Discovery of Nanomolar Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse *N*-(Substituted)glycine Peptoid Library

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Screening a diverse, combinatorial library of ca. 5000 synthetic dimer and trimer *N*-(substituted)glycine "peptoids" yielded novel, high-affinity ligands for 7-transmembrane G-protein-coupled receptors. The peptoid library was efficiently assembled using readily available chemical building blocks. The choice of side chains was biased to resemble known ligands to 7-transmembrane G-protein-coupled receptors. All peptoids were screened in solution-phase, competitive radioligand-binding assays. Peptoid trimer CHIR 2279 binds to the α_1-adrenergic receptor with a K_i of 5 nM, and trimer CHIR 4531 binds to the μ-opiate receptor with a K_i of 6 nM. This represents the first example of the discovery of high-affinity receptor ligands from a combinatorial library of non-natural chemical entities.

Introduction

Increasing pressures in today's global pharmacoeconomic climate demand more efficient methods for identifying and optimizing drug candidates. Traditionally, screening of natural products from fermentation broths, plants, and marine organisms, as well as synthetic intermediates from industrial compound collections, has yielded substantial numbers of pharmaceutical leads.[1] More recently, both chemists and molecular biologists have developed molecular diversity technologies that are dramatically accelerating the process of lead discovery.[2] Combinatorial synthesis methods are used to rapidly generate large compound collections suitable for screening against a wide variety of biological targets.

To date, the molecular diversity field has primarily focused on the preparation and screening of nucleic acid,[3-5] synthetic peptide,[6-12] and recombinant peptide libraries.[13-15] These biopolymers are well suited to generate combinatorial libraries because of their efficient synthesis chemistries. Although biopolymer libraries have yielded moderate- to high-affinity ligands to antibodies,[6-14] cellular receptors,[16,17] and even to proteins with no known natural peptide or nucleic acid ligands,[15,18,19] the natural biopolymers rarely make appropriate drug candidates, partly because of their poor pharmacokinetic properties.[20]

In order for combinatorial libraries to directly provide viable drug candidates, chemists are extending molecular diversity concepts to synthetic oligomers with non-natural backbones[21-25] as well as to nonoligomeric structures.[26,27] In contrast to biopolymer libraries, the synthetic polymers should be stable to proteases and nucleases. Furthermore, because an abundant variety of building blocks and structural scaffolds can be used, greater diversity is theoretically obtainable.

We previously reported the synthesis of oligo-*N*-(substituted)glycines (NSGs) or "peptoids" as a new class of polymer designed for drug discovery.[23] These oligomers are resistant to proteases (manuscript in preparation) and can be assembled via automated synthesis. We also reported a new, highly efficient synthetic route to oligo-NSGs[28] that can incorporate any of thousands of readily available primary amines as building blocks. Equimolar mixtures of NSG peptoids are prepared automatically by a custom robotic instrument[29] which employs the resin-splitting method of solid-phase synthesis.[8,9,30,31] We now report the design, synthesis, and screening of a diverse, ca. 5000-component NSG peptoid library and the discovery of potent ligands that bind to therapeutically important receptors in the 7-transmembrane G-protein-coupled receptor (7TM/GPCR) family.

Design of the Peptoid Library

The efficient assembly of diverse NSG peptoid libraries from readily available starting materials,[28] coupled with automated equimolar mixture synthesis technology,[29] has opened up a tremendous new source of inexpensive compounds for screening. Since the number of potentially available compounds is greater than the number that can be efficiently and accurately screened in solution-phase competition assays, several experimental design criteria were imposed. Bioavailable compounds typically have molecular weights below ca. 600 Da, so the library was limited to dimers and trimers. For robotics convenience, the mixture design was limited to a basis set of fewer than 25 different monomers. The complexity of each mixture was limited to 500 compounds in order to simplify subsequent deconvolution to identify the individual high-affinity compounds.

Since we desired potent ligands to a wide variety of 7TM/GPCRs, a survey was made of known ligands. The amino acid sequences of this class of receptors are highly homologous,[32] and compounds that bind to different receptors in this class frequently share common functional groups. This information was used to focus the library toward known pharmacophores. Analysis of the structural fragments from known 7TM/GPCR ligands suggested that each compound in a trimer library might best contain at least one aromatic hydrophobic side chain and one hydrogen bond-donating side chain. The remaining side chain was chosen from a diverse set of

