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SAR Matrices Enable Discovery of Mixed Efficacy μ-Opioid Receptor Agonist Peptidomimetics with Simplified Structures through an Aromatic-Amine Pharmacophore

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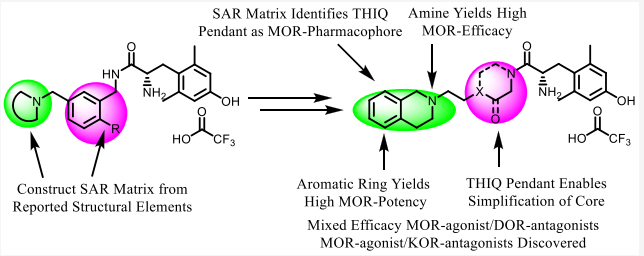
ABSTRACT: We previously described the development of potent μ-opioid receptor (MOR)-agonist/δ-opioid receptor (DOR)-antagonist peptidomimetic ligands as an approach toward effective analgesics with reduced side effects. In this series, a tetrahydroquinoline (THQ) or substituted phenyl is employed to link two key pharmacophore elements, a dimethyltyrosine amino acid and typically an aromatic pendant. Using new and previously reported analogues, we constructed a structure−activity relationship (SAR) matrix that probes the utility of previously reported amine pendants. This matrix reveals that the MOR-agonist/DOR-antagonist properties of these ligands do not change when a tetrahydroisoquinoline (THIQ) pendant is used, despite removal of substituents on the core phenyl ring. Based on this observation, we retained the THIQ pendant and replaced the phenyl core with simpler aliphatic chain structures. These simpler analogues proved to be potent MOR-agonists with high variability in their effects at the DOR and the κ-opioid receptor (KOR). These data show that the amine of the THIQ pendant may be a novel pharmacophore element that favors high MOR-efficacy, whereas the aromatic ring of the THIQ pendant may produce high MOR-potency. Combined, the two pharmacophores within the THIQ pendant may be a structurally efficient means of converting opioid peptides and peptidomimetics into potent and efficacious MOR-agonists.



KEYWORDS: *μ-Opioid receptor, δ-opioid receptor, κ-opioid receptor, peptidomimetics, structure−activity relationship, bifunctional ligands*

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INTRODUCTION

The opioid receptors are an important family of G protein-coupled receptors (GPCRs) that affect various behavioral processes in the central nervous system. Activation of the μ-opioid receptor (MOR) yields analgesia, euphoria, and symptoms associated with drug addiction.[1,2] Stimulation of the δ-opioid receptor (DOR) is known to produce convulsions, while activation of the κ-opioid receptor (KOR) is known to produce dysphoria.[1,2] These opioid receptors are acted on by endogenous peptides, and structure−activity relationship (SAR) studies of these peptides found that the first four residues are responsible for opioid receptor activation whereas subsequent residues are responsible for receptor selectivity.[3] This led to the development of selective opioid peptides such as DAMGO,[4] DPDPE,[5] and DIPP-NH_2[Ψ],[6] which are used to study the opioid receptors. Frequently, the exploration of opioid peptide SAR has produced increasingly bulky and complicated structures to induce their effects.

Mixed efficacy opioid ligands, i.e., ligands that afford varying degrees of activation across different opioid receptors, have recently become an increasingly attractive avenue of research,[7−9] as targeting multiple opioid receptors simultaneously has been shown to produce altered pharmacological profiles compared to activation of a single opioid receptor type.[10] MOR-agonists in conjunction with DOR-antagonists have been shown to reduce the addictive side effects of MOR-activation while retaining their analgesic properties.[11−16] MOR-agonist/DOR-agonists have also shown these beneficial effects,[17−19] in addition to reduced convulsions associated with DOR-activation.[20]

In the course of our development of a MOR-agonist/DOR-antagonist peptidomimetic series with a reduced abuse profile,[21−29] we found that the core tetrahydroquinoline structure in this series was a major metabolic liability. As such, we opted to simplify the core to a benzene ring.[30] Short chain ethers attached to this core aromatic ring provided the greatest improvement in stability while producing a MOR-agonist/

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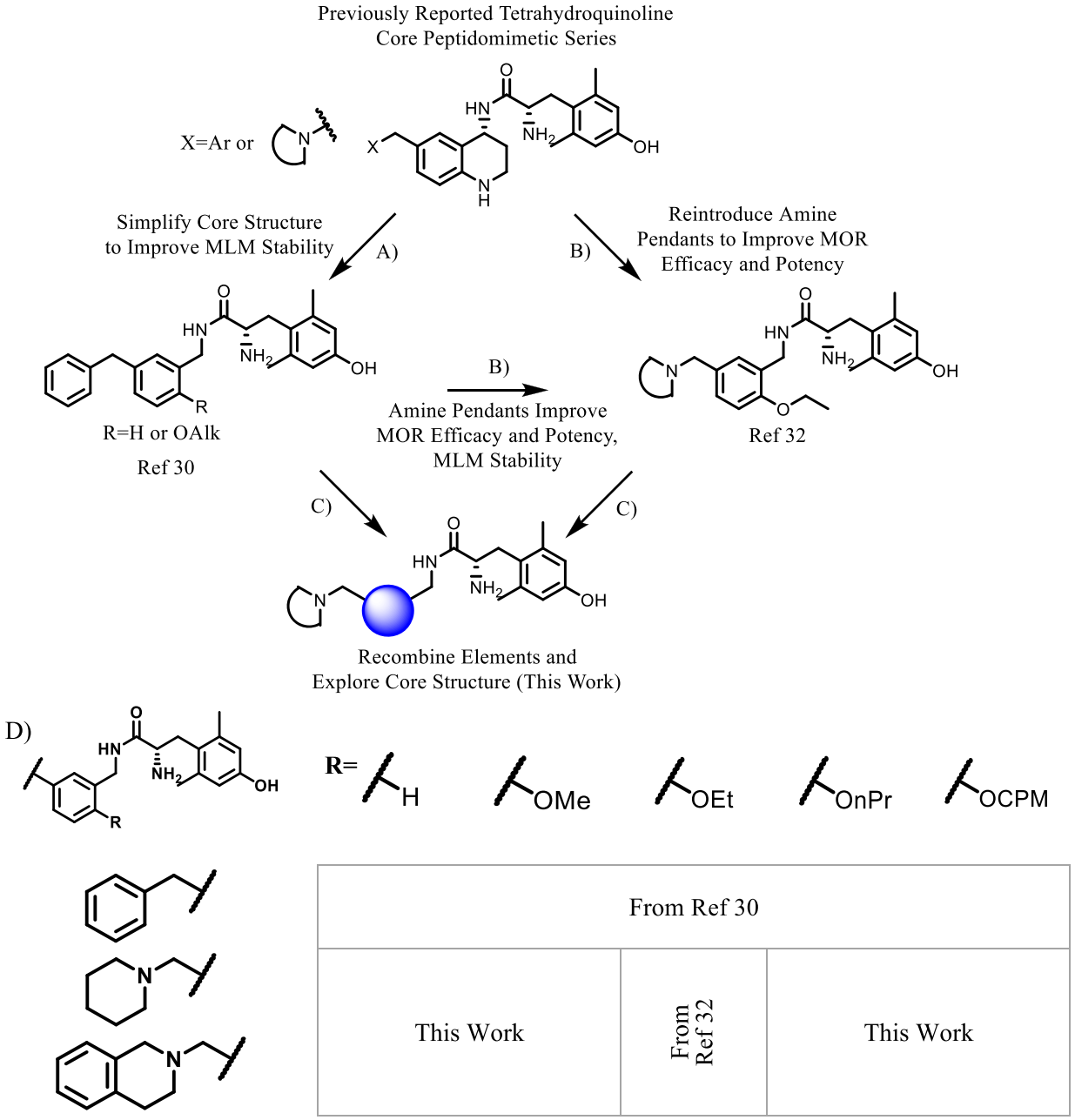

Figure 1. Design rationale that led to the initial SAR study reported in this work. This work stems from two previously reported SAR campaigns aimed at (A) converting a metabolically unstable tetrahydroquinoline core to a more stable aromatic core and (B) fully restoring lost MOR-efficacy, MOR-potency, and improving their stability through the use of amine pendants. The data from these two studies lead to (C) the current study that aims to demonstrate how the incorporation of these amine pendants into the peptidomimetics allows us to alter the core structure. Initially, this was manifested in (D) the construction of an SAR matrix aimed at understanding the interplay between the amine pendants and the aromatic R groups.

DOR-antagonist profile (Figure 1A). However, the ability of the compounds to operate as MOR-agonists was highly dependent on the size of the ether, and MOR-potency and efficacy were limited compared to previous analogues. Since we were interested in improving MOR-efficacy, MOR-potency, and metabolic stability further, we reexamined our original THQ core series for modifications that might be useful. In that series, there were cyclic amine pendant analogues that improved MOR-efficacy while reducing the cLogP of the ligands.[23] Since lowering cLogP is known to reduce metabolism by CYP enzymes,[31] we took the ether with the greatest level of MOR-agonism and replaced the benzyl pendant with a cyclic amine (Figure 1B). This was found to improve MOR-efficacy while also improving the metabolic stability of these ligands.[32] Furthermore, the addition of an aromatic ring onto this cyclic amine pendant improved MOR-potency at the cost of some of the improved metabolic stability.

Given the considerable improvement in MOR-potency and MOR-efficacy that these amine pendants yielded in our series, we opted to retain some of these amine pendants and again varied the core structure using modifications from our initial benzyl core study (Figure 1C). We were interested in the degree to which the MOR-agonism of this series can be maintained with these amine pendants without further structural additions to the peptidomimetic. To narrow down our syntheses, two example amine pendants were selected, namely, the piperidine and the

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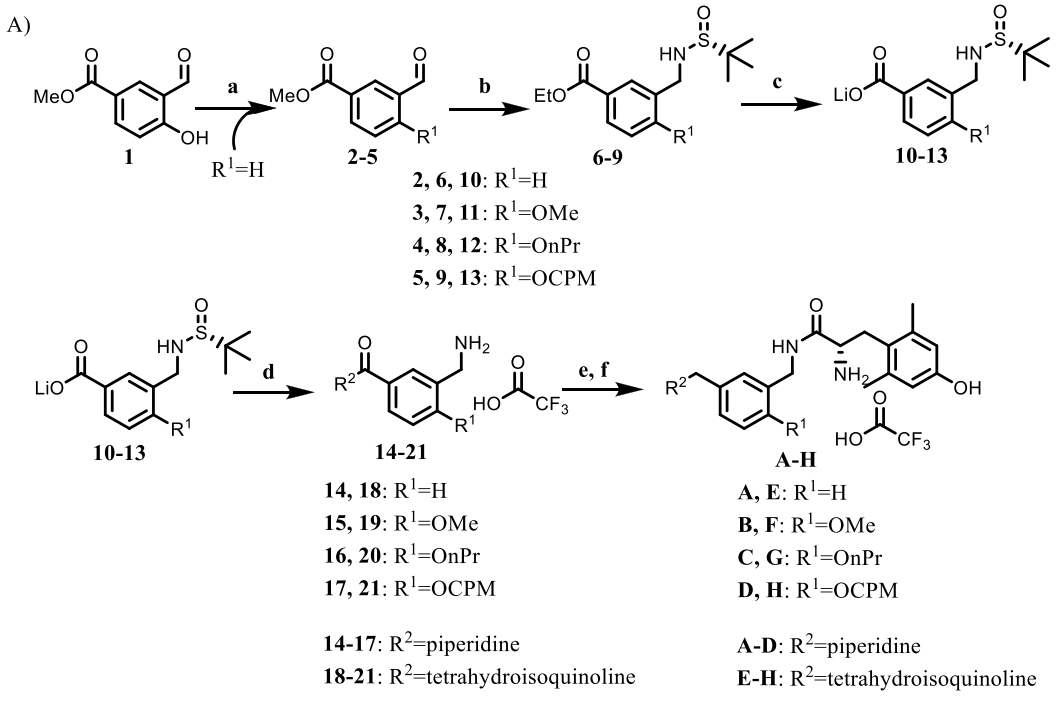
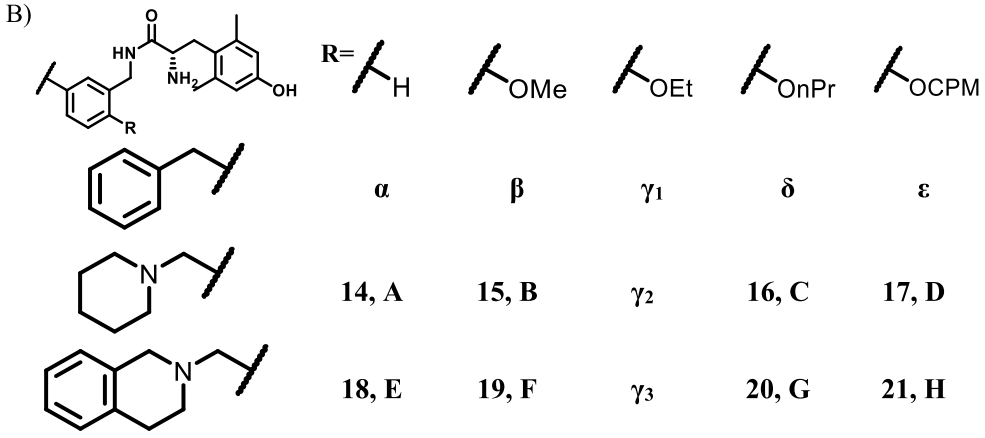
Scheme 1. (A) Synthesis of Aromatic Core Analogues to Produce SAR Matrices and (B) Analogue Codes for the Intermediate Amides and Final Compounds for Each Aromatic Core Modification and Amine Pendant Organized into Matrices[a]





[a]Previously reported compounds are encoded with Greek letters. Reaction conditions: (a) MeI or Alk-Br, K_2CO_3, DMF. (b) (1) (*R*)-(+)-2-methyl-2-propanesulfinamide, $Ti(OEt)_4$, THF; (2) $NaBH_4$. (c) LiOH, THF, EtOH, H_2O. (d) (1) 1,2,3,4-tetrahydroisoquinoline, NMM, PyBOP, DMF; (2) conc. HCl, dioxane. (e) BH_3*Me_2S, THF, 75 C°. (f) (1) DiBocDMT, DIEA, PyBOP, 6-Cl-HOBt, DMF; (2) TFA, DCM.

tetrahydroisoquinoline (THIQ) structures. The THIQ pendant was chosen as it showed the highest MOR-affinity and potency of those previously reported, whereas the piperidine pendant was selected due to its enhanced metabolic stability. These pendants were then synthesized with three previously reported core ethers (methyl, n-propyl, cyclopropylmethyl) and an unmodified aromatic core, yielding a total of eight new analogues. These analogues were compiled into an SAR matrix aimed at producing a tighter SAR campaign that addresses the following questions: To what degree can these new amine pendants stimulate MOR? How dependent is this activity on their respective aromatic core modifications?

