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Convergent Synthesis of Complex Diketopiperazines Derived from Pipecolic Acid Scaffolds and Parallel Screening against GPCR Targets

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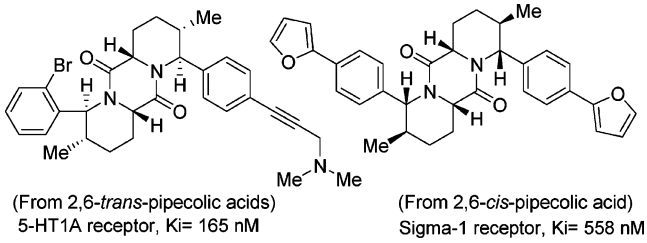

A convergent approach to highly functionalized diketopiperazines (DKPs) using enantioenriched pipecolic acids is described. Scandium triflate-catalyzed [4 + 2] aza-annulation was employed to produce stereochemically well-defined building blocks. A resin "catch and release" strategy was devised to convert annulation products to pipecolic acid monomers. Complex diketopiperazines were efficiently assembled utilizing one-pot cyclodimerization of pipecolic acids. Massively parallel screening of the complex DKPs against a panel of molecular targets identified novel ligands for a number of G-protein-coupled receptors (GPCRs).

Introduction

The diketopiperazine (DKP) subunit is embedded in a number of natural products with complex architectures and diverse biological activities (cf. Figure 1).[1] In addition to total synthesis efforts, DKPs have gained considerable attention from medicinal chemists, and several DKP-based enzyme inhibitors have been developed.[2,3] While proline-derived DKPs are abundant and extensively studied, the corresponding DKPs derived from pipecolic acid[4] largely remain under-explored. We recently reported the stereoselective synthesis of both 2,6-*cis* and 2,6-*trans* trisubstituted tetrahydropyridines employing intramolecular crotylation of imines.[5] This reaction provides direct access to diverse enantioenriched pipecolic acid building blocks which may be dimerized to afford complex diketopiperazines with considerable structural

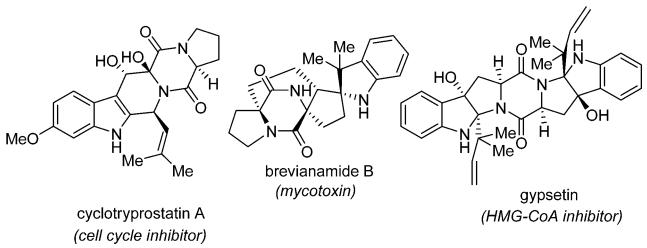

FIGURE 1. Representative diketopiperazine natural products.

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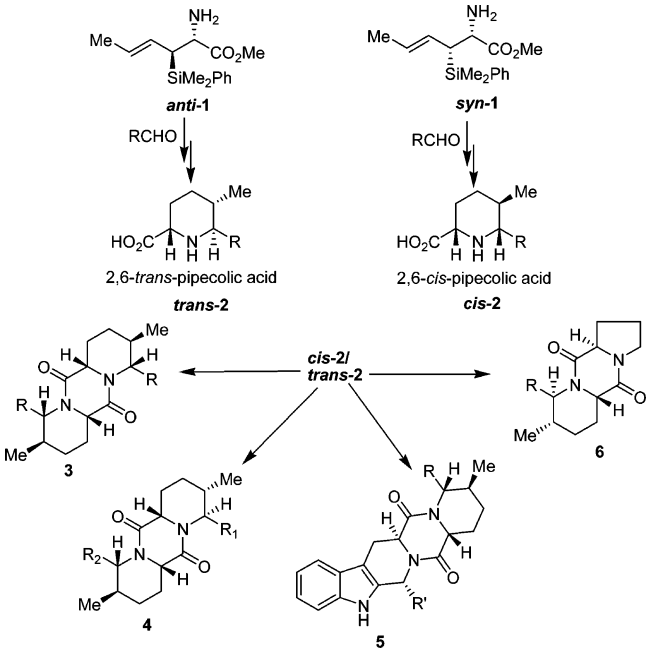
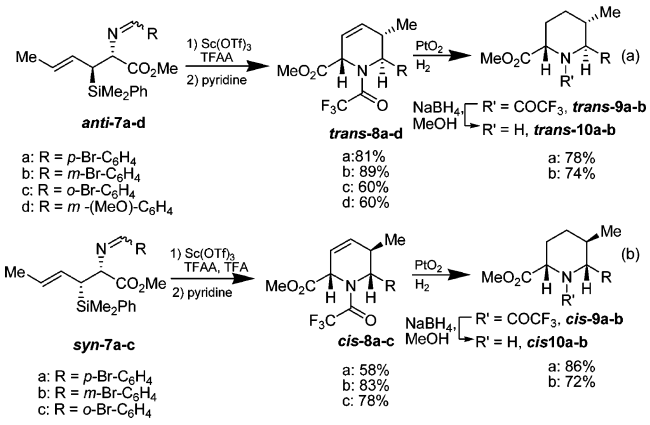


FIGURE 2. Synthetic approach toward complex DKPs.

and stereochemical variation. Herein, we report the convergent synthesis of diketopiperazines derived from highly complex pipecolic acid cores as well as parallel screening of the complex DKPs against a panel of G-protein-coupled receptors (GPCRs).

Our strategy for the convergent synthesis of complex diketopiperazines is outlined in Figure 2. Intramolecular cyclization of the imine derived from a 2,3-*anti* aminosilane (***anti*-1**) and an aldehyde, followed by further elaboration, should provide access to 2,6-*trans*-pipecolic acids, ***trans*-2**. In a similar manner, beginning with 2,3-*syn*-aminosilane, (***syn*-1**), the corresponding 2,6-*cis*-pipecolic acids, ***cis*-2**, may be obtained. Use of bromobenzaldehydes in the initial imine formation step with **1** permits the synthesis of diverse pipecolate esters by subsequent cross-coupling. The derived pipecolic acids may be homo- or heterodimerized to access complex DKPs.[6] We wished to evaluate the feasibility of direct cyclodimerization[7] or cyclooligomerization[8] of pipecolic acids, or pipecolic acids with other amino acids, for convergent access to diverse complex DKPs. For example, homo- or heterodimerization of pipecolic acids may lead to DKPs of general structures **3** and **4**, respectively,

SCHEME 1. Sc(OTf)$_3$-Catalyzed Cyclization to Pipecolate Esters



while heterodimerization of pipecolic acids with other α-amino acids (e.g., *β*-carbolines and proline) could result in diverse DKPs of general structures **5** and **6**, respectively. In addition, stereochemical diversity[9] of the complex DKPs may be obtained by variation of diastereomeric and enantiomeric relationships in the corresponding pipecolic acid core structures.

Results and Discussion

Development of a Catalytic System for Aza-annulation. Our initial protocol[5] for conducting intramolecular crotylation of imines **7** required 2−4 equiv of $TiCl_4$ at −78 °C. The excessive amounts of titanium tetrachloride led to thick emulsions of titanium oxide at the workup stage on larger scales. Although standard protocols can be employed to break such emulsions, the tedious workup was found to be troublesome both on larger scale and during parallel synthesis efforts. To overcome this limitation and in an effort to identify a catalytic aza-annulation process, we evaluated a number of transition and rare-earth metal based Lewis acids[10] for promoting room temperature cyclization of imine ***anti*-7a** (derived from condensation of ***anti*-1** and *p*-bromobenzaldehyde). Initial screening using 1 equiv of Lewis acid[11] indicated that Sc(OTf)$_3$ was effective for promoting intramolecular cyclization of *anti*-imino silane ***anti*-7a**. However, use of catalytic amounts of Sc(OTf)$_3$ (0.2 equiv) resulted in incomplete conversion. After further optimization, we found that a combination of trifluoroacetic anhydride (TFAA, 1.2 equiv) and Sc(OTf)$_3$ (0.2−0.5 equiv), followed by addition of pyridine (2.5 equiv) upon reaction completion, enhanced the yield of product ***trans*-8a** (Scheme 1a).[12]

Control experiments with only TFAA showed no reactivity in the aza-annulation. Although a detailed understanding of this

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(11) The following Lewis acids were surveyed and found to be less effective: Bi(OTf)$_3$, La(OTf)$_3$, Eu(OTf)$_3$, $HfCl_4$, Zr(O*i*Pr)$_4$, Dy(OTf)$_3$, La(OTf)$_3$, and Yb(OTf)$_3$.

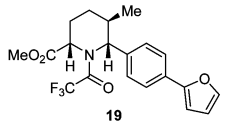
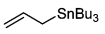
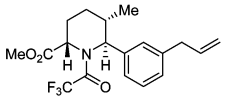
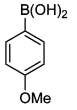
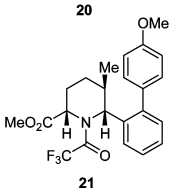
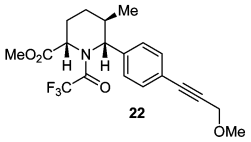
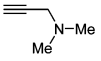
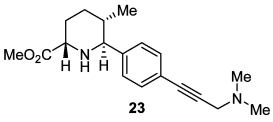
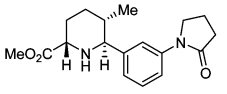
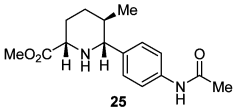
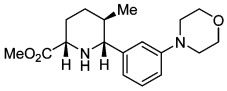
(12) See Supporting Information for complete experimental details.

catalytic system awaits further experiments, we presume that TFAA plays a dual role to both activate scandium triflate and acylate the initially formed product to promote catalytic turnover. Scandium triflate has been reported to act as an effective catalyst for acylation of alcohols, and the complex between scandium and acid anhydride is proposed to be an intermediate.[13] Following this protocol, imines ***anti*-7b−d** also cyclized efficiently to the corresponding tetrahydropyridines ***trans*-8b−d**.

