Large scale synthesis of the Cdc42 inhibitor secramine A and its inhibition of cell spreading

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We describe a large scale synthesis of secramine A. Consistent with its ability to inhibit activation of the small GTPase Cdc42, we find that secramine A inhibits cell spreading, a process previously shown to be Cdc42-dependent.

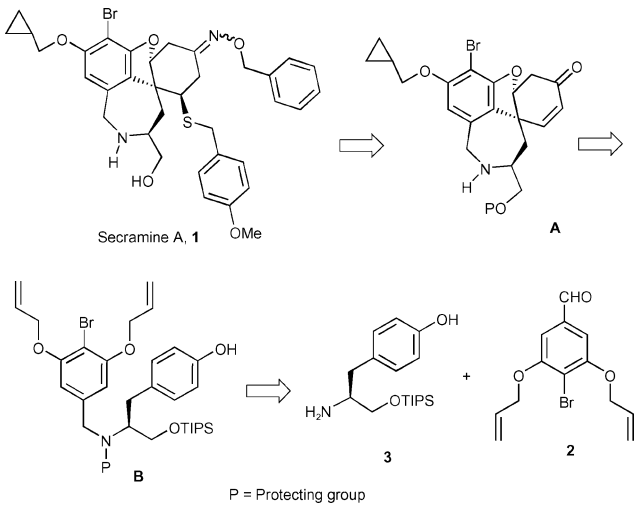
Introduction

The synthesis and phenotypic screening of natural product-like libraries has emerged as a promising approach to reagent and drug discovery.[1] This strategy led to the discovery of secramine[2] (later renamed secramine A[3]) (**1**), a synthetic analog of galanthamine that inhibits some forms of membrane traffic out of the Golgi apparatus. Secramine A emerged from a library of ~2500 compounds that were synthesized by mimicking the biosynthesis of galanthamine on a solid support.[2] Recently, Kirchhausen, Shair and coworkers reported that secramine A inhibits activation of the Rho GTPase Cdc42, a protein involved in membrane traffic, by a mechanism dependent upon the guanine dissociation inhibitor RhoGDI.[3] RhoGDI1 shuttles Cdc42 between the cytosol and intracellular membranes.[4] Through precise subcellular localization and the binding and hydrolysis of GTP, Cdc42 contributes to numerous cellular processes, including polarization, migration and spreading.[5] Many of these events stem from the ability of Cdc42 to regulate actin polymerization and *in vitro*, secramine A inhibits Cdc42-dependent actin polymerization.[3] As shown in Scheme 1, the most salient features in the synthesis of **1** are the bis-cyclization of intermediate **B**, followed by a Mitsunobu reaction to give **A**, which then undergoes a conjugate addition and hydroxylamine formation. Intermediate **B** was prepared by reductive amination of the tyrosine derivative **3** with substituted benzaldehyde **2**. In view of its distinctive biological activity, we sought a synthesis protocol to generate large amounts of biological active secramine A by improving the low-yielding, small-scale (<50 mg) synthesis of the original procedure (3.8% yield from **4**).

Here we report a scaled-up synthesis of secramine A, including some attempted variations to our synthetic route and the ability of secramine A to inhibit cell spreading.

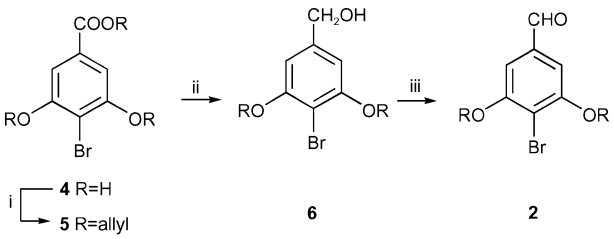
Results and discussion

Our synthesis strategy maintained the biomimetic approach used in the initial secramine A synthesis, but focused on improved purification, reduction of chromatographic steps and altering the synthetic route towards an early stage intermediate. First we optimized the synthesis of precursors **2** and **3**.



Scheme 1 Retrosynthesis of secramine A (**1**).

Because both precursors are polar compounds, we minimized chromatography in the preliminary stages. We obtained aldehyde **2** by recrystallization from methanol in 75% overall yield (3 steps) from 4-bromo-3,5-dihydroxybenzoic acid (**4**) without the use of chromatography (Scheme 2). Similarly, we prepared amine **3** in five successive steps before purification by column chromatography in 40% overall yield (Scheme 3). Both compounds **2** and **3** have been synthesized in ~20 g scale.



Scheme 2 Synthesis of bisallyloxy-4-bromobenzaldehyde (**2**). *Reagents and conditions*: (i) allyl bromide K_2CO_3, DMF, rt, overnight; (ii) LAH, ether, 0 °C, 2 h; (iii) PCC, CH_2Cl_2, rt, 3 h. Overall yield: 75% from **4**.

With substantial amounts of **2** and **3** in hand, the successive steps in the synthesis of secramine A were based on small modifications

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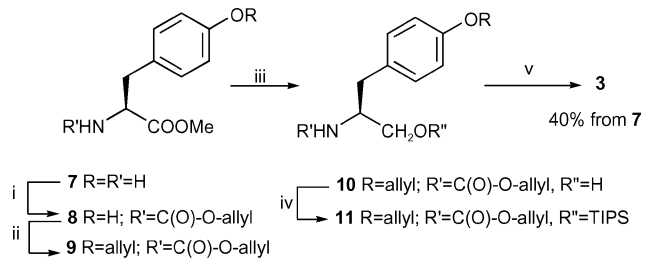
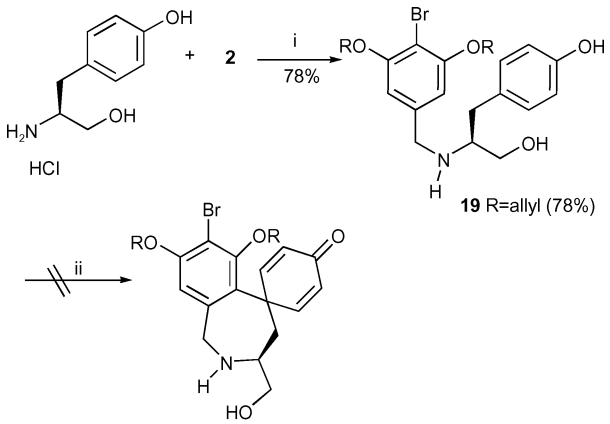


Scheme 3 Synthesis of 4-[(*S*)-2-amino-3-(triisopropylsilanoxyl)propyl]-phenol (**3**). *Reagents and conditions*: (i) allyl chloroformate, i-Pr_2NEt, THF–CH_2Cl_2 2 : 1, 0 °C, 2 h; (ii) allyl bromide, K_2CO_3, DMF, rt, overnight; (iii) LiCl, $NaBH_4$, THF–EtOH, rt, overnight; (iv) TIPSOTf, i-Pr_2NEt, CH_2Cl_2, 0 C, 2 h; (v) $Pd(PPh_3)_4$, morpholine, THF, 47 °C, overnight.

of the published procedure.[2] This new synthesis produced **1** (800 mg) in 9.0% overall yield from **4** as a 1 : 1.6 mixture of oxime isomers (Scheme 4).

