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Unpredictable Stereochemical Preferences for Mu Opioid Receptor Activity in an Exhaustively Stereodiversified Library of 1,4-Enediols

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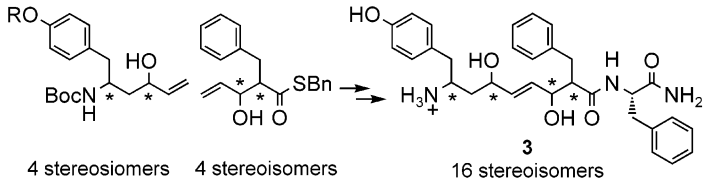
ABSTRACT



Using olefin cross-metathesis, we synthesized a novel stereodiversified library of compounds 3 containing a *trans*-1,4-enediol. Screening this library for mu opioid receptor (MOR) affinity identified multiple high-affinity ligands and revealed that the stereochemical configuration varied widely among those ligands having the highest affinity. It was not possible to predict the configurations of the most active compounds 3 on the basis of the configuration of endomorphin-2, a known MOR peptide ligand, validating the diversity-based approach to ligand discovery.

Diversity-based approaches for discovering bioactive small molecules are most attractive when the biologic properties of the molecules are least predictable.[1] Approaches to molecular diversity have mostly focused on constitutional variation, often starting with a fixed, cyclic scaffold.[2] We are investigating a complementary approach wherein stereochemical diversity of densely branched acyclic molecules generates geometric variation among library members.[3]

We recently reported the synthesis of an exhaustively stereodiversified library of 1,5 enediols of structure **2**,[4] based on biasing elements from endomorphin-2 (**1**),[5] a highly potent and selective mu opioid receptor (MOR)[6] peptide agonist. Screening of this library for MOR affinity identified several active stereoisomers. The most potent, (*S*,*S*,*S*,*R*)-**2**,[7] had a K_i of 8.8 nM in a radioligand competitive binding assay, with 57- and 150-fold selectivity for MOR over the delta opioid receptor (DOR) and kappa opioid receptor (KOR), respectively. This compound acted as a partial agonist for MOR in functional assays. Interestingly, the stereochemical configuration of **2** strongly impacted the affinity and selectivity. The five stereoisomers exhibiting the highest affinity had an 18-fold range in MOR affinity with a 17-

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[†] These authors contributed equally to this work.

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(7) All stereochemical labels are given in the C2 → C8 direction as shown in compounds **2** and **3**.

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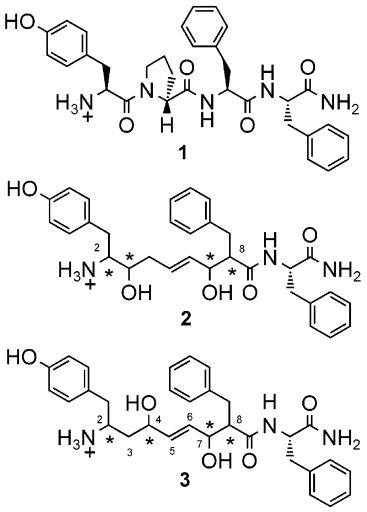
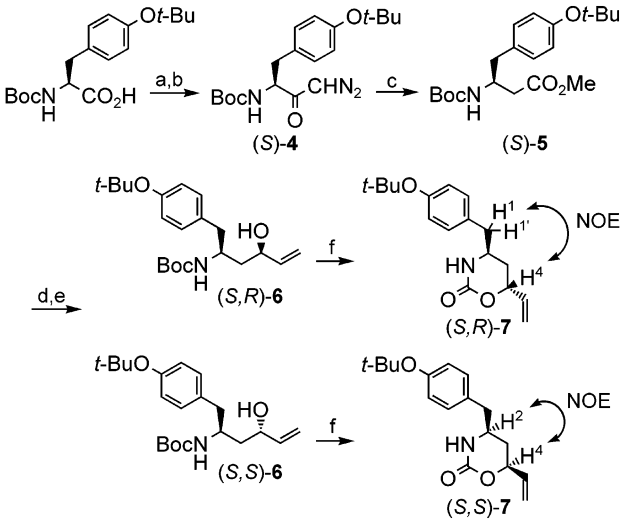
and 9-fold range in selectivity for MOR over DOR and KOR, respectively. These ligands all conserved the (*S*)-configuration at C2, and four of the five conserved the (*R*)-configuration at C8, corresponding to the configuration of **1** at the respective positions. In an effort to discover MOR ligands with improved agonist activity, we sought to synthesize a new class of stereodiverse compounds **3** containing a 1,4-enediol moiety.



