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Article

Evaluation of Amide Bioisosteres Leading to 1,2,3-Triazole Containing Compounds as GPR88 Agonists: Design, Synthesis, and Structure−Activity Relationship Studies

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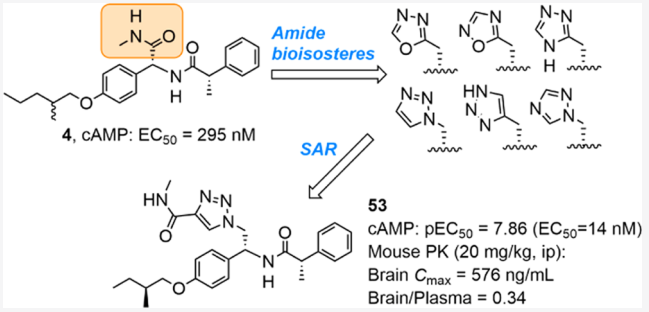
ABSTRACT: The orphan receptor GPR88 has been implicated in a number of striatal-associated disorders, yet its endogenous ligand has not been discovered. We have previously reported that the amine functionality in the 2-AMPP-derived GPR88 agonists can be replaced with an amide (e.g., **4**) without losing activity. Later, we have found that the amide can be replaced with a bioisosteric 1,3,4-oxadiazole with improved potency. Here, we report a further study of amide bioisosteric replacement with a variety of azoles containing three heteroatoms, followed by a focused structure−activity relationship study, leading to the discovery of a series of novel 1,4-disubstituted 1*H*-1,2,3-triazoles as GPR88 agonists. Collectively, our medicinal chemistry efforts have resulted in a potent, efficacious, and brain-penetrant GPR88 agonist **53** (cAMP EC_{50} = 14 nM), which is a suitable probe to study GPR88 functions in the brain.



INTRODUCTION

GPR88 is a class A G-protein-coupled receptor (GPCR) for which the endogenous ligand is yet to be discovered.[1] Since its discovery two decades ago, the GPR88 receptor has evoked significant interest due to its predominant expression in both striatonigral and striatopallidal pathways of the striatum.[2] GPR88 transcriptional profiling[3−7] and genetic knockout (KO)[8−19] studies have provided evidence of GPR88's involvement in a variety of striatal-associated disorders, including Parkinson's disease, schizophrenia, and drug addiction. For example, GPR88's expression in rodents was altered upon their exposure to antipsychotic,[3,4] antidepressant,[5] or drugs of abuse.[6] Additionally, GPR88 KO studies found its key role as a regulator of the dopaminergic system that linked this receptor to the motor-control and reward system.[8,9] In this regard, there is a direct link between GPR88 and alcohol addiction as GPR88 KO mice were prone to higher voluntary alcohol intake compared to the wild-type (WT) mice, while their consumptions of water and palatability were intact.[11] Furthermore, human genetic studies revealed that the GPR88 gene is linked to schizophrenia and several childhood cognitive genetic disorders including speech delay, learning disabilities, and chorea.[20,21] Collectively, these research studies demonstrate that GPR88 is a novel drug target that can potentially meet current unmet demands for treating various neuropsychiatric and neurodegenerative diseases.

Despite the continuous efforts and progress on multiple fronts, GPR88 has not been deorphanized to date.[22] In this vein, our medicinal chemistry campaign to understand the signaling mechanism and biological functions of GPR88 showed that 2-PCCA [(1*R*,2*R*)-2-(pyridin-2-yl)cyclopropane carboxylic acid ((2*S*,3*S*)-2-amino-3-methylpentyl)-(4′-propyl-biphenyl-4-yl)amide, **1**, Figure 1] can activate GPR88 via a $G\alpha_i$-coupled signaling pathway in the functional cAMP assay in GPR88 overexpressing CHO cells.[23,24] Afterward, our systematic modifications of the 2-PCCA scaffold have resulted in the discovery of a brain-penetrant agonist, RTI-13951-33 (**2**), that enabled *in vivo* studies of GPR88 in operant alcohol self-administration in rats, demonstrating that GPR88 agonists are potential drug candidates for the treatment of alcohol addiction.[25] Other than the 2-PCCA scaffold, the 2-AMPP [(2*S*)-*N*-((1*R*)-2-amino-1-(4-(2-methylpentyloxy)phenyl)-ethyl)-2-phenylpropanamide (**3**, Figure 1)] scaffold has also shown promise for GPR88 agonist development.[15,26,27] It has been illustrated that the 2-AMPP scaffold can tolerate

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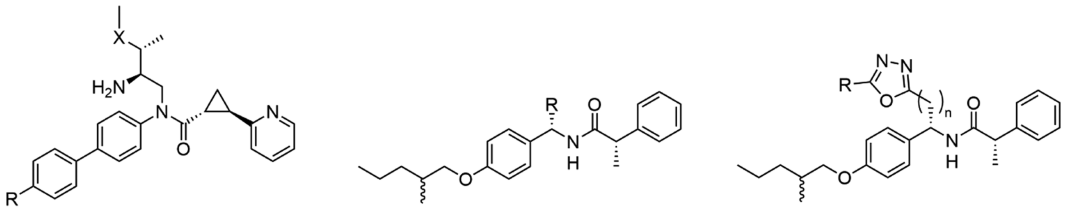
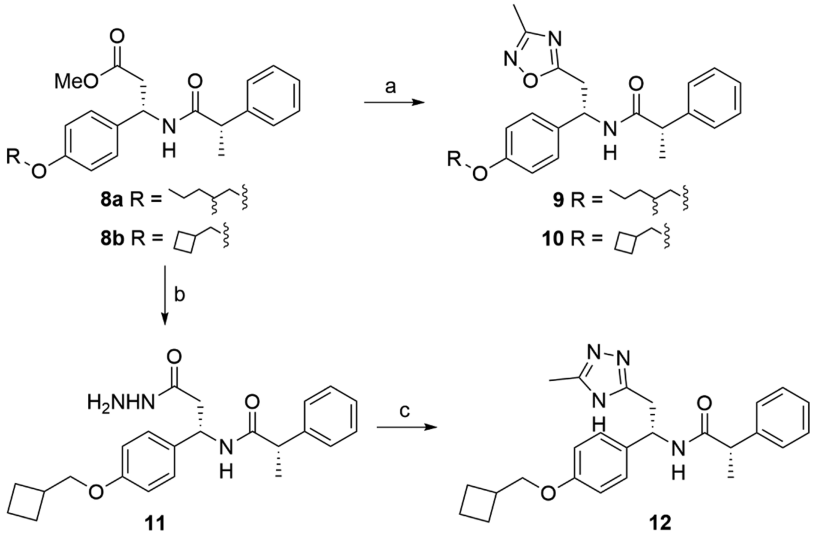


Figure 1. Selected GPR88 agonists 2-PCCA (**1**), RTI-13951-33 (**2**), 2-AMPP (**3**), and **4**–**7** and their cAMP EC_{50} values.

Scheme 1. Synthesis of Target Compounds 9, 10, and 12[a]



[a]Reagents and conditions: (a) NaH, *N*′-hydroxyethanimidamide, tetrahydrofuran (THF), 0 °C to rt, 4 h; (b) hydrazine, EtOH, reflux, 3 h; (c) CH_3CN, K_2CO_3, *n*-BuOH, 150 °C, 5 h.

modifications at three distinct sites and the amino group can be replaced by amide (**4**), among other functional groups, with improved potency.[26,28] Recently, we have reported our rational modifications of **4** by investigating the structure–activity relationship (SAR) at the alkoxy side chain and the *N*-methyl amide functionality.[28] Although replacement of the *N*-methyl amide group with a bioisosteric 1,3,4-oxadiazole moiety provided **5** with a moderate potency, notable improvement in potency was achieved upon the addition of a methylene linker between the oxadiazole moiety and the benzylic carbon (e.g., **6** and **7**, Figure 1). 5-Amino-1,3,4-oxadiazole **7** had an EC_{50} of 59 nM in the cAMP assay using CHO-GPR88 cells, which is 5-fold more potent than **4**. Additionally, **7** had GPR88-specific agonist signaling activity when tested in the [^{35}S]GTPγS binding assay using striatal membranes prepared from WT mice and GPR88 KO mice.[28] However, *in vitro* ADME and pharmacokinetic (PK) assessments revealed that 1,3,4-oxadiazole analogues have poor aqueous solubility and limited blood–brain barrier (BBB) permeability, thus requiring further optimization to improve druglike properties. It is worthy to note that **7** has high lipophilicity (clog *P* = 4.2) and polar surface area (PSA = 103.3), both of which are above the recommended threshold (CNS drugs usually have clog *P* in the range 2–4[29] and PSA less than 90 Å^2).[30] Given that both potency and brain bioavailability continue to be the major challenges for developing an *in vivo* agonist probe for the GPR88 receptor, we planned to further modify the 2-AMPP scaffold by fine-tuning potency and physicochemical properties, such as clog *P* and PSA, to overcome the aforementioned challenges.

Bioisosteric replacement continues to prevail as an indispensable tool in medicinal chemists' toolbox.[31] Due to the prevalence of amide functionality in a wide range of chemical probes and drugs, amide chemistry engenders tremendous interest to medicinal chemists. Further, due to the enzymatic lability of the amide functional group *in vivo*, the improvement of metabolic stability of this moiety using a number of bioisosteric replacements has been documented.[31,32] In addition to our initially explored 1,3,4-oxadiazoles, several other heterocycles such as 1,2,4-oxadiazole, 1,2,4-triazole, 1,2,3-triazole, and tetrazole have also been shown to perform as amide-mimetics.[31]

Both 1,2,4- and 1,2,3-triazoles are well documented as nonclassical five-membered heterocyclic amide bioisosteres, although application of 1,2,3-triazoles is much more prevalent.[31,33] Depending on the substitution pattern, 1,2,3-triazoles can mimic both *trans*- and *cis*-amide bonds.[34] Because 1,4-disubstituted 1,2,3-triazoles imitate the *trans*-amide bond, their applications as amide replacements are much more widespread than the 1,5-disubstituted 1,2,3-triazoles that mimic the *cis*-amide.[31] The 1,2,3-triazoles can be easily synthesized via "click reaction" of the readily accessible alkynes and azides using mild reaction conditions.[35–38] In addition, the 1,2,3-triazole motif is capable of forming hydrogen-bond, dipole–dipole or π-

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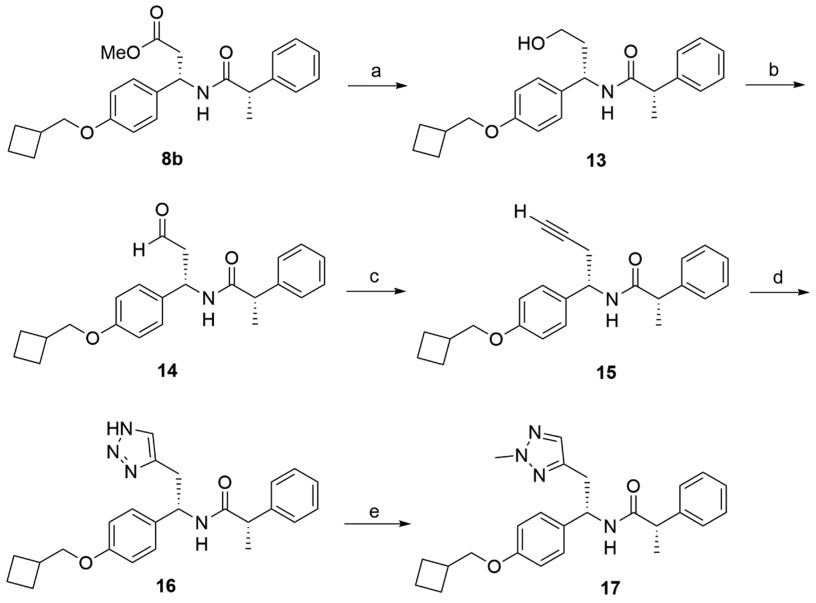
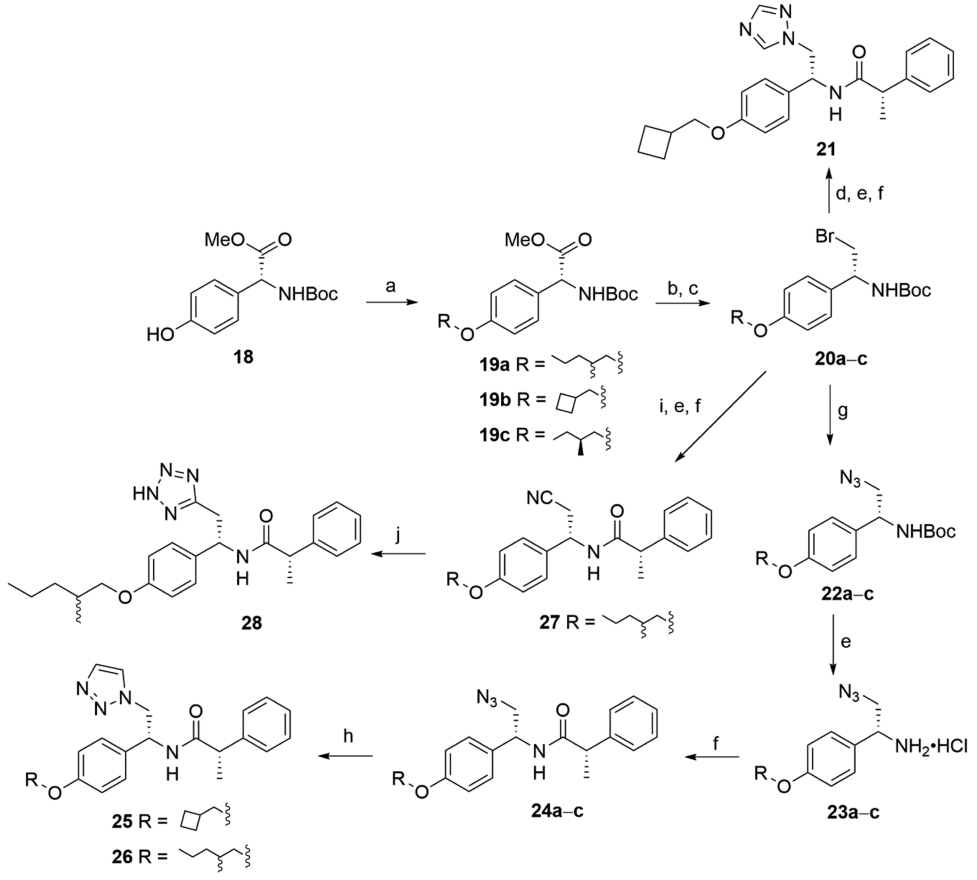
Scheme 2. Synthesis of Target Compounds 16 and 17[a]



[a]Reagents and conditions: (a) $NaBH_4$, LiCl, THF, 0 °C to rt, 3 h; (b) DMP, DCM, rt, 3 h; (c) dimethylacetyldiazophosphonoacetate, K_2CO_3, MeOH, rt, 3 h; (d) TMS−N_3, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *N*,*N*-dimethylformamide (DMF)/H_2O (9:1), 90 °C, 5 h; (e) CH_3I, K_2CO_3, DMF, rt, overnight.