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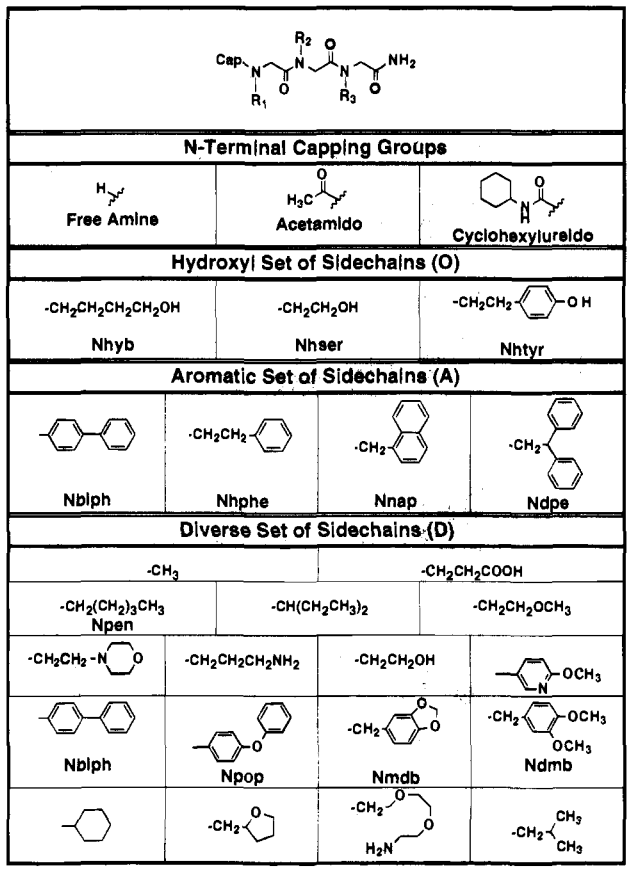

Figure 1. Trimer library backbone, side chain, and N-terminal cap structures. The combinatorial peptoid library was assembled from three sets of *N*-(substituted)glycine monomers: A is a set of four aromatic monomers, O is a set of three hydroxylic monomers, and D is a set of 17 diverse monomers.

chemical functionalities, thus presenting the key substituents in a variety of geometries and chemical environments to maximize the chances of favorable interactions with a target receptor. Finally, several different N-terminal capping groups were included, and the carboxy-terminus was fixed as a primary amide.

Accordingly, three sets of monomers were chosen (Figure 1) from commercially available amines: a set of four aromatic hydrophobic monomers, a set of three hydroxylic monomers, and a set of 17 diverse monomers, designated A, O, and D, respectively. Since set D included one member from each of the other two sets, the total basis set contained 22 amines. The amino terminus was left as a free amine or capped as the acetamide or cyclohexylurea.

For the aromatic set, *N*-(4-biphenylyl)glycine was chosen because biphenyls occur in AT_1 antagonists.[33] Similarly, the diphenylmethyl functionality is common in ligands for adrenergic,[34,35] AT_2,[36] muscarinic,[37] neurotensin,[38] neurokinin$_1$,[39,40] adenosine,[41] and endothelin[42] receptors. The naphthyl framework is found in adrenergic[35] and CCK[43] ligands, and the phenethyl or benzyl functionalities are common in a wide variety of CNS ligands.[44] Monomers for the hydroxylic set were similarly chosen. Aliphatic hydroxyls often occur in adrenergic,[34] muscarinic,[37] AT_1,[33] oxytocin,[45] and endothelin[46] ligands; phenols are found in endothelin[46] and oxytocin[45] ligands. The diverse monomer set was chosen to cover a wide range of characteristics: charge, hydrophobicity, aromaticity, hydrogen bonding, size, shape, flexibility, and degree of branching.

Table 1. Complete Library of the 18 Initial Mixtures[a]

H- = free amine	Ac- = acetamido	Chu- = cyclohexylureido
H-AOD-NH_2	Ac-AOD-NH_2	Chu-AOD-NH_2
H-ADO-NH_2	Ac-ADO-NH_2	Chu-ADO-NH_2
H-DAO-NH_2	Ac-DAO-NH_2	Chu-DAO-NH_2
H-DOA-NH_2	Ac-DOA-NH_2	Chu-DOA-NH_2
H-ODA-NH_2	Ac-ODA-NH_2	Chu-ODA-NH_2
H-OAD-NH_2	Ac-OAD-NH_2	Chu-OAD-NH_2

[a] Each of the 18 initial mixtures was made from all combinations drawn from the six permutations of three sets of amines, times three N-terminal endings (see Figure 1). Thus, each trimer contains at least one aromatic and one hydroxylic group. Mixture H-ODA-NH_2 showed the greatest inhibition of [^{3}H]prazosin binding to the α_1-adrenergic receptor, and mixture H-ADO-NH_2 showed the greatest inhibition of [^{3}H]DAMGO binding to the μ-opiate receptor.

The complete library consisted of 18 mixtures, generated from the six permutations of the three monomer sets, times three N-termini (Table 1). Thus, each of the 18 combinatorial mixtures included 3O × 4A × 17D = 204 peptoid trimers. This strategy guaranteed that each trimer contained at least one aromatic and one hydroxylic side chain. In addition, all possible dimers were included, adding 12, 51, or 68 additional compounds to pools with N-terminal D, A, and O groups, respectively. Thus, each mixture contained either 216, 255, or 272 compounds.

