Use of Biomimetic Diversity-Oriented Synthesis to Discover Galanthamine-Like Molecules with Biological Properties beyond Those of the Natural Product

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Natural products are central to biology and medicine, serving as pharmaceutical leads, drugs,[4] and powerful reagents for studying cell biology.[5] To date, libraries based upon natural products have been synthesized primarily for the purpose of improving the known biological and pharmacokinetic properties of the parent natural products.[6,7] In contrast, we have used diversity-oriented synthesis to construct a library based on a natural product, galanthamine (**2**, Figure 1), with the goal of discovering molecules that exhibit biological effects beyond those previously associated with the natural product. Although **2** is a potent acetylcholinesterase inhibitor,[8] our aim was not to improve this activity. Galanthamine was selected because it offered a range of functionality for diversity-generating reactions, it presented a rigid polycyclic core that might lower the potential entropy penalty associated with protein binding, and it allowed for the use of powerful biomimetic reactions in the synthesis.[9] In this communication, we report a biomimetic solid-phase synthesis of 2527 molecules based on the alkaloid natural product galanthamine and the identification of a molecule from the library that perturbs the secretory pathway in mammalian cells[10]—a process unrelated to the acetylcholinesterase inhibitory activity of **2**.

Our library synthesis strategy took advantage of efficient biomimetic reactions (**3** → **4**),[11] paralleling the biosynthesis of the natural product (**1** → **2**).[12] Following biomimetic solid-phase synthesis of the core structure **4**, four diversity-generating reactions were performed to complete the library synthesis (Figure 1).

The library synthesis commenced with attachment of a tyrosine derivative to 500−600μm high capacity (1.43 mmol/g) polystyrene beads through a Si−O bond to generate **5** upon deprotection (Scheme 1).[13] Reductive amination[14] and protecting-group adjustments produced **7**. Exposure of **7** to $PhI(OAc)_2$[15] afforded **8** which was then converted to **9** via Pd-mediated deprotection and spontaneous cyclization. For the library synthesis, building blocks were selected that reacted in >80% yield and as a group possessed diverse physical characteristics. (Figure 2). The first diversity step was accomplished by coupling the phenol of **9** with five primary alcohols to afford **10** (Scheme 1).[16] Treatment of **10** with thiols in the presence of ^{n}BuLi afforded **11** as a single diastereomer.[17] The nitrogen of **11** was either acylated or alkylated, providing compounds that would be neutral or positively charged, respectively, at physiological pH. The last diversification step involved treatment of **12** with hydrazines and hydroxylamines, generating **13**.[18]

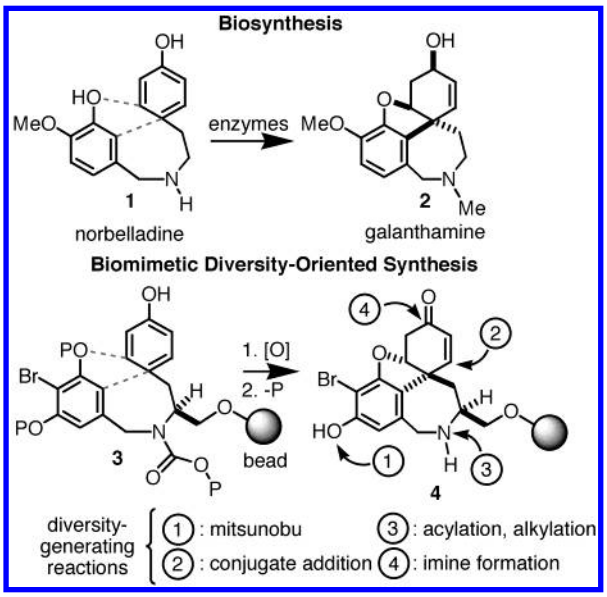

Figure 1. Biomimetic diversity-oriented synthesis parallels the biosynthesis of galanthamine.

