JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

pubs.acs.org/JACS

Design, Synthesis, and Validation of a β-Turn Mimetic Library Targeting Protein−Protein and Peptide−Receptor Interactions

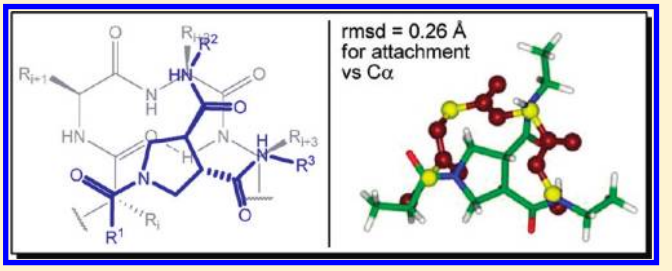
Landon R. Whitby,[†] Yoshio Ando,[†] Vincent Setola,[‡] Peter K. Vogt,[†] Bryan L. Roth,[‡] and Dale L. Boger*[,†]

[†]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

[‡]NIMH Psychoactive Drug Screening Program, University of North Carolina Chapel Hill, Genetic Medicine Building, Chapel Hill, North Carolina 27514, United States

Supporting Information

ABSTRACT: The design and synthesis of a β-turn mimetic library as a key component of a small-molecule library targeting the major recognition motifs involved in protein−protein interactions is described. Analysis of a geometric characterization of 10 245 β-turns in the protein data bank (PDB) suggested that *trans*-pyrrolidine-3,4-dicarboxamide could serve as an effective and synthetically accessible library template. This was confirmed by initially screening select compounds against a series of peptide-activated GPCRs that recognize a β-turn structure in their endogenous ligands. This validation study was highlighted by identification of both nonbasic and basic small molecules with high affinities (K_i = 390 and 23 nM, respectively) for the κ-opioid receptor (KOR). Consistent with the screening capabilities of collaborators and following the design validation, the complete library was assembled as 210 mixtures of 20 compounds, providing a total of 4200 compounds designed to mimic all possible permutations of 3 of the 4 residues in a naturally occurring β-turn. Unique to the design and because of the C_2 symmetry of the template, a typical 20 × 20 × 20-mix (8000 compounds prepared as 400 mixtures of 20 compounds) needed to represent 20 variations in the side chains of three amino acid residues reduces to a 210 × 20-mix, thereby simplifying the library synthesis and subsequent screening. The library was prepared using a solution-phase synthetic protocol with liquid−liquid or liquid−solid extractions for purification and conducted on a scale that insures its long-term availability for screening campaigns. Screening the library against the human opioid receptors (KOR, MOR, and DOR) identified not only the activity of library members expected to mimic the opioid receptor peptide ligands but also additional side-chain combinations that provided enhanced receptor binding selectivities (>100-fold) and affinities (as low as K_i = 80 nM for KOR). A key insight to emerge from the studies is that the phenol of Tyr in endogenous ligands bearing the H-Tyr-Pro-Trp/Phe-Phe-NH_2 β-turn is important for MOR binding but may not be important for KOR (accommodated, but not preferred) and that the resulting selectivity for KOR observed with its removal can be increased by replacing the phenol OH with a chlorine substituent, further enhancing KOR affinity.



INTRODUCTION

The interaction of proteins to form higher-order complexes is critical to nearly all biological processes, including cellular signaling.[1] Despite their central role in cellular signaling, such protein−protein interactions have emerged relatively slowly as viable small-molecule therapeutic targets.[2] This can be attributed to the fact that protein−protein interfaces do not typically bind endogenous small-molecule ligands that could provide lead structures for drug discovery programs and that the interfaces often also present physical challenges to small-molecule binding. These challenges are generally thought to come in the form of the noncontiguous binding regions within the interacting proteins as well as an often relatively large and/or flat binding interface. However, systematic case studies of selected protein−protein interactions have revealed that there is typically a small cluster of key residues near the center of the interface that contributes the majority of the recognition or binding affinity.[3] Significantly and although endogenous small-molecule ligands may not exist, many if not most protein−protein interactions are mediated by three main recognition motifs (α-helix, β-turn, and β-strand).[4] Consequently, an attractive approach for the discovery of modulators of protein−protein interactions is to mimic the key interaction residues using small-molecule mimetics of these three major recognition motifs.

A related class of interactions in which small-molecule mimetics of peptide secondary structure have already proven valuable is the peptide−receptor interactions, the best characterized example of which is the interaction of the peptide-activated G protein-coupled receptors (GPCRs) with their endogenous

Received: March 7, 2011
Published: May 24, 2011

peptide ligands. Significantly, GPCRs comprise the targets of over one-third of the currently registered pharmaceuticals, although only a subset of GPCRs are peptide-activated. Although the peptide ligands of this subset of GPCRs are usually flexible and able to adopt variable secondary structures, the active conformation recognized by the receptor typically involves a turn structure (β- or γ-turn).[5] Due to this pervasive recognition pattern, small-molecule mimetics of turn structures (typically β-turn)[5] and peptides with turn constraints have been successful in targeting this class of receptors.[5,6] As the roles of these receptors have emerged, small-molecule scaffolds capable of mimicking peptide turn structures continue to be valuable in defining the residues and secondary structure responsible for the binding recognition and affinity, useful in validating new drug targets, and central to the subsequent development of new therapeutics.[6b]

Over the past decade, we have enlisted a simple solution-phase library synthesis protocol, complementary to more conventional solid-phase techniques, for the generation of libraries capable of targeting protein−protein or protein−DNA interactions.[7] The protocol employs acid/base liquid−liquid or liquid−solid extractions for the purification of products (>95% pure irrespective of the reaction efficiency) and offers the advantages of a less limiting scale, expanded repertoire of chemical reactions (use of heterogeneous catalysts and reagents), direct production of soluble intermediates and final products for assay, and the lack of required linking, attachment and detachment, or capping steps. The approach is amenable to convergent synthetic strategies, the synthesis of mixture libraries, or the use of dynamic libraries. Notably, a number of effective small-molecule modulators of protein−protein[7−12] or protein−DNA interactions[13] have been identified by screening the libraries prepared to date.

Recently, we initiated a program to expand our current library of 95 000 compounds[2f,7] with the preparation of a comprehensive small-molecule library designed to mimic the three major recognition motifs that mediate protein−protein interactions, namely, the α-helix, the β-turn, and the β-strand. Three libraries built around templates designed to mimic each secondary structure and substituted with all triplet combinations of groups representing the 20 natural amino acid side chains would contain a member capable of mimicking the key interaction residues of many, if not most, protein−protein interactions.[4] Such a library would provide a powerful tool to interrogate protein−protein interaction networks in order to validate new therapeutic targets, to provide therapeutic lead compounds, and to afford modulators of biological processes for study. The screening of the comprehensive library would not only provide lead structures for many protein−protein interaction targets even if the nature of the interaction is unknown, but it can also be expected to yield key insights into the recognition motif and key residues mediating the interaction.[14] Screening of the library would also provide an initial comprehensive structure−activity relationship (SAR) study for subsequent iterative lead optimization.[14] Notably, the β-turn component of this library would be particularly valuable for the protein- and peptide-activated GPCRs that recognize turn structures in their endogenous ligands.[4,5] To date, we have disclosed the design, synthesis, and validation of an 8000-membered α-helix mimetic component of this library,[15] and we disclose herein the second component of this comprehensive library, a β-turn mimetic library targeting protein−protein and peptide−receptor interactions.

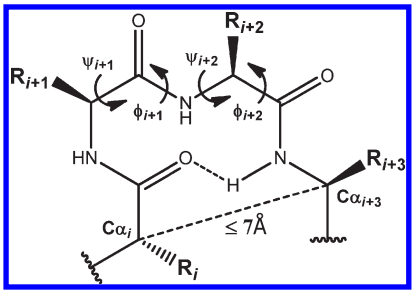


Figure 1. Schematic diagram of a β-turn.

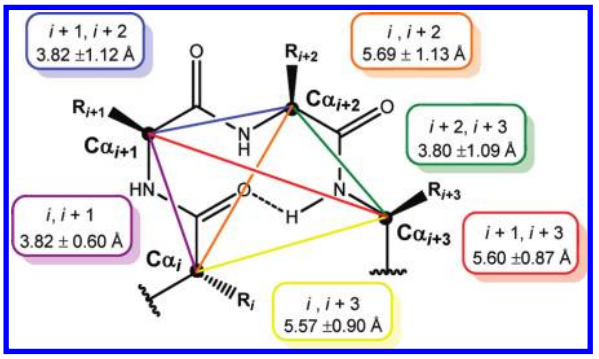


Figure 2. Mean and standard deviations of the Cα distances taken from a set of 10 245 β-turns in the PDB.

RESULTS AND DISCUSSION

***β*-Turn Mimetic Design.** The β-turn is one of the three main secondary structural motifs found in proteins and peptides and occurs where the polypeptide strand reverses direction. As illustrated in Figure 1, the β-turn consists of four amino acid residues designated i to $i + 4$ in which the distance between $C\alpha_i$ and $C\alpha_{i+3}$ is ≤7 Å. Several different types of β-turns exist, depending on the dihedral angles ψ and φ of the $i + 1$ and $i + 2$ residues.

Small-molecule mimetics of β-turns have been extensively investigated and utilized to discover compounds that can mimic or disrupt β-turn-mediated recognition events.[16,17] An ideal β-turn mimetic scaffold around which to build a screening library would be constrained to approximate the correct geometric display of the amino acid side-chain functionality found within a β-turn, be sufficiently flexible to allow the side chains to approximate the side-chain vectors of the many turn types, and be amenable to robust library synthesis.