Synthesis of the Peptoid Library

The original method[23] for the synthesis of oligomeric NSG peptoids is analogous to standard solid-phase methods for peptide synthesis. A recent synthetic advance[28] has enabled the assembly of each *N*-(substituted)glycine monomer from two readily available "submonomers" in the course of extending the NSG polymer (Figure 2). Each cycle of monomer addition consists of an acylation step and a nucleophilic displacement step. The backbone is introduced via an acylation with bromoacetic acid, and the side chains are introduced via a displacement of bromine by a wide variety of primary amines.

Rather than attempting to fully characterize the completed library, we chose to establish the oligomerization efficiency of each submonomer. Thus, prior to library synthesis, each amine submonomer was individually tested for its ability to be efficiently incorporated into a test oligomer. Only aliphatic hydroxyl, amine, and acid side chains required protection. Equimolarity of the mixtures was achieved by using the resin-splitting method[8,9,30,31] on an automated synthesizer.[29] The inherent ±3% deviation in the splitting of the resin by the isopycnic slurry method[29] does not significantly

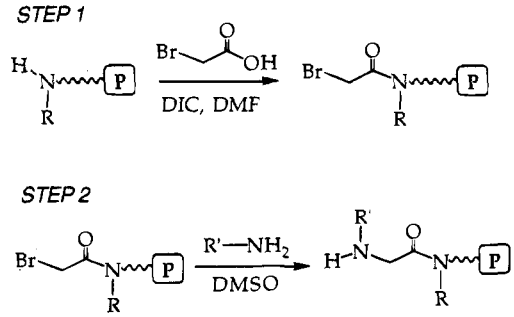

Figure 2. Synthesis of NSG peptoid oligomer libraries by a solid-phase submonomer synthesis that uses a two-step monomer addition cycle.[28]

affect the equimolarity of the library in this case, since only trimers were made. Monomer addition reactions were carried out simultaneously in separate reaction vessels, after which all portions were combined. High reagent concentrations and long reaction times were used, ensuring that all reactions proceeded to completion (even for the less nucleophilic amines like 4-aminobiphenyl). Since a very large number of resin beads ($>5 \times 10^6$) was used for the synthesis of a small number of compounds, <5000, significant deviations from equimolarity owing to Poisson distribution effects during resin-splitting were unlikely. Thus, although the final library can be only qualitatively characterized (by reversed-phase HPLC and mass spectrometry fingerprinting), the quality control prior to library synthesis ensures that the library will be quite close to its intended composition.

The complete library was generated through six independent syntheses, each corresponding to a different permutation of the three monomer basis sets (O, A, and D). Each synthesis was obtained by performing three cycles of resin-splitting. Dimers were simultaneously generated by including a solvent blank in each N-terminal basis set. After the last step, each mixture was separated into three equal portions for N-terminal capping, generating the 18 initial pools.

Discovery of Potent Adrenergic and Opiate Receptor Ligands

The 7TM/GPCRs have perhaps yielded more major pharmaceuticals than any other family of therapeutic targets to date.[47] Among these are various non-peptide adrenergic receptor ligands for treating asthma, congestion, glaucoma, heart failure, hypertension,[34] and benign prostatic hyperplasia.[48] Various peptide and non-peptide opiate receptor ligands are useful in alleviating pain, suppressing cough, and decreasing gut motility.[49] To demonstrate the utility of NSG libraries in the *de novo* discovery of different types of pharmaceutical leads, we screened the libraries against both the adrenergic and opiate receptors. Screening was performed with equimolar mixtures of soluble peptoids in competitive radioligand-binding assays. Individual active peptoid components were identified by iterative resynthesis of successively smaller subpools (deconvolution).[8,50]

The original 18 NSG pools were assayed against an α_1-adrenergic receptor preparation (of unknown subtype composition) (Figure 3a). After each round of screening, the pools showing greatest inhibition of [^{3}H]prazosin binding[51] were selected for the next round of deconvolution. Thus, when the H-ODA-NH_2 pool was identified as the most inhibitory of the original set of 18 pools, it was resynthesized as a set of four subpools, each containing 68 compounds of the form H-X-DA-NH_2, where X represents one of the three side chains from the hydroxylic set or a solvent blank. As seen in Figure 3b, a single pool with Nhtyr in the N-terminal position was responsible for the activity in the more complex parent pool. The peptoids from this subpool were similarly deconvoluted and assayed as 17 mixtures of four compounds, each of the form H-Nhtyr-X-A-NH_2, with X now representing each of the 17 side chains from the diverse set (Figure 3c). Two mixtures, with Nbiph or Npop in the middle position, showed significant

