2,6-Dimethyltyrosine Analogues of a Stereodiversified Ligand Library: Highly Potent, Selective, Non-Peptidic μ Opioid Receptor Agonists

Bryce A. Harrison,† Gavril W. Pasternak,‡ and Gregory L. Verdine*,†

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, and Laboratory of Molecular Neuropharmacology, Memorial Sloan Kettering Cancer Center, New York, New York 10021

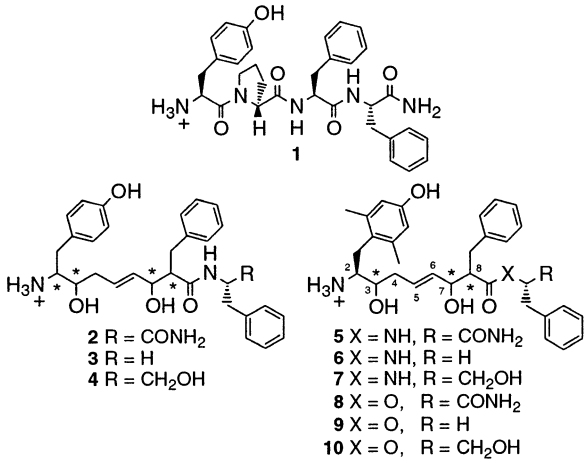
Received November 11, 2002

Abstract: We recently reported the use of an exhaustively stereodiversified library based on endomorphin-2 (**1**) to discover μ opioid receptor (MOR) ligands of type **2**−**4**. Here, we report the synthesis and evaluation of 2,6-dimethyltyrosine analogues **5**−**10**. These analogues showed improved affinity for MOR relative to **2**−**4**. In the cases of **5** and **6**, we synthesized and evaluated five stereoisomers of each, thereby discovering stereoisomers with unexpected potency, selectivity, and efficacy. These results illustrate the utility of acyclic, stereodiverse libraries.

Introduction. Screening of libraries of diverse compounds is a powerful approach to the discovery of small molecules with desired biologic properties.[1] Currently, many libraries are synthesized by attaching varied side chains to a rigid, cyclic scaffold.[2] Although these libraries incorporate a large amount of structural diversity, the single fixed scaffold may limit the functional diversity of the library. To complement this approach, we are investigating the use of acyclic, highly stereodiverse libraries.[3] We believe that acyclic stereocontrol will confer on each stereoisomer a unique conformational profile, thereby modulating its interaction with the biologic target.

In recently reported results,[4] we prepared an exhaustively stereodiversified library of potential μ opioid receptor (MOR) ligands **2**, based on biasing elements from endomorphin 2 (**1**),[5] a highly selective MOR peptide agonist. **2** contained a non-peptidic backbone with a dense array of stereocenters and a rigidifying olefin that may generate geometric diversity within the library. Screening of the 16 stereoisomers of **2** for MOR activity identified several active configurations. Additional analogues **3** and **4** were synthesized that also had high affinity for MOR. The most potent of these compounds, (*S*,*S*,*S*,*R*)-**2**, (*S*,*S*,*S*,*R*)-**3**, and (*S*,*S*,*S*,*R*)-**4**,[6] had K_i of 8.8−21 nM for MOR, 57- to 170-fold selectivity for MOR over the δ opioid receptor (DOR), and 86- to 600-fold selectivity for MOR over the κ opioid receptor (KOR). The five most active stereoisomers of **2** exhibited an 18-fold range in affinity for MOR and a 17- and 9-fold range in selectivity for MOR versus DOR and KOR, respectively. In functional assays, these compounds were partial agonists for MOR.[7]

For many peptide-based MOR ligands with N-terminal tyrosine residues, 2,6-dimethyltyrosine analogues show improved affinity for MOR.[8] In addition, these analogues are often highly potent agonists for MOR. However, efforts to convert these peptides to non-peptide ligands have met with only partial success. In one example, replacement of the amide bond C terminal to 2,6-dimethyltyrosine with peptide isosteres abolished activity in vitro and in vivo.[9] In an effort to discover compounds with improved affinity, selectivity, and efficacy at MOR, we synthesized the 2,6-dimethyltyrosine analogues (*S*,*S*,*S*,*R*)-**5** through -**10**. Additionally, to investigate the impact of stereochemical diversity of differing backbone structures, we prepared five stereoisomers each of **5** and **6**.



