Chemical tools to modulate endocannabinoid biosynthesis

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Discovery of DH376, a 2,4-substituted triazole urea, as a potent and selective inhibitor for diacylglycerol lipases

Based on

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Introduction

Compound libraries that contain a 1,2,3-triazole urea scaffold have previously been applied to the discovery of potent inhibitors of diverse serine hydrolases, such as diacylglycerol lipase-β (DAGLβ), α,β-hydrolase domain (ABHD) 6/11, DDHD2, APEH and PAFAH2.[1-5] 1,2,3-Triazole ureas constitute a versatile chemotype for the covalent, irreversible and selective inhibition of serine hydrolases. They contain an electrophilic carbonyl group with tunable reactivity as well as a scaffold to introduce functional groups conferring enzyme potency and/or specificity. 1,2,3-Triazole ureas irreversibly inhibit serine hydrolases via carbamoylation of the active-site serine alcohol. Some reported triazole urea inhibitors were proven to be potent and selective for specific serine hydrolases both in cells and mouse models, and are effective chemical probes to study the biological function of serine hydrolases in diverse

biological systems.[1,2] For example, KT109 (**1**), a selective and *in vivo* active DAGLβ inhibitor, reduces 2-arachidonoylglycerol (2-AG), arachidonic acid and eicosanoid levels in peritoneal macrophages of lipopolysaccharide (LPS)-treated mice and significantly decreases the pro-inflammatory cytokine, tumour necrosis factor α (TNFα) in LPS-treated mice.[4]

Two isoforms of DAGL exist, and that are expressed in a tissue-dependent manner. Both isoforms, termed DAGLα and DAGLβ, employ a Ser-His-Asp catalytic triad characteristic for serine hydrolases to hydrolyse ester bond of diacylglycerol (DAG) in a *sn*-1 specific manner. DAGLα and DAGLβ share extensive homology, but differ in size: DAGLα is about 120 kDa and DAGLβ is around 70 kDa.[6,7] DAGLα is the principal regulator of 2-AG formation in the nervous system, where it controls the activity of this endocannabinoid, which activates the cannabinoid CB_1 receptor, as a retrograde messenger at neuronal synapses. DAGLβ in turn is the dominant enzyme for 2-AG production in the periphery during inflammation.[8,9]

To study the function of DAGLs in a temporal and dynamic manner, *in vivo*-active inhibitors of these enzymes would be of great value. Particularly, a CNS-active chemical probe is required for DAGLα (mainly expressed in the brain) that can be used to acutely perturb 2-AG production in the central nervous system. The known DAGL inhibitors can be classified into six different chemotypes: α-ketoheterocycles, glycine sulfonamides (both reversible, competitive DAGL inhibitor classes), bis-oximino-carbamates, β-lactones, fluorophosphonates and 1,2,3-triazole ureas (the latter four being mechanism-based and irreversible).[4,10-14] These inhibitors have been used to study the function of 2-AG in cellular models and brain slice preparations, but they lack selectivity over serine hydrolases, potency and/or chemical properties required for central activity. Of note, with the exception of the α-ketoheterocycles, all DAGL inhibitors reported to date also inhibit ABHD6, which also involved in the hydrolysis of partial 2-AG.

KT109 was selected as a suitable starting point for the rational design of new, potent and selective inhibitors for DAGLα, because it inhibits DAGLα with an IC_{50} of 2.3 μM in a competitive activity-based protein profiling (ABPP) assay.[4] In a first round of optimization, KT109 was converted into **38** (DH376), a highly potent, *in vivo* active compound that inhibits DAGLα in a time- and dose-dependent manner in mouse brain. Using **38**, as well as the structurally distinct compound DO34, functional studies on DAGLα in nervous system were performed (which will be described in Chapter 3 in detail).[15]

In this chapter, a full account of the discovery and development of DH376 (**38**) as an inhibitor of DAGLα is described. The influence of regioselectivity of the 1,4- and 2,4-triazole moiety, the nature of the substituents on the triazole core, the chirality of the benzylpiperidine and the substituent pattern of the piperidine ring on DAGLα and ABHD6 activity was systematically investigated. To this end, an enantioselective synthesis route to obtain both enantiomers of 2-benzylpiperidine and their derivatives was developed. Additionally, competitive activity-based protein profiling was employed to evaluate the selectivity profiles of the 1,2,3-triazole ureas in mouse brain membrane proteome. Finally, the cellular activity of DH376 in Neuro2A cells was determined.

Table 1. pIC_{50} values of compounds **1-6** against DAGLα and DAGLβ as determined by the colorimetric assay with PNP butyrate as substrate and competitive ABPP assay. Data represent average values ± SEM; n = 4 per group for substrate assays, and n = 3 per group for ABPP assays.

	Substrate assay (PNP butyrate)		**ABPP** (DH379)	
	hDAGLα	mDAGLβ	hDAGLα	hDAGLβ
1	8.9±0.1	7.1±0.2	8.1±0.1	8.2±0.1
2	7.2±0.1	4.9±0.3	6.2±0.1	6.0±0.1
3	7.6±0.1	7.1±0.1	6.8±0.1	6.2±0.1
4	8.6±0.1	7.9±0.1	7.8±0.1	7.6±0.1
5	7.7±0.1	4.7±0.2	6.7±0.1	6.1±0.1
6	5.4±0.1	N.A.	N.A.	N.A.

Results and Discussion

Discovery of 2,4-substituted 1,2,3-triazole urea as new chemotype of DAGLα inhibitor

In the search for CNS-active DAGLα inhibitors, a rational design drug discovery approach was employed in which KT109 (**1**) and a closely related analogue, ML226 (**2**), served as starting points (structures are shown in Figure 1). KT109 is a peripherally restricted DAGLβ inhibitor with 60-fold selectivity over DAGLα. ML226 in turn, is a potent, cellular and *in vivo* active ABHD11 inhibitor with excellent physicochemical properties.[1,4] First, the activity of KT109 and ML226 on HEK293T membranes overexpressing human DAGLα and mouse DAGLβ were tested in a colorimetric assay using *para*-nitrophenylbutyrate (PNP) as a surrogate substrate (Figure 1b, c; Table 1).[10,11] In this assay, KT109 inhibites mouse DAGLβ with a pIC_{50} of 7.1±0.2, which is consistent with previously reported in a gel-based ABPP assay using HT-01 as a chemical probe (pIC_{50} = 7.4)[4]. However, KT109 (pIC_{50} = 8.9±0.1) was much more potent on human DAGLα in the assay, than previously reported in a gel-based ABPP assay using HT-01 as a chemical probe (pIC_{50} = 5.6)[4]. The difference might be due to the weak labeling efficiency of HT-01 for DAGLα. In contrast, ML226 demonstrated weak DAGLα activity with a pIC_{50} of 7.2±0.1 and poor DAGLβ activity in the PNP-assay. At first sight this is in line with the previously reported preference of DAGLβ for 1,4-regioisomers of the triazole ureas over the corresponding 2,4-regioisomers.[16] ML226 lacks, however, the 2-benzyl substituent on the piperidine moiety thus blocking an appropriate comparison between the two inhibitors. Therefore, compounds **3-6** were synthesized as hybrid structures harbouring elements of both KT109 and ML226. To this end, the triazole building