To investigate fully the impact of stereochemical diversity, we prepared 16 stereoisomers of **3** using a modular approach, beginning with the synthesis of the four stereoisomers **6** (Scheme 1).[8] Boc-L-Tyr(*t*-Bu)-OH was subjected to Arndt−Eistert homologation to give methyl ester (*S*)-**5** by way of diazoketone (*S*)-**4**.[9] Compound (*S*)-**5** was reduced to the aldehyde with DIBAL-H and reacted with vinylmagnesium bromide to give (*S*,*R*)-**6** and (*S*,*S*)-**6**, separable by flash chromatography, in a 1.6:1 ratio in 75% combined yield. To determine the relative configurations of **6**, these compounds were cyclized to **7** by treatment with KHMDS in THF. In (*S*,*R*)-**7**, a 1.4% NOE was observed between H^4 and $H^1/H^{1'}$ and a 0% NOE between H^4 and H^2, while in (*S*,*S*)-**7**, a 2.3% NOE was observed between H^4 and H^2 and a 0% NOE between H^4 and $H^1/H^{1'}$. Starting with Boc-D-Tyr(tBu)-OH, (*R*,*S*)-**6** and (*R*,*R*)-**6** were synthesized by the same procedure.

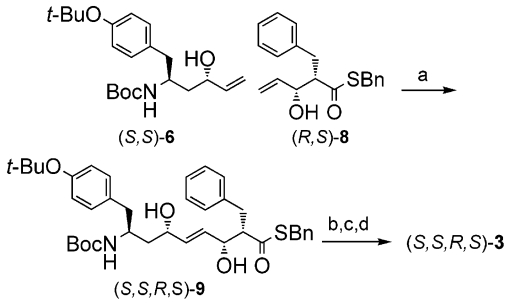
Scheme 1. Synthesis of **6**[a]



[a] Reagents and conditions: (a) $ClCO_2iBu$, *N*-methylmorpholine, THF; (b) CH_2N_2, THF, 85% for two steps; (c) $PhCO_2Ag$, NEt_3, MeOH; (d) DIBAL-H, THF, 43% for two steps; (e) vinyl-MgBr, THF, 1.6:1 ratio of (*S*,*R*)-**6** to (*S*,*S*)-**6**, 75%; (f) KHMDS, THF, 92−96%.

Compounds **6** were coupled with compounds **8**[4] by olefin cross-metathesis[10] to give **9** (Scheme 2). Our previously reported synthesis of **2** featured a olefin cross-metathesis between an allylic and a homoallylic alcohol;[4] however, the synthesis of **9** represented our first efforts at the cross-metathesis of two allylic alcohols in a stereodiverse manner. In this system, both metathesis partners have a similar substitution pattern around the olefin, which could reduce the selectivity of the coupling. Moreover, the increased steric congestion around the olefin could hinder the metathesis reaction. The coupling was attempted first with different ratios of **6** and **8** using second generation Grubbs catalyst[11] in refluxing CH_2Cl_2. A 1:1 or 5:1 ratio of **6** to **8** gave low yields of **9**. However, a 1:5 ratio of **6** to **8** gave **9** in 30−56% yield for eight diastereomers. In an optimized procedure, **6** was added slowly by syringe pump to excess **8** in the presence of second generation Grubbs catalyst in refluxing CH_2Cl_2. Using only a 1:2 ratio of **6** to **8**, this procedure gave **9** in 51−81% yield for the eight diastereomers. By a combination of these methods, we completed the synthesis of all 16 stereoisomers of **9**.

Scheme 2. Synthesis of **3**[a]



[a] Reagents and conditions: (a) $Cl_2(PCy_3)(IMesH_2)RuCHPh$, CH_2Cl_2, 40 °C, 53%; (b) LiOH, H_2O_2, THF, H_2O, 79%; (c) HBTU, HOBt, DIPEA, NMP, Phe-NH−Rink amide AM resin; (d) 95% TFA.