Chemistry. 5−**10** were synthesized using our previously reported olefin cross-metathesis methodology.[4] Commercially available Fmoc-L-2,6-dimethyltyrosine was converted to (*S*)-**11** in 69% yield by protecting group manipulations involving methyl ester protection of the carboxylic acid, *tert*-butyl ether protection of the phenol,[10] and exchange of the Fmoc for a Boc protecting group (Scheme 1). (*S*)-**11** was reduced to the aldehyde with DIBAL-H and allylated with allylmagnesium bromide to give (*S*,*S*)-**12** and (*S*,*R*)-**12** in 65% yield in a 1:1.4 ratio, separable by flash chromatography.

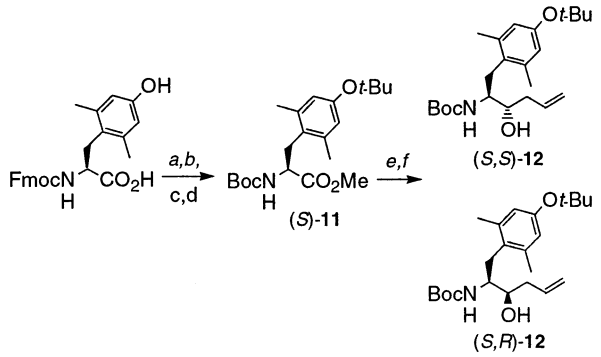
(*S*,*S*)-**12** was coupled with excess (*S*,*R*)-**13**, (*R*,*R*)-**13**, and (*R*,*S*)-**13**[4] in three parallel olefin cross-metathesis reactions using the second-generation Grubbs olefin metathesis catalyst in refluxing CH_2Cl_2 (Scheme 2).[11] The crossed metathesis products were hydrolyzed to the free acids with LiOH and H_2O_2 to give three stereoisomers of **14**. Comound (*S*,*R*)-**12** was coupled with excess (*S*,*R*)-**13** and (*R*,*R*)-**13** by the same reaction sequence to give two more stereoisomers of **14**. The yields for the five stereoisomers of **14** ranged from 50% to 59% from **12**.

Target compounds **5**−**9** were synthesized from **14** by coupling with the desired amine or alcohol, followed by deprotection. Compounds **5** were prepared in parallel in 48−63% yield by HBTU/HOBT promoted coupling of the five stereoisomers of **14** with solid-supported phenylalanine, followed by TFA deprotection and HPLC purification. Compounds **6** were prepared in parallel in

* To whom correspondence should be addressed. E-mail: verdine@chemistry.harvard.edu. Phone: 617-495-5323. Fax: 617-495-8755.
† Harvard University.
‡ Memorial Sloan Kettering Cancer Center.

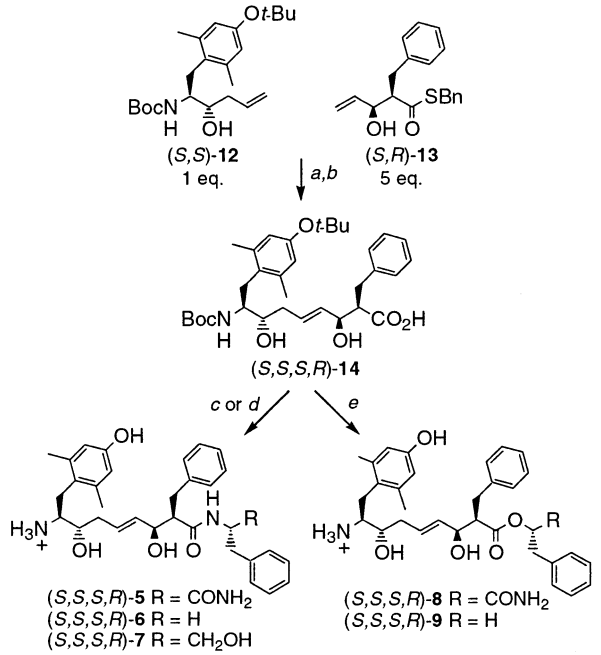
10.1021/jm025608s CCC: $25.00
Published on Web 01/29/2003