Scheme 3. Synthesis of Target Compounds 21, 25, 26, and 28[a]



[a]Reagents and conditions: (a) PPh_3, diethyl azodicarboxylate (DEAD), alcohol, THF, rt, overnight; (b) $NaBH_4$, LiCl, EtOH/THF (1:1), rt, 5 h; (c) PPh_3, CBr_4, THF, rt, overnight; (d) 1,2,4-triazole, K_2CO_3, DMF, rt, overnight; (e) 4 M HCl in dioxane, DCM, rt, overnight; (f) (*S*)-2-phenylpropionic acid, HBTU, TEA, MeCN, rt, 5 h; (g) NaN_3, DMF, rt, overnight; (h) acetylene, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH/H_2O, rt, overnight; (i) $Bu_4N^+CN^-$, THF, 50 °C, 2 h; (j) NaN_3, $ZnBr_2$, 1,4-dioxane, reflux, 48 h.

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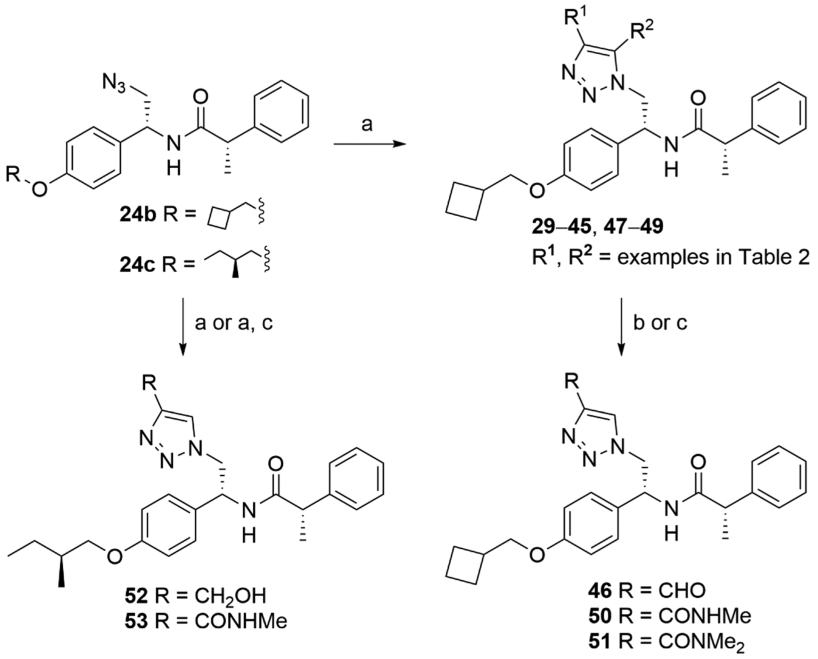
Scheme 4. Synthesis of Target Compounds 29−53[a]



[a](a) Alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH/H_2O, rt, overnight or alkyne, toluene, heat; (b) Dess−Martin periodinane (DMP), dichloromethane (DCM), rt, 2 h; (c) (i) NaOH, THF/H_2O, rt, 2 h, then HCl; (ii) $MeNH_2 \cdot HCl$ or $Me_2NH \cdot HCl$, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), triethylamine (TEA), MeCN, rt, overnight.

stacking interactions with the receptor and is not prone to metabolic degradation.[33,34] As a result, the 1,2,3-triazole motif offers a rapid and efficient way to expand SAR for hit-to-lead optimization. Herein, we report our further optimization of the 2-AMPP scaffold by replacing the 1,3,4-oxadiazole moiety in **6** (Figure 1) with other azole heterocycles, especially 1,4-disubstituted 1*H*-1,2,3-triazoles, leading to highly potent and efficacious brain-penetrant GPR88 agonists.

RESULTS AND DISCUSSION

Chemistry. The newly designed GPR88 agonists were synthesized following procedures depicted in Schemes 1−4. All final products were characterized by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry (HRMS) and determined to be >95% pure by high-performance liquid chromatography (HPLC) analyses. The reaction yield is presented in the Experimental Section. As shown in Scheme 1, reaction of esters **8a**,**b** with *N*′-hydroxyethanimidamide in the presence of NaH afforded 1,2,4-oxadiazoles **9** and **10**. On the other hand, treatment of **8b** with hydrazine afforded the corresponding hydrazide **11**, which was then reacted with acetonitrile in the presence of K_2CO_3 in *n*-butanol at 150 °C to furnish 3-substituted 5-methyl-4*H*-1,2,4-triazole **12**.

1,2,3-Triazoles **16** and **17** were synthesized in accordance with the reactions in Scheme 2. Reduction of ester **8b** with $LiBH_4$, prepared freshly from $NaBH_4$ and LiCl, afforded alcohol **13**, which was oxidized subsequently by Dess−Martin periodinane (DMP) in dichloromethane (DCM) to give aldehyde **14**. The aldehyde was converted into the terminal alkyne by treating **14** with Ohira's reagent to provide **15**. The alkyne was subjected to standard copper-catalyzed azide-alkyne cycloaddition (CuAAC) conditions with TMS−N_3 to provide the desired 4-substituted 1*H*-1,2,3-triazole **16**. Methylation of the triazole N−H with iodomethane gave 2-methyl-2*H*-1,2,3-triazole **17** as the major product. The structure of compound **17** was confirmed by NMR studies, including the heteronuclear multiple bond correlation (HMBC) experiment (see the Supporting Information).

Synthesis of 1-substituted 1*H*-1,2,4-triazole **21**, 1-substituted 1*H*-1,2,3-triazoles **25** and **26**, and 5-substituted 2*H*-1,2,3,4-tetrazole **28** is depicted in Scheme 3. *O*-alkylation of **18**[28] with 2-methylpentanol, cyclobutylmethanol, or (*S*)-2-methylbutanol under Mitsunobu conditions afforded ethers **19a**, **19b**, and **19c**, respectively. The ester in **19a**−**c** was reduced with freshly prepared $LiBH_4$ to give the corresponding alcohol, which was converted into bromides **20a**−**c** by treatment with CBr_4. The bromo group in **20b** was substituted with 1,2,4-triazole followed by Boc deprotection with 4 M HCl in dioxane and subsequent amide coupling with (*S*)-2-phenylpropionic acid to furnish 1,2,4-triazole **21**. An S_N2 displacement of the bromo group in **20a**−**c** with NaN_3 in DMF provided azides **22a**−**c**. Removal of the Boc group with HCl led to the ammonium salts **23a**−**c**, which were subsequently subjected to HBTU-mediated coupling with (*S*)-2-phenylpropionic acid to give amides **24a**−**c**. Click reaction of **24b** and **24a** with acetylene provided 1,2,3-triazoles **25** and **26**, respectively. Bromide **20a** was subjected to an S_N2 reaction with tetrabutylammonium cyanide followed by Boc deprotection and HBTU-mediated coupling of the resulting amine with (*S*)-2-phenylpropionic acid to give nitrile **27**. Compound **27** was reacted with NaN_3 in the presence of $ZnBr_2$ to provide tetrazole **28**.

Synthesis of 1,4-disubstituted 1*H*-1,2,3-triazoles **29**−**53** is depicted in Scheme 4. Cyclization of azide **24b** with an appropriate alkyne under CuAAC or thermal conditions provided 1,2,3-triazoles **29**−**45** and **47**−**49**. Dess−Martin oxidation of the 4-hydroxymethyl group in **39** gave aldehyde **46**. The ester in **48** was converted into the corresponding carboxylic acid, which was subjected to HBTU-mediated coupling with methylamine or dimethylamine to furnish

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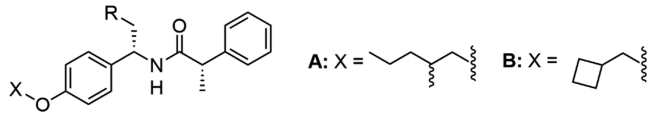
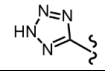
Table 1. Biological Data of Amide Bioisosteres[c]



Compound	Structure	R	clog P[a]	cAMP pEC$_{50}$ (EC$_{50}$, nM)[b]	E_{max}[c]
2			3.3	7.35 ± 0.02 (45)	100
6	A		4.5	6.83 ± 0.08 (148)	95 ± 3
9	A		5.7	6.88 ± 0.07 (132)	101 ± 2
10	B		4.8	6.49 ± 0.01 (324)	102 ± 2
12	B		3.5	6.30 ± 0.05 (501)	107 ± 3
16	B		4.4	6.45 ± 0.03 (355)	96 ± 5
17	B		4.2	6.43 ± 0.05 (372)	90 ± 5
21	B		4.0	6.75 ± 0.06 (178)	95 ± 6
25	B		4.3	7.02 ± 0.07 (95)	101 ± 2
26	A		5.2	7.22 ± 0.05 (60)	101 ± 4
28	A		5.3	Not active	N.D.

[a]clog P was calculated using Instant JChem 5.4.0 (ChemAxon Ltd.). [b]pEC$_{50}$ values are mean ± standard error of at least three independent experiments performed in duplicate. [c]E_{max} value is % of RTI-13951-33 maximal signal (mean ± standard error).

amides **50** and **51**, respectively. Likewise, cyclization of azide **24c** with propargyl alcohol under CuAAC conditions gave **52**. Finally, cyclization of azide **24c** with ethyl propiolate, followed by functional group transformations of the ester yielded the *N*-methyl amide **53** by employing conditions analogous to the synthesis of **50**.

Pharmacological Evaluation and SAR Study. Our Lance time-resolved fluorescence resonance energy transfer (TR-FRET) cAMP assay with stable CHO-GPR88 cells was used to assess the agonist potency (EC$_{50}$) and efficacy (E_{max}) at the GPR88 receptor as described previously.[24,28] In these assays, RTI-13951-33 (**2**, Figure 1) was used as the reference compound and had a collectively average EC$_{50}$ of 45 nM (n > 100). E_{max} values (%) of test compounds were evaluated with RTI-13951-33 as 100%. In the previous SAR study of 2-AMPP, we revealed that GPR88 agonist activity correlates with lipophilicity of the compounds;[28] thus, the clog P values of all newly designed compounds were also calculated. Given the improvement in potency with 1,3,4-oxadiazole as the bioisostere of amide (**6** vs **4**, Figure 1),[28] we first explored whether the 1,3,4-oxadiazole moiety can be replaced with other five-membered heterocycles with the goal of identifying the optimal balance between potency and lipophilicity. The data are summarized in Table 1. The previously reported 1,3,4-oxadiazole **6**[28] is included to highlight SAR comparison. In this SAR, both 2-methylpentyl and cyclobutylmethyl groups were used in the alkoxy side chain as the cyclobutylmethyl analogue has a lower lipophilicity (in general an order of magnitude of clog P) than that of the 2-methylpentyl counterpart. As it can be seen from Table 1, replacement of the 5-methyl-1,3,4-oxadiazole moiety in **6** with a 3-methyl-1,2,4-oxadizaole led to an equally potent compound **9** (EC$_{50}$ = 132 nM); however, it has a significantly higher clog P than that of **6** (5.7 vs 4.5). Attempts to lower clog P by substituting the 2-methylpentyl side chain with a cyclobutylmethyl group led to a 2-fold less potent compound **10**. Holding the cyclobutylmethyl side chain in analogue **10** constant, we next introduced 5-methyl-4*H*-1,2,4-triazole to give **12**, resulting in further loss of activity (EC$_{50}$ = 501 nM). On the contrary, 1*H*-1,2,3-triazole **16** and 2-methyl-2*H*-1,2,3-triazole **17** were moderately active with EC$_{50}$ values of 355 and 372 nM, respectively, but offered no advantage in terms of lipophilicity. An improvement in potency was realized when a 1*H*-1,2,4-triazole moiety was used as the amide bioisostere to give **21**, which had a comparable potency

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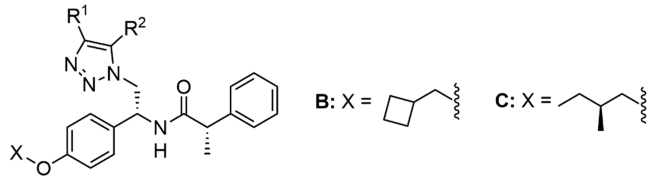
Table 2. Biological Data of 1,2,3-Triazoles[c]



Compound	Structure	R^1	R^2	clog P[a]	cAMP pEC$_{50}$ (EC$_{50}$, nM)[b]	E_{max}[c]
25	B	H	H	4.3	7.02 ± 0.07 (95)	101 ± 2
29	B	Methyl	H	4.4	7.05 ± 0.05 (89)	93 ± 2
30	B	Methyl	Methyl	4.6	6.34 ± 0.03 (457)	107 ± 5
31	B	Ethyl	H	5.1	6.58 ± 0.03 (263)	96 ± 2
32	B	*n*-Propyl	H	5.6	6.74 ± 0.04 (182)	105 ± 5
33	B	*i*-Propyl	H	5.7	6.44 ± 0.04 (363)	101 ± 8
34	B	*t*-Butyl	H	6.2	6.22 ± 0.01 (603)	100 ± 1
35	B	Hexyl	H	6.9	6.04 ± 0.07 (912)	93 ± 1
36	B	Cyclopropyl	H	5.2	6.61 ± 0.08 (245)	88 ± 1
37	B	Cyclohexyl	H	6.5	6.47 ± 0.05 (339)	83 ± 2
38	B	Phenyl	H	6.3	6.74 ± 0.01 (182)	84 ± 1
39	B	$HOCH_2$	H	3.6	7.04 ± 0.02 (91)	91 ± 2
40	B	$MeOCH_2$	H	4.2	6.55 ± 0.04 (282)	94 ± 1
41	B	$AcOCH_2$	H	4.0	6.78 ± 0.02 (166)	101 ± 3
42	B	NH_2CH_2	H	3.5	5.96 ± 0.05 (1096)	81 ± 2
43	B	$MeNHCH_2$	H	3.9	5.70 ± 0.09 (1995)	95 ± 8
44	B	Me_2NCH_2	H	4.3	6.15 ± 0.06 (708)	103 ± 9
45	B	CH_3CH_2O	H	5.1	6.39 ± 0.05 (407)	96 ± 2
46	B		H	4.7	7.13 ± 0.08 (74)	98 ± 2
47	B		H	4.2	6.94 ± 0.06 (115)	95 ± 3
48	B		H	4.8	6.78 ± 0.08 (166)	82 ± 6
49	B		H	3.5	7.04 ± 0.06 (91)	87 ± 1
50	B		H	3.7	7.40 ± 0.04 (40)	85 ± 3
51	B		H	4.0	6.41 ± 0.08 (389)	96 ± 1
52	C	$HOCH_2$	H	4.1	7.11 ± 0.03 (77)	94 ± 2
53	C		H	4.2	7.86 ± 0.08 (14)	92 ± 3

[a]clog P was calculated using Instant JChem 5.4.0 (ChemAxon Ltd.). [b]pEC$_{50}$ values are mean ± standard error of at least three independent experiments performed in duplicate. [c]E_{max} value is % of RTI-13951-33 maximal signal (mean ± standard error).