Compounds **9** were hydrolyzed to the free acids and coupled with phenylalanine supported on Rink Amide AM

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Table 1. MOR Affinity, Selectivity, and Efficacy of Compounds **1** and **3**[a]

compound	K_i MOR[b] (nM)	K_i DOR[c] (μM) (K_i DOR/K_i MOR)	K_i KOR[d] (μM) (K_i KOR/K_i MOR)	% GTP-γ-S bound[e]
endomorphin-2 (**1**)	1.0 ± 0.1	25 ± 1 (25 000)	10.4 ± 8.6 (10 000)	100 ± 6
(*S,S,R,S*)-**3**	14 ± 1	1.2 ± 0.1 (82)	0.45 ± 0.06 (32)	25 ± 5
(*S,R,R,S*)-**3**	28 ± 6	2.5 ± 3 (89)	1.2 ± 0.6 (42)	48 ± 8
(*S,R,S,S*)-**3**	42 ± 5	2.5 ± 0.3 (60)	3.1 ± 2.6 (74)	57 ± 12
(*S,R,S,R*)-**3**	32 ± 6	0.74 ± 0.04 (23)	0.29 ± 0.1 (9)	37 ± 7
(*R,R,S,S*)-**3**	38 ± 13	1.2 ± 0.1 (30)	0.88 ± 0.55 (23)	30 ± 6
(*R,S,R,R*)-**3**	20 ± 3	2.5 ± 0.3 (125)	0.27 ± 0.03 (14)	68 ± 10

[a] Errors on measurements represent two standard deviations from the mean (95% confidence interval). [b] Competitive binding assay with ^{3}H-DAMGO for hMOR-1 stably transfected into CHO cells. [c] Competitive binding assay with ^{3}H-DPN for hDOR-1 stably transfected into HEK-293 cells. [d] Competitive binding assay with ^{3}H-U-69 593 for KOR in guinea pig cerebellum preparation. [e] 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 **3** (10 μM), expressed as a percentage of DAMGO-induced GTP-γ-^{35}S-specific binding.

resin. The product was cleaved from the resin, deprotected with 95% TFA, and purified by reverse-phase HPLC to give 16 stereoisomers of **3**.

Compounds **3** were screened for MOR affinity at 1 μM concentration in a competitive binding assay with ^{3}H-DAMGO (Figure 1).[12] The stereoisomers of **3** showed

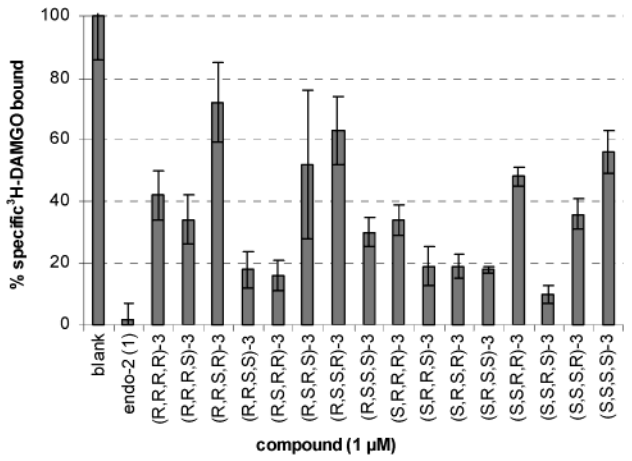

Figure 1. Competitive binding assay with ^{3}H-DAMGO (1.3 nM) for hMOR-1 stably transfected into CHO cells. Specific binding was defined by the difference in radioligand bound between assays with no ligand (blank) and with 10 μM naloxone. Error bars represent two standard deviations from the mean (95% confidence interval) on triplicate measurements.