blocks and the final compounds were synthesized as previously reported.[4,15] Interestingly, compound **4**, a 2-benzylpiperidine urea of a 2,4-triazole with a 1,1-diphenylmethanol substituent at the 4-position (as in ML226), showed the highest DAGLα and DAGLβ inhibitory activity with pIC_{50} of 8.6±0.1 and 7.9±0.1, respectively. Its 1,4-regioisomer (compound **3**) is 10-fold less potent. This indicates that 2,4-triazole is the preferred regioisomer for DAGLα and DAGLβ inhibition (Figure 1d, e; Table 1). Hybrid compounds (**5** and **6**) with an ethyl substituent at the 2-position of the piperidine ring appeared less potent than KT109, which suggests that the benzyl substituent is required to address an additional lipophilic pocket near the active site in the enzymes (Figure 1d, e). Competitive ABPP assays were next employed to confirm the inhibitory activities of compounds **1-6** against recombinant human DAGLα/β. DAGL-tailored activity-based probe DH379 (which will be described in Chapter 3) was used for these studies.[15] The results of gel-based ABPP assay were in line with the above PNP-assay that KT109 potently inhibited DAGLα and DAGLβ labeling by DH379, and compound **4** showed the highest potency against human DAGLα and DAGLβ among the hybrid compounds (Figure 1f and g).

The contribution of the phenyl groups of the 1,1-diphenylmethanol substituent in compound **4** to DAGLα inhibition was next investigated (Table 2). To this end, the phenyl substituents were replaced by cyclohexyl (**7**, **8**); removed one (**9**) or both (**10**) phenyl groups; replaced them by a pyridyl (**11**) or introduced fluorine atoms (**12**). The biochemical assay revealed *para*-fluoro substituted inhibitor (**12**) as the most potent agent in this series against hDAGLα with a pIC_{50} of 9.0, which suggested that its increased lipophilicity and/or electron withdrawing effect is beneficial. The ~100-fold drop in potency of the more polar (compared to lead **4**) pyridyl-containing compound (**11**), suggested that lipophilicity is more important than electron withdrawing properties. Indeed, the lipophilic interactions of the phenyl groups are essential features of the DAGLα inhibitor, because their removal led to a 160-2000 fold decrease in potency (**8**-**10**), whereas retaining two bulky cyclohexyl groups (**7**) resulted in only a 40-fold drop in potency. A role for pi-sigma/cation interactions can, however, also not be excluded. A 10-fold decrease in potency was observed when the tertiary alcohol group was methylated, as in compound (**13**), suggesting that a hydrogen bond donor is important (or alternatively, the grafted methyl group has a steric clash with the enzyme). To reduce the lipophilicity, 2-benzyl substituent of the piperidine ring was substituted for a phenoxymethyl- (**14**, **15**) or (4-fluoro)phenoxymethyl group (**16**, **17**) and polar methoxy substituents on the phenyl ring were introduced as well (**18**, **19**). These substitutions were tolerated (Table 2), but led to a five-fold reduced activity.

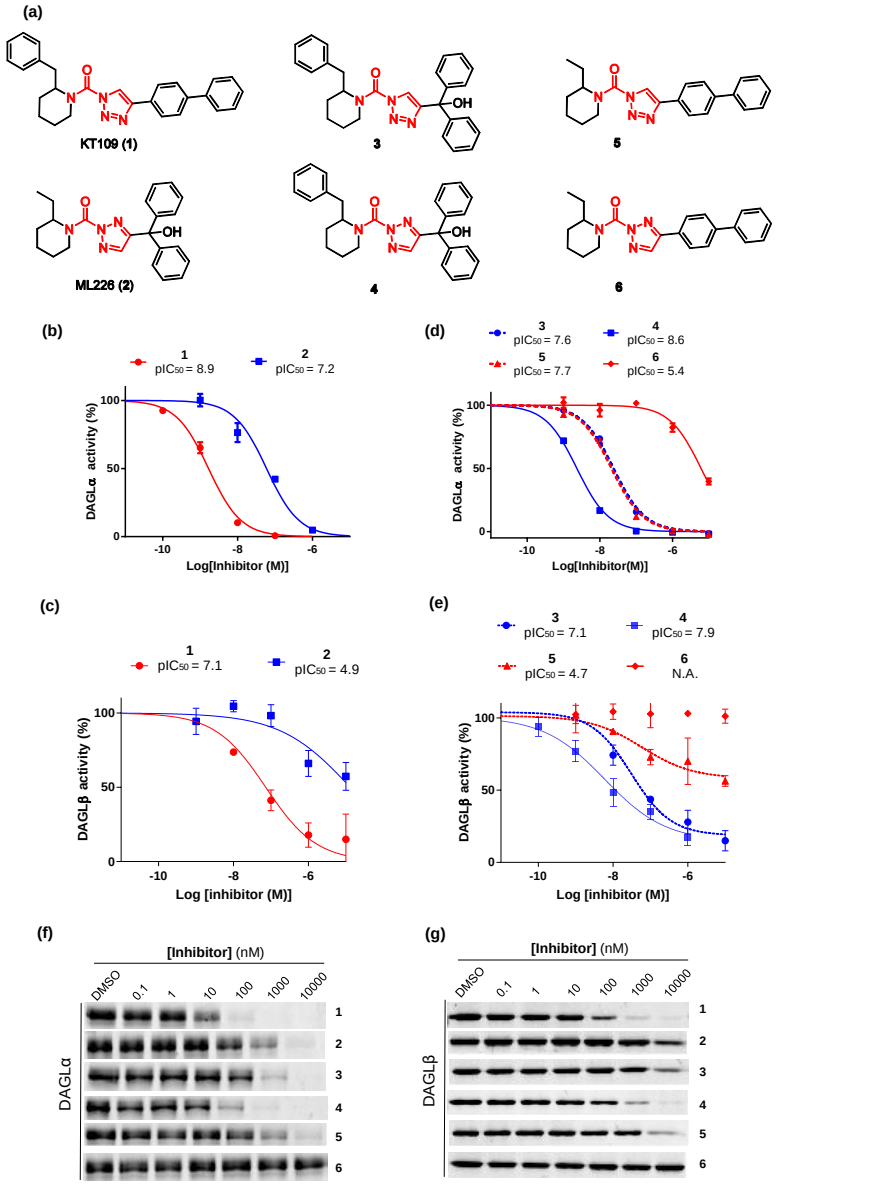
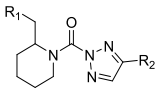
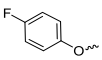
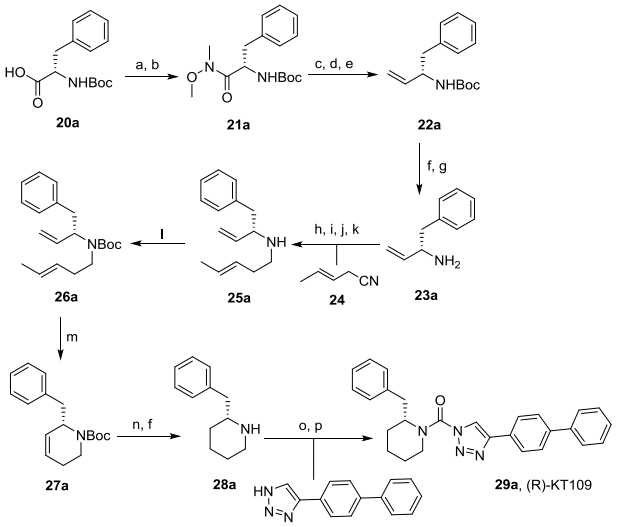