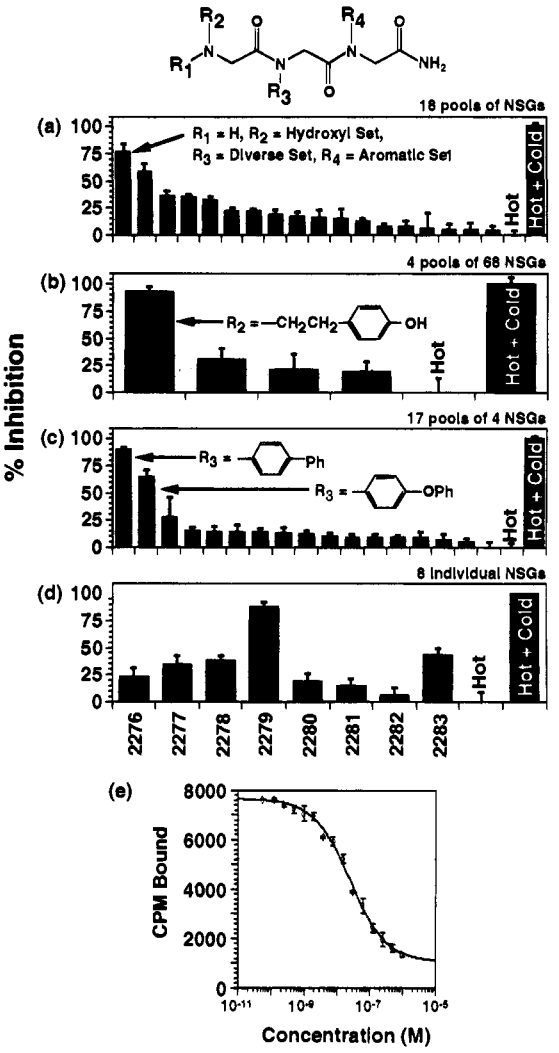

Figure 3. Identification of high-affinity ligands. High-affinity ligands for the α_1-adrenergic receptor were identified from a biased peptoid library by assaying the original 18 pools (at 100 nM per peptoid) for binding[51] and tracing the binding activity to individual compounds by iterative resynthesis and screening of smaller subpools (at 1 μM per peptoid). Assays were performed in duplicate; error bars indicate the range of values.

activity. The remaining eight peptoids that comprised these last two pools were resynthesized and assayed individually (Figure 3d). Compound CHIR 2279, from the more inhibitory of the two preceding pools, was the most potent, with a K_i of 5 ± 3 nM (Figure 3e). Closely related compounds (Figure 4a), CHIR 2276 and CHIR 2283, had K_i values of 310 and 140 nM, respectively.

The original 18 NSG pools were similarly assayed against an opiate receptor preparation (of unknown subtype composition) (Figure 5a). After each round of screening, the pool showing the greatest inhibition of [^{3}H]DAMGO (μ-specific) binding[52] was selected for the next round of deconvolution. The H-ADO-NH_2 pool was identified as the most inhibitory initial mixture and was resynthesized as a set of five subpools, each containing 51 compounds of the form H-X-DO-NH_2, where X represents one of the four side chains from the aromatic set or a solvent blank. The mixture with Ndpe in the N-terminal position was responsible for most of the activity seen in the more complex mixture (Figure 5b). The peptoids from this pool were deconvoluted and assayed as 17 subpools of three compounds, each of the

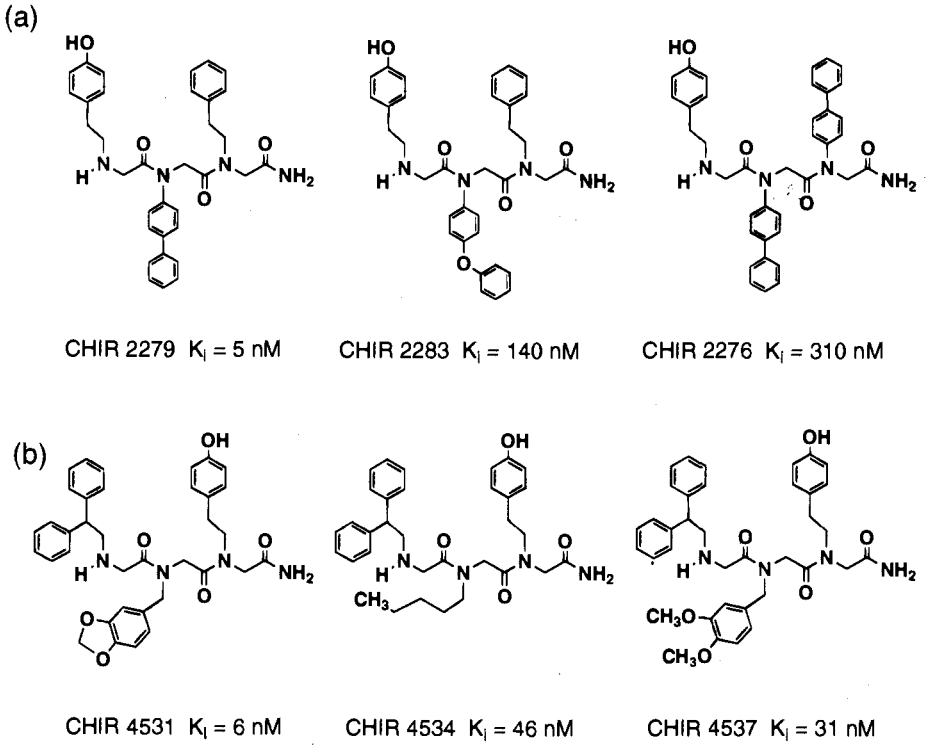

Figure 4. High-affinity ligands for the (a) α_1-adrenergic and (b) μ-specific opiate receptors discovered from a combinatorial peptoid library.