Scheme 1. Synthesis of **12**[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH; (b) isobutylene (5 psi), H_2SO_4, CH_2Cl_2, 71%, two steps; (c) $NHMe_2$, THF; (d) Boc_2O, NEt_3, THF, 97%, two steps; (e) DIBAL-H, toluene, −78 °C; (f) allylMgBr, THF, Et_2O, 0 °C, 27% (*S*,*S*)-**12**, 38% (*S*,*R*)-**12**, two steps.

Scheme 2. Synthesis of **5**−**9**[a]

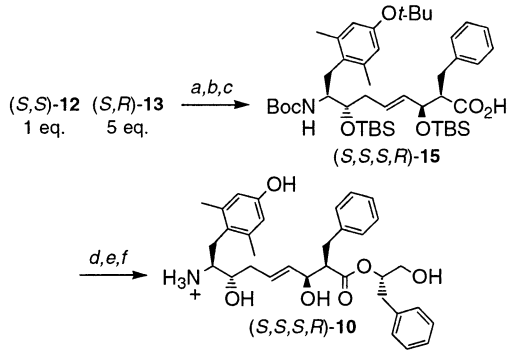


[a] Reagents and conditions: (a) $Cl_2(PCy_3)(IMesH_2)RuCHPh$, CH_2Cl_2, 40 °C; (b) LiOH, H_2O_2, THF, H_2O, 54%, two steps; (c) for **5**, HBTU, HOBT, DIPEA, NMP, Phe-NH−Rink amide AM resin, then 95% TFA, 63%; (d) for **6** and **7**, EDCI, HOBT, NEt_3, (*S*)-$H_2N(CHR)CH_2Ph$, CH_2Cl_2, then 95% TFA, 52% for **6**, 51% for **7**; (e) EDCI, DMAP, (*S*)-$HO(CHR)CH_2Ph$ (10−20 equiv), CH_2Cl_2, then 95% TFA, 21% for **8**, 20% for **9**.

up to 87% optimized yield by solution-phase EDCI/HOBT mediated coupling of the five stereoisomers of **14** with phenehtylamine, followed by TFA deprotection and HPLC purification. By use of a similar procedure, (*S*,*S*,*S*,*R*)-**7** was synthesized in 51% yield from (*S*,*S*,*S*,*R*)-**14** and phenylalanol.

Ester compounds **8**−**10** posed a more difficult challenge in that the hydroxyls in **14** would compete with other alcohols for coupling to the activated acid. Nevertheless, by use of an excess of the desired alcohol, (*S*,*S*,*S*,*R*)-**14** was coupled with phenethyl alcohol and (*S*)-$PhCH_2(CHOH)CONH_2$ using EDCI and DMAP to give a mixture of products. The mixture was deprotected with TFA, and the desired product was isolated by HPLC to give (*S*,*S*,*S*,*R*)-**8** and (*S*,*S*,*S*,*R*)-**9** in 21% and 20% yield, respectively. However, this approach was not

Scheme 3. Synthesis of **10**[a]



[a] Reagents and conditions: (a) $Cl_2(PCy_3)(IMesH_2)RuCHPh$, CH_2Cl_2, 40 °C; (b) TBSCl, imidazole, DMF; (c) LiOH, H_2O_2, THF, H_2O, MeOH, 52%, three steps; (d) (*S*)-$PhCH_2(CHOH)CH_2OTBS$, EDCI, DMAP, CH_2Cl_2; (e) 48% HF (aq), MeCN; (f) 95% TFA, 23%, three steps.

