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Extensively Stereodiversified Scaffolds for Use in Diversity-Oriented Library Synthesis

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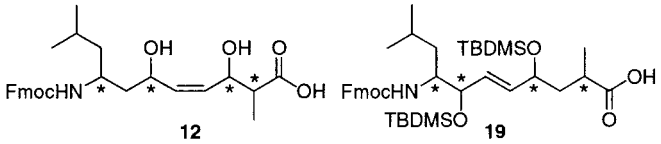
ABSTRACT



The syntheses of stereodiverse libraries of 12 and 19 are reported, where each asterisk represents an independently varied stereocenter. These scaffolds provide additional templates for investigations of geometric diversity in library syntheses. Libraries of these *N*-Fmoc-amino acids were further functionalized by incorporation into a peptide sequence, demonstrating the utility of 12 and 19 as building blocks for diversity oriented synthesis.

The synthesis of libraries of small molecules is an important activity in the discovery of novel compounds with important biologic functions. Pharmaceutical companies have long utilized libraries of analogues in their efforts to enhance or optimize initial lead compounds. More recently, the field of chemical genetics has sought to identify compounds for all biologic receptors, a goal that requires the synthesis and screening of structurally unique compounds.[1] An important criterion in the design of small molecule libraries is the incorporation of diversity among the library members to maximize the potential for any one library member to exhibit potent biologic activity. The literature abounds with examples where diversity is probed through functional group variation, often through the appendage of side chains (R groups) from a fixed, cyclic scaffold.[2] In a complementary approach, diversity can also be probed through geometric variation of the scaffold itself, although this strategy has not been as widely investigated.[3] A truly exhaustive diversification strategy would encompass both functional group and geometric variation.

Our laboratory has been developing systems that explore geometric diversity through extensive stereochemical variation of acyclic scaffolds (Figure 1).[4,5] It is expected that individual stereoisomers will have distinct conformational preferences and thus unique three-dimensional structures.[6] Peptide chimeras containing scaffold **1** embedded in their sequence show a remarkable distribution of hydrophobicities between stereoisomers as measured by retention times on a reversed-phase HPLC column[4] and a pronounced stereochemical dependence on their ability to inhibit the aspartyl protease renin.[6] Stereochemically diverse small molecules containing scaffolds **2**−**4** have been screened for binding to

(1) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. Mitchison, T. J. *Chem. Biol.* **1994**, *1*, 3−6.

(2) See, for example: Pelish, H. E.; Westwood, N. J.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 6740−6741. Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; Cao, G. Q.; Affleck, R. L.; Lillig, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 9954−9967. Tan, D. S.; Foley, M. A.; Shair, M. D.; Schreiber, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8565−8566. Bunin, B. A.; Plunkett, M. J.; Ellman, J. A.; Bray, A. M. *New J. Chem.* **1997**, *21*, 125−130.

(3) See, for example: Paterson, I.; Temal-Laieb, T. *Org. Lett.* **2002**, *4*, 2473−2476. Misske, A. M.; Hoffmann, H. M. R. *Chem. Eur. J.* **2000**, *6*, 3313−3320. Paterson, I.; Scott, J. P. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1003−1014. Annis, D. A.; Helluin, O.; Jacobsen, E. N. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1907−1909. Sutherlin, D. P.; Armstrong, R. W. *J. Org. Chem.* **1997**, *62*, 5267−5283.

(4) Gierasch, T. M.; Chytil, M.; Didiuk, M. T.; Park, J. Y.; Urban, J. J.; Nolan, S. P.; Verdine, G. L. *Org. Lett.* **2000**, *2*, 3999−4002.

(5) Harrison, B. A.; Verdine, G. L. *Org. Lett.* **2001**, *3*, 2157−2159.

(6) Michielin, O.; Zoete, V.; Gierasch, T. M.; Eckstein, J.; Napper, A.; Verdine, G.; Karplus, M. *J. Am. Chem. Soc.* **2002**, *124*, 11131−11141.