form H-Ndpe-X-O-NH_2, with X now representing side chains from the diverse set. Three pools, with Ndmb, Npen, or Nmdb in the middle position, showed significant activity (Figure 5c). The remaining nine peptoids (CHIR 4529–CHIR 4537) comprising these last three pools were synthesized and assayed individually (Figure 5d). Compound CHIR 4531, from the most inhibitory of the three preceding pools, was the most potent, with a K_i value of 6 ± 2 nM (Figure 5e). The related compounds (Figure 4b), CHIR 4537 and CHIR 4534, have K_i values of 31 and 46 nM, respectively.

Discussion

A convergence of technologies has made the generation and screening of diverse synthetic libraries a viable drug discovery tool. The peptoid approach offers several distinct advantages over the use of natural biopolymer libraries. For example, it can directly provide relevant drug candidates—the conversion of a lead into a biologically stable structure can be avoided. The oligo-NSGs are synthesized in high yield by a practical, polymer-supported method that uses commercially available starting materials. A robotic apparatus fully automates the splitting of resins so that defined mixtures of precise composition can be rapidly prepared. The chemistry permits the incorporation of a wider variety of side chain structures than are found in the standard amino acid and nucleotide side chains, affording high diversity[53] without resorting to high molecular weight or large numbers of compounds.

Much of the prior work in this field has centered on screening random libraries containing enormous numbers of natural biopolymers.[2] Here, a small, focused, combinatorial library with non-natural building blocks provided novel, high-affinity ligands for two pharmaceutically relevant receptors. The effectiveness of this approach is the result of the library design criteria, the efficiency of the oligomerization chemistry, and the ability to include a wide variety of structural fragments into the peptoids. Some monomers were chosen to mimic known 7TM/GPCR pharmacophores, while others were chosen to be maximally diverse. This strategy allowed for efficient searching because there was little chemical redundancy among library components, yet each compound was forced to contain relevant structural fragments.[54] This approach allowed screening with smaller libraries, increasing the accuracy of the screen and reducing the work needed to identify high-affinity ligands. The use of encoded libraries,[11,12,55,56] support-bound libraries,[7,9] or phage libraries[13–15] facilitates the screening of larger libraries but also involves the covalent attachment of the drug candidates to a solid matrix or to the code molecule which can interfere with binding. By screening the peptoids as equimolar mixtures in solution, quantitative binding data[31] can be obtained without interferences from a solid support.

Low molecular weight tripeptoids were discovered for both the α_1-adrenergic receptor and the μ-opiate receptor. The α_1-adrenergic ligand CHIR 2279 (MW = 565 g/mol, K_i = 5 nM) competes with prazosin (K_i of 0.2 nM),[57] a small molecule heterocycle (Figure 6a). The endogenous ligands for this receptor (Figure 6a) are the non-peptidic neurotransmitters epinephrine (K_i of 2–14 μM)[57] and norepinephrine (K_i of 0.5–4 μM).[57] Although epinephrine and CHIR 2279 share a substituted phenethylamine, the binding of CHIR 2279 is clearly not due to the tyramine residue alone, as evidenced by the fact that deshydroxy analogs of CHIR 2279 bind with no loss of affinity (K_i of 4 nM). Furthermore, over 135 other peptoids containing an N-terminal tyramine did not bind significantly in the initial screen of the library, and tyramine itself did not compete for binding at a concentration of 10 μM (data not shown).

The opiate ligand CHIR 4531 (MW = 623 g/mol, K_i =

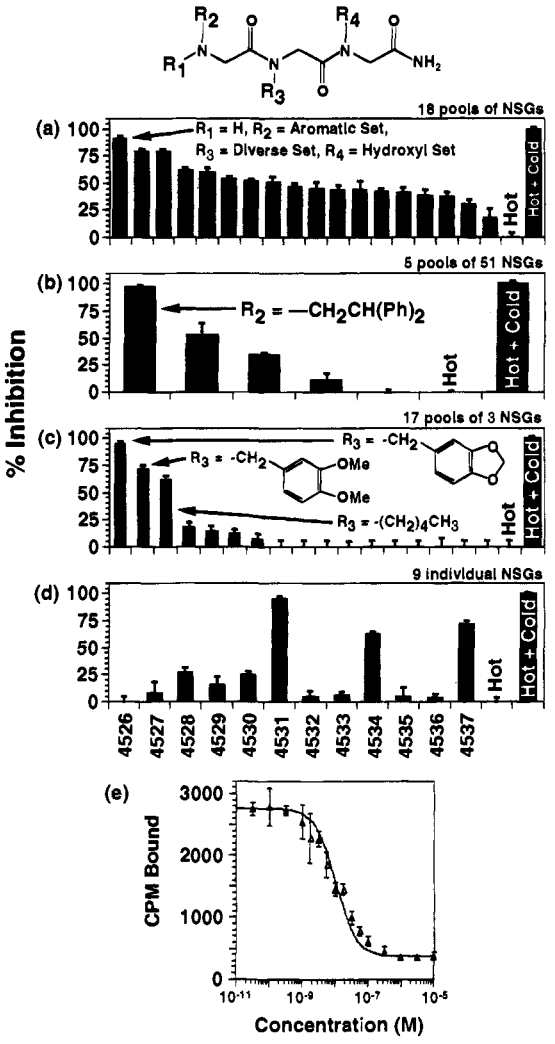

Figure 5. Discovery of μ-specific opiate receptor ligands. Assays were performed at 100 nM per peptoid[52] and done in (a–d) duplicate, where error bars indicate the range of values, or (e) quadruplicate, where error bars indicate standard deviations.

