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High-Affinity Mu Opioid Receptor Ligands Discovered by the Screening of an Exhaustively Stereodiversified Library of 1,5-Enediols

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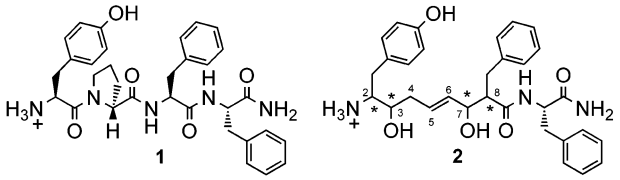
The discovery of small organic molecules with potent biologic activity is a key goal of life science research.[1] Success in this arena is frequently dependent on access to libraries of diverse chemical compounds.[2] Diversity may be incorporated into these libraries through the attachment of diverse functional groups to a fixed, cyclic scaffold.[3] A complementary approach is to use stereochemical variation and acyclic stereocontrol to generate geometric diversity among the members of the library.[4]

To illustrate the latter concept, we have focused our efforts on the discovery of nonpeptidic ligands for peptide receptors. A fertile ground for such efforts lies in the discovery of ligands for the opioid class of G-protein coupled receptors,[5] which are found in the nervous system and mediate the sensation of pain.[6] An endogenous ligand for the mu opioid receptor (MOR) is the tetrapeptide endomorphin-2 (**1**), a potent agonist of MOR with high (10^4-fold) selectivity for MOR over the delta opioid receptor (DOR) and the kappa opioid receptor (KOR).[7]

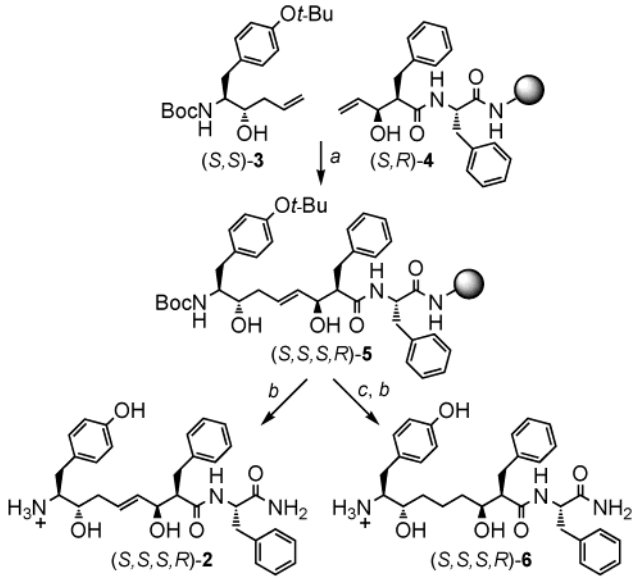


Stereodiverse, nonpeptidic compounds **2** are designed to target MOR. In compounds **2**, the N-terminal tripeptide unit of **1** has been replaced by a nonpeptidic, stereodiverse unit incorporating a 1,5-enediol moiety. The dense array of stereocenters combined with the rigidifying olefin in **2** are intended to generate geometric diversity. An explicit goal of this study was to investigate how such geometric diversification impacts binding to MOR. Here we report the synthesis of an exhaustively stereodiversified library comprising 16 stereoisomers of **2** and the screening of these compounds for binding to MOR. Furthermore, we report diversification of the central hydrocarbon linkage and of the residual C-terminal amino acid.

Ligands **2** were synthesized in parallel using a solid-phase cross metathesis approach (Scheme 1).[8] Resin-bound **4** was treated with $Cl_2(PCy_3)(IMesH_2)RuCHPh$[9] and an excess of **3** to give **5**, which was then deprotected and cleaved from the resin with 95% TFA. Although the yield for this process was modest (24−38%), the products were obtained in good purity.

Sixteen stereoisomers of **2** were screened at a concentration of 10 μM for competitive binding to MOR. Several stereoisomers showed significant binding under these conditions; K_i values for the five most active compounds were then determined in competitive binding assays with ^{3}H-labeled DAMGO (Table 1).[10]

Scheme 1 [a]



[a] Reagents and conditions: (a) $Cl_2(PCy_3)(IMesH_2)RuCHPh$, CH_2Cl_2, 25 °C, 25%; (b) 95% TFA; (c) TPSH, piperidine, NMP, 100%.

The stereoisomer with the highest affinity, (*S*,*S*,*S*,*R*)-**2**,[11] exhibited a K_i of 8.8 nM, within an order of magnitude of the K_i measured for **1** (1.2 nM) under the same conditions. Variation of configuration at even a single stereocenter of (*S*,*S*,*S*,*R*)-**2** had a strong impact on binding affinity. For example, inversion of only the C-7 hydroxyl [(*S*,*S*,*S*,*R*)-**2** → (*S*,*S*,*R*,*R*)-**2**] resulted in a 3-fold loss in affinity. Inversion of only the C-3 hydroxyl [(*S*,*S*,*S*,*R*)-**2** → (*S*,*R*,*S*,*R*)-**2**] gave an 18-fold reduction in binding affinity. Inversion of either the C-2 or the C-8 aryl groups resulted in significant reduction in affinity at 10 μM (not shown in Table 1). Most of the high-affinity ligands preserved the stereochemical configuration corresponding to that of a natural L-amino acid for the aryl side chains; however, (*S*,*S*,*R*,*S*)-**2**, with the nonnatural configuration at the C-8 benzyl side chain, exhibited the fourth-highest affinity among the stereoisomers of **2**.