In contrast to the *anti*-imino silane series, cyclization of the corresponding *syn*-imino silanes proved to be more challenging, underscoring the subtle effect of relative stereochemistry on the cyclization. For example, when imino silane ***syn*-7a** was subjected to the aforementioned annulation protocol described above, less than 30% of the cyclized product was isolated with disappointing diastereoselectivity (***cis*-8a**: C-2 epimer of ***cis*-8a** = ∼1:1) (cf. Scheme 1b). After extensive screening of additives, we found that addition of trifluoroacetic acid (0.4 equiv) improved the yield and diastereoselectivity for the aza-annulation process. Imino silanes ***cis*-8a−c** were cyclized in the presence of $Sc(OTf)_3$ (0.4 equiv), TFAA (1.2 equiv), and TFA (0.4 equiv) (Scheme 1b). Initial results are consistent with a reaction process which may be catalyzed by a combination of Lewis and Brønsted acids.[14] Finally, tetrahydropyridines **8** were subjected to hydrogenation with PtO_2 (Adam's catalyst) in ethyl acetate to afford *N*-acyl methyl pipecolate building blocks **9** in high yield. Treatment of **9** with $NaBH_4$ at 0 °C cleanly provided pipecolate methyl esters **10** without over-reduction of the methyl ester moiety.[15]

Diversification of Pipecolate Scaffolds. With both the *cis* and *trans* building blocks accessible, diversification was next undertaken. We utilized the arylbromide functionality in the building blocks as a handle to append alkynes,[16] arenes,[17] amines,[18] and amides[19] using various strategies (Table 1). For example, when ***cis*-9a** was subjected to a Stille reaction[17] with furyl stannane **11**, the coupled product **19** was readily formed (entry 1). However, *ortho*-bromoaryl building blocks **8c** showed poor reactivity in most coupling reactions presumably due to steric hindrance. On the other hand, *para*- and *meta*-bromo-substituted building blocks showed high reactivity, affording complete conversion in all cases examined. However, with *N*-trifluoroacetyl-protected pipecolates **9**, epimerization (20−50%) of the stereocenter adjacent to the ester was a significant problem during cross-couplings.[20] Interestingly, when pipecolates **10** containing a free amine were employed, cross-couplings to afford substituted pipecolates proceeded without epimerization.

TABLE 1. Diversification of Pipecolic Esters by Cross-Coupling

entry	aryl bromide	cross-coupling partner	Product[a]
1[b]	*cis*-9a	 **11**	 **19**
2[b]	*trans*-9b	 **12**	 **20**
3[c]	*cis*-9c	 **13**	 **21**
4[d]	*cis*-9a	 **14**	 **22**
5[d]	*trans*-10a	 **15**	 **23**
6[e]	*trans*-10b	 **16**	 **24**
7[e]	*cis*-10a	 **17**	 **25**
8[f]	*cis*-10b	 **18**	 **26**

[a] Crude products from this step were directly submitted to ester hydrolysis by "catch and release"; combined yield for the two steps is reported in Figure 3. [b] Tributylstannane (1.2 equiv), $Pd(PPh_3)_4$ (10%), THF (0.3 M), microwave, 100 °C, 30 min, 150 W. [c] Boronic acid (2.0 equiv), $Pd_2(dba)_3$ (10%), 2-(dicyclohexylphosphino)biphenyl (20%), K_3PO_4 (2 equiv), toluene (3.0 equiv), 90 °C, 15 h. [d] Alkyne (2.5 equiv), $Pd(PPh_3)_4$ (10%), PPh_3 (20%), CuI (10%), Et_3N/DMF (v/v, 1/2, 0.3 M), 90 °C, 15 h. [e] Amide (1.2 equiv), CuI (20%), *N*,*N*′-dimethyl-1,2-ethanediamine (40%), K_2CO_3 (2 equiv), toluene (0.5 M), 100 °C, 15 h. [f] Amine (1.5 equiv), $Pd_2(dba)_3$ (5%), 2-(dicyclohexylphosphino)biphenyl (20%), K_3PO_4 (2 equiv), DME (0.5 M), 100 °C, 15 h.

This significant difference is likely due to the decreased acidity of the proton on the stereocenter α to the methyl ester when the amine is not protected as trifluoroacetamide, which makes the substrates less vulnerable to deprotonation under basic conditions. Alternatively, differences in epimerization may be a result of differences in barriers for interconversion of conformers in the free amines **10** and the *N*-trifluoroacetylated amines **9**. Crude products from cross-coupling reactions were directly subjected to ester hydrolysis by a "catch and release"

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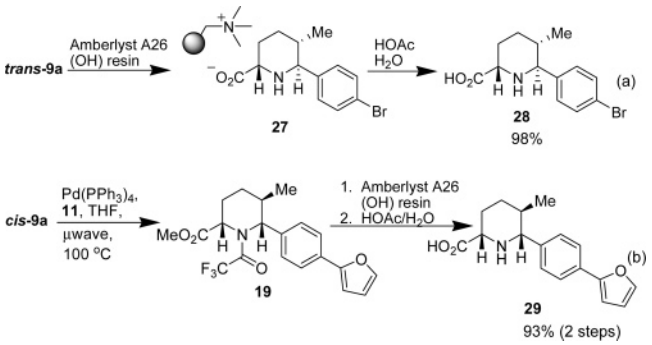
SCHEME 2. Resin "Catch and Release" approach for Pipecolic Acid Synthesis



process that also enabled product purification (vide infra). Product yields were determined after this purification protocol.

A Resin "Catch and Release" Approach for Pipecolic Acid Synthesis and Purification. Amino acids typically require laborious chromatographic purification using highly polar solvent systems.[21] Our convergent approach to complex DKPs required access to numerous pipecolic acid building blocks in high diastereo- and enantioenriched form. Accordingly, we considered developing a "streamlined"[22] approach to pipecolic acids using a resin catch and release strategy (Scheme 2).[23]

After considerable experimentation, we found that Amberlyst A26 hydroxide resin[24] hydrolyzes the pipecolate methyl ester while simultaneously effecting deprotection of the trifluoroacetamide. The liberated pipecolic acid is concomitantly captured by the basic resin. For example, *N*-trifluoroacetylated methyl pipecolate ***trans*-9a** was treated with Amberlyst A26 hydroxide resin in a mixture of methanol, THF, and water. After shaking the mixture for 10 h, the resin was filtered and washed with a solution of acetic acid in water (v/v 1:1) to afford the pipecolic acid **28** in quantitative yield and excellent purity. This catch and release process was conducted directly on the crude reaction mixtures for pipecolate scaffolds that were subjected to cross-coupling reactions. For example, ***cis*-9a** was subjected to Stille reaction with stannane **11** to provide coupled product **19**. The crude product **19** was subjected to Amberlyst A26 hydroxide resin, and the liberated pipecolic acid was captured by the basic resin. The impurities were then removed by washing the resin with THF and methylene chloride. The free acid **29** was then released from the resin by further washing it with acetic acid: water (v/v 1:1). Representative structures and yields of the pipecolic acids synthesized by this process are shown in Figure 3. Thus, this catch and release process not only enables the purification of crude products from cross-coupling reactions but also provides a convenient approach to hydrolyze *N*-trifluoroacetylated amino esters and related compounds that is likely to have further applications in complex synthesis.

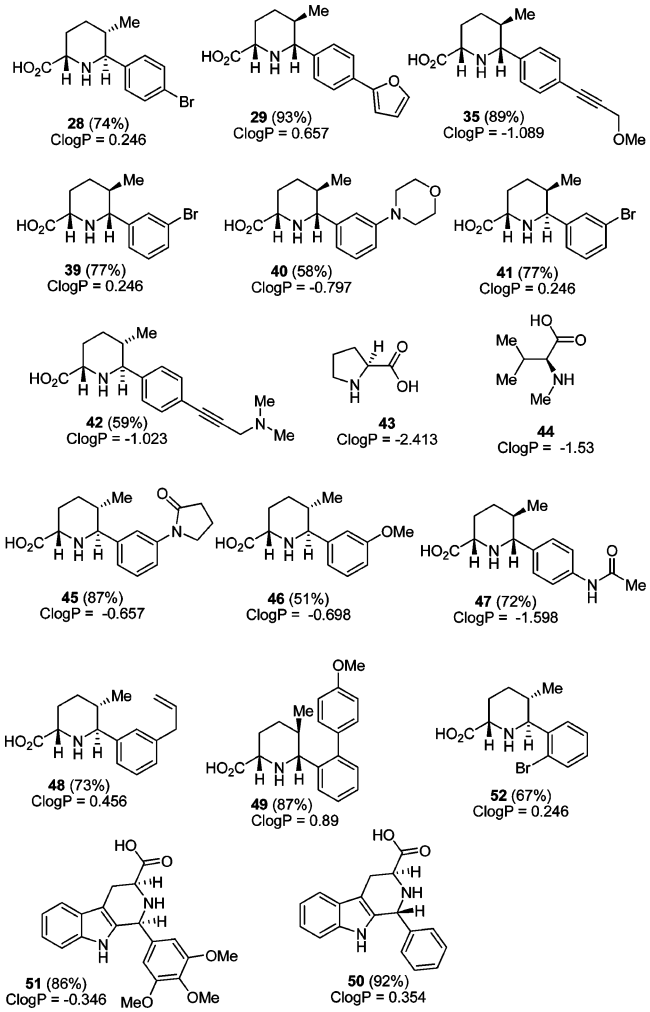


FIGURE 3. Pipecolic acids used for cyclodimerization and their *C*(log *P*) values. Yields for β-carbolines and pipecolic acids are after one and two steps, respectively. See Supporting Information for details.