Table 1. Binding Affinity for MOR

compd	K_i(MOR)[a] (nM)
1	0.69 ± 0.09

	K_i(MOR)[a] (nM) for different configurations				
compd	*S*,*S*,*S*,*R*	*S*,*R*,*S*,*R*	*S*,*S*,*R*,*R*	*S*,*R*,*R*,*R*	*S*,*S*,*R*,*S*
5	0.17 ± 0.04	1.2 ± 0.2	0.42 ± 0.05	1.6 ± 0.5	1.2 ± 0.2
6	0.29 ± 0.03	0.24 ± 0.02	0.37 ± 0.07	0.72 ± 0.13	0.64 ± 0.04
7	0.16 ± 0.03				
8	0.45 ± 0.03				
9	0.24 ± 0.02				
10	0.44 ± 0.01				

[a] Competitive binding assay with ^{3}H-DAMGO for hMOR-1 stably transfected into CHO cells. Shown are the mean values ± standard deviation of triplicate measurements.

successful for the synthesis of (*S*,*S*,*S*,*R*)-**10** presumably because of increased steric hindrance of the coupling. Consequenlty, (*S*,*S*,*S*,*R*)-**10** was prepared in 23% yield by coupling of TBS-protected (*S*,*S*,*S*,*R*)-**15** with (*S*)-$PhCH_2(CHOH)CH_2OTBS$ using EDCI and DMAP, followed by HF and TFA deprotection and HPLC purification (Scheme 3).

Results and Discussion. 5−**10** were assayed for their binding affinity to MOR using a competitive binding assay with ^{3}H-DAMGO (Table 1).[12] In this assay, the 2,6-dimethyltyrosine analogues exhibited increased affinity for MOR relative to **2**−**4**. Compounds (*S*,*S*,*S*,*R*)-**5** through -**7** bound MOR with K_i values of 0.16−0.29 nM. Replacing the central amide in these structures with an ester resulted in less than a 3-fold loss of affinity for (*S*,*S*,*S*,*R*)-**8** and (*S*,*S*,*S*,*R*)-**10** and no loss of affinity for (*S*,*S*,*S*,*R*)-**9**. (*S*,*S*,*S*,*R*)-**5**, -**6**, -**7**, and -**9** all bound MOR with significantly higher affinity ($p < 0.05$)[13] than **1** (K_i = 0.69 nM).

The five stereoisomers of **5** showed a 9-fold range in affinity (0.17−1.6 nM) with the same configuration (*S*,*S*,*S*,*R*) favored as in **2**. This stereoisomer showed significantly higher affinity ($p < 0.001$) than (*S*,*R*,*S*,*R*)-, (*S*,*R*,*R*,*R*)-, and (*S*,*S*,*R*,*S*)-**5.** However, the five stereoisomers of **6** showed only a 3-fold range in affinity (0.24−0.72 nM) with no clearly favored configuration ($p > 0.05$ for comparisons among (*S*,*S*,*S*,*R*)-, (*S*,*R*,*S*,*R*)-, and (*S*,*S*,*R*,*R*)-**5**). The reduced impact of stereochemical variation in **6** compared to **5** may be derived from the increased flexibility of the phenethylamine moiety in **6** that may allow improved access of the aryl ring to a

Table 2. Binding Affinity for DOR

compd	K_i(DOR)[a] (nM) (K_i(DOR/K_i(MOR))
1	25000 ± 1000 (36000)

	K_i(DOR)[a] (nM) ((K_i(DOR)/K_i(MOR)) for different configurations				
compd	*S,S,S,R*	*S,R,S,R*	*S,S,R,R*	*S,R,R,R*	*S,S,R,S*
5	40 ± 1 (230)	46 ± 8 (38)	36 ± 3 (86)	85 ± 6 (53)	230 ± 10 (190)
6	99 ± 6 (340)	123 ± 4 (510)	26 ± 1 (70)	66 ± 1 (91)	119 ± 16 (190)
7	54 ± 4 (340)				
8	21 ± 2 (46)				
9	56 ± 2 (230)				
10	17 ± 3 (39)				

[a] Competitive binding assay with ^{3}H-diprenorphine for hDOR-1 stably transfected into HEK-293 cells. Shown are the mean values ± standard deviation of duplicate measurements.