In answering these questions, we found that incorporation of the THIQ pendant into these analogues produced potent and efficacious MOR-agonist/DOR antagonist ligands whose properties were insensitive to modifications on the core aromatic structure. With this surprising discovery, we opted then to remove the core aromatic structure and replace it with simple alkyl, heteroalkyl, and peptide structures to link the THIQ pharmacophore to the 2′6′-dimethyltyrosine pharmacophore. This produced a series of potent and efficacious MOR-agonists that were either selective for MOR or were mixed efficacy MOR/DOR or MOR/KOR ligands. Herein, the two SAR campaigns that describe the development of the THIQ pharmacophore are discussed.

RESULTS AND DISCUSSION

The Effect of Aromatic Core Substituents on Amine Pendant Analogues. The syntheses of these new analogues followed the same synthetic pathway described previously[32] and are shown in Scheme 1A. Here, different ethers were introduced by alkylating methyl 3-formyl-4-hydroxybenzoate (**1**) using

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Table 1. Binding Affinity Matrix of Aromatic Core Compounds at MOR, DOR, and KOR (in nM)[c]

		α, A, E	β, B, F	$\gamma_1, \gamma_2, \gamma_3$	δ, C, G	ε, D, H
		R= H	OMe	OEt	OnPr	OCPM
MOR		1.0±0.2[a]	3.6±0.1[a]	3.60±0.52[a]	0.91±0.06[a]	2.7±0.6[a]
		9.3±1.3	7.5±2.2	5.0±1.5[b]	4.6±0.2	6.5±1.2
		0.23±0.02	0.16±0.05	0.23±0.04[b]	0.22±0.04	0.05±0.01
DOR		14.7±0.6[a]	21.5±4.5[a]	4.81±0.89[a]	5.3±1.4[a]	13.9±1.8[a]
		143±25	41±16	15.7±4.3[b]	20.1±2.1	11.2±4.0
		4.8±1.0	3.0±0.8	2.4±0.5[b]	1.4±0.3	3.6±0.9
KOR		410±47[a]	610±100[a]	1180±120[a]	390±150[a]	319±51[a]
		82.8±7.3	23.5±7.3	101±14[b]	460±170	325±35
		46.3±9.7	28.4±3.1	44.2±4.6[b]	202±18	23.5±3.6
Select		1:15:410[a]	1:6.0:170[a]	1:8.5:420[a]	1:5.8:430[a]	1:5.1:120[a]
		1:15:8.9	1:5.5:3.1	1:3.1:20[b]	1:4.4:100	1:1.7:50
		1:21:200	1:19:180	1:10:192[b]	1:6.4:920	1:71:470

>500 nM	499-100 nM	99-50 nM	49-10 nM	<10 nM

[a]From ref 30. [b]From ref 32. [c]Binding affinities (K_i) were obtained by competitive displacement of radiolabeled [^{3}H]diprenorphine in membrane preparations from CHO cells expressing single human opioid receptors. Included are previously reported benzyl pendant (a) and ethyl ether (b) analogues for comparison. Selectivity was calculated by dividing the K_i of each receptor by the K_i at MOR for a given compound. All data are from three separate experiments, performed in duplicate unless otherwise noted. These data are reported as the average ± standard error of the mean. Cells colored in progressively darker shades of blue indicate progressively higher binding affinity.

appropriate alkyl iodides or bromides. These ethers, in addition to methyl 3-formylbenzoate (**2**), were then treated with Ellman's chiral auxiliary to introduce a protected amine in place of the aldehyde. The subsequent ethyl ester was then cleaved using LiOH and used as a common intermediate for incorporation of our two selected amine pendants. These pendants were incorporated into each aromatic core scaffold using PyBOP, and the Ellman auxiliary was removed with conc. HCl and purified by reversed-phase chromatography, yielding the intermediate as a TFA salt. The subsequent tertiary amide was then reduced with BH_3*Me_2S at 75 °C and the primary amine was coupled to Boc-protected 2′,6′-dimethyltyrosine. The synthesis was completed by deprotection of the Boc group using TFA. The analogues synthesized in this manner are organized into matrices (Scheme 1B) that include previously reported analogues to probe this SAR space.

We began our SAR analyses evaluating the binding affinity of these new analogues (Table 1) at MOR, DOR, and KOR to determine selectivity. Included also are previously reported analogues (Greek letters) placed in positions appropriate for their structure. Finally, the cells that correspond to each analogue are color coded with darker shades of blue to illustrate higher levels of binding affinity to illustrate trends within these data. Regarding MOR-binding affinity, two trends readily emerge. The first is that there is a relatively flat binding landscape within all three pendants across different R-groups, in which there exists a maximum of a 5-fold spread in binding affinity. The second trend is that the piperidine pendant (**A**−**D**, γ_2) possesses single-digit nanomolar binding affinity, whereas the THIQ pendant (**E**−**H**, γ_3) yields a log improvement in affinity into the subnanomolar range. The previously reported benzyl pendant analogues ($\alpha, \beta, \gamma_1, \delta, \varepsilon$) possess binding affinity values between those of the piperidine and THIQ pendants.

DOR-binding affinity in the piperidine pendants favored progressively larger ethers and ranged from low triple-digit nanomolar (**A**) to low double-digit nanomolar (**B**−**D**, γ_2)

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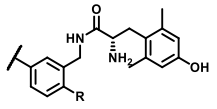
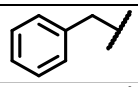
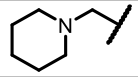
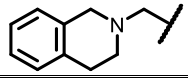
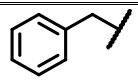
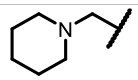
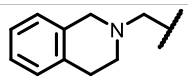
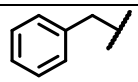
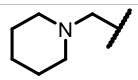
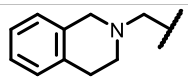
Table 2. Potency Matrix of Aromatic Core Compounds at MOR, DOR, and KOR (in nM)[c]



		α, A, E	β, B, F	γ1, γ2, γ3	δ, C, G	ε, D, H
		R= H	OMe	OEt	OnPr	OCPM
MOR		DNS[a]	264±21[a]	72±14[a]	67.5±9.9[a]	71±13[a]
		>740	740±330	585±73[b]	183±46	377±63
		6.1±3.1	2.9±0.5	1.9±0.5[b]	2.6±1.3	1.6±0.5
DOR		DNS[a]	DNS[a]	DNS[a]	DNS[a]	DNS[a]
		DNS	143±76	129±20[b]	117±45	158±68
		490±170	5.0±0.9	DNS[b]	DNS	DNS
KOR		DNS[a]	DNS[a]	DNS[a]	DNS[a]	DNS[a]
		DNS	DNS	DNS[b]	DNS	DNS
		DNS	>1000	DNS[b]	DNS	DNS

>500 nM	499-100 nM	99-50 nM	49-10 nM	<10 nM

[a]From ref 30. [b]From ref 32. [c]Potency data were obtained using agonist induced stimulation of [^{35}S] GTPγS binding in CHO cells expressing single human opioid receptors. Potency is represented as EC_{50} (nM). Included are previously reported benzyl pendant (a) and ethyl ether (b) analogues for comparison. All data are from three separate experiments, performed in duplicate unless otherwise noted. These data are reported as the average ± standard error of the mean. Cells colored in progressively darker shades of blue indicate progressively higher potency. DNS = Does not stimulate.

affinity values. However, there was little variance in DOR-binding affinity with the THIQ pendant, with all analogues showing low nanomolar binding affinities. At KOR, a reverse trend is observed with the piperidine pendants in that improved affinity was observed with the shorter core analogues, particularly the methyl ether (**B**). The THIQ pendants also presented a flat SAR at KOR in the double-digit nanomolar range, though an exception exists with the lower affinity *n*-propyl ether analogue (**G**). Notably, both amine-containing pendants possessed a significantly higher binding affinity for KOR over the benzyl pendant. The binding affinities between MOR and DOR tended to become more similar as the size of the core modification increased across all pendants, with the notable exception here being analogue **H**, likely due to its exceptional MOR affinity. Finally, the differences in binding affinity between MOR and KOR across all pendants reached a minimum with the methyl ethers and a maximum with the *n*-propyl ethers. Interestingly, the benzyl pendant and THIQ pendant possessed more similar binding affinity ratios to each other than to the piperidine pendant. Within the piperidines, the smaller analogues in fact had greater binding affinity to KOR than to DOR.

In addition to the binding affinity data collected above, the compounds were analyzed for their potency (Table 2) and relative efficacy (Table 3) at MOR, DOR, and KOR. For the piperidine pendants, potency at MOR was drastically lower than that of THIQ pendants. Furthermore, the piperidines were also less potent than the benzyl pendants and like the benzyl pendants, their potency was also dependent on the identity of the aromatic R-group. Conversely, the THIQ pendants possessed consistent single-digit nanomolar potency regardless of the identity of the aromatic R-group. Curiously, both amine pendants possessed very high MOR-efficacy, save piperidine **A**, whose measured maximal effect was likely limited by its low MOR-potency. This is in stark contrast to the benzyl pendant analogues;, whose degree of maximal stimulation was highly dependent on the identity of the aromatic R-group. In total, these potency and relative efficacy data illustrate that the amine may be an important pharmacophore for acquiring high MOR-efficacy, whereas the reattachment of the aromatic ring as exemplified in the THIQ pendant may be an important pharmacophore for acquiring high MOR-potency.

At DOR, the piperidine analogues generally functioned as weak partial agonists, a contribution that appears to come from the presence of an ether substituent. Where present, potency consistently was around 100 nM and maximal stimulation values were between 18 and 40% of the peptidic agonist DPDPE. The THIQ pendant was found to produce moderate DOR efficacy with an unmodified core (analogue **E**), though the potency of this analogue was particularly weak. These analogues generally possessed no activity at KOR, though some very modest activity was present with analogue **F**.

Elimination of the Aromatic Core. Given the surprisingly potent and efficacious MOR-agonist/DOR-antagonists the

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Table 3. Efficacy Matrix of Aromatic Core Compounds at MOR, DOR, and KOR (in %)[c]

		α, A, E	β, B, F	γ1, γ2, γ3	δ, C, G	ε, D, H
		R= H	OMe	OEt	OnPr	OCPM
MOR		DNS[a]	37.2±1.7[a]	75.6±5.8[a]	54.9±4.0[a]	37.5±1.3[a]
		>49	87.4±2.7	77.5±5.8[b]	98.0±3.5	104±5.6
		126±20	102±7	94.6±3.9[b]	110±4.1	93.3±4.0
DOR		DNS[a]	DNS[a]	DNS[a]	DNS[a]	DNS[a]
		DNS	37.2±6.2	36.8±6.7[b]	39.9±6.5	17.7±4.4
		57±6	29.8±3.5	DNS[b]	DNS	DNS
KOR		DNS[a]	DNS[a]	DNS[a]	DNS[a]	DNS[a]
		DNS	DNS	DNS[b]	DNS	DNS
		DNS	>15	DNS[b]	DNS	DNS
<30	30-50	51-70	71-90	>90		

[a]From ref 30. [b]From ref 32. [c]Relative efficacy data were obtained using agonist-induced stimulation of [^{35}S] GTPγS binding in CHO cells expressing human opioid receptors. Efficacy is represented as percent maximal stimulation relative to standard agonist DAMGO (MOR), DPDPE (DOR), or U69,593 (KOR) at 10 μM. Included are previously reported benzyl pendant (a) and ethyl ether (b) analogues for comparison. All data were from three separate experiments, performed in duplicate unless otherwise noted. These data are reported as the average ± standard error of the mean. DNS = Does not stimulate.

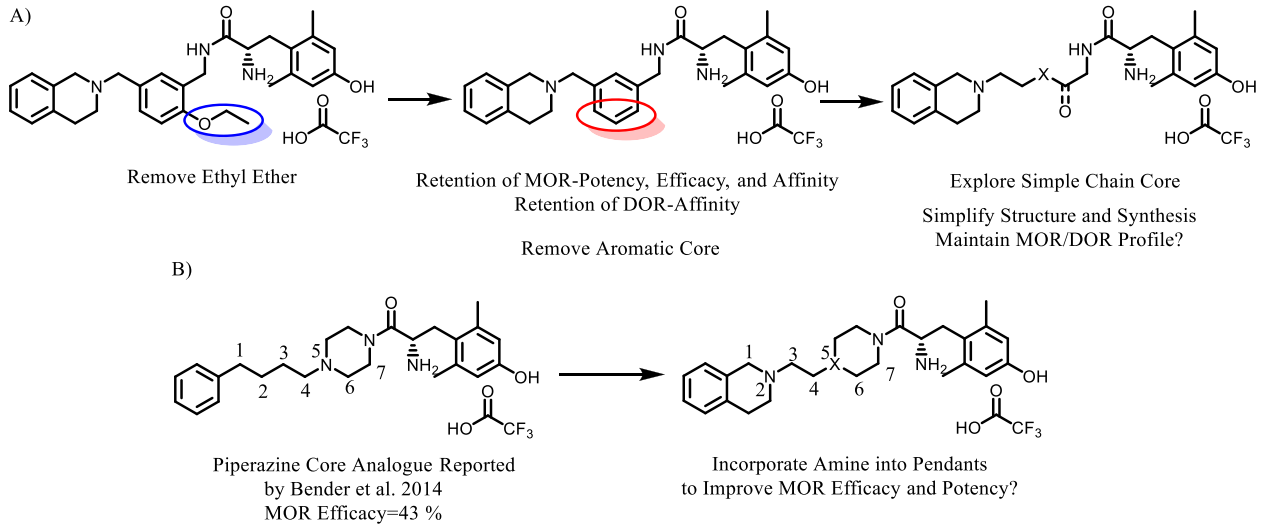

Figure 2. (A) Design of analogues with elimination of the aromatic core. (B) Design of more rigid analogues that use the THIQ pendant. These were inspired by previously described piperazine core analogues.[36]

THIQ pendants produced, we next examined the ability of the THIQ pendants to produce this MOR/DOR profile in simpler structures. As such, we opted to remove the entire core aromatic ring and replace it with simple alkyl or peptide chains (Figure 2A). The feasibility of this approach was reinforced by the fact that many opioid ligands are peptides that frequently use glycine

