (s, 3H), 4.98 (t, J =8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 14.1, 22.5, 28.0, 31.3, 31.3, 37.8, 52.8, 80.1, 171.4, 174.0; HRMS (ESI): calcd for $C_{10}H_{17}NO_2Se$, $[M+Na]^+$ 286.0322; found: 286.0294.

Methyl 2-pentylselenazole-4-carboxylate (10i)

The title compound was obtained in 93% yield as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.88 (t, J = 6.8 Hz, 3H), 1.31−1.42 (m, 4H), 1.74-1.82 (m, 2H), 3.04 (t, J = 7.8 Hz, 2H), 3.91 (s, 3H), 8.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 14.0, 22.5, 30.7, 31.3, 37.2, 52.6, 134.6, 147.2, 162.3, 179.9; HRMS (ESI): calcd for $C_{12}H_{19}NO_2Se$, $[M+Na]^+$ 284.0166; found: 284.0151, $[M+K]^+$ 299.9905; found: 299.9899.

Methyl 2-heptylselenazoline-4-carboxylate (9j)

The title compound was obtained in 65% yield as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.87 (t, J = 9.0 Hz, 3H), 1.27−1.31 (m, 8H), 1.58-1.66 (m, 2H), 2.61 (t, J = 7.6 Hz, 3H), 3.64 (d, J = 9.6 Hz, 2H), 3.80 (s, 3H), 4.98 (t, J = 12.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 14.2, 22.7, 28.3, 29.1, 29.1, 31.3, 31.8, 37.8, 52.8, 80.1, 171.4, 173.9; HRMS (ESI): calcd for $C_{12}H_{21}NO_2Se$, $[M+H]^+$ 292.0816; found: 292.0803, $[M+Na]^+$ 314.0635; found: 314.0626.

Methyl 2-heptylselenazole-4-carboxylate (10j)

The title compound was obtained in 90% yield as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.86 (t, J = 6.4 Hz, 3H), 1.27−1.44 (m, 8H), 1.75-1.82 (m, 2H), 2.61 (t, J = 7.6 Hz, 3H), 3.05 (t, J = 7.8 Hz, 2H), 3.92 (s, 3H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 14.2, 22.7, 29.1, 29.1, 31.0, 31.7, 37.3, 52.6, 134.6, 147.2, 162.3, 179.9; HRMS (ESI): calcd for $C_{12}H_{19}NO_2Se$, $[M+Na]^+$ 312.0479; found: 312.0482, $[M+K]^+$ 328.0218; found: 328.0203.

(*S*)-Methyl 2-(1-(*tert*-butyldiphenylsilyloxy)ethyl)selenazoline-4-carboxylate (9k)

The title compound was obtained in 77% yield (*R*/*S* = 55:45) as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 1.11(s, 4.05H,), 1.12 (s, 4.95H), 1.23(d, J = 6.4 Hz,1.35H), 1.28 (d, J = 6.4 Hz, 1.65H), 3.47(d, J = 9.6 Hz, 1.1H), 3.53(dd, J = 8.8 Hz, J = 2.0 Hz, 0.9H), 3.78(s, 1.35H), 3.79(s, 1.65H), 4.67−4.75 (m, 1H), 4.91(t, J = 9.6 Hz, 0.45H), 5.(dt, J = 9.6 Hz, J = 0.8 Hz, 0.55H), 7.32−7.48 (m, 6H), 7.34-7.44 (m, 6H), 7.65-7.0 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 19.4, 23.4, 23.5, 27.0, 28.6, 28.7, 52.8, 72.1, 72.2, 80.5, 80.7, 127.8, 127.8, 127.8, 130.0,130.0, 130.0, 132.8, 132.9, 133.7, 133.8, 135.9, 136.0, 136.1, 136.1, 171.3, 171.4, 181.5, 182.1; HRMS (ESI): calcd for $C_{23}H_{29}NO_3SeSi$, $[M+Na]^+$ 498.0980; found: 498.0962.

(*S*)-Methyl 2-(1-(*tert*-butyldiphenylsilyloxy)ethyl)selenzole-4-carboxylate (10k)

The title compound was obtained in 90% yield as colorless oil. $[\alpha]_D^{25}$ -31.0 (*c* 0.85, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 1.13 (s, 9H), 1.35 (d, J = 6.4 Hz, 3H), 3.91(s, 3H), 5.12(q, J = 6.4 Hz, 1H), 7.32−7.48 (m, 6H), 7.62 (d, J = 7.2 Hz, 2H), 7.70 (d, J = 6.8 Hz, 2H), 8.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 19.4, 25.5, 27.1(3), 52.6, 72.8, 127.9(2), 128.0(2), 130.2, 130.3, 132.5, 133.5, 134.5, 135.9(2), 136.0(2), 147.9, 162.5, 186.9; HRMS (ESI): calcd for $C_{23}H_{27}NO_3SeSi$, $[M+Na]^+$ 496.0823; found: 496.0839, $[M+K]^+$ 512.0562; found: 512.0577.