Figure 1. (a) The structures of 1,2,3-triazole ureas **1**-**6**. (b, d) Concentration-dependent inhibition of recombinant human DAGLα by compounds **1**-**6** as measured with a colorimetric assay based on the hydrolysis of PNP butyrate from DAGL-transfected HEK293T cells. (c, e) Concentration-dependent inhibition of recombinant mouse DAGLβ by compounds **1**-**6** as measured with the PNP butyrate substrate assay. Data represent average values ± SEM; n = 4 per group. (f, g) Representative fluorescent gel-based competitive ABPP with compounds **1**-**6** against recombinant human DAGLα and DAGLβ by tailored activity-based probe DH379 (1 µM, 30 min).

Table 2. Structure-activity relationship of triazole ureas with N2-isomers as leaving group. Inhibition of recombinant human DAGLα or ABHD6 was measured by the indicated colorimetric assay based on PNP or 2-AG substrate assay, repectively. Data represent average values ± SEM; n = 4 per group.



Entry	R_1	R_2	pIC_{50}±SEM (DAGLα)	pIC_{50}±SEM (ABHD6)
7			7.0±0.1	5.1±0.2
8			5.8±0.1	5.5±0.1
9			6.4±0.1	5.5±0.1
10			5.3±0.2	<5
11			6.8±0.1	5.5±0.2
12			9.0±0.1	7.6±0.1
13			7.9±0.2	<5
14			8.1±0.1	6.8±0.1
15			8.3±0.1	6.7±0.1
16			8.2±0.1	6.6±0.1
17			8.3±0.1	6.7±0.1
18			8.5±0.5	6.8±0.2
19			8.3±0.1	6.6±0.1



Scheme 1. Enantioselective synthesis of (*R*)-KT109 (**29a**) Reagents and conditions: (a) Me(OMe)NH·HCl; (b) EDCI, NMM; (c) $LiAlH_4$; (d) H_3O^+; (e) $(Ph)_3P{=}CH_2$, 86% (**22a**, based on **20a**); (f) MeOH, HCl; 70% (**28a**) (g) NaOH, 89%; (h) **24**, diethyl ether, DIBAL-H, -80 °C to 0 °C; (i) MeOH, -90 °C; (j) amine **23a** (3 equiv), r.t., 20h; (k) $NaBH_4$, 0 °C to r.t., 5h, 44%; (l) Boc_2O, Et_3N, THF, 50 °C, 20h, 92%; (m) Grubbs I cat. 4 mol%, DCM, reflux, 48h, 68%; (n) H_2, Pd/C, MeOH; (o) DIPEA, triphosgene, THF, 0 °C; (p) DIPEA, DMAP, triazole , THF, 60 °C, 30%.

(*R*)-KT109 is the most active DAGL inhibitor

Previously, the eutomer of KT109 was found to be 100-fold more potent than the distomer against DAGLβ.[16] The absolute configuration of the eutomer (and distomer) was, however, not assigned. To determine whether (*R*)-KT109 (**29a**) or (*S*)-KT109 (**29b**) is the most potent enantiomer, a enantioselective synthesis route (Scheme 1) was develeoped. The synthesis of the separate enantiomers of KT109 began with the preparation of chiral amine **23a** in four steps from commercially available Boc-protected L-phenylalanine **20a**.[17] Amine **23a** was reacted with 3-pentene nitrile **24** to give secondary amine **25a** via a one-pot DIBAL-H reduction-transimination-$NaBH_4$ reduction sequence.[18] Subsequent Boc-protection of the amine, ring-closing metathesis, hydrogenation and Boc-deprotection led to key chiral 2-benzylpiperidine building block **28a**. Direct coupling of the chiral piperidine with 4-([1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazole using triphosgene provided final compound **29a** in >95% e.e. as determined by chiral HPLC. The synthesis of enantiomer **29b** proceeded in a similar fashion using chiral amine **23b** (see experimental section, Scheme 5).

To correlate the activity of the compounds with their stereochemistry, both enantiomers and a 1:1 mixture were tested in the colorimetric surrogate PNP-substrate assay using HEK293T membranes expressing recombinant human

DAGLα. Compound **29a** proved to be the eutomer with a pIC_{50} of 9.1±0.1, while compound **29b** showed ~100-fold less activity (pIC_{50} of 7.4±0.1) (Figure 2a) in the PNP-assay. The 1:1 racemic mixture demonstrated a pIC_{50} of 8.2±0.1. A real-time, fluorescence-based assay was also employed to test the activity of the inhibitors on DAGLα-mediated hydrolysis of its natural substrate 1-stearoyl-2-arachidonoly-*sn*-glycerol.[19] Again, compound **29a** was the most active DAGLα inhibitor with a pIC_{50} of 7.6±0.1 (Figure 2b). Since ABHD6 was previously reported as an off-target of KT109, the activities of both enantiomers (**29a** and **29b**) against human ABHD6 were also tested.[11,20] Compound **29a** (pIC_{50} 8.6±0.1) was ~100-fold more potent than **29b** (pIC_{50} 6.2±0.1) (Figure 2c), which reveals that the inhibitory activity for both DAGLα and ABHD6 resides in the (*R*)-enantiomer. To assess the activity and selectivity of compounds **29a** and **29b** on endogenously expressed DAGLα in mouse brain membrane proteome, a competitive ABPP method with MB064 was used.[10] Consistent with the biochemical assays, compounds **29a** and **29b** were found to block DAGLα labeling by MB064 with pIC_{50} of 8.1±0.1 and 6.2±0.1, respectively (Figure 2d and e). Additionally, both **29a** and **29b** showed same selectivity profile (with ABHD6 as only identified off-target) in mouse brain membrane proteome determined by a broad-spectrum TAMRA-FP probe.