We envisioned a β-turn mimetic scaffold that could be substituted with all triplet combinations of groups representing the 20 natural amino acid side chains and that was amenable to substitution using our solution-phase library synthesis protocol. This would enable production of a library of pure compounds (>95%) on a scale that would permit virtually unlimited screening opportunities and enable efficient optimization of lead compounds through the rapid synthesis of subsequent lead optimization libraries. In order to identify a template that would allow the appropriate geometric display of the amino acid side-chain functionality, we utilized a geometric analysis of the mean distances found between α-carbon centers in a set of 10 245 β-turns in the protein data bank (PDB, Figure 2).[18,19]

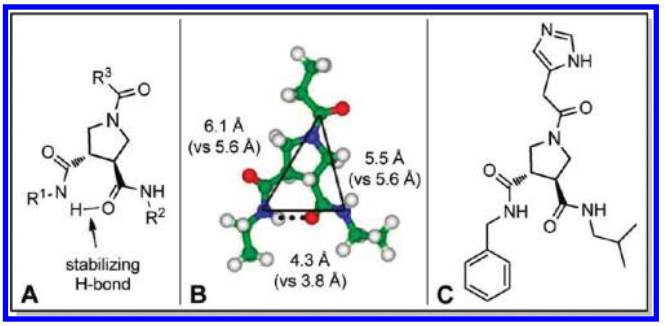


Figure 3. (A) The *trans*-pyrrolidine-3,4-dicarboxamide template. (B) Low-energy conformation and measured distances between substituted centers of a *trans*-pyrrolidine-3,4-dicarboxamide with ethyl substituents. (C) A *trans*-pyrrolidine-3,4-dicarboxamide substituted with groups representing the side chains of Leu, Phe, and His.

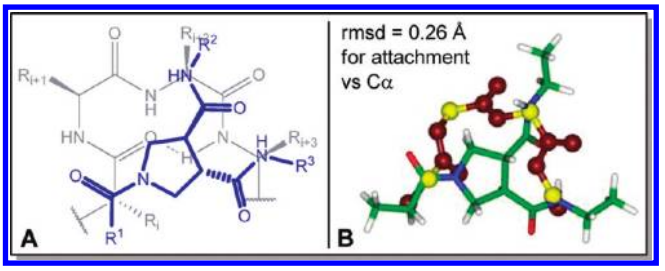
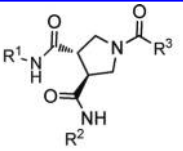


Figure 4. (A) Overlay of a potential mode of β-turn mimicry by a trisubstituted *trans*-pyrrolidine-3,4-dicarboxamide. (B) Overlay and calculated rmsd value for attachment of the three substituted centers of the template with three α-carbons in a type-I β-turn.

Recognizing that one of the turn amino acids ($i + 1$ or $i + 2$) often serves a structural rather than a recognition role (e.g., Pro or Gly),[4,18] we sought to mimic Cα triplets in which either $C\alpha_{i+1}$ or $C\alpha_{i+2}$ is omitted (i.e., $C\alpha_i$, $C\alpha_{i+2}$, $C\alpha_{i+3}$ or $C\alpha_i$, $C\alpha_{i+1}$, $C\alpha_{i+3}$). From this analysis, we found that *trans*-pyrrolidine-3,4-dicarboxamide could serve as a synthetically accessible template upon which to display the amino acid side-chain groups (Figure 3A). Rigidified by an intramolecular H-bond, the lowest-energy conformation of the *trans*-pyrrolidine-3,4-dicarboxamide has substituted centers that conform closely to the triangle geometries of the Cα triplets found in Figure 2 in which either $C\alpha_{i+1}$ or $C\alpha_{i+2}$ is omitted (Figure 3B). An overlay of the low-energy conformation with the peptide backbone of a type-I β-turn (Figure 4A and B) demonstrates a potential mode of mimicry and yielded a rmsd value of 0.26 Å for ethyl substituted centers versus the Cα triplet $C\alpha_i$, $C\alpha_{i+2}$, $C\alpha_{i+3}$. In line with the design, the noncovalent constraints that stabilize the lowest-energy conformer also permit a degree of flexibility to allow the compound to adopt variable H-bond donor/acceptor patterns (e.g., which the carbonyl is exo or endo in the pseudo-7-member ring) and permit the attached side chains to approximate the correct vector display of amino acid side chains in a wide range of β-turn structures.[18] Significantly, the *trans*-pyrrolidine-3,4-dicarboxamide can be substituted using amide coupling reactions and possesses a simplifying C_2 symmetry axis that allows all triplet combinations of 20 different side chains to be accomplished with only 4200 compounds (versus 8000). Thus, in addition to potentially serving as a β-turn mimetic accurately matching the triangle geometries of the Cα triplets of a β-turn while flexibly



compound substituents			binding affinity K_i (nM)[a]	
R^1/R^2	R^3		MOR(h)	KOR(h)
Phe/Phe	Tyr	(1)	>10,000	1,735
Phe/Phe	HoTyr	(2)	>10,000	1,700
Phe/Phe	Tyr(Alkyl)	(3)	2,200	270
Phe/HoPhe	Tyr	(4)	>10,000	5,600
Phe/Phe(4Cl)	Tyr	(5)	>10,000	800
Phe/Nap	Tyr	(6)	>10,000	530
Trp/Phe	Tyr	(7)	>10,000	1000
Trp/Phe	HoTyr	(8)	930	390
Trp/Phe	Tyr(Alkyl)	(9)	2,700	23
Trp/HoPhe	Tyr	(10)	820	420
Trp/Nap	Tyr	(11)	2,600	770
Trp/Lys	Tyr	(12)	3,000	2,500
Phe/Val	Tyr	(13)	>10,000	>10,000
Phe/Val	HoTyr	(14)	>10,000	>10,000
Phe/Val	Tyr(Alkyl)	(15)	1,900	6,700
Thr/Lys	Tyr(Alkyl)	(16)	>10,000	4,800
Phe/Ala	Tyr	(17)	>10,000	>10,000
Phe/Ala	HoTyr	(18)	>10,000	>10,000
Phe/Ala	Tyr(Alkyl)	(19)	>10,000	>10,000
HoPhe/Ala	Tyr	(20)	>10,000	>10,000
Phe(4Cl)/Ala	Tyr	(21)	>10,000	>10,000
Nap/Ala	Tyr	(22)	>10,000	>10,000

Figure 5. Screening data of compounds against μ-opioid receptor (MOR) and κ-opioid receptor (KOR).

accommodating the vector displays of side chains in a wide range of β-turn structures,[18] it also possesses a unique simplifying C_2 symmetry and is functionalized in a manner amenable to library synthesis.

Evaluation and Validation of the Design against Peptide-Activated GPCRs: The Opioid Receptors. In order to establish the ability of substituted *trans*-pyrrolidine-3,4-dicarboxamides to modulate β-turn-mediated recognition events and before embarking on a comprehensive library synthesis, the evaluation of a select series of compounds against a representative set of peptide-activated GPCRs that are thought to recognize β-turns in their endogenous ligands was conducted. The μ-opioid receptor (MOR) is a well-known clinical target for the treatment of pain. MOR selective opiate analgesics such as morphine remain the drugs of choice for the treatment of severe pain, but their use is limited by the well-characterized side effects of respiratory depression, desensitization with chronic use (tolerance), and development of dependence. The additional two members of the opioid receptor family, the κ-opioid and δ-opioid receptors (KOR and DOR), have also been investigated as analgesic targets. DOR agonists produce analgesia but may also exhibit side effects including convulsions. Activation of the KOR in the central nervous system produces analgesia, though it is generally accompanied by dysphoria, hallucinations, and sedation. Targeting of peripheral KOR, however, has emerged as a potentially

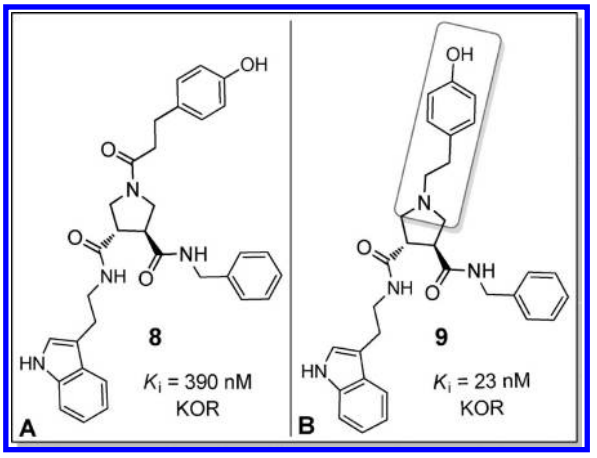


Figure 6. (A,B) Structures of high-affinity KOR-selective compounds **8** and **9**. The tyramine pharmacophore found in the "message" structure of opioid compounds and peptides is highlighted in (B).

promising treatment for inflammatory and visceral pain as well as arthritis.[20]