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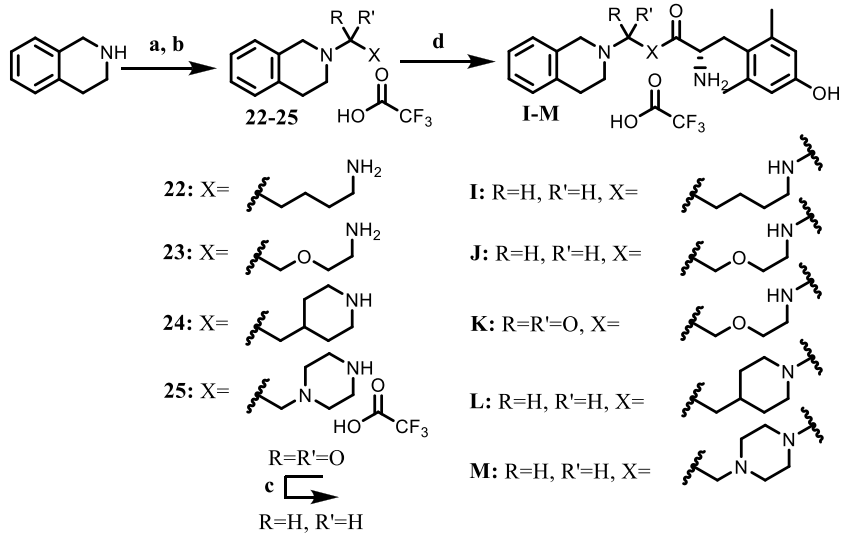
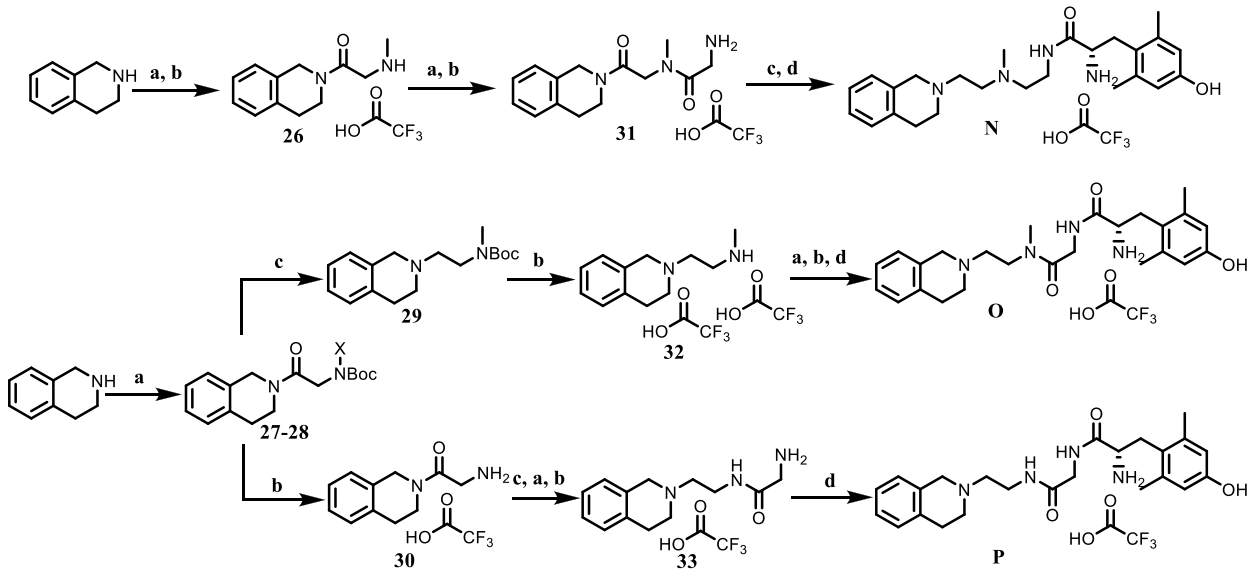
Scheme 2. Synthesis of Analogues I−M[a]



[a](A) 1. HO_2C-X-Boc, NMM, PyBOP, DMF. (B) Conc. HCl, dioxane, or TFA, DCM. (C) BH_3*Me_2S, THF, 65 C°. (D) (1) DiBocDMT, DIEA, PyBOP, 6-Cl-HOBt, DMF; (2) TFA, DCM.

Scheme 3. Synthesis of Peptide Core Analogues N−P[a]



[a](A) (1) $HO_2C-CH_2-NXBoc$, NMM, PyBOP, DMF. (B) Conc. HCl, dioxane, or TFA, DCM. (C) BH_3*Me_2S, THF, 65 C°. (D) (1) DiBocDMT, DIEA, PyBOP, 6-Cl-HOBt, DMF; (2) TFA, DCM.

or D-alanine residues[4,33−35] to link the tyrosine and phenylalanine pharmacophores.[3] In conjunction with these analogues, we previously reported a series of piperazine and piperidine core analogues that terminate in a simple benzene ring (Figure 2B).[36] Notably, the MOR-efficacy of a representative analogue in this prior study peaked at 43% of the full agonist DAMGO. We then hypothesized that the efficacy of these structures may be improved by replacing the terminal benzene ring with the amine-containing THIQ pendant.

The synthesis of many of these new analogues required very few steps and most are described in Scheme 2. These include a simple alkyl chain, a chain possessing an ether, and the piperidine and piperazine core structures aimed at reintroducing conformational restrictions. An amide analogue **K** was also synthesized to confirm that the compound efficacy produced at MOR is a product of the amine itself. The synthesis here began with peptide coupling of tetrahydroisoquinoline to an appropriate Boc-protected δ-amino acid. The Boc group was removed with conc. HCl, and the tertiary amide was reduced with BH_3*Me_2S at 65 °C if necessary. The primary amine was then coupled to Boc-protected 2′,6′-dimethyltyrosine, at which point the Boc-groups were removed with TFA, yielding the final peptidomimetics.

The analogues that contained a peptide bond or amine in the chain required a modified procedure that is described in Scheme 3. Analogue **N**, which possesses a tertiary amine, was synthesized using peptide coupling of tetrahydroisoquinoline to N-Boc sarcosine using PyBOP, at which point the Boc group was

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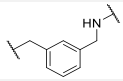
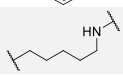
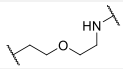
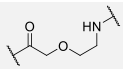
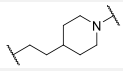
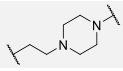
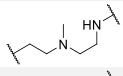
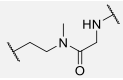
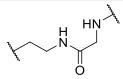
Table 4. Binding Affinity of Simple Core Compounds at MOR, DOR, and KOR[a]

		*Binding Affinity, K_i (nM)*			*Selectivity*
Name	Structure	MOR	DOR	KOR	MOR:DOR:KOR
E		0.23±0.02	4.8±1.0	46.3±9.7	1:21:200
I		1.5±0.3	142±16	44.2±5.6	1:95:29
J		0.64±0.19	113±21	45±12	1:177:65
K		1.28±0.68	17.5±5.7	12.5±2.5	1:14:9.8
L		1.64±0.62	130±17	21.0±5.9	1:79:13
M		3.59±0.40	16.6±4.9	173±20	1:4.6:48
N		1.70±0.39	77±45	8.5±1.1	1:45:5
O		24±11	24.7±5.0	780±570	1:1.0:33
P		1.32±0.38	7.3±1.5	107±35	1:5.5:81

[a]Binding affinities (K_i) and selectivities were obtained as described in Table 1. Included is **E** for comparison. Selectivity was calculated by dividing the K_i of each receptor by the K_i at MOR for a given compound. All data are from three separate experiments, performed in duplicate unless otherwise noted. These data are reported as the average ± standard error of the mean.

removed with conc. HCl. This was then coupled to N-Boc glycine and deprotected using the same method. Both peptide bonds were then reduced with BH_3*Me_2S and the primary amine was then coupled to Boc-protected 2′,6′-dimethyltyrosine, at which point the Boc-groups were removed with TFA, yielding **N**.

Two additional analogues containing peptide bonds in the chain were also synthesized. These two analogues largely used the same steps as those for the simpler analogues in Scheme 2 but used slightly different synthetic strategies. As described in Scheme 3, tetrahydroisoquinoline was coupled to N-Boc protected glycine or sarcosine. These intermediates were isolated before Boc deprotection, at which point the synthetic schemes diverged. The sarcosine intermediate **27** was then subject to reduction with borane before removal of the Boc group, yielding intermediate **29**. This was coupled to glycine and deprotected. No extensive purification of this intermediate was performed, as this analogue had poor UV absorbance properties and the impurities present were peptide coupling side products, which were present in the subsequent coupling to DMT. As such, DMT coupling was performed on this impure mixture and subsequent deprotection yielded the *N*-methyl amide analogue **O**. For the glycine intermediate **28**, Boc deprotection was performed before reduction, as the secondary carbamate was unstable under these reduction conditions. This was then coupled to an additional glycine residue, yielding intermediate **33** with better UV absorbance properties than its sarcosine counterpart. After Boc deprotection, the intermediate was then coupled to DMT, yielding **P** after final Boc deprotection.

The binding affinities of these analogues were determined and are shown in Table 4. For comparison, analogue **E** is included. Regarding MOR-binding, each analogue in this new subseries possessed single-digit or subnanomolar binding-affinity except for the *N*-methyl amide analogue **O**. At DOR, elimination of the core aromatic ring produced well over a 10-fold drop in affinity. The exceptions here are the rigid piperazine **M** and the two amide analogues **O** and **P**. KOR-binding affinity does not change significantly from the aromatic core analogue **E**, the only exceptions being the *N*-methyl amide analogue **O**, which has low KOR-affinity, and the tertiary amine analogue **N**, which has improved KOR-affinity compared to **E**. Notably, this series generated analogues that deviate from our original MOR/DOR bifunctional profile and instead appear to promote a MOR/KOR profile. This is illustrated with the alkyl (**I**), piperidine (**L**), and tertiary amine (**N**) analogues. Finally, the ether analogue (**J**) possessed a more monofunctional MOR selective profile.

The potency and efficacy relative to standard full agonists of these analogues were also screened at each of these receptors and are shown in Table 5. Significantly, each of the amine pendant compounds possessed moderate to high MOR-efficacy. The amide analogue **K**, while showing significantly reduced MOR-efficacy compared to its amine counterpart **J**, retained upward of 48% stimulation compared to the standard DAMGO. Curiously, removal of the aromatic ring did not produce a significant drop in MOR-potency upon simplification, even when the amine is converted to an amide as with analogue **K**. Losses in potency only occurred with the analogues that possessed a reformed ring structure in the core (**L** and **M**) or upon possession of an *N*-methyl substituent (**N** and **O**).

DOR efficacy was low in this series. Analogues that did stimulate DOR were weak partial agonists with low potency and efficacy, and included the ether (**J**), tertiary amine (**N**), and the

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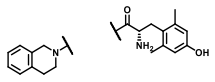
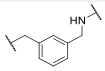
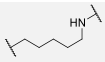
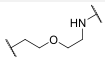
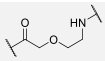
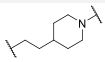
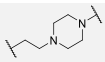
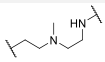
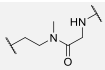
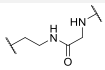
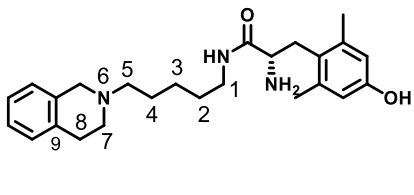
Table 5. Potency and Efficacy of Simple Core Compounds at MOR, DOR, and KOR[a]

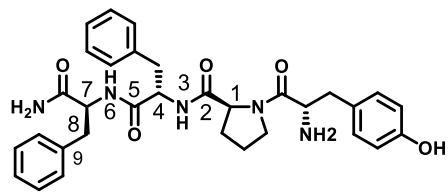


		Potency, EC_{50} (nM)			*Efficacy (% Stimulation)*		
Name	Structure	MOR	DOR	KOR	MOR	DOR	KOR
E		6.1±3.1	490±170	DNS	126±20	57±6	DNS
I		15.4±1.3	DNS	DNS	80.8±4.5	DNS	DNS
J		9.6±3.1	243±23	1060±180	90.7±3.6	27.4±8.2	22.0±3.8
K		2.29±0.37	DNS	DNS	49±11	DNS	DNS
L		68±19	DNS	DNS	73±12	DNS	DNS
M		56±17	DNS	DNS	70±16	DNS	DNS
N		24±10	1630±250	1220±260	80.0±9.5	28.4±2.1	43.0±2.5
O		568±56	DNS	DNS	53±17	DNS	DNS
P		6.0±1.0	99±16	DNS	86.6±3.3	36.5±1.2	DNS

[a]Potency and efficacy data were obtained as described in Tables 2 and 3. Potency is represented as EC_{50} (nM) and efficacy as percent maximal stimulation relative to standard agonist DAMGO (MOR), DPDPE (DOR), or U69,593 (KOR) at 10 μM. Included is **E** for comparison. All data are from three separate experiments, performed in duplicate unless otherwise noted. These data are reported as the average ± standard error of the mean. DNS = Does Not Stimulate.



Analogue I
Molecular Weight: 409.5740
MOR EC_{50}=15.4±1.3 nM
MOR %Stim=80.8±4.5 %



Endomorphin-2
Molecular Weight: 571.6780
MOR EC_{50}=138±31 nM[a]
MOR %Stim=104±4 %[a]

Figure 3. Structural comparison between the opioid peptide Met-enkephalin and analogue **I**. Relative positions of the amine and aromatic ring from the tyrosine pharmacophore are numbered. (a) From ref 37.

most potent secondary amide analogue (**P**). At KOR, these analogues either could not stimulate this receptor or were weak partial agonists with very low potency (**J** and **N**).

The high MOR-potency in most of these simplified analogues illustrate that the aromatic part of the THIQ pendant is an important pharmacophore for MOR-potency, and tolerates a variety of core structures, including those previously reported. The exception here is the *N*-methyl amide analogue **O**, whose reduced potency is likely due to the combination of both the *N*-methyl group and the carbonyl, as each of these elements alone had a modest or no effect on MOR-potency (**N** and **P** respectively).

We opted to compare MOR-potency and efficacy of the simplest analogue **I** to one of the simpler endogenous opioid peptides endomorphin-2 (Figure 3).[37] Here, each position moving away from the tyrosine residue was numbered, and the amine of analogue **I** was found to be in the same position as the amido nitrogen of the fourth position phenylalanine residue. Furthermore, the aromatic ring of the THIQ pendant lines up nicely with the aromatic ring of the same phenylalanine. A

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phenylalanine is typical of many opioid peptides and is a key pharmacophore element necessary for opioid receptor activation.[3] As such, the enhancements in MOR-potency we observed in the THIQ pendants are consistent with classical opioid peptide SAR.

However, the amines represent a deviation from this classical SAR, as this nitrogen is tied up in an amide structure that is largely conserved throughout these opioid peptides. The conversion to an amine in this position may be an efficient means of acquiring high MOR-efficacy in these structurally simplified peptidomimetics. This is illustrated by comparing analogues **J** and **K** (Table 5), and the previously reported benzyl pendant analogues and amine pendant analogues presented in the matrices (Table 3). The conversion of the amide in **K** to an amine in **J** results in considerably increased efficacy at MOR (from 49% to 90.7%), while the pendant amines presented in Table 3 express higher MOR-efficacy than their benzyl pendant counterparts As such, the reduction of the amide to an amine may prove effective in the development of future high efficacy MOR-agonist peptides. Furthermore, the results illustrated in Tables 4 and 5 point toward the development of selective MOR-agonists or bifunctional MOR-agonists that may also be produced with small deviations in structure.