(EC$_{50}$ = 178 nM) to the 5-methyl-1,3,4-oxadiazole **6**. Exchanging of 1*H*-1,2,4-triazole in **21** with a 1*H*-1,2,3-triazole led to further improvement of potency. The 1*H*-1,2,3-triazole analogues **25** and **26** with EC$_{50}$ values of 95 and 60 nM, respectively, emerged as the most potent and efficacious (both with E_{max} = 101% relative to RTI-13951-33) agonists from the

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heterocyclic bioisosteres investigated. Finally, tetrazole **28** was completely inactive which was expected as 1,2,3,4-tetrazoles are better known as carboxylic acid bioisosteres. Overall, this SAR suggests that a variety of azoles containing three heteroatoms can serve as amide bioisosteres in the 2-AMPP scaffold with moderate to high GPR88 agonist activity. Considering both potency and synthetic feasibility for a rapid SAR expansion, the 1*H*-1,2,3-triazole **25** was chosen as the starting point for further optimization.

The SAR of the modified 1*H*-1,2,3-triazole is shown in Table 2, again holding the cyclobutylmethyl side chain constant. The 1,2,3-triazole moiety in **25** tolerated a substitution at the 4-position as the 4-methyl analogue **29** (EC_{50} = 89 nM) was equipotent to **25** but did not tolerate a substitution at the 5-position as the 4,5-dimethyl analogue **30** (EC_{50} = 457 nM) was 5-fold less potent. The C-5 hydrogen atom of 1,2,3-triazoles is known to be a hydrogen-bond donor.[31] In this case, the lack of tolerance of a substitution at C-5 is presumably due to the necessity of a hydrogen-bond donor at this position. Consequently, a range of alkyl and aryl functional substitutions at C-4 were synthesized keeping the C-5 unsubstituted. In general, the GPR88 activity decreased with the increase in size of the substitutions with a preferred rank order of methyl (**29**) > ethyl (**31**) ≈ *n*-propyl (**32**) > *i*-propyl (**33**) > *t*-butyl (**34**) > *n*-hexyl (**35**). Interestingly, the C-4 position was well tolerated with a cycloalkyl group (e.g., cyclopropyl **36** and cyclohexyl **37**) or a phenyl group (**38**), all of which had EC_{50} values less than 350 nM. In the further SAR studies, substitutions with different electronic properties (e.g., hydroxyl, amino, electron-donating, and electron-withdrawing groups) were probed. Addition of a hydroxymethyl group at C-4 (**39**) had similar activity to **25** (EC_{50} = 91 vs 95 nM) but reduced the clog *P* by almost a unit. On the other hand, when the hydroxyl group in **39** was capped with a methyl ether (**40**) or an acetyl ester (**41**), it led to approximately 2- to 3-fold loss of activity. In addition, all of the aminomethyl analogues **42**−**44** (EC_{50} = 700−2000 nM) suffered from substantial loss of activity compared with **25** (EC_{50} = 95 nM). An ethoxy group directly linked to the C-4 (**45**, EC_{50} = 407 nM) was also not favored, indicating that incorporation of an electron-donating group (EDG) has a detrimental effect on the GPR88 activity. On the contrary, substitution of C-4 with an electron-withdrawing group (EWG), such as aldehyde (**46**), ketone (**47**), ester (**48**), and amides (primary amide **49** and *N*-methyl amide **50** but not *N*,*N*-dimethyl amide **51**), had a favorable effect on activity leading to compounds with EC_{50} values either comparable or better than that of **25**. Among these, the *N*-methyl amide **50**, with an EC_{50} of 40 nM, was the most potent compound. In addition, compound **50** has a favorable clog *P* of 3.7 which is within the 2−4 range for potential BBB penetration.[29] Given that both hydroxymethyl **39** (EC_{50} = 91 nM, clog *P* = 3.6) and *N*-methyl amide **50** (EC_{50} = 40, clog *P* = 3.7) have a good balance of potency and lipophilicity, we designed and synthesized compounds **52** and **53**, in which an (*S*)-2-methylbutyl group was used as the alkoxy side chain. The (*S*)-2-methylbutyl analogues have been previously shown to have superior agonist activity compared to the cyclobutylmethyl analogues but have lower lipophilicity than the 2-methylpentyl analogues.[28] In agreement with the previously reported SAR, compounds **52** and **53** were more potent than **39** and **50**, respectively. Compound **53** had an EC_{50} of 14 nM, being the most potent compound in this study and is 3-fold more potent than RTI-13951-33 in the functional cAMP assay (Figure 2).

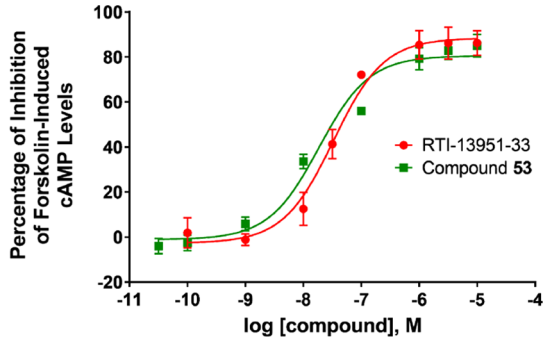

Figure 2. Concentration−response curves of RTI-13951-33 and **53** in the GPR88 Lance TR-FRET cAMP assay. The TR-FRET signal (665 nm) was converted to fmol cAMP by interpolating from the standard cAMP curve. Percent inhibition of forskolin-induced (300 nM) cAMP levels was plotted against the log of compound concentration, and data were fit to a three-parameter logistic curve to generate EC_{50} values. Each data point is the mean ± standard deviation (S.D.) of at least three independent experiments performed in duplicate.

Because GPR88 is highly expressed in the striatum, we further characterized **53** in the [^{35}S]GTPγS binding assays using striatal membrane preparations from WT mice and GPR88 KO mice to confirm its agonist activity and GPR88-specificity. RTI-13951-33 was used as the reference compound and had an EC_{50} of 2.9 μM, which is 5-fold less potent than the previously reported EC_{50} of 535 nM.[25] The variation in potency is likely due to the different batch of striatal membranes with different GPR88 expression levels. Nevertheless, compound **53** also significantly enhanced [^{35}S]GTPγS binding activity to a similar extent as RTI-13951-33, with an EC_{50} of 8.9 μM (E_{max} = 270%, n = 3) in WT mouse striatal membranes (Figure 3). This activity was confirmed using

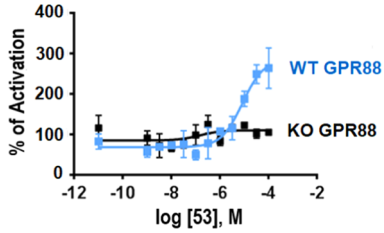

Figure 3. [^{35}S]GTPγS binding of compound **53** in wild-type (WT) mouse striatal membranes vs GPR88 KO mouse striatal membranes. The data are the means of triplicate measurements with standard deviation shown as error bars and are representative from three independent experiments.

striatal membranes from commercial C57BL/6J mice with an EC_{50} of 6.5 μM (E_{max} = 320%, n = 4). Importantly, the compound at a concentration of 100 μM was inactive in striatal membranes prepared from GPR88 KO mice, demonstrating a GPR88-specific G-protein signaling activity in the striatum.

BBB Permeability and PK Profile of Compound 53. Considering both GPR88 agonist activity and druglike properties (e.g., clog *P* and PSA, Table 3), compounds **39**, **50**, **52**, and **53** were selected for further characterization. The BBB penetration remains a significant challenge for the development of GPR88 modulators since sufficient brain exposure is necessary to modulate the target receptor which is predominantly expressed in the brain. Thus, selected compounds were first screened through a bidirectional

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Table 3. Physicochemical Properties and BBB Permeability of Compounds 39, 50, 52, and 53

	desired value	39	50	52	53
cAMP EC_{50} (nM)		91	40	77	14
clog P[a]	2−4	3.6	3.7	4.1	4.2
PSA[a]	<90	89.3	98.1	89.3	98.1
P_{app} (10^{-6} cm/s) A-to-B	>3	6.7	10.9	10.6	13.3
P_{app} (10^{-6} cm/s) B-to-A		35.1	23.6	31.9	23.5
efflux ratio	<2.5	5.2	2.2	3.0	1.8

[a]clog P and PSA were calculated using Instant JChem 5.4.0 (ChemAxon Ltd.).

transport assay using Madin−Darby canine kidney (MDCK) cells expressing the human multidrug resistance protein 1 (MDR1) gene before selecting and testing a lead compound in the PK assessment. As shown in Table 3, all compounds demonstrated good apical to basolateral apparent permeability P_{app} (A → B) values of 6−13 × 10^{-6} cm/s, which is higher than the recommended threshold of 3 × 10^{-6} cm/s.[39] Both hydroxymethyl analogues **39** and **52** are likely *P*-glycoprotein (*P*gp) substrates as indicated by their high efflux ratios ($P_{B\to A}/P_{A\to B}$). On the other hand, both *N*-methyl amides **50** and **53** have efflux ratios within the desired value of <2.5.[40]

Compound **53**, with the best potency and BBB permeability, was further evaluated in a mouse PK study using a single dose of 20 mg/kg by intraperitoneal (ip) injection. The results are summarized in Table 4. Compound **53** had a good plasma

Table 4. PK Profile of Compound 53 in Mice

	mouse PK (20 mg/kg, ip)				
	plasma			brain	
compound	C_{max} (ng/mL)	$t_{1/2}$ (h)	CL (mL/min/kg)	C_{max} (ng/mL)	B/P ratio
53	1670	5.6	21	576	0.34

exposure after ip and reached plasma C_{max} of 1670 ng/mL at 2 h. The compound had acceptable metabolic stability with a half-life of 5.6 h and clearance (CL) of 21 mL/mg/kg. The C_{max} in the brain was 576 ng/mL (1.2 μM), which is ∼100 times its cAMP EC_{50} of 14 nM, indicating that **53** has sufficient brain penetration to modulate GPR88 functions when administered intraperitoneally. The brain-to-plasma ratio was 0.34.

CONCLUSIONS

In summary, our optimization efforts to develop novel and potent small molecule GPR88 agonists based on the 2-AMPP scaffold in this study have unraveled interesting SAR trends. We have demonstrated that 1,2,3-triazoles can bioisosterically replace the amide functionality as the key pharmacophore in the 2-AMPP scaffold with a good balance of potency and lipophilicity. Compound **53** emerged as the most potent compound in this study and had an EC_{50} of 14 nM in the cell-based functional cAMP assay and GPR88-specific agonist activity in the mouse striatal tissues. In addition, **53** exhibited a good BBB permeability and had a good *in vivo* PK profile. Taken together, our data suggest that **53** is a suitable agonist probe for elucidating GPR88 functions and for evaluating GPR88's therapeutic potential for the treatment of striatal-associated disorders such as Parkinson's disease, schizophrenia, and drug addiction.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* All solvents and chemicals were reagent grade. Unless otherwise mentioned, all reagents and solvents were purchased from commercial vendors and used as received. Flash column chromatography was carried out on a Teledyne ISCO CombiFlash Rf system using prepacked columns. Solvents used include EtOAc, hexanes, MeOH, and DCM. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz) or a Bruker AVANCE III HD 700 MHz spectrometer and were determined in $CDCl_3$ or CD_3OD with tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to the reference signal and coupling constant (J) values are reported in hertz (Hz). Nominal mass spectra were obtained using an Agilent InfinityLab MSD single quadrupole mass spectrometer system (ESI). High-resolution mass spectra (HRMS) were obtained using Agilent 1290 Infinity UHPLC-6230 TOF mass spectrometer (ESI). Thin-layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light or iodine staining. CMA80 for column chromatography is a mixture of 80:18:2 chloroform/MeOH/NH_4OH. All final compounds were greater than 95% pure as determined by HPLC on a Waters 2695 Separation Module equipped with a Waters 2996 Photodiode Array Detector and a Phenomenex Synergi 4 mm Hydro-RP 80A C18 250 mm × 4.6 mm column using a flow rate of 1 mL/min starting with 1 min at 5% solvent B, followed by a 15 min gradient of 5−95% solvent B, followed by 9 min at 95% solvent B (solvent A, H_2O with 0.1% TFA; solvent B, acetonitrile with 0.1% TFA and 5% H_2O; absorbance monitored at 280 nm). All of the synthesized target compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS, and determined to be >95% pure by HPLC analyses.