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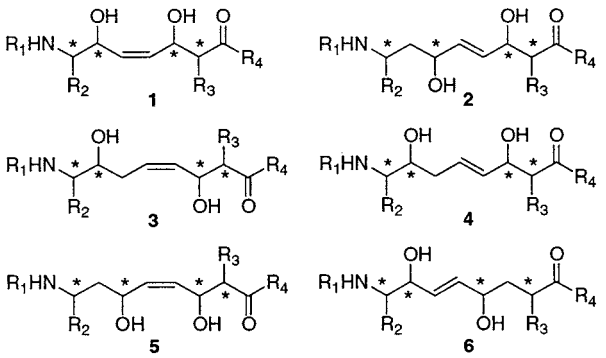


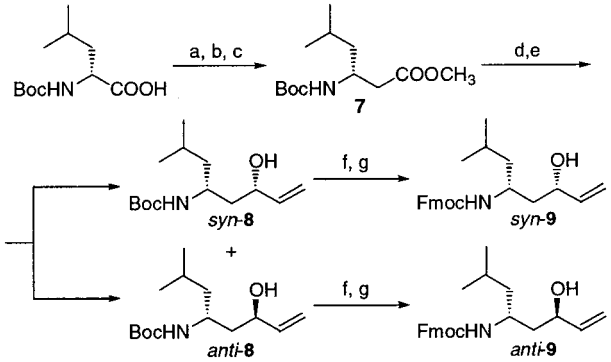
Figure 1. Extensively stereodiversified acyclic scaffolds for use in library synthesis. Each scaffold represents all possible stereoisomers where each asterisk represents an independently varied stereocenter.

the μ opioid receptor (MOR), resulting in the discovery of individual stereoisomers that bind the MOR with nanomolar affinity.[7]

In conjunction with our efforts to extend the range of stereodiversified libraries, we sought to synthesize scaffolds **5** and **6**, which contain both the 1,4-enediol moiety of scaffolds **1** and **2** and are isoatomic in their backbone length with scaffolds **2**−**4**. Additionally, it was imagined that the syntheses might parallel that of scaffolds **1**−**4**, which involve the coupling of stereodiverse C-terminal intermediates and stereodiverse N-terminal intermediates using olefin metathesis. Herein, we report the synthesis of scaffolds **5** and **6** for the case where R_2 = *i*-Bu and R_3 = Me, employing a ring-closing metathesis strategy for scaffold **5** and a cross-metathesis strategy for scaffold **6**.[8]

The synthesis of scaffold **5** relied on *N*-Boc-leucine as a chiral source (Scheme 1). Thus, *N*-Boc-D-leucine was converted to its corresponding diazoketone and subjected to Wolff rearrangement to produce the known methyl ester **7**.[9]

Scheme 1[a]



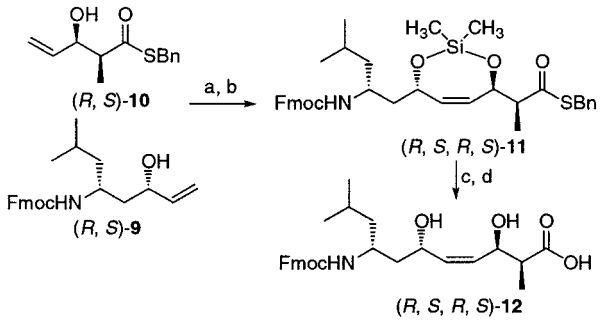
[a] Key: (a) *N*-methylmorpholine, $ClCO_2$-*i*-Bu, THF, −20 °C; (b) CH_2N_2, THF, −20 °C, 81% two steps; (c) $PhCO_2Ag$, Et_3N, MeOH, quant; (d) DIBAL-H, toluene, −78 °C, 80%; (e) vinyl-MgBr, Et_2O, −70 → −40 °C, 68% (2.2:1 syn/anti); (f) HCl, dioxane; (g) Fmoc-OSu, DIPEA, CH_2Cl_2, 78% syn, 70% anti (two steps).

Methyl ester **7** was reduced to the aldehyde using DIBAL-H and reacted with vinylmagnesium bromide to provide a 2.2:1 ratio of *syn*/*anti*-**8**. The diastereomers were separated by silica gel chromatography, and the N-protecting group was converted from Boc to Fmoc to provide *syn*-**9** and *anti*-**9**.