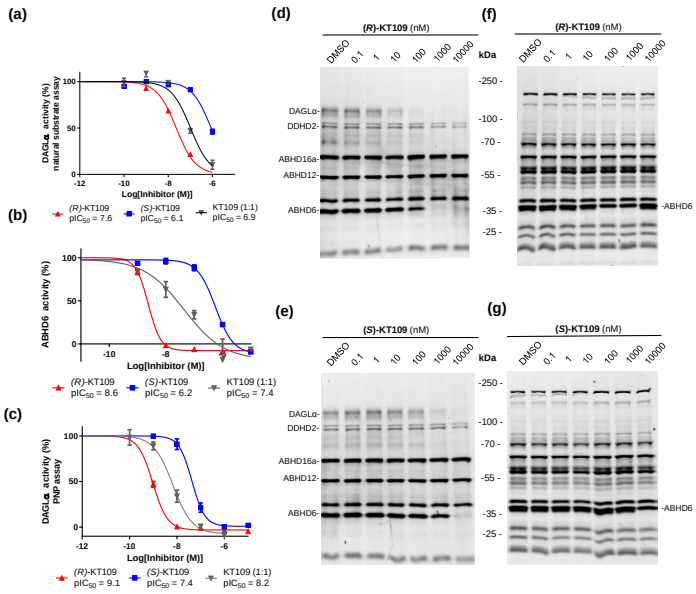
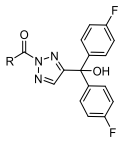
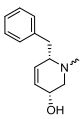
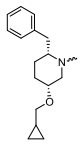
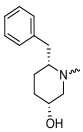
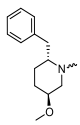
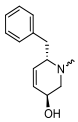
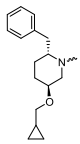
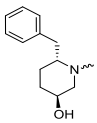
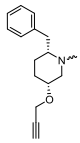
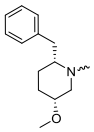


Figure 2. Characterization of both enantiomers of KT109 as DAGLα inhibitors: (a) Concentration-dependent inhibition of recombinant hDAGLα by (*R*)-KT109 (**29a**), (*S*)-KT109 (**29b**) and racemic KT-109 (1:1) as measured with a colorimetric assay based on the hydrolysis of PNP butyrate. (b) Concentration-dependent inhibition of recombinant hDAGLα by (*R*)-KT109 (**29a**), (*S*)-KT109 (**29b**) and racemic KT-109 (1:1) as measured with a SAG substrate assay from DAGLα-transfected HEK293T cells. (c) Concentration-dependent inhibition of hABHD6 by (*R*)-KT109 (**29a**), (*S*)-KT109 (**29b**) and racemic KT-109 (1:1) as measured with a 2-AG

substrate assay. Data represent average values ± SEM; n = 4 per group. (d, e) Representative fluorescent gel-based competitive ABPP with (*R*)-KT109 (**29a**) and (*S*)-KT109 (**29b**) in mouse brain proteome by tailored activity-based probe MB064 (0.25 µM, 30 min). (f-g) Selectivity profiles of *(R)*-KT109 (f) and (*S*)-KT109 (g) across mouse brain serine hydrolases as determined by competitive ABPP using broad-spectrum probe FP-TAMRA (0.5 µM, 20 min). Of note, in these gel profiles for FP-TAMRA labeling, ABHD6 and MAGL signals were not resolved, and DAGLs are not visualized.

Table 3. Structure-activity relationship of N2-triazole urea isomers with functionalized chiral pure (2-benzyl)-piperidine staying groups. Inhibition of recombinant human DAGLα or ABHD6 was measured by the indicated colorimetric assay based on PNP or 2-AG substrate assay, repectively. Data represent average values ± SEM; n = 4 per group.



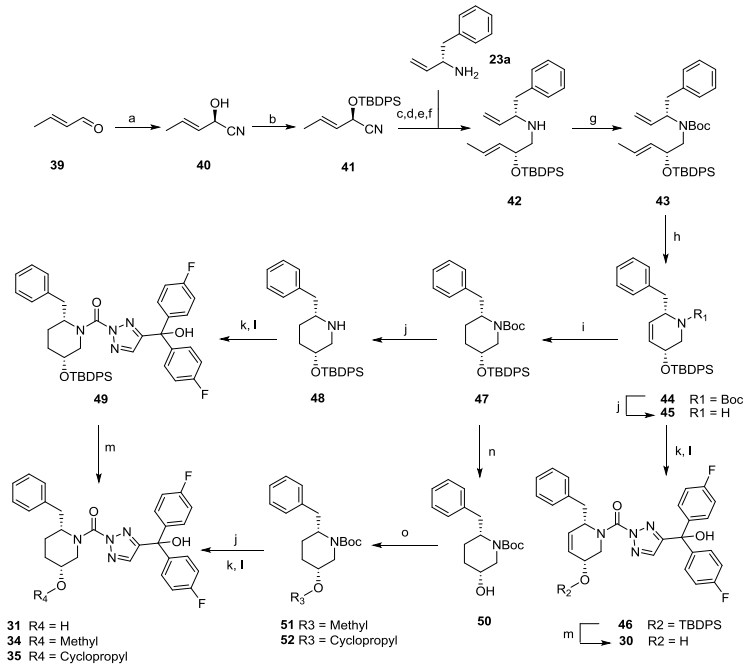
Entry	R	pIC_{50} (DAGLα)	pIC_{50} (ABHD6)	Entry	R	pIC_{50} (DAGLα)	pIC_{50} (ABHD6)
30		7.9±0.1	7.4±0.1	**35**		9.2±0.1	7.4±0.1
31		7.7±0.1	6.8±0.1	**36**		8.1±0.1	6.9±0.2
32		7.7±0.1	6.6±0.1	**37**		7.7±0.1	5.6±0.1
33		5.2±0.1	6.7±0.2	**38** (DH376)		8.9±0.1	8.6±0.2
34		9.1±0.1	7.3±0.1				

Discovery of highly potent DAGL inhibitors

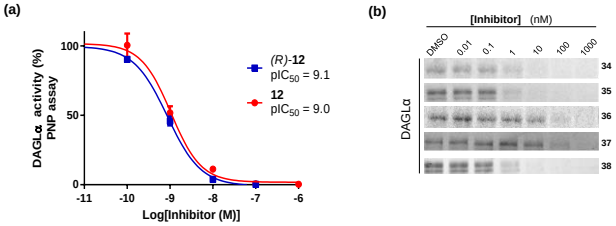
Having discovered that the (*R*)-enantiomer is the most active compound in the 1,4-triazole series, this knowledge was transferred to the 2,4-triazole series. To this end, the chiral amine building block **23a** was coupled to the triazole scaffold to provide compound **(*R*)-12**. **(*R*)-12** was found to have a pIC_{50} of 9.1±0.1, which was slightly higher than the racemic mixture (Figure 3a). To improve solubility and to mimic the natural substrate diacylglycerol, several new analogues were designed with a chiral hydroxyl group at the C-5 position (Scheme 2 and Scheme S.4 in experimental section; **30-33**). The chiral, diastereomers were synthesized according to Scheme 2. In brief, cyanohydrin **40** was enzymatically produced by the almond (*R*)-hydroxynitrile lyase using crotonic aldehyde **39** as a substrate.[21] After silyl protection of the alcohol, key intermediate **44** was generated by the same strategy as described for the synthesis of **29a**. After *N*-Boc deprotection, and optional hydrogenation, compounds **45** and **48** were coupled to the 1,2,3-triazole building block, yielding *O*-silyl protected intermediates **46** and **49**. Deprotection gave compounds **30** and **31**. Further alkylation of intermediate **50**, *N*-Boc deprotection and coupling with 1,2,3-triazole building block afforded compounds **34** and **35**. Compounds **32**, **33**, **36** and **37** were synthesized in the same fashion as described for the corresponding diastereoisomers (See experimental Scheme 6). Compounds **30**-**38** were tested in the PNP-assay and found that the free alcohol derivatives **30**-**33** are less potent than compound **12** (Table 1 and 2). Capping the secondary hydroxyl group with an alkyl moiety yielded (ultra)potent inhibitors. For example, compounds **34** and **35** demonstrated picomolar activity with pIC_{50} values of 9.1±0.1 and 9.2±0.1, respectively (Table 3). Comparison of the diastereoisomers (**34** vs **36**; **35** vs **37**) revealed that the back isomer at C-5 is the active diastereomer (**34**, **35**) (with ~10-fold higher potency). To visualize target engagement, a propargyl at C-5 was introduced, which serves as a ligation handle to introduce reporter groups by copper catalyzed azide-alkyne cycloaddition (or "click"-chemistry). This yielded inhibitor **38** (DH376) with a pIC_{50} = 8.9±0.1.