Two highly potent and selective endogenous peptide ligands of the MOR are endomorphin-1 (H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2).[21] Additional endogenous agonists include Met-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) and Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), which show high affinity for MOR and DOR, their longer peptide precursors β-endorphin and dynorphin, as well as their C-terminus-truncated neoendorphin peptides that display high affinity for the KOR.[20] Although some uncertainty remains, there is considerable evidence that the active conformation of the endomorphins is a β-turn, including activities observed by analogues that incorporate turn constraints and appropriately substituted β-turn mimetics.[22] Therefore, we prepared a small series of pyrrolidine-3,4-dicarboxamide-derived compounds designed to mimic the three side-chain residues in the pharmacophores of the endomorphins and enkephalins along with several Ala negative controls (**17**−**22**) and measured their binding (K_i values) to MOR, KOR and DOR (Figure 5). The highest affinity for the MOR was exhibited by compound **10** with the R^1/R^2 combination of Trp/HoPhe and R^3 of Tyr (K_i = 820 nM) followed by compound **8** with R^1/R^2 of Trp/Phe and R^3 of HoTyr (K_i = 930 nM). In addition to exhibiting submicromolar activity using a simple template, the activity reflected a combination of side-chain residues found in the endogenous endorphin ligands. Moreover, comparisons among the compounds examined demonstrated that the best affinity to MOR was observed when single carbon extensions of key side chains (HoPhe or HoTyr) were incorporated into the structure, and this influenced our selection of side chains incorporated into the full library design. Interestingly, the compounds showed much higher affinities for the KOR than for the MOR, highlighted by **8** and **9** with K_i values measured at 390 and 23 nM, respectively (Figure 6). The identification of **9** as the most active compound is easy to appreciate considering that the R^3 Tyr side chain is attached to the pyrrolidine nitrogen via an alkyl rather than acyl linkage, giving rise to the familiar tyramine group bearing a free amine found in the "message" structure of nearly all opioid analgesics and peptides (Figure 6B).[20a] However, even its *N*-acyl variant lacking the basic nitrogen (**8**, K_i = 390 nM) proved remarkably effective. Selectivity for KOR binding was observed

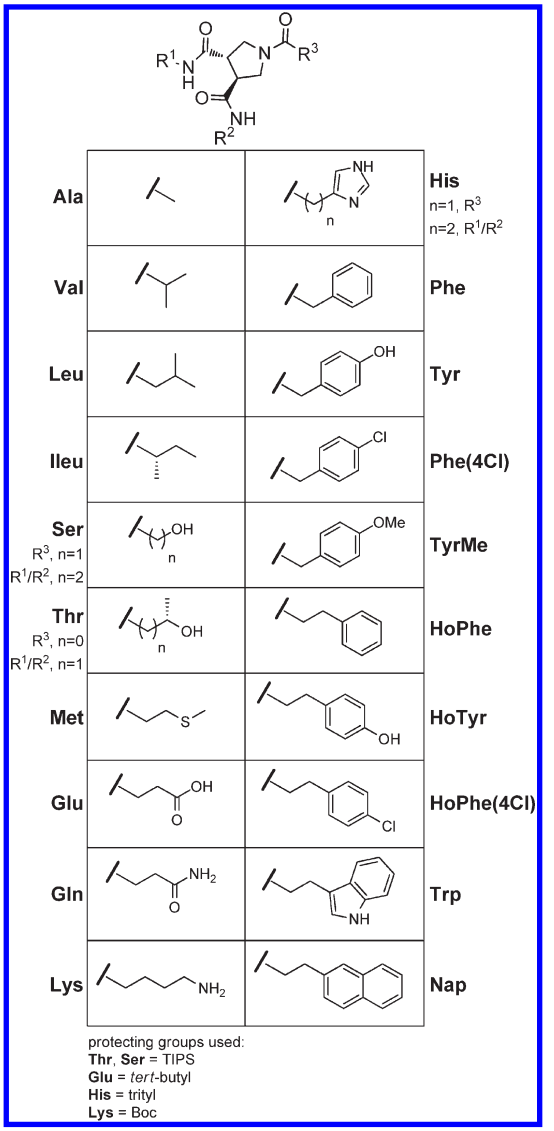
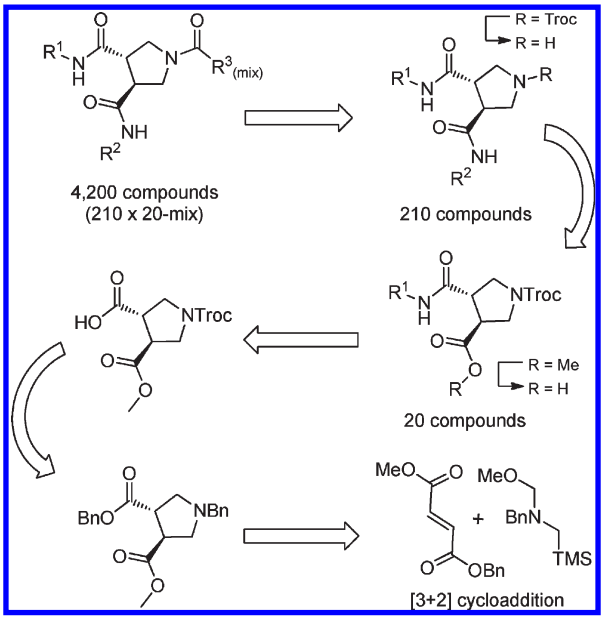


Figure 7. The 20 groups used in the synthesis of the β-turn mimetic library.

with **9** (~100 fold), and this trend was observed with nearly all compounds tested. The origin of this selectivity is currently undefined, but its consistency across the range of compounds tested suggests that there are intrinsic properties to the *trans*-pyrrolidine-3,4-dicarboxamide template, including its three-dimensional projection of side chains or interactions made by the template itself, that favor KOR binding. Nevertheless, the high nanomolar affinities of compounds **8** and **10** indicate that the *trans*-pyrrolidine-3,4-dicarboxamide template can effectively mimic the active turn structure of the endomorphins.

Synthesis of the β-Turn Mimetic Library. Convinced that the pyrrolidine-3,4-dicarboxamide template would prove to be a valuable β-turn mimetic and having established subtle design features from the exploratory studies, the synthesis of the comprehensive β-turn mimetic library was initiated. The 20 amino acid side-chain groups utilized to construct the library along with corresponding protecting groups are provided in Figure 7. By substituting each position of the *trans*-pyrrolidine-3,4-dicarboxamide template with all combinations of these 20 groups, a total of 4200 compounds are produced representing all possible

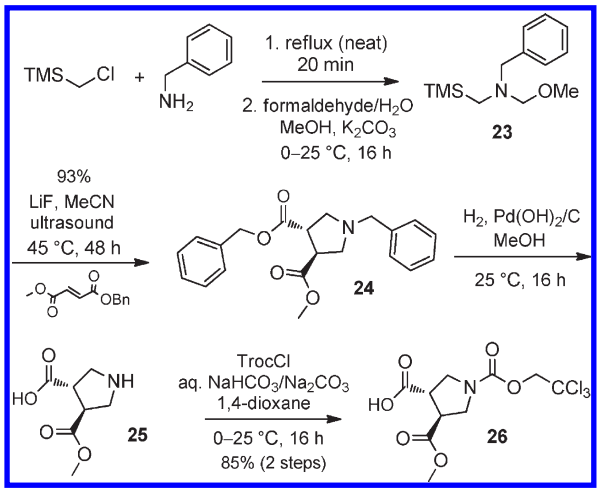
Scheme 1. Retrosynthetic Plan for Construction of the *β*-Turn Mimetic Library

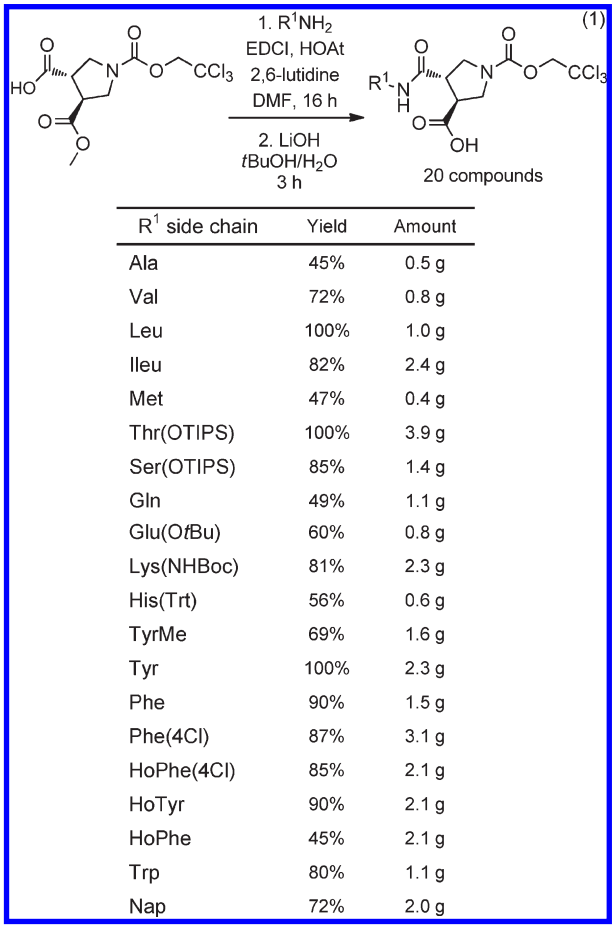


permutations of 3 of the 4 residues in a naturally occurring β-turn. Due to the perceived degeneracy of incorporating the side chains of both aspartic and glutamic acid as well as asparigine and glutamine, only the side chains of glutamic acid (Glu) and glutamine (Gln) were used in the library. Similarly, an arginine side chain was omitted, but the side chain of lysine (Lys) was incorporated. No attempt to incorporate a cysteine side chain was made due to anticipated stability and storage problems. Finally, the side chains of glycine and proline were omitted due to their expected minor role in recognition events. These six natural amino acid side-chain omissions were replaced with additional unnatural aromatic side-chain groups that often dominate protein−protein and protein−peptide interactions or with groups that represent a one carbon extension of such residues. Thus, *O*-methyl tyrosine (TyrMe), naphthyl (Nap), and 4-chlorophenylalanine [Phe(4Cl)] were included in the library as well as the side chains of the one carbon extension residues homophenylalanine (HoPhe), homotyrosine (HoTyr), and 4-chlorohomophenylalanine [HoPhe(4Cl)]. Having selected the 20 groups to incorporate into the library, they were used in their terminal amine form (for incorporation into the R^1/R^2 positions) or terminal carboxylic acid form (for incorporation into the R^3 position) for the library synthesis.