The library was prepared as a single copy (1 bead per library member), arrayed in 384-well plates (1 bead per well), and detached from the solid-support with HF-pyridine (**13** → **14**).

Following completion of the synthesis, the presence of 2527 out of 2946 (86%) potential compounds was confirmed by mass spectrometry.[19] Evaporation of the cleavage reaction solution and resuspension in 7 μL of DMSO afforded 2527 stock solutions for biological screening.

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(16) "Skip codon" (Figure 2) refers to the absence of a building block which renders the core structure functionality a diversity element.
(17) Observed by NMR and correlated with molecular modeling. See Supporting Information.
(18) All combinations of **11** were synthesized in parallel for quality control. Following a pool step, R_3 and R_4 building blocks were incorporated in parallel to generate **13**. See Supporting Information.

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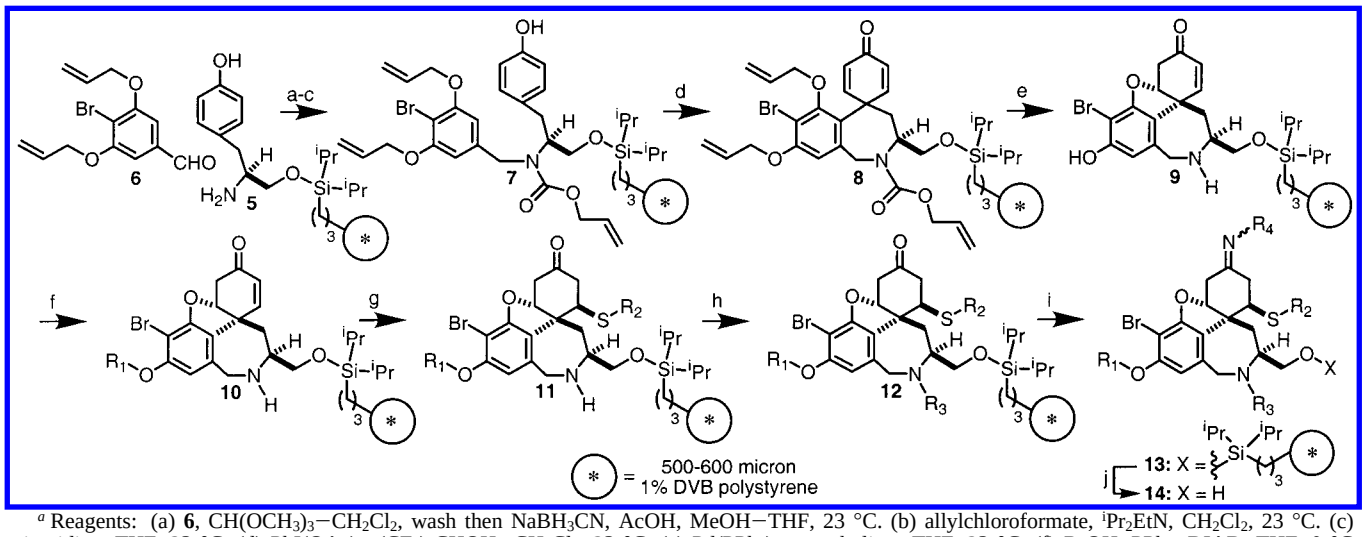
Scheme 1[a]



[a] Reagents: (a) **6**, $CH(OCH_3)_3-CH_2Cl_2$, wash then $NaBH_3CN$, AcOH, MeOH−THF, 23 °C. (b) allylchloroformate, $^iPr_2EtN$, CH_2Cl_2, 23 °C. (c) piperidine, THF, 23 °C. (d) $PhI(OAc)_2$, $(CF_3)_2CHOH-CH_2Cl_2$, 23 °C. (e) $Pd(PPh_3)_4$, morpholine−THF, 23 °C. (f) R_1OH, PPh_3, DIAD, THF, 0 °C (2×). (g) R_2SH, 2,6-lutidine, ^nBuLi, THF 0 → 40 °C. (h) R_3CHO, AcOH, MeOH−THF, then $NaBH_3CN$ in MeOH, 23 °C or R_3COCl, 2,6-lutidine, CH_2Cl_2, 23 °C or R_3NCO, CH_2Cl_2, 23 °C, (i) R_4NH_2, AcOH, MeOH−CH_2Cl_2, 23 °C. (j) HF−pyridine, THF, 23 °C then TMSOMe.