Activity and selectivity on endogenous DAGLα and ABHD6 in brain membrane proteome

To determine the activity and selectivity of the inhibitors in native proteomes, the most potent chiral inhibitors **34-38** were incubated for 30 min with mouse brain membrane homogenates and a gel-based ABPP-assay using ABPs MB064 and TAMRA-FP was performed. All compounds block DAGLα labeling in a concentration-dependent manner. Complete blockade of DAGLα was already observed at 10 nM for compounds **34**, **35** and **38**, whereas the diastereoisomers **36** and **37** were less active (Figure 3b and Figure S.3). Compound **35** inhibited labeling of DAGLα and ABHD6 with pIC_{50} of 8.7±0.1 and 6.5±0.1, respectively. This indicated that **35** was ~160-fold selective over ABHD6 (Figure 3c). Of note, compound **38** showed ~126 fold selectivity over ABHD6. No additional off-targets were identified using FP-TAMRA as a probe (Figure 4).



Scheme 2. Enantioselective synthesis of 1,2,3 triazole ureas **30**, **31**, **34** and **35.** Reagents and conditions: (a) HCN, EtOAc, 0.1 M aq. citrate buffer, pH 5.4, hydroxynitrile lyases, 83%; (b) TBDPS-Cl, imidazole, DMF, 0 ^{o}C, 94%; (c) diethyl ether, DIBAL-H, -80 ^{o}C to 0 ^{o}C; (d) MeOH, -90 ^{o}C; (e) (*S*)-amine (**23a**) (3 equiv), r.t., 20h; (f) $NaBH_4$; (g) Boc_2O, Et_3N, THF, 50 ^{o}C, 20h; (h) Grubbs G1 cat. 4 mol%, DCM, reflux, 48h, 72% (**44**, based on **41)**; (i) hydrazine, $CuSO_4$, EtOH, 0 ^{o}C to 70 ^{o}C, 65% (**47**); (j) 25% TFA, DCM, r.t.; (k) DIPEA, triphosgene, THF, 0 ^{o}C; (l) DIPEA, DMAP, triazole, THF, 60 ^{o}C, 28% (**34**, based on **51**), 22% (**35**, based on **52**); (m) HF-pyridine, THF : pyridine = 1:1 (v/v), 24% (**30**, based on **44**), 16% (31, based on **47**); (n) TBAF, THF, r.t., 72%; (o) NaH, corresponding bromide, 83% (**51**), 81% (**52**).



(c)

pIC_{50} ABPP (MB064)	**34**	**35**	**36**	**37**	**38** (DH376)
DAGLα	8.7±0.1	8.7±0.1	7.1±0.1	7.1±0.1	9.2±0.1
ABHD6	7.3±0.1	6.5±0.1	6.4±0.1	<5	7.1±0.1
Selectivity fold	~25	~160	~5	~145	~126

Figure. 3 (a) Concentration-dependent inhibition of hDAGLα by **12** and **(*R*)-12** as measured with a colorimetric assay based on the hydrolysis of PNP butyrate. Data represent average values ± SEM; n = 4 per group. (b) Representative fluorescent gel-based competitive ABPP with **34-38** in mouse brain proteome by activity-based probe MB064 (0.25 µM, 30 min). (c) pIC_{50} ± SEM and selectivity of compounds **34-38** against DAGLα and ABHD6 as determined by competitive ABPP (n = 3 per group).

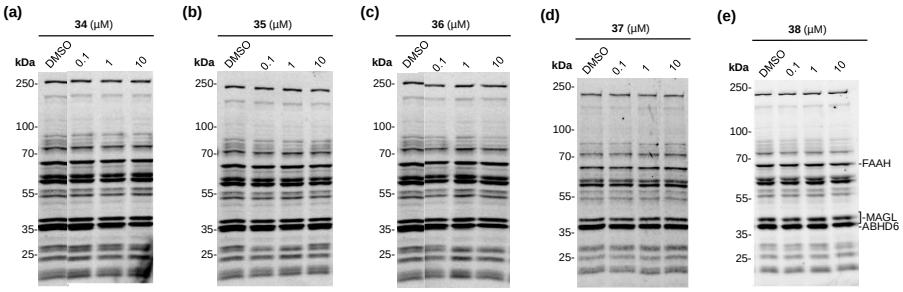


Figure 4. Selectivity profiles of compounds **34-38** across mouse brain serine hydrolases as determined by competitive ABPP using broad-spectrum probe FP-TAMRA (0.5 µM, 20 min).

DH376 reduces 2-AG levels in Neuro2A cells

A mouse neuroblastoma cell line (Neuro2A), known to express both DAGLα and DAGLβ, was used to evaluate the cellular activity of DH376.[22,23] Neuro2A cells were incubated with a range of concentrations of DH376 for 1h, lysed and analyzed by competitive gel-based ABPP using DH379 as an activity-based probe to establish cellular target engagement. DH376 blocked the labeling of DAGLβ in Neuro2A cells with low-nanomolar potency (IC_{50} = 1.3 nM, Figure 5a,b). As expected, DH376 also prevented the labeling of ABHD6 (Figure 5a,b), but did not inhibit the labeling of any of the other serine hydrolase as demonstrated in a competitive gel-based ABPP using the broad-spectrum probe TAMRA-FP (Figure 5c). Next, the effect of DH376 on the cellular levels of 2-AG was determined using targeted lipidomics. Neuro 2A cells treated for 1h with DH376 (1 µM) strongly reduced cellular 2-AG levels (Figure 5d), whereas no effect on anandamide was observed (Figure 5e).

