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A Modular Synthetic Approach toward Exhaustively Stereodiversified Ligand Libraries

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ABSTRACT

This report describes a modular approach to the synthesis of stereodiversified natural product-like libraries. Monomers 2 and 3 were coupled in parallel by silyl-tethered olefin metathesis to generate all 16 stereoisomers of *cis*-enediols 1. All 16 stereoisomers were incorporated into chimerae having flanking peptidic segments. These chimerae exhibited a broad range of hydrophobicities, raising the possibility that stereochemical variation might be used to tune the pharmacologic properties of small molecules.

A major challenge of the post-genomic era will be to discover potent and selective ligands to the large number of macromolecular receptors having unclear or unknown biological function.[1] The chemical genetics approach toward this problem entails the synthesis and screening of diverse organic molecules to identify those that have an observable phenotypic effect on cells.[2] To date, design and synthesis of small-molecule libraries has primarily focused on achieving diversity through functional group variation among members of the library.[3] The alternative and complementary strategy of varying functional group presentation through extensive stereochemical diversification has received much less attention.[4,5] Indeed, the only case of true library generation by exhaustive stereochemical diversification is the panel of all 32 polyketide stereoisomers individually synthesized by Patterson and co-workers.[6] Although this case represents a triumph of asymmetric synthesis, it also illustrates the difficulties of achieving exhaustive stereochemical coverage

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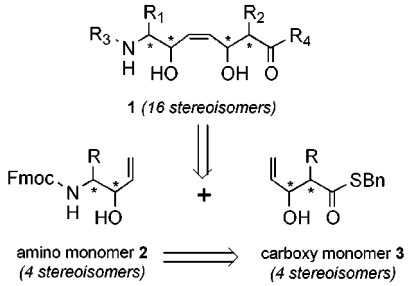
while also enabling variation of functional groups. Here we report a modular approach to the synthesis of libraries of natural product-like molecules comprising all possible stereoisomers. This approach lays the groundwork for the generation of small molecule libraries bearing extensive variation in both stereochemistry and functional groups.

From the standpoint of biological potency, we considered it important for the library members to possess a semirigid acyclic framework rich in stereogenic centers, with a hydrocarbon framework presenting both hydrogen-bonding functionality and variable side chain ("R") groups. For reasons of synthetic practicality, we sought a route that would allow stringent stereochemical control, be broadly inclusive of functional groups, and converge on the final products through a late-stage carbon−carbon bond-coupling step. Among the candidate systems considered a priori, we selected the *cis*-enediol unit **1** (Scheme 1) for development in the initial phase of this study.

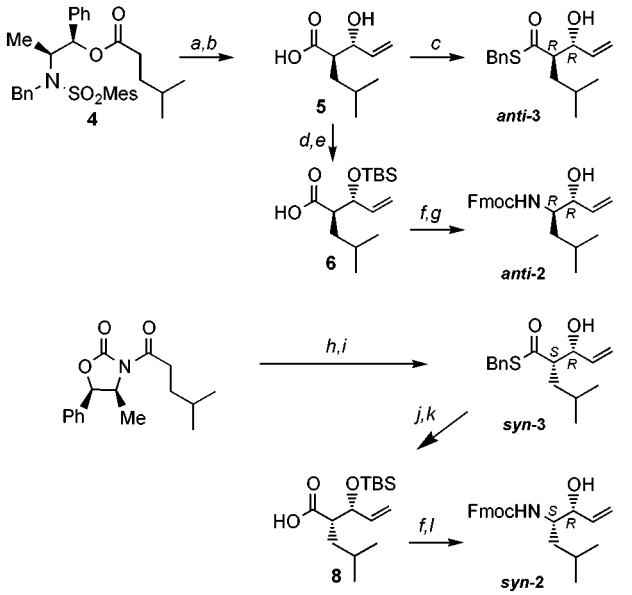
Scheme 1. Retrosynthetic Scheme for the Synthesis of Stereodiversified Libraries Containing the *cis*-Enediol Module **1**



The internal framework of **1** presents two independently diversifiable "R" groups and two hydroxyls, which can interact with receptors through nonpolar and hydrogen-bonding interactions, respectively. Chemically differentiated ends in **1** allow for the sequential attachment of two additional "R" groups. The presence of four sp^3-hybridized stereogenic centers in **1** gives rise to an ensemble of 16 stereoisomers, each of which can be accessed individually by stereocontrolled synthesis. Although the acyclic framework allows some degree of conformational freedom, significant restrictions are imposed by 1,3-allylic strain across the *cis*-configurated olefin and by torsional strain between adjacent tertiary carbons. This results in strong and unique conformational preferences for each of the 16 stereoisomers;[7] such preorganization lowers the entropic cost of receptor recognition and enhances its specificity. The entire stereochemical ensemble covers a broad range of conformational space, increasing the likelihood that one or more members will be chemically complementary to a receptor pocket.