*(2S)-N-[(1S)-2-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-{4-[(2-methylpentyl)oxy]phenyl}ethyl]-2-phenylpropanamide (**9**).* To a solution of **8a**[28] (80 mg, 0.19 mmol) in dry THF (10 mL) at 0 °C under nitrogen were added *N*′-hydroxyethanimidamide (57.6 mg, 0.78 mmol), NaH (8.5 mg, 0.21 mmol), and molecular sieves (3 Å, 300 mg). After stirring at room temperature for 3 h, the reaction was quenched with saturated aqueous NH_4Cl solution and diluted with EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (2 × 30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% EtOAc in hexanes afforded **9** (65 mg, 77% yield) as a waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.19 (m, 5H), 6.91 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 6.45 (d, J = 8.3 Hz, 1H), 5.43 (dd, J = 14.2, 6.0 Hz, 1H), 3.72 (dt, J = 15.0, 7.5 Hz, 1H), 3.68−3.55 (m, 2H), 3.29 (qd, J = 15.4, 6.0 Hz, 2H), 2.29 (s, 3H), 2.00−1.71 (m, 1H), 1.50 (t, J = 7.1 Hz, 3H), 1.47−1.09 (m, 4H), 0.98 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.4, 166.8, 158.8, 141.1, 131.4, 128.9, 127.7, 127.3, 127.0, 114.7, 73.2, 49.9, 47.1, 35.7, 32.8, 32.6, 20.0, 18.2, 17.0, 14.3, 11.5; HRMS (ESI) m/z calcd for $C_{26}H_{33}N_3O_3$ $[M + H]^+$ 436.2595, m/z found 436.2596; HPLC, t_R 18.56 min.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(3-methyl-1,2,4-oxadiazol-5-yl)ethyl]-2-phenylpropanamide (**10**).* The procedure for the synthesis of **9** was followed starting with **8b**[28] to afford **10** (75% yield) as a white waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.19 (m, 5H), 6.91 (d, J = 8.7 Hz, 2H), 6.79−6.64 (m, 2H), 6.46 (d, J = 8.3 Hz, 1H), 5.53−5.32 (m, 1H), 3.84 (d, J = 6.7 Hz, 2H), 3.61 (q, J = 7.2 Hz, 1H), 3.29 (qd, J = 15.4, 6.0 Hz, 2H), 2.72 (dt, J = 14.7, 7.5 Hz, 1H), 2.29 (s, 3H), 2.20−2.01 (m, 2H), 2.01−1.67 (m, 4H), 1.51 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.4, 166.8, 158.8, 141.1, 131.4, 128.9, 127.7, 127.3, 127.0, 114.7, 72.1, 49.9, 47.1, 34.6, 32.6, 24.8, 18.5, 18.2, 11.5; HRMS (ESI) m/z calcd for $C_{25}H_{29}N_3O_3$ $[M + H]^+$ 420.2282, m/z found 420.2280; HPLC, t_R 17.22 min.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(5-methyl-4H-1,2,4-triazol-3-yl)ethyl]-2-phenylpropanamide (**12**).* To a solution of **11**[28] (50 mg, 0.13 mmol) in *n*-BuOH (5 mL) at room temperature were added K_2CO_3 (52.4 mg, 0.38 mmol) and CH_3CN (0.2 mL). The

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reaction was heated at 150 °C in a sealed tube for 5 h. After cooling to room temperature, H_2O (10 mL) and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **12** (26 mg, 49% yield) as a waxy white solid. The product was a tautomeric mixture as judged by NMR studies. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.17 (m, 5H), 7.17−7.09 (m, 1H), 7.04 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 6.75 (d, J = 8.7 Hz, 1H), 6.67 (d, J = 8.7 Hz, 1H), 5.46−5.13 (m, 1H), 3.82 (dd, J = 11.9, 6.7 Hz, 2H), 3.54 (dq, J = 14.4, 7.1 Hz, 1H), 3.10 (dd, J = 15.0, 6.1 Hz, 2H), 2.70 (dq, J = 14.6, 7.5 Hz, 1H), 2.32 (s, 1.6H), 2.25 (s, 1.4H), 2.18−2.01 (m, 2H), 2.01−1.69 (m, 4H), 1.43 (dd, J = 7.1, 3.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 173.9, 158.6, 158.4, 141.3, 141.1, 133.0, 132.9, 128.7, 128.6, 127.6, 127.5, 127.3, 127.1, 127.0, 114.6, 114.5, 72.1, 72.1, 51.4, 51.2, 47.1, 46.9, 34.6, 33.7, 33.6, 24.8, 18.5, 18.4, 18.2, 12.7, 12.7; HRMS (ESI) m/z calcd for $C_{25}H_{30}N_4O_2$ $[M + H]^+$ 419.2442, m/z found 419.2434; HPLC, t_R 14.07 min.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-3-hydroxypropyl]-2-phenylpropanamide (**13**).* To a stirred suspension of $NaBH_4$ (120 mg, 3.16 mmol) in EtOH (5 mL) at 0 °C was added LiCl (134 mg, 3.16 mmol). The mixture was stirred at 0 °C for 30 min. After that, a solution of **8b** (500 mg, 1.26 mmol) in THF (5 mL) was added to the above reaction at 0 °C. After stirring at room temperature for 6 h, the reaction was quenched with saturated NH_4Cl solution (10 mL) followed by addition of H_2O. The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−50% EtOAc in hexanes afforded **13** (465 mg, quantitative yield) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.13 (m, 5H), 6.99 (d, J = 8.7 Hz, 2H), 6.86−6.61 (m, 2H), 5.87 (d, J = 7.5 Hz, 1H), 5.10 (td, J = 10.3, 3.9 Hz, 1H), 3.88 (d, J = 6.7 Hz, 2H), 3.71−3.45 (m, 3H), 3.35 (m, 1H), 2.92−2.49 (m, 1H), 2.24−1.62 (m, 8H), 1.54 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 158.6,140.9, 133.2, 128.9, 127.6, 127.4, 127.3, 114.7, 72.1, 58.9, 49.9, 47.1, 38.4, 34.6, 24.8, 18.6, 18.4; MS (ESI) m/z 368.0 $[M + H]^+$.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-3-oxopropyl]-2-phenylpropanamide (**14**).* To a solution of **13** (300 mg, 0.85 mmol) in DCM (10 mL, saturated with H_2O) at 0 °C was added Dess−Martin periodinane (756 mg, 1.78 mmol) in two portions over 15 min. After stirring at 0 °C for 14 h, the reaction was diluted with H_2O and poured into a mixture of $Na_2S_2O_3$ (1.5 g) in $NaHCO_3$ (20 mL, 80% saturated in H_2O). The layers were separated, and the aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layers were washed with ice-cold $NaHCO_3$ (10 mL), brine (2 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−50% EtOAc in hexanes afforded **14** (95 mg, 31% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82−9.49 (m, 1H), 7.48−7.14 (m, 5H), 6.98 (d, J = 8.6 Hz, 2H), 6.83−6.69 (m, 2H), 5.85 (d, J = 7.9 Hz, 1H), 5.40 (dd, J = 14.3, 6.8 Hz, 1H), 3.87 (d, J = 6.7 Hz, 2H), 3.55 (q, J = 7.1 Hz, 1H), 3.00−2.79 (m, 2H), 2.73 (dt, J = 14.9, 7.5 Hz, 1H), 2.19−2.04 (m, 2H), 2.04−1.75 (m, 4H), 1.50 (d, J = 7.2 Hz, 3H); MS (ESI) m/z 366.4 $[M + H]^+$.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]but-3-yn-1-yl]-2-phenylpropanamide (**15**).* A solution of **14** (73.5 mg, 0.20 mmol) and dimethylacetyldiazophosphonoacetate (82 μL, 0.54 mmol) in anhydrous MeOH (5 mL) at room temperature was treated with K_2CO_3 (97.3 mg, 0.70 mmol) and stirred for 2 h. The reaction was partitioned between EtOAc and aqueous $NaHCO_3$ (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% EtOAc in hexanes afforded **15** (65 mg, 89% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51−7.20 (m, 5H), 7.02 (d, J = 8.6 Hz, 2H), 6.83−6.70 (m, 2H), 5.84 (d, J = 8.3 Hz, 1H), 5.10 (dt, J = 8.2, 5.6 Hz, 1H), 3.88 (d, J = 6.7 Hz, 2H), 3.62 (q, J = 7.2 Hz, 1H), 2.85−2.48 (m, 3H), 2.21−2.02 (m, 2H), 2.02−1.73 (m, 5H), 1.53 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 157.9, 140.4, 131.5, 128.1, 126.9, 126.5, 113.7, 79.1, 76.4, 71.3, 70.5, 49.3, 46.4, 33.8, 24.8, 24.0, 17.7, 17.4; MS (ESI) m/z 362.4 $[M + H]^+$.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(1H-1,2,3-triazol-4-yl)ethyl]-2-phenylpropanamide (**16**).* To a stirred solution of **15** (29 mg, 0.08 mmol) in DMF/H_2O (3 mL, 9:1, v/v) at room temperature were added $CuSO_4·5H_2O$ (10 mg, 0.03 mmol), sodium ascorbate (20 mg, 0.08 mmol), and TMS−N_3 (21 μL, 0.16 mmol). The reaction was heated at 90 °C for 5 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **16** (24 mg, 75% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.44 (s, 1H), 7.30−7.11 (m, 5H), 7.07 (d, J = 8.6 Hz, 2H), 6.75 (d, J = 8.7 Hz, 2H), 5.17 (t, J = 7.6 Hz, 1H), 3.86 (d, J = 6.6 Hz, 2H), 3.64 (q, J = 7.1 Hz, 1H), 3.17 (d, J = 7.6 Hz, 2H), 2.82−2.59 (m, 1H), 2.18−2.02 (m, 2H), 2.02−1.70 (m, 4H), 1.35 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.2, 160.0, 142.8, 134.7, 129.5, 128.6, 128.4, 127.9, 115.5, 73.2, 53.8, 47.4, 36.1, 32.5, 25.7, 19.4, 18.7; HRMS (ESI) m/z calcd for $C_{24}H_{28}N_4O_2$ $[M + H]^+$ 405.2285, m/z found 405.2277; HPLC, t_R 15.37 min.

*(2S)-N-[(1S)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(2-methyl-2H-1,2,3-triazol-4-yl)ethyl]-2-phenylpropanamide (**17**).* To a solution of **16** (16 mg, 0.04 mmol) in DMF (2 mL) at room temperature were added K_2CO_3 (11 mg, 0.08 mmol) and CH_3I (2.5 μL, 0.04 mmol). After stirring at room temperature overnight, the reaction was quenched with H_2O (1 mL) and diluted with EtOAc (5 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 mL). The combined organic layers were washed with H_2O (3 × 5 mL), brine (3 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **17** (7 mg, 42% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.14 (m, 5H), 7.02 (s, 1H), 6.85 (d, J = 8.6 Hz, 2H), 6.77−6.64 (m, 2H), 6.10 (d, J = 7.9 Hz, 1H), 5.21 (dd, J = 13.9, 6.3 Hz, 1H), 4.05 (s, 3H), 3.86 (d, J = 6.7 Hz, 2H), 3.58 (q, J = 7.2 Hz, 1H), 3.03 (qd, J = 14.8, 6.1 Hz, 2H), 2.83−2.65 (m, 1H), 2.25−2.04 (m, 2H), 2.02−1.73 (m, 4H), 1.48 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 158.4, 144.2, 141.4, 133.5, 133.1, 128.8, 127.8, 127.2, 127.1, 114.5, 72.1, 51.8, 47.1, 41.4, 34.6, 32.0, 24.8, 18.5, 18.2; HRMS (ESI) m/z calcd for $C_{25}H_{30}N_4O_2$ $[M + H]^+$ 419.2442, m/z found 419.2434; HPLC, t_R 16.96 min.

*Methyl (2R)-2-{[(tert-Butoxy)carbonyl]amino}-2-{4-[(2-methylpentyl)oxy]phenyl}acetate (**19a**).* To a solution of **18**[28] (5.0 g, 17.78 mmol) in THF (100 mL) at room temperature were added PPh_3 (7.93 g, 30.21 mmol) and 2-methylpentanol (4.41 mL, 35.55 mmol). This was followed by slow addition of DEAD (6.13 mL, 30.21 mmol) maintaining the reaction temperature below 35 °C. After stirring at room temperature overnight, the reaction was quenched with H_2O (50 mL) and diluted with EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine (3 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% EtOAc in hexanes afforded **19a** (6.50 g, quantitative yield) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 5.63 (d, J = 7.0 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 3.78 (dd, J = 8.9, 5.8 Hz, 1H), 3.73−3.60 (m, 4H), 1.97−1.79 (m, 1H), 1.55−1.28 (m, 12H), 1.28−1.07 (m, 1H), 1.00 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 159.4, 154.8, 128.7, 128.3, 114.8, 79.8, 73.1, 57.0, 52.4, 35.7, 32.8, 28.2, 20.0, 16.9, 14.2; MS (ESI) m/z 388.4 $[M + Na]^+$.

*Methyl (2R)-2-{[(tert-Butoxy)carbonyl]amino}-2-[4-(cyclobutylmethoxy)phenyl]acetate (**19b**).* The procedure for the synthesis of **19a** was followed starting with **18** and cyclobutylmethanol to afford **19b** (98% yield) as a colorless oil: ^{1}H

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NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 5.90 (d, J = 7.3 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 3.86 (d, J = 6.6 Hz, 2H), 3.63 (s, 3H), 2.86−2.61 (m, 1H), 2.25−2.01 (m, 2H), 1.96−1.69 (m, 4H), 1.40 (s, 9H); MS (ESI) m/z 372.4 $[M + Na]^+$.

*Methyl (2R)-2-{[(tert-Butoxy)carbonyl]amino}-2-{4-[(2S)-2-methylbutoxy]phenyl}acetate (**19c**).* The procedure for the synthesis of **19a** was followed starting with **18** and (*S*)-2-methylbutanol to afford **19c** (quantitative yield) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, J = 8.7 Hz, 2H), 6.92−6.78 (m, 2H), 5.49 (t, J = 23.9 Hz, 1H), 5.25 (d, J = 7.3 Hz, 1H), 3.79 (dd, J = 9.0, 6.0 Hz, 1H), 3.75−3.64 (m, 4H), 1.94−1.73 (m, 1H), 1.67−1.49 (m, 1H), 1.43 (s, 9H), 1.33−1.12 (m, 2H), 1.00 (d, J = 6.7 Hz, 3H), 0.93 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 159.4, 154.8, 128.7, 128.3, 114.8, 80.0, 72.9, 57.0, 52.5, 34.6, 28.3, 26.1, 16.5, 11.2; MS (ESI) m/z 374.4 $[M + Na]^+$.