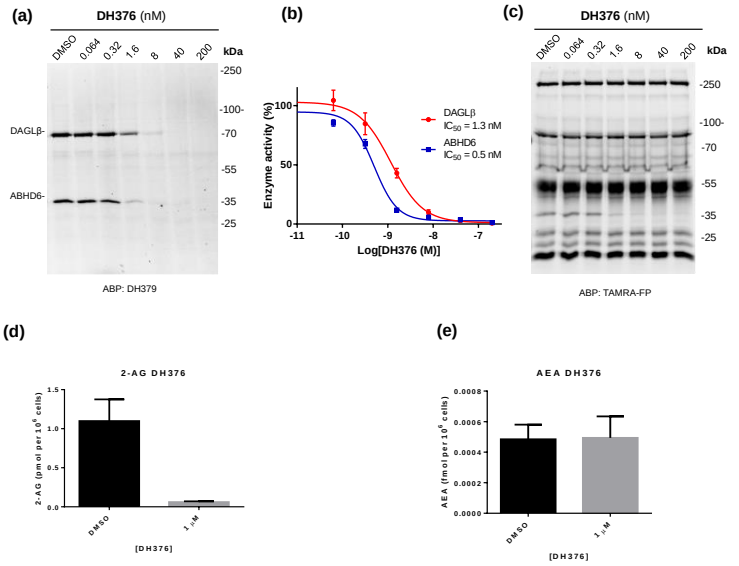


Figure 5. *In situ* activity of DH376. (a) Neuro2A cells were treated with various concentrations of DH376 for 1h at 37 °C, lysed and analyzed by gel-based competitive ABPP using ABP DH379 (1 uM, 30 min). (b) *In situ* concentration-dependent inhibition curves for endogenous DAGLβ and ABHD6 as measured by competitive ABPP using DH379 as probe. DH376 (pIC_{50} = 8.9±0.1 for DAGLβ, 9.3±0.1 for ABHD6). Data represent average values ± SEM, n=3 per group. (c) Selectivity profile of DH376 in Neuro 2A cells was determined by competitive ABPP using a broad-spectrum ABP TAMRA-FP (500 nM, 30 min). (d-e) *In situ* treatment of Neuro2A cells (1h, 37 °C) with DH376 (1 µM) abolished 2-AG levels (d), while keeping anandamide (AEA) levels constant (e) (mean ± SEM; n = 4).

Conclusion

In summary, the enantioselective synthesis and structure-activity relationship (SAR) studies of 2,4-regioisomers of 1,2,3-triazole ureas as a new chemotype of DAGLα inhibitors were described in this chapter. (*R*)-benzylpiperidine substituted triazole ureas were found to constitute the active enantiomer for DAGLα inhibition as measured in biochemical assays and activity-based protein profiling. (*R*)-KT109 (**29a**) was shown to be a potent DAGLα inhibitor. The investigations culminated in the discovery of compound **38** (DH376), a cellular-active DAGL inhibitor.

Experimental section

Experimental Procedures: Biochemistry

Cloning Procedures

For the preparation of the different constructs, full length human cDNA was purchased from Source Bioscience and cloned into mammalian expression vector pcDNA3.1, containing genes for ampicillin and neomycin resistance. DAGLα and ABHD6 constructs were obtained as reported previously.[10,15] Plasmids were isolated from transformed XL-10 Z-competent cells (Maxi Prep, Qiagen) and verified by Sanger sequencing (BaseClear). The sequences were confirmed by sequence analysis at the Leiden Genome Technology Centre.

Cell culture and membrane preparation

Cell culture was performed as previously reported.[15] In brief, HEK293T cells were grown in DMEM with stable glutamine and phenolred (PAA or Sigma) with 10% New Born Calf serum, penicillin and streptomycin. Cells were passaged every 2-3 days by resuspending in medium and seeding them to appropriate confluence. Membranes were prepared from transiently transfected HEK293T cells. One day prior to transfection 10^7 cells were seeded in a 15 cm petri dish. Cells were transfected by the addition of a 3:1 mixture of polyethyleneimine (60 µg) and plasmid DNA (20 µg) in 2 mL serum free medium. The medium was refreshed after 24h, and after 72h the cells were harvested by suspending them in 20 mL medium. The suspension was centrifuged for 10 min at 1000 rpm, and the supernatant was removed. The cell pellet was stored at -80 ^{o}C until use.

Cell pellets were thawed on ice and suspended in lysis buffer A (20 mM HEPES, 2 mM DTT, 0.25 M sucrose, 1 mM $MgCl_2$, 1x Cocktail (Roche complete EDTA free), 25 U/mL benzonase). The suspension was homogenized by polytrone (3 × 7 sec) and incubated for 30 min on ice. The suspension was subjected to ultracentrifugation

(93,000 × g, 30 min, 4 ^{o}C, Beckman Coulter, Type Ti70 rotor) to yield the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was resuspended in lysis buffer B (20 mM HEPES, 2 mM DTT, 1x Cocktail (Roche cOmplete EDTA free)). The protein concentration was determined with Quick Start Bradford reagent (BioRad) or QubitTM fluorometric quantitation (Life Technologies). The protein fractions were diluted to a total protein concentration of 1 mg/mL and stored in small aliquots at -80 ^{o}C until use.

Biochemical DAGL activity assay

The biochemical hDAGLα assay was performed as reported previously.[10] In brief, the biochemical hDAGLα activity assay is based on the hydrolysis of para-nitrophenylbutyrate (PNP-butyrate) by membrane preparations from HEK293T cells transiently transfected with hDAGLα. Reactions were performed in 50 mM pH 7.2 HEPES buffer with 0.05 µg/µL final protein concentration hDAGLα transfected protein.

Natural substrate based fluorescence assay (DAGLα and ABHD6)

The natural substrate assay was performed as reported previously.[11,19] Standard assay conditions: 0.2 U/mL glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO) and horseradish peroxidase (HRP), 0.125 mM ATP, 10 µM Amplifu™Red, 5% DMSO in a total volume of 200 µL. For ABHD6, the assay additionally contained 25 µM 2-AG and 0.5% acetonitrile, with a final protein concentration of 40 µg/mL. For DAGLα, the assay additionally contained 5 µg/mL MAGL-overexpressing membranes, 100 µM SAG and 0.0075% (w/v) Triton X-100, with a final protein concentration of 50 µg/mL.

Preparation of mouse brain membrane proteome

Mouse brain membrane proteome preparation was performed as previously reported.[15] In brief, mouse brains were isolated according to guidelines approved by the ethical committee of Leiden University (DEC#10095). Mouse brains were Dounce homogenized in pH 7.2 lysis buffer A (20 mM HEPES pH 7.2, 2 mM DTT, 1 mM $MgCl_2$, 25 U/mL Benzonase) and incubated for 5 min on ice, followed by low speed spin (2,500 × g, 3 min, 4 ^{o}C) to remove debris. The supernatant was subjected to ultracentrifugation (100,000 × g, 45 min, 4 ^{o}C, Beckman Coulter, Type Ti70 rotor) to yield the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was resuspended in storage buffer B (20 mM HEPES pH 7.2, 2 mM DTT). The total protein concentration was determined with Quick Start Bradford reagent (Bio-Rad) or QubitTM fluorometric quantitation (Life Technologies). Membranes and supernatant were flash frozen in liquid nitrogen and stored in aliquots at -80 ^{o}C until use.

Activity based protein profiling in mouse brain

Mouse brain proteome (2 mg/mL, 19.5 µL) was incubated with DMSO or inhibitor in 0.5 µL DMSO for 30 min at r.t. and subsequently incubated with 250 nM (final

concentration) ABP MB064, or 500 nM (final concentration) ABP TAMRA-FP for 20 min at r.t. before the reaction was quenched with standard 3x Laemmli sample buffer. The gels were scanned using a ChemiDoc MP system and analyzed using Image Lab 4.1.