6 nM) competes with DAMGO (K_i of 4 nM),[52] a pentapeptide. Endogenous ligands for the opiate receptors (Figure 6b) can be either low molecular weight heterocycles such as morphine (K_i of 2 nM)[58] or peptide hormones such as enkephalin (K_i of 20 nM).[58] In both the adrenergic and opiate cases, the NSG peptoid trimers represent a novel class of ligands.

The results here demonstrate that screening receptor-targeted synthetic combinatorial libraries of NSG peptoids is an effective general approach for discovering novel ligands to both peptide- and non-peptide-binding receptors. In the examples shown here, low molecular weight, achiral NSG peptoid trimers are potent competitors for chiral endogenous ligands. The peptoids are a class of structurally diverse organic molecules that are well suited for the high-throughput synthesis of non-natural molecular diversity and can directly provide relevant high-affinity drug candidates. The drug discovery technologies and principles presented here are compatible with most existing biological assays and can be adapted to accommodate a variety of diversity-generating chemistries. This combination of technologies should dramatically accelerate the drug discovery process.

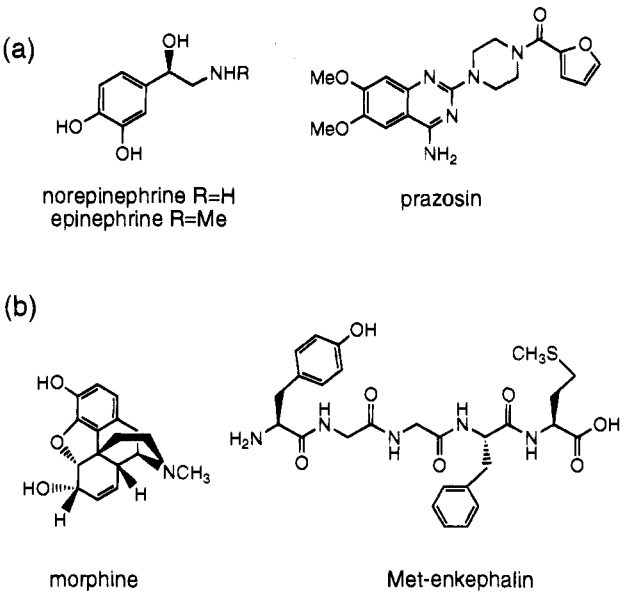

Figure 6. Known ligands for the (a) α_1-adrenergic and (b) opiate receptors.

Experimental Section

Solvents and reagents were obtained from commercial suppliers and used without further purification. Oligomer synthesis was performed on a Rink amide poly(styrene) resin (1% cross-linked, 100–200 mesh). All solid-phase reactions were performed at room temperature in glass vessels equipped with a coarse frit. Agitation of the resin–reagent slurry was performed at every step and achieved by the periodic bubbling of argon gas through the frit. Filtration of the resin–reagent suspension through the frit was achieved by the application of vacuum. Splitting of the resin into equimolar portions was performed by distributing equal volumes of an isopycnic slurry of the resin in 3:2 dichloroethane:DMF.

Individual NSG oligomers were analyzed by reversed-phase HPLC on C-18 columns (Vydac, 5 μm, 300 Å, 4.5 × 250 mm^2). A linear gradient of 0–80% B in 40 min was used at a flow rate of 1 mL/min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in CH_3CN). Mass spectra were analyzed in a glycerol matrix by liquid matrix secondary ion mass spectrometry on a VG Analytical ZAB 2SE mass spectrometer at Mass Search (Modesto, CA).

2-[(Triisopropylsilyl)oxy]ethylamine (1). A solution of triisopropylsilyl chloride (50.0 g, 259 mmol) in dichloromethane (50 mL) was added dropwise under N_2 at 0 °C to a vigorously stirred solution of ethanolamine (158 g, 2.59 mol) in dichloromethane (150 mL), and stirring was continued for 1 h at 0 °C. The mixture was allowed to warm to ambient temperature and stirred for an additional 16 h. The crude mixture was poured into water (300 mL), the layers were separated, and the organic layer was washed with water (2 × 100 mL) and brine (100 mL). The aqueous layers were re-extracted with dichloromethane (1 × 100 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was filtered over Na_2SO_4 (3.0 g) to yield 55.2 g (98%) of a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (t, J = 7 Hz, 2H, CH_2), 2.76 (t, J = 7 Hz, 2H, CH_2), 1.42 (br s, 2H, NH_2), 1.04 (m, 21H, CH_3 and isopropyl).

