

Benzofuran-Derived Cyclic β -Amino Acid Scaffold for Building a Diverse Set of Flavonoid-Like Probes and the Discovery of a Cell Motility Inhibitor

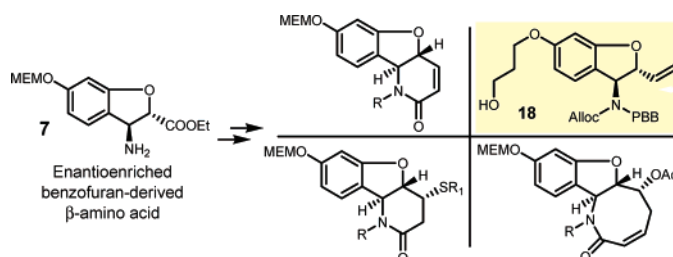
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ABSTRACT



We report here a practical, enantioselective synthesis of benzofuran-derived, cyclic *trans*- β -amino acid scaffold. In two cases, tricyclic derivatives having six- and eight-membered unsaturated lactams were obtained from this versatile scaffold. To explore the biological applications, these compounds were subjected to cell-based assays, using NIH3T3 mouse cells to examine their potency as cell motility inhibitors and identified 18 as a potent cell motility inhibitor ($IC_{50} \approx 40 \mu M$ in chamber cell migration assay).

With the growing interest in the use of small molecules as chemical modulators of biomacromolecular interactions, a ready access to natural product-like structurally complex compounds with diverse architectures that could exhibit highly specific biological responses comparable to bioactive natural products has also risen.^{1–5} Toward this objective, here

we describe a highly practical enantioselective synthesis of a new class of benzofuran-derived β -amino acids, 1 and 2, which could further be utilized in generating a diverse set of flavonoid-inspired, different bicyclic and tricyclic architectures. The benzofuran sub-structure is commonly found in a wide variety of flavonoids that exhibit a wide range of biological responses.^{6–8}

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(1) Perez, L.; Danishefsky, S. J. *ACS Chem. Biol.* **2007**, *2*, 159.

(2) Tolliday, N.; Clemons, P. A.; Ferraiolo, P.; Koehler, A. N.; Lewis, T. A.; Li, X.; Schreiber, S. L.; Gerhard, D. S.; Eliasof, S. *Cancer Res.* **2006**, *66*, 8935.

(3) Schreiber, S. L. *Nat. Chem. Biol.* **2005**, *1*, 64.

(4) Koch, M. A.; Schuffenhauer, A.; Scheck, M.; Wetzel, S.; Casaulta, M.; Odermatt, A.; Ertl, P.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17272.

(5) Tan, D. S. *Nat. Chem. Biol.* **2005**, *1*, 74.

(6) Maurya, R.; Yadav, P. P. *Nat. Prod. Rep.* **2005**, *22*, 400.

(7) Baumann, B.; Bohnenstengel, F.; Siegmund, D.; Wajant, H.; Weber, C.; Herr, I.; Debatin, K.-M.; Proksch, P.; Wirth, T. *J. Biol. Chem.* **2002**, *277*, 44791.

Because of their ability to generate “druggable”-forms of artificial peptides and peptidomimetics due to resistance to the enzymatic degradation, the use of β -amino acid scaffolds is well-documented in the literature.^{9–11} In addition to this, there are also examples of highly potent bioactive natural products (e.g., taxol) that contain the β -amino acid functionality. The cyclic β -amino acids have also been utilized as building blocks leading to the generation of bioactive architectures.^{12–14}

With the objective of developing the high-throughput synthesis of benzofuran flavonoid-inspired compounds, we have developed a practical enantioselective synthesis of a new class of cyclic β -amino acid having the benzofuran scaffold. Through combining these two important features (i.e., benzofuran scaffold and cyclic β -amino acid functionality), we were interested in generating a wide-variety of benzofuran-derived compounds that could be subjected to biological evaluation, in particular, in the search of small molecule modulators of cell migration. In post angiogenesis state of cancer, tumor cell invasion is one of the most critical steps prior to metastasis¹⁵ and the latter is responsible for about 90% death among all cancer patients.^{16–18} Thus, there is a strong interest in finding small molecules that could function as cell migration inhibitor and further could slow the cancer invasion to prevent metastasis.^{19–22}