ABPP inhibitor activity measurements

The percentage of activity remaining was determined by measuring the integrated optical intensity of the fluorescent protein bands using Image Lab 4.1. The relative intensity was compared to the vehicle-treated proteins, which were set to 100%. IC_{50} values were determined by plotting a log (inhibitor) vs. normalized response (Variable slope) dose-response curve generated using Prism software (Graphpad Prism 5.0).

Synthesis

General Remarks. Reagents were purchased from Sigma Aldrich, Acros or Merck and used without further purification unless noted otherwise. Some reactions were performed using oven or flame-dried glassware and dry solvents. All moisture sensitive reactions were performed under an argon atmosphere. Traces of water were removed from starting compounds by co-evaporation with toluene.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400 (^{1}H) and 101 (^{13}C) MHz, or on a Bruker DMX-600 spectrometer 600 (^{1}H) and 150 (^{13}C) MHz using $CDCl_3$, CD_3OD or $(CD_3)_2SO$ as solvent, unless stated otherwise. Chemical shift values are reported in ppm with tetramethylsilane or solvent resonance as the internal standard ($CDCl_3$, δ 7.26 for ^{1}H, δ 77.16 for ^{13}C; CD_3OD, δ 3.31 for ^{1}H, δ 49.00 for ^{13}C; $(CD_3)_2SO$, δ 2.50 for ^{1}H, δ 39.52 for ^{13}C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants *J* (Hz), and integration. High-resolution mass spectra (HRMS) were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile 50/50 (v/v) and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 ^{o}C) with resolution R = 60,000 at m/z 400 (mass range m/z = 150-2,000) and dioctylphthalate (m/z = 391.28428) as a “lock mass”. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Gemmi C_{18} 50x4.60 mm column (detection at 200-600 nm), coupled to a Finnigan LCQ Adantage Max mass spectrometer with ESI. The applied buffers were H_2O, MeCN and 1.0% TFA in H_2O (0.1% TFA end concentration). HPLC purification was performed on a preparative LC-MS system (Agilent 1200 series) with an Agilent 6130 Quadruple MS detector. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). Flash chromatography was performed using SiliCycle silica gel type SilicaFlash P60 (230 – 400 mesh). TLC

analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm. Compounds were visualized using either Seebach's reagent (a mixture of phosphomolybdic acid (25 g), cerium (IV) sulfate (7.5 g), H_2O (500 mL) and H_2SO_4 (25 mL)) or a $KMnO_4$ stain (K_2CO_3 (40 g), $KMnO_4$ (6 g), H_2O (600 mL) and 10% NaOH (5 mL)).

Analysis of Compound Purity by LC/MS. Compound purity was determined by an LCQ Adventage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C_{18} (Gemini, 4.6 mm x 50 mm, 3 µm particle size, Phenomenex) equipped with buffer A: H_2O, B: acetonitrile (MeCN) and C: 1% aqueous TFA. All final compounds were determined to be above 95% pure by this method.

(4-([1,1'-Biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-benzylpiperidin-1-yl)methanone (1, KT109). A solution of 2-benzylpiperidine (90.0 mg, 0.513 mmol) in THF was treated with DIPEA (0.269 mL, 1.54 mmol) and bis(trichloromethyl) carbonate (76.0 mg, 0.257 mmol) and the reaction mixture was stirred for 30 min at 0 ^oC. After that the reaction mixture was poured into water and extracted with ethyl acetate (3 x 10 mL).The organic layer was washed with water, brine and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The intermediate was dissolved in THF and DIPEA (0.269 mL, 1.54 mmol), DMAP (62.7 mg, 0.513 mmol) and 4-([1,1'-biphenyl]-4-yl) -1*H*-1,2,3-triazole (125 mg, 0.565 mmol) were added to the solution. The mixture was stirred for 2h at 60 ^oC and poured into saturated aqueous NH_4Cl solution (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL), washed with water, brine, dried over $MgSO_4$ and filtered. The solvents are removed under reduced pressure to yield the crude triazole urea as a mixture of N1- and N2-carbamoylated regioisomers. The N1-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford KT109 (**1**) (99.0 mg, 0.234 mmol, 46% yield). LC-MS m/z: calculated for $C_{27}H_{26}N_4O$ $[M+H]^+$ 423.22, found: 423.04. ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (br s, 2H), 7.74 – 7.60 (m, 4H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.42 – 7.34 (m, 1H), 7.22 (br s, 4H), 7.01 (br s, 1H), 4.86 (br s, 1H), 4.37 (br d, *J* = 13.6 Hz, 1H), 3.43 – 3.18 (m, 2H), 2.70 (br s, 1H), 2.07 – 1.67 (m, 6H).

(2-Ethylpiperidin-1-yl)(4-(hydroxydiphenylmethyl)-2*H*-1,2,3-triazol-2-yl)methanone (2, ML226). The title compound was synthesized from 2-ethylpiperidine (0.057 mL, 0.430 mmol) and diphenyl(1*H*-1,2,3-triazol-4-yl)methanol (90.0 mg, 0.358 mmol) according to the procedures described for compound **1**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea ML226 (76.0 mg, 0.195 mmol, 54% yield). LC-MS m/z: calculated for $C_{23}H_{26}N_4O_2$ $[M+H]^+$ 391.49, found: 391.14. ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.39 – 7.24 (m, 10H), 4.22 (br s, 1H), 3.98 – 3.03 (m, 3H), 1.84 – 1.50 (m, 8H), 1.11 – 0.58 (m, 3H).

(2-Benzylpiperidin-1-yl)(4-(hydroxydiphenylmethyl)-1*H*-1,2,3-triazol-1-yl)methanone (3). A solution of 2-benzylpiperidine (50.0 mg, 0.285 mmol) in THF (4 mL) was

treated with DIPEA (0.249 mL, 1.426 mmol) and bis(trichloromethyl) carbonate (42.3 mg, 0.143 mmol) and the reaction mixture was stirred for 30 min at 0 °C. The mixture was poured into water and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The intermediate was dissolved in a mixture of THF (8 mL) and DIPEA (0.249 mL, 1.426 mmol), DMAP (34.9 mg, 0.285 mmol) and diphenyl(1H-1,2,3-triazol-4-yl)methanol (71.7 mg, 0.285 mmol) were added to the solution. The mixture was stirred for two hours at 60 °C and poured into a saturated aqueous NH_4Cl solution. The mixture was extracted with ethyl acetate (3 x 20 mL), washed with water, brine, dried over $MgSO_4$ and filtered. The solvents were removed under reduced pressure to yield the crude triazole urea as a mixture of N1- and N2-carbamoylated regioisomers. The N1-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 1,4-triazole urea **3** (29.7 mg, 0.066 mmol, 23% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{28}N_4O_2$ $[M+H]^+$ 453.2285, found: 453.2283. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 10H), 7.04 – 6.82 (m, 5H), 4.76 (s, 1H), 4.28 (br s, 1H), 3.71 (br s, 1H), 3.33 – 3.21 (m, 2H), 2.63 (br s, 1H), 2.00 – 1.66 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.28, 137.90, 129.03, 128.78, 128.18, 127.76, 127.29, 127.23, 127.22, 126.80, 123.76, 69.63, 57.47, 40.46, 36.50, 29.18, 25.32, 18.75.