Methodology Development for Complex Diketopiperazine Synthesis. A typical approach for synthesis of diketopiperazines involves cyclization of the corresponding dipeptides.[3b] Our preliminary studies showed that the highly hindered 2,6-disubstituted structure of the pipecolic acids created difficulties for a stepwise coupling process (Scheme 3). For example, all attempts to acylate ***trans*-9a** with a Boc-protected amino acid (for example, Boc-protected phenylalanine) provided less than 25% yield of the desired dipeptide product **30**, presumably due to steric encumbrance of the *trans*-pipecolate core.[25] In addition, subsequent Boc deprotection of the dipeptide with TMSOTf[26] afforded only the methyl pipecolate ***trans*-9a**.

On the other hand, pipecolic acid **28** successfully reacted with another amino acid methyl ester in the presence of the free amine. Hydrolysis with LiOH afforded the dipeptide acid **31** in moderate yield. However, subsequent cyclization provided the diketopiperazine **32** as a mixture of two diastereomers (1:1) by epimerization presumably as a result of active ester formation.[27]

(21) Mota, A. J.; Castellanos, E.; Juaristi, E. *Org. Prep. Proc. Int.* **2003**, *35*, 414.

(22) For a comprehensive review of polymeric reagents and scavengers, see: Ley, S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815.

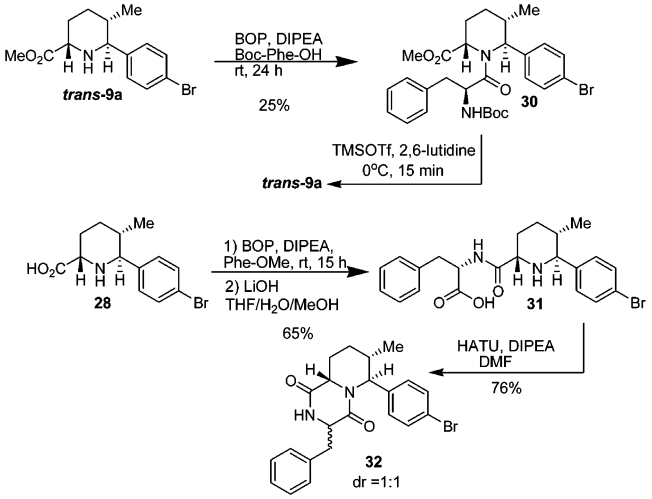
(23) (a) Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2574. (b) Siegel, M. G.; Hahn, P. J.; Dressman, B. A.; Fritz, J. E.; Grunwell, J. R.; Kaldor, S. W. *Tetrahedron Lett.* **1997**, *38*, 3357. For reviews on catch and release using polymer resins, see: (c) Kirschning, A.; Monenschein, H.; Wittenberg, R. *Chem.—Eur. J.* **2000**, *6*, 4445. (d) Thompson, L. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 324. (d) Kirschning, A.; Wittenberg, R. *Organic Synthesis Highlights V* **2003**, 265.

(24) (a) Reed, L. A., III; Risbood, P. A.; Goodman, L. *J. Chem. Soc., Chem. Commun.* **1981**, *15*, 760. (b) Kulkarni, B.; Ganesan, A. *Chem. Commun.* **1998**, *7*, 785. (c) Morwick, T. M. *J. Comb. Chem.* **2006**, *8*, 649.

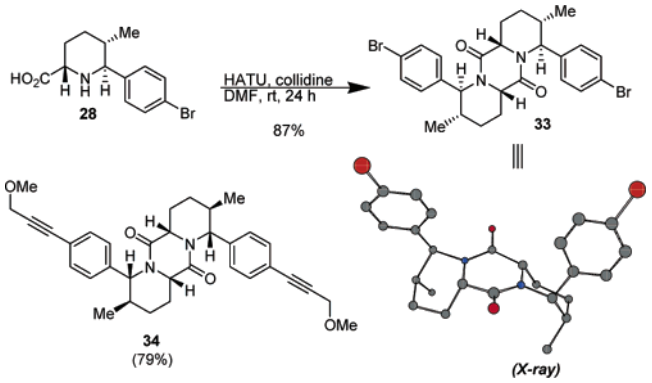
(25) Sebahar, P. R.; Osada, H.; Usui, T.; Williams, R. W. *Tetrahedron* **2002**, *58*, 6311.

(26) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870.

SCHEME 3. Attempted Stepwise Diketopiperazine Synthesis

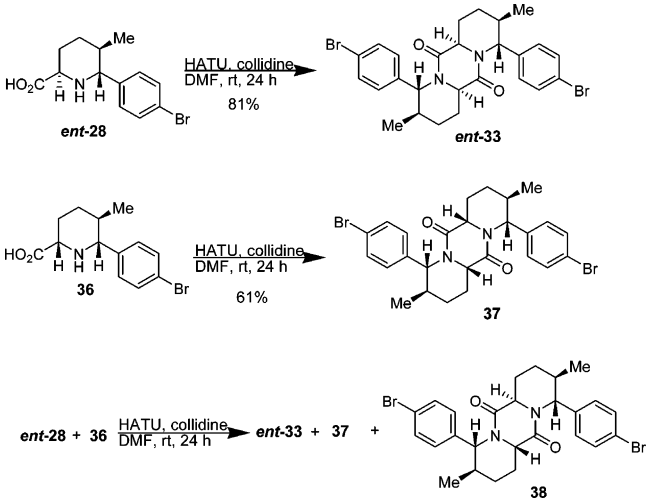


SCHEME 4. Homodimerization of Pipecolic Acids



In light of the problems experienced with stepwise coupling, we turned our attention to a one-pot cyclodimerization process to prepare complex diketopiperazines without relying on a protection−deprotection sequence.[28] Treatment of pipecolic acid **28** with BOP and DIPEA afforded diketopiperazine **33** in 50% yield. Other coupling conditions were tested (e.g., ACTU, HATU), and the best result was obtained when the combination of HATU[29] and collidine was used in DMF as solvent (87% yield; Scheme 4). X-ray crystal structure analysis confirmed the structure of homodimer **33**. The solid state structure of **33** showed a diaxial arrangement of the aryl and methyl groups likely due to $A^{1,3}$ strain with the cyclic amide.[30] In a similar manner, homodimer **34** was prepared in 79% yield from the corresponding pipecolic acid **35** (cf. Figure 3). Isolation of DKP **30** in 87% yield and rigorous structural verification by X-ray crystal structure analysis indicates that the HATU-mediated dimerization of the pipecolic acids to DKPs does not suffer from epimerization of the α-stereocenter.

SCHEME 5. Control Experiments to Probe for Epimerization



To confirm this hypothesis, we homodimerized pipecolic acids ***ent*-28**, its C2 epimer **36**, and heterodimerized a 1:1 mixture of ***ent*-28** and **36** (Scheme 5). Homo-cyclodimerizations of the *trans*- (***ent*-28**) and the *cis* (**36**)-pipecolic acids provided the corresponding DKPs ***ent*-33** and **37** in 81 and 61% isolated yields, respectively, after purification by preparative HPLC.[12,31] Heterodimerization of a mixture of ***ent*-28** and **36** produced the homodimers ***ent*-33** and **37** along with the heterodimer **38**.

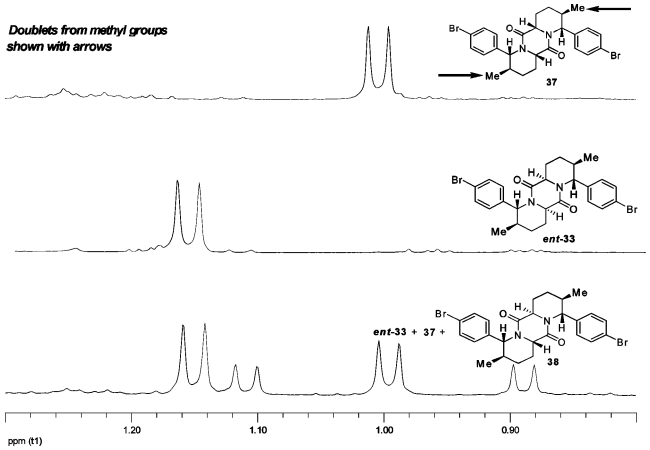


FIGURE 4. Expanded crude ^{1}H NMR spectra of homo- and heterodimerizations of ***ent*-28** and **33**.

Expanded segments (1.3−0.8 ppm) of the ^{1}H NMR spectra from the crude reaction mixtures of all the three dimerizations are shown in Figure 4. This region of the proton NMR spectrum shows the doublet resonances from the 2° methyl groups (see arrows in Figure 4). The methyl resonances of ***ent*-33** and **37** in the ^{1}H NMR spectrum of the crude reaction mixture from heterodimerization of ***ent*-28** and **36** can be readily identified by comparison to authentic spectra. By elimination, the methyl resonances of **38** were identified as the doublets at 1.12 and 0.90 ppm. These two resonances were absent in the ^{1}H NMR

(27) For epimerization during peptide segment coupling, see: (a) Sieber, P.; Kamber, B.; Hartman, A.; Johl, A.; Riniker, B.; Rittel, W. *Helv. Chim. Acta* **1977**, *60*, 27. (b) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: Berlin, 1984.