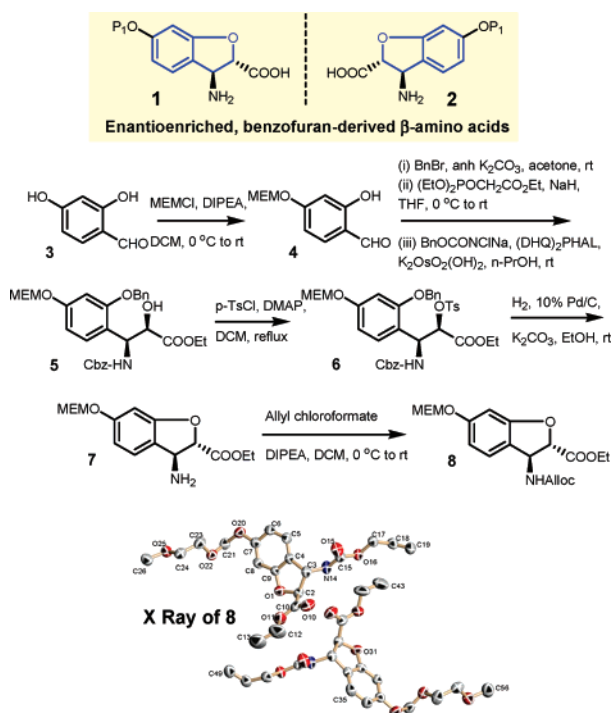
To develop the enantioselective synthesis of benzofuran derived cyclic β -amino acid scaffolds, 2,4-dihydroxybenzaldehyde (**3**) was subjected to a selective stepwise phenolic hydroxyl protections as -OMEM and -OBn (Scheme 1). Following the two carbon chain extension by Wittig–Horner reaction, it was then subjected to Sharpless aminohydroxy-

lation reaction,^{23,24} which worked very well even on a large scale (15.0 gram scale) and the *N*-protected amino hydroxyl product, **5** was obtained in 75% yield (ee > 95%). The hydroxyl group was then tosylated to obtain **6**, which to our delight, under hydrogenation conditions in the presence of a mild base (K_2CO_3), afforded the benzofuran derivative **7** following deprotection of the amino and the phenolic hydroxyl groups and the nucleophilic displacement of -OTs by the phenolic hydroxyl generated in situ. This process is highly simple, practical and both enantiomers of β -amino acids could be easily obtained in large quantities depending on the chirality of the ligand used for the aminohydroxylation reaction. The *N*-alloc protection of this novel cyclic β -amino acid **7** gave **8** as the crystalline solid that was further confirmed by X-ray crystallographic studies.

As a test study (Scheme 2), compound **7** was converted to **9** in a few steps that included *N*-alkylation (*N*-para-bromobenzyl = NPBB) of the benzylic nitrogen, followed by *N*-alloc protection and conversion of the carboxyl moiety to aldehyde by sequential reduction ($LiBH_4$) to alcohol, followed by oxidation (Dess–Martin periodinane) of the hydroxyl derivative. Using the ring closing metathesis as the key reaction, the next step was to explore the synthesis of different tricyclic architectures from compound **9**.

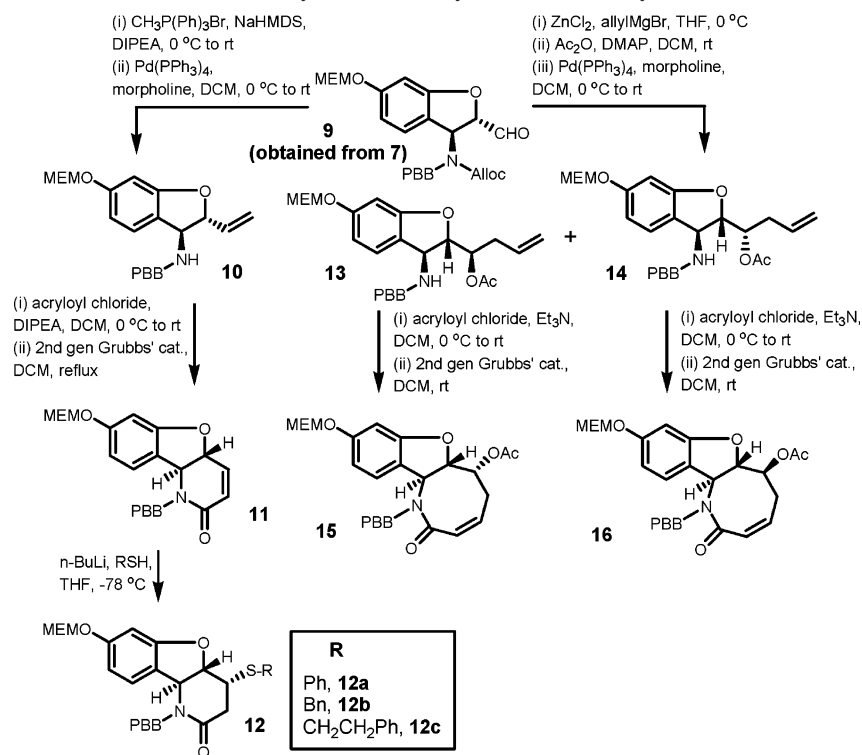
In one study, the Wittig olefination of the aldehyde, followed by *N*-alloc removal gave the secondary benzyl amine **10**, which was then *N*-acryloylated to obtain the precursor for the ring closing metathesis for developing the six-membered unsaturated lactam ring moiety. This was nicely achieved in good yields by the use of 10 mol % second generation Grubbs’ catalyst, to obtain compound **11**. To explore the unsaturated lactam functionality as a potential diversity generating site, it was then independently subjected to three thiols (e.g., $PhSH$, $BnSH$, $PhCH_2CH_2SH$) producing the 1,4-thiol adducts **12a**, **12b**, and **12c** as single diastereomers.