Table 3. Binding Affinity for KOR

compd	K_i(KOR)[a] (nM) ((K_i(KOR)/K_i(MOR)
1	10400 ± 4300 (15000)

	K_i(KOR)[a] (nM) ((K_i(KOR)/K_i(MOR)) for different configurations				
compd	*S,S,S,R*	*S,R,S,R*	*S,S,R,R*	*S,R,R,R*	*S,S,R,S*
5	22 ± 5 (130)	15 ± 4 (13)	21 ± 2 (49)	25 ± 7 (16)	50 ± 8 (41)
6	42 ± 14 (140)	51 ± 1 (210)	64 ± 7 (170)	69 ± 10 (96)	60 ± 16 (94)
7	69 ± 7 (430)				
8	126 ± 33 (280)				
9	260 ± 110 (1100)				
10	85 ± 1 (190)				

[a] Competitive binding assay with ^{3}H-U-69,593 for KOR in guinea pig cerebellum preparation. Shown are the mean values ± standard deviation of two or more replicates.

binding pocket for certain stereoisomers. Most notably, (*S,R,S,R*)-**6** showed a 5-fold improvement in affinity relative to (*S,R,S,R*)-**5**. Interestingly, (*S,S,S,R*)-**6** did not show this improvement and had an affinity very close to that of (*S,R,S,R*)-**6**. These two stereoisomers differ only in the configuration of the C-3 hydroxyl, suggesting perhaps that the configuration of this hydroxyl influences the conformation of the C-terminal aryl moiety in **5** more so than in **6**.

5−**10** also were assayed for affinity for DOR and KOR to determine their selectivity for MOR. Although these compounds did not achieve the level of selectivity of **1**, they did demonstrate good levels of selectivity for MOR over DOR and KOR (Tables 2 and 3). (*S,S,S,R*)-**5** through -**7** had 230- to 340-fold selectivity for MOR over DOR and 130- to 430-fold selectivity for MOR over KOR. Relative to (*S,S,S,R*)-**5** through -**7**, the ester series (*S,S,S,R*)-**8** through -**10** generally exhibited decreased selectivity for MOR over DOR but increased selectivity for MOR over KOR. (*S,S,S,R*)-**9** showed the significantly ($p < 0.05$) highest selectivity for MOR over KOR (1100-fold) of any of the compounds **5**−**10**.

The five stereoisomers of **5** had a 6-fold and 10-fold range in selectivity for MOR over DOR and KOR, respectively. MOR affinity largely determined the selectivity because the affinity for DOR and KOR varied little among the stereoisomers with the exception of (*S,S,R,S*)-**5**, which showed significantly reduced affinity for DOR ($p < 0.001$) and reduced affinity for KOR. The five stereoisomers of **6** showed a 7-fold range in selectivity for MOR over DOR, and (*S,R,S,R*)-**6** exhibited the significantly ($p < 0.01$) the highest selectivity for MOR over DOR (510-fold) of any of the compounds **5**−**10**. However, the stereoisomers of **6** had only a 2-fold range

Table 4. MOR Activation

compd	% GTP-γ-^{35}S bound[a] (EC_{50} (nM))
1	100 ± 3 (31 ± 3)

	% GTP-γ-^{35}S bound[a] (EC_{50} (nM)) for different configurations				
compd	*S,S,S,R*	*S,R,S,R*	*S,S,R,R*	*S,R,R,R*	*S,S,R,S*
5	35 ± 2	14 ± 2	75 ± 5 (7.9 ± 0.5)	43 ± 4	32 ± 3
6	40 ± 1	49 ± 2 (5.8 ± 0.3)	48 ± 5	27 ± 4	52 ± 3
7	44 ± 3				
8	47 ± 2				
9	79 ± 3 (4.2 ± 0.4)				
10	62 ± 2				

[a] Specific binding of GTP-γ-^{35}S by G proteins in CHO membrane preparations stably transfected with hMOR-1 in the presence of GDP and **1** or **5**−**10** (10 μM), expressed as a percentage of DAMGO-induced GTP-γ-^{35}S specific binding. Shown are the mean values ± standard deviation of quadruplicate measurements.