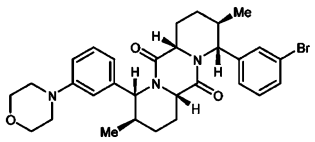
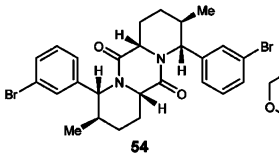
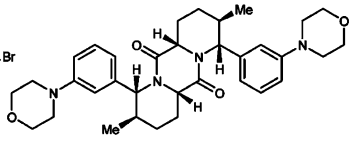
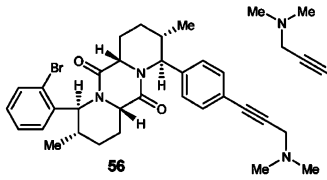
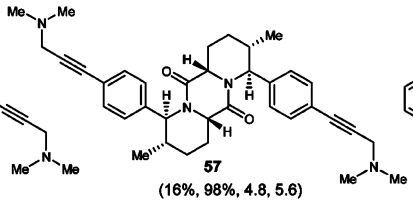
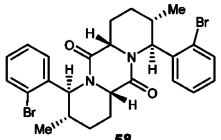
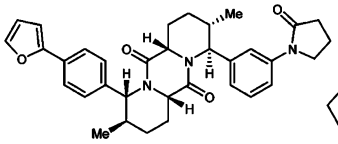
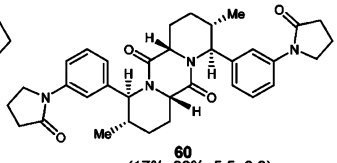
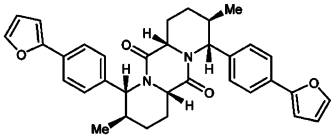
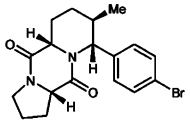
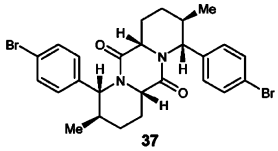
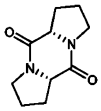
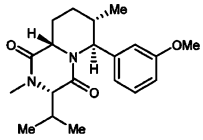
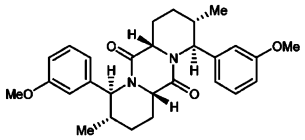
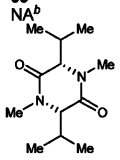
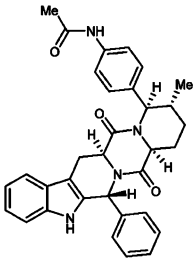
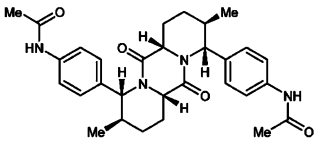
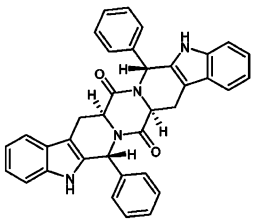
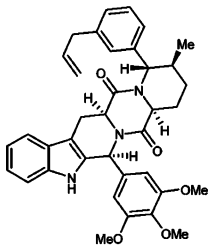
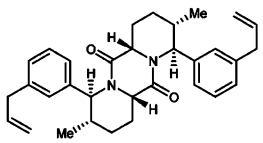
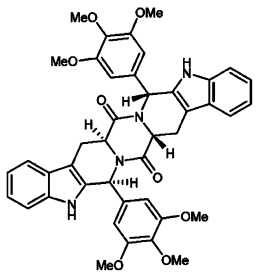
(28) For an example of DKP synthesis by coupling of unprotected amino acids, see: Santagada, V.; Fiorino, F.; Perissutti, E.; Severino, B.; Terracciano, S.; Cirino, G.; Caliendo, G. *Tetrahedron Lett.* **2003**, *44*, 1145.

(29) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1995**, *60*, 3561. (c) Humphrey, J. M.; Aggen, J. B.; Chamberlin, A. R. *J. Am. Chem. Soc.* **1996**, *118*, 11759. (d) Ohshima, T.; Gnanadesikan, V.; Shibuguchi, T.; Fukuta, Y.; Nemoto, T.; Shibasaki, M. *J. Am. Chem. Soc.* **2003**, *125*, 11206.

(30) Hoffmann, R. W. *Chem. Rev.* **1989**, *89*, 1841.

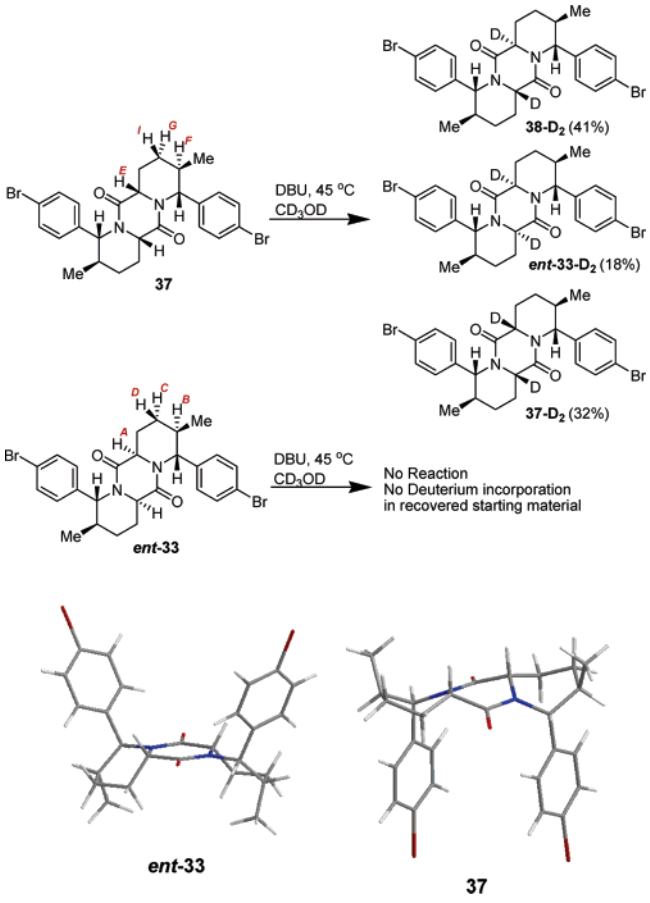
(31) (a) Blom, K. F.; Sparks, R.; Doughty, J.; Everlof, G. J.; Haque, T.; Combs, A. P. *J. Comb. Chem.* **2003**, *5*, 670. (b) Rosentreter, U.; Huber, U. *J. Comb. Chem.* **2004**, *6*, 159.

TABLE 2. Representative DKPs Produced by Cyclodimerization (yield, purity, *C*(log *P*), retention time (min))[a]

entry	heterodimer	homodimer	homodimer
1	 **53** (25%, 98%, 6.2, 7.9)	 **54** (16%, 98%, 7.3, 8.7)	 **55** (14%, 98%, 5.2, 7.0)
2	 **56** (23%, 98%, 6.9, 1.8)	 **57** (16%, 98%, 4.8, 5.6)	 **58** (14%, 98%, 7.3, 8.3)
3	 **59** (31%, 98%, 6.8, 7.9)	 **60** (17%, 98%, 5.5, 6.8)	 **61** (13%, 98%, 8.1, 8.6)
4	 **62** (22%, 98%, 3.7, 4.3)	 **37** (13%, 98%, 7.3, 7.8)	 **63** NA[b]
5	 **64** (39%, 98%, 3.8, 6.2)	 **65** (26%, 98%, 5.4, 7.6)	 **66** NA[b]
6	 **67** (39%, 98%, 3.8, 6.2)	 **68** (12%, 98%, 3.6, 7.8)	 **69** (7%, 98%, 5.5, 9.1)
7	 **70** (25%, 98%, 5.9, 6.8)	 **71** (10%, 98%, 7.7, 7.4)	 **72** (7%, 98%, 4.1, 6.5)

[a] Products were isolated from crude reaction mixtures by preparative HPLC with MS/ELSD-triggered fraction collection. The numbers in parentheses denote isolated yield, HPLC purity, *C*(log *P*) and retention time in analytical HPLC (5:95 acetonitrile/water to 95:5 acetonitrile/water in 5 min; run time = 10 min), respectively. [b] Not detected by ELSD, therefore not collected.

SCHEME 6. Reactivity of *cis-cis* (37) and *trans-trans* (*ent*-33) DKPs toward DBU-Mediated Epimerization Conditions



spectra of the crude ***ent*-33** and **37**, indicating that the HATU-mediated cyclodimerization proceeded with negligible epimerization.