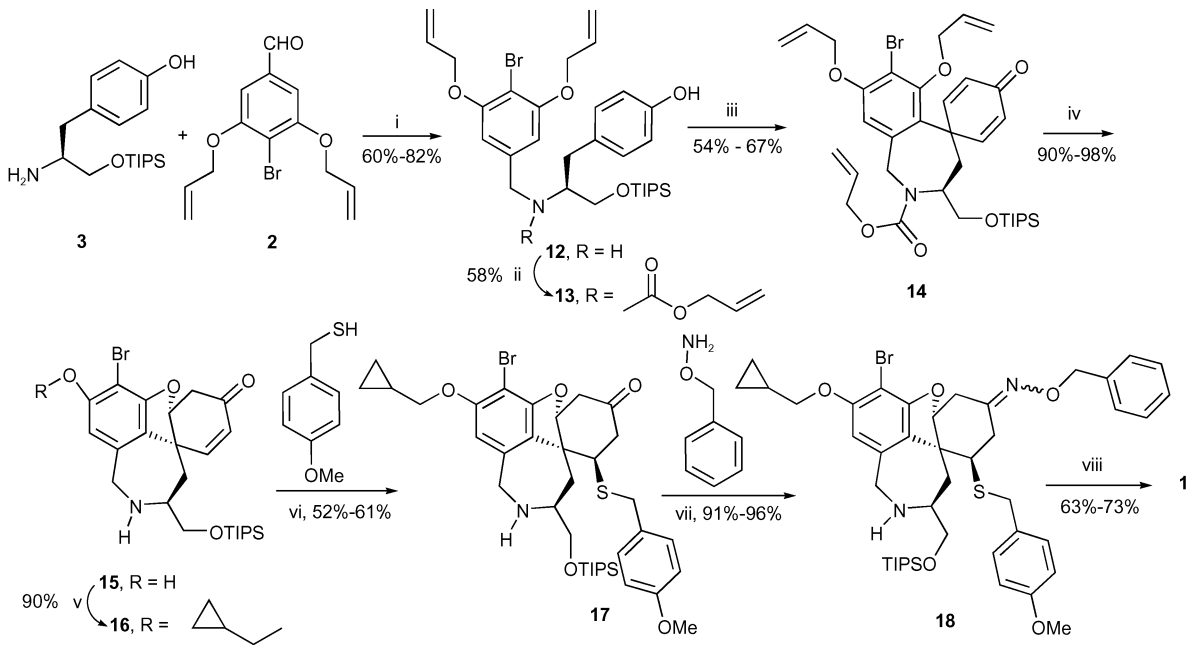
Having accomplished a practical synthesis of **1**, we explored other synthetic approaches that could bypass or reduce the number of protection–deprotection steps. Accordingly, we first investigated the use of commercially available D-tyrosinol. The reductive amination of tyrosinol with aldehyde **2** proceeded efficiently, but the resulting unprotected vicinal amino alcohol **19** did not undergo the desired oxidative intramolecular phenolic coupling and gave a complex mixture of compounds (Scheme 5). The amine and alcohol moieties of **19** likely act as nucleophiles (intra- or intermolecular) leading to a mixture of products. Also, these moieties may subsequently react with the presumed spirodienone intermediate, leading to undesired heterocyles.

The vicinal aminoalcohol group of **19** was then protected as an oxazolidine (compounds **20**–**22**, Scheme 6). In each case,

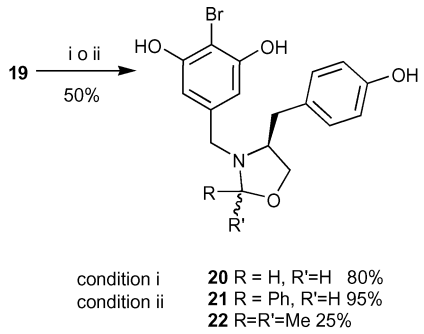


Scheme 5 Attempted oxidative intramolecular phenolic coupling of **19**. *Reagents and conditions*: (i) $NaBH_3CN$, MEOH, NEt_3, 0 °C to rt, overnight, (ii) $PhI(OAc)_2$, HFIPA, 0 °C, 3 h.

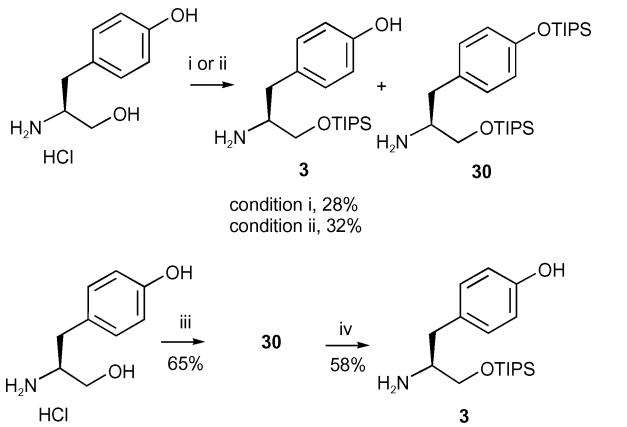
however, the oxidative intramolecular phenolic coupling reaction of compounds **20**, **21** and **22** still furnished intractable mixtures. In the original hypervalent iodine-mediated intramolecular phenolic coupling reaction, the amino group was protected as its corresponding amide or carbamate.[2,6] We therefore attempted to protect the vicinal amino alcohol as a cyclic carbamate. The reaction of diphenyl carbonate with amino alcohol **19** produced cyclic carbamate **23** in high yield (Scheme 7), and the next successive steps—oxidative intramolecular phenolic coupling reaction, deprotection, Mitsunobu and condensation reactions—proceeded efficiently to yield the cyclic carbamate protected secramine **28** (Scheme 7). However, deprotection of **28** using Katz's method (ethylenediamine–THF)[5] at rt failed. Increasing the temperature led to a complex reaction mixture. We then



Scheme 4 Synthesis of secramine A (**1**). *Reagents and conditions*: (i) $NaBH_3CN$, MeOH, AcOH, 0 °C to rt overnight; (ii) allyl chloroformate, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 8 h; (iii) $PhI(OAc)_2$, HFPIA, 0 °C, 3 h; (iv) $Pd(PPh_3)_4$, morpholine, THF, rt, overnight; (v) cyclopropyl methanol, PPh_3, DIAD or DEAD, THF, 0 °C, 2 h; (vi) 2,6-lutidine, THF, 0 °C for 15 min, then *n*-BuLi, 0 °C to rt, 1 h; (vii) 2,6-lutidine, CH_2Cl_2, rt, 1 h; (viii) HF–pyridine, THF, rt, 2 h.



Scheme 6 Protection of **19** as its oxazolidine. *Reagents and conditions*: for **20**–**21**: (i) RCHO, MS 4 Å, CH_2Cl_2, rt, overnight; for **22**: (ii) dimethoxypropane, reflux, 2 d.



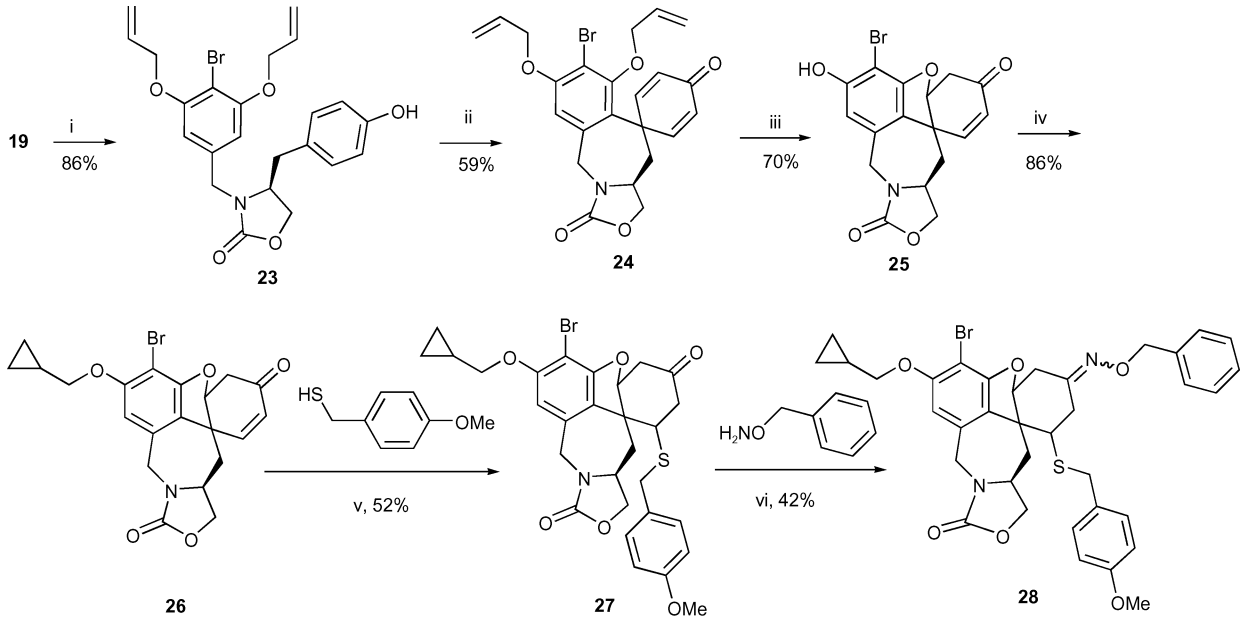
Scheme 8 Synthesis of **3** by a protection and deprotection sequence. *Reagents and conditions*: (i) TIPSOTf (1 eq.), Et_3N, CH_2Cl_2, 0 °C to rt, 24 h; (ii) TIPSOTf (2 eq.), Et_3N, CH_2Cl_2, 0 C to rt, 24 h; (iii) TIPSOTf (3 eq.), Et_3N, CH_2Cl_2, 0 °C to rt, 24 h; (iv) TBAF (1 eq.), −78 °C to rt, THF, 1 h.

opted for the deprotection of the cyclic carbamate at an earlier stage (using compounds **25**, **26** and **27**).[7] Katz's method[8] or more vigorous conditions (KOH–EtOH–reflux)[9] led to either recovery of starting material or complex reaction products and therefore we abandoned this strategy.