Methyl 2-((R)-1-((tert-butoxycarbonyl)amino)-2-phenylethyl)selenazoline-4-carboxylate (9l)

The title compound was obtained in 62% yield as a pale yellow oil (*R*/*S* = 32:68). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 28.4, 30.9, 31.2, 39.6, 39.7, 52.9, 56.5, 80.1, 80.2, 80.4, 127.0, 127.1, 128.5, 128.6, 129.7, 129.8, 136.1, 136.3, 155.0, 155.1, 170.9, 171.1, 175.3, 176.3; HRMS (ESI): calcd for $C_{18}H_{24}N_2O_4Se$, $[M+H]^+$ 413.0980; found: 413.0973.

Methyl 2-((*R*)-1-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)selenazole-4-carboxylate (10l)

The title compound was obtained in 91% yield as pale yellow oil. $[\alpha]_D^{25}$ 5.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 1.38 (s, 9H), 3.29 (bs, 1H), 3.38-3.42 (m, 1H), 3.95 (s, 3H), 5.25 (bs, 2H), 7.14-7.16 (m, 2H), 7.21-7.30 (m, 3H), 8.83 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 128.4, 41.2, 52.6, 56.6, 80.6, 127.2, 128.8, 129.6, 135.2, 136.3, 148.0, 155.2, 162.3, 181.2; HRMS (ESI): calcd for $C_{18}H_{22}N_2O_4Se$, $[M+Na]^+$ 433.0642; found: 433.0654.

Methyl 2-(2,3,5-tri-*O*-benzyl-*β*-D-ribofuranosyl)selenazoline-4-carboxylate (14)

To a solution of acid **13** (434 mg, 1 mmol) in anhydrous acetonitrile (10 mL) were added Et_3N (202 mg, 2 mmol) and pentafluorophenyl diphenylphosphate (FDP, 416 mg, 1 mmol) at room temperature. After stirring for 5 min, *β*-azido diselenide **6** (104 mg, 0.25 mmol) and Ph_3P (524 mg, 2 mmol) was added into the solution. The reaction mixture was stirred at 70 ℃ for another 5h, and the solvent was removed under diminished pressure. The residue was purified by FC (silica gel, hexanes/EtOAc 7:1) to give compound **14** (70%, *R*/*S* = 58:42,) as yellow oil. ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 29.3, 29.4, 52.7, 52.7, 70.1, 70.2, 72.1, 72.1, 72.3, 72.4, 77.6, 78.0, 78.0, 80.6, 80.7, 80.8, 80.9, 81.6, 81.9, 83.6, 83.6, 127.6, 127.7, 127.9, 127.9, 127.9, 128.0, 128.3, 128.4, 128.4, 137.6, 137.7, 137.9, 138.3, 138.3, 171.1, 171.1, 176.2, 176.6; HRMS (ESI): calcd for $C_{31}H_{33}NO_6Se$, $[M+H]^+$ 596.1551; found: 596.1543.

Methyl 2-(2,3,5-tri-*O*-benzyl-*β*-D-ribofuranosyl)selenazole-4-carboxylate (15)

To a solution of **14** (100 mg, 0.17 mmol) in 1, 2-dichloroethane (5 mL) was added activated MnO_2 (870 mg, 1 mmol). The mixture was refluxed for 8 h under a nitrogen atmosphere, then filtered and the filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel (EtOAc/hexane 2:1) to give compound **15** (85 mg, 85%) as colorless oil. $[\alpha]_D^{25}$ 105.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.67 (dd, $J_{4',5a'}$ = 3.8 Hz, $J_{4',5a'}$ = 4.8 Hz, 1H, $H_{5a'}$), 3.85 (dd, $J_{4',5b'}$ = 2.4 Hz, $J_{5a',5b'}$ = 10.8 Hz, 1H, $H_{5b'}$), 4.00 (s, 3H, OCH_3), 4.04 (dd, $J_{3',2'}$ = 7.6 Hz, $J_{3',4'}$ = 4.8 Hz, 1H,

$H_{3'}$), 4.04 (dd, $J_{3',2'}$ = 3.0 Hz, $J_{3',4'}$ = 4.8 Hz, 1H, $H_{3'}$), 4.41-4.45 (m, 2H, CH_2, $H_{4'}$), 4.68-4.56 (m, 4H, CH_2), 4.79 (d, J = 11.6 Hz, 1H, CH_2), 4.89 (d, J = 12.0 Hz, 1H, CH_2), 5.40 (d, $J_{1',2'}$ = 2.8 Hz, 1H, $H_{1'}$), 7.28-7.39 (m, 13H, Ph), 7.47-7.48 (m, 2H, Ph), 8.88 (s, 1H, $H_{1'}$); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 52.5, 69.3, 72.2, 72.4, 73.5, 77.3, 81.3, 81.7, 84.5, 127.8, 127.9, 128.0, 128.5, 128.5, 135.5, 137.6, 137.8, 138.2, 162.4, 180.7; HRMS (ESI): calcd for $C_{31}H_{31}NO_6Se$, $[M+K]^+$ 632.0954; found: 632.0981.