*tert-Butyl N-[(1R)-2-Bromo-1-{4-[(2-methylpentyl)oxy]phenyl}-ethyl]carbamate (**20a**).* This compound was synthesized according to our previously reported procedure.[26]

*tert-Butyl N-[(1R)-2-Bromo-1-[4-(cyclobutylmethoxy)phenyl]-ethyl]carbamate (**20b**).* To a suspension of $NaBH_4$ (541 mg, 14.30 mmol) in EtOH (20 mL) at 0 °C under nitrogen was added LiCl (607 mg, 14.30 mmol). After stirring at 0 °C for 10 min, a solution of **19b** (2.0 g, 5.72 mmol) in THF (20 mL) was added. The reaction mixture was stirred at room temperature for 3 h and quenched with saturated NH_4Cl solution (30 mL), followed by addition of H_2O (30 mL). The mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (2 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded the intermediate alcohol (1.7 g, 92% yield) as a waxy white solid: MS (ESI) m/z 344.2 $[M + Na]^+$. To a solution of the intermediate alcohol (1.6 g, 4.98 mmol) in THF (40 mL) at room temperature were added PPh_3 (1.96 g, 7.47 mmol) and CBr_4 (2.48 g, 7.47 mmol). After stirring at room temperature overnight, the mixture was filtered, and the filtrate was concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% EtOAc in hexanes afforded **20b** (1.1 g, 58% yield) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.6 Hz, 2H), 6.96−6.81 (m, 2H), 5.07 (s, 1H), 4.93 (s, 1H), 3.91 (d, J = 6.7 Hz, 2H), 3.76−3.43 (m, 2H), 2.95−2.61 (m, 1H), 2.27−2.03 (m, 2H), 2.03−1.77 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.0, 155.0, 131.3, 127.5, 114.7, 80.0, 72.1, 54.5, 37.1, 34.6, 28.3, 24.8, 18.6; MS (ESI) m/z 406.2 $[M + Na]^+$ (^{79}Br), 408.2 $[M + Na]^+$ (^{81}Br).

*tert-Butyl N-[(1R)-2-Bromo-1-{4-[(2S)-2-methylbutoxy]phenyl}-ethyl]carbamate (**20c**).* The procedure for the synthesis of **20b** was followed starting with **19c** to afford **20c** (60% yield over 2 steps) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.24−7.10 (m, 2H), 6.95−6.79 (m, 2H), 5.08 (br s, 1H), 4.93 (br s, 1H), 3.87−3.51 (m, 4H), 1.98−1.75 (m, 1H), 1.66−1.48 (m, 1H), 1.44 (s, 9H), 1.35−1.17 (m, 1H), 1.00 (d, J = 6.7 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 155.0, 131.3, 127.5, 114.7, 80.0, 72.9, 54.6, 37.1, 34.7, 28.3, 26.1, 16.5, 11.3; MS (ESI) m/z 408.2 $[M + Na]^+$ (^{79}Br), 410.2 $[M + Na]^+$ (^{81}Br).

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(1H-1,2,4-triazol-1-yl)ethyl]-2-phenylpropanamide (**21**).* To a solution of **20b** (35 mg, 0.09 mmol) in DMF (2 mL) at room temperature were added 1,2,4-triazole (12.4 mg, 0.14 mmol) and K_2CO_3 (38 mg, 0.27 mmol). After stirring at room temperature overnight, the reaction was quenched with H_2O (2 mL) and diluted with EtOAc (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (5 mL). The combined organic layers were washed with brine (3 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded the intermediate *N*-Boc triazole (33 mg, 97% yield) as a white solid: MS (ESI) m/z 373.4 $[M + H]^+$. Removal of the Boc protecting group of this material (33 mg, 0.09 mmol) was achieved by treatment with 4 M HCl in dioxane (0.27 mL) at room temperature overnight. The solvent was removed under reduced pressure to give the corresponding amine HCl salt. To a suspension of the salt in MeCN (2 mL) at room temperature were added HBTU (61 mg, 0.14 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (56 μL, 0.27 mmol), and (*S*)-2-phenylpropionic acid (18 mg, 0.14 mmol). After stirring at room temperature overnight, the reaction was quenched with H_2O (5 mL) and diluted with EtOAc (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (5 mL). The combined EtOAc layers were washed with brine (3 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **21** (25 mg, 46% yield over three steps) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.54 (s, 1H), 7.45−7.16 (m, 5H), 6.95−6.52 (m, 5H), 5.46−5.14 (m, 1H), 4.41 (ddd, J = 19.2, 14.0, 4.6 Hz, 2H), 3.83 (d, J = 6.6 Hz, 2H), 3.63 (q, J = 7.2 Hz, 1H), 2.89−2.53 (m, 1H), 2.25−2.03 (m, 2H), 2.03−1.76 (m, 4H), 1.50 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 158.9, 152.1, 144.0, 141.0, 129.7, 128.9, 127.7, 127.4, 126.9, 114.8, 72.1, 53.9, 52.9, 47.1, 34.6, 24.8, 18.5, 17.9; HRMS (ESI) m/z calcd for $C_{24}H_{28}N_4O_2$ $[M + H]^+$ 405.2285, m/z found 405.2269; HPLC, t_R 15.72 min.

*tert-Butyl N-[(1R)-2-Azido-1-{4-[(2-methylpentyl)oxy]phenyl}-ethyl]carbamate (**22a**).* This compound was synthesized according to our previously reported procedure.[26]

*tert-Butyl N-[(1R)-2-Azido-1-[4-(cyclobutylmethoxy)phenyl]-ethyl]carbamate (**22b**).* To a solution of **20b** (1.0 g, 2.60 mmol) in DMF (15 mL) at room temperature was added NaN_3 (677 mg, 10.41 mmol). After stirring at room temperature overnight, the reaction was quenched by H_2O (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (2 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **22b** (870 mg, 97% yield) as a waxy white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.04 (br s, 1H), 4.93 (br s, 1H), 3.91 (d, J = 6.7 Hz, 2H), 3.74−3.45 (m, 2H), 2.93−2.53 (m, 1H), 2.26−2.04 (m, 2H), 2.04−1.77 (m, 4H), 1.44 (s, 9H); MS (ESI) m/z 369.4 $[M + Na]^+$.

*tert-Butyl N-[(1R)-2-Azido-1-{4-[(2S)-2-methylbutoxy]phenyl}-ethyl]carbamate (**22c**).* The procedure for the synthesis of **22b** was followed starting with **20c** to afford **22c** (92% yield) as a waxy white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.7 Hz, 2H), 6.96−6.79 (m, 2H), 4.97 (br s, 1H), 4.80 (br s, 1H), 3.76 (ddd, J = 26.1, 9.0, 6.3 Hz, 2H), 3.60 (d, J = 5.4 Hz, 2H), 1.97−1.74 (m, 1H), 1.69−1.49 (m, 1H), 1.44 (s, 9H), 1.36−1.14 (m, 1H), 1.00 (d, J = 6.7 Hz, 3H), 0.94 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 155.0, 131.1, 127.6, 114.8, 80.0, 72.9, 55.7, 53.7, 34.7, 28.3, 26.1, 16.5, 11.3; MS (ESI) m/z 371.2 $[M + Na]^+$.

*(1R)-2-Azido-1-{4-[(2-methylpentyl)oxy]phenyl}ethan-1-amine Hydrochloride (**23a**).* This compound was synthesized according to our previously reported procedure.[26]

*(1R)-2-Azido-1-[4-(cyclobutylmethoxy)phenyl]ethan-1-amine Hydrochloride (**23b**).* To a solution of **22b** (870 mg, 2.51 mmol) in DCM (20 mL) at room temperature was added 4 M HCl in dioxane (6.3 mL). After stirring at room temperature overnight, the mixture was concentrated under reduced pressure to afford **23b** (740 mg, quantitative yield) as a residue, which was used for the next step without purification.

*(1R)-2-Azido-1-{4-[(2S)-2-methylbutoxy]phenyl}ethan-1-amine Hydrochloride (**23c**).* The procedure for the synthesis of **23b** was followed starting with **22c** to afford **23c** (quantitative yield) as a residue, which was used for the next step without purification.

*(2S)-N-[(1R)-2-Azido-1-{4-[(2-methylpentyl)oxy]phenyl}ethyl]-2-phenylpropanamide (**24a**).* This compound was synthesized according to our previously reported procedure.[26]

*(2S)-N-[(1R)-2-Azido-1-[4-(cyclobutylmethoxy)phenyl]ethyl]-2-phenylpropanamide (**24b**).* To a solution of **23b** (0.8 g, 2.83 mmol) in MeCN (30 mL) at 0 °C were added HBTU (1.61 g, 4.24 mmol), Et_3N (2.37 mL, 16.98 mmol), and (*S*)-2-phenylpropionic acid (425 mg, 2.83 mmol). After stirring at room temperature 5 h, the reaction was quenched with saturated $NaHCO_3$ (20 mL) and diluted with EtOAc (30 mL). The organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers

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were washed with brine (2 × 30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−50% EtOAc in hexanes afforded **24b** (0.97 g, 91% yield) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.17 (m, 5H), 6.96 (d, J = 8.6 Hz, 2H), 6.84−6.64 (m, 2H), 5.91 (d, J = 7.9 Hz, 1H), 5.23−4.95 (m, 1H), 3.87 (d, J = 6.6 Hz, 2H), 3.69−3.42 (m, 3H), 2.90−2.57 (m, 1H), 2.21−2.03 (m, 2H), 2.03−1.71 (m, 4H), 1.51 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 159.0, 141.1, 130.5, 129.0, 127.6, 127.5, 127.4, 114.8, 72.1, 55.1, 52.1 47.1, 34.6, 24.8, 18.6, 18.3; MS (ESI) m/z 379.2 $[M + H]^+$.

*(2S)-N-[(1R)-2-Azido-1-{4-[(2S)-2-methylbutoxy]phenyl}ethyl]-2-phenylpropanamide (**24c**).* The procedure for the synthesis of **24b** was followed starting with **23c** to afford **24c** (1.96 g, quantitative yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.23 (m, 5H), 6.96 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 5.80 (d, J = 7.8 Hz, 1H), 5.22−4.94 (m, 1H), 3.87−3.41 (m, 5H), 1.90−1.75 (m, 1H), 1.63−1.41 (m, 4H), 1.33−1.15 (m, 1H), 1.04−0.85 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 159.0, 141.0, 130.4, 129.0, 127.6, 127.5, 127.4, 114.8, 72.9, 55.1, 52.1, 47.1, 34.7, 26.1, 18.3, 16.5, 11.3; MS (ESI) m/z 381.2 $[M + H]^+$.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**25**).* To a stirred solution of **24b** (100 mg, 0.26 mmol) in MeOH/H_2O (5 mL, 1:1, v/v) at room temperature were added K_2CO_3 (55 mg, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (66 mg, 0.26 mmol), sodium ascorbate (53 mg, 0.26 mmol), and trimethylsilyl acetylene (56 μL, 0.40 mmol). After stirring at room temperature for 14 h, the reaction was treated with NH_4OH/NH_4Cl (5 mL, 1:9, v/v) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% CMA80 in DCM afforded **25** (59 mg, 55% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.39−7.16 (m, 6H), 6.81 (d, J = 8.6 Hz, 2H), 6.72 (d, J = 8.6 Hz, 2H), 6.47 (d, J = 7.9 Hz, 1H), 5.36 (dd, J = 12.2, 7.0 Hz, 1H), 4.65 (qd, J = 14.0, 5.7 Hz, 2H), 3.84 (d, J = 6.6 Hz, 2H), 3.57 (q, J = 7.1 Hz, 1H), 2.88−2.57 (m, 1H), 2.21−2.04 (m, 2H), 2.00−1.70 (m, 4H), 1.45 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 159.0, 140.9, 133.6, 129.5, 128.9, 127.5, 127.3, 127.2, 124.5, 114.9, 72.1, 54.2, 52.7, 47.0, 34.6, 24.8, 18.5, 18.1; HRMS (ESI) m/z calcd for $C_{24}H_{28}N_4O_2$ $[M + H]^+$ 405.2285, m/z found 405.2278; HPLC, t_R 16.08 min.

*(2S)-N-[(1R)-1-{4-[(2-Methylpentyl)oxy]phenyl}-2-(1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**26**).* The procedure for the synthesis of **25** was followed starting with **24a** to afford **26** (20% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 0.7 Hz, 1H), 7.40−7.08 (m, 6H), 6.88−6.60 (m, 4H), 6.30 (d, J = 7.9 Hz, 1H), 5.46−5.26 (m, 1H), 4.66 (qd, J = 14.0, 5.6 Hz, 2H), 3.82−3.49 (m, 3H), 2.00−1.77 (m, 1H), 1.50−1.14 (m, 7H), 0.98 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.1, 140.8, 133.6, 129.3, 128.9, 127.6, 127.3, 127.2, 124.5, 114.8, 73.3, 54.2, 52.8, 47.0, 35.7, 32.8, 20.0, 18.1, 16.9, 14.2; HRMS (ESI) m/z calcd for $C_{25}H_{32}N_4O_2$ $[M + H]^+$ 421.2598, m/z found 421.2594; HPLC, t_R 17.37 min.

*(2S)-N-[(1S)-2-Cyano-1-{4-[(2-methylpentyl)oxy]phenyl}ethyl]-2-phenylpropanamide (**27**).* To a solution of **20a** (200 mg, 0.50 mmol) in THF (5 mL) at room temperature was added tetrabutylammonium cyanide (336 mg, 1.25 mmol). The reaction was heated at 50 °C for 2 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. Flash column chromatography of crude product on silica gel using 0−50% EtOAc in hexanes to afford N-Boc protected cyano intermediate (150 mg, 87% yield). Removal of the Boc protecting group of this material (35 mg, 0.10 mmol) was achieved by treatment with TFA:DCM (1:2, 1 mL). After stirring at 0 °C for 1 h, the reaction was quenched with cold saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 20 mL). The organic layer was washed with brine (2 × 20 mL), dried (Na_2SO_4), and concentrated to provide the intermediate amine (25.5 mg). To a solution of the amine (25.5 mg, 0.10 mmol) in MeCN (2 mL) at 0 °C were added HBTU (59 mg, 0.15 mmol), Et_3N (43 μL, 0.31 mmol), and (*S*)-2-phenylpropionic acid (17 mg, 0.11 mmol). After stirring at room temperature for 5 h, the reaction was quenched with H_2O and diluted with EtOAc (10 mL). The layers were separated, and the organic layer was washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−50% EtOAc in hexanes afforded **27** (30 mg, 77% yield) as a brown residue: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.21 (m, 5H), 7.05 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.81 (d, J = 7.0 Hz, 1H), 5.24−4.99 (m, 1H), 3.88−3.51 (m, 3H), 3.12−2.91 (m, 1H), 2.81 (dd, J = 16.7, 4.6 Hz, 1H), 1.92 (dd, J = 12.6, 6.2 Hz, 1H), 1.53 (d, J = 7.2 Hz, 3H), 1.50−1.10 (m, 4H), 0.99 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 159.5, 140.7, 129.7, 129.0, 127.6, 127.5, 127.2, 117.0, 115.1, 73.3, 49.3, 47.0, 35.7, 32.8, 24.4, 20.0, 18.3, 16.9, 14.3; MS (ESI) m/z 379.2 $[M + H]^+$.