The diastereomers were assigned by analysis of the X-ray crystal structure of the diacylated product obtained by Fmoc removal of *syn*-**9** and treatment of the resulting amino alcohol with TFAA.[10] The remaining two isomers of N-terminal intermediate **9** were produced in an identical way from *N*-Boc-L-leucine. This route differs from our previous reported stereoselective syntheses of C-terminal and N-terminal intermediates in that it proceeds nonselectively; however, the efficiency resulting from obtaining both isomers in one reaction sequence is an attractive alternative when isomer separation and identification are feasible.

A ring-closing metathesis strategy was employed to couple N-terminal intermediates **9** to C-terminal partners **10**[11] to produce spatially separated stereoisomers of acid **12** (Scheme 2).[4,5] All stereoisomers of intermediates **9** and **10** were

Scheme 2[a]



[a] Key: Me_2SiCl_2, pyridine, 74%; (b) $Cl_2(PCy_3)(IMesH_2)$-Ru=CHPh, toluene, 75 °C, 75%; (c) HF/pyridine, THF, 84%; (d) LiOH, H_2O_2 pH 8.5, THF, 97%. Configurations are reported from left to right for clarity.

available; thus, all 16 stereoisomers of **12** were synthesized. The sequence is detailed here for one specific isomer pairing. The thioester (*R*,*S*)-**10** was monosilylated with excess Me_2SiCl_2, and the N-terminal intermediate (*R*,*S*)-**9** was added to generate only the heterotethered product, which was then subjected to olefin metathesis using 10% $Cl_2(PCy_3)(IMesH_2)$-Ru=CHPh[12] to produce selectively the *Z*-olefin product (*R*,*S*,*R*,*S*)-**11** in 56% yield over two steps. Metathesis product (*R*,*S*,*R*,*S*)-**11** was desilylated with HF−pyridine and hydrolyzed (LiOH, H_2O_2, pH 8.5) to acid (*R*,*S*,*R*,*S*)-**12** with retention of the base-labile Fmoc group in 81% yield over

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(9) Johnson, R. L.; Verschoor, K. *J. Med. Chem.* **1983**, *26*, 1457−1462.

(10) Details are provided in the Supporting Information.

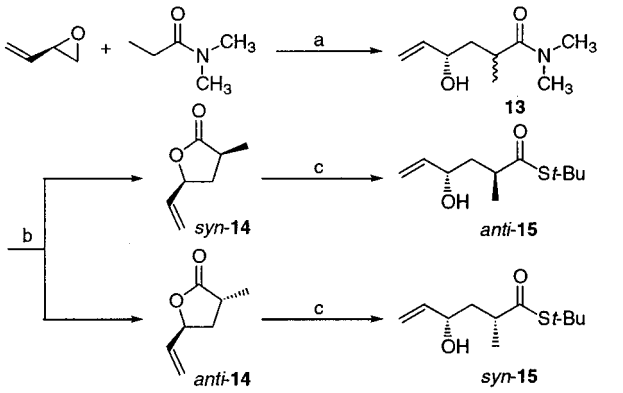
(11) Synthesized as reported in ref 4. Complete details and spectroscopic information are provided in the Supporting Information.

(12) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18−29.

two steps. This stereodiverse set of *N*-Fmoc-amino acids **12** represents a defined version of scaffold **5**, which can be further diversified at either end.

The synthesis of scaffold **6** relied on enantiomerically enriched butadiene monoxide[13] to define the absolute stereochemistry of the C-terminal intermediates **14** and **15** (Scheme 3). Nucleophilic epoxide opening of butadiene monoxide can

Scheme 3[a]



[a] Key: (a) LDA, LiCl, THF, 0 °C; (b) H_2SO_4, dioxane, 95 °C, 60%, two steps, 1:1 syn/anti; (c) *t*-BuSH−$AlMe_3$, CH_2Cl_2, 4 °C, 78% anti, 70% syn.