Our synthesis of TIPS-protected tyrosinol **3** (Scheme 3) needed five steps because of the number of protection–deprotection steps employed. If this number of steps could be reduced, then the synthesis of **1** would be greatly simplified. Collington and coworkers[10] have reported the selective deprotection of alcoholic and phenolic silyl ethers. Following their protocol, we protected both the alcohol and phenol using an excess of TIPSOTf in 55% yield (Scheme 8).

Selective deprotection conditions (1 eq. TBAF–THF) cleaved the TIPS group on the phenol group and compound **3** was the only product isolated. Its ^1H and ^{13}C data matched those recorded for the same compound using the sequence shown in Scheme 3.

Further proof of the success of this approach was an NMR experiment in which NaOH was added to a solution of **3** in $CDCl_3$. A significant upfield shift of the aromatic proton took place, indicating that the deprotection had taken place on the phenol TIPS group. Hence, using this strategy, the synthesis of **3** was carried out in one (Scheme 8, top) or two simple steps (Scheme 8, bottom). Following our synthesis of secramine A, we tested its biological activity. As expected, secramine A inhibited the Golgi apparatus to plasma membrane transport of $VSVG^{ts}$-EGFP in monkey kidney epithelial (BS–C-1) cells as previously described[3] (data not shown). We subsequently tested whether secramine A inhibited cell spreading. In culture, at a low density of plating, BS–C-1 cells spread out to a flattened, fried-egg shaped morphology within 35 min of plating (Fig. 1a, vehicle panels). This spreading is thought to be mediated by integrins, a family of transmembrane proteins that form contacts with the extracellular matrix



Scheme 7 The synthesis of **28**, a carbamate protected derivative of secramine A (**1**). *Reagents and conditions*: (i) $(PhO)_2CO$, CH_3CN, 60 °C, 18 h; (ii) $PhI(OAc)_2$, morpholine, THF, rt, overnight; (iv) cyclopropyl methanol, PPh_3, DIAD or DEAD, THF, 0 °C, 2 h; (v) 2,6-lutidine, THF, 0 °C for 15 min, then *n*-BuLi, 0 °C to rt, 1 h; (vi) 2,6-lutidine, CH_2Cl_2, rt, 1 h.

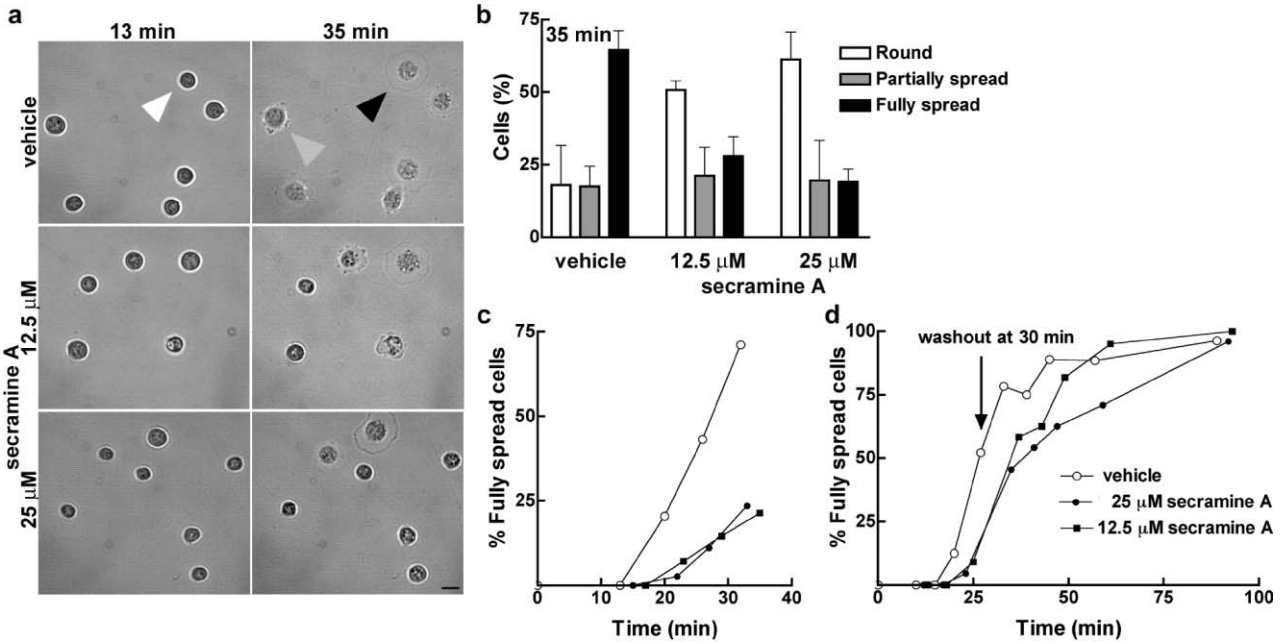


Fig. 1 Effect of secramine A on cell spreading. 1 h after trypsinization, BS–C-1 cells were mixed with vehicle (1% DMSO) or secramine A (**1**) and allowed to adhere and spread on plastic. The cells were visualized live by phase contrast microscopy (20× magnification) at regular intervals and scored as fully spread, partially spread, or round. (A) Representative images of the same cells at 13 and 35 min after deposition on plastic. Bar is 20 μm. The shapes of the cells were designated as previously described:[11] round indicates that they did no spread; partially spread indicates some thinning of the outer boundaries of the cells and fully spread corresponds to cells with a clear fried-egg shaped morphology. White arrowhead indicates a cell scored as round and the black arrowhead indicates the same cell scored as fully spread. The gray arrowhead indicates another cell that was scored as a partially spread cell. (B) Quantification of cell morphology at 35 min. Values are means ± SD from two independent experiments in which >20 cells were scored per condition. (C) Representative time course for cell spreading. (D) Representative time course for cell spreading upon washout of secramine A (**1**) at 30 min.

to regulate adhesion and subsequent morphological changes. Integrins were shown to mediate cell spreading through activation of the Rho GTPases Rac and Cdc42.[11] Rac and Cdc42 regulate the formation of distinct actin-dependent structures through the binding and hydrolysis of GTP. Introduction of an activated form of Cdc42 (a mutant unable to hydrolyze GTP) into cells stimulates the extension of long, thin membrane projections called filopodia.[12] In contrast, introduction of an activated form of Rac (a mutant unable to hydrolyze GTP) into cells stimulates the extension of thin, broad membrane sheets called lamelipodia and ruffles.[12] Overexpression of dominant negative mutants of either Cdc42 or Rac (mutants unable to bind to GTP) caused a marked inhibition of cell spreading.[11] Indeed, incubation with secramine A inhibited cell spreading. BS–C-1 cells were dispensed onto plastic surfaces, adhered and spread; secramine A inhibited this cell spreading process (see the Experimental section) (Fig. 1).

The cells were imaged periodically and designated as round, partially spread and fully spread as previously described[11] (Fig. 1a). As indicated in Fig. 1b, vehicle-treated cells fully spread to greater than 70% in 35 min, whereas greater than 50% remained round in the presence of secramine A. This cell spreading process was reversible upon washout of secramine A at 30 min (Fig. 1c, d).