*(2S)-N-[(1S)-1-{4-[(2-Methylpentyl)oxy]phenyl}-2-(2H-1,2,3,4-tetrazol-5-yl)ethyl]-2-phenylpropanamide (**28**).* To a solution of **27** (20 mg, 0.05 mmol) in 1,4-dioxane (3 mL) at room temperature were added NaN_3 (4.1 mg, 0.06 mmol) and $ZnBr_2$ (18 mg, 0.08 mmol). The reaction was refluxed for 48 h. After cooling to room temperature, the mixture was acidified to pH ∼1 with 1 N HCl. The solution was extracted with EtOAc (4 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 7% MeOH in DCM afforded **28** (15 mg, 68% yield) as an off-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.31−7.13 (m, 5H), 7.05 (d, J = 8.6 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 5.31 (t, J = 7.1 Hz, 1H), 3.86−3.53 (m, 3H), 3.47−3.35 (m, 2H), 1.96−1.73 (m, 1H), 1.58−1.09 (m, 7H), 0.98 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.2, 160.2, 156.1, 142.8, 133.6,129.5, 128.6, 128.4, 127.9, 115.5, 74.2, 52.8, 47.3, 36.9, 34.1, 31.4, 21.1, 18.7, 17.3, 14.6; HRMS (ESI) m/z calcd for $C_{24}H_{31}N_5O_2$ $[M + H]^+$ 422.2551, m/z found 422.2540; HPLC, t_R 16.35 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-methyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**29**).* To a solution of **24b** (80 mg, 0.21 mmol) in *t*-BuOH:H_2O (5 mL, 1:1, v/v) at room temperature were added $CuSO_4{\cdot}5H_2O$ (21.1 mg, 0.08 mmol), sodium ascorbate (41.8 mg, 0.21 mmol), and propyne bubbled into the reaction via a needle. After stirring at room temperature overnight, the reaction was treated with NH_4OH/NH_4Cl (5 mL, 1:9, v/v) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% CMA80 in DCM afforded **29** (71 mg, 80% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.07 (m, 5H), 6.96 (s, 1H), 6.83 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.7 Hz, 2H), 6.41 (d, J = 7.9 Hz, 1H), 5.43−5.12 (m, 1H), 4.56 (qd, J = 14.0, 5.8 Hz, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.56 (q, J = 7.1 Hz, 1H), 2.87−2.54 (m, 1H), 2.25 (s, 3H), 2.19−2.02 (m, 2H), 2.02−1.74 (m, 4H), 1.45 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 159.0, 143.1, 140.9, 129.7, 128.9, 127.5, 127.3, 122.2, 114.8, 72.1, 54.1, 52.8, 47.0, 34.6, 24.8, 18.5, 18.1, 10.7; HRMS (ESI) m/z calcd for $C_{25}H_{30}N_4O_2$ $[M + H]^+$ 419.2442, m/z found 419.2435; HPLC, t_R 16.07 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4,5-dimethyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**30**).* A solution of **24b** (50 mg, 0.13 mmol) and 2-butyne (0.1 mL, 1.3 mmol) in toluene (2 mL) was heated at 125 °C for 18 h in a sealed tube. After cooling to room temperature, the mixture was concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−100% EtOAc in hexanes afforded **30** (38 mg, 63% yield) as a white waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.15 (m, 5H), 6.83 (d, J = 7.8 Hz, 1H), 6.77−6.61 (m, 4H), 5.40−5.18 (m, 1H), 4.41 (ddd, J = 20.8, 14.2, 5.3 Hz, 2H), 3.84 (d, J = 6.7 Hz, 2H), 3.58 (q, J = 7.1 Hz, 1H), 2.89−2.50 (m, 1H), 2.17 (s, 3H), 2.15−1.80 (m, 6H), 1.79 (d, J = 4.8 Hz, 3H), 1.45 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 159.0, 141.1, 140.4, 130.1, 128.8, 127.5, 127.2, 127.1, 114.8, 72.2, 52.3, 52.1, 46.9, 34.6, 24.8, 18.5, 18.1, 10.2, 7.2; HRMS (ESI) m/z calcd for $C_{26}H_{32}N_4O_2$ $[M + H]^+$ 433.2598, m/z found 433.2592; HPLC, t_R 16.06 min.

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*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-ethyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**31**).* To a solution of **24b** (51 mg, 0.13 mmol) in MeOH (5 mL) were added sodium ascorbate (30 mg, 0.16 mmol), $CuSO_4{\cdot}5H_2O$ (30 mg, 0.13 mmol), 2-pentynoic acid (15 μL, 0.15 mmol), and Cs_2CO_3 (33 mg, 0.1 mmol). The reaction was heated at 60 °C for 2 h. After cooling to room temperature, the mixture was filtered through a short of pad of Celite, the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC on silica gel eluted with 30% EtOAC/hexanes to afford **31** (36 mg, 60% yield) as a white waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.14 (m, 5H), 6.96 (s, 1H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.40 (d, *J* = 7.8 Hz, 1H), 5.32 (td, *J* = 7.3, 4.7 Hz, 1H), 4.57 (qd, *J* = 14.0, 5.8 Hz, 2H), 3.85 (d, *J* = 6.6 Hz, 2H), 3.56 (q, *J* = 7.2 Hz, 1H), 2.85−2.49 (m, 3H), 2.21−2.01 (m, 2H), 2.01−1.65 (m, 4H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 149.7, 140.9, 129.7, 128.9, 127.5, 127.2, 121.3, 114.8, 72.1, 54.22, 52.8, 47.0, 34.6, 24.8, 18.9, 18.5, 18.1, 13.7; HRMS (ESI) *m*/*z* calcd for $C_{26}H_{32}N_4O_2$ $[M + H]^+$ 433.2598, *m*/*z* found 433.2591; HPLC, t_R 16.40 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-propyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**32**).* The procedure for the synthesis of **29** was followed starting with **24b** and 1-pentyne to afford **32** (80% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.14 (m, 5H), 6.94 (s, 1H), 6.80 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 6.52 (d, *J* = 7.9 Hz, 1H), 5.48−5.14 (m, 1H), 4.56 (qd, *J* = 14.0, 5.7 Hz, 2H), 3.84 (d, *J* = 6.6 Hz, 2H), 3.57 (q, *J* = 7.1 Hz, 1H), 2.82−2.64 (m, 1H), 2.59 (t, *J* = 7.5 Hz, 2H), 2.23−2.03 (m, 2H), 2.04−1.77 (m, 4H), 1.70−1.50 (m, 2H), 1.44 (d, *J* = 7.1 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 148.0, 141.0, 129.7, 128.8, 127.5, 127.2, 121.8, 114.8, 72.1, 54.2, 52.7, 46.9, 34.6, 27.5, 24.8, 22.6, 18.5, 18.2, 13.6; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{34}N_4O_2$ $[M + H]^+$ 447.2755, *m*/*z* found 447.2750; HPLC, t_R 17.17 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-[4-(propan-2-yl)-1H-1,2,3-triazol-1-yl]ethyl]-2-phenylpropanamide (**33**).* The procedure for the synthesis of **29** was followed starting with **24b** and 3-methyl-1-butyne to afford **33** (56% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.12 (m, 5H), 6.92 (s, 1H), 6.83−6.65 (m, 4H), 6.34 (d, *J* = 7.8 Hz, 1H), 5.39−5.23 (m, 1H), 4.62 (dd, *J* = 14.0, 4.4 Hz, 1H), 4.50 (dd, *J* = 14.0, 6.9 Hz, 1H), 3.85 (d, *J* = 6.7 Hz, 2H), 3.55 (q, *J* = 7.1 Hz, 1H), 3.09−2.91 (m, 1H), 2.82−2.60 (m, 1H), 2.19−2.02 (m, 2H), 2.02−1.74 (m, 4H), 1.45 (d, *J* = 7.2 Hz, 3H), 1.23 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 154.5, 141.0, 129.7, 128.9, 127.5, 127.2, 127.2, 120.4, 114.8, 72.1, 54.3 52.8, 47.0, 34.6, 25.7, 24.8, 22.5, 22.5, 18.5, 18.1; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{34}N_4O_2$ $[M + H]^+$ 447.2755, *m*/*z* found 447.2748; HPLC, t_R 17.08 min.

*(2S)-N-[(1R)-2-(4-tert-Butyl-1H-1,2,3-triazol-1-yl)-1-[4-(cyclobutylmethoxy)phenyl]ethyl]-2-phenylpropanamide (**34**).* The procedure for the synthesis of **29** was followed starting with **24b** and 3,3-dimethyl-1-butyne to afford **34** (80% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.20 (m, 5H), 6.91 (s, 1H), 6.82−6.66 (m, 4H), 6.43−6.27 (m, 1H), 5.41−5.23 (m, 1H), 4.55 (ddd, *J* = 21.0, 14.0, 5.7 Hz, 2H), 3.85 (d, *J* = 6.7 Hz, 2H), 3.55 (q, *J* = 7.1 Hz, 1H), 2.83−2.60 (m, 1H), 2.21−2.02 (m, 2H), 2.02−1.76 (m, 4H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 157.5, 141.0, 129.8, 128.8, 127.5, 127.2, 119.8, 114.8, 72.2, 54.3, 52.8, 47.0, 34.6, 30.6, 30.3, 24.8, 18.5, 18.2; HRMS (ESI) *m*/*z* calcd for $C_{28}H_{36}N_4O_2$ $[M + H]^+$ 461.2911, *m*/*z* found 461.2905; HPLC, t_R 17.56 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-hexyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**35**).* The procedure for the synthesis of **29** was followed starting with **24b** and 1-octyne to afford **35** (90% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.15 (m, 5H), 6.93 (s, 1H), 6.75 (dd, *J* = 21.9, 8.8 Hz, 4H), 6.44 (d, *J* = 7.9 Hz, 1H), 5.44−5.21 (m, 1H), 4.56 (ddd, *J* = 20.8, 14.0, 5.6 Hz, 2H), 3.84 (d, *J* = 6.6 Hz, 2H), 3.57 (q, *J* = 7.1 Hz, 1H), 2.84−2.66 (m, 1H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.23−2.02 (m, 2H), 2.02−1.69 (m, 4H), 1.63−1.48 (m, 2H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.28 (s, 6H), 0.88 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 148.2, 141.0, 129.7, 128.9, 127.5, 127.2, 127.2, 121.8, 114.8, 72.1, 54.3, 52.7, 47.0, 34.6, 31.5, 29.4, 28.8, 25.5, 24.8, 22.5, 18.5, 18.1, 14.0; HRMS (ESI) *m*/*z* calcd for $C_{30}H_{40}N_4O_2$ $[M + H]^+$ 489.3224, *m*/*z* found 489.3218; HPLC, t_R 19.07 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-cyclopropyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**36**).* The procedure for the synthesis of **29** was followed starting with **24b** and cyclopropyl acetylene to afford **36** (80% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.18 (m, 5H), 6.92 (s, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 8.7 Hz, 2H), 6.36 (d, *J* = 7.7 Hz, 1H), 5.30 (dd, *J* = 12.0, 7.1 Hz, 1H), 4.54 (qd, *J* = 14.0, 5.8 Hz, 2H), 3.86 (d, *J* = 6.6 Hz, 2H), 3.55 (q, *J* = 7.0 Hz, 1H), 2.86−2.56 (m, 1H), 2.24−2.03 (m, 2H), 2.03−1.69 (m, 5H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.03−0.82 (m, 2H), 0.82−0.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 150.1, 140.9, 129.7, 128.9, 127.5, 127.3, 120.7, 114.8, 72.1, 54.2, 52.7, 47.0, 34.6, 24.8, 18.5, 18.2, 7.7, 7.6, 6.5; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{32}N_4O_2$ $[M + H]^+$ 445.2598, *m*/*z* found 445.2592; HPLC, t_R 16.73 min.

*(2S)-N-[(1R)1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**37**).* The procedure for the synthesis of **29** was followed starting with **24b** and cyclohexyl acetylene to furnish **37** (80% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.14 (m, 5H), 6.91 (s, 1H), 6.75 (q, *J* = 8.8 Hz, 4H), 6.41 (d, *J* = 7.9 Hz, 1H), 5.43−5.18 (m, 1H), 4.56 (ddd, *J* = 21.0, 14.0, 5.7 Hz, 2H), 3.85 (d, *J* = 6.7 Hz, 2H), 3.55 (q, *J* = 7.1 Hz, 1H), 2.90−2.56 (m, 2H), 2.21−2.04 (m, 2H), 2.03−1.58 (m, 10H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.40−1.19 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 153.5, 141.0, 129.8, 128.8, 127.5, 127.2, 120.6, 114.8, 72.2, 54.3, 52.8, 47.0, 35.1, 34.6, 32.9, 32.9, 26.0, 24.8, 18.5, 18.2; HRMS (ESI) *m*/*z* calcd for $C_{30}H_{38}N_4O_2$ $[M + H]^+$ 487.3068, *m*/*z* found 487.3062; HPLC, t_R 18.26 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**38**).* The procedure for the synthesis of **29** was followed starting with **24b** and phenylacetylene to afford **38** (74% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.1 Hz, 2H), 7.47−7.37 (m, 3H), 7.37−7.15 (m, 6H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.26 (d, *J* = 7.7 Hz, 1H), 5.38 (dd, *J* = 12.1, 6.8 Hz, 1H), 4.68 (qd, *J* = 14.1, 5.8 Hz, 2H), 3.85 (d, *J* = 6.7 Hz, 2H), 3.58 (q, *J* = 7.1 Hz, 1H), 2.72 (dt, *J* = 14.5, 7.3 Hz, 1H), 2.21−2.01 (m, 2H), 2.01−1.66 (m, 4H), 1.47 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 159.1, 147.6, 140.8, 130.4, 129.4, 128.9, 128.8, 128.2, 127.5, 127.3, 127.3, 125.8, 120.6, 115.0, 72.1, 54.4, 52.9, 47.1, 34.6, 24.8, 18.5, 18.1; HRMS (ESI) *m*/*z* calcd for $C_{30}H_{32}N_4O_2$ $[M + H]^+$ 481.2598, *m*/*z* found 481.2591; HPLC, t_R 17.70 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]ethyl]-2-phenylpropanamide (**39**).* The procedure for the synthesis of **29** was followed starting with **24b** and propargyl alcohol to afford **39** (75% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52−7.14 (m, 6H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.28 (d, *J* = 7.9 Hz, 1H), 5.33 (dd, *J* = 12.6, 7.2 Hz, 1H), 4.77−4.49 (m, 4H), 3.85 (d, *J* = 6.6 Hz, 2H), 3.56 (q, *J* = 7.1 Hz, 1H), 2.73 (dt, *J* = 14.8, 7.5 Hz, 1H), 2.51 (br s, 1H), 2.22−2.02 (m, 2H), 2.02−1.77 (m, 4H), 1.45 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 159.1, 147.5, 140.8, 129.3, 128.9, 127.5, 127.3, 127.3, 122.6, 114.9, 72.1, 56.5, 54.2, 52.8, 47.0, 34.6, 24.8, 18.5, 18.1; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{30}N_4O_3$ $[M + H]^+$ 435.2391, *m*/*z* found 435.2385; HPLC, t_R 14.49 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-[4-(methoxymethyl)-1H-1,2,3-triazol-1-yl]ethyl]-2-phenylpropanamide (**40**).* The procedure for the synthesis of **29** was followed starting with **24b** and methoxyethyne to afford **40** (75% yield) as a white solid: ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.31 (t, *J* = 7.5 Hz, 2H), 7.28−7.24 (m, 1H), 7.22 (d, *J* = 5.9 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.36 (d, *J* = 7.8 Hz, 1H), 5.41−5.29 (m, 1H), 4.65 (dd, *J* = 14.1, 4.6 Hz, 1H), 4.58 (dd, *J* = 14.1, 7.0 Hz, 1H), 4.52−4.44 (m, 2H), 3.85 (d, *J* = 6.6 Hz, 2H), 3.57 (q, *J* = 7.1 Hz, 1H), 3.32 (s, 3H), 2.82−2.64 (m, 1H), 2.16 − 2.05 (m, 2H), 2.00−1.87 (m, 2H), 1.87−

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1.75 (m, 2H), 1.46 (d, J = 7.2 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.8, 159.0, 144.9, 140.9, 129.4, 128.9, 127.5, 127.3, 127.2, 123.5, 114.8, 72.1, 65.7, 58.1, 54.3, 52.7, 47.0, 34.5, 24.8, 18.5, 18.2; HRMS (ESI) m/z calcd for $C_{26}H_{32}N_4O_3$ $[M + H]^+$ 449.2547, m/z found 449.2543; HPLC, t_R 15.60 min.