The retrosynthetic analysis for the construction of the library is shown in Scheme 1. We anticipated that the final 4200 compound library would be obtained by acylation of 210 individual *trans*-pyrrolidine-3,4-dicarboxamides at the pyrrolidine nitrogen using an equimolar mixture of the carboxylic acids of the 20 selected side chains (210 × 20-mix). The order of the side-chain introductions (amide couplings; R^1, R^2, and R^3) and the location of the 20-mix functionalization (R^3) were dictated by the C_2 symmetry of the template and the simplifying opportunity that it presented for the number of compounds required to represent all 20 × 20 × 20-mix combinations (210 individual R^1/R^2 combinations × 20-mix for R^3). The 210 individual amines were anticipated to be derived from their 2,2,2-trichloroethoxycarbonyl (Troc) protected precursors, which were individually synthesized starting from the mono methyl ester

Scheme 2





R^1 side chain	Yield	Amount
Ala	45%	0.5 g
Val	72%	0.8 g
Leu	100%	1.0 g
Ileu	82%	2.4 g
Met	47%	0.4 g
Thr(OTIPS)	100%	3.9 g
Ser(OTIPS)	85%	1.4 g
Gln	49%	1.1 g
Glu(O*t*Bu)	60%	0.8 g
Lys(NHBoc)	81%	2.3 g
His(Trt)	56%	0.6 g
TyrMe	69%	1.6 g
Tyr	100%	2.3 g
Phe	90%	1.5 g
Phe(4Cl)	87%	3.1 g
HoPhe(4Cl)	85%	2.1 g
HoTyr	90%	2.1 g
HoPhe	45%	2.1 g
Trp	80%	1.1 g
Nap	72%	2.0 g

Figure 8. Synthesized 20 monoamides with coupling yields and product amounts.

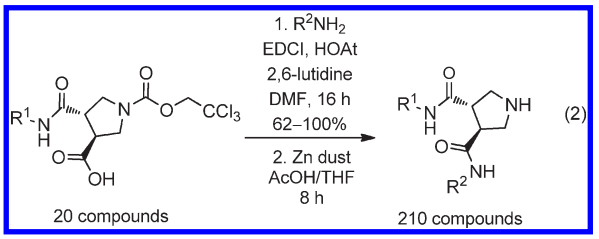
of *trans*-*N*-Troc-pyrrolidine-3,4-dicarboxylic acid utilizing a straightforward amide coupling, methyl ester hydrolysis, and amide coupling reaction sequence. In turn, the mono methyl ester was to be prepared from the *N*-benzylpyrrolidine mixed benzyl methyl ester using hydrogenolysis of the benzyl group followed by Troc protection of the free amine. Finally, the *trans*-*N*-benzylpyrrolidine-3,

4-dicarboxylic acid benzyl methyl ester could be accessed using a [3 + 2] dipolar cycloaddition between the mixed fumarate ester and the azomethine ylide precursor *N*-benzyl-*N*-methoxymethyl-*N*-(trimethylsilyl)methylamine.[23]

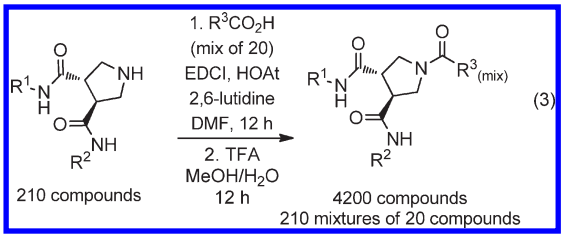
The synthesis of the mono methyl ester of *trans*-*N*-Troc-pyrrolidine-3,4-dicarboxylic acid (**26**) is shown in Scheme 2. The azomethine ylide precursor *N*-benzyl-*N*-methoxymethyl-*N*-(trimethylsilyl)methylamine (**23**) was prepared as described[23] by condensing benzylamine with chloromethyltrimethylsilane to yield *N*-benzyl-*N*-(trimethylsilyl)methylamine, followed by treatment with formaldehyde/H_2O/MeOH in the presence of K_2CO_3. This material was employed in a [3 + 2] dipolar cycloaddition with the mixed fumarate ester in the presence of LiF and under ultrasound conditions to give **24** in multigram quantities.[23] This material was further elaborated to the amino acid **25** by hydrogenolysis removal of the benzyl groups [H_2, $Pd(OH)_2$] followed by Troc protection of the pyrrolidine nitrogen using 2,2,2-trichloroethyl chloroformate to provide **26**.

With the starting template in hand, the library diversification began with the first amide coupling using 20 primary amines under EDCI/HOAt-mediated coupling conditions to provide the monoamides (eq 1 and Figure 8), which were fully characterized by ^{1}H and ^{13}C NMR, IR, and HRMS (Supporting Information). Methyl ester saponification (LiOH) provided the individual 20 monosubstituted carboxylic acids in high yields.

The next stage in the library synthesis entailed preparation of the 210 *trans*-pyrrolidine-3,4-dicarboxamides (eq 2). The Troc-protected dicarboxamides were prepared by amide coupling (EDCI, HOAt, 2,6-lutidine, DMF, 25 °C, 14 h), and this enabled isolation and purification of the products (62−100% yield) using acid/base liquid−liquid extractions in which all reagents, reagent byproducts, and any unreacted starting materials were removed. A diagonal set of 20 diamides of the full 210 matrix, representing a member of each of the monamides and each of the R^2 amine coupling reactions, was fully characterized (^{1}H and ^{13}C NMR, IR, and HRMS), confirming both the compound structure and purity (>95%) (Supporting Information). The subsequent Troc deprotection was conducted using activated zinc nanopowder (20 equiv) in 2:1 THF/AcOH (25 °C, 8 h).[24] Following zinc treatment, the compounds were isolated by filtration through Celite to remove the zinc and removal of the solvent in vacuo with a toluene azeotrope. Residual AcOH was completely removed by dissolving the compound in MeOH and passing it through a small column of basic silica gel (Chromatorex NH, Fuji Silysia Ltd.). This procedure provided highly pure amines (>95%) in good yields (product yields and amounts are in the Supporting Information) and avoided the use of an aqueous base extraction, which was found to result in low product recovery in certain cases due to the aqueous solubility. As before, a representative but different diagonal set of 20 compounds of the full 210 matrix was fully characterized by ^{1}H and ^{13}C NMR, IR, and HRMS (Supporting Information).



The final library was obtained by coupling (EDCI, HOAt, 2,6-lutidine, DMF, 25 °C, 12 h) each of the 210 amines with an equimolar mixture of the carboxylic acids of the 20 groups shown in Figure 10 (eq 3). To ensure that each of the 20 carboxylic acids was fully consumed to yield an equimolar mixture of 20 products, 1.2 equiv of the free amine was employed in each coupling reaction. The excess starting amine was subsequently removed upon completion of the reaction using aqueous acid extractions. The final global deprotection of all side-chain protecting groups was accomplished by treatment with 3:1:1 TFA/MeOH/H_2O (25 °C). Using this condition, complete removal of Boc, *tert*-butylester, TIPS, and trityl groups was observed in 12−16 h. We then verified that all final products in 20 representative final mixtures were detected by MS using a diagonal 20/210 matrix characterization (see Supporting Information).



To confirm the quality of the construction of the library as 20 compound mixtures, a representative final library mixture was selected for comparison by HPLC to an authentic equimolar mixture prepared from the individually synthesized compounds (Figure 9). Although a separation of all 20 mixture components is not possible on a single HPLC run, the nearly identical detection

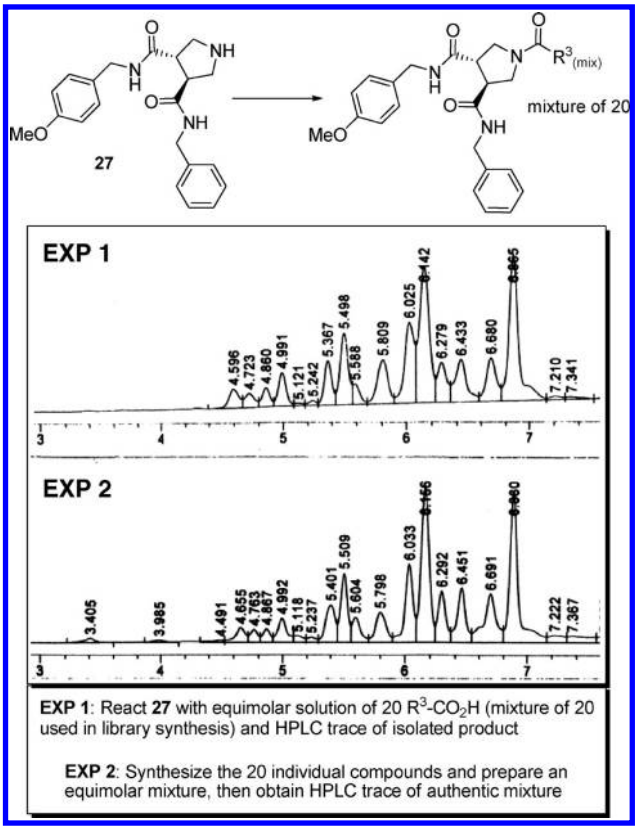


Figure 9. HPLC comparison of library (EXP 1) and authentic (EXP 2) equimolar mixture of the final 20 compounds derived from **27**. HPLC conditions: linear gradient, 0−90% MeCN in H_2O over 7 min and then 90% MeCN for 10 min at a flow rate of 0.75 mL/min.