Conclusions

In summary, we have developed a synthetic sequence using reaction conditions amenable to a scaled-up synthesis of secramine A, which can also be applied to other analogs. Furthermore, selective deprotection of the TIPS group further shortens the synthesis. Secramine A made with the improved synthesis is bioactive and was used to provide a complementary approach to corroborate the importance of Rho GTPase activation in cell spreading.

Experimental

General

NMR spectra were recorded on Varian Inova 500 (500 MHz) instruments. ^{1}H and ^{13}C spectra were recorded at 500 and 126 MHz, respectively, using $CDCl_3$ as a solvent. The chemical shifts are reported in δ (ppm) values relative to $CHCl_3$ (7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). Coupling constants are reported in hertz (Hz). All air and/or moisture sensitive reactions were carried out under an argon atmosphere. Dry solvents (tetrahydrofuran, ether, CH_2Cl_2 and DMF) were purchased from Aldrich and were additionally purified on PureSolv PS-400-4 purification system (Innovative Technology, Inc.). All other reagents and solvents were employed without further purification. The products were purified by Biotage FLASH+ system or Chromatotron (thin layer chromatograph system). TLC was developed on Merck silica gel 60 F254 aluminum sheets.

3,5-Bisallyloxy-4-bromobenzoic acid allyl ester (5). To a solution of 4-bromo-3,5-dihydroxybenzoic acid **4** (20.5 g, 87.5 mmol)

in DMF 120 mL at rt was added solid K_2CO_3 (60.5 g, 438 mmol) during stirring. To the suspension was added allylbromide (30 mL, 358 mmol) dropwise over 40 min, then the reaction mixture was stirred for another 18 h at rt, then the reaction mixture was poured into water (500 mL), after stirring for 30 min, the water layer was decanted and the solid was filtered and washed with water, then the residue solid was dissolved in ether (200 mL), dried over Na_2SO_4, condensation of the solvent gave the crude ester **5** (30 g). The crude ester was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (s, 2H), 6.12–5.98 (m, 3H), 5.51 (dd, J = 17.6, 1.6 Hz, 2H), 5.40 (dd, J = 17.2, 1.2 Hz, 2H), 5.32 (dd, J = 10.2, 1.2 Hz, 2H), 5.30 (dd, J = 17.6, 1.6 Hz, 2H), 4.81 (d, J = 6.0 Hz, 2H), 4.66 (d, J = 3.4 Hz, 4H).[13]

(3,5-Bisallyloxy-4-bromophenyl)methanol (6). Lithium aluminium hydride (7 g, 175 mmol) was added to anhydrous ether (150 mL) slowly, the mixture was cooled to 0 °C, under stirring the solution of the above crude ester **5** (30 g, *ca.* 87 mmol) which was dissolved in anhydrous THF (150 mL) was added slowly, after completion of addition, the reaction mixture was allowed to warm to rt, then the reaction mixture was stirred for 4 h at rt, then saturated aqueous potassium sodium tartrate was added dropwise until bubbling ceased (25 ml, over 30 min), then the mixture was stirred overnight, filtered, the solid was washed with ether (150 mL), the combined organic phase was washed with water and brine, dried over Na_2SO_4, distillation of the solvent in vacuum gave the crude alcohol **6** (26 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.65 (s, 2H), 6.10–6.00 (m, 2H), 5.48 (dd, J = 17.4, 1.6 Hz, 2H), 5.29 (dd, J = 10.6, 1.6 Hz, 2H), 4.62 (s, 2H), 4.60 (d, J = 5.2 Hz, 4H).[13]

Bisallyloxy-4-bromobenzaldehyde (2). To a suspension of pyridinium chlorochromate (34.7 g, 161 mmol) and celite (34.7 g) and sodium acetate hydrate (5.44 g, 40 mmol) in CH_2Cl_2 (300 mL) at 0 °C, the solution of crude alcohol **6** (26 g, *ca.* 87 mmol) in CH_2Cl_2 (120 mL) was added slowly. After completion, the reaction mixture was stirred for another 2 h at 0 °C. Then hexane (300 mL) was added, stirred for 30 min at rt, filtered, the solid was washed by CH_2Cl_2, the combined organic solution was distilled off, the residue was recrystallized from hot methanol (50 mL) to give 19.5 g pure aldehyde **2** (75%, 3 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.02 (s, 2H), 6.18–6.02 (m, 2H), 5.50 (dd, J = 17.4, 2.0 Hz, 2H), 5.33 (dd, J = 10.6, 1.2 Hz, 2H), 4.70 (ddd, J = 5.2, 1.6, 1.6 Hz, 4H).[13]

(*S*)-2-Allyoxycarbonylamino-3-(4-hydroxyphenyl)propionic acid methyl ester (8). To a solution of L-tyrosine methyl ester **7** (23.0 g, 99.3 mmol) in THF (100 mL) and CH_2Cl_2 (100 mL) at rt was added diisopropylethylamine (50 mL) during stirring. The solution was cooled to 0 °C (some solid formed when the solution was cooled to 0 °C) and allylchoroformate (11.97 g, 99.3 mmol), was added dropwise. The reaction mixture was stirred at 0 °C for 2 h, after that saturated ammonium chloride solution (100 mL) was added, then most solvent was removed under reduced pressure. The aqueous suspension was extracted by EtOAc (2 × 200 mL), the organic layer was combined and was washed first by water and then by brine, and then dried by Na_2SO_4, condensation of the solvent gave the crude product 30 g. The crude product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.95 ((d, J = 7.9 Hz, 2H), 6.71 (d, J = 8.0 Hz, 2H), 5.92–5.83 (m, 1H), 5.30–5.18 (m, 4H), 4.65–4.60 (m, 1H), 4.53 (d, J = 5.2 Hz, 2H), 3.71 (s, 3H), 3.04–3.00 (m, 2H).[13]

(*S*)-2-Allyoxycarbonylamino-3-(4-allyloxyphenyl)propionic acid methyl ester (9). To a solution of crude **8** (30.0 g, *ca.* 107 mmol) in DMF (170 mL) at rt was added solid K_2CO_3 (29.6 g, 214 mmol) then allylbromide (10.2 mL, 116 mmol) was added dropwise. The suspension was stirred overnight at rt; the reaction mixture was poured into water (500 mL) under stirring and then extracted with a mixture of EtOAc (300 mL) and hexane (100 mL). The organic layer was combined and washed first by water and then by brine, and then dried with Na_2SO_4. Condensation of the solvent gave the crude product 30 g. The crude product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.00 ((d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 5.99–5.86 (m, 1H), 5.85–5.80 (m, 1H), 5.44 (d, J = 8.0 Hz, 1H), 5.36 (dd, J = 17.4, 1.2 Hz, 1H), 4.56 (dd, J = 14.2, 6.0 Hz, 1H), 4.51 (d, J = 5.6, 2H), 4.45 (d, J = 5.2 Hz, 2H), 3.66 (s, 3H), 3.03 (dd, J = 14.4, 5.2 Hz, 1H), 2.96 (dd, J = 14.0, 6.4 Hz, 1H).[13]

[(*S*)-2-(4-Allyloxyphenyl)-1-hydroxymethylethyl]carbamic acid allyl ester (10). To a solution of crude **9** (30.0 g, *ca.* 94 mmol) in THF (170 mL) at rt was added solid LiCl (7.95 g, 94 mmol), sodium borohydride (7.18 g, 188 mmol) and then ethanol (200 mL). The suspension was stirred overnight at rt and then saturated ammonium chloride (100 mL) was added. Most of the solvent was removed in reduced pressure, the residue was extracted EtOAc (2 × 150 mL), the organic layer was combined and washed first by water and then by brine, and then dried by Na_2SO_4. Condensation of solvent gave the crude product (30 g). The crude product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.09–6.00 (m, 1H), 5.92–5.84 (m, 1H), 5.40 (dd, J = 15.6, 2.0 Hz, 1H), 5.28 (dd, J = 10.4, 1.2 Hz, 1H), 5.27 (dd, J = 17.2, 1.6 Hz, 1H), 5.20 (dd, J = 10.0, 1.6, 1H), 4.97 (d, J = 7.2 Hz, 1H), 4.54–4.50 (m, 4H), 3.89–3.86 (m, 1H), 3.68–3.65 (b, 1H), 3.57 (b, 1H), 2.80 (d, J = 7.2 Hz, 2H), 2.29 (br-s, 1H).[13]