CONCLUSION

The two SAR campaigns illustrated here describe the development of a potentially novel pharmacophore element that can be used in the development of future opioid peptides and peptidomimetics. The SAR matrices that describe the first campaign illustrate that an aromatic ring as represented in the THIQ pendant produces high MOR-potency when compared to the corresponding piperidine pendant. Furthermore, the high MOR-efficacy in both the piperidine and THIQ pendant analogues compared to the previously reported benzyl pendant illustrate that the amine in these pendants is responsible for this efficacy. In this first series, the analogues acted as potent bifunctional MOR-agonist/DOR antagonist ligands.

Given the promising profile the THIQ pendants offered, we pursued a second SAR campaign aimed at simplifying the peptidomimetics while simultaneously exploring the scope and power that the THIQ pendants offer to our peptidomimetic series. These modifications consistently produced potent and efficacious MOR-agonists. Simultaneously, we found that slight changes to the core structure can produce selective MOR-agonists, bifunctional MOR-agonist/DOR-antagonists, and bifunctional MOR/KOR analogues. Overall, the amine component of the THIQ pendant may be a structurally efficient means of introducing MOR-efficacy in opioid peptides and peptidomimetics. Furthermore, the linking segment between the THIQ pendant and the dimethyltyrosine pharmacophore can be varied to fine-tune selectivity and enable monofunctional or bifunctional opioid effects that can be used to develop future opioid therapeutics.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* All reagents and solvents were obtained commercially and were used without further purification. Intermediates were purified by flash chromatography using a Biotage Isolera One instrument. Most purification methods utilized a hexanes/ethyl acetate solvent system in a Biotage SNAP KP-Sil column, with a linear gradient between 0 and 100% ethyl acetate. Reversed-phase column chromatography using a linear gradient of 0% to 100% solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) using a Biotage SNAP Ultra C18 column was utilized for some intermediate amine salts. Purification of final compounds was performed using a Waters semipreparative HPLC with a Vydac protein and peptide C18 reversed-phase column, using a linear gradient of 0% to 100% solvent B in solvent A at a rate of 1%/min, monitoring UV absorbance at 230 nm. The purity of final compounds was assessed using a Waters Alliance 2690 analytical HPLC instrument with a Vydac protein and peptide C18 reversed-phase column. A linear gradient (gradient A) of 0% to 70% solvent B in solvent A in 70 min, measuring UV absorbance at 230 nm was used to determine purity. All final compounds used for testing were ≥95% pure, as determined by analytical HPLC. ^{1}H NMR and ^{13}C NMR data were obtained on a 500 or 400 MHz Varian spectrometer using $CDCl_3$, CD_3OD, DMSO-*d6*, or D_2O as solvents. The identities of final compounds were verified by mass spectrometry using an Agilent 6130 LC−MS mass spectrometer in positive ion mode, or an Agilent 6230 TOF HPLC-MS in the positive ion mode.

General Procedure for the Synthesis of 6-position Ethers (Procedure A). To a flame-dried flask containing methyl 3-formyl-4-hydroxybenzoate (**1**) was added 3 equiv of potassium carbonate. The flask was purged with argon, and 4 mL of DMF was added. Three equivalents of an alkyl iodide or bromide was then added, and the solution was stirred at room temperature overnight. The solution was then concentrated in vacuo, partitioned between ethyl acetate and saturated sodium carbonate, and extracted with ethyl acetate. The organic layers were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo, yielding the desired ether.

General Procedure for Ellman Reductions (Procedure B). A flamed-dried round-bottom flask containing 1 equiv of aldehyde and 3 equiv of (R)-(+)-2-methyl-2-propanesulfinamide was attached to a reflux condenser and flushed with argon. Four mL of THF was added and cooled to 0 °C. Six equivalents of titanium(IV) ethoxide was added, followed by an additional 4 mL of THF. The solution was stirred and heated to 75 °C overnight with TLC monitoring until all ketone or aldehyde was consumed. A separate flame-dried flask containing 6 equiv of sodium borohydride was flushed with argon. A volume of 4 mL of THF was added, at which point the solution was cooled to −78 °C. The solution containing Ellman adduct was cooled to room temperature and slowly transferred to the sodium borohydride solution via syringe. This final solution was then allowed to warm to room temperature and stirred for 2 h, at which point the reaction mixture was quenched with methanol to consume the sodium borohydride, followed by DI water to precipitate the titanium. The solution was vacuum filtered, and the precipitate was washed with ethyl acetate. The filtrate was the concentrated in vacuo and purified via column chromatography (0−100% EtOAc in hexanes).

General Procedure for the Saponification of Esters (Procedure C). To a flask containing 1 equiv of the desired ester was added 7 equiv of LiOH, 2 mL of THF, 2 mL of EtOH, and 2 mL of H_2O. The reaction was stirred overnight under ambient atmosphere and temperature. Upon completion, the solvent was concentrated in vacuo, suspended in acetone, and filtered. The precipitate was washed with additional acetone, and the filtrate was concentrated in vacuo, yielding the saponified product as a lithium carboxylate.

General Procedure for Amine Pendant Attachment and Cleavage of Ellman Auxiliaries (Procedure D). To a flask containing 1 equiv of the lithium carboxylate or carboxylic acid was added 1 equiv of PyBOP and 1 equiv of the desired amine. The flask was flushed with argon, DMF was added as solvent, and 10 equiv of *N*-methylmorpholine was added. The reaction was stirred overnight, at which point it was concentrated in vacuo and purified via column chromatography (0−10% methanol in DCM). To the protected amine was immediately added 2 mL of dioxane and 0.2 mL concentrated HCl. The solution was stirred at room temperature for 1 min and concentrated in vacuo. The ensuing salt was triturated with diethyl ether, and then was purified using a reversed-phase chromatography (0−100% B in A), yielding the product as a TFA salt.

General Procedure for the Reduction of Amides (Procedure E). To a dried flask containing 1 equiv of the desired amide under argon was added THF and 7 equiv of 2 M BH_3*Me_2S complex in THF. The reaction was heated at 75 °C for 3 h, at which point the reaction was

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quenched with MeOH and heated for an additional 15 min. The reaction was then cooled, concentrated in vacuo, and was used in Procedure F without further purification.

General Procedure for the Coupling of 2′,6′-Dimethyltyrosine to Functionalized Amine Salts (Procedure F). To a dried flask containing the amine under argon was added 3 mL of DMF and 10 equiv of Hunig's base. One equivalent of PyBOP and 1 equiv of 6-Cl-HOBt were added, followed by a 1 equiv of doubly Boc protected 2′,6′-dimethyltyrosine in 1.5 mL of DMF. The solution was stirred overnight at room temperature, concentrated in vacuo, and purified via semipreparative reversed-phase HPLC (0.1% TFA in water: 0.1% TFA in acetonitrile). Then 2 mL of TFA and 2 mL of DCM were added, and the solution was stirred for an additional hour. The reaction mixture was concentrated in vacuo and purified via an additional semipreparative reversed-phase HPLC (0.1% TFA in water: 0.1% TFA in acetonitrile). The product was concentrated in vacuo and lyophilized overnight to yield the final peptidomimetic.

General Procedure for Coupling of Tetrahydroisoquinoline Analogues to N-Boc Protected Carboxylic Acids (Procedure G). To a flask containing 1 equiv of N-Boc protected carboxylic acid was added 1 equiv of PyBOP and 1 equiv of tetrahydroisoquinoline or the tetrahydroquinoline analogue. The flask was flushed with argon, DMF was added as the solvent, and 10 equiv of *N*-methylmorpholine was added. The reaction was stirred overnight, at which point it was concentrated in vacuo. The residue was then partitioned between EtOAc and sat. Na_2CO_3 and extracted with EtOAc, the organic layers were combined, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (0−5% MeOH in DCM, or 0−66% EtOAc in hexanes) yielded the coupled product.

General Procedure for the Deprotection of Boc-Groups Using Concentrated HCl (Procedure Ha). To the Boc protected compound was added 2−6 mL of dioxane and 0.2−0.6 mL concentrated HCl. The solution was stirred at room temperature for 1−5 min and concentrated in vacuo. The ensuing salt was suspended in solvent A and was either concentrated in vacuo and triturated with hexanes, or purified by reversed-phase chromatography (0−100% B in A), yielding the product as a TFA salt.

General Procedure for the Deprotection of Boc-Groups Using TFA (Procedure Hb). To the Boc protected compound was added 2 mL of TFA and 2 mL of DCM. The solution was stirred at room temperature for 1−5 min and concentrated in vacuo. The ensuing salt was either continued without further purification or suspended in solvent A and was purified via reversed-phase chromatography (0−100% B in A), yielding the product as a TFA salt.

*Methyl 4-Methoxy-3-formylbenzoate (**3**).* See Procedure A: 160 mg (0.88 mmol) of methyl 3-formyl-4-hydroxybenzoate (**1**), 368 mg (2.66 mmol, 3.00 equiv) of K_2CO_3, 170 μL (388 mg, 2.73 mmol, 3.08 equiv) of MeI, 4 mL of DMF. Compound **3** (162 mg, yield = 94%) was isolated as a yellow solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 10.44 (s, 1H), 8.49 (d, *J* = 2.3 Hz, 1H), 8.23 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 1H), 4.00 (s, 3H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 188.84, 165.96, 164.71, 137.10, 130.67, 124.44, 122.92, 111.54, 56.06, 52.12.

*Methyl 4-Propoxy-3-formylbenzoate (**4**).* See Procedure A: 168 mg (0.93 mmol) of methyl 3-formyl-4-hydroxybenzoate (**1**), 388 mg (2.81 mmol, 3.01 equiv) of K_2CO_3, 260 μL (352 mg, 2.86 mmol, 3.07 equiv) of nPrBr, 4 mL of DMF. Compound **4** (188 mg, yield = 91%) was isolated as a colorless oil that turns to a white solid on standing. ^{1}H NMR (500 MHz, chloroform-*d*) δ 10.44 (s, 1H), 8.43 (d, *J* = 2.3 Hz, 1H), 8.15 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 1H), 4.07 (t, *J* = 6.4 Hz, 2H), 3.86 (s, 3H), 1.87 (h, *J* = 7.2 Hz, 2H), 1.05 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 188.78, 165.94, 164.41, 136.98, 130.24, 124.36, 122.56, 112.27, 70.44, 52.03, 22.30, 10.41.

*Methyl 4-(Cyclopropylmethoxy)-3-formylbenzoate (**5**).* See Procedure A: 181 mg (1.00 mmol) of methyl 3-formyl-4-hydroxybenzoate (**1**), 417 g (3.02 mmol, 3.00 equiv) of K_2CO_3, 290 μL (404 mg, 2.99 mmol, 2.98 equiv) of cyclopropylmethyl bromide, 4 mL of DMF. Compound **5** (233 mg, yield = 99%) was isolated as a yellow oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 10.41 (s, 1H), 8.35 (d, *J* = 2.3 Hz, 1H), 8.07 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 3.91 (d, *J* = 6.9 Hz, 2H), 3.80 (s, 3H), 1.31−1.20 (m, 1H), 0.65−0.56 (m, 2H), 0.32 (dt, *J* = 6.2, 4.8 Hz, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 188.81, 165.84, 164.29, 136.86, 130.06, 124.34, 122.48, 112.43, 73.58, 51.97, 9.83, 3.18.

*Ethyl (R)-3-(((tert-Butylsulfinyl)amino)methyl)benzoate (**6**).* See Procedure B: Step 1: 220 mg (1.34 mmol) of methyl 3-formylbenzoate (**2**), 490 mg (4.04 mmol, 3.02 equiv) of (*R*)-(+)-2-methyl-2-propanesulfinamide, 1.7 mL (1.8 g, 8.1 mmol, 6.1 equiv) of $Ti(OEt)_4$, 5 + 5 mL THF. Step 2: 331 mg (8.75 mmol, 6.08 equiv) of sodium borohydride in 5 mL of THF. Compound **6** (278 mg, yield = 73%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.93 (t, *J* = 1.8 Hz, 1H), 7.86 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.46 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.32 (td, *J* = 7.7, 2.5 Hz, 1H), 4.33−4.18 (m, 4H), 3.80 (dd, *J* = 6.9, 4.6 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 1.4 Hz, 9H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.24, 139.00, 132.41, 130.68, 128.97, 128.66, 128.57, 60.95, 55.94, 48.79, 22.63, 14.24.

*Ethyl (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-methoxybenzoate (**7**).* See Procedure B: Step 1: 234 mg (1.21 mmol) of **3**, 441 mg (3.64 mmol, 3.02 equiv) of (*R*)-(+)-2-methyl-2-propanesulfinamide, 1.55 mL (1.69 g, 7.39 mmol, 6.14 equiv) of $Ti(OEt)_4$, 4 + 4 mL of THF. Step 2: 274 mg (7.24 mmol, 6.01 equiv) of sodium borohydride in 4 mL THF. Compound **7** (374 mg, yield = 99%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.96−7.90 (m, 2H), 6.84 (d, *J* = 9.3 Hz, 1H), 4.36 (dd, *J* = 14.3, 5.6 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.16 (dd, *J* = 14.3, 7.6 Hz, 1H), 3.85 (s, 3H), 3.73 (t, *J* = 6.6 Hz, 1H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.17 (s, 9H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.19, 160.92, 131.07, 130.63, 127.08, 122.66, 109.83, 60.65, 55.88, 55.58, 44.92, 22.56, 21.94, 14.31.

*Ethyl (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-propoxybenzoate (**8**).* See Procedure B: Step 1: 181 mg (0.81 mmol) of **4**, 299 mg (2.5 mmol, 3.0 equiv) of (*R*)-(+)-2-methyl-2-propanesulfinamide, 1025 μL (1115 mg, 4.9 mmol, 6.0 equiv) of $Ti(OEt)_4$, 4 + 4 mL of THF. Step 2: 187 mg (4.9 mmol, 6.1 equiv) of sodium borohydride in 4 mL THF. Compound **8** (272 mg, yield = 98%) was isolated as a yellow oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.89 (d, *J* = 2.2 Hz, 1H), 7.86 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 4.32 (dd, *J* = 14.4, 5.5 Hz, 1H), 4.23 (q, *J* = 7.0 Hz, 2H), 4.13 (dd, *J* = 14.3, 7.7 Hz, 1H), 3.91 (t, *J* = 6.4 Hz, 2H), 3.76 (t, *J* = 6.6 Hz, 1H), 1.75 (h, *J* = 7.4 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.13 (s, 9H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.11, 160.35, 130.90, 130.46, 127.11, 122.34, 110.40, 69.73, 60.20, 55.74, 44.88, 22.52, 22.40, 14.25, 10.52.