Retrosynthetically **1** was envisioned to arise from two modular building blocks, amino monomer **2** and carboxy monomer **3**, via ruthenium-mediated olefin metathesis.[8] This modular construction approach, which takes advantage of the chemical orthogonality and functional group tolerance of olefin metathesis, is intended to allow expedient construction of libraries varied in both stereochemistry and side chain functionality. Here we demonstrate this synthetic approach for the example in which both R_1 and R_2 are isobutyl groups (Schemes 2 and 3).

Scheme 2[a]



[a] Key: (*a*) 2.0 equiv of Cx_2BOTf, TEA, THF, 0 → −78 °C, then CH_2CHCHO −78 → 0 °C; (*b*) 0.5 M NaOH in MeOH:H_2O (4:1); (*c*) $PhCH_2SH$, DCC, cat. DMAP, CH_2Cl_2, 0 °C; (*d*) 2.4 equiv of TBDMSCl, imidazole, DMF; (*e*) THF:MeOH:H_2O (2:1:1); (*f*) $(PhO)_2PON_3$, TEA, MeCN, reflux, then cat. CuCl, 9-fluorenemethanol, MeCN; (*g*) AcOH:THF:H_2O (2:1:1), 55 °C; (*h*) 1.1 equiv of Bu_2BOTf, *i*-Pr_2NEt, CH_2Cl_2, 0 → −78 °C, then CH_2CHCHO, −78 → 0 °C; (*i*) $PhCH_2SLi$, $AlMe_3$, CH_2Cl_2 0 °C; (*j*) 1.2 equiv of TBDMSCl, imidazole, DMF; (*k*) LiOOH, THF:MeOH:H_2O (3:1:1), 0 → 20 °C, then aqueous 1.0 M Na_2SO_3; (*l*) AcOH:THF:H_2O (2:1:1), 45 °C.

Monomers **3** were accessed in a stereocontrolled fashion via either Evans[9] (*syn*) or Masamune[10] (*anti)* chiral auxiliary-controlled aldol chemistry. Monomers **2** were obtained stereospecifically from **3** though the Curtius rearrangement,[11,12] thus providing an efficient and convenient overall process for monomer generation. Coupling monomers **2** and **3** via olefin metathesis raises the challenge of generating only heterocoupled products while controlling the olefin geometry. Both issues were resolved by employing a ring-closing metathesis (RCM) strategy.[13]

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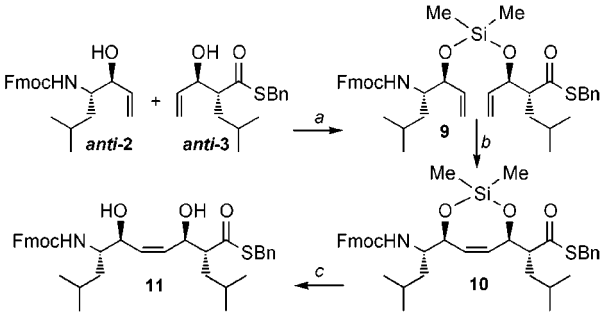
(12) The isocyanate intermediate can also be trapped with lithium *tert*-butoxide to yield the Boc-protected version of the amino monomer. See the Supporting Information for further details.

Scheme 3[a]



[a] Key: (*a*) Me_2SiCl_2 in pyridine, then *anti*-**3** in pyridine; (*b*) $Cl_2(IMes)(PCy_3)Ru{=}CHPh$, toluene, 70 °C; (*c*) HF/pyridine, THF, 0 → 20 °C.

Table 1. RCM of **9** Mediated by a Thermostable Metathesis Catalyst[a]

configuration (N → C)	isolated yield (%)	catalyst (%)	allylic substitution
RSSR	69	15	*trans*
RRRR	69	10	*trans*
RRRS	69	10	*trans*
RSSS	67	15	*trans*
RSRS	73	10	*cis*
RRSS	83	10	*cis*
RRSR	70	10	*cis*
RSRR	79	5	*cis*

[a] Reactions were carried out under argon for 1 h in toluene (2.5 mM with respect to catalyst) at 75 °C.

Monomer **3** was monosilylated[14] using excess Cl_2SiMe_2, separated from the excess silylating agent in vacuo, and then reacted with 1 equiv of monomer **2** to produce the heterotethered product **9** in yields ranging from 60 to 90%, with the major side products being monosilylated monomer **3** and unreacted monomer **2**. The strongest determinant of tethering efficiency was the relative stereochemistry of the carboxy monomer. *Syn* monomers **3** consistently gave lower yields (60−65%) while the *anti* monomers proceeded in 85−90% yield. Variations in the reaction conditions, including reaction time, temperature, and order of monomer silylation, had no effect on the reaction progress.