[(*S*)-2-(4-Allyloxybenzyl)-2-(triisopropylsilanyl)ethyl]carbamic acid allyl ester (11). To a solution of crude **10** (7.07 g, *ca.* 24.3 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added diisopropylethylamine 9.43 g (29.2 mmol) and triisopropylsilyltriflate 8.94 g (29.2 mmol) dropwise with stirring. The reaction mixture was stirred at 0 °C for 2 h and then warmed to rt, then saturated ammonium chloride (80 mL) was added, the aqueous suspension was extracted by EtOAc (2 × 200 mL), the organic layer was combined and washed first by water and then by brine, and then dried by Na_2SO_4. Condensation gave crude **11** (11 g). The crude product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.11 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.0 Hz, 2H), 6.08–6.00 (m, 1H), 5.92–5.82 (m, 1H), 5.39 (dd, J = 17.2, 1.6 Hz, 1H), 5.27–5.22 (m, 2H), 5.17 (dd, J = 10.0, 0.8 Hz, 1H), 4.95 (d, J = 8.4 Hz, 1H), 4.52–4.48 (m, 4H), 3.80 (br s, 1H), 3.60 (d, J = 3.6 Hz, 2H), 2.81 (d, J = 7.2 Hz, 2H), 1.10–1.00 (m, 21H).[13]

4-[(*S*)-2-Amino-3-(triisopropylsilanoxyl)propyl]phenol (3). To a solution of crude **11** (10.8 g, 24.3 mmol) in THF 100 mL at rt was added morpholine 20 mL, then tetrakis(triphenylphosphine)palladium(0) (280 mg, 0.24 mmol) in the dark. The solution was warmed to 47 °C and then stirred overnight.

Condensation of the solvent gave the crude product. Purification by flash chromatography (30% EtOAc–hexane to 100% EtOAc) afforded **3** (3.05 g, 40% overall yield from **7**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01 (d, $J = 8.0$ Hz, 2H), 6.71 (d, $J = 8.0$ Hz, 2H), 4.50 (br s, 2H), 3.75 (dd, $J = 10.0$, 4.0 Hz, 1H), 3.61 (dd, $J = 10.0$, 7.0 Hz, 1H), 3.19 (br s, 1H), 2.79 (dd, $J = 13.3$, 6.0 Hz, 1H), 2.64 (dd, $J = 13.8$, 7.5 Hz, 1H), 1.14–1.01 (m, 21H).[13]

4-[(*S*)-2-(3,5-Bisallyloxy-4-bromobenzylamino)-3-(triisopropylsilanyloxy)propyl]phenol (12). A solution of **3** (3.49 g, 10.79 mmol) and aldehyde **2** (3.20 g, 10.79 mmol) was cooled to 0 °C, then acetic acid (7.5 mL) was added, then a solution of $NaBH_3CN$ 0.745 g (11.8 mmol, 1.1 eq.) in methanol (50 mL) was added dropwise. After addition, the reaction mixture warmed to rt and stirred overnight. Then Et_3N (10 mL) was added, most of the solvent was removed under reduced pressure and brine (100 mL) and saturated $NaHCO_3$ (30 mL) were added. The mixture was extracted by EtOAc (150 mL × 2). The organic layers were combined and dried over Na_2SO_4. Purification by flash chromatography (10–40% EtOAc–hexane) afforded **12** (5.29 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.98 (d, $J = 8.0$ Hz, 2H), 6.69 (d, $J = 8.0$ Hz, 2H), 6.45 (s, 2H), 6.09–5.99 (m, 2H), 5.46 (dd, $J = 17.3$, 1.5 Hz, 2H), 5.27 (dd, $J = 11.0$, 1.5 Hz, 2H), 4.49 (d, $J = 5.0$ Hz, 4H), 3.80 (d, $J = 13.0$ Hz, 1H), 3.75 (d, $J = 13.5$, 1H), 3.68–3.61 (m, 2H), 2.93–2.88 (m, 1H), 2.76–2.67 (m, 2H), 1.05–1.00 (m, 21H).[13]

(3,5-Bisallyloxy-4-bromobenzyl)-[(*S*)-2-(4-hydroxyphenyl)-1-(triisopropylsilanyloxymethyl)ethyl]carbamic acid allyl ester (13). To a solution of **12** (5.20 g, 8.62 mmol) in THF 150 mL at 0 °C was added 2,6-lutidine (1.38 g, 8.62 mmol), then allylchloroformate (1.25 g, 10.34 mmol) and warmed to rt. After 8 h, saturated aqueous NH_4Cl (100 mL) was added, THF was removed under reduced pressure, and the aqueous suspension was extracted with EtOAc. The organics were combined, dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (10–30% EtOAc in hexane) afforded **13** as a white solid (3.46 g, 58%); The rt ^{1}H NMR was complex due to presence of amide group, ^{1}H NMR (400 MHz, $(CD_3)_2SO$, 80 °C) δ 8.90 (s, 1H), 6.88 (d, $J = 8$, 2H), 6.639 (d, $J = 8.4$, 2H) 6.55 (s, 2H), 6.05–5.97 (m, 2H), 5.96–5.83 (m, 1H), 5.41 (dd, $J = 19.2$, 1.4 Hz, 2H), 5.24 (dd, $J = 11.2$, 1.4 Hz 2H), 5.22–5.13 (m, 2H), 4.52 (br s, 6H), 4.37 (d, $J = 16$ Hz, 1H), 4.23 (d, $J = 16.4$ Hz, 1H), 4.12 (br s, 1H), 3.70 (m, 2H), 2.77 (dd, $J = 18.4$, 13.6 Hz, 2H), 1.30–0.81 (m, 21H).[13]

Spiro-[5*H*-2-benzazepine-5,1′-[2,5]cyclohexadiene]-2(1*H*) carboxylic acid, 7-bromo-3,4-dihydro-3-(triisopropylsilanyloxymethyl)-4′-oxo-6,8-bis(2-propenyloxy)-, 2-propenyl ester [(3*S*)-14]. Compound **13** (3.46 g, 5.03 mmol) was dissolved in the HFIPA (12 mL) and cooled to 0 °C. Then $PhI(OAc)_2$ (1.95 g, 6.04 mmol) dissolved in CH_2Cl_2 (12 mL) was added and the reaction mixture was stirred for 3 h. Then the reaction was quenched by $NaHCO_3$ solution, extracted by EtOAc, and the organic layer was dried over Na_2SO_4. Flash chromatography (10–30% EtOAc in hexane) afforded **14** (2.32 g, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) At rt, 2 rotamers are present in a ratio of 1 : 1.1, δ 7.08–7.04 (m, 2H), 6.96–6.92 (m, 2H), 6.60 (s, 1H), 6.51 (s, 1H), 6.35–6.31 (m, 2H), 6.22–6.19 (m, 2H), 6.09–6.03 (m, 2H), 5.96–5.78 (m, 4H), 5.50 (dd, $J = 1.25$, 17.8 Hz, 2H), 5.35–5.08 (m, 10H), 4.90 (d, $J = 16.5$ Hz, 1H), 4.80 (d, $J = 22$ Hz, 1H), 4.74 (d, $J = 22$ Hz, 1H), 4.68–4.56 (m, 9H), 4.52–4.42 (m, 2H), 4.3–4.27 (m, 1H), 4.22–4.16 (m, 1H), 4.14–4.06 (m, 2H), 3.92 (dd, $J = 4$, 10 Hz, 1H) 3.83 (dd, $J = 4.75$, 10 Hz, 1H), 2.99 (dd, $J = 15$, 14 Hz, 1H), 2.86 (dd, $J = 13$, 15 Hz, 1H), 1.75 (dd, $J = 4.5$, 15 Hz), 1.70 (dd, $J = 4$, 15 Hz, 1H), 1.10–1.03 (m, 42H).[13]