Synthesis of Complex DKPs by Hetero-cyclodimerization of Pipecolic Acids. Having established a route to the dimerization of pipecolic acids that proceeds without epimerization, we carried out cyclodimerizations to access complex DKPs. Hetero-cyclodimerization typically affords a statistical mixture of homo- and heterodimers.[7] We wished to design a heterodimerization array affording products readily separated by reverse-phase HPLC. Thus, we selected 17 α-amino acid monomers comprised of pipecolic acids ($n = 13$), β-carbolines ($n = 2$), (*S*)-proline **43**, and (*S*)-*N*-methyl valine **44** (Figure 3). We anticipated that monomer pairings with a sufficient difference in *C*(log *P*) should result in homo- and heterodimers with corresponding differences in log *P* and HPLC retention time.[32] Monomer pairs were incubated using the standard reaction conditions optimized for homodimerization (cf. Scheme 3). Each reaction mixture was worked up and evaluated by analytical LC/MS/ELSD.[12]

In all cases, separations predicted by *C*(log *P*) values[32] were observed. Reaction mixtures were subsequently purified by preparative HPLC/MS/ELSD to afford pure homo- and heterodimers. In general, the ratio of three dimers formed in each cyclodimerization was roughly 1:2:1 with the heterodimer as the major product. Alternating the stereochemical combinations of the monomers, such as *cis*/*cis*, *cis*/*trans*, and *trans*/*trans* (entries 1−3, Table 2), did not noticeably change the product distribution. Notably, dimerization of pipecolic acid with relatively less hindered proline or *N*-methyl valine proceeded in the similar manner, affording both heterodimeric and homodimeric DKPs. Formation of homodimers **60** and **63** was observed by crude ^{1}H NMR analysis, but these compounds were not collected during HPLC purification. All other dimers were successfully purified with the total yield varying from 35 to 65%, and each product isolated with a minimum purity of 98% based on LC/ELSD analysis.

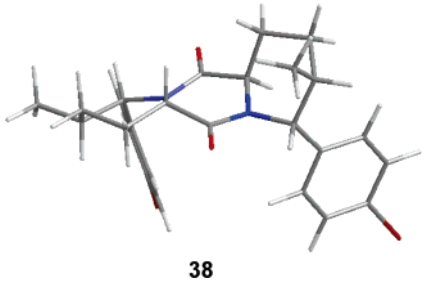


FIGURE 5. Calculated ground state structure of *cis-trans* DKP **38**.

Reactivity and Conformational Analysis of DKPs. Having synthesized a collection of DKPs with considerable structural and stereochemical variation, we were interested in exploring the chemical reactivity of the stereochemically well-defined DKPs. To this end, we subjected the DKPs ***ent*-33** and **37** to typical enolization conditions (KHMDS or LiHMDS) and attempted alkylation of the resulting enolate with a number of electrophiles (allyl iodide, allyl bromide, methyl triflate, and methyl iodide).[33] In some experiments, trace amount of products was detected by LC-MS analysis. However, despite significant efforts, the yields of alkylated products could not be improved to satisfactory levels. During these investigations, we observed that, under strongly basic conditions, *cis-cis* DKP **37** underwent decomposition (presumably through the enolate), while the *trans-trans* DKP ***ent*-33** was stable. To further investigate the differences in enolization of stereoisomeric DKPs, we subjected the *cis-cis* DKP **37** and the *trans-trans* DKP ***ent*-33** to DBU in CD_3OD at 45 °C for 6 h (Scheme 6).[34] The *cis-cis* DKP **37** gave the *cis-trans* DKP **38-D$_2$**, *trans-trans* DKP ***ent*-33-D$_2$**, and the *cis-cis* DKP **37-D$_2$** in 41, 18, and 32% isolated yields, respectively.[12] However, the *trans-trans* DKP ***ent*-33** did not undergo any reaction under these conditions, and the recovered starting material had no deuterium incorporation confirming that the *trans-trans* DKP did not undergo enolization. To understand the differences in reactivity, we carried out conformational searches of DKPs ***ent*-33** and **37** using a molecular mechanics force field.[35] Ground state conformations of the two stereoisomeric DKPs are shown in Scheme 6. The following observations

(32) (a) Hanai, T.; Koizumi, K.; Kinoshita, T. *J. Liq. Chrom. Relat. Technol.* **2000**, *23*, 363. (b) Djakovic-Sekulic, T.; Acanski, M.; Perisic-Janjic, N. *J. Chromatogr. B* **2001**, *766*, 67. (c) Xing, L.; Glen, R. C. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 796. (d) Eros, D.; Kovesdi, I.; Orfi, L.; Novak-Takacs, K.; Acsady, G.; Keri, G. *Curr. Med. Chem.* **2002**, *9*, 1819.

(33) For a recent publication describing enolization/alkylation of DKPs, see: Davies, S. G.; Rodriguez-Solla, H.; Tamayo, J. A.; Cowley, A. R.; Concellon, C.; Garner, A. C.; Parkes, A. L.; Smith, A. D. *Org. Biomol. Chem.* **2005**, *3*, 1435.

(34) For an example of DBU-mediated epimerization of an α-alkoxy ester, see: Kadota, I.; Kadowaki, C.; Yoshida, N.; Yamamoto, Y. *Tetrahedron Lett.* **1998**, *39*, 6369.

(35) All calculations were performed using *Spartan 04* for Windows; Wavefunction: Irvine, CA.

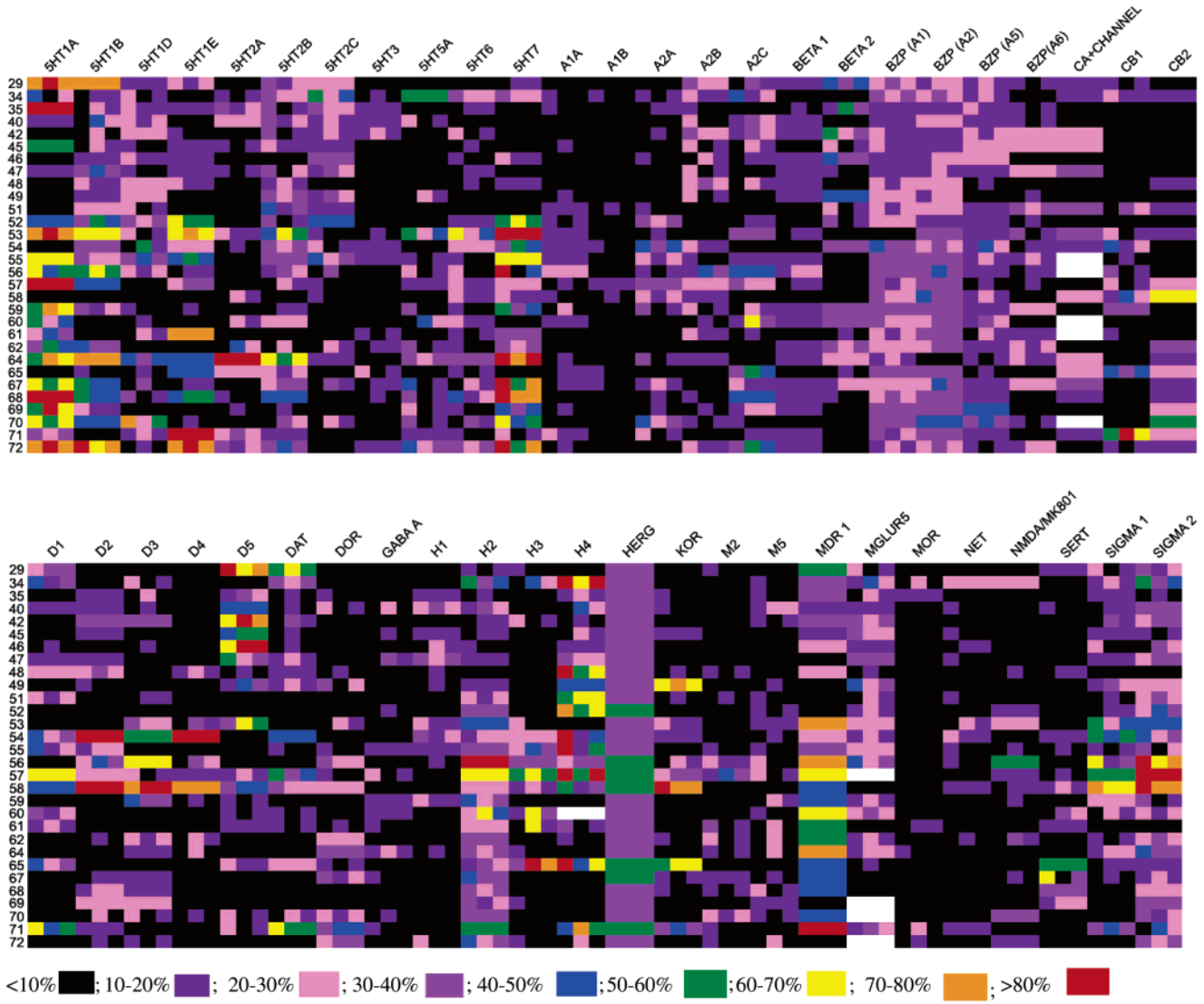


FIGURE 6. Results of the initial screen (% inhibition or activation) in the form of a heat map. Compound numbers and the names of molecular targets are shown along *Y*- and *X*-axes, respectively.

were made from the calculated ground state conformations of the two DKPs which were later supported experimentally. The exterior rings of the *trans-trans* DKP ***ent*-33** adopt chair-like conformations, and the dihedral angle between the C−H^A bond (cf. structure of ***ent*-33** in Scheme 6) and the adjacent carbonyl group is 57°, which is far from the ideal angle of 90° required for enolization.

This calculated dihedral angle deviates only 9° from the observed dihedral angle of 48° in the X-ray crystal structure of enantiomer **33**.[12] On the other hand, calculations suggest that the exterior rings of *cis-cis* DKP **37** adopt boat-like conformations, and the dihedral angle between the C−H^E bond (cf. structure of **37** in Scheme 6) and the adjacent carbonyl group measures 97°, which is reasonable for an effective overlap of the σ^* of the C−H^E bond and the π orbitals of the carbonyl group to promote enolization.