Ligands **2** were assayed against DOR and KOR, to determine MOR selectivity (Table 2). Ligands **2** exhibited stereochemically dependent selectivity for MOR, although none was as selective as **1**. (*S*,*S*,*S*,*R*)-**2**, the highest affinity MOR ligand, was 57- and 150-fold selective for MOR over DOR and KOR, respectively, while (*S*,*R*,*S*,*R*)-**2** was only 5- and 18-fold selective. Interestingly,

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Table 1. Binding Affinity for MOR

	K_i MOR (nM)[a,b]				
1	1.2 ± 0.1 Configuration:				
	S,S,S,R	*S,S,R,R*	*S,R,R,R*	*S,R,S,R*	*S,S,R,S*
2	8.8 ± 0.7	25 ± 5	67 ± 38	160 ± 40	79 ± 23
6	98 ± 31	95 ± 61	120 ± 30	190 ± 20	
9	370 ± 150	74 ± 12	400 ± 110	260 ± 70	
10	21 ± 1	29 ± 8	53 ± 6		
11	10 ± 2	16 ± 1	28 ± 5		
12	20 ± 1	22 ± 4	37 ± 1		
13	37 ± 8				

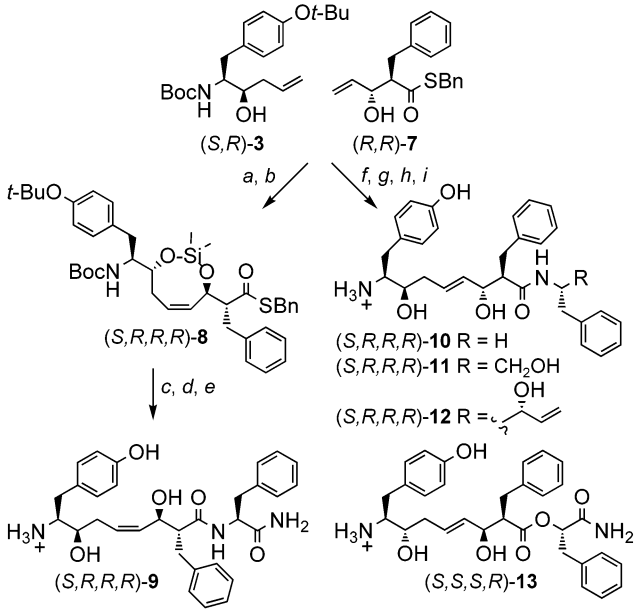
[a] Competitive binding assay with ^{3}H-DAMGO for hMOR-1 stably transfected into CHO cells. [b] Errors represent 95% confidence interval.

Table 2. Selectivity of Ligands for MOR versus DOR and KOR

	K_i DOR[a]/K_i MOR, K_i KOR[b]/K_i MOR				
1	10 000, 9000 Configuration:				
	S,S,S,R	*S,S,R,R*	*S,R,R,R*	*S,R,S,R*	*S,S,R,S*
2	57, 150	45, 48	21, 18	5, 18	86, 16
6	22, 40	31, 8	26, 13	21, 35	
9	6, 16	58, 22	23, 15	39, 7	
10	170, 86	42, 120	55, 53		
11	110, 600	39, 180	34, 120		
12	34, 53	28, 35	15, 26		
13	24, 160				

[a] Competitive binding assay with ^{3}H-DPDPE for hDOR-1 stably transfected into HEK-293 cells. [b] Competitive binding assay with ^{3}H-U-69 593 for KOR in guinea pig cerebellum preparation.

Scheme 2 [a]



[a] Reagents and conditions: (a) $SiMe_2Cl_2$, pyridine, 60%; (b) $Cl_2(PCy_3)$-$(IMesH_2)$RuCHPh, toluene, 95 °C, 89%; (c) HF·pyridine, THF, 0 °C, 85%; (d) LiOH, H_2O_2, THF, H_2O, 100%; (e) SPPS, 66%; (f) $Cl_2(PCy_3)(IMesH_2)$RuCHPh, CH_2Cl_2, 40 °C; (g) LiOH, H_2O_2, THF, H_2O, 44% for two steps; (h) EDCI, HOBT, TEA, DMF, RNH_2; (i) TFA, CH_2Cl_2.

(*S,S,R,S*)-**2**, with the unnatural configuration at the benzyl side chain, had the highest MOR over DOR selectivity (86-fold).