6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-one, 3-bromo-4*a*,5,9,10,11,12-hexahydro-2-hydroxy-10-(triisopropylsilanyloxymethyl)-[(4*aR*,8*aR*,10*S*)-15]. To a solution of **14** (2.30 g, 3.35 mmol) in THF (50 mL) was added morpholine (2.92 g, 33.5 mmol) followed by solid $Pd(PPh_3)_4$ (116 mg, 0.1 mmol) and PPh_3 (26.4 mg, 0.01 mmol) at rt in the dark. After stirring overnight, the solution was concentrated under reduced pressure and purified by flash chromatography (10–100% EtOAc–hexane) to obtain **15** as a solid (1.723 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (d, $J = 15$ Hz, 1H), 6.33 (s, 1H), 6.0 (d, $J = 15$ Hz, 1H), 4.85 (br s, 1H), 4.04 (d, $J = 15$ Hz, 1H), 3.96 (d, $J = 15$ Hz, 1H), 3.70 (dd, $J = 10$, 5 Hz, 1H), 3.62 (dd, $J = 10$, 10 Hz, 1H), 3.3–3.2 (m, 1H), 3.15 (dd, $J = 17.5$, 2.5 Hz, 1H), 2.75 (dd, $J = 17.5$, 2.5 Hz, 1H), 1.95–1.9 (m, 2H), 1.20–1.00 (m, 21H).[13]

6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-one, 3-bromo-2-(cyclopropylmethoxy)-4*a*,5,9,10,11,12-hexahydro-10-(triisopropysilanyloxymethyl)- [(4*aR*,8*aR*,10*S*)-16]. To a solution of **15** (1.72 g, 3.30 mmol) in THF (30 mL) was added PPh_3 (1.041 g, 3.96 mmol) and cyclopropylmethylalcohol (0.356 g, 4.95 mmol). The solution was cooled to 0 °C and DIAD (0.866 g, 4.29 mmol) was added with stirring. After 2 h, the solution was concentrated under reduced pressure and purified by flash chromatography (0–50% EtOAc–hexane) to obtain **16** (1.85 g, 97%). $R_f = 0.29$ (50% EtOAc–hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.84 (d, $J = 10.4$ Hz, 1H), 6.24 (s, 1H), 6.0 (d, 10.4 Hz, 1H), 4.74 (br s, 1H), 4.04 (d, $J = 15.6$ Hz, 1H), 3.98 (d, $J = 16.6$ Hz, 1H), 3.84 (dd, $J = 6.4$, 10 Hz, 1H), 3.78 (dd, $J = 6.8$, 6.6 Hz), 3.67–3.57 (m, 2H), 3.22–3.16 (m, 1H), 3.14 (d, $J = 16$ Hz, 1H), 2.72 (dd, $J = 3.4$, 17.8 Hz, 1H), 2.01 (d, $J = 12.8$ Hz, 1H), 1.69 (dd, $J = 12.4$, 12.4 Hz, 1H), 1.2–1.0 (m, 22H), 0.62–0.57 (m, 2H), 0.36–0.33 (m, 1H).[13]

6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-one, 3-bromo-2-(cyclopropylmethoxy)-4*a*,5,7,8,9,10,11,12-octahydro-10-(triisopropylsilanyloxymethyl)-8-[[(4-methoxyphenyl)methyl]thio]- [(4*aR*,8*aR*,10*S*)-17]. To a solution of **16** (1.85 g, 3.21 mmol) in THF (30 mL) at 0 °C was added 4-methoxybenzyl mercaptan (0.643 g, 4.17 mmol) and 2,6-lutidine (0.446 g, 4.17 mmol). After 15 min, *n*-BuLi (0.05 mL of 2.5 M solution in hexane, 0.125 mmol) was added and the solution was warmed to rt. After 1 h, anhydrous acetic acid (0.05 mL, 0.87 mmol) was added, the solution was concentrated under reduced pressure, and purified by flash chromatography (0–40% EtOAc–hexane) to obtain compound **17** (1.20 g, 52%). $R_f = 0.5$ (50% EtOAc–hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, $J = 8.5$ Hz, 2H), 6.84 (d, $J = 8.5$ Hz, 2H), 6.18 (s, 1H), 4.71 (dd, $J = 2.75$, 2.75 Hz, 1H), 3.84 (dd, $J = 6.5$, 9.5 Hz, 1H), 3.80 (s, 3H), 3.79 (dd, $J = 6.5$, 9.5 Hz, 1H), 3.74 (d, $J = 13.7$ Hz, 1H), 3.73–3.70 (m, 1H), 3.60 (d, $J = 13.7$ Hz, 1H), 3.52 (dd, $J = 8.5$, 8.5 Hz, 1H), 3.37 (d, $J = 15$ Hz, 1H), 3.14–3.08 (m, 2H), 3.05 (dd, $J = 2.75$, 18.3 Hz, 1H), 2.97 (dd, $J = 2.75$, 18.3 Hz, 1H), 2.61 (dd, $J = 4$, 17 Hz, 1H), 2.40 (dd, $J = 3$, 17), 2.08 (d, $J = 13$ Hz, 1H), 1.46 (dd, $J = 17$, 17 Hz), 1.30–1.26 (m, 1H), 1.16–1.07 (m, 21H), 0.64–0.61 (m, 2H), 0.38–0.34 (m, 2H).[13]

6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-one, 3-bromo-2-(cyclopropylmethoxy)-4*a*,5,7,8,9,10,11,12-octahydro-10-(triisopropysilanyloxymethyl)-8-[[(4-methoxyphenyl)methyl]thio]-, *O*-(phenylmethyl)oxime [(4*aR*,8*aR*,10*S*)-18]. To a solution of compound **17** (1.20 g, 1.64 mmol) in CH_2Cl_2 (2 mL) was added *O*-benzylhydroxylamine hydrochloride (392 mg, 2.46 mmol) and 2,6-lutidine (350 mg, 3.28 mmol) in CH_2Cl_2 (10 mL) at rt. After 10 min, anhydrous acetic acid (0.12 mL, 2.1 mmol) was added. After 1 h, the solution was concentrated under reduced pressure and immediately purified by flash chromatography (5–30% EtOAc–hexane) to obtain **18** as a 1.6 : 1 mixture of isomers (1.32 g, 96%) as a white solid. (0–2% MeOH–CH_2Cl_2). $R_f = 0.5$ (66% EtOAc–hexane). The isomer mixture can be isolated by careful chromatograph separation if a single isomer of secramine A is needed, this separation should be done before TIPS removal. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, $J = 7.5$ Hz, 2H), 7.36 (dd, $J = 7.7$, 7.7 Hz, 2H), 7.30 (d, $J = 6.5$ Hz), 7.17 (d, $J = 8.5$ Hz, 2H), 6.81 (d, $J = 9$ Hz, 2H), 6.16 (s, 1H), 5.20 (d, $J = 13$ Hz, 1H), 5.15 (d, $J = 12.5$ Hz, 1H), 4.57 (dd, $J = 2.5$, 2.5 Hz, 1H), 3.83 (dd, $J = 6.75$, 9.75 Hz, 1H), 3.79 (s, 3H), 3.78 (dd, $J = 6.75$, 9.75 Hz, 1H), 3.71 (br s, 1H), 3.63 (d, $J = 13$ Hz, 1H), 3.53 (d, $J = 14$ Hz, 1H), 3.51 (dd, $J = 2.5$, 18.2 Hz, 1H), 3.44 (d, $J = 15$ Hz, 1H), 3.23 (br s, 1H), 3.02 (br s, 1H), 2.80 (dd, $J = 2.5$, 18 Hz), 2.55 (dd, $J = 3.75$, 11.25 Hz, 1H), 2.34 (d, $J = 10$ Hz, 1H), 1.97 (d, $J = 13$ Hz, 1H), 1.29–1.25 (m, 2H), 1.16–1.07 (m, 21H), 0.63–0.61 (m, 2H), 0.37–0.34 (m, 2H).[13]