To demonstrate that these calculated structures are relevant under solution-phase reaction conditions, we performed ^{1}H NMR experiments to measure the relevant three-bond coupling constants. If the *cis-cis* DKP **37** adopts a boat-like conformation for the exterior rings, one medium and one large coupling constants are expected when H^F couples to H^I and H^G (H^F−C−C−H^I and H^F−C−C−H^G dihedral angles are 119 and 2.9°, respectively).[36] The observed coupling constants between H^F−H^I and H^F−H^G were 4.3 and 13.3 Hz, supporting the calculated structure. In a similar manner, if the *trans-trans* DKP ***ent*-33** adopts a chair-like conformation for the outer rings, one would expect both H^B−H^C and H^B−H^D coupling constants to be medium/small (H^B−C−C−H^C and H^B−C−C−H^D dihedral angles are 43 and 73°, respectively). Once again, the observed coupling constants (4.4 and 4.3 Hz) are consistent with the predicted dihedral angles.

We also carried out a conformational search of the *cis-trans* DKP **38** using the molecular mechanics force field;[35] the calculated ground state conformation is shown in Figure 5. The exterior six-membered ring with 2,6-*cis* (pipecolic acid numbering) disubstitution adopts a half-chair-like conformation, while the other exterior ring with the 2,6-*trans* disubstitution adopts a chair-like conformation. The loss of symmetry is also apparent

(36) http://www.spectroscopynow.com/FCKeditor/UserFiles/File/specNOW/HTML%20files/proton-proton2.htm (accessed August 23, 2006).

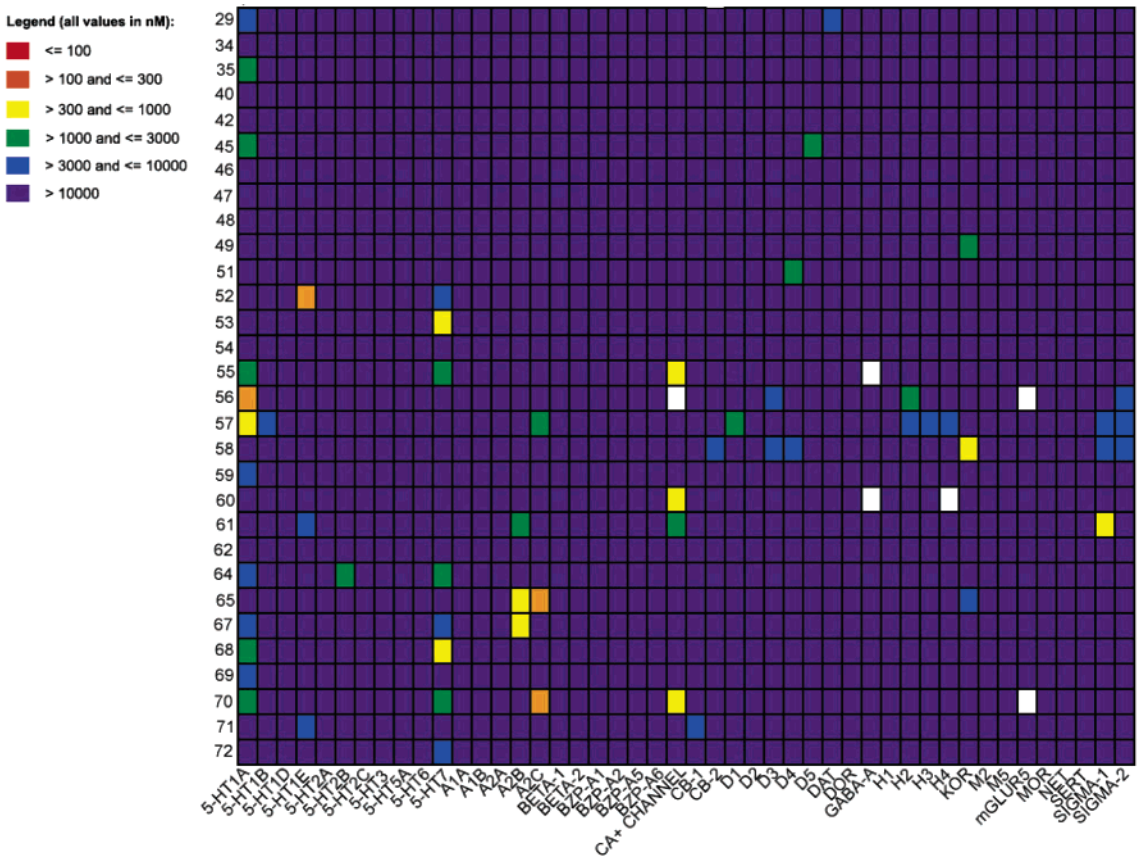


FIGURE 7. K_i values. Compound numbers and the names of molecular targets are shown along *Y*- and *X*-axes, respectively.

from the proton NMR spectrum of this compound. For example, the two doublets corresponding to the methyl resonances appear at 1.12 and 0.90 ppm. In any event, our initial studies indicate that stereochemical variations have a profound effect on the three-dimensional conformations of complex DKPs which is reinforced by their chemical reactivity toward enolization.

Parallel Screening of Complex DKPs against GPCR Targets. We and others have recently proposed that the massively parallel screening of chemical libraries against large numbers of molecular targets should assist in the rapid identification of novel chemical probes for biological processes.[37−40] Ideally, one would like to screen novel chemical scaffolds against every "druggable target" in the genome in a massively parallel process, but the technology does not currently exist which would allow such parallel screening against all conceivable druggable targets. Instead, we have suggested focusing on that portion of the proteome encoding receptors (e.g., the "receptorome") for the identification of both novel and useful chemical probes.[37] Using this approach, we have discovered and validated an extraordinary diversity of molecular targets for biological phenomena, including viral entry,[41] hallucinogenic drug actions,[42] and drug-induced cardiac valvulopathy.[43−45] Because G-protein-coupled receptors (GPCRs) continue to represent targets for therapeutic drug discovery, and because they are tractable to parallel screening efforts, we have focused our efforts on screening this portion of the receptorome for molecular and biological probe development. On the basis of the efficiency of this method for molecular probe development, we attempted a screen of the complex diketopiperazines against a large panel of molecular targets, including GPCRs, ion channels, and transporters. Diketopiperazines are an attractive group of compounds for such screening as prior studies have discovered that they can have a diversity of biological actions. These include their use as potential oxytocin receptor antagonists,[46] as purinergic P2Y receptor antagonists,[47] and as neuroprotective

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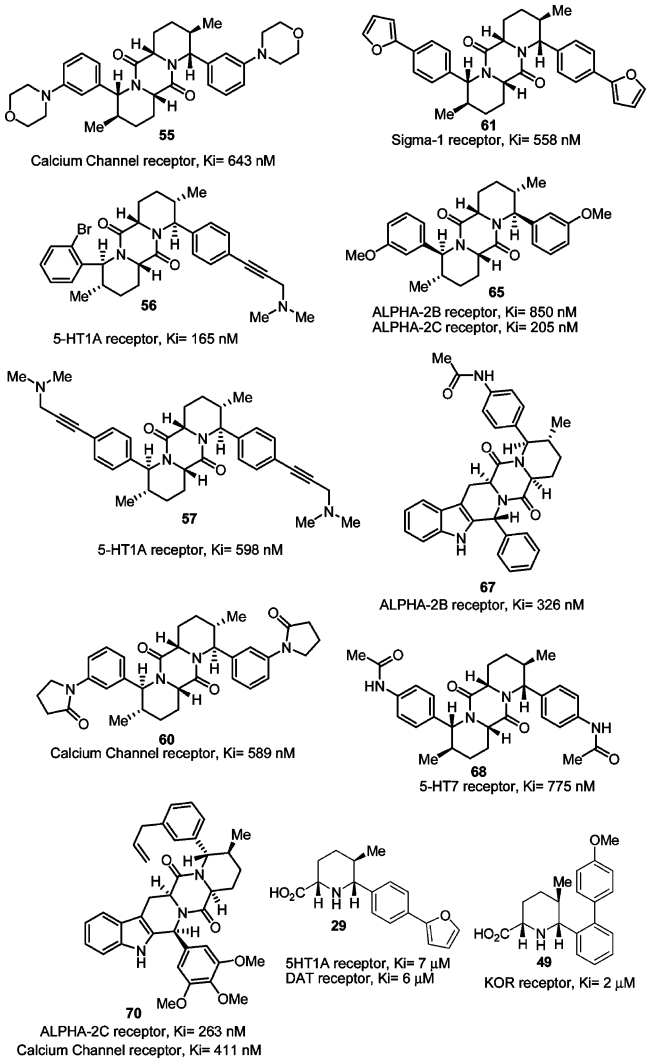

FIGURE 8. Structures of select compounds exhibiting biological activity.

compounds.[48] Indeed, one of us (B.L.R.) has previously reported in collaboration with others that a novel diketopiperazine enhances motor and cognitive recovery following traumatic brain injury,[49] although the molecular target for this compound is unknown. Accordingly, we screened the complex diketopiperazines produced in the present study against a large collection of molecular targets. We report the discovery that many of these compounds show apparent selectivity for selected molecular targets. Importantly, these discoveries would not likely have been made in an efficient manner using conventional techniques of screening wherein single molecular targets are probed with libraries of hundreds of thousands of chemically diverse compounds.