4-[(Triisopropylsilyl)oxy]butylamine (2). A solution of triisopropylsilyl chloride (43.2 g, 224 mmol) in dichloromethane (50 mL) was added dropwise under N_2 at 0 °C to a vigorously stirred solution of 4-amino-1-butanol (100 g, 1.12 mol) in dichloromethane (25 mL), and stirring was continued for 1 h at 0 °C. The mixture was allowed to warm to ambient temperature and stirred for an additional 16 h. The crude mixture was poured into water (300 mL), the layers were separated, and the organic layer was washed with water (2 × 100 mL) and brine (100 mL). The aqueous layers were re-extracted with dichloromethane (1 × 100 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was

removed *in vacuo*. The residue was filtered over Na_2SO_4 (3.0 g) to yield 48.0 g (88%) of a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (t, 2H, CH_2), 2.70 (t, 2H, CH_2), 1.80 (br s, 2H), 1.52 (m, 4H), 1.04 (m, 21 H, CH_3 and isopropyl).

2-Amino-2′-[(*tert*-butoxycarbonyl)amino]ethylene Glycol Diethyl Ether (3). To a solution of 2,2′-(ethylenedioxy)-bis[ethylamine] (83 mL, 0.55 mol) in *p*-dioxane (400 mL) was added dropwise at ambient temperature over 6 h a solution of di-*tert*-butyl dicarbonate (20.4 g, 0.093 mol) in *p*-dioxane (100 mL). The mixture was stirred overnight and then concentrated *in vacuo*. The residue was dissolved in water (100 mL) and rinsed with dichloromethane (5 × 200 mL). The combined organic layers were rinsed with saturated aqueous NaCl (4 × 100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give 22.8 g (99%) of a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.18 (br s, 1H, N*H*Boc), 3.60 (s, 4H), 3.50–3.54 (m, 4H), 3.30 (m, 2H), 2.87 (t, 2H), 1.53 (br s, 2H, NH_2), 1.43 (s, 9H, *t*-Bu).

Monomer Evaluation. Reactivity of the amine submonomers was determined prior to mixture synthesis. Each of the 22 amines was incorporated into an individual NSG pentamer synthesized with the sequence Nphe-X-Nphe-X-Nphe (X = *N*-(substituted)glycine of test amine). Criteria for acceptance[59] were confirmation of the expected molecular ion by mass spectrometry, purity of at least 75% as determined by reversed-phase C-18 HPLC, and a minimum yield of 50%.

Library Synthesis. Each of the six mixture syntheses was performed with 1.8 mmol of Rink amide poly(styrene) resin (0.45 mmol/g). Resin-bound amines (0.1 mmol) were bromoacetylated by *in situ* activation with DIC. To the oligomer–resin was added a DMF solution of bromoacetic acid (0.6 M, 1.7 mL) followed by a DMF solution of DIC (3.2 M, 0.4 mL). The reaction mixture was agitated for 30 min at ambient temperature and drained, and the reaction was repeated once more. Resin-bound bromoacetamides (0.1 mmol) were displaced by addition of the amine as a solution in DMSO (1–2 M, 2.0 mL, 2 h, ambient temperature), except for methylamine which was used as a 40% aqueous solution. Each trimer–resin mixture was split into three aliquots to which different capping groups were added. Capping conditions (0.60 mmol): Trimer–resin was acetylated with acetic anhydride (0.5 M) and DIEA (0.5 M) in DMF (24 mL total, 30 min, ambient temperature); cyclohexylurea capping groups were added with a solution of cyclohexyl isocyanate (1.0 M) in DMF (24 mL, 1 h, ambient temperature). Mixtures were cleaved from the resin (0.6 mmol) by treatment with 95% TFA/5% water (25 mL, 15 min, ambient temperature) followed by washing and lyophilization (2×) from acetic acid.

[*N*-[2-(4-Hydroxyphenyl)ethyl]glycyl]-[*N*-(4-biphenylyl)-glycyl]-*N*-(2-phenylethyl)glycinamide (CHIR 2279). A 180 mL vessel was charged with Rink amide resin (5.4 g, 3.7 mmol). The resin was briefly swelled in DMF (144 mL) with gentle agitation and drained. The 9-fluorenylmethoxycarbonyl (Fmoc) group was then removed by treatment with 20% piperidine/DMF (144 mL, 1 × 5 min followed by 1 × 20 min). The resin was then washed with DMF (7 × 144 mL). The remainder of the trimer was synthesized by performing three cycles of acylation with bromoacetic acid and displacement with an amine.

Acylation conditions: Resin-bound amines were bromoacetylated by *in situ* activation with DIC. To the oligomer–resin was added a DMF solution of bromoacetic acid (0.6 M, 62 mL) followed by a DMF solution of DIC (3.2 M, 15 mL). The reaction mixture was agitated for 30 min at ambient temperature. The mixture was drained, and the reaction was repeated once. The resin was washed with DMF (3 × 144 mL).