*{1-[(2R)-2-[4-(Cyclobutylmethoxy)phenyl]-2-[(2S)-2-phenylpropanamido]ethyl]-1H-1,2,3-triazol-4-yl}methyl Acetate (**41**).* The procedure for the synthesis of **29** was followed starting with **24b** and propargyl acetate to afford **41** (70% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.10 (m, 5H), 6.82 (d, J = 8.7 Hz, 2H), 6.77−6.64 (m, 2H), 6.28 (d, J = 7.9 Hz, 1H), 5.41−5.25 (m, 1H), 5.11 (s, 2H), 4.62 (qd, J = 14.0, 5.8 Hz, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.56 (q, J = 7.1 Hz, 1H), 2.90−2.51 (m, 1H), 2.22−2.06 (m, 2H), 2.04 (s, 3H), 1.99−1.75 (m, 4H), 1.46 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 170.7, 159.1, 142.7, 140.8, 128.9, 127.5, 127.3, 127.2, 124.8, 114.9, 72.1, 57.4, 54.3, 52.8, 47.0, 34.6, 24.8, 20.8, 18.5, 18.1; HRMS (ESI) m/z calcd for $C_{27}H_{32}N_4O_4$ $[M + H]^+$ 477.2496, m/z found 477.2489; HPLC, t_R 16.20 min.

*(2S)-N-[(1R)-2-[4-(Aminomethyl)-1H-1,2,3-triazol-1-yl]-1-[4-(cyclobutylmethoxy)phenyl]ethyl]-2-phenylpropanamide Hydrochloride (**42**).* The procedure for the synthesis of **29** was followed starting with **24b** and propargylamine to afford **42** (75% yield) as a white solid: ^{1}H NMR [free base] (300 MHz, $CDCl_3$) δ 7.43−7.16 (m, 5H), 7.12 (s, 1H), 6.83 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 8.7 Hz, 2H), 6.30 (d, J = 7.8 Hz, 1H), 5.33 (dd, J = 11.9, 6.9 Hz, 1H), 4.60 (qd, J = 14.0, 5.8 Hz, 2H), 3.98−3.73 (m, 4H), 3.57 (q, J = 7.2 Hz, 1H), 2.91−2.55 (m, 1H), 2.23−2.02 (m, 2H), 2.02−1.70 (m, 4H), 1.46 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.1, 149.5, 140.9, 129.5, 128.9, 127.5, 127.3, 127.2, 121.7, 114.9, 72.1, 54.3, 52.8, 47.0, 37.5, 34.6, 24.8, 18.5, 18.2; HRMS (ESI) m/z calcd for $C_{25}H_{31}N_5O_2$ $[M + H]^+$ 434.2551, m/z found 434.2542; HPLC, t_R 13.06 min. The sample was converted into the HCl salt.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-{4-[(methylamino)methyl]-1H-1,2,3-triazol-1-yl}ethyl]-2-phenylpropanamide (**43**).* The procedure for the synthesis of **29** was followed starting with **24b** and *N*-methylpropargylamine to afford **43** (80% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44−7.20 (m, 5H), 7.16 (s, 1H), 6.81 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.8 Hz, 2H), 6.37 (d, J = 7.9 Hz, 1H), 5.50−5.25 (m, 1H), 4.60 (qd, J = 14.0, 5.7 Hz, 2H), 3.84 (d, J = 6.6 Hz, 2H), 3.78 (s, 2H), 3.58 (q, J = 7.1 Hz, 1H), 2.80−2.63 (m, 1H), 2.38 (s, 3H), 2.23−2.04 (m, 2H), 2.04−1.76 (m, 4H), 1.51−1.39 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 159.0, 146.2, 140.9, 129.5, 128.9, 127.5, 127.3, 127.2, 122.8, 114.9, 72.1, 54.3, 52.7, 47.0, 46.3, 35.6, 34.6, 24.8, 18.5, 18.2; HRMS (ESI) m/z calcd for $C_{26}H_{33}N_5O_2$ $[M + H]^+$ 448.2707, m/z found 448.2700; HPLC, t_R 13.41 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-{4-[(dimethylamino)methyl]-1H-1,2,3-triazol-1-yl}ethyl]-2-phenylpropanamide (**44**).* The procedure for the synthesis of **29** was followed starting with **24b** and *N*,*N*-dimethylpropargylamine to afford **44** (75% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.11 (m, 5H), 7.07 (s, 1H), 6.67 (dd, J = 20.0, 8.7 Hz, 4H), 6.31 (d, J = 7.9 Hz, 1H), 5.42−5.14 (m, 1H), 4.53 (ddd, J = 20.6, 14.0, 5.5 Hz, 2H), 3.77 (d, J = 6.6 Hz, 2H), 3.60−3.31 (m, 3H), 2.78−2.49 (m, 1H), 2.13 (s, 6H), 2.10−1.98 (m, 2H), 1.93−1.64 (m, 4H), 1.40 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 157.3, 143.0, 139.2, 127.7, 127.2, 125.8, 125.5, 125.4, 122.0, 113.1, 70.4, 52.8, 52.4, 50.9, 45.3, 43.1, 32.8, 23.1, 16.8, 16.4; HRMS (ESI) m/z calcd for $C_{27}H_{35}N_5O_2$ $[M + H]^+$ 462.2864, m/z found 462.2855; HPLC, t_R 14.07 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-ethoxy-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**45**).* The procedure for the synthesis of **29** was followed starting with **24b** and ethoxyethyne to afford **45** (80% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.09 (m, 5H), 6.84 (d, J = 8.7 Hz, 2H), 6.79−6.70 (m, 2H), 6.68 (s, 1H), 6.29 (d, J = 7.9 Hz, 1H), 5.42−5.19 (m, 1H), 4.51 (qd, J = 14.0, 5.9 Hz, 2H), 4.28−4.06 (m, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.56 (q, J = 7.1 Hz, 1H), 2.85−2.50 (m, 1H), 2.24−2.03 (m, 2H), 2.03−1.73 (m, 4H), 1.46 (d, J = 7.2 Hz, 3H), 1.37 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 160.6, 159.1, 140.9, 129.5, 128.9, 127.5, 127.3, 127.2, 114.9, 107.2, 72.1, 66.3, 55.0, 52.6, 47.0, 34.6, 24.8, 18.5, 18.2, 14.7; HRMS (ESI) m/z calcd for $C_{26}H_{32}N_4O_3$ $[M + H]^+$ 449.2547, m/z found 449.2541; HPLC, t_R 16.69 min.

*(2S)-N-[(1R)-1-[4-(Cyclobutylmethoxy)phenyl]-2-(4-formyl-1H-1,2,3-triazol-1-yl)ethyl]-2-phenylpropanamide (**46**).* To a solution of alcohol **39** (30 mg, 0.07 mmol) in DCM (2 mL, saturated with H_2O) was added Dess−Martin periodinane (44 mg, 0.10 mmol). After stirring at room temperature for 3 h, the reaction was treated with saturated $NaHCO_3$ (3 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−10% MeOH in DCM afforded **46** (21 mg, 70% yield) as a thick oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.74 (s, 1H), 7.45−7.12 (m, 5H), 6.78 (dd, J = 20.6, 8.8 Hz, 4H), 6.06 (d, J = 7.5 Hz, 1H), 5.34 (dd, J = 12.9, 6.2 Hz, 1H), 4.88−4.52 (m, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.59 (q, J = 7.2 Hz, 1H), 2.81−2.55 (m, 1H), 2.20−2.01 (m, 2H), 2.01−1.73 (m, 4H), 1.49 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.6, 174.0, 159.4, 147.3, 140.6, 129.0, 128.5, 127.5, 127.2, 126.2, 115.1, 72.1, 54.6, 53.0, 47.1, 34.5, 24.8, 18.5, 18.0; HRMS (ESI) m/z calcd for $C_{25}H_{28}N_4O_3$ $[M + H]^+$ 433.2230, m/z found 433.2230; HPLC, t_R 15.85 min.

*(2S)-N-[(1R)-2-(4-Acetyl-1H-1,2,3-triazol-1-yl)-1-[4-(cyclobutylmethoxy)phenyl]ethyl]-2-phenylpropanamide (**47**).* The procedure for the synthesis of **29** was followed starting with **24b** and but-3-yn-2-one to afford **47** (50% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.37−7.15 (m, 5H), 6.83 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 8.8 Hz, 2H), 6.10 (d, J = 7.8 Hz, 1H), 5.34 (dd, J = 12.6, 6.8 Hz, 1H), 4.84−4.44 (m, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.58 (q, J = 7.2 Hz, 1H), 2.83−2.68 (m, 1H), 2.65 (s, 3H), 2.21−2.02 (m, 2H), 2.02−1.77 (m, 4H), 1.48 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 173.9, 159.3, 147.8, 140.7, 129.0, 127.5, 127.4, 127.2, 126.3, 115.1, 72.1, 54.5, 52.9, 47.1, 34.5, 27.1, 24.8, 18.5, 18.1; HRMS (ESI) m/z calcd for $C_{26}H_{30}N_4O_3$ $[M + H]^+$ 447.2391, m/z found 447.2381; HPLC, t_R 16.33 min.

*Ethyl 1-[(2R)-2-[4-(Cyclobutylmethoxy)phenyl]-2-[(2S)-2-phenylpropanamido]ethyl]-1H-1,2,3- triazole-4-carboxylate (**48**).* The procedure for the synthesis of **29** was followed starting with **24b** and ethyl propiolate to afford **48** (77% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.38−7.12 (m, 5H), 6.84 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 8.7 Hz, 2H), 6.21 (d, J = 7.8 Hz, 1H), 5.34 (dd, J = 12.6, 6.9 Hz, 1H), 4.85−4.59 (m, 2H), 4.39 (q, J = 7.1 Hz, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.57 (q, J = 7.1 Hz, 1H), 2.86−2.59 (m, 1H), 2.22−2.03 (m, 2H), 2.03−1.72 (m, 4H), 1.47 (d, J = 7.2 Hz, 3H), 1.39 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 160.5, 159.2, 140.7, 140.0, 129.0, 127.5, 127.4, 127.3, 115.1, 72.1, 61.3, 54.5, 52.9, 47.0, 34.5, 24.8, 18.5, 18.1, 14.3; HRMS (ESI) m/z calcd for $C_{27}H_{32}N_4O_4$ $[M + H]^+$ 477.2496, m/z found 477.2489; HPLC, t_R 16.72 min.

*1-[(2R)-2-[4-(Cyclobutylmethoxy)phenyl]-2-[(2S)-2-phenylpropanamido]ethyl]-1H-1,2,3-triazole-4-carboxamide (**49**).* The procedure for the synthesis of **29** was followed starting with **24b** and propiolamide to afford **49** (76% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H), 7.32−7.08 (m, 7H), 6.82 (d, J = 8.7 Hz, 2H), 5.42 (dd, J = 9.1, 5.7 Hz, 1H), 4.83−4.63 (m, 2H), 3.88 (d, J = 6.6 Hz, 2H), 3.62 (q, J = 7.2 Hz, 1H), 2.89−2.51 (m, 1H), 2.22−2.03 (m, 2H), 2.03−1.73 (m, 4H), 1.32 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 164.7, 160.6, 143.6, 142.6, 131.2, 129.5, 128.9, 128.3, 128.2, 128.0, 115.8, 73.2, 55.3, 54.1, 47.4, 36.1, 25.7, 19.3, 18.7; HRMS (ESI) m/z calcd for $C_{25}H_{29}N_5O_3$ $[M + H]^+$ 448.2343, m/z found 448.2341; HPLC, t_R 15.68 min.