*Ethyl (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-(cyclopropylmethoxy)benzoate (**9**).* See Procedure B: Step 1: 230 mg (0.98 mmol) of **5**, 359 mg (2.96 mmol, 3.02 equiv) of (*R*)-(+)-2-methyl-2-propanesulfinamide, 1.25 mL (1.36 g, 5.96 mmol, 6.07 equiv) of $Ti(OEt)_4$, 4 + 4 mL of THF. Step 2: 225 mg (4.26 mmol, 5.95 equiv) of sodium borohydride in 4 mL of THF. Compound **9** (333 mg, yield = 96%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.97−7.87 (m, 2H), 6.79 (d, *J* = 8.5 Hz, 1H), 4.42 (dd, *J* = 14.3, 5.7 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.20 (dd, *J* = 14.3, 7.8 Hz, 1H), 3.91−3.81 (m, 3H), 1.34 (t, *J* = 7.1 Hz, 4H), 1.29−1.20 (m, 2H), 1.19 (s, 10H), 0.66−0.58 (m, 2H), 0.36−0.29 (m, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.23, 160.41, 131.01, 130.59, 127.24, 122.48, 110.58, 72.99, 60.65, 55.83, 45.46, 22.59, 14.33, 10.10, 3.24, 3.20.

*Lithium (R)-3-(((tert-Butylsulfinyl)amino)methyl)benzoate (**10**).* See Procedure C: 285 mg (1.01 mmol) of **6**, 143 mg (5.97 mmol, 5.94 equiv) of LiOH, 2 mL of THF, 2 mL of EtOH, and 2 mL of H_2O. The compound was suspended and filtered in EtOH instead of acetone. Compound **10** (240 mg, yield = 91%) was isolated as a white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.92 (d, *J* = 1.8 Hz, 1H), 7.85 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.45−7.39 (m, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 4.34 (d, *J* = 14.4 Hz, 1H), 4.26 (d, *J* = 14.4 Hz, 1H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, methanol-d_4) δ 173.83, 138.51, 137.91, 129.60, 128.53, 127.93, 127.48, 55.72, 48.76, 21.79.

*Lithium (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-methoxybenzoate (**11**).* See Procedure C: 181 mg (0.58 mmol) of **7**, 86 mg (3.59 mmol, 6.21 equiv) of LiOH, 2 mL of THF, 2 mL of EtOH, and 2 mL of H_2O. Compound **11** (128 mg, yield = 76%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.95−7.88 (m, 2H), 6.93 (d, *J* = 8.4 Hz, 1H), 4.32 (d, *J* = 14.4 Hz, 1H), 4.22 (d, *J* = 14.4 Hz, 1H),

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3.87 (s, 3H), 1.22 (s, 9H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 173.83, 159.18, 130.41, 130.20, 129.69, 125.95, 109.03, 55.64, 54.63, 44.23, 21.74.

*Lithium (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-propoxybenzoate (**12**).* See Procedure C: 272 mg (0.80 mmol) of **8**, 114 mg (4.76 mmol, 6.0 equiv) of LiOH, 2 mL of THF, 2 mL of EtOH, and 2 mL of H_2O. Compound **12** (226 mg, yield = 89%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.92 (d, J = 2.2 Hz, 1H), 7.87 (dd, J = 8.5, 2.2 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 4.35 (d, J = 14.3 Hz, 1H), 4.23 (d, J = 14.3 Hz, 1H), 4.01 (t, J = 6.4 Hz, 2H), 1.85 (h, J = 7.4 Hz, 2H), 1.22 (s, 9H), 1.08 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 173.91, 158.57, 130.34, 130.11, 129.48, 125.96, 109.75, 69.35, 55.63, 44.22, 22.32, 21.73, 9.67.

*Lithium (R)-3-(((tert-Butylsulfinyl)amino)methyl)-4-(cyclopropylmethoxy)benzoate (**13**).* See Procedure C: 333 mg (0.94 mmol) of **9**, 135 mg (5.64 mmol, 5.98 equiv) of LiOH, 2 mL of THF, 2 mL of EtOH, and 2 mL of H_2O. Compound **13** (271 mg, yield = 87%) was isolated as a white amorphous solid. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.93 (d, J = 2.2 Hz, 1H), 7.88 (dd, J = 8.5, 2.2 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 4.37 (d, J = 14.3 Hz, 1H), 4.25 (d, J = 14.4 Hz, 1H), 3.90 (d, J = 7.2 Hz, 2H), 1.30 (dddd, J = 11.6, 8.0, 4.7, 1.2 Hz, 2H), 1.23 (s, 12H), 0.67−0.59 (m, 2H), 0.41−0.35 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 173.84, 158.58, 130.38, 130.15, 129.60, 126.06, 110.05, 72.54, 55.65, 44.55, 21.77, 9.87, 2.28, 2.27.

*(3-(Piperidine-1-carbonyl)phenyl)methanaminium Trifluoroacetate (**14**).* See Procedure D: Step 1:20 mg (0.077 mmol) of **10**, 41 mg (0.079 mmol, 1.03 equiv) of PyBOP, 20 μL (17 mg, 0.20 mmol, 2.6 equiv) of piperidine, 90 μL (83 mg, 0.82 mmol, 10.7 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL conc. HCl. Compound **14** (15 mg, yield = 59%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.60−7.51 (m, 2H), 7.47 (d, J = 1.8 Hz, 1H), 7.44 (dt, J = 7.1, 1.6 Hz, 1H), 3.72 (d, J = 5.9 Hz, 2H), 3.37 (t, J = 5.4 Hz, 2H), 1.76−1.63 (m, 4H), 1.54 (s, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.04, 136.87, 133.77, 129.94, 129.13, 126.96, 126.91, 48.62, 42.94, 42.53, 26.13, 25.28, 23.97.

*(2-Methoxy-5-(piperidine-1-carbonyl)phenyl)methanaminium Trifluoroacetate (**15**).* See Procedure D: Step 1: 23 mg (0.079 mmol) of **11**, 42 mg (0.081 mmol, 1.02 equiv) of PyBOP, 20 μL (17 mg, 0.20 mmol, 2.5 equiv) of piperidine, 90 μL (83 mg, 0.82 mmol, 10.4 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL conc. HCl. Compound **15** (23 mg, yield = 80%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.41 (d, J = 2.1 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 4.13 (s, 2H), 3.97 (s, 3H), 3.69 (br s, 2H), 3.44 (br s, 2H), 1.74−1.51 (m, 6H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.18, 158.97, 129.82, 129.49, 128.10, 121.24, 110.47, 55.08, 48.43, 38.67, 24.01, 20.05.

*(5-(Piperidine-1-carbonyl)-2-propoxyphenyl)methanaminium Trifluoroacetate (**16**).* See Procedure D: Step 1: 25 mg (0.078 mmol) of **12**, 41 mg (0.079 mmol, 1.0 equiv) of PyBOP, 20 μL (17 mg, 0.20 mmol, 2.6 equiv) of piperidine, 90 μL (83 mg, 0.82 mmol, 10.5 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL conc. HCl. Compound **16** (24 mg, yield = 79%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.46 (dd, J = 8.5, 2.2 Hz, 1H), 7.41 (d, J = 2.1 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 4.14 (s, 2H), 4.11 (t, J = 6.6 Hz, 2H), 3.68 (br s, 2H), 3.44 (br s, 2H), 1.89 (h, J = 7.4 Hz, 2H), 1.75−1.61 (m, 4H), 1.55 (br s, 2H), 1.08 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.32, 158.30, 129.73, 129.29, 127.79, 121.27, 111.30, 70.03, 38.34, 23.98, 21.89, 20.07, 9.34.

*(2-(Cyclopropylmethoxy)-5-(piperidine-1-carbonyl)phenyl)methanaminium Trifluoroacetate (**17**).* See Procedure D: Step 1:31 mg (0.094 mmol) of **13**, 49 mg (0.094 mmol, 1.01 equiv) of PyBOP, 20 μL (17 mg, 0.20 mmol, 2.2 equiv) of piperidine, 100 μL (92 mg, 0.91 mmol, 9.7 equiv) of NMM, and 4.5 mL of DMF. Step 2:2 mL of dioxane and 0.2 mL conc. HCl. Compound **17** (25 mg, yield = 69%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.45 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 7.12 (d, J = 8.4 Hz, 1H), 4.17 (s, 2H), 4.00 (d, J = 6.9 Hz, 2H), 3.68 (br s, 2H), 3.44 (br s, 2H), 1.72 (br s, 2H), 1.64 (br s, 2H), 1.56 (br s, 2H), 1.42−1.30 (m, 1H), 0.71−0.61 (m, 2H), 0.48−0.35 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.20, 158.30, 129.68, 129.29, 127.96, 121.38, 111.51, 73.30, 38.45, 24.01, 9.50, 2.24.

*(3-(1,2,3,4-Tetrahydroisoquinoline-2-carbonyl)phenyl)-methanaminium Trifluoroacetate (**18**).* See Procedure D: Step 1: 16 mg (0.061 mmol) of **10**, 36 mg (0.069 mmol, 1.13 equiv) of PyBOP, 20 μL (21 mg, 0.16 mmol, 2.6 equiv) of 1,2,3,4-tetrahydroisoquinoline, 80 μL (74 mg, 0.73 mmol, 11.9 equiv) of NMM, and 5 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL of conc. HCl. Compound **18** (17 mg, yield = 73%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, 50 °C, methanol-d_4) δ 7.62−7.41 (m, 5H), 7.17 (s, 3H), 4.77 (br s, 2H), 4.18 (s, 2H), 3.68 (br s, 2H), 2.92 (br s, 2H). ^{13}C NMR (101 MHz, 50 °C, methanol-d_4) δ 170.64,136.84, 133.78, 132.44, 130.11, 129.20, 128.32, 127.16, 127.00, 126.57, 126.18, 42.63.

*(2-Methoxy-5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-phenyl)methanaminium Trifluoroacetate (**19**).* See Procedure D: Step 1: 19 mg (0.065 mmol) of **11**, 34 mg (0.065 mmol, 1.0 equiv) of PyBOP, 40 μL (42 mg, 0.32 mmol, 4.8 equiv) of 1,2,3,4-tetrahydroisoquinoline, 75 μL (69 mg, 0.68 mmol, 10.0 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL of conc. HCl. Compound **19** (23 mg, yield = 86%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, 50 °C, methanol-d_4) δ 7.56 (dd, J = 8.5, 2.2 Hz, 1H), 7.47 (d, J = 2.1 Hz, 1H), 7.20−7.13 (m, 4H), 7.07 (br s, 1H), 4.74 (br s, 2H), 4.15 (s, 2H), 3.98 (s, 3H), 3.80 (br s, 2H), 2.92 (t, J = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, 50 °C, methanol-d_4) δ 159.24, 134.20, 132.60, 130.00, 129.60, 128.31, 128.17, 126.52, 126.14, 121.34, 110.66, 55.13, 38.75.

*(2-Propoxy-5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-phenyl)methanaminium Trifluoroacetate (**20**).* See Procedure D: Step 1: 30 mg (0.094 mmol) of **12**, 49 mg (0.094 mmol, 1.0 equiv) of PyBOP, 20 μL (21 mg, 0.16 mmol, 1.7 equiv) of 1,2,3,4-tetrahydroisoquinoline, 110 μL (101 mg, 1.0 mmol, 10.7 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL of conc. HCl. Compound **20** (33 mg, yield = 80%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, 50 °C, methanol-d_4) δ 7.53 (dd, J = 8.5, 2.2 Hz, 1H), 7.48 (d, J = 2.1 Hz, 1H), 7.21−7.12 (m, 4H), 7.07 (br s, 1H), 4.73 (br s, 2H), 4.17 (s, 2H), 4.14 (t, J = 6.6 Hz, 2H), 3.80 (br s, 2H), 2.92 (br t, J = 6.1 Hz, 2H), 1.90 (h, J = 7.2 Hz, 2H), 1.08 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, 50 °C, Methanol-d_4) δ 158.55, 134.20, 132.61, 129.88, 129.46, 128.31, 127.98, 126.53, 126.14, 121.42, 111.50, 70.23, 38.43, 21.90, 9.22.

*(2-(Cyclopropylmethoxy)-5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)phenyl)methanaminium Trifluoroacetate (**21**).* See Procedure D: Step 1: 28 mg (0.085 mmol) of **13**, 46 mg (0.089 mmol, 1.0 equiv) of PyBOP, 20 μL (21 mg, 0.16 mmol, 1.9 equiv) of 1,2,3,4-tetrahydroisoquinoline, 100 μL (92 mg, 0.91 mmol, 10.8 equiv) of NMM, and 4 mL of DMF. Step 2: 2 mL of dioxane and 0.2 mL of conc. HCl. Compound **21** (30 mg, yield = 79%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, 50 °C, methanol-d_4) δ 7.52 (dd, J = 8.5, 2.1 Hz, 1H), 7.48 (d, J = 1.9 Hz, 1H), 7.20−6.97 (m, 5H), 4.73 (s, 2H), 4.19 (s, 2H), 4.04 (d, J = 7.0 Hz, 2H), 3.80 (s, 2H), 2.92 (t, J = 6.1 Hz, 2H), 1.41−1.29 (m, 1H), 0.77−0.60 (m, 2H), 0.42 (qd, J = 4.6, 2.3 Hz, 2H). ^{13}C NMR (101 MHz, 50 °C, methanol-d_4) δ 158.58, 134.19, 132.61, 129.86, 129.42, 128.31, 128.04, 126.52, 126.14, 121.52, 111.81, 73.45, 38.59, 9.53, 2.22.

*(S)-3-(4-Hydroxy-2,6-dimethylphenyl)-1-oxo-1-((3-(piperidin-1-ylmethyl)benzyl)amino)propan-2-aminium Trifluoroacetate (**A**).* See Procedures E and F: 16 mg (0.048 mmol) of **14**, 170 μL (0.34 mmol, 7.08 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 90 μL (67 mg, 0.52 mmol, 10.7 equiv) of *N*,*N*-diisopropylethylamine, 29 mg (0.056 mmol, 1.16 equiv) of PyBOP, 9 mg (0.053 mmol, 1.10 equiv) of 6-Cl-HOBt, 21 mg (0.051 mmol, 1.07 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mLof DCM. Compound **A** (11.5 mg, yield = 47%) was isolated as a white solid. (MS)EI: 396.3 (M + H). Retention time: 15.67 min. ^{1}H NMR (500 MHz, deuterium oxide) δ 7.95 (t, J = 6.0 Hz, 1H), 7.33−7.18 (m, 2H), 6.96 (s, 1H), 6.90 (d, J = 7.2 Hz, 1H), 6.28 (s, 2H), 4.38−4.28 (m, 1H), 4.13 (d, J = 13.2 Hz, 1H), 4.06 (d, J = 13.2 Hz, 1H), 3.95−3.82 (m, 2H), 3.29 (t, J = 12.4 Hz, 2H), 3.03 (dd, J = 13.9, 11.8 Hz, 1H), 2.94 (dd, J = 14.0, 5.0 Hz, 1H), 2.78 (qd, J = 13.1, 3.0 Hz, 2H),

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1.93 (s, 6H), 1.78 (d, J = 12.7 Hz, 2H), 1.67 (dt, J = 13.4, 3.3 Hz, 1H), 1.60−1.46 (m, 2H), 1.32 (qt, J = 12.6, 3.5 Hz, 1H).