For these studies, the resources of the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH−PDSP) were used essentially as previously detailed.[42,50−52] In

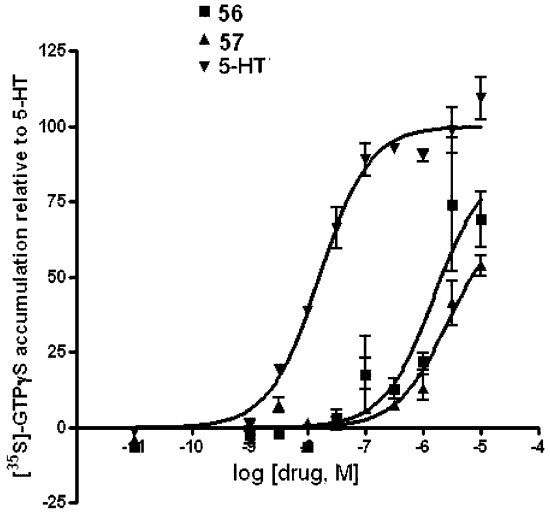

FIGURE 9. Representative ^{35}S-GTPγS binding curve for 5-HT1A receptor-active compounds.

brief, a large collection of cloned human molecular targets were individually expressed and assays devised for molecular target-based screening[42,53] with initial screens performed at a final concentration of 10 μM in quadruplicate. Complete protocols for the assays have been previously published and are also available online (http://pdsp.case.edu/). The results of the initial screens are shown in Figure 6 wherein the extent of activity (e.g., inhibition or activation) is graphically displayed as a "heat map".[12] Where significant activity was measured, secondary screens were performed wherein K_i values were calculated using test compound concentrations from 1 to 10 000 nM and calculated using GraphPad Prizm 4.0 (Figure 7). Compound numbers and names of the molecular targets are shown along *Y*- and *X*-axes, respectively.

Structures of selected compounds with high biological activity are shown in Figure 8. Of note in these initial studies is the apparent influence of the stereochemistry of the DKP core on selectivity for particular GPCR receptors.[54] For example, hybrid DKPs derived from combination of complex pipecolic acids and β-carbolines (e.g., **67** and **70**) show selectivity for α-receptors. The *cis-cis* DKPs **55**, **61**, and **68** show preferences for 5HT-7, calcium channel, and σ-receptors with selectivity dependent on aryl ring modifications. The *trans-trans* DKPs, such as **56** and **57**, show preferences for 5-HT1A and KOR receptors. Some complex pipecolic acids also exhibited modest biological activity against GPCR targets. For example, pipecolic acids **29** (Figure 8), **45** and **35** (Figure 3), showed low micromolar activity against the 5HT1A receptor, while the 2-bromophenyl-substituted acid **52** (Figure 3), showed high affinity for 5HT1E and 5HT7 receptors. Interestingly, the 2,6-*cis*-pipecolic acid **49** with the electron-rich biphenyl substituent

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was selective for the KOR receptor with a binding constant of 2 μM. For selected compounds, tertiary screens were performed wherein the ability of the compounds to activate selected molecular targets via ^{35}S-GTPγS binding was measured.

Of note, **56** and **57** (Figure 8), were partial agonists at 5-HT1A receptors with EC_{50} values of 2.5 and 1.5 μM, respectively (Figure 9). All other compounds tested were antagonists. These studies reinforce the use of stereochemical diversity to influence receptor selectivity[54] and the potential of complex DKPs as ligands for a wide array of biological targets.

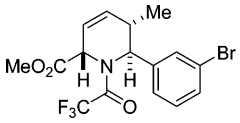
Conclusion

We have developed a convergent approach to rapidly synthesize highly functionalized diketopiperazines with complex pipecolic acids as core structures. The approach involves a scandium triflate-catalyzed in situ aza-annulation and protection which is readily adaptable to gram scale synthesis. The application of a resin catch and release strategy using Amberlyst hydroxide resin further streamlined access to various modified pipecolic acids, tetrahydro-β-carbolines, and other amino acids which are difficult to handle with traditional solution phase synthesis. One-pot cyclodimerization, combined with automated LC-MS purification, was efficiently utilized for the parallel synthesis of complex diketopiperazines. Massively parallel screening of the complex DKPs against a panel of molecular targets identified novel ligands for a number of G-protein-coupled receptors. Further studies toward the synthesis of complex libraries via convergent approaches, including alternative dimerization strategies, are in progress and will be reported in due course.

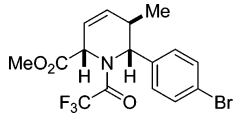
Experimental Section[55]

General Procedure 1: Synthesis of Imine *anti*-7b and Sc(OTf)$_3$-Catalyzed Aza-annulation to *trans*-8b. Magnesium sulfate (3.47 g, 28.8 mmol) was added to a solution of ***anti*-1** (2.0 g, 7.2 mmol) and 3-bromobenzaldehyde (1.33 g, 7.2 mmol) in dichloromethane (24 mL). The suspension was stirred at room temperature for 30 min and filtered through a Celite pad. The filtrate was concentrated and dried under vacuum to afford the crude imine product ***anti*-7b** as a yellow oil which was used without further purification. Trifluoroacetic anhydride (1.22 mL, 8.6 mmol) was added dropwise to Sc(OTf)$_3$ (710 mg, 1.4 mmol) and stirred at room temperature for 10 min. A solution of crude ***anti*-7b** (7.2 mmol) in dichloromethane (24 mL) was added dropwise via cannula. After stirring at room temperature for 15 h, the solution was cooled to −78 °C. Pyridine (1.46 mL, 18 mmol) and trifluoroacetic anhydride (0.6 mL, 4.3 mmol) were added. After stirring at −78 °C for 2 h, the reaction mixture was warmed to room temperature and quenched by the addition of water (30 mL). The two-layer mixture was separated, and the aqueous layer was extracted with dichloromethane (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography with ethyl acetate in hexane (from 0 to 30%) gave the desired product ***trans*-8b** as a colorless oil (2.6 g, 89% yield).

(2*S*,5*S*,6*S*)-Methyl-6-(3-bromophenyl)-1-(2,2,2-trifluoroacetyl)-1,2,5,6-tetrahydro-5-methylpyridine-2-carboxylate (*trans*-8b): colorless oil; $[\alpha]_D^{20}$ −69.8° (c = 2.0, $CHCl_3$); IR (thin film) ν_{max} 2956, 2360, 1754, 1699, 1434, 1276, 1199, 1036, 789 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 7.6 Hz, 1H), 7.20 (s, 1H), 7.18 (t, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 5.96 (t, J = 8.8 Hz, 1H), 5.79 (dd, J = 3.6, 9.6 Hz, 1H), 5.16 (br s, 1H), 5.01 (d, J = 4.0 Hz, 1H), 3.79 (s, 3H), 2.84−2.77 (m, 1H), 1.45 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 158.9, 144.2, 131.8, 130.8, 130.3, 128.4, 123.9, 122.9, 120.1, 60.4, 57.6, 53.6, 53.0, 36.9, 20.0; HRMS (CI/NH_3) m/z calcd for $C_{16}H_{15}F_3NO_3$ 405.0187, found 405.0170.



General Procedure 2: Synthesis of Imine *syn*-7a and Sc(OTf)$_3$-Catalyzed Aza-annulation to *cis*-8a. Magnesium sulfate (3.16 g, 26.2 mmol) was added to a solution of ***syn*-1** (1.82 g, 6.6 mmol) and 4-bromobenzaldehyde (1.24 g, 6.6 mmol) in dichloromethane (20 mL). The suspension was stirred at room temperature for 30 min and filtered through a Celite pad. The filtrate was concentrated and dried under vacuum to afford the crude imine product ***syn*-7a** as a yellow oil which was used without further purification. Trifluoroacetic anhydride (2.3 mL, 16.0 mmol) and trifluoroacetic acid (0.25 mL, 3.3 mmol) were added to Sc(OTf)$_3$ (1.3 g, 2.6 mmol) and sonicated for 10 min. A solution of crude ***syn*-7a** (6.6 mmol) in dichloromethane (20 mL) was added dropwise via cannula. After stirring at room temperature for 15 h, the solution was cooled to −78 °C. Pyridine (1.3 mL, 16.0 mmol) was added. After stirring at −78 °C for 2 h, the reaction mixture was warmed to room temperature and quenched by addition of water (30 mL). The two-layer mixture was separated, and the aqueous layer was extracted with dichloromethane (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography with ethyl acetate in hexane (from 0 to 30%) gave the desired product ***cis*-8a** as a colorless oil (1.54 g, 58% yield).



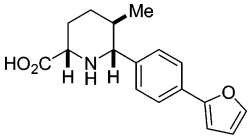
(2*S*,5*R*,6*R*)-Methyl-6-(4-bromophenyl)-1-(2,2,2-trifluoroacetyl)-1,2,5,6-tetrahydro-5-methylpyridine-2-carboxylate (*cis*-8a): colorless oil (ca. 1:1 rotamers, * = rotamer A, ** = rotamer B); $[\alpha]^{20}_D$ −44.0° (c = 0.36, $CHCl_3$); IR (thin film) ν_{max} 3349, 2963, 2878, 1765, 1490, 1442, 1138, 1075, 817 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.38 (m, 4H), 7.31−7.28 (m, 4H), 6.25−6.22* (m, 1H), 6.16−6.12** (m, 1H), 6.02−5.96 (m, 2H), 5.78* (s, 1H), 5.45−5.44** (m, 1H), 5.07** (s, 1H), 4.98−4.96* (m, 1H), 3.33** (s, 3H), 3.22* (s, 3H), 2.88−2.76 (m, 2H), 1.20** (d, J = 6.8 Hz, 3H), 1.15* (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 167.8, 158.4, 157.2, 137.1, 136.2, 131.7, 131.4, 131.3, 131.1, 130.5, 130.3, 122.4, 122.1, 121.3, 120.2, 118.3, 118.0, 115.4, 115.1, 59.2, 56.3, 54.1, 52.7, 52.5, 33.0, 32.1, 19.9, 19.2; HRMS (CI/NH_3) m/z calcd for $C_{16}H_{15}F_3NO_3$ 405.0187, found 405.0170.