varying degrees of affinity for MOR, displacing 28−90% of ^{3}H-DAMGO from MOR. The six most potent ligands from this screen were assayed at multiple concentrations to obtain MOR binding curves for K_i determination (Table 1). These experiments identified (*S,S,R,S*)-**3** as the highest affinity ligand with a K_i of 14 nM, about 14-fold less active than **1**, and the six highest affinity ligands showed a 3-fold range in affinity. Most interestingly, the stereochemical preference for MOR affinity was not predictable from the configuration of **1**, as only one of the six ligands had both the (*S*)-configuration at C2 and the (*R*)-configuration at C8. In fact, the (*S*)-configuration at C8 was present in four of the six ligands. Moreover, the effects of stereochemical changes to the ligands were unpredictable as well. For example, inverting C2, C7, or C8 of (*S,S,R,S*)-**3**, the highest affinity ligand, greatly reduced affinity. However, inverting C4 [(*S,S,R,S*)-**3** → (*S,R,R,S*)-**3**] resulted in only a 2-fold reduction in affinity. Compound (*S,R,S,S*)-**3** was the least sensitive to stereochemical changes; inverting C2, C7, or C8 actually improved MOR affinity. Compound (*R,S,R,R*)-**3** was the most sensitive to stereochemical changes; inverting any one stereocenter significantly reduced the affinity. Overall, no stereochemical configuration at any of the stereocenters was conserved among all six of the highest affinity ligands. This result suggests that binding affinity resulted from the combined impact of multiple stereocenters on the ligand−receptor interaction.

To determine MOR selectivity, the six most potent ligands were screened for affinity for DOR and KOR (Table 1). The ligands showed 23−125-fold selectivity for MOR over DOR

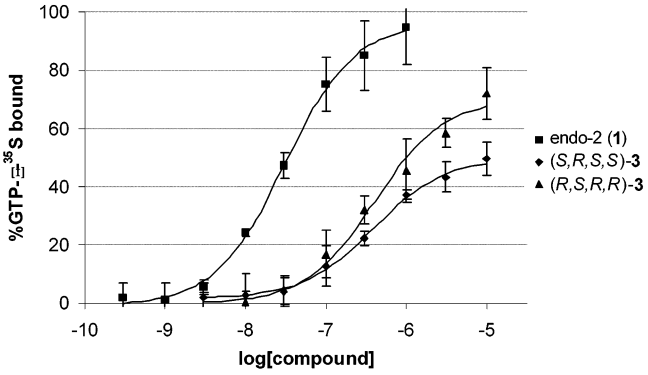

Figure 2. Specific binding of GTP-γ-^{35}S by G-proteins in CHO membrane preparations stably transfected with hMOR-1, in the presence of GDP and **1** or **3** at multiple concentrations, expressed as a percentage of DAMGO-induced GTP-γ-^{35}S specific binding. Error bars represent two standard deviations from the mean (95% confidence interval) on triplicate measurements.

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and 9−74-fold selectivity for MOR over KOR. Selectivity did not correlate highly with MOR affinity, with the most potent ligand (*S*,*S*,*R*,*S*)-**3** exhibiting only average selectivity (82- and 32-fold selective for MOR over DOR and KOR).

The six highest affinity ligands **3** also were tested for the ability to induce MOR-mediated GTP-γ-^{35}S binding by G-proteins in CHO membrane preparations (Table 1). At 10 μM, compounds **3** induced 25−68% of the GTP-γ-^{35}S binding induced by the full MOR agonist DAMGO, indicating that compounds **3** are partial agonists for MOR. Of the ligands tested, (*R*,*S*,*R*,*R*)-**3** (68%) and (*S*,*R*,*S*,*S*)-**3** (57%) had the highest activity in the GTP-γ-^{35}S assay. Activation curves were measured for these two compounds (Figure 2), which gave EC_{50} values of 390 ± 110 and 390 ± 40 nM for (*R*,*S*,*R*,*R*)-**3** and (*S*,*R*,*S*,*S*)-**3**, respectively.

In conclusion, we developed an efficient olefin cross-metathesis procedure for accessing a new class of stereodiverse 1,4-enediol compounds, **3**. Screening of these compounds for MOR activity identified multiple potent and selective partial agonists. Although stereochemical diversity did not impact the properties of these compounds as greatly as in **2**, the effects of stereochemical variation in **3** on MOR affinity, selectivity, and efficacy were not predictable on the basis of either the configuration of **1** or the properties of any given stereoisomer of **3**. These results illustrate the need to sample stereochemistry broadly when attempting to identify bioactive small molecules having multiple stereocenters.

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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.

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