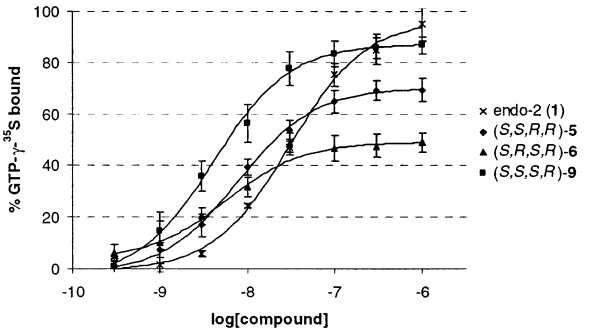


Figure 1. Specific binding of GTP-γ-^{35}S by G proteins in CHO membrane preps stably transfected with hMOR-1, in the presence of GDP and **1**, (*S,S,R,R*)-**5**, (*S,R,S,R*)-**6**, or (*S,S,S,R*)-**9** at various concentrations, expressed as a percentage of DAMGO-induced GTP-γ-^{35}S specific binding. Shown are the mean values ± standard deviation of triplicate measurements.

in selectivity for MOR over KOR. Once again, the flexibility of the phenethylamine moiety may result in similar affinity for the KOR for the stereoisomers.

Finally, **5**−**10** were tested at 10 μM for the ability to induce MOR-mediated binding of GTP-γ-35S to G proteins in CHO membrane preparations (Table 4). At this concentration, the compounds should fully saturate the receptors, allowing comparison of the maximal ability of the compounds to activate the receptor, a measure of its efficacy. Compounds (*S,S,S,R*)-**5** through -**8** showed reduced ability to induce GTP-γ-^{35}S binding (35−47%) compared to (*S,S,S,R*)-**2** through -**4** (49−75%); however, analogues (*S,S,S,R*)-**9** and (*S,S,S,R*)-**10** maintained good levels of induction (62−79%). Among the stereoisomers of **5**, (*S,S,R,R*)-**5** demonstrated the significantly ($p < 0.001$) highest efficacy (75%). Similar to the trend in affinities, the stereoisomers of **6** all showed similar, moderate activities in the GTP-γ-^{35}S assay.

We selected several of the most interesting compounds, **1**, (*S,S,R,R*)-**5**, (*S,R,S,R*)-**6**, and (*S,S,S,R*)-**9**, to obtain concentration-dependent activity curves for MOR-mediated GTP-γ-^{35}S binding (Figure 1). In these experiments, (*S,S,R,R*)-**5**, (*S,R,S,R*)-**6**, and (*S,S,S,R*)-**9** exhibited maximal binding of 70 ± 1%, 49.1 ± 0.1%, and 87 ± 1%, respectively, compared to 96 ± 2% for **1**. Moreover, the curves revealed that the EC_{50} values for (*S,S,R,R*)-**5**, (*S,R,S,R*)-**6**, and (*S,S,S,R*)-**9** (4.2−7.9 nM)

were considerably lower than the EC_{50} value of **1** (31 ± 3 nM). These assays indicated that, like **2**−**4**, 2,6-dimethyltyrosine analogues **5**−**10** are partial agonists for MOR.

Conclusions. Starting from structure **1** and using both stereochemical and structural variation, we have developed a new class of highly potent MOR agonists. The 2,6-dimethyltyrosine analogues **5**−**10** showed improved affinity for MOR relative to the tyrosine analogues **2**−**4**. Stereochemical variation of **5** and **6** impacted the properties of these ligands less than in **2**; nevertheless, it enabled the discovery of (*S*,*R*,*S*,*R*)-**6**, with unexpectedly high affinity and selectivity, and (*S*,*S*,*R*,*R*)-**5**, with unexpectedly high efficacy. While previous research has shown that isosteric replacements of amide bonds in 2,6-dimethyltyrosine peptide ligands may eliminate MOR activity,[9] our diversity-based approach has discovered completely non-peptidic, polyketide-like MOR partial agonists such as (*S*,*S*,*S*,*R*)-**9**, with improved potency relative to **1** and good selectivity and efficacy. These results suggest that stereodiverse acyclic libraries will be useful for discovering non-peptide ligands for multiple receptor types.