*(S)-3-(4-Hydroxy-2,6-dimethylphenyl)-1-((2-methoxy-5-(piperidin-1-ylmethyl)benzyl)amino)-1-oxopropan-2-aminium Trifluoroacetate (**B**).* See Procedures E and F: 23 mg (0.063 mmol) of **15**, 220 μL (0.44 mmol, 6.93 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 110 μL (82 mg, 0.63 mmol, 9.95 equiv) of *N*,*N*-diisopropylethylamine, 33 mg (0.063 mmol, 1.00 equiv) of PyBOP, 13 mg (0.077 mmol, 1.21 equiv) of 6-Cl-HOBt, 28 mg (0.068 mmol, 1.08 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **B** (9.1 mg, yield = 27%) was isolated as a white solid. 426.3 (M + H). Retention time: 16.13 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.94 (t, J = 6.0 Hz, 1H), 7.41 (dd, J = 8.4, 2.3 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.33 (s, 2H), 4.35 (dd, J = 14.5, 6.8 Hz, 1H), 4.22−4.12 (m, 3H), 3.89 (dd, J = 11.7, 4.4 Hz, 1H), 3.74 (s, 3H), 3.42 (t, J = 12.3 Hz, 2H), 3.12 (dd, J = 13.7, 11.8 Hz, 1H), 2.96−2.84 (m, 3H), 2.04 (s, 6H), 1.93 (d, J = 14.9 Hz, 2H), 1.84 (d, J = 13.7 Hz, 1H), 1.78−1.66 (m, 2H), 1.50 (qt, J = 12.5, 3.7 Hz, 1H).

*(S)-3-(4-Hydroxy-2,6-dimethylphenyl)-1-oxo-1-((5-(piperidin-1-ylmethyl)-2-propoxybenzyl)amino)propan-2-aminium Trifluoroacetate (**C**).* See Procedures E and F: 24 mg (0.062 mmol) of **16**, 220 μL (0.44 mmol, 7.15 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 110 μL (82 mg, 0.63 mmol, 10.3 equiv) of *N*,*N*-diisopropylethylamine, 28 mg (0.061 mmol, 1.00 equiv) of PyBOP, 11 mg (0.065 mmol, 1.05 equiv) of 6-Cl-HOBt, 26 mg (0.063 mmol, 1.03 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **C** (4 mg, yield = 12%) was isolated as a white solid. (MS)EI: 454.3 (M + H). Retention time: 21.64 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.66 (t, J = 5.8 Hz, 1H), 7.39 (dd, J = 8.4, 2.3 Hz, 1H), 7.12 (d, J = 2.3 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.35 (s, 2H), 4.39 (dd, J = 14.7, 6.8 Hz, 1H), 4.24−4.11 (m, 3H), 3.98−3.82 (m, 3H), 3.41 (d, J = 13.6 Hz, 2H), 3.12 (dd, J = 13.7, 11.9 Hz, 1H), 2.98−2.83 (m, 3H), 2.04 (s, 6H), 1.94 (d, J = 10.4 Hz, 2H), 1.84 (d, J = 13.5 Hz, 1H), 1.79−1.65 (m, 4H), 1.50 (qt, J = 12.6, 3.4 Hz, 1H), 1.01 (t, J = 7.4 Hz, 3H).

*(S)-1-((2-(Cyclopropylmethoxy)-5-(piperidin-1-ylmethyl)benzyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**D**).* See Procedures E and F: 25 mg (0.062 mmol) of **17**, 220 μL (0.44 mmol, 7.10 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 110 μL (82 mg, 0.63 mmol, 10.2 equiv) of *N*,*N*-diisopropylethylamine, 32 mg (0.061 mmol, 0.99 equiv) of PyBOP, 12 mg (0.071 mmol, 1.14 equiv) of 6-Cl-HOBt, 25 mg (0.061 mmol, 0.98 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL TFA and 2 mL DCM. Compound **D** (1.8 mg, yield = 5%) was isolated as a white solid. (MS)EI: 466.3 (M + H). Retention time: 22.01 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.63 (t, J = 5.9 Hz, 1H), 7.37 (dd, J = 8.4, 2.3 Hz, 1H), 7.12 (d, J = 2.3 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.34 (s, 2H), 4.42 (dd, J = 14.7, 6.8 Hz, 1H), 4.24−4.13 (m, 3H), 3.90 (dd, J = 11.7, 4.4 Hz, 1H), 3.78 (d, J = 6.9 Hz, 2H), 3.47−3.37 (m, 3H), 3.13 (dd, J = 13.6, 11.9 Hz, 2H), 2.95 (dd, J = 14.1, 4.3 Hz, 2H), 2.93−2.82 (m, 2H), 2.05 (s, 6H), 1.97−1.91 (m, 2H), 1.84 (d, J = 13.6 Hz, 1H), 1.72 (p, J = 12.7 Hz, 2H), 1.51 (dddd, J = 16.9, 13.0, 8.2, 3.7 Hz, 1H), 1.16 (dddd, J = 15.5, 12.3, 7.6, 4.6 Hz, 1H), 0.59 (dd, J = 8.1, 1.8 Hz, 2H), 0.30 (pd, J = 4.2, 2.8 Hz, 2H).

*(S)-1-((3-((3,4-Dihydroisoquinolin-2(1H)-yl)methyl)benzyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**E**).* See Procedures E and F: 11 mg (0.029 mmol) of **18**, 100 μL (0.20 mmol, 6.92 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 50 μL (37 mg, 0.29 mmol, 9.93 equiv) of *N*,*N*-diisopropylethylamine, 17 mg (0.033 mmol, 1.13 equiv) of PyBOP, 5 mg (0.029 mmol, 1.02 equiv) of 6-Cl-HOBt, 12 mg (0.029 mmol, 1.01 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **E** (5.0 mg, yield = 31%) was isolated as a white solid. (MS)EI: 444.3 (M + H). Retention time: 20.66 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.31 (t, J = 5.7 Hz, 1H), 7.44 (d, J = 6.3 Hz, 2H), 7.37−7.25 (m, 3H), 7.20 (s, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.06 (d, J = 6.6 Hz, 1H), 6.45 (s, 2H), 4.50−4.34 (m, 5H), 4.17 (dd, J = 15.1, 4.2 Hz, 1H), 3.89 (dd, J = 11.6, 4.7 Hz, 1H), 3.76 (s, 1H), 3.42 (s, 1H), 3.25−3.14 (m, 3H), 3.01 (dd, J = 13.8, 4.8 Hz, 1H), 2.19 (s, 6H).

*(S)-1-((5-((3,4-Dihydroisoquinolin-2(1H)-yl)methyl)-2-methoxybenzyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**F**).* See Procedures E and F: 22 mg (0.054 mmol) of **19**, 190 μL (0.38 mmol, 7.09 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 100 μL (74 mg, 0.57 mmol, 10.7 equiv) of *N*,*N*-diisopropylethylamine, 28 mg (0.054 mmol, 1.00 equiv) of PyBOP, 10 mg (0.059 mmol, 1.10 equiv) of 6-Cl-HOBt, 23 mg (0.056 mmol, 1.05 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **F** (3.4 mg, yield = 11%) was isolated as a white solid. (MS)EI: 474.3 (M + H). Retention time: 20.56 min. ^{1}H NMR (500 MHz, deuterium oxide) δ 7.39 (dd, J = 8.5, 2.3 Hz, 1H), 7.25−7.19 (m, 1H), 7.19−7.14 (m, 3H), 7.03 (d, J = 7.5 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.11 (s, 2H), 4.32 (d, J = 13.5 Hz, 3H), 4.24 (s, 2H), 3.88 (d, J = 14.1 Hz, 1H), 3.84 (dd, J = 11.6, 5.1 Hz, 1H), 3.52 (s, 5H), 3.07 (t, J = 6.4 Hz, 2H), 2.96−2.81 (m, 2H), 1.76 (s, 6H).

*(S)-1-((5-((3,4-Dihydroisoquinolin-2(1H)-yl)methyl)-2-propoxybenzyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**G**).* See Procedures E and F: 33 mg (0.075 mmol) of **20**, 260 μL (0.52 mmol, 6.93 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 130 μL (96 mg, 0.75 mmol, 9.95 equiv) of *N*,*N*-diisopropylethylamine, 39 mg (0.075 mmol, 1.00 equiv) of PyBOP, 13 mg (0.077 mmol, 1.02 equiv) of 6-Cl-HOBt, 31 mg (0.076 mmol, 1.01 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **G** (7 mg, yield = 15%) was isolated as a white solid. (MS)EI: 502.3 (M + H). Retention time: 26.01 min. ^{1}H NMR (500 MHz, deuterium oxide) δ 7.51 (q, J = 7.5 Hz, 1H), 7.39 (dt, J = 8.6, 3.1 Hz, 1H), 7.27−7.15 (m, 4H), 7.05 (t, J = 7.9 Hz, 1H), 6.92 (dd, J = 8.5, 3.0 Hz, 1H), 6.17 (s, 2H), 4.39−4.19 (m, 6H), 3.99−3.84 (m, 3H), 3.75−3.62 (m, 3H), 3.36−3.25 (m, 1H), 3.13−3.02 (m, 2H), 2.96−2.78 (m, 2H), 1.72 (s, 6H), 1.47 (dtdd, J = 27.3, 14.1, 6.8, 2.1 Hz, 2H), 0.81 (t, J = 7.4 Hz, 3H).

*(S)-1-((2-(Cyclopropylmethoxy)-5-((3,4-dihydroisoquinolin-2(1H)-yl)methyl)benzyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**H**).* See Procedures E and F: 30 mg (0.067 mmol) of **21**, 230 μL (0.46 mmol, 6.91 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of F: 120 μL (89 mg, 0.69 mmol, 10.3 equiv) of *N*,*N*-diisopropylethylamine, 35 mg (0.067 mmol, 1.01 equiv) of PyBOP, 11 mg (0.065 mmol, 0.97 equiv) of 6-Cl-HOBt, 27 mg (0.066 mmol, 0.99 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of F: 2 mL of TFA and 2 mL of DCM. Compound **H** (7.0 mg, yield = 17%) was isolated as a white solid. (MS)EI: 514.3 (M + H). Retention time: 26.36 min. ^{1}H NMR (500 MHz, deuterium oxide) δ 7.63 (q, J = 7.3 Hz, 1H), 7.38 (dq, J = 8.4, 3.1, 2.7 Hz, 1H), 7.28−7.13 (m, 4H), 7.05 (t, J = 8.4 Hz, 1H), 6.89 (dd, J = 8.5, 2.7 Hz, 1H), 6.16 (s, 2H), 4.43−4.17 (m, 5H), 4.00 (dt, J = 13.9, 4.1 Hz, 1H), 3.87 (dt, J = 10.6, 5.2 Hz, 1H), 3.71 (dd, J = 12.3, 5.3 Hz, 1H), 3.56 (d, J = 7.1 Hz, 2H), 3.38−3.25 (m, 1H), 3.18−3.03 (m, 2H), 3.02−2.76 (m, 2H), 1.72 (s, 6H), 0.99−0.85 (m, 1H), 0.52−0.36 (m, 2H), 0.27−0.02 (m, 2H).

*5-(3,4-Dihydroisoquinolin-2(1H)-yl)-5-oxopentan-1-aminium Trifluoroacetate (**22**).* See Procedures G and Ha: 85 mg (0.39 mmol) of 5-((*tert*-butoxycarbonyl)amino)pentanoic acid, 204 mg (0.39 mmol, 1.0 equiv) of PyBOP, 50 μL (53 mg, 0.39 mmol, 1.0 equiv) of 1,2,3,4-tetrahydroisoquinoline, 430 μL (396 mg, 3.9 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−50% EtOAc in hexanes) and used without further purification. Procedure Ha: 5 mL of dioxane and 0.5 mL of conc. HCl were added, and the solution was concentrated in vacuo after 5 min. Compound **22** (99 mg, yield = 73%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.23−7.09 (m, 4H), 4.69 (s, 1H), 4.68 (s, 1H), 3.78 (t, J = 6.0 Hz, 1H), 3.74 (t, J = 6.0 Hz, 1H), 2.93 (p, J = 6.4 Hz, 3H), 2.84 (t, J = 6.0 Hz, 1H), 2.55 (q, J = 6.6 Hz, 2H), 1.76−1.67 (m, 4H). ^{13}C NMR (126 MHz, methanol-d_4) δ 172.33, 172.29, 160.32, 160.02, 134.68, 134.26, 132.96, 132.60, 128.24, 127.98, 126.60, 126.38, 126.18, 126.08, 126.02, 125.81, 117.21, 114.90, 43.94, 43.15, 39.96, 38.96, 32.12, 31.87, 28.82, 27.93, 26.74, 26.70, 21.30.

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*2-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethoxy)ethan-1-aminium Trifluoroacetate (**23**).* See Procedures G and Ha: 94 mg (0.43 mmol) of 2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)acetic acid, 223 mg (0.43 mmol, 1.0 equiv) of PyBOP, 60 μL (63 mg, 0.47 mmol, 1.1 equiv) of 1,2,3,4-tetrahydroisoquinoline, 470 μL (432 mg, 4.3 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM) and used without further purification. Procedure Ha: 5 mL of dioxane and 0.5 of mL conc. HCl were added, and the solution was concentrated in vacuo after 5 min. Compound **23** (121 mg, yield = 81%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.21−7.12 (m, 4H), 4.69 (s, 1H), 4.55 (s, 1H), 4.43 (s, 1H), 4.42 (s, 1H), 3.82−3.74 (m, 3H), 3.59 (t, J = 6.0 Hz, 1H), 3.15 (q, J = 4.5 Hz, 2H), 2.91 (t, J = 6.0 Hz, 1H), 2.85 (t, J = 6.0 Hz, 1H). ^{13}C NMR (101 MHz, methanol-d_4) δ 169.39, 169.35, 161.16, 160.80, 160.44, 160.08, 134.54, 134.14, 132.52, 131.97, 128.26, 127.98, 126.70, 126.50, 126.26, 126.15, 126.03, 125.87, 120.64, 117.75, 114.86, 68.20, 45.19, 43.79, 41.68, 39.89, 39.22, 28.46, 27.74.