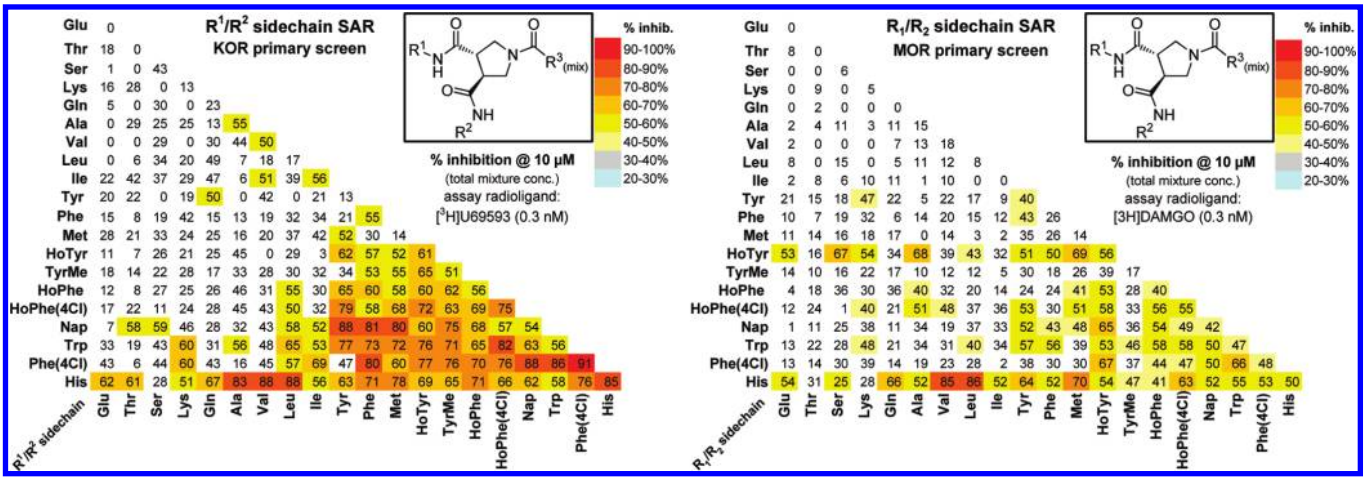


Figure 10. (A) % inhibition of [^{3}H]U69593 binding for the indicated R^1/R^2 side-chain combinations in the screening of the 210 mixtures against the KOR. (B) % inhibition of [^{3}H]DAMGO binding for the indicated R^1/R^2 side-chain combinations in the screening of the 210 mixtures against the MOR.

R^1/R^2 sidechains	% inhibition		
	KOR	MOR	DOR
Trp/Phe	73	56	25
Trp/Phe(4Cl)	86	66	26
Phe(4Cl)/Phe(4Cl)	91	48	18

Figure 11. Mixtures selected for deconvolution.

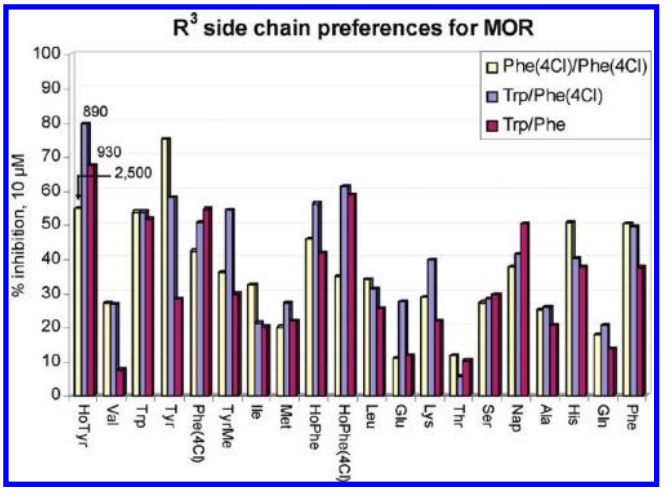


Figure 12. Screening results (% inhibition at 10 μM) for the three series of deconvolution compounds against MOR along with measured K_i values (nM) (versus [^{3}H]DAMGO).

profiles displayed by the two mixtures confirms that not only are all 20 compounds present in the library mixture but that they must be present in amounts that approach equimolar.

Screening the Library against the Opioid Receptors. The entire library composed of 210 mixtures of 20 compounds (210 wells) was screened at 10 μM (total concentration, 0.5 μM per compound) for activity at opioid receptors in radioligand binding assays at human cloned receptors (KOR, MOR, and DOR). The summary of the screening results for the KOR and MOR is shown in Figure 10. The overall trends against the three receptors proved consistent with our previous single-compound results in exhibiting an intrinsic binding selectivity of the *trans*-pyrrolidine-3,4-dicarboxamides for the KOR, followed by the MOR and then DOR (data not shown). A clear SAR trend is evident from the primary screening results even without the examination of individual compounds, illustrating that hydrophobic aromatic side chains generally dominate the interaction with the opioid receptors, with Phe(4Cl), Trp, and Nap showing strong inhibition against the KOR and the HoTyr, Trp, and HoPhe(4Cl) side chains ranking among the best against the MOR. The exception to this trend with both the KOR and MOR was the potent inhibition observed with a basic His side chain in the R^1/R^2 position, with a particularly potent R^1/R^2 combination being His and a bulky aliphatic residue (Val or Leu).

Deconvolution of Selected Mixtures and Screening of Individual Compounds. On the basis of the primary screening data for the library and with the recognition β-turn sequence of the endomorphins (H-Tyr-Pro-Trp/Phe-Phe-NH_2) in mind, three related mixtures were selected for deconvolution (Figure 11). The series containing R^1/R^2 side chains Trp and Phe is the closest representative within the library of the endomorphin-1 sequence and contains compound **8**, which exhibited the measured K_i values of 390 and 930 nM for KOR and MOR, respectively. The Trp/Phe(4Cl) series represents a closely related series to the Trp/Phe series, but the addition of the 4-Cl substituent enhanced binding of the mixture to both the KOR (86 versus 73% inhibition) and the MOR (66 versus 56% inhibition). Its deconvolution could be expected to yield individual compounds with enhanced affinity to both receptors while also allowing a further evaluation of the β-turn mimicry of the compounds. The final series chosen for deconvolution, the Phe(4Cl)/Phe(4Cl) series, was the mixture that displayed the highest affinity against the KOR (91% inhibition) and also the greatest difference between KOR and MOR affinity (91 versus 48%). These three mixtures were deconvoluted by resynthesis of the individual compounds from the three archived penultimate intermediates using the conditions found in eq 3 to yield 60 individual compounds for screening.

The 60 individual compounds were screened at 10 μM for activity at the KOR and MOR via the radioligand binding assays. Because of the lower activity at the MOR, where the % inhibition was not pegged at levels approaching 90% for each series, the

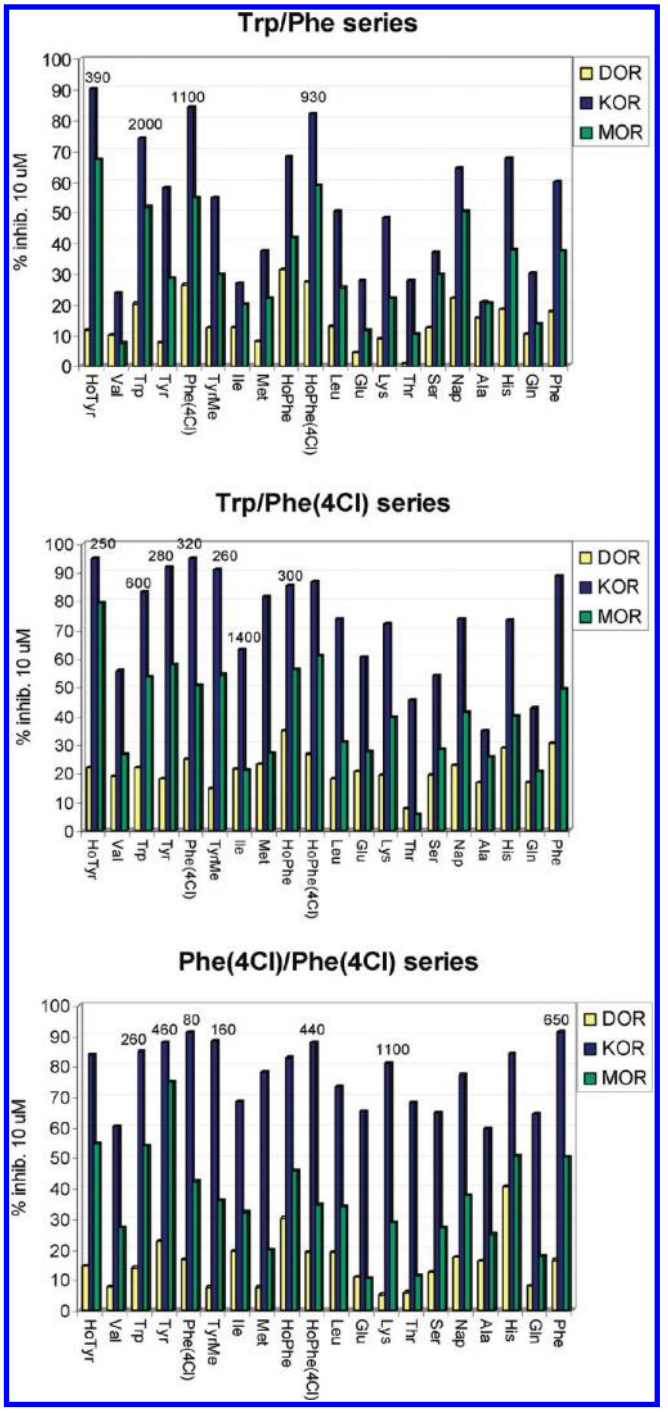


Figure 13. Screening results (% inhibition at 10 μM) for the three series of deconvolution compounds against KOR (versus [^{3}H]U69593), MOR (versus [^{3}H]DAMGO), and DOR (versus [^{3}H]DADLE) along with measured K_i values (nM) against KOR.