6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-one, 3-bromo-2-(cyclopropylmethoxy)-4*a*,5,7,8,9,10,11,12-octahydro-10-(hydroxymethyl)-8-[[(4-methoxyphenyl)methyl]thio], *O*-(phenylmethyl)oxime, (4*aR*,8*aR*,10*S*)-secramine A (1). To a solution of **18** (1.32 g, 1.58 mmol, 1 eq.) in THF (10 mL) in a high density polyethylene vial was added HF–pyridine (3 mL) slowly with stirring. After 2 h, the solution was concentrated under reduced pressure and purified by flash chromatography (0–5% MeOH–CH_2Cl_2) to obtain **1** 0.79 g (yield 73%, 1.6 : 1 mixture of isomers) white solid. $R_f = 0.24$ (5% MeOH–CH_2Cl_2). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) major isomer: δ 7.39–7.29 (m, 5H), 7.19 (d, $J = 8.5$ Hz, 2H) 6.84 (d, $J = 8.5$ Hz, 2H), 6.38 (s, 1H), 5.11 (d, $J = 12.5$ Hz, 1H), 5.08 (d, $J = 12.5$ Hz, 1H), 4.60 (dd, $J = 3$, 3 Hz, 1H), 4.54 (br s, 1H), 3.85 (dd, $J = 7$, 6.5 Hz, 1H), 3.79 (dd, $J = 7.5$, 6.5 Hz, 1H), 3.72 (s, 3H), 3.89 (d, $J = 12.5$ Hz, 1H), 3.68 (d, $J = 16$ Hz, 1H), 3.57 (d, $J = 13$ Hz, 1H), 3.37–3.24 (m, 3H), 3.14–3.10 (m, 1H), 3.07 (br s, 1H), 2.99–2.93 (m, 1H), 2.63 (d, $J = 19$ Hz, 1H), 2.07 (d, $J = 15.5$ Hz, 1H), 1.90 (d, $J = 13.5$ Hz), 1.27–1.16 (m, 2H), 0.56–0.52 (m, 2H), 0.32–0.28 (m, 2H).[13]

Synthesis of 4-[(*S*)-2-amino-3-(triisopropylsilanoxyl)propyl]-phenol (3) through direct protection. To a solution of tyrosinol hydrochloride (206 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TIPSOTf (612 mg, 2.0 mmol), Et_3N (253 mg, 3.5 mmol), then the solution was warmed to rt and then stirred for 24 h. The reaction mixture was quenched by sat. NH_4Cl solution and extracted by ethyl acetate, the organic layer was dried over Na_2SO_4. Condensation of the solvent gave the crude product. Purification by flash chromatography (30% EtOAc–hexane to 100% EtOAc) afforded **3** (103 mg, 32%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01 (d, $J = 8.0$ Hz, 2H), 6.71 (d, $J = 8.5$ Hz, 2H), 4.50 (br s, 2H), 3.75 (dd, $J = 10.0$, 3.5 Hz, 1H), 3.61 (dd, $J = 9.5$, 6.5 Hz, 1H), 3.19 (br s, 1H), 2.79 (dd, $J = 13.3$, 6.0 Hz, 1H), 2.64 (dd, $J = 13.8$, 7.5 Hz, 1H), 1.05 (m, 21H).

(*S*)-1-(Triisopropylsilyloxy)-3-(4-(triisopropylsilyloxy)phenyl)-propan-2-amine (30). To a solution of tyrosinol hydrochloride (206 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TIPSOTf (918 mg, 3.0 mmol), Et_3N (505 mg, 5.0 mmol), the solution was warmed to rt and then stirred for 24 h. The reaction mixture was quenched by sat. NH_4Cl solution and extracted by ethyl acetate, the organic layer was dried over Na_2SO_4, condensation of the solvent gave the crude product. Purification by flash chromatography (10–50% EtOAc in hexane) afforded **30** (311 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.05 ((d, $J = 8.0$ Hz, 2H), 6.81 (d, $J = 8.0$ Hz, 2H), 3.66–3.64 (m, 2H), 3.52–3.49 (m, 2H), 3.08–3.05 (m, 1H), 2.74–2.70 (m, 1H), 2.52–2.48 (m, 1H), 1.54 (br s, 2H), 1.28–1.22 (m, 6H), 1.11–1.02 (m, 36H).

4-[(*S*)-2-Amino-3-(triisopropylsilanoxyl)propyl]phenol (3) (through selective deprotection of 30)

To a solution of **30** (239 mg, 0.5 mmol) in THF (3 mL) at −78 °C was added TBAF (1 M solution in THF, 0.5 mL, 0.5 mmol) the solution was warmed to 0 °C slowly, then reaction mixture was quenched by sat. NH_4Cl solution and extracted by ethyl actate, the organic layer was dried over Na_2SO_4. Condensation of the solvent gave the crude product. Purification by flash chromatography (30% EtOAc–hexane to 100% EtOAc) afforded **3** (93 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01 (d, $J = 8.0$ Hz, 2H), 6.71 (d, $J = 8.5$ Hz, 2H), 4.50 (br s, 2H), 3.75 (dd, $J = 10.0$, 3.5 Hz, 1H), 3.61 (dd, $J = 9.5$, 6.5 Hz, 1H), 3.19 (br s, 1H), 2.79 (dd, $J = 13.3$, 6.0 Hz, 1H), 2.64 (dd, $J = 13.8$, 7.5 Hz, 1H), 1.05 (m, 21H).

(*S*)-4-(2-(3,5-bis(allyloxy)-4-bromobenzylamino)-3-hydroxypropyl)phenol (19). A solution of tyrosinol hydrochloride (814 mg, 4.0 mmol) and aldehyde **2** (1.32 g, 4.45 mmol) in methanol (20 mL) was cooled to 0 °C, then a solution of $NaBH_3CN$ (315 mg, 5.0 mmol) in methanol (20 mL) was added dropwise. After addition, the reaction mixture warmed to rt and stirred overnight. Then Et_3N (5 ml) was added, most of the solvent was removed under reduced pressure and brine (100 mL) and saturated $NaHCO_3$ (30 mL) were added. The mixture was extracted by EtOAc (50 mL × 2). The organic layers were combined and dried over Na_2SO_4. Purification by flash chromatography (50%–100% EtOAc in hexane) afforded **19** (1.40 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.98 (d, $J = 8.4$ Hz, 2H), 6.74 (d, $J = 8.4$ Hz, 2H), 6.41 (s, 2H), 6.11–5.99 (m, 2H), 5.52–5.44 (m, 2H), 5.31–5.27 (m, 2H), 4.54 (td, $J = 4.8$, 1.6 Hz, 4H), 3.71 (s, 2H), 3.41–3.36 (m, 1H), 2.91–2.89 (m, 2H), 2.73–2.68 (m, 2H).

(*S*)-4-((3-(3,5-bis(allyloxy)-4-bromobenzyl)-2,2-dimethyloxazolidin-4-yl)methyl)phenol (22). A solution of compound **19** (700 mg, 1.56 mmol), TsOH monohydrate (12 mg, 0.06 mmol) and 2,2-dimethoxypropane (2.5 mL) was refluxed for 2 d, then saturated $NaHCO_3$ (30 mL) were added to quench the reaction. The mixture was extracted by EtOAc (50 mL × 2). The organic layers were combined, washed by brine and dried over Na_2SO_4. Purification by flash chromatography (10–50% EtOAc in hexane) afforded **22** (192 mg, 25%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.97 (d, $J = 8.4$ Hz, 2H), 6.69 (d, $J = 8.4$ Hz, 2H), 6.43 (s, 2H), 6.10–5.97 (m, 2H), 527 (dq, $J = 10.4$, 1.5 Hz, 4H), 4.52–4.49 (m, 4H), 3.77 (s, 2H), 3.42–3.36 (m, 2H), 3.16 (s, 2H), 2.94–2.91 (m, 1H), 2.70

(d, $J = 6.8$ Hz, 2H), 1.32 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 24.4, 37.3, 48.5, 51.4, 57.9, 62.4, 69.7, 100.1, 106.0, 115.4, 117.5, 130.3, 132.7, 140.7, 154.6, 156.1.