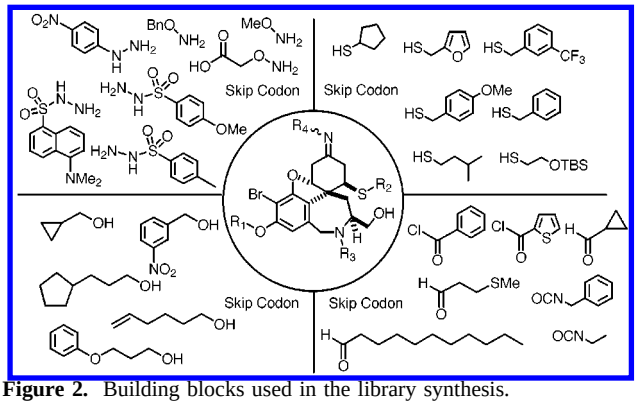


Figure 2. Building blocks used in the library synthesis.

Natural products, such as brefeldin A,[20] are powerful reagents for studying the secretory pathway, a process in cells that shuttles proteins from the endoplasmic reticulum (ER) to the plasma membrane via the Golgi apparatus (GA). In an effort to expand the limited pool of molecules that perturb this process,[21] our library was screened using a cell-based, phenotypic assay. The fluorescent fusion protein VSVG-GFP was used to monitor the ability of individual library members to block protein trafficking (Figure 3).[22] Screening at ∼750 nM identified **15** as a potent inhibitor of VSVG-GFP movement from the GA to the plasma membrane. In analogy to the yeast *sec* mutants[23] and as a result of its secondary amine and the phenotype it induces, we have named this compound secramine. Cell-based experiments with secramine confirmed its ability to block protein trafficking from the GA to the plasma membrane at 2 μM.[24] Notably, galanthamine (**2**) had no observable effect on the secretory pathway at up to 100 μM.

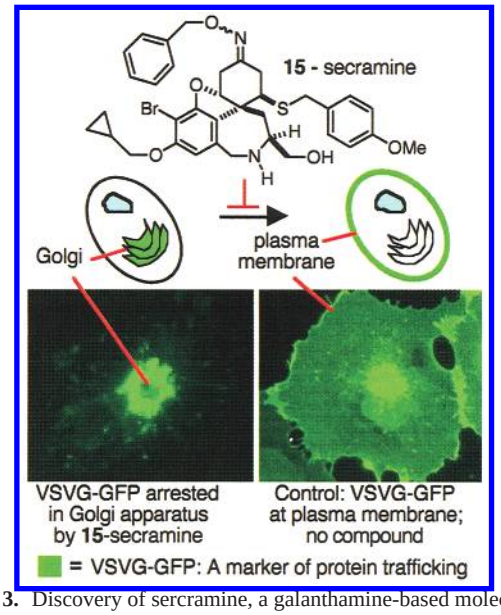


Figure 3. Discovery of sercramine, a galanthamine-based molecule that perturbs protein trafficking.

In conclusion, a library of 2527 molecules based upon galanthamine was synthesized using solid-phase biomimetic reactions. Even though the library was based on a natural product that had no effect on the secretory pathway, diversity-oriented synthesis combined with phenotypic screening was successful in identifying an active molecule that may emerge as an important probe reagent for exploring protein trafficking. The success of this synthesis and screen underscores the power of this approach to discover small molecules with utility in cell biology.[25]

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Supporting Information Available: Syntheses of solid-phase precursors, library synthesis protocols, MS analysis for the structural assignment of all 2527 library members, LC analysis for purity assessment, and representative compound synthesis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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