general trends were clearest in examining its results. The R^3 side-chain preferences for the individual compounds against the MOR are shown in Figure 12. According to the proposed mode of β-turn mimicry by the compounds and the sequence of the MOR-selective endomorphins, Tyr or HoTyr would be expected to be the favored side chain at the R^3 position if mimicry of an endomorphin β-turn is being achieved. Consistent with this expectation, the screening demonstrated that either

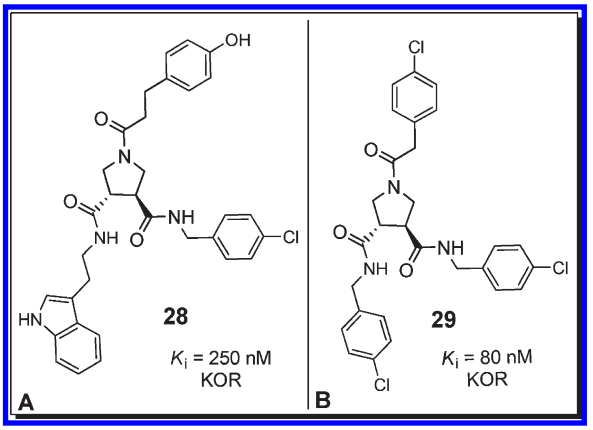


Figure 14. Chemical structures of compounds with the highest affinity for KOR from the Trp/Phe(4Cl) series (**28**) and the Phe(4Cl)/Phe(4Cl) series (**29**).

HoTyr or Tyr was the favored R^3 substituent in all three series, with HoTyr performing best in the Trp/Phe and Trp/Phe(4Cl) series and Tyr yielding the most potent compound in the Phe(4Cl)/Phe(4Cl) series. Beautifully, and if the turn recognition motif were unknown, these results would represent the identification of the Tyr-XXX-Trp-Phe and Tyr-XXX-Phe-Phe β-turn motifs. This result also reinforced our earlier decision to incorporate single carbon extensions of key side chains such as Tyr into the library. In general, other aromatic R^3 side chains were less active but roughly equal in potency, and all were typically more active than compounds bearing the aliphatic or charged side chains regardless of the series.

The data from each of the three deconvolution series are shown in Figure 13. Clear from these comparisons is the greatest activity against the KOR (KOR > MOR > DOR) for all three series and that the activity of the three series generally follows the order of Phe(4Cl)/Phe(4Cl) > Trp/Phe(4Cl) > Trp/Phe, as observed in the original mixture screening results (Figure 11). The excellent activity of **8** (K_i = 390 nM) used originally to test the design and found in the Trp/Phe series was improved with the replacement of Phe with Phe(4Cl) in the Trp/Phe(4Cl) series providing **28** (K_i = 250 nM, Figure 14A), and this series provided several related compounds that exhibited K_i's of <300 nM. Finally, the most active Phe(4Cl)/Phe(4Cl) series not only showed a greater activity for the KOR, but it also exhibited a greater selectivity for the KOR versus the MOR or DOR, as inferred from the original mixture screening results (Figure 11). The most potent compound in this series for the KOR was **29** (K_i = 80 nM, Figure 14B), which was found to be >100-fold selective versus the MOR or DOR (K_i > 10 000 nM).

As detailed earlier, the trends for MOR binding illustrated that the HoTyr [Trp/Phe, Trp/Phe(4Cl)] or Tyr [Phe(4Cl)/Phe(4Cl)] were the clearly favored R^3 substituents in all three series, indicative of a prominent or productive role for the Tyr free phenol. Other aromatic R^3 side chains were less active and roughly equal in potency (Figure 12). This unique preference for HoTyr or Tyr and the presence of the free phenol were not observed with KOR. While still preferring a third aryl R^3 side-chain substituent [Phe(4Cl), HoTyr > Tyr = Tyr(Me), Phe > HoPhe(4Cl), > Trp, HoPhe > Nap] and accommodating the free phenol of Tyr, it no longer exhibits a phenol preference (Figure 15). Without over interpreting the minor differences

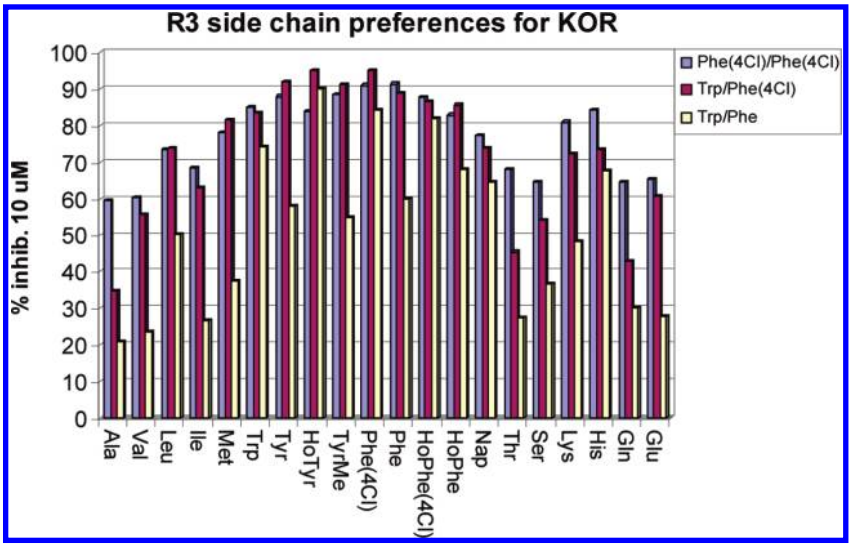


Figure 15. Screening results (% inhibition at 10 μM) for the three series of deconvolution compounds against KOR (versus [^{3}H]U69593).

in the measured and sometimes pegged assay values, there was virtually no difference in Tyr and Tyr(Me) for each of the three series, indicating that protection of the phenol as a methyl ether has no impact in binding affinity, and both were nearly identical to Phe itself in each of the three series. Among the best of the R^3 substituents in nearly each of the three series was Phe(4Cl) and HoPhe(4Cl). In addition to suggesting that the Tyr free phenol of the endomorphins and enkephalins may not be important to KOR binding,[25] it also suggests that a way to enhance KOR versus MOR and DOR binding selectivity is to remove this pharmacophore phenol OH. Like the behavior of **29**, further enhancements in KOR affinity may be achieved by utilizing Phe(4Cl), resulting in higher affinity and even more selective KOR ligands.

CONCLUSIONS

The solution-phase synthesis of a β-turn mimetic library as the second component of a general small-molecule library targeting the key recognition motifs involved in protein−protein interactions is described. Using a geometric characterization of β-turns in the PDB, the *trans*-pyrrolidine-3,4-dicarboxamide was proposed and subsequently found to serve as an effective and synthetically accessible library β-turn mimetic template, and this was initially validated by screening test compounds against a series of peptide-activated GPCRs that recognize β-turn structure in their ligands. The screening of selected compounds designed to mimic the β-turn pharmacophore of the peptide ligands of the opioid receptors demonstrated that appropriately substituted *trans*-pyrrolidine-3,4-dicarboxamides could replace the peptide backbone for effective display of side-chain groups in a β-turn. This validation was highlighted by identification of both nonbasic and basic small molecules with high affinity (K_i = 390 and 23 nM, respectively) for the KOR. The library was assembled using a solution-phase protocol to provide 210 mixtures of 20 compounds for a total of 4200 compounds designed to mimic all possible permutations of 3 of the 4 residues in a naturally occurring β-turn. Even if the recognition motif is unknown or unrecognized, the library screening should provide lead structures, provide insights into the nature of the interaction (β-turn), and identify the key amino acid residues responsible for the protein−protein or peptide−receptor interaction. Additionally, the use of such a comprehensive library can take advantage of the principles of selection to discover compounds with improved properties (e.g., of affinity, selectivity) over those that mimic the endogenous protein or peptide ligands. The screening of the complete library against the opioid receptors demonstrated that not only could the expected activity be observed with library mixtures containing the compounds shown to mimic the opioid receptor endogenous peptide ligands but that additional side-chain combinations could be identified with even higher receptor binding affinities and selectivity, providing new insights into the β-turn recognition events.