To investigate the role of the olefin in MOR binding, four stereoisomers of reduced olefin ligands **6** (Scheme 1) and four stereoisomers of cis-configurated olefin ligands **9** (Scheme 2) were synthesized and assayed for MOR affinity and selectivity. Compounds **6** were prepared by hydrogenation of **5** on the solid phase using TPSH,[12] followed by deprotection, and ligands **9** were prepared by our previously reported solution phase, silyl-tethered ring-closing metathesis method. Ligands **6** generally exhibited reduced binding affinity and selectivity for MOR relative to **2** having the same configuration, presumably owing to their reduced conformational preorganization. Isomerization of the central C=C bond from trans (**2**) to cis (**9**) resulted in a significant loss of binding affinity.

To investigate the role of the C-terminal Phe in MOR binding of **2**, three stereoisomers of **10**−**12** and one stereoisomer of **13** were synthesized and screened for MOR affinity and selectivity (Scheme 2). Monomers **3** and **7** were coupled by solution phase cross metathesis,[8] followed by hydrolysis of the thioester, amidation, and deprotection. Analogues **10** and **11** contain only one amide bond, yet had similar or higher affinity and selectivity for MOR than **2**. For example, (*S,S,S,R*)-**11** had a K_i value for MOR of 10 nM, and 110- and 600-fold selectivity for MOR over DOR and KOR, respectively. Compounds **12** and **13** also retained significant MOR affinity and selectivity, indicating that C-terminal modifications are well tolerated.

In conclusion, screening of an exhaustively stereodiversified library has resulted in the identification of novel, nonpeptidic ligands for the MOR. The best of these ligands, (*S,S,S,R*)-**2**, -**10**, and -**11**, bind MOR with low nanomolar affinity and 57−600-fold selectivity for MOR over other opioid receptors. Functional assays show that these compounds are partial agonists for MOR (data not shown), and we are currently searching for derivatives showing full agonist activity. The results provide encouraging signs that stereochemical diversity will be a valuable strategy for the discovery of nonpeptidic ligands for peptide receptors.

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

References

(1) (a) Mitchison, T. J. *Chem. Biol.* **1994**, *1*, 3−6. (b) Schreiber, S. L. *Bioorg. Med. Chem.* **1998**, *6*, 1127−1152.
(2) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (b) Stockwell, B. R. *Nat. Rev. Genet.* **2000**, *1*, 116−125.
(3) For examples, see: (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; Cao, G.-Q.; Affleck, R. L.; Lillig, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 9954−9967. (b) Tan, D. S.; Foley, M. A.; Shair, M. D.; Schreiber, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8565−8566. (c) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A.; Bray, A. M. *New J. Chem.* **1997**, *21*, 125−130.
(4) (a) Gierasch, T. M.; Chytil, M.; Didiuk, M. T.; Park, J. Y.; Urban, J. J.; Nolan, S. P.; Verdine, G. L. *Org. Lett.* **2000**, *2*, 3999−4002. (b) Harrison, B. A.; Verdine, G. L. *Org. Lett.* **2001**, *3*, 2157−2159. (c) Annis, D. A.; Helluin, O.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1998**, *37*, 1907−1909. (d) Paterson, I.; Scott, J. P. *J. Chem. Soc., Perkins Trans. 1* **1999**, 1003−1014. (e) Paterson, I.; Channon, J. A. *Tetrahedron Lett.* **1992**, *33*, 797−800.
(5) (a) Dooley, C. T.; Houghten, R. A. *Biopolymers* **1999**, *51*, 379−390. (b) Hruby, V. J.; Agnes, R. S. *Biopolymers* **1999**, *51*, 391−410.
(6) (a) Standifer, K. M.; Pasternak, G. W. *Cell Signalling* **1997**, *9*, 237−248. (b) Pasternak, G. W. *Life Sci.* **2001**, *68*, 2213−2219.
(7) (a) Zadina, J. E.; Hackler, L.; Ge, L.-J.; Kastin, A. J. *Nature* **1997**, *386*, 499−502. (b) Zadina, J. E.; Martin-Schild, S.; Gerall, A. A.; Kastin, A. J.; Hackler, L.; Ge, L.-J.; Zhang, X. *Ann. N.Y. Acad. Sci.* **1999**, *897*, 136−144.
(8) (a) Kingsbury, C. L.; Mehrman, S. J.; Takacs, J. M. *Curr. Org. Chem.* **1999**, *3*, 497−555. (b) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012−3043. (c) Blackwell, H. E.; O'Leary, D. J.; Chatterjee, A. K.; Washenfelder, R. A.; Bussmann, D. A.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 58−71.
(9) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953−956.
(10) Pasternak, G. W. *Mod. Methods Pharmacol.* **1990**, *6*, 1−17.
(11) All configurations are given in the C2 → C8 direction.
(12) (a) Cusack, N. J.; Reese, C. B.; Risius, A. C.; Roozpeikar, B. *Tetrahedron* **1976**, *32*, 2157−2162. (b) Lacombe, P.; Castagner, Y. G.; Ruel, R. *Tetrahedron Lett.* **1998**, *39*, 6785−6786.

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