Displacement conditions: Resin-bound bromoacetamides were displaced by the addition of the amine as a solution in DMSO (1–2 M, 62 mL). The reaction mixture was agitated at ambient temperature for 2 h. The reaction mixture was drained, and the resin was washed with DMF (3 × 144 mL). Phenethylamine and 4-aminobiphenyl were used at 2.0 M concentration, and tyramine was used at 1.0 M (to avoid solubility problems).

After completion of the synthesis, the resin was washed with CH_2Cl_2 (3 × 144 mL) and air-dried for 5 min. The full length trimer was cleaved from the resin (3.7 mmol) by treatment with 95% TFA/5% water (200 mL) at ambient temperature for 15 min. The resin was then washed with 95% TFA/5% water (2 × 20 mL) and water (1 × 10 mL). The filtrates were pooled, and the solvent was removed *in vacuo*. The residue was dissolved in glacial acetic acid (100 mL) and lyophilized (2×) to afford a light yellow powder (1.7 g, 82% yield). The purity of the crude product was determined to be 94% by reversed-phase HPLC (MH^+ = 566): ^{1}H NMR (300 MHz, D_2O) δ 7.25–7.68 (m, 7H, arom H), 6.45–7.12 (m, 11H, arom H), 3.95 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.47 (t, J = 7 Hz, 2H, CH_2), 3.35 (s, 2H, CH_2), 3.05 (t, J = 7 Hz, 2H, CH_2), 2.70 (t, J = 7 Hz, 2H, Ar-CH_2), 2.66 (t, J = 7 Hz, 2H, Ar-CH_2). Anal. ($C_{34}H_{36}N_4O_4{\cdot}CF_3CO_2H{\cdot}0.5\ H_2O$) C, H, N.

[*N*-(2,2-Diphenylethyl)glycyl]-[*N*-[3,4-(methylenedioxy)-benzyl]glycyl]-*N*-[2-(4-hydroxyphenyl)ethyl]glycinamide (CHIR 4531). This compound was prepared in a similar fashion to CHIR 2279 except on a larger scale. The trimer was synthesized on 62 g of Rink amide resin (23.5 mmol) using tyramine, piperonylamine, and 2,2-diphenylethylamine as the amine submonomers. After workup and lyophilizations as described above, 14.2 g (88%) of a light yellow powder was obtained. The purity of the crude product was determined to be 93% by reversed-phase HPLC (MH^+ = 624). The ^{1}H NMR of CHIR 4531 shows a 1:1 mixture of conformers at 25 °C in D_2O: ^{1}H NMR (300 MHz, D_2O) δ 7.05–7.28 (m, 10 H, arom H), 6.28–6.90 (m, 7H, arom H), 5.76 and 5.82 (2s, 2H, dioxolane CH_2), 4.13 and 4.25 (2t, J = 7 Hz, 2H, tyramine NCH_2), 3.30–4.00 (m, 11H, aliph CH and CH_2), 2.58 and 2.60 (2t, J = 7 Hz, 2H, Ar-CH_2). Anal. ($C_{36}H_{36}N_4O_6{\cdot}CF_3CO_2H{\cdot}H_2O$) C, H, N.

Receptor-Binding Assays. The α_1-adrenergic[51] and μ-opiate[52] receptor-binding assays were performed by measuring competitive displacement of radioligand from rat brain membrane preparations. Rat forebrains were homogenized and washed in 50 mM Tris, pH 7.5, containing 20 mM NaCl, 5 mM EGTA, 2 mM $MgCl_2$, 21 μg/mL aprotinin, 0.5 mg/L leupeptin, 0.7 mg/L pepstatin, and 0.2 mM PMSF; 50 μL of membrane (10 mg/mL protein) was dispensed into 1 mL of 50 mM Tris, pH 7.5, containing the radioligand and the peptoid mixture. After incubation for 1 h at ambient temperature, the reactions were quenched with 3 mL of ice-cold incubation buffer and then the mixtures were rapidly filtered over Whatman GF/B glass fiber filters. Filters were washed three times with 3 mL of additional ice-cold buffer, soaked overnight in 5 mL of Beckman ReadySafe scintillation cocktail, and then counted for 1 min in a Wallac 1409 liquid scintillation counter. K_is were determined via nonlinear least squares regression fitting of the competition binding data to single-site binding isotherms.

The α_1-adrenergic receptor assays were performed using 0.5 nM [^{3}H]prazosin as the radioligand; nonspecific binding was determined in the presence of 5 μM unlabeled prazosin. The μ-opiate receptor assays were performed using 1 nM [^{3}H]-DAMGO; nonspecific binding was determined in the presence of 1 μM naloxone. For both receptor assays, the initial 18 peptoid pools were assayed at 100 nM per peptoid. Resynthesized subpools were assayed at 1 μM per peptoid (α_1-adrenergic) or 100 nM per peptoid (μ-opiate).

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