Scheme 1. Synthesis of Optically Enriched Benzofuran Scaffold



- (8) Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. *J. Med. Chem.* **2002**, 45, 1741.
- (9) Liu, M.; Sibi, M. P. *Tetrahedron* **2002**, 58, 7991.
- (10) Sibi, M. P.; Patil, K. *Angew. Chem., Int. Ed.* **2004**, 43, 1235.
- (11) Sibi, M. P.; Itoh, K. *J. Am. Chem. Soc.* **2007**, 129, 8064.
- (12) Porter, E. A.; Wang, X.; Lee, H. S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, 404, 565.
- (13) Fulop, F. *Chem. Rev.* **2001**, 101, 2181.
- (14) Karlsson, A. J.; Pomerantz, W. C.; Weisblum, B.; Gellman, S. H.; Palecek, S. P. *J. Am. Chem. Soc.* **2006**, 128, 12630.
- (15) Woodhouse, E. C.; Chuaqui, R. F.; Liotta, L. A. *Cancer* **1997**, 80, 1529.
- (16) Wittekind, C.; Neid, M. *Oncology* **2005**, 69 Suppl 1, 14.
- (17) Fidler, I. J. *Differentiation* **2002**, 70, 498.
- (18) Fidler, I. J. *Semin. Cancer Biol.* **2002**, 12, 89.
- (19) Metaferia, B. B.; Chen, L.; Baker, H. L.; Huang, X. Y.; Bewley, C. A. *J. Am. Chem. Soc.* **2007**, 129, 2434.
- (20) Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, 126, 1038.
- (21) Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, 126, 11326.
- (22) Fenteany, G.; Zhu, S. *Curr. Top. Med. Chem.* **2003**, 3, 593.
- (23) Li, G.; Angert, H. H.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, 35, 2813.
- (24) Li, G.; Chang, H.-T.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, 35, 451.

Scheme 2. Synthesis of Tricyclic Derivatives by RCM



In a second study, the aldehyde **9** was subjected to Lewis acid-catalyzed Grignard reaction giving two allylic alcohols as a mixture of separable diastereomers. Each isomer was then independently subjected to the protection of the hydroxyl groups. Following this, the *N*-alloc removal afforded amines, **13** and **14**, which were then acryloylated on their benzylic nitrogens to provide the precursor for the ring closing metathesis. Interesting to note was the observation that in the presence of 5 mol % of second generation Grubbs' catalyst, the unsaturated eight membered ring lactams, **15** and **16** were formed from these precursors very easily and in high yields. The RCM reaction was independent of the stereochemistry of the allylic hydroxyl group.

Following the success with the above studies in solution, our next goal was to develop this approach on the solid phase. While repeating a solution-phase reaction protocol in solid phase, it is often necessary to anchor the molecules onto the resin through a suitable spacer. With this in mind, we attached compound **8** to a 3-carbon spacer using its phenolic oxygen to obtain compound **18** (Scheme 3, the synthesis details are provided in the supporting material). This would serve as a starting material for the six-membered lactam ring formation on the solid phase.

At this stage, before developing the solid-phase synthesis program, all benzofuran-based compounds (25 in total)