Acknowledgment. We thank Loriann Mahurter and Claire Neilan for expert assistance. B.A.H. is the recipient of a National Science Foundation predoctoral fellowship. This work was supported by a gift from Enanta Pharmaceuticals (to G.L.V.) and a grant from NIH (to G.W.P.).

Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Schreiber, S. L. Target-Oriented and Diversity-Oriented Organic Synthesis in Drug Discovery. *Science* **2000**, *287*, 1964−1969. (b) Stockwell, B. R. Chemical Genetics: Ligand-Based Discovery of Gene Function. *Nat. Rev. Genet.* **2000**, *1*, 116−125.

(2) (a) Pelish, H. E.; Westwood, N. J.; Feng, Y.; Kirchhausen, T.; Shair, M. D. Use of Biomimetic Diversity-Oriented Synthesis To Discover Galanthamine-like Molecules with Biological Properties beyond Those of the Natural Product. *J. Am. Chem. Soc.* **2001**, *123*, 6740−6741. (b) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; et al. Natural Product-like Combinatorial Libraries Based on Privileged Structures. 2. Construction of a 10000-Membered Benzopyran Library by Directed Split-and-Pool Chemistry Using NanoKans and Optical Encoding. *J. Am. Chem. Soc.* **2000**, *122*, 9954−9967. (c) Tan, D. S.; Foley, M. A.; Shair, M. D.; Schreiber, S. L. Stereoselective Synthesis of over Two Million Compounds Having Structural Features both Reminiscent of Natural Products and Compatible with Miniaturized Cell-Based Assays. *J. Am. Chem. Soc.* **1998**, *120*, 8565−8566.

(3) (a) Harrison, B. A.; Verdine, G. L. The Synthesis of an Exhaustively Stereodiversified Library of *cis*-1,5 Enediols by Silyl-Tethered Ring-Closing Metathesis. *Org. Lett.* **2001**, *3*, 2157−2159. (b) Gierasch, T. M.; Chytil, M.; Didiuk, M. T.; Park, J. Y.; Urban, J. J.; et al. A Modular Synthetic Approach toward Exhaustively Stereodiversified Ligand Libraries. *Org. Lett.* **2000**, *2*, 3999−4002. (c) Michielin, O.; Zoete, V.; Gierasch, T. M.; Eckstein, J.; Napper, A.; et al. Conformational Analysis of a Stereochemically Complete Set of Cis-enediol Peptide Analogues. *J. Am. Chem. Soc.* **2002**, *124*, 11131−11141.

(4) Harrison, B. A.; Gierasch, T. M.; Neilan, C.; Pasternak, G. W.; Verdine, G. L. High-Affinity μ Opioid Receptor Ligands Discovered by the Screening of an Exhaustively Stereodiversified Library of 1,5-Enediols. *J. Am. Chem. Soc.* **2002**, *124*, 13352−13353.

(5) (a) Zadina, J. E.; Hackler, L.; Ge, L.-J.; Kastin, A. J. A Potent and Selective Endogenous Agonist for the Mu-Opiate Receptor. *Nature* **1997**, *386*, 499−502. (b) Zadina, J. E.; Martin-Schild, S.; Gerall, A. A.; Kastin, A. J.; Hackler, L.; et al. Endomorphins: Novel Endogenous Mu-Opiate Receptor Agonists in the Regions of High Mu-Opiate Receptor Density. *Ann. N. Y. Acad. Sci.* **1999**, *897*, 136−144.

(6) Stereochemical labels for all compounds refer to the configuration at C2, C3, C7, and C8, in that order, as labeled in compound **5**.

(7) Compounds (*S*,*S*,*S*,*R*)-**2**, **3**, and **4** exhibited 71 ± 5%, 49 ± 4%, and 75 ± 4% activation of MOR in GTP-γ-^{35}S assays as described in the footnote to Table 4.