ASSOCIATED CONTENT

Supporting Information. Full experimental details on GPCR screening conditions and the library synthesis along with single-compound characterizations of all 20 monoamides (eq 1: ^{1}H and ^{13}C NMR, IR, and HRMS), a diagonal set of 20 Troc-diamides of the full 210 matrix representing a member of each of the monamides and each of the R^2 amine coupling reactions (eq 2: ^{1}H and ^{13}C NMR, IR, and HRMS), confirming the compound structure and purity (>95%), a table summarizing the LCMS characterization of an additional 50 Troc-diamides, establishing their isolation (MS) and purity, compound characterizations of a second but different diagonal set of 20 compounds in the full 210 matrix of diamide amines (eq 2: ^{1}H and ^{13}C NMR, IR, and HRMS), MS characterization of a diagonal set of 20 of the final 210 mixtures, confirming the presence of all 20 components, the characterization of 33 individual final triamides constituting deconvolution samples (^{1}H NMR, MS), and three tables summarizing the isolated amounts and yields of each component in the three stages of the library synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
boger@scripps.edu

■ ACKNOWLEDGMENT

We gratefully acknowledge the financial support of the National Institutes of Health (CA078045, D.L.B. and P.K.V.; R01DA017204, R01DA027170, the Michael Hooker Distinguished Professorship and the NIMH Psychoactive Drug Screening Program Contract, B.L.R.). L.R.W. is a Skaggs Fellow.

■ REFERENCES

(1) Stites, W. E. *Chem. Rev.* **1997**, *97*, 1233–1250.

(2) Reviews of small-molecule modulators of protein−protein interactions: (a) Ross, N. T.; Katt, W. P.; Hamilton, A. D. *Philos. Trans. R. Soc. London, Ser. A* **2010**, *368*, 989–1008. (b) Arkin, M. R.; Whitty, A. *Curr. Opin. Chem. Biol.* **2009**, *13*, 284–290. (c) Wilson, A. J. *Chem. Soc. Rev.* **2009**, *38*, 3289–3300. (d) Berg, T. *Curr. Opin. Drug Discov. Dev.* **2008**, *11*, 666–674. (e) Fry, D. C. *Curr. Protein Pept. Sci.* **2008**, *9*, 240–247. (f) Wells., J. A.; McClendon, C. L. *Nature* **2007**, *450*, 1001–1009. (g) Perez de Vega, M. J.; Martin-Martinez, M.; Genzalez-Muniz, R. *Curr. Top. Med. Chem.* **2007**, *7*, 33–62. (h) Hershberger, S. J.; Lee, S.; Chmielewski, J. *Curr. Top. Med. Chem.* **2007**, *7*, 928–942. (i) Fry, D. C. *Biopolymers* **2006**, *84*, 535–552. (j) Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130–4163. (k) Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Discovery* **2004**, *3*, 301–317. (l) Boger, D. L.; Desharnais, J.; Capps, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 4138–4176. (m) Toogood, P. L. *J. Med. Chem.* **2002**, *45*, 1543–1558. (n) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479–2494.

(3) Moreira, I. S.; Fernandez, P. A.; Ramos, M. J. *Proteins: Struct., Funct., Bioinf.* **2007**, *68*, 803–812.

(4) Che, Y.; Brooks, B. R.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **2006**, *20*, 109–130.

(5) (a) Ruiz−Gomez, G.; Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, PR1–PR41. (b) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793–826.

(6) (a) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431–7448. (b) Blakeney, J. S.; Reid, R. C.; Le, G. T.; Fairlie, D. P. *Chem. Rev.* **2007**, *107*, 2960–3041.

(7) Solution-phase preparation of libraries: (a) Boger, D. L.; Lee, J. K.; Goldberg, J.; Jin, Q. *J. Org. Chem.* **2000**, *65*, 1467–1474. (b) Boger, D. L.; Goldberg, J.; Satoh, S.; Ambroise, Y.; Cohen, S. B.; Vogt, P. K. *Helv. Chim. Acta* **2000**, *83*, 1825–1845. (c) Boger, D. L.; Chai, W.; Jin., Q. *J. Am. Chem. Soc.* **1998**, *120*, 7220–7225. (d) Boger, D. L.; Chai, W. *Tetrahedron* **1998**, *54*, 3955–3970. (e) Boger, D. L.; Ducray, P.; Chai, W.; Jiang, W.; Goldberg, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2339–2344. (f) Boger, D. L.; Goldberg, J.; Jiang, W.; Chai, W.; Ducray, P.; Lee, J. K.; Ozer, R. S.; Andersson, C.-M. *Bioorg. Med. Chem.* **1998**, *6*, 1347–1378. (g) Boger, D. L.; Chai, W.; Ozer, R. S.; Andersson, C.-M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 463–468. (h) Boger, D. L.; Ozer, R. S.; Andersson, C.-M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1903–1908. (i) Cheng, S.; Comer, D. D.; Williams, J. P.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567–2573. (j) Cheng, S.; Tarby, C. M.; Comer, D. D.; Williams, J. P.; Caporale, L. H.; Boger, D. L. *Bioorg. Med. Chem.* **1996**, *4*, 727–737. (k) Boger, D. L.; Tarby, C. M.; Caporale, L. H. *J. Am. Chem. Soc.* **1996**, *118*, 2109–2110. (l) Chen, Y.; Bilban, M.; Foster, C. A.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5431–5440. Reviews: (m) Boger, D. L.; Goldberg, J. *Bioorg. Med. Chem.* **2001**, *9*, 557–562. (n) Boger, D. L. *Bioorg. Med. Chem.* **2003**, *11*, 1607–1613.

(8) (a) Silletti, S.; Kessler, J.; Goldberg, J.; Boger, D. L.; Cheresh, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 119–124. (b) Boger, D. L.; Goldberg, J.; Silletti, S.; Kessler, T.; Cheresh, D. A. *J. Am. Chem. Soc.* **2001**, *123*, 1280–1288.

(9) Goldberg, J.; Jin, Q.; Ambroise, Y.; Satoh, S.; Desharnais, J.; Capps, K.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 544–555.

(10) (a) Berg, T.; Cohen, S. B.; Desharnais, J.; Sonderegger, C.; Maslyar, D. J.; Goldberg, J.; Boger, D. L.; Vogt, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3830–3835. (b) Lu, X.; Vogt, P. K.; Boger, D. L.; Lunec, J. *Oncol. Rep.* **2008**, *19*, 825–830. (c) Shi, J.; Stover, J. S.; Whitby, L. R.; Vogt, P. K.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6038–6041.

(11) (a) Ambroise, Y.; Yuspan, B.; Ginsberg, M. H.; Boger, D. L. *Chem. Biol.* **2002**, *9*, 1219–1226. (b) Capps, K. J.; Humiston, J.; Dominique, R.; Hwang, I.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2840–2844. (c) Chang, M. W.; Giffin, M. J.; Muller, R.; Savage, J.; Lin, Y. C.; Hong, S.; Jin, W.; Whitby, L. R.; Elder, J. H.; Boger, D. L.; Torbett, B. E. *Biochem. J.* **2010**, *429*, 527–532. (d) Eubanks, L. M.; Hixon, M. S.; Jin, W.; Hong, S.; Clancy, C. M.; Tepp, W. H.; Malizio, C. J.; Goodnough, M. C.; Barbieri, J. T.; Johnson, E. A.; Boger, D. L.; Dickerson, T. J.; Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A* **2007**, *104*, 2602–2607.

(12) (a) Lee, A. M.; Rojek, J. M.; Spiropoulou, C. F.; Gunderson, A.; Jin, W.; Shaginian, A.; York, J.; Nunberg, J. H.; Boger, D. L.; Oldstone, M. B. A.; Kunz, S. *J. Biol. Chem.* **2008**, *283*, 18734–18742. (b) Whitby, L. R.; Lee, A. M.; Kunz, S.; Oldstone, M. B. A.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3771–3774.

(13) (a) Stover, J. S.; Shi, J.; Jin, W.; Vogt, P. K.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 3342–3348. (b) Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382–6394. (c) Boger, D. L.; Schmitt, H.; Fink, B. E.; Hedrick, M. P. *J. Org. Chem.* **2001**, *66*, 6654–6661. (d) Boger, D. L.; Fink, B. F.; Brunette, S. R.; Tse, W.; Hedrick, M. P. *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891. (e) Tse, W.; Boger, D. L. *Acc. Chem. Res.* **2004**, *37*, 61–69. (f) Hauschild, K. E.; Stover, J. S.; Boger, D. L.; Ansari, A. Z. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3779–3782.

(14) Unlike a peptide library, such mimetic templates not only identify key residues mediating the interaction, but they may be used to define the binding recognition motif (α-helix, β-turn, β-strand) and may serve as stable in vitro and in vivo probes of biological processes needed for target validation and as lead compounds for optimization in therapeutics.

(15) (a) Shaginian, A.; Whitby, L. R.; Hong, S.; Hwang, I.; Farooqi, B.; Searcey, M.; Chen, J.; Vogt, P. K.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 5564–5572. For a recent application, see: (b) Ambrus, G.; Whitby, L. R.; Singer, E. L.; Trott, O.; Choi, E.; Olson, A. J.; Boger, D. L.; Gerace, L. *Bioorg. Med. Chem.* **2010**, *18*, 7611–7620. For related designs, see: (c) Plante, J. P.; Burnley, T.; Malkova, B.; Webb, M. E.; Warriner, S. L.; Edwards, T. A.; Wilson, A. J. *Chem. Commun.* **2009**, 5091–5093. (d) Plante, J.; Campbell, F.; Malkova, B.; Kilner, C.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2008**, *6*, 138–146. (e) Campbell, F.; Plante, J. P.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2010**, *8*, 2344–2351. (f) Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 535–539. (g) Lee, T.; Ahn, J. *ACS Comb. Sci.* **2011**, *13*, 107–111.