produce three regioisomeric products, resulting from S_N2 attack (primary or secondary carbon) or S_N2' attack. Initially, it was thought that the relative preference for these three products might be modulated by the choice of enolate. However, ester and thioester enolates gave low yields of the epoxide-opened products due to competing Claisen condensation.[14] In contrast, the enolate derived from *N*,*N*-dimethylpropionamide exclusively gave products resulting from epoxide opening.[15] Further screening of reaction conditions found that lower temperatures (0 °C) favored S_N2 attack at the primary carbon over the secondary carbon and addition of excess LiCl disfavored competing S_N2' attack. Thus, treatment of (*R*)-butadiene monoxide with the enolate derived from *N*,*N*-dimethylpropionamide and an excess of LiCl at 0 °C produced a separable 2:1 mixture of regioisomers resulting from S_N2 attack where the major product **13** was generated as a 1:1 mixture of diastereomers at the α-carbon. Diastereomers **13** were treated with H_2SO_4 in dioxane at 95 °C to form lactones *syn*-**14** and *anti*-**14**.[16] The diastereomers were separated by silica gel chromatography and their relative configurations established by comparison of the magnitude of their vicinal coupling constants to known disubstituted lactones.[17] The remaining two isomers of **14** were produced in an identical manner from (*S*)-butadiene monoxide. In analogy to the synthesis of the N-terminal intermediate **9**, the efficiency of this nonselective route and ease of separation and identification of the *cis* and *trans* lactones **14** made this an attractive alternative to a stereoselective synthesis.[18]

Lactone **14** was converted to thioester **15** by treatment with *t*-BuSH−$AlMe_3$.[19] It was immediately evident that the γ-hydroxy thioester was not stable in its unprotected form, as evidenced by the partial reversion of **15** to **14** upon exposure to silica gel chromatography,[20] although this could be minimized by addition of Et_3N to the solvent. Thioester *anti*-**15** was successfully tethered to *syn*-**16**[21] according to the procedure described above and subjected to RCM to provide a *Z*-olefin product **17** analogous to **11** in modest (37%) yield (Figure 2). Removal of the silyl tether proceeded

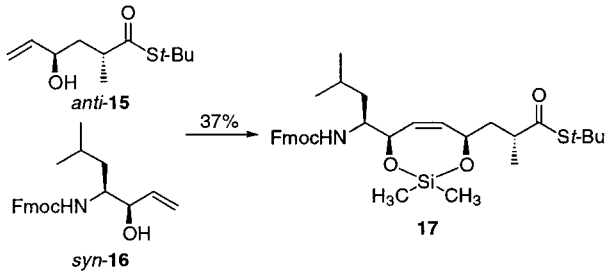


Figure 2. Initial coupling of **15** with N-terminal partner **16** to produce tethered, metathesized **17**.

without difficulty, although attempts to hydrolyze the thioester to an acid did lead to relactonization. It became apparent that the γ-hydroxy group would need to be protected in order for the carbonyl group to be useful as a point of diversification and that the Me_2Si tether was not robust enough for this purpose.

In light of these results, a modified route was developed based on a cross-metathesis strategy, which would allow for protection of the problematic γ-hydroxy group (Scheme 4). Lactone **14** was employed as a metathesis partner for the N-terminal partner **16**, in a cross-metathesis reaction to provide **18** (Scheme 4). Ratios of **14**/**16** from 3:1 → 1:2 were screened in the metathesis reaction to determine what conditions would maximize the yield of the desired heterocoupled product. These investigations revealed that the ratio of homocoupled and heterocoupled products was statistically determined, as would be expected from two similar olefin partners. While it was possible to get higher yields of the desired product (based on the limiting partner) by using an

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(17) Hussain, S. A. M. T.; Ollis, W. D.; Smith, C.; Stoddart, J. F. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1480−1492. Myers, A. G.; McKinstry, L. *J. Org. Chem.* **1996**, *61*, 2428−2440.

(18) A stereoselective route was explored based on Myers' pseudoephedrine methodology (ref 18); however, the reaction of (*S*)-butadiene monoxide with the enolate derived from (+)-pseudoephedrine (a stereochemically "matched" case) provided a modest 4: 1 ratio of *trans*/*cis* lactones.