General Procedure 3: Stille Cross-Coupling of *cis*-9a and Furyl Stannane: Tetrakis(triphenylphosphine)palladium(0) (113 mg, 0.1 mmol) was weighed into a 10 mL microwave reaction tube followed by addition of THF (3 mL) and ***cis*-9a** (400 mg, 1 mmol). After addition of 2-(tributylstannyl)furan (420 mg, 1.2 mmol), the microwave tube was sealed and subjected to microwave irradiation (150 W, 100 °C) for 30 min. TLC showed completion of the reaction. The solution was concentrated and then dissolved in acetonitrile (10 mL). Hexane (5 mL) was used to extract the tin-containing byproducts and then discarded. The acetonitrile solution was finally concentrated and filtered though a silica gel plug with ethyl acetate to afford **19**.

General Procedure 4: Amberlyst Resin-Mediated Catch and Release Protocol to Pipecolic Acid 29: Amberlyst hydroxide resin (3.5 g, 14.7 mmol, Aldrich, loading 4.2 mmol/g) was weighed into a 40 mL vial, then a solution of the crude product **19** (1 mmol) in

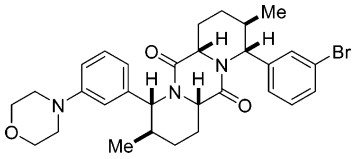
(55) All isolated new compounds gave satisfactory ^{1}H and ^{13}C NMR, IR, and HRMS data. Reported yields are after chromatographic purification unless otherwise mentioned. Experimental details for other compounds, copies of ^{1}H and ^{13}C NMR spectra, X-ray crystal structure analysis for compound **33**, and data for Figures 6 and 7 are provided in the Supporting Information.

THF (10 mL) was added into the vial followed by addition of methanol (10 mL) and water (10 mL). The vial was tightly sealed and placed on a mechanical shaker. After 15 h, the resin was filtered and washed with methanol and dichloromethane alternatively (10 mL × 3) in a filtration tube equipped with frit and a stopcock. A solution of acetic acid and water (1/1, v/v, 20 mL) was added to mix with the resin in the filtration tube, and the cleavage solution was collected into a vial. The AcOH/H_2O cleavage step was repeated three more times. All four cleavage solutions were concentrated, dried on Genevac, and combined to give the final product **29** as a yellow solid (260 mg, 93% yield).

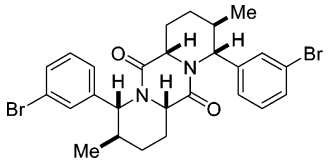


(2*S*,5*R*,6*R*)-6-(4-(Furan-2-yl)phenyl)-5-methylpiperidine-2-carboxylic acid 29: this compound was synthesized from ***cis*-9a** using general procedures 3 and 4; yield 93% (two steps); yellow solid, mp 152−155 °C; $[\alpha]_D^{20}$ −3.9° (*c* = 0.45, MeOH); IR (thin film) ν_{max} 3416, 2928, 2818, 2555, 2361, 1615, 1396, 1277, 1010, 738 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 1.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 3.6 Hz, 1H), 6.53 (dd, *J* = 1.6, 3.6 Hz, 1H), 3.82 (d, *J* = 10.8 Hz, 1H), 3.63 (dd, *J* = 3.2, 12.8 Hz, 1H), 2.39−2.35 (m, 1H), 2.17−2.06 (m, 2H), 1.93−1.82 (m, 1H), 1.56−1.46 (m, 1H), 0.75 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.8, 154.5, 144.1, 136.0, 133.2, 130.2, 125.3, 113.1, 107.3, 67.6, 62.2, 35.0, 33.6, 27.9, 18.6; HRMS (CI/NH_3) *m*/*z* calcd for $C_{17}H_{20}NO_3$ 286.1365, found 286.1401.

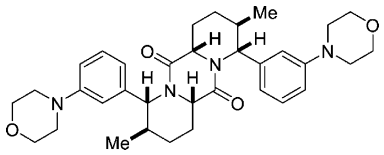
General Procedure 5. Hetero-cyclodimerization of Pipecolic Acids 39 and 40: To remove the trace amounts of water/methanol, pipecolic acids **39** and **40** were individually mixed with toluene and concentrated on Genevac EZ-2 evaporator. Dry **39** (29.7 mg, 0.1 mmol) and **40** (30.4 mg, 0.1 mmol) were added to a 1-dram vial. A 0.25 M solution of HATU in DMF (0.8 mL, 0.2 mmol) and a 3 M solution of collidine in DMF (0.2 mL, 0.6 mmol) were added. After stirring at room temperature for 19 h, the reaction mixture was concentrated on a Genevac EZ-2 evaporator. The residue obtained was treated with saturated aqueous ammonium chloride solution (8 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic solution was washed with aqueous saturated sodium bicarbonate (15 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude products were separated by preparative HPLC (*t* = 0 min, 80:20 water:acetonitrile to *t* = 10 min, 20:80 water:acetonitrile to *t* = 20 min, 20:80 water:acetonitrile; flow rate = 10 mL/min) with MS/ELSD triggered fraction collection. The desired fractions were concentrated in a Genevac EZ-2 evaporator to afford heterodimer **53** (16 mg, 28%), homodimers **54** (9 mg, 16%), and **55** (8 mg, 14%).



Heterodimer 53: yield, 28%; oil, $[\alpha]_D^{20}$ −71.2° (*c* 1.0, acetone); IR (thin film) ν_{max} 2958, 2854, 1652, 1600, 1492, 1383, 1263, 1172, 838 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 6.79 (s, 1H), 6.76 (d, *J* = 8.0 Hz, 1 H), 6.63 (d, *J* = 7.2 Hz, 1H), 4.64 (d, *J* = 6.8 Hz, 1H), 4.54 (d, *J* = 6.8 Hz, 1H), 4.01 (dd, *J* = 3.2, 5.2 Hz, 1H), 3.98 (dd, *J* = 3.2, 4.8 Hz, 1H), 3.83 (t, *J* = 4.8 Hz, 4H), 3.14−3.12 (m, 4H), 2.27−2.17 (m, 3H), 2.00−1.89 (m, 4H), 1.85−1.71 (m, 3H), 1.06 (d, *J* = 6.4 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 170.9, 151.3, 146.5, 145.0, 130.1, 130.0, 129.6, 129.3, 125.1, 122.7, 117.6, 114.1, 67.1, 62.6, 62.4, 56.1, 56.0, 49.5, 36.4, 36.1, 27.2, 27.0, 24.1, 23.7, 21.8, 21.1; HRMS (CI/NH_3) *m*/*z* calcd for $C_{30}H_{36}BrN_3O_3$ 565.1940, found 565.1954.



Homodimer 54: yield, 16%; oil, $[\alpha]_D^{20}$ −94.0° (*c* 2.54, ethyl acetate); IR (thin film) ν_{max} 2959, 2926, 2855, 1680, 1453, 1380, 1321, 1240, 686 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.20 (t, *J* = 7.2 Hz, 2H), 7.08 (d, *J* = 7.2 Hz, 2H), 4.49 (d, *J* = 6.4 Hz, 2H), 3.95 (dd, *J* = 5.2, 13.2 Hz, 2H), 2.26−2.20 (m, 2H), 1.95−1.85 (m, 4H), 1.76−1.66 (m, 2H), 1.27−1.21 (m, 2H), 1.00 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 146.4, 130.4, 130.1, 129.4, 124.9, 122.7, 62.6, 56.2, 36.5, 27.3, 24.2, 21.0.



Homodimer 55: yield, 14%; oil; $[\alpha]_D^{20}$ −25.8° (*c* 0.55, acetone); IR (thin film) ν_{max} 3476, 2959, 2850, 1673, 1600, 1453, 1377, 1239, 1119, 995, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, *J* = 8.0 Hz, 2H), 6.75−6.73 (m, 4H), 6.64 (d, *J* = 7.2 Hz, 2H), 4.76 (d, *J* = 5.6 Hz, 2H), 4.04 (dd, *J* = 4.8, 12.4 Hz, 2H), 3.79−3.76 (m, 8H), 3.10−3.07 (m, 8H), 2.23−2.16 (m, 2H), 2.14−2.07 (m, 2H), 1.99−1.81 (m, 4H), 1.29−1.20 (m, 2H), 1.08 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 151.4, 145.0, 129.4, 117.7, 113.9, 113.8, 67.1, 62.0, 55.8, 49.3, 35.9, 26.6, 23.1, 22.2; HRMS (CI/NH_3) *m*/*z* calcd for $C_{34}H_{45}N_4O_4$ 573.3363, found 573.3497.

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Supporting Information Available: Experimental procedures, compound characterization data, including X-ray crystal data for **33** (CIF), chromatographic separation of products of cyclodimerization, and biological data for Figures 6 and 7. This material is available free of charge via the Internet at http://pubs.acs.org.

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