(16) α-helix mimetic library: (a) Lu, F.; Chi, S.-W.; Kim, D.-H.; Han, K.-H.; Kuntz, I. D.; Guy, R. K. *J. Comb. Chem.* **2006**, *8*, 315–325. (b) Antuch, W.; Menon, S.; Chen, Q.-Z.; Lu, Y.; Sakamuri, S.; Beck, B.; Schauer-Vukašinović, V.; Agarwal, S.; Hess, S.; Dömling, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1740–1743. β-turn mimetic library: (c) Mieczkowski, A.; Koźmński, W.; Jurczak, J. *Synthesis* **2010**, *2*, 221–232. (d) Lee, J. Y.; Im, I.; Webb, T. R.; McGrath, D.; Song, M.; Kim, Y. *Bioorg. Chem.* **2009**, *37*, 90–95. (e) Angell, Y.; Chen, D.; Brahimi, F.; Saragovi, H. U.; Burgess, K. *J. Am. Chem. Soc.* **2008**, *30*, 556–565. β-strand or β-sheet mimetic library: (f) Fuchi, N.; Doi, T.; Cao, B.; Kahn, M.; Takahashi, T. *Synlett* **2002**, 285–289. (g) Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Hill, T. A.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, 32–69. Universal peptidomimetics: (h) Ko, E.; Liu, J.; Perez, L. M.; Lu, G.; Schaefer, A.; Burgess, K. *J. Am. Chem. Soc.* **2011**, *133*, 462–477.

(17) (a) Ko, E.; Burgess, K. *Org. Lett.* **2011**, *13*, 980–983. (b) Liu, J.; Brahimi, F.; Saragovi, H. U.; Burgess, K. *J. Med. Chem.* **2010**, *53*, 5044–5048. (c) Beierle, J. M.; Horne, W. S.; van Maarseveen, J. H.; Waser, B.; Reubi, J. C.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 4725–4729. (d) Lesma, G.; Landoni, N.; Pilati, T.; Sacchetti, A.; Silvani, A. *J. Org. Chem.* **2009**, *74*, 8098–8105. (e) Fustero, S.; Mateu, N.; Albert, L.; Acena, J. L. *J. Org. Chem.* **2009**, *74*, 4429–4432. (f) Gentilucci, L.; Cardillo, G.; Tolomelli, A.; De Marco, R.; Garelli, A.; Spampinato, S.; Sparta, A.; Juaristi, E. *ChemMedChem* **2009**, *4*, 517–523. (g) De Wachter, R.; Brans, L.; Ballet, S.; Van den Eynde, I.; Feytens, D.; Keresztes, A.; Toth, G.; Urbanczyk-Lipkowska, Z.; Tourwe, D. *Tetrahedron* **2009**, *65*, 2266–2278. (h) Sanudo, M.; Garcia-Valverde, M.;

Marcaccini, S.; Delgado, J. J.; Rojo, J.; Torroba, T. *J. Org. Chem.* **2009**, *74*, 2189–2192. (i) Chen, D.; Brahimi, F.; Angell, T.; Li, Y.; Moscowicz, J.; Saragovi, H. U.; Burgess, K. *ACS Chem. Biol.* **2009**, *4*, 769–781. (j) Lomlin, L.; Einsiedel, J.; Heinemann, F. W.; Meyer, K.; Gmeiner, P. *J. Org. Chem.* **2008**, *73*, 3608–3611. (k) Lesma, G.; Meschini, E.; Recca, T.; Sacchetti, A.; Silvani, A. *Tetrahedron* **2007**, *63*, 5567–5578. (l) Che, Y.; Brooks, B. R.; Riley, D. P.; Reaka, A. J. H.; Marshall, G. R. *Chem. Biol. Drug. Des.* **2007**, *69*, 99–110. (m) Danieli, E.; Trabocchi, A.; Menchi, G.; Guarna, A. *Eur. J. Org. Chem.* **2007**, *10*, 1659–1668. (n) Pappo, D.; Kashman, Y. *Org. Lett.* **2006**, *8*, 1177–1179. (o) Yamanaka, T.; Ohkubo, M.; Kato, M.; Kawamura, Y.; Nishi, A.; Hosokawa, T. *Synlett* **2005**, *4*, 631–634. (p) Smith, A. B.; Charnley, A. K.; Mesaros, E. F.; Kikuchi, O.; Wang, W.; Benowitz, A.; Chu, C.; Feng, J.; Chen, K.; Lin, A.; Cheng, F.; Taylor, L.; Hirschmann, R. *Org. Lett.* **2005**, *7*, 399–402. (q) Wels, B.; Kruijtzer, J. A. W.; Garner, K. M.; Adan, R. A. H.; Liskamp, R. M. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 287–290. Reviews: (r) Smith, A. B.; Charnley, A. K.; Hirschmann, R. F. *Acc. Chem. Res.* **2011**, *44*, 180–193. (s) Hirschmann, R. F.; Nicolaou, K. C.; Angeles, A. R.; Chen, J. S.; Smith, A. B. *Acc. Chem. Res.* **2009**, *42*, 1511–1520. (t) Brakch, N.; El Abida, B.; Rholam, M. *Cent. Nerv. Syst. Agents Med. Chem.* **2006**, *6*, 163–173. (u) Eguchi, M.; Kahn, M. *Mini-Rev. Med. Chem.* **2002**, *2*, 447–462. (v) Suat Kee, K.; Seetharama, D. S. *Curr. Pharm. Des.* **2003**, *9*, 1209–1224. (w) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826–835.

(18) (a) Garland, S. L.; Dean, P. M. *J. Comput.-Aided Mol. Des.* **1999**, *13*, 469–483. (b) Garland, S. L.; Dean, P. M. *J. Comput.-Aided Mol. Des.* **1999**, *13*, 485–498.

(19) For applications see: (a) Webb, T. R.; Jiang, L.; Sviridov, S.; Venegas, R. E.; Vlaskina, A. V.; McGrath, D.; Tucker, J.; Wang, J.; Deschenes, A.; Li, R. *J. Comb. Chem.* **2007**, *9*, 704–710. (b) Chianelli, D.; Kim, Y. C.; Lvovskiy, D.; Webb, T. R. *Bioorg. Med. Chem.* **2003**, *11*, 5059–5068.

(20) (a) Kane, B. E.; Svensson, B.; Ferguson, D. M. *AAPS J.* **2006**, *8*, 126–137. (b) Aldrich, J. V.; McLaughlin, J. P. *AAPS J.* **2009**, *11*, 312–322. (c) Eguchi, M. *Med. Res. Rev.* **2004**, *24*, 182–212. (d) Wang, Y.; Sun, J.; Tao, Y.; Chi, Z.; Liu, J. *Acta Pharmacol. Sin* **2010**, *31*, 1065–1070. (e) Bruchas, M. R.; Land, B. B.; Chavkin, C. *Brain Res.* **2010**, *1314*, 44–55. (f) Bruijnzeel, A. W. *Brain Res. Rev.* **2009**, *62*, 127–146.

(21) (a) Zadina, J. E.; Hackler, L.; Ge, L. J.; Kastin, A. J. *Nature* **1997**, *386*, 499–502. For recent reviews, see: (b) Keresztes, A.; Borics, A.; Toth, G. *ChemMedChem* **2010**, *5*, 1176–1196. (c) Janecka, A.; Staniszewska, R.; Fichna, J. *Curr. Med. Chem.* **2007**, *14*, 3201–3208. (d) Fichna, J.; Janecka, A.; Costentin, J.; Do Rego, J. *Pharmacol. Rev.* **2007**, *59*, 88–123.

(22) (a) Tomboly, C.; Ballet, S.; Feytens, D.; Kover, K. E.; Borics, A.; Lovas, S.; Al-Khrasani, M.; Furst, Z.; Toth, G.; Benyhe, S.; Tourwe, D. *J. Med. Chem.* **2008**, *51*, 173–177. (b) Eguchi, M.; Shen, R. Y. W.; Shea, J. P.; Lee, M. S.; Kahn, M. *J. Med. Chem.* **2002**, 1395–1398. (c) Leitgeb, B.; Szekeres, A.; Toth, G. *J. Pept. Res.* **2003**, *62*, 145–157. (d) Leitgeb, B.; Otvos, F.; Toth, G. *Biopolymers* **2003**, *68*, 497–511. (e) Leitgeb, B.; Toth, G. *Eur. J. Med. Chem.* **2005**, *40*, 674–686. (g) Bedini, A.; Baiula, M.; Gentilucci, L.; Tolomelli, A.; De Marco, R.; Spampinato, S. *Peptides* **2010**, *31*, 2135–2140. Review: Gentilucci, L.; Tolomelli, A. *Curr. Top. Med. Chem.* **2004**, *4*, 105–121.(f) Mallareddy, J. R.; Borics, A.; Keresztes, A.; Kover, K. E.; Tourwe, D.; Toth, G. *J. Med. Chem.* **2011**, *54*, 1462–1472.

(23) (a) Fray, A. H.; Meyers, A. I. *J. Org. Chem.* **1996**, *61*, 3362–3374. (b) Padwa, A.; Dent, W. *Org. Synth.* **1989**, *67*, 133–140.

(24) Matsuura, F.; Yasumasa, H.; Takayuki, S. *Tetrahedron* **1994**, *50*, 265–274.

(25) (a) Bennett, M. A.; Murray, T. F.; Aldrich, J. V. *J. Med. Chem.* **2002**, *45*, 5617–5619. (b) Frankowski, K. J.; Ghosh, P.; Setola, V.; Tran, T. B.; Roth, B. L.; Aube, J. *ACS Med. Chem. Lett.* **2010**, *1*, 189–193.