*4-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)piperidin-1-ium Hydrochloride (**24**).* See Procedures G and Ha: 75 mg (0.31 mmol) of 2-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)acetic acid, 161 mg (0.31 mmol, 1.0 equiv) of PyBOP, 40 μL (42 mg, 0.32 mmol, 1.0 equiv) of 1,2,3,4-tetrahydroisoquinoline, 340 μL (313 mg, 3.1 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM) and used without further purification. Procedure Ha: 5 mL of dioxane and 0.5 mL of conc. HCl were, added and the solution was concentrated in vacuo after 1 min. Compound **24** (86 mg, yield = 95%) was isolated as an off white amorphous solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.22−7.11 (m, 4H), 4.70 (s, 1H), 4.68 (s, 1H), 3.78 (t, J = 6.0 Hz, 1H), 3.75 (t, J = 5.9 Hz, 1H), 3.37 (ddd, J = 12.8, 6.3, 3.1 Hz, 2H), 3.05−2.94 (m, 2H), 2.92 (t, J = 5.9 Hz, 1H), 2.84 (t, J = 6.0 Hz, 1H), 2.49 (t, J = 7.3 Hz, 2H), 2.14 (ddtt, J = 14.4, 10.7, 6.9, 3.8 Hz, 1H), 2.05−1.95 (m, 2H), 1.55−1.41 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.84, 170.78, 160.78, 160.49, 134.67, 134.24, 132.94, 132.62, 128.26, 127.99, 126.62, 126.38, 126.19, 126.08, 126.03, 125.80, 117.46, 115.14, 43.92, 43.73, 43.71, 43.26, 39.96, 38.72, 38.52, 30.66, 30.63, 28.87, 28.46, 28.42, 27.92.

*1-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)piperazine-1,4-diium Trifluoroacetate (**25**).* See Procedures G and Ha: 78 mg (0.32 mmol) of 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)acetic acid, 172 mg (0.33 mmol, 1.0 equiv) of PyBOP, 50 μL (53 mg, 0.39 mmol, 1.2 equiv) of 1,2,3,4-tetrahydroisoquinoline, 350 μL (322 mg, 3.2 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM) and used without further purification. Procedure Ha: 5 mL of dioxane and 0.5 mL conc. HCl were added and the solution was concentrated in vacuo after 5 min. Compound **25** (156 mg, quantitative yield) was isolated as a white waxy solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.25−7.09 (m, 4H), 4.70 (s, 1H), 4.63 (s, 1H), 4.40 (s, 2H), 3.79 (t, J = 6.1 Hz, 1H), 3.69−3.57 (m, 9H), 2.96 (t, J = 6.0 Hz, 1H), 2.87 (t, J = 6.1 Hz, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 163.34, 163.26, 161.82, 161.54, 161.25, 134.44, 134.05, 132.07, 131.59, 128.26, 128.05, 126.90, 126.70, 126.39, 126.31, 126.07, 125.99, 117.61, 115.29, 57.02, 56.93, 49.41, 49.38, 45.84, 44.05, 42.45, 40.69, 40.31, 28.35, 27.64.

*2-(3,4-Dihydroisoquinolin-2(1H)-yl)-N-methyl-2-oxoethan-1-aminium Trifluoroacetate (**26**).* See Procedures G and Ha: 104 mg (0.55 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine, 288 mg (0.55 mmol, 1.0 equiv) of PyBOP, 70 μL (74 mg, 0.55 mmol, 1.0 equiv) of 1,2,3,4-tetrahydroisoquinoline, 600 μL (552 mg, 5.5 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM) and used without further purification. Procedure Ha: 6 mL of dioxane and 0.6 mL of conc. HCl were added, and the solution was concentrated in vacuo after 5 min. Compound **26** (132 mg, yield = 75%) was isolated as a colorless oil that turned purple overtime. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.23−7.10 (m, 4H), 4.69 (s, 1H), 4.60 (s, 1H), 4.16 (s, 1H), 4.15 (s, 1H), 3.79 (t, J = 6.1 Hz, 1H), 3.63 (t, J = 6.0 Hz, 1H), 2.94 (t, J = 6.0 Hz, 1H), 2.85 (t, J = 6.0 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 164.05, 164.01, 161.03, 160.74, 160.46, 160.16, 134.42, 134.00, 132.18, 131.60, 128.27, 128.05, 126.81, 126.61, 126.33, 126.24, 126.02, 125.92, 119.71, 117.40, 115.09, 112.77, 49.10, 49.00, 45.56, 43.93, 42.14, 40.08, 32.25, 28.34, 27.67.

*tert-Butyl (2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-(methyl)carbamate (**27**).* See Procedure G: 99 mg (0.52 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine, 271 mg (0.52 mmol, 1.0 equiv) of PyBOP, 70 μL (74 mg, 0.55 mmol, 1.1 equiv) of 1,2,3,4-tetrahydroisoquinoline, 580 μL (534 mg, 5.3 mmol, 10 equiv) of NMM, and 4 mL of DMF. The product was purified by column chromatography (0−50% EtOAc in hexanes), yielding compound **27** (152 mg, yield = 95%) as a colorless oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.22−7.02 (m, 4H), 4.71 (d, J = 9.6 Hz, 1H), 4.57 (s, 1H), 4.13 (d, J = 6.2 Hz, 1H), 4.05 (s, 1H), 3.84−3.77 (m, 1H), 3.67−3.59 (m, 1H), 2.92 (d, J = 5.4 Hz, 3H), 2.85 (dt, J = 15.7, 5.5 Hz, 2H), 1.46 (s, 6H), 1.39 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 167.55, 167.46, 156.22, 134.87, 133.97, 133.23, 132.16, 129.01, 128.85, 128.28, 126.94, 126.62, 126.57, 126.36, 126.07, 125.96, 79.95, 51.02, 50.56, 50.44, 46.29, 44.36, 42.43, 39.93, 35.46, 29.30, 28.36, 28.29.

*tert-Butyl (2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-carbamate (**28**).* See Procedure G: 91 mg (0.52 mmol) of (*tert*-butoxycarbonyl)glycine, 270 mg (0.52 mmol, 1.0 equiv) of PyBOP, 70 μL (74 mg, 0.55 mmol, 1.1 equiv) of 1,2,3,4-tetrahydroisoquinoline, 570 μL (524 mg, 5.2 mmol, 10 equiv) of NMM, and 4 mL of DMF. The product was purified by column chromatography (0−66% EtOAc in hexanes), yielding compound **28** (136 mg, yield = 90%) as a yellow oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.22−7.03 (m, 4H), 5.59 (s, 1H), 4.71 (s, 1H), 4.52 (s, 1H), 4.02 (dd, J = 8.5, 4.4 Hz, 2H), 3.81 (t, J = 6.0 Hz, 1H), 3.58 (t, J = 5.9 Hz, 1H), 2.86 (dt, J = 22.9, 6.0 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 167.29, 155.83, 134.70, 133.80, 132.83, 131.68, 128.84, 128.30, 127.12, 126.75, 126.70, 126.55, 126.53, 126.11, 79.61, 45.93, 44.38, 42.64, 42.48, 42.03, 40.06, 29.09, 28.34, 28.29.

*tert-Butyl (2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)(methyl)-carbamate (**29**).* See Procedure E: 48 mg (0.16 mmol) of **27**, 550 μL (1.1 mmol, 7.0 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL THF. The concentrated product was purified by column chromatography (0−33% EtOAc in hexanes), yielding compound **29** (30 mg, yield = 66%) as a colorless oil. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.26−7.13 (m, 3H), 7.02 (d, J = 7.1 Hz, 1H), 4.23 (d, J = 15.6 Hz, 1H), 3.96−3.58 (m, 3H), 3.35−2.78 (m, 9H), 1.40 (s, 4H), 1.34 (s, 5H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 129.75, 128.59, 127.60, 127.36, 127.00, 126.78, 80.00, 79.82, 62.10, 61.03, 54.96, 54.79, 54.10, 53.87, 44.29, 43.94, 35.01, 34.70, 28.32, 24.04.

*2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethan-1-aminium Trifluoroacetate (**30**).* Procedure Ha: 54 mg (0.19 mmol) of **28**, 5 mL of dioxane and 0.5 mL of conc. HCl were added, and the solution was concentrated in vacuo after 5 min. Compound **30** (32 mg, yield = 57%) was isolated as a pinkish white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.24−7.10 (m, 4H), 4.69 (s, 1H), 4.60 (s, 1H), 4.04 (s, 1H), 4.03 (s, 1H), 3.78 (t, J = 6.1 Hz, 1H), 3.64 (t, J = 6.0 Hz, 1H), 2.94 (t, J = 6.0 Hz, 1H), 2.86 (t, J = 6.1 Hz, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 164.95, 164.90, 161.74, 161.46, 161.18, 160.89, 134.48, 134.04, 132.13, 131.57, 128.28, 128.07, 126.91, 126.69, 126.40, 126.32, 126.08, 125.98, 119.96, 117.64, 115.32, 113.00, 45.64, 44.15, 42.23, 40.39, 28.33, 27.66.

*2-((2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)(methyl)-amino)-2-oxoethan-1-aminium Trifluoroacetate (**31**).* See Procedures G and Ha: 47 mg (0.15 mmol) of **26**, 80 mg (0.15 mmol, 1.0 equiv) of PyBOP, 29 mg (0.17 mmol, 1.1 equiv) of N-(tert-butoxycarbonyl)glycine, 170 μL (156 mg, 1.5 mmol, 10 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM). Procedure Ha: 5 mL of dioxane and 0.5 mL conc. of HCl were added, and the solution was concentrated in vacuo after 5 min. Compound **31** (24 mg, yield = 43%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.23−7.12 (m, 4H), 4.71−4.66 (m, 2H), 4.45−4.40 (m, 2H), 4.00 (s, 2H), 3.79 (q, J = 6.0, 5.6 Hz, 1H), 3.71 (td, J = 6.0, 1.8 Hz, 1H), 3.11−3.02 (m, 3H), 2.98−2.96 (m, 1H), 2.90−2.81 (m, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 167.14, 166.71, 160.08, 159.78, 134.59, 134.22, 133.52, 132.63, 128.81, 128.23, 127.97, 127.93, 127.26, 126.68, 126.58, 126.50, 126.30, 126.24, 126.15, 126.00, 125.89, 117.07, 114.76, 55.11,

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49.47, 49.32, 45.76, 44.24, 44.08, 42.29, 42.23, 41.87, 40.16, 39.64, 39.47, 34.68, 34.63, 34.27, 28.49, 27.82, 27.25.

*2-(3,4-Dihydroisoquinolin-2(1H)-yl)-N-methylethan-1-aminium Trifluoroacetate (**32**).* See Procedure Ha: 30 mg (0.16 mmol) of **29**, 5 mL of dioxane, and 0.5 mL of conc. HCl were added, and the solution was concentrated in vacuo after 5 min. The residue was then partitioned between EtOAc and sat. Na_2CO_3 and then extracted with EtOAc. The organic layers were then combined, dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was suspended in solvent A, concentrated in vacuo, and triturated with hexanes. Compound **32** (28 mg, yield = 89%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.33−7.25 (m, 3H), 7.22−7.18 (m, 1H), 4.53 (s, 2H), 3.71−3.63 (m, 4H), 3.63−3.53 (m, 2H), 3.24 (t, J = 6.4 Hz, 2H), 2.82 (s, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 130.47, 128.46, 128.18, 128.15, 127.09, 126.93, 126.88, 126.43, 126.38, 53.31, 53.24, 52.14, 50.94, 50.47, 50.22, 49.57, 42.85, 42.24, 32.58, 27.17, 24.88.

*2-((2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)amino)-2-oxoethan-1-aminium Trifluoroacetate (**33**).* See Procedures E, G, and Hb: 24 mg (0.079 mmol) of **30**, 280 μL (0.56 mmol, 7.1 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL THF. Procedure G: 43 mg (0.083 mmol, 1.0 equiv) of PyBOP, 14 mg (0.080 mmol, 1.0 equiv) of *N*-(*tert*-butoxycarbonyl)glycine, 90 μL (83 mg, 0.82 mmol, 10 equiv) of NMM, and 4 mL of DMF. Procedure Hb: 2 mL of TFA and 2 mL of DCM were added, and the solution was concentrated in vacuo after 1 min. The residue was then purified by reversed-phase chromatography. Compound **33** (16 mg, yield = 58%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.35−7.23 (m, 3H), 7.20 (d, J = 7.2 Hz, 1H), 4.55 (br s, 2H), 3.99−3.68 (m, 5H), 3.65−3.40 (m, 3H), 3.22 (s, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 167.41, 160.53, 160.24, 159.94, 130.59, 128.43, 128.09, 127.21, 126.85, 126.41, 117.24, 114.93, 55.08, 53.22, 49.88, 40.13, 33.96, 24.78.

*(S)-1-((5-(3,4-Dihydroisoquinolin-2(1H)-yl)pentyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**I**).* See Procedures E and F: 30 mg (0.087 mmol) of **22**, 300 μL (0.60 mmol, 6.9 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of Procedure F: 150 μL (111 mg, 0.86 mmol, 10 equiv) of *N*,*N*-diisopropylethylamine, 45 mg (0.086 mmol, 1.0 equiv) of PyBOP, 16 mg (0.094 mmol, 1.1 equiv) of 6-Cl-HOBt, 38 mg (0.093 mmol, 1.1 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **I** (13.1 mg, Yield = 29%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.80 (t, J = 5.7 Hz, 1H), 7.36−7.24 (m, 3H), 7.22 (d, J = 7.4 Hz, 1H), 6.51 (s, 2H), 4.60 (dd, J = 14.5, 7.7 Hz, 1H), 4.32 (t, J = 14.4 Hz, 1H), 3.90−3.75 (m, 2H), 3.40 (dd, J = 25.6, 15.6 Hz, 1H), 3.31−3.10 (m, 9H), 3.00 (dd, J = 13.8, 4.8 Hz, 1H), 2.97−2.87 (m, 1H), 2.27 (s, 6H), 1.81−1.65 (m, 2H), 1.35 (dp, J = 9.1, 6.5 Hz, 2H), 1.05 (dtt, J = 21.2, 13.2, 6.7 Hz, 2H). (MS)EI: 410.3 (M + H). Retention time: 19.34 min.

*(S)-1-((2-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethoxy)ethyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**J**).* See Procedures E and F: 41 mg (0.12 mmol) of **23**, 420 μL (0.84 mmol, 7.1 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of Procedure F: 210 μL (156 mg, 1.2 mmol, 10 equiv) of *N*,*N*-diisopropylethylamine, 63 mg (0.12 mmol, 1.0 equiv) of PyBOP, 21 mg (0.12 mmol, 1.0 equiv) of 6-Cl-HOBt, 50 mg (0.12 mmol, 1.0 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **J** (11.1 mg, yield = 18%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.34−7.25 (m, 3H), 7.21 (d, J = 7.5 Hz, 1H), 6.50 (s, 2H), 4.56 (s, 1H), 4.37 (s, 1H), 3.89 (dd, J = 11.4, 4.9 Hz, 1H), 3.83−3.72 (m, 2H), 3.69 (ddd, J = 11.7, 7.7, 4.2 Hz, 1H), 3.50−3.34 (m, 5H), 3.29−3.16 (m, 4H), 3.01 (dd, J = 13.8, 4.9 Hz, 1H), 2.26 (s, 6H). (MS)EI: 413.3 (M + H). Retention time: 17.78 min.