Scheme 3. Bicyclic Derivative Needed for the Solid-Phase Synthesis

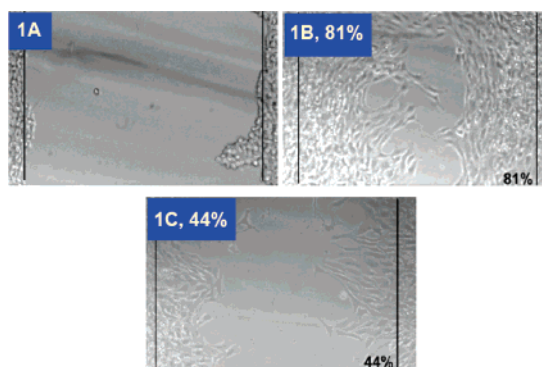
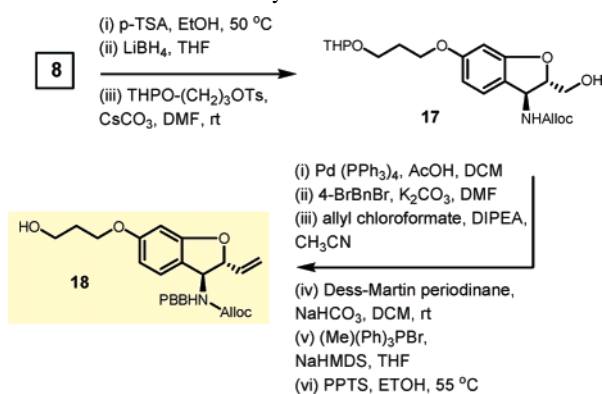


Figure 1. (A) fresh wound; (B) healing in the presence of DMF; (C) healing in the presence of DMF and small molecule, **18**.

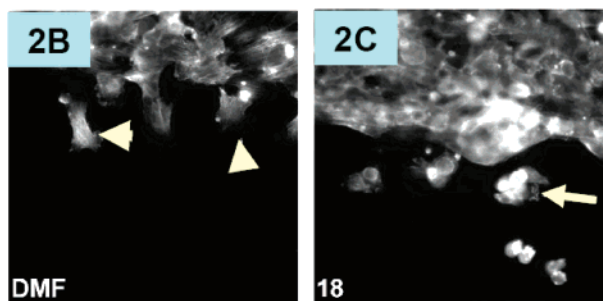
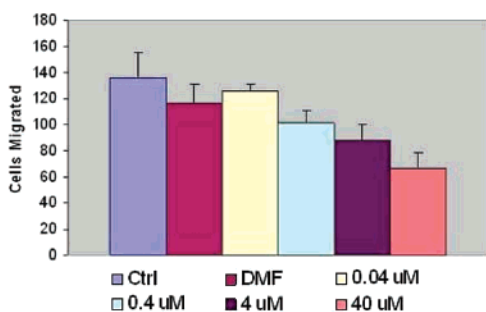


Figure 2. (A) Chamber cell migration assay. Dose response curve of **18** with NIH3T3 cell lines. (B) and (C) Staining experiments. NIH3T3 cell lines wounded in the presence of DMF (B) and **18** (C) were stained for actin fibers. Cells treated with **18** display membrane ruffles (arrow) but lacked extensive lamellipodia (arrowhead) and stress fibers.

obtained and 450 natural product-like small molecules from the group were subjected to explore their potency as cell motility inhibitors through different cellular assays. In one study, the effect of these compounds was investigated on the migration of NIH 3T3 mouse cells which are routinely utilized in studying the cell motility.

The effects were assessed in wound-healing assay²⁵ and quantitative Boyden chamber cell migration assay.²⁶

The NIH 3T3 cells were grown to confluence and then manually scratched with a pipette tip to create a wound

(Figure 1A). The cells were then allowed to close the wound overnight in the presence of the small molecules or DMF as control. To our pleasant surprise, only 44% wound closure was observed in the presence of **18** (Figure 1C) whereas in the control experiment, the wound was closed by 81% (Figure 1B). These findings were further confirmed by the quantitative Boyden chamber cell migration assay. Performed with the same cell line in this assay, cells were treated with different concentrations of **18** and then plated in the upper chamber. Cells that migrated to the underside of the chamber filter were then enumerated. Dose response curves on NIH3T3 showed that the IC₅₀ for compound **18** was approximately 40 μ M (Figure 2A) where 50% inhibition of motility was attained (see the Supporting Information).

To investigate the potential effect of **18** on actin dynamics, NIH3T3 cells were wounded and stained for filamentous actin using rhodamine-labeled phalloidin. Although some membrane ruffling was observed, cells at the wound edge showed a marked reduction in lamellipodial extension and actin stress fibers following treatment with compound **18**, suggesting that it impairs actin dynamics (Figure 2C).

To summarize, the present study that involves the generation of benzofuran natural product-inspired architectures has resulted in the discovery of a new class of small molecule modulators of cell motility. Further, investigations are warranted to obtain a better understanding of the mechanism of action of small molecule, **18**, and will be reported as the new information becomes available.

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Supporting Information Available: Full characterization of compounds and additional information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(25) Liang, C. C.; Park, A. Y.; Guan, J. L. *Nat. Protoc.* **2007**, 2, 329.
(26) Carter, S. B. *Nature* **1967**, 213, 256.