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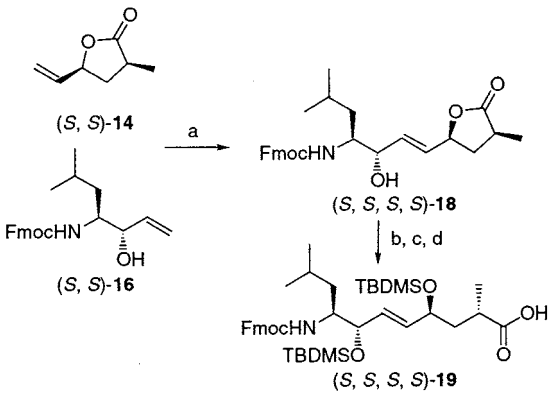
(20) Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. *J. Org. Chem.* **1985**, *50*, 4615−4625.

(21) Available as detailed in ref 4.

Scheme 4[a]



[a] Key: (a) $Cl_2(PCy_3)(IMesH_2)Ru{=}CHPh$, CH_2Cl_2, 45 °C, 58%; (b) *t*-BuSH−$AlMe_3$, CH_2Cl_2, 4 °C; (c) TBDMS-Cl, imidazole, DMF, 74%, two steps; (d) LiOH, H_2O_2, pH 8.5, THF, 70%. Configurations are reported from left to right for clarity.

excess of one olefin, atom economy argued for a 1:1 ratio of olefin partners. This was a viable option due to the easy separation of the heterocoupled product **18** from each of the homocoupled products. All four stereoisomers of **14** were available as well as two diastereomers of **16** (syn and anti), which allowed for the parallel synthesis of all 8 diastereomers of **18** (and therefore **19**).

As a specific example, 1.0 equiv of (*S*,*S*)-**14** and 1.2 equiv of (*S*,*S*)-**16** were exposed to 5% $Cl_2(PCy_3)(IMesH_2)Ru{=}CHPh$ in refluxing CH_2Cl_2 for 1 h to provide (*S*,*S*,*S*,*S*)-**18** in 58% yield (crude NMR showed >95:5 *E*/*Z* olefin). The lactone was converted to the γ-hydroxy thioester using *t*-BuSH−$AlMe_3$ and immediately silylated with TBDMS-Cl to prevent relactonization. The thioester was hydrolyzed to the acid (LiOH, H_2O_2, pH 8.5) to produce (*S*,*S*,*S*,*S*)-**19** in 52% yield over three steps. This stereodiverse set of TBDMS-protected *N*-Fmoc-amino acids is suitable for further diversification at either end, allowing for the synthesis of libraries containing scaffold **6**.

To demonstrate how these two *N*-Fmoc-amino acids **12** and **19** might be incorporated into larger structures, we chose to append amino acid residues at the R_1 and R_4 sites to produce peptide chimeras **20** and **21** (Figure 3), reminiscent of the APP cleavage site recognized by the aspartyl protease β-secretase.[22]

This was accomplished using standard solid-phase peptide synthesis techniques, where the library of acids **12** or **19** were

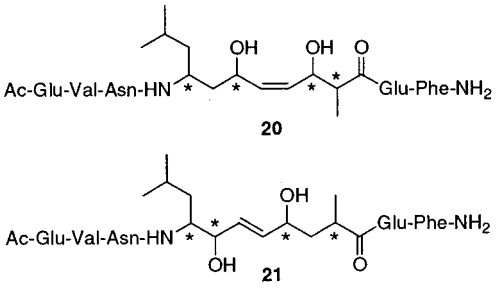


Figure 3. Structures of the libraries of peptide chimeras incorporating stereodiversified scaffolds **5** and **6**. Each asterisk indicates a stereocenter that is independently varied among the library members.

coupled in a manner similar to that used for the α-amino acids (HATU, HOAt, DIPEA, and NMP). The peptide chimeras were simultaneously cleaved from the solid support and deprotected using 85% trifluoroacetic acid. The peptide chimeras were purified by reversed-phase HPLC to produce 16 individual isomers represented by **20** and 8 individual isomers represented by **21**.

In conclusion, we have reported the parallel synthesis of libraries containing **12** and **19** where diversity arises from extensive stereochemical variation. All possible diastereomers of **12** and **19** were synthesized and successfully incorporated into a larger peptide fragment to demonstrate how they might be further functionalized. This extends the number of scaffolds available as platforms for exploring the effects of stereochemical diversity in library synthesis to include **5** and **6**.

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Supporting Information Available: Experimental details and characterization data regarding the preparation of synthetic intermediates and products. This material is available free of charge via the Internet at http://pubs.acs.org.

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