*1-[(2R)-2-[4-(Cyclobutylmethoxy)phenyl]-2-[(2S)-2-phenylpropanamido]ethyl]-N-methyl-1H-1,2,3-triazole-4-carboxamide (**50**).* A solution of ester **48** (50 mg, 0.10 mmol) and NaOH (4.6 mg, 0.11 mmol) in MeOH/H_2O (1 mL, 1:1) was stirred at room temperature for 2 h. The reaction was acidified with 1 N HCl and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford the corresponding acid (45 mg, 95% yield) as a white solid: MS (ESI) m/z 449 $[M + H]^+$. The carboxylic acid (50 mg, 0.11 mmol) was then

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dissolved in DMF (4 mL), followed by addition of EDCI (32.1 mg, 0.17 mmol), HOBt (22.6 mg, 0.17 mmol), DIPEA (99.6 μL, 0.55 mmol) and methylamine hydrochloride (11.3 mg, 0.17 mmol). After stirring at room temperature for 24 h, the mixture was diluted with EtOAc (5 mL) and treated with saturated $NaHCO_3$ (2 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine (4 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0−30% CMA80 in DCM afforded **50** (42 mg, 82% yield) as a white solid: ^{1}H NMR (700 MHz, CD_3OD) δ 8.21 (s, 1H), 7.27−7.16 (m, 5H), 7.15−7.10 (m, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.42 (dd, J = 9.4, 5.5 Hz, 1H), 4.74 (ddd, J = 23.4, 13.9, 7.5 Hz, 2H), 4.58 (s, 1H), 3.89 (d, J = 6.5 Hz, 2H), 3.63 (q, J = 7.1 Hz, 1H), 2.93 (d, J = 4.9 Hz, 3H), 2.76−2.65 (m, 1H), 2.23−2.07 (m, 2H), 2.06−1.77 (m, 4H), 1.32 (d, J = 7.1 Hz, 3H); ^{13}C NMR (175 MHz, CD_3OD) δ 173.6, 160.1, 157.6, 140.8, 139.7, 128.2, 126.6, 126.0, 125.4, 125.0, 124.7, 112.8, 70.2, 52.3, 51.1, 44.4, 33.1, 23.1, 22.8, 16.4, 15.8; HRMS (ESI) m/z calcd for $C_{26}H_{31}N_5O_3$ $[M + H]^+$ 462.2500, m/z found 462.2649; HPLC, t_R 15.14 min.

*1-[(2R)-2-[4-(Cyclobutylmethoxy)phenyl]-2-[(2S)-2-phenylpropanamido]ethyl]-N,N-dimethyl-1H-1,2,3-triazole-4-carboxamide (**51**).* The procedure for the synthesis of **50** was followed starting with **48** and dimethylamine hydrochloride to afford **51** (75% yield) as a sticky solid: ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.34−7.28 (m, 2H), 7.27−7.19 (m, 4H), 6.83 (d, J = 8.6 Hz, 2H), 6.74 (d, J = 8.7 Hz, 2H), 6.23 (d, J = 7.8 Hz, 1H), 5.38−5.27 (m, 1H), 4.66 (ddd, J = 20.9, 14.0, 5.8 Hz, 2H), 3.85 (d, J = 6.6 Hz, 2H), 3.58 (q, J = 7.2 Hz, 1H), 3.48 (s, 3H), 3.09 (d, J = 6.1 Hz, 3H), 2.80−2.64 (m, 1H), 2.17−2.05 (m, 2H), 2.01−1.88 (m, 2H), 1.88−1.73 (m, 2H), 1.47 (d, J = 7.2 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.8, 161.2, 159.2, 144.3, 140.9, 128.9, 128.8, 127.6, 127.4, 127.2, 114.9, 72.0, 54.3, 52.8, 47.0, 38.7, 36.3, 34.5, 24.8, 18.6, 18.5, 18.2; HRMS (ESI) m/z calcd for $C_{27}H_{33}N_5O_3$ $[M + H]^+$ 476.2656, m/z found 476.2649; HPLC, t_R 15.47 min.

*(2S)-N-[(1R)-2-[4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl]-1-{4-[(2S)-2-methylbutoxy]phenyl} ethyl]-2-phenylpropanamide (**52**).* The procedure for the synthesis of **29** was followed starting with **24c** and propargyl alcohol to afford **52** (63% yield) as a waxy white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44−7.11 (m, 6H), 6.90 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.7 Hz, 2H), 6.61 (d, J = 8.1 Hz, 1H), 5.35 (dd, J = 14.2, 6.4 Hz, 1H), 4.75−4.50 (m, 4H), 3.83−3.59 (m, 3H), 3.54 (q, J = 7.1 Hz, 1H), 2.33 (br s, 1H), 1.97−1.68 (m, 1H), 1.67−1.46 (m, 1H), 1.40 (d, J = 7.1 Hz, 3H), 1.33−1.14 (m, 1H), 0.98 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 174.1, 159.1, 147.8, 140.9, 129.4, 128.8, 127.5, 127.4, 127.3, 122.8, 114.8, 72.90, 56.2, 54.2, 52.8, 46.8, 34.7, 26.1, 18.2, 16.5, 11.3; HRMS (ESI) m/z calcd for $C_{25}H_{32}N_4O_3$ $[M + H]^+$ 437.2547, m/z found 437.2529; HPLC, t_R 15.47 min.

*N-Methyl-1-[(2R)-2-{4-[(2S)-2-methylbutoxy]phenyl}-2-[(2S)-2-phenylpropanamido]ethyl]-1H-1,2,3-triazole-4-carboxamide (**53**).* The procedure for the synthesis of **50** was followed starting with **24c** and ethyl propiolate to afford **53** (27% yield over 3 steps) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.21 (d, J = 1.4 Hz, 1H), 7.18 (ddd, J = 15.8, 9.6, 6.8 Hz, 7H), 6.91−6.66 (m, 2H), 5.41 (dd, J = 9.1, 5.7 Hz, 1H), 4.83−4.64 (m, 3H), 3.87−3.67 (m, 2H), 3.62 (q, J = 7.0 Hz, 1H), 2.92 (s, 3H), 1.91−1.68 (m, 1H), 1.65−1.42 (m, 1H), 1.29 (dd, J = 13.4, 7.4 Hz, 4H), 0.99 (d, J = 6.7 Hz, 3H), 0.94 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 160.6, 143.8, 142.6, 131.1, 129.5, 128.9, 128.3, 128.0, 127.6, 115.8, 73.9, 55.2, 54.1, 47.4, 36.0, 27.1, 26.1, 18.7, 16.8, 11.6; HRMS (ESI) m/z calcd for $C_{26}H_{33}N_5O_3$ $[M + H]^+$ 464.2656, m/z found 464.2650; HPLC, t_R 15.97 min.

Pharmacology. *Materials.* Cell culture materials were purchased from Fisher SSI. Forskolin was purchased from Sigma-Aldrich. The Lance *Ultra* kit (TRF0262) was purchased from PerkinElmer.

Lance Ultra cAMP Assay Using Stable PPLS-HA-GPR88 CHO Cells. All cAMP assays were performed using our previously published methods.[24] Stimulation buffer containing 1X Hank's balanced salt solution (HBSS), 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 0.1% bovine serum albumin (BSA) stabilizer, and 0.5 mM final IBMX was prepared and titrated to pH 7.4 at room temperature. Serial dilutions of the test compounds (5 μL) and 300 nM forskolin (5 μL), both prepared at 4× the desired final concentration in 2% DMSO/stimulation buffer, were added to a 96-well white 1/2 area microplate (PerkinElmer). A cAMP standard curve was prepared at 4× the desired final concentration in stimulation buffer and 5 μL was added to the assay plate. Stable PPLS-HA-GPR88 CHO cells were lifted with versene and spun at 270*g* for 10 min. The cell pellet was resuspended in stimulation buffer and 4000 cells (10 μL) were added to each well except wells containing the cAMP standard curve. After incubating for 30 min at room temperature, Eu-cAMP tracer and u*LIGHT-anti-cAMP* working solutions were added per the manufacturer's instructions. After incubation at room temperature for 1 h, the TR-FRET signal (ex 337 nm) was read on a CLARIOstar multimode plate reader (BMG Biotech, Cary, NC).

Data Analysis. The TR-FRET signal (665 nm) was converted to fmol cAMP by interpolating from the standard cAMP curve. Fmol cAMP was plotted against the log of compound concentration and data were fit to a three-parameter logistic curve to generate EC_{50} values (Prism, version 6.0, GraphPad Software, Inc., San Diego, CA). The E_{max} value for each test compound relative to the control compound RTI-13951-33 was calculated with the equation % control E_{max} = (maximal test compound signal/maximal control signal) × 100.

[^{35}S]GTPγS Binding Assay. [^{35}S]GTPγS binding assays were performed on membrane preparations from wild-type (WT) mice or GPR88 KO mice, following our previously published methods.[15,16] To assess [^{35}S]GTPγS binding in the whole striatal region, brains were quickly removed after cervical dislocation and the whole striatal region was dissected out, frozen, and stored at −80 °C until use. Membranes were prepared by homogenizing brain samples in ice-cold 0.25 M sucrose solution 10 vol (mL/g wet weight of tissue). The obtained suspensions were then centrifuged at 2500*g* for 10 min. Supernatants were collected and diluted 10 times in buffer containing 50 mM TrisHCl (pH 7.4), 3 mM $MgCl_2$, 100 mM NaCl, and 0.2 mM EGTA, and then centrifuged at 23 000*g* for 30 min. The pellets were homogenized in 800 μL of ice-cold sucrose solution (0.32 M), aliquoted, and kept at −80 °C. For [^{35}S]GTPγS binding assays, 2 μg of protein was used per well. Samples were incubated with and without the test compound for 1 h at 25 °C in an assay buffer containing 30 mM GDP and 0.1 nM [^{35}S]GTPγS. Bound radioactivity was quantified using a liquid scintillation counter. Nonspecific binding was defined as binding in the presence of 10 μM GTPγS; basal binding refers to binding in the absence of the agonist. Data were expressed as a mean percentage of activation above the basal binding. GTPγS binding by agonist was plotted with X axis representing concentration and Y axis representing the percentage of activation against background. EC_{50} values were calculated using GraphPad Prism software. The E_{max} is expressed as percentage of activation above the basal binding, which is set as 100%, and the basal binding refers to binding in the absence of the agonist.

Bidirectional MDCK-MDR1 Permeability Assay. MDCK-MDR1 cells at passage 17 were seeded onto permeable polycarbonate supports in 12-well Costar Transwell plates and allowed to grow and differentiate for 3 days. On day 3, culture medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS)) was removed from both sides of the transwell inserts, and cells were rinsed with warm HBSS. After the rinse step, the chambers were filled with warm transport buffer (HBSS containing 10 mM HEPES, 0.25% BSA, pH 7.4), and the plates were incubated at 37 °C for 30 min prior to Trans Epithelial Electric Resistance (TEER) measurements.

The buffer in the donor chamber (apical side for A-to-B assay, basolateral side for B-to-A assay) was removed and replaced with the working solution (10 μM test article in transport buffer). The plates were then placed at 37 °C under light agitation. At designated time points (30, 60, and 90 min), an aliquot of transport buffer from the receiver chamber was removed and replenished with fresh transport

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buffer. Samples were quenched with ice-cold acetonitrile containing internal standard and then centrifuged to pellet protein. Resulting supernatants are further diluted with 50/50 acetonitrile/water (water only for atenolol) and submitted for LC-MS/MS analysis. Reported apparent permeability (P_{app}) values were calculated from single determination. Atenolol and propranolol were tested as low and moderate permeability references. Bidirectional transport of digoxin was assessed to demonstrate *P*gp activity/expression.

The apparent permeability (P_{app}, measured in cm/s) of a compound is determined according to the following formula: $P_{app} = (dQ/dt)/(AC_i \times 60)$, where dQ/dt is the net rate of appearance in the receiver compartment, A is the area of the Transwell measured in cm^2 (1.12 cm^2), C_i is the initial concentration of compound added to the donor chamber, and 60 is the conversion factor for minute to seconds.

Pharmacokinetic Analysis. Male C57BL/6NCrl mice were procured from Charles River Laboratories at 8−9 weeks of age. The animals were allowed to acclimate to the vivarium for a period of 1 week. Each mouse was administered a single 20 mg/kg dose of **53** through IP injection in a 10 mL/kg dosing volume. Doses were formulated in 2% NMP in canola oil. Three animals were sacrificed at each of the selected time points (0.25, 0.5, 1, 2, 4, 6, 8, and 24 s) post-dose. Blood was collected through the abdominal vena cava for plasma analysis, and whole-body perfusion was performed with phosphate-buffered saline (pH 7.4). Brains were collected following perfusion.

Sample Preparations. Plasma (40 μL), MeCN (10 μL), and Reserpine (150 μL, 100 ng/mL) in MeCN with 0.1% formic acid were vortexed and centrifuged at 4000 RPM for 10 min. The supernatant (50 μL) was diluted with 50 μL of H_2O prior to LC/MS/MS analysis. The brain was homogenized with 50:50 EtOH:H_2O (1:5, v/v) using a Geno Grinder. 40 μL of the homogenate, 10 μL of MeCN, and 150 μL of 100 ng/mL Reserpine in MeCN with 0.1% formic acid were vortexed and centrifuged at 4000 RPM for 10 min. The supernatant (50 μL) was diluted with 50 μL of H_2O prior to LC/MS/MS analysis.

LC/MS/MS Analysis. LC/MS/MS was conducted using an Applied Biosystems API 4000 coupled with an Agilent 1100 HPLC system. Chromatography was performed with a Phenomenex Luna C18 (50 mm × 2 mm, 5 μm) column with C18 guard cartridge. Mobile phases were (A) 0.1% formic acid and 10 mM ammonium formate in H_2O and (B) 0.1% formic acid and 10 mM ammonium formate in MeOH. Initial conditions were 10% B and held for 0.5 min, followed by a linear gradient to 95% B over 3.5 min; 95% B was held for 2.9 min before returning to initial conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01075.

Molecular formula strings with biological data (CSV)

^1H NMR, ^{13}C NMR, and HPLC analysis results of target compounds; structural assignment of compound **17** (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; 2-AMPP, (2*S*)-*N*-((1*R*)-2-amino-1-(4-(2-methyl-pentyloxy)-phenyl)ethyl)-2-phenylpropanamide; cAMP, cyclic adenosine monophosphate; BBB, blood−brain barrier; CHO, Chinese hamster ovary; CL, clearance; CNS, central nervous system; CuAAC, copper-catalyzed azide-alkyne cycloaddition; DEAD, diethyl azodicarboxylate; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMP, Dess−Martin periodinane; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDG, electron-donating group; EWG, electron-withdrawing group; ESI, electrospray ionization; GPCR, G-protein-coupled receptor; GPR88, G-protein-coupled receptor 88; HA, human influenza hemagglutinin; HBSS, Hanks balanced salt solution; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); HMBC, heteronuclear multiple bond correlation; HOBt, hydroxybenzotriazole; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; IP, intraperitoneal; KO, knockout; MDCK, Madin−Darby canine kidney; MDR1, multidrug resistance

protein 1; NMR, nuclear magnetic resonance; MS, mass spectroscopy; 2-PCCA, (1*R*,2*R*)-2-(pyridin-2-yl)cyclopropane carboxylic acid ((2*S*,3*S*)-2-amino-3-methylpentyl)-(4′-propyl-biphenyl-4-yl)amide; *P*gp, *P*-glycoprotein; PK, pharmacokinetic; PPLS, pre-prolactin leader sequence; PSA, polar surface area; SAR, structure−activity relationship; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; TMS, trimethylsilyl; TR-FRET, time-resolved fluorescence resonance energy transfer; WT, wild-type

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