*(S)-1-((2-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-oxoethoxy)ethyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**K**).* See Procedure F: Step 1: 36 mg (0.10 mmol) of **23**, 180 μL (134 mg, 1.0 mmol, 10 equiv) of *N*,*N*-diisopropylethylamine, 56 mg (0.11 mmol, 1.0 equiv) of PyBOP, 19 mg (0.11 mmol, 1.1 equiv) of 6-Cl-HOBt, 44 mg (0.11 mmol, 1.0 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2: 2 mL of TFA and 2 mL of DCM. Compound **K** (15.1 mg, yield = 27%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.14 (dt, J = 22.7, 5.2 Hz, 1H), 7.23−7.11 (m, 4H), 6.46 (s, 1H), 6.43 (s, 1H), 4.67 (s, 1H), 4.55 (d, J = 2.7 Hz, 1H), 4.16 (qd, J = 15.0, 6.9 Hz, 2H), 3.85 (dd, J = 11.5, 4.9 Hz, 1H), 3.83−3.70 (m, 1H), 3.63−3.54 (m, 1H), 3.54−3.48 (m, 1H), 3.37−3.31 (m, 1H), 3.28−3.15 (m, 3H), 3.04−2.83 (m, 3H), 2.25 (s, 2H), 2.24 (s, 4H). (MS)EI: 427.3 (M + H). Retention time: 26.81 min.

*(S)-1-(4-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)piperidin-1-yl)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**L**).* See Procedures E and F: 25 mg (0.067 mmol) of **24**, 250 μL (0.50 mmol, 7.4 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of Procedure F: 120 μL (89 mg, 0.69 mmol, 10 equiv) of *N*,*N*-diisopropylethylamine, 35 mg (0.067 mmol, 1.0 equiv) of PyBOP, 13 mg (0.077 mmol, 1.1 equiv) of 6-Cl-HOBt, 29 mg (0.071 mmol, 1.1 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **L** (2.8 mg, yield = 8%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.37−7.15 (m, 4H), 6.56 (s, 1H), 6.49 (s, 1H), 4.66−4.44 (m, 3H), 4.38−4.20 (m, 1H), 3.79 (s, 1H), 3.28−3.10 (m, 5H), 3.10−3.00 (m, 1H), 2.87 (td, J = 12.9, 2.6 Hz, 1H), 2.50 (dtd, J = 44.8, 13.0, 2.4 Hz, 1H), 2.29 (s, 4H), 2.23 (s, 2H), 1.73 (dd, J = 33.7, 13.1 Hz, 2H), 1.67−1.38 (m, 3H), 1.21 (d, J = 13.2 Hz, 1H), 1.17−1.00 (m, 1H), 0.84 (qd, J = 13.0, 4.7 Hz, 1H), −0.30 − −0.63 (m, 1H). (MS)EI: 436.3 (M + H). Retention time: 20.44 min.

*(S)-1-(4-(2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)piperazin-1-yl)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**M**).* See Procedures E and F: 70 mg (0.14 mmol) of **25**, 720 μL (1.4 mmol, 10 equiv) of 2 M BH_3*Me_2S in THF, and 5 mL of THF. Step 1 of Procedure F: 250 μL (186 mg, 1.4 mmol, 10 equiv) of *N*,*N*-diisopropylethylamine, 76 mg (0.15 mmol, 1.0 equiv) of PyBOP, 25 mg (0.15 mmol, 1.0 equiv) of 6-Cl-HOBt, 59 mg (0.14 mmol, 1.0 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 2.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **M** (2.9 mg, yield = 3.7%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.35−7.23 (m, 3H), 7.22−7.12 (m, 1H), 6.52 (s, 2H), 4.53 (dd, J = 12.1, 4.4 Hz, 1H), 4.46 (s, 2H), 3.66 (d, J = 13.4 Hz, 1H), 3.60 (t, J = 6.4 Hz, 2H), 3.54 (t, J = 10.5 Hz, 1H), 3.39 (t, J = 6.1 Hz, 2H), 3.24−3.17 (m, 3H), 3.16−3.10 (m, 1H), 3.07 (dd, J = 13.8, 4.4 Hz, 1H), 2.80 (q, J = 6.4 Hz, 2H), 2.69−2.57 (m, 2H), 2.43−2.33 (m, 2H), 2.26 (s, 6H), 1.64−1.52 (m, 1H). (MS)EI: 437.3 (M+H). Retention time: 17.60 min.

*(S)-1-((2-((2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)(methyl)amino)ethyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**N**).* See Procedures E and F: 24 mg (0.064 mmol) of **31**, 300 μL (0.60 mmol, 9.4 equiv) of 2 M BH_3*Me_2S in THF, and 4 mL of THF. Step 1 of Procedure F: 110 μL (82 mg, 0.63 mmol, 9.9 equiv) of *N*,*N*-diisopropylethylamine, 35 mg (0.67 mmol, 1.1 equiv) of PyBOP, 13 mg (0.077 mmol, 1.2 equiv) of 6-Cl-HOBt, 28 mg (0.068 mmol, 1.1 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **N** (4.9 mg, yield = 14%) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.34−7.25 (m, 3H), 7.20 (d, J = 7.4 Hz, 1H), 6.51 (s, 2H), 4.49 (s, 2H), 3.84 (dd, J = 11.3, 5.1 Hz, 1H), 3.73−3.58 (m, 6H), 3.57−3.47 (m, 1H), 3.29−3.18 (m, 4H), 3.14 (dt, J = 11.9, 5.7 Hz, 1H), 3.01 (ddd, J = 20.8, 13.6, 6.0 Hz, 2H), 2.83 (s, 3H), 2.25 (s, 6H). (MS)EI: 425.3 (M+H). Retention time: 16.72 min.

*(S)-1-((2-((2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)(methyl)amino)-2-oxoethyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**O**).* See Procedures G, Hb, and F: 28 mg (0.092 mmol) of **32**, 47 mg (0.0.90 mmol, 0.98 equiv) of PyBOP, 18 mg (0.10 mmol, 1.1 equiv) of *N*-(*tert*-butoxycarbonyl)-glycine, 100 μL (92 mg, 0.91 mmol, 9.9 equiv) of NMM, and 4 mL of DMF. The intermediate was purified by column chromatography (0−5% MeOH in DCM), and 25 mg of intermediate was isolated as a crude mixture with tri(pyrrolidin-1-yl)phosphine oxide (approximately 2.5:1 phosphine oxide:intermediate by NMR). No repurification was performed due to poor UV absorbance. Procedure Hb: 25 mg (approximately 0.029 mmol) of crude, 2 mL of TFA, and 2 mL of DCM were added, and the solution was concentrated in vacuo after 5 min. No purification by reversed-phase chromatography was performed. Step 1 of Procedure F: 50 μL (37 mg, 0.29 mmol, 1.0 equiv) of *N*,*N*-

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diisopropylethylamine, 16 mg (0.031 mmol, 1.1 equiv) of PyBOP, 7 mg (0.041 mmol, 1.4 equiv) of 6-Cl-HOBt, 13 mg (0.32 mmol, 1.1 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL of TFA and 2 mL of DCM. Compound **O** (10.1 mg, yield = 20% over four steps) was isolated as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.35−7.22 (m, 3H), 7.19 (d, J = 7.4 Hz, 1H), 6.48 (s, 2H), 4.67 (s, 1H), 4.42 (s, 1H), 4.16−3.74 (m, 6H), 3.60−3.41 (m, 3H), 3.25−3.14 (m, 3H), 3.05 (s, 3H), 3.01 (dd, J = 14.1, 5.3 Hz, 1H), 2.24 (s, 6H). (MS)EI: 439.3 (M + H). Retention time: 16.60 min.

*(S)-1-((2-((2-(3,4-Dihydroisoquinolin-2(1H)-yl)ethyl)amino)-2-oxoethyl)amino)-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropan-2-aminium Trifluoroacetate (**P**).* See Step 1 of Procedure F: 15 mg (0.043 mmol) of **33**, 80 μL (59 mg, 0.46 mmol, 11 equiv) of *N*,*N*-diisopropylethylamine, 23 mg (0.044 mmol, 1.0 equiv) of PyBOP, 7 mg (0.041 mmol, 0.96 equiv) of 6-Cl-HOBt, 19 mg (0.046 mmol, 1.1 equiv) of Boc-O-Boc-L-2′,6′-dimethyltyrosine, 3 + 1.5 mL of DMF. Step 2 of Procedure F: 2 mL TFA and 2 mL DCM. Compound **P** (7.3 mg, yield = 31%) was isolated as a white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.38−7.24 (m, 3H), 7.21 (d, J = 6.6 Hz, 1H), 6.49 (s, 2H), 4.62 (s, 1H), 4.41 (s, 1H), 4.03−3.83 (m, 3H), 3.69 (s, 2H), 3.56 (d, J = 16.8 Hz, 1H), 3.41 (t, J = 5.8 Hz, 3H), 3.27−3.14 (m, 3H), 3.02 (dd, J = 14.0, 5.2 Hz, 1H), 2.24 (s, 6H). (MS)EI: 425.3 (M + H). Retention time: 16.30 min.

In Vitro Pharmacology. *Cell Lines and Membrane Preparations.* All tissue culture reagents were purchased from Gibco Life Sciences (Grand Island, NY) unless otherwise noted. Chinese hamster ovary (CHO) cells stably expressing human MOR (CHO-MOR), DOR (CHO-DOR), or KOR (CHO-KOR) (a generous gift from Larry Toll) were used for all in vitro assays. Cells were grown to confluence at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM-F12) containing 10% fetal bovine serum and 5% penicillin/streptomycin. Membranes were prepared by washing confluent cells three times with ice cold phosphate buffered saline (0.9% NaCl, 0.61 mM Na_2HPO_4, 0.38 mM KH_2PO_4, pH 7.4). Cells were detached from the plates by incubation in warm harvesting buffer (20 mM HEPES, 150 mM NaCl, 0.68 mM EDTA, pH 7.4) and pelleted by centrifugation at 1600 rpm for 3 min. The cell pellet was suspended in ice-cold 50 mM Tris-HCl buffer, pH 7.4, and homogenized with a Tissue Tearor (Biospec Products, Inc., Bartlesville, OK) for 20 s. The homogenate was centrifuged at 15 000 rpm for 20 min at 4 °C. The pellet was rehomogenized in 50 mM Tris-HCl with a Tissue Tearor for 10 s, followed by recentrifugation. The final pellet was resuspended in 50 mM Tris-HCl and frozen in aliquots at −80 °C. Protein concentration was determined via a BCA protein assay (Thermo Scientific Pierce, Waltham, MA) using bovine serum albumin as the standard.

Radioligand Competition Binding Assays. Radiolabeled compounds were purchased from PerkinElmer (Waltham, MA). Opioid ligand binding assays were performed by competitive displacement of 0.2 nM [^{3}H]-diprenorphine (250 μCi, 1.85 TBq/mmol) by the peptidomimetic from membrane preparations containing opioid receptors as described above. The assay mixture, containing membranes (20 μg protein/tube) in 50 mM Tris-HCl buffer (pH 7.4), 0.2 nM [^{3}H]-diprenorphine and various concentrations of test peptidomimetic, was incubated at room temperature on a shaker for 1 h to allow binding to reach equilibrium. The samples were rapidly filtered through Whatman GF/C filters using a Brandel harvester (Brandel, Gaithersburg, MD) and washed three times with 50 mM Tris-HCl buffer, pH 7.4. Bound radioactivity on dried filters was determined by liquid scintillation counting, after saturation with EcoLume liquid scintillation cocktail, in a MicroBeta 2450 (PerkinElmer, Waltham, MA). Nonspecific binding was determined using 10 μM naloxone. The results presented are the mean ± standard error (SEM) from at least three separate assays performed in duplicate. K_i (nM) values were calculated using nonlinear regression analysis to fit a logistic equation to the competition data using GraphPad Prism, ver. 8.4 (GraphPad Software Inc., La Jolla, CA).

[^{35}S]-GTPγS Binding Assays. Agonist stimulation of [^{35}S]guanosine 5′-*O*-[γ-thio]triphosphate ([^{35}S]-GTPγS, 1250 Ci, 46.2 TBq/mmol) binding to G protein was measured as described previously.[38] Briefly, membranes (10 μg of protein/well) were incubated for 1 h at 25 °C in GTPγS buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4) containing 0.1 nM [^{35}S]-GTPγS, 30 μM guanosine diphosphate (GDP), and varying concentrations of test peptidomimetic. G protein activation following receptor activation by peptidomimetic was compared with 10 μM of the standard compounds [D-Ala2,*N*-MePhe4,Gly-ol]enkephalin (DAMGO) at MOR, D-Pen2,5-enkephalin (DPDPE) at DOR, or U69,593 at KOR. The reaction was terminated by vacuum filtration through GF/C filters that were washed five times with GTPγS buffer. Bound radioactivity was measured as described above. The results are presented as the mean ± standard error (SEM) from at least three separate assays performed in duplicate; potency (EC_{50} (nM)) and percent stimulation were determined using nonlinear regression analysis with GraphPad Prism, as above.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.0c00693.

Molecular formula strings (XLSX)

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Author Contributions

Synthesis was carried out by S.P.H. and C.E.L. The research project was designed by S.P.H. and H.I.M. In vitro assays were carried out by J.P.A., J.J.T., D.M.M., and A.C.B., and supervised by J.R.T. This manuscript was written through contributions by all authors.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

BCA, bicinchoninic acid; BH_3*Me_2S, borane-dimethyl sulfide complex; CHO, Chinese hamster ovary; 6-Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DAMGO, [D-Ala^2-*N*-Me-Phe^4-Gly-ol]-enkephalin; DI, deionized; DiBocDMT, N-Boc-O-Boc-2′,6′-dimethyl-L-tyrosine; DIEA, N,N-diisopropylethylamine; DMEM, Dulbecco's modified Eagle's medium; DMT, 2′,6′-dimethyl-L-tyrosine; DNS, does not stimulate; DOR, δ-opioid receptor; DPDPE, [D-Pen^2-D-Pen^5]enkephalin; EtBr, ethyl bromide; GTPγS, guanosine 5′-*O*-[γ-thio]triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KOR, κ-opioid receptor; MLM, mouse liver microsome; MOR, μ-opioid receptor; NLX, naloxone; NMM, *N*-methyl morpholine; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; SEM, standard error of the mean; THIQ, 1,2,3,4-tetrahydroisoquinoline; THQ, 1,2,3,4-tetrahydroquinoline

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