Although the Grubbs catalyst[15] has been used to form seven-membered siloxane rings of the type found in **10**,[16] this catalyst proved inefficient with our system. The conversion of **9** to **10** required stoichiometric amounts of Grubbs catalyst and gave low yields (<30%) and poor mass balances (<60%).[17] The difficulty of the present RCM operation presumably arises from the steric crowding and high density of heteroatom functionality in our substrates. Following recent reports of improved product conversion by a thermostable metathesis catalyst,[18] we tested the ability of this new catalyst to perform RCM on our substrates (**9** → **10**) and observed dramatically improved results. On a 25−100 mg scale, 5−15 mol % of catalyst at 75 °C gave yields in the range of 65−85% after removal of the silyl tether to produce the 16 stereodiversified units **11**, with exclusive formation of the *Z*-configurated olefin (Table 1). Although the yields varied somewhat from run to run, we noted a consistent influence of substituent stereochemistry on the reaction efficiency. Specifically, closure of rings with *cis* substitution at the allylic carbons required lower catalyst loadings (5−10%) and furnished higher yields (70−85%) than the corresponding *trans*-substituted rings (10−15% and 65−70%, respectively). A likely explanation for this effect is that both allylic substituents on the seven-membered siloxane ring are pseudoequatorial in the *cis* case, while one is pseudoaxial and the other pseudoequatorial in the *trans* case.

Stereodiversified units **11** are suitably equipped for chemoselective functionalization at the amino and carboxy terminal ends. To explore one potentially useful elaboration, we chose to synthesize chimerae having **11** flanked by peptidic functionality. The 16 stereoisomeric units **11** were activated for carboxy-terminal functionalization by conversion to the corresponding 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one esters.[19] These activated esters were coupled under standard conditions to the N-terminus of a tripeptide (C-Lys-His-Ile-N) immobilized on Rink Amide AM resin. Following capping and Fmoc deprotection, five additional residues were coupled (C-His-Phe-Pro-His-Pro-N), the N-terminus was acetylated, and the chimeric products were deprotected and released from the resin under standard conditions. HPLC analysis of the crude products revealed in each case a single major product,[20] which was shown by electrospray ionization mass spectrometry to possess the mass (662.0 or 662.1 AMU, M + 2H) expected of the chimerae **12**. Importantly, the *cis*-enediol units were found to withstand the strongly acidic conditions (95% trifluoroacetic acid, 3 h, room temperature) employed in peptide cleavage and deprotection. These results demonstrate that unit **1** can be cleanly functionalized at both ends, thereby producing an ensemble of stereochemically diversified chimerae.

The members of the ensemble **12** differ only in their stereochemistry at the four asymmetric centers of the

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(17) The following parameters were varied widely: reaction time and temperature, solvent, substrate and catalyst concentration, catalyst loading, order of addition, rigor of oxygen exclusion, tether structure.

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(20) None of the minor products had the mass of **12**, but instead had lower masses, indicating that they arose from incomplete coupling rather than racemization of the stereodiversified unit.

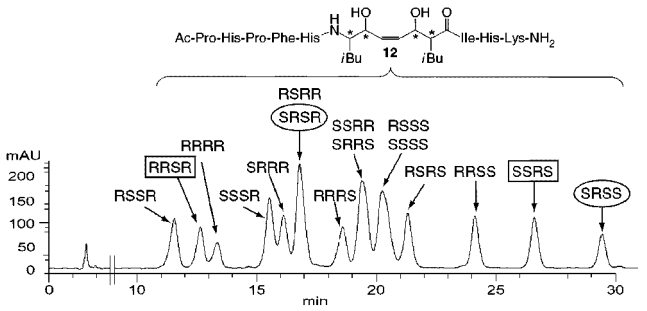

Figure 1. HPLC trace of the ensemble of 16 chimerae **12**, which differ only in the stereochemistry within the *cis*-enediol unit (asterisks). The stereochemical designations (e.g., RRRR) correspond to their order in the structure above, reading from left to right. The HPLC run is a linear gradient at 1 mL/min from 18 to 33% (v/v) MeCN in H_2O with 0.1% (v/v) trifluoroacetic acid over 35 min using a Beckmann 5 μm C_{18} column (4.6 mm $\times$ 15 cm), with UV detection at 220 nm.

embedded *cis*-enediol unit. To assess the impact of this stereochemical diversity on a pharmacologically relevant physical property of the molecules, we compared their hydrophobicities by measuring retention times on a reversed-phase C18 HPLC column.

As is evident in Figure 1, the stereochemistry of **1** has a surprisingly large effect on the overall hydrophobicity of the chimerae **12**. For example, inverting the configuration at a single stereogenic center alters the retention time by as much as 9 min (SRSR, ~17 min → SRSS, ~26 min). Even though enantiomeric *cis*-enediols **11** exhibit identical retention times, these give rise to diastereomeric pairs of chimerae **12** having pronounced differences in their hydrophobicities (RRSR, ~13 min → SSRS, ~27 min). The observation that stereochemistry strongly impacts hydrophobicity raises the exciting prospect of using stereochemical variation not only to optimize affinity but also to tune the pharmacologic properties of small molecule ligands.

Here we have described a modular synthetic approach toward the construction of small molecule libraries having exhaustive stereochemical variation at every sp^3-hybridized center. Nature has made exquisite use of stereochemical variation to fine-tune the biological activity of small molecules. The present studies aim to emulate this aspect of Nature in the creation of potent ligands for chemical genetic studies.

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

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