(*S*)-3-(3,5-bis(allyloxy)-4-bromobenzyl)-4-(4-hydroxybenzyl)-oxazolidin-2-one (23). A solution of compound **19** (1.0 g, 2.24 mmol), Diphenyl carbonate (1.2 g, 5.6 mmol) and acetonitrile (10 mL) was refluxed for 18 h, then saturated $NaHCO_3$ (30 mL) was added to quench the reaction. The mixture was extracted with EtOAc (50 mL × 2). The organic layers were combined was washed by brine and dried over Na_2SO_4. Purification by flash chromatography (10–50% EtOAc in hexane) afforded **23** (0.91 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.90 ((d, $J = 8.5$ Hz, 2H), 6.78 (d, $J = 8.4$ Hz, 2H), 6.39 (s, 2H), 6.09–5.99 (m, 2H), 5.47 (ddd, $J = 17.3$, 1.7, 1.5 Hz, 2H), 5.29 (ddd, $J = 10.5$, 2.4, 1.5 Hz, 1H), 4.70 (d, $J = 15.2$ Hz, 1H), 4.59–4.56 (m, 4H), 4.19–4.09 (m, 2H), 4.03–3.97 (m, 2H), 3.81–3.75 (m, 1H), 2.96 (dd, $J = 13.8$, 5.2 Hz, 1H), 2.60 (dd, $J = 13.8$, 8.1 Hz, 1H).

Synthesis of intermediates 24 to 28

Compound **23** (0.620 mg, 1.31 mmol) was dissolved in the HFIPA (4 mL) and cooled to 0 °C. Then a solution of $PhI(OAc)_2$ (505 mg, 1.57 mmol), dissolved in CH_2Cl_2 (12 mL) was added at 0 °C and the reaction mixture was stirred for 3 h at same temperature. Then the reaction was quenched by $NaHCO_3$ solution, extracted by EtOAc, and organic layer was dried over Na_2SO_4. Flash chromatography (10–50% EtOAc in hexane) afforded compound **24** as colorless oil (400 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–7.36 (m, 2H), 6.73 (s, 1H), 6.41–6.37 (m, 1H), 6.17–6.13 (m, 1H), 6.10–6.04 (m, 1H), 6.01–5.85 (m, 1H), 5.53–5.47 (m, 1H), 5.36–5.32 (m, 1H), 5.29–5.21 (m, 3H), 4.82 (d, $J = 16.0$ Hz, 1H), 4.66–4.62 (m, 2H), 4.57–4.42 (m, 2H), 4.26–4.23 (m, 1H), 4.13–4.05 (m, 2H), 3.91–3.84 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 185.0, 157.0, 156.1, 155.4, 147.7, 136.7, 131.8, 131.7, 128.8, 122.1, 119.0, 118.2, 112.6, 109.0, 74.5, 69.8, 67.3, 55.2, 48.5, 47.3, 46.6

25: To a solution of **24** (127 mg, 0.27 mmol) in THF (3 mL) was added morpholine (234 mg, 2.7 mmol), followed by solid $Pd(PPh_3)_4$ (2.3 mg, 0.018 mmol) and PPh_3 (26.4mg, 0.01 mmol) at rt in the dark. After stirring overnight, the solution was concentrated under reduced pressure and purified by flash chromatography (10–50% EtOAc–hexane) to obtain compound **25** as a solid (90 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (dd, $J =$ 10.3, 1.9 Hz, 1H), 6.11 (d, $J = 10.3$ Hz, 1H), 4.88 (d, $J = 16.4$ Hz, 1H), 4.84–4.82 (m, 1H), 4.58–4.52 (m, 1H), 4.33–4.28 (m, 2H), 4.00–3.96 (m, 1H), 3.75–3.68 (m, 1H), 3.22 (d, $J = 17.8$ Hz, 1 H), 2.76 (dd, $J = 3.7$ Hz, 17.9 Hz, 1H), 2.19–2.16 (m, 2H).

26: To a solution of **25** (70 mg, 0.179 mmol) in THF (2.0 mL) was added PPh_3 (56 mg, 0.215 mmol) and cyclopropylmethylalcohol (17 mg, 0.233 mmol). The solution was cooled to 0 °C and DIAD (38.5 mg, 0.179 mmol) was added with stirring. After 2 h, the solution was concentrated under reduced pressure and purified by flash chromatography (10–50% EtOAc–hexane) to obtain **26** (68.5 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (dd, $J = 10.4$, 2.0 Hz, 1H), 6.37 (s, 1H), 6.04 (d, $J = 10.4$ Hz, 1H), 4.85 (d, $J =$ 16.4 Hz, 1H), 4.51 (t, $J = 8.5$ Hz, 1H), 4.36–4.29 (m, 2H), 3.96–3.92 (m, 1H), 3.85 (dd, $J = 6.8$, 1.3 Hz, 2H), 3.18 (d, $J = 18.0$ Hz, 1H), 2.76 (dd, $J = 18.0$ Hz, 1H), 2.06–2.22 (m, 1H), 0.66–0.60 (m, 2H), 0.38–0.34 (m, 2H).

27: To a solution of **26** (60 mg, 0.134 mmol, 1 eq.) in THF (2 mL) at 0 °C was added 4-methoxybenzyl mercaptan (27 mg, 0.174 mmol) and 2,6-lutidine (18 mg, 0.174 mmol). After 15 min, *n*-BuLi (0.005 mL of 2.5M solution in hexane, 0.008 mmol) was added and the solution was warmed to rt. After 1 h, anhydrous acetic acid (0.01 mL) was added, the solution was concentrated under reduced pressure, and purified by flash chromatography (10–40% EtOAc–hexane) to obtain compound **27** (30 mg, 52%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, $J = 8.6$ Hz, 2H), 6.89 (d, $J = 8.6$ Hz, 2H), 6.30 (s, 1H), 4.72 (t, $J = 2.7$ Hz, 1H), 4.46 (d, $J = 15.8$ Hz, 1H), 4.34 (t, $J = 7.8$ Hz, 1H), 3.85–3.75 (m, H), 3.55 (d, $J = 13.8$ Hz, 1H), 3.00 (d, $J = 2.8$ Hz, 2H), 2.65 (dd, $J = 17.2$, 3.5 Hz, 1H), 2.53 (s, 1H), 2.38 (dd, $J = 17.2$, 2.9 Hz, 1H), 2.24 (dd, $J = 1.8$, 13.3 Hz, 1H), 1.75–1.85 (m, 1H), 0.67–0.60 (m, 2H), 0.39–0.34 (m, 2H).

28: To a solution of **27** (10 mg, 0.0167 mmol) in CH_2Cl_2 (1 mL) was added *O*-benzylhydroxylamine hydrochloride (5.0 mg, 0.03 mmol) and 2,6-lutidine (3.2 mg, 0.03 mmol) in CH_2Cl_2 (10 mL) at rt. After 10 min, anhydrous acetic acid (0.12 mL, 2.1 mmol, 5 eq.) was added. After 1 h, the solution was concentrated under reduced pressure and immediately purified by flash chromatography (5–30% EtOAc–hexane) to obtain compound **28** as a 1.6 : 1 mixture of isomers (5 mg, 42%) as a white solid. (0–2% MeOH–CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.33 (m, 5H), 7.15 (d, $J = 8.6$ Hz, 2H), 6.85 (d, $J = 8.6$ Hz, 2H), 6.27 (s, 1H), 5.20–5.12 (m, 1H), 4.57–4.49 (m, 1H), 4.46 (d, $J = 15.8$ Hz, 1H), 4.37 (t, $J = 8.4$ Hz, 1H), 4.40–4.34 (m, 1H), 3.80 (s, 3H), 3.72–3.50 (m, 1H).

Procedure for cell spreading assay

Secramine A was stored as 20 mM aliquots at −20 °C. Confluent BS-C-1 cells were trypsinized, washed, and resuspended in culture media containing 10% heat-inactivated FBS at 37 °C. After 1 h, the cells were pelleted and resuspended in DMEMα with 2% Nu-serum (BD Biosciences) at 37 °C. The cells were mixed 1 : 1 in the same media containing 2× vehicle (2% DMSO) or 2× secramine A (50 μM or 25 μM) an plated in 6-well plates with transparent bottom at low density (~100 000 cells per well). For washout experiments, the imaging media was diluted ~3-fold with DMEMα containing 10% FBS and exchanged with DMEMα containing 10% FBS. Live cell imaging was done using phase contrast microscopy at 20× magnification starting 5 min after plating. >20 cells per condition were imaged for each subsequent time point using a computer-control inverted Zeiss 200M microscope under control of Slidebook (Intelligent Imaging Innovations, Inc.). Experiments were performed in duplicate and scoring was not carried out in a blinded fashion.

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