Methyl 2-*β*-D-Ribofuranosylselenazole-4-carboxylate (16)

To a solution of compound **15** (50 mg, 0.084mmol) in $CHCl_3$ (1 mL) was added methanesulfonic acid (0.06 mL, 0.963 mmol). The reaction mixture was stirred at room temperature for 8 h, then neutralized with NaOH (0.13 mL aqueous solution, 0.963 mmol) and concentrated. The solid was washed with a co-solvent ($CHCl_3$/MeOH, 3 mL, v/v 2:1) and filtered through Celite, and the residue was purified by FC (silica gel, methanol/EtOAc 1:10) to give compound **16** (24 mg, 90%) as colorless solid. m.p. 113-114 °C (lit. m.p. 113–115 °C); $[\alpha]_D^{25}$ -10.0 (*c* 0.5, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 3.71 (dd, J = 2.8 Hz, J = 12.0 Hz, 1H), 3.82 (dd, J = 4.2 Hz, J = 12.0 Hz, 1H), 3.90 (s, 3H), 4.02−4.08 (m, 2H), 3.47 (d, J = 4.8 Hz, 1H), 4.98 (d, J = 4.4 Hz, 1H), 9.05 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 52.9, 63.4, 72.9, 78.6, 86.4, 86.4, 136.8, 148.8, 163.6, 183.1; HRMS (ESI): calcd for $C_{10}H_{13}NO_6Se$, $[M+Na]^+$ 345.9806; found: 345.9796.

2-*β*-D-Ribofuranosylselenazole-4-carboxamide（Selenazofurin 1）

Compound **16** (20 mg) was treated with an ammonia saturated methanol (10 mL) at 0 ºC for 5 h. The mixture was evaporated off *in vacuo*, and the residue was washed with chloroform (2 mL x 3), then purified by FC (silica gel, methanol/EtOAc 1:5) to give selenazofurin **1** (19 mg, quantitative yield) as colorless solid. m.p. 133-134 ºC (lit. m.p. 131–133 ºC); $[\alpha]_D^{25}$ -4.6 (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, MeOD), δ (ppm) 3.71 (dd, $J_{4,5a'}$ = 4.4 Hz, $J_{5a',5b'}$ = 12 Hz, 1H, $H_{5a'}$), 3.79 (dd, $J_{4,5b'}$ = 2.8 Hz, $J_{5a',5b'}$ = 12 Hz, 1H, $H_{5b'}$), 4.16 (t, J = 4.8 Hz, 1H, $H_{2'}$), 4.09-4.05 (m, 2H, $H_{3'}$, $H_{4'}$), 4.97 (d, J = 5.2 Hz, 1H, $H_{1'}$), 8.86 (s, H_5, 1H); ^{13}C NMR (100 MHz, MeOD), δ (ppm) 63.6, 73.1, 78.5, 86.1, 86.7, 132.4, 152.0, 166.3, 181.7; HRMS (ESI): calcd for $C_9H_{12}N_2O_5Se$, $[M+Na]^+$ 330.9809; found: 330.9824.

4.3 Cells and cytotoxicity assays

Three human cell lines, A549, K562 and HCT-116 were cultured in RPMI 1640 medium (GIBCO-Invitrogen, NY) with 10% fetal bovine serum (FBS) and supplemented with glutamine (2 mmol L^{-1}), penicillin G (100 µg mL^{-1}), and streptomycin (100 µg mL^{-1}) at 37 °C under 5% CO_2. MTT assay was carried out on K562 (chronic erythroleukaemia), A549 and HCT 116 cells to test the cytotoxicity of

Selenazofurin. The cells under study were harvested, counted and seeded into a 96 well plate at a density of 3000–5000 cells per well depending on their doubling times. The cells were allowed to adhere for 24 h at 37 °C and 5% CO_2. After 24 h incubation, the culture media was removed, 100 µL of test chemical dissolved in dimethyl sulphoxide (DMSO) and culture medium was added to the cells, culture medium containing DMSO only (without test compounds) was used for the control cells. Then, the cells were incubated with compounds for 48 h under the same conditions. After 48 h, the culture medium containing compounds was removed and 100 µL of culture media containing 5 mg mL^{-1} MTT (Thiazoyl blue tetrazolium bromide) was added to each well and cells were further incubated for 4 hours. After 4 hours incubation the media containing MTT was aspirated and 100 µL DMSO was added to each well to solubilize the crystallized formazan product. The plates were read on a plate reader at 570 nm and a reference wavelength of 630 nm. The absorbance readings for 630 nm were subtracted from the 570 nm readings and the results were adjusted by dividing the average by the DMSO control to adjust for any toxicity that may have occurred in this control treatment set. The percentage inhibition was calculated based on 100−[(mean OD of treated cell × 100)/mean OD of vehicle treated cells (DMSO)]. The IC_{50} values were calculated using Probit Software. All the tests were repeated in at least three independent experiments.

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Supporting Information

Supplementary data related to this article can be found at

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