(8) (a) Schiller, P. W.; Fundytus, M. E.; Merovitz, L.; Weltrowska, G.; Nguyen, T. M.-D.; et al. The Opioid μ Agonist/μ Antagonist DIPP-NH_2[Ψ] Produces a Potent Analgesic Effect, No Physical Dependence, and Less Tolerance than Morphine in Rats. *J. Med. Chem.* **1999**, *42*, 3520−3526. (b) Schiller, P. W.; Nguyen, T. M.-D.; Berezowska, I.; Dupuis, S.; Weltrowska, G.; et al. Synthesis and in Vitro Opioid Activity Profiles of DALDA Analogues. *Eur. J. Med. Chem.* **2000**, *35*, 895−901. (c) Hansen, D. W.; Stapelfeld, A.; Savage, M. A.; Reichman, M.; Hammond, D. L.; et al. Systematic Analgesic Activity and δ-Opioid Selectivity in [2,6-dimethyl-Tyr1, D-Pen2, D-Pen5]enkephalin. *J. Med. Chem.* **1992**, *35*, 684−687. (d) Chandrakumar, N. S.; Stapelfeld, A.; Beardsley, P. M.; Lopez, O. T.; Drury, B.; et al. Analogs of the δ Opioid Receptor Selective Cyclic Peptide [cyclic][2-D-Penicillamine, 5-D-penicillamine]-enkephalin: 2′,6′-Dimethyltyrosine and Gly3-Phe4 Amide Bond Isostere Substitutions. *J. Med. Chem.* **1992**, *35*, 2928−2938. (e) Balboni, G.; Guerrini, R.; Salvadori, S.; Bianchi, C.; Rizzi, D.; et al. Evaluation of the Dmt-Tic Pharmacophore: Conversion of a Potent δ-Opioid Receptor Antagonist into a Potent δ Agonist and Ligands with Mixed Properties. *J. Med. Chem.* **2002**, *45*, 713−720. (f) Salvadori, S.; Guerrini, R.; Balboni, G.; Bianchi, C.; Bryant, S. D.; et al. Further Studies on the Dmt-Tic Pharmacophore: Hydrophobic Substituents at the C-Terminus Endow δ Antagonists To Manifest μ Agonism or μ Antagonism. *J. Med. Chem.* **1999**, *42*, 5010−5019.

(9) (a) Chandrakumar, N. S.; Yonan, P. K.; Stapelfeld, A.; Savage, M.; Rorbacher, E.; et al. Preparation and Opioid Activity of Analogues of the Analgesic Dipeptide 2,6-Dimethyl-L-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide. *J. Med. Chem* **1992**, *35*, 223−233. (b) Pitzele, B. S.; Hamilton, R. W.; Kudla, K. D.; Tsymbalov, S.; Stapelfeld, A.; et al. Enkephalin Analogs as Systemically Active Antinociceptive Agents: O- and N-Alkylated Derivatives of the Dipeptide Amide L-2,6-Dimethyltyrosyl-*N*-(3-phenylpropyl)-D-alaninamide. *J. Med. Chem.* **1994**, *37*, 888−896.

(10) Adamson, J. G.; Blaskovich, M. A.; Groenevelt, H.; Lajoie, G. A. Simple and Convenient Synthesis of *tert*-Butyl Ethers of Fmoc-serine, Fmoc-threonine, and Fmoc-tyrosine. *J. Org. Chem.* **1991**, *56*, 3447−3449.

(11) (a) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. Synthesis and Activity of a New Generation of Ruthenium-Based Olefin Metathesis Catalysts Coordinated with 1,3-Dimesityl-4,5-dihydroimidazol-2-ylidene Ligands. *Org. Lett.* **1999**, *1*, 953−956. (b) Blackwell, H. E.; O'Leary, D. J.; Chatterjee, A. K.; Washenfelder, R. A.; Bussmann, D. A.; et al. New Approaches to Olefin Cross-Metathesis. *J. Am. Chem. Soc.* **2000**, *122*, 58−71. (c) Fürstner, A. Olefin Metathesis and Beyond. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012−3043.

(12) Pasternak, G. W. Radioligand Binding. *Mod. Methods Pharmacol.* **1990**, *6*, 1−17.

(13) Statisitcal comparisons for this and subsequent data were determined by performing one-way ANOVA followed by Tukey's post hoc analysis.

JM025608S