(2-Benzylpiperidin-1-yl)(4-(hydroxydiphenylmethyl)-2*H*-1,2,3-triazol-2-yl)methanone (4). The title compound was isolated from the mixture of compound **3**. The N2-carbamoyl triazole regioisomer was purified by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **4** (39.0 mg, 0.086 mmol, 30% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{28}N_4O_2$ $[M+H]^+$ 453.2285, found: 453.2284. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.35 – 7.27 (m, 10H), 7.22 – 6.97 (m, 5H), 4.75 – 4.17 (m, 1H), 3.68 (br s, 1H), 3.26 – 3.19 (m, 1H), 3.10 – 3.15 (m, 1H), 2.93 (br s, 1H), 1.79 – 1.60 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.48, 149.55, 145.04, 137.91, 135.14, 129.07, 128.64, 128.21, 127.84, 127.22, 126.64, 69.61, 53.28, 42.44, 36.31, 26.05, 25.24, 18.71.

(4-([1,1'-Biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-ethylpiperidin-1-yl)methanone (5). The title compound was synthesized from 2-ethylpiperidine (0.059 mL, 0.442 mmol) and 4-([1,1'-biphenyl]-4-yl)-1H-1,2,3-triazole (98.0 mg, 0.442 mmol) according to the procedures described for compound **3**. The N1-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 1,4-triazole urea **5** (48.0 mg, 0.133 mmol, 30% yield). HRMS [ESI+] m/z: calculated for $C_{22}H_{24}N_4O$ $[M+H]^+$ 361.2023, found: 361.2022. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.66 – 7.61 (m, 2H), 7.47 – 7.44 (m, 2H), 7.38 -7.34 (m, 1H), 4.55 (q, *J* = 6.6 Hz, 1H), 4.29 (br d, *J* = 13.4 Hz, 1H), 3.20 (br s, 1H), 1.87 – 1.66 (m, 8H), 0.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.54, 141.42, 140.52, 128.93, 128.71, 127.70, 127.63, 127.08, 126.35, 120.95, 55.26, 53.87, 42.83, 28.15, 25.74, 22.60, 18.86, 10.70.

(4-([1,1'-Biphenyl]-4-yl)-2*H*-1,2,3-triazol-2-yl)(2-ethylpiperidin-1-yl)methanone (6).

The title compound was isolated from the mixture of compound **5**. The N2-carbamoyl triazole regioisomer was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **6** (36.0 mg, 0.100 mmol, 23% yield). HRMS [ESI+] m/z: calculated for $C_{22}H_{24}N_4O$ $[M+H]^+$ 361.2023, found: 361.2024. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 7.7 Hz, 2H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 1H), 4.26 (br s, 2H), 3.16 (br s, 1H), 1.88 – 1.63 (m, 8H), 0.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.64, 142.12, 140.36, 132.95, 128.96, 128.25, 127.77, 127.70, 127.10, 126.90, 53.85, 43.21, 42.24, 28.01, 25.94, 22.78, 18.93, 10.76.

(2-Benzylpiperidin-1-yl)(4-(dicyclohexyl(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (7). The title compound was synthesized from 2-benzylpiperidine (73.6 mg, 0.42 mmol) and dicyclohexyl(2*H*-1,2,3-triazol-4-yl) methanol (122 mg, 0.462 mmol). According to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **7** (31.0 mg, 0.067 mmol, 16% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{40}N_4O_2$ $[M+H^+]$ 465.3224, found: 465.3221. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.37 – 7.04 (m, 5H), 4.59 (br s, 1H), 3.81 – 3.68 (m, 1H), 3.28 (td, *J* = 13.3, 2.9 Hz, 1H), 3.10 (dd, *J* = 13.3, 6.4 Hz, 1H), 2.99 (t, *J* = 11.6 Hz, 1H), 1.93 – 1.59 (m, 16H), 1.45 – 1.34 (m, 2H), 1.27 – 1.15 (m, 4H), 1.10 – 0.98 (m, 4H), 0.84 – 0.72 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.64, 149.83, 137.99, 134.24, 129.10, 128.60, 126.64, 78.22, 67.99, 43.88, 42.45, 36.10, 29.71, 27.22, 26.52, 26.42, 26.21, 25.46, 18.78.

(2-Benzylpiperidin-1-yl)(4-(1-hydroxycyclohexyl)-2*H*-1,2,3-triazol-2-yl)methanone (8). The title compound was synthesized from 2-benzylpiperidine (88.0 mg, 0.500 mmol) and 1-(2*H*-1,2,3-triazol-4-yl)cyclohexan-1-ol (92 mg, 0.550 mmol). According to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **8** (61.0 mg, 0.17 mmol, 34% yield). HRMS [ESI+] m/z: calculated for $C_{21}H_{28}N_4O_2$ $[M+H^+]$ 369.2285, found: 369.2288. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.36 – 7.09 (m, 5H), 4.63 (br s, 1H), 4.14 (br s, 1H), 3.29 (td, *J* = 13.2, 2.9 Hz, 1H), 3.16 (dd, *J* = 13.3, 6.1 Hz, 1H), 3.06 – 2.98 (m, 1H), 2.29 (br s, 1H), 2.02 – 1.55 (m, 16H), 1.45 – 1.33 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.07, 149.78, 138.07, 132.99, 129.11, 128.59, 126.61, 69.77, 53.47, 42.23, 38.01, 36.04, 26.62, 25.47, 25.25, 21.79, 18.78.

(2-Benzylpiperidin-1-yl)(4-(1-hydroxy-1-phenylethyl)-2*H*-1,2,3-triazol-2-yl)methanone (9). The title compound was synthesized from 2-benzylpiperidine (88.0 mg, 0.500 mmol) and 1-phenyl-1-(1*H*-1,2,3-triazol-4-yl)ethan-1-ol (104 mg, 0.550 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **9** (25.0 mg, 0.064 mmol, 13% yield). HRMS [ESI+] m/z: calculated for $C_{23}H_{26}N_4O_2$ $[M+H^+]$ 391.2129, found: 391.2131. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 3.5 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.42 – 7.34 (m, 2H), 7.34 – 7.30 (m, 1H), 7.29 – 7.17 (m, 5H), 4.76 – 4.31 (m, 2H), 3.30 (td, *J* = 13.4, 2.8 Hz, 1H), 3.15 (dd, *J* = 13.3,

6.2 Hz, 1H), 3.08 – 2.95 (m, 1H), 2.86 (br s, 1H), 2.02 (d, *J* = 2.5 Hz, 3H), 1.90 – 1.57 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.82, 138.03, 133.69, 129.12, 128.66, 128.45, 127.57, 126.67, 125.15, 72.39, 63.05, 35.57, 30.64, 25.48, 18.81.

(2-Benzylpiperidin-1-yl)(4-(2-hydroxypropan-2-yl)-2*H*-1,2,3-triazol-2-yl)methanone (10). The title compound was synthesized from 2-benzylpiperidine (88.0 mg, 0.500 mmol) and 2-(2*H*-1,2,3-triazol-4-yl)propan-2-ol (69.9 mg, 0.550 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **10** (17.0 mg, 0.052 mmol, 11% yield). HRMS [ESI+] m/z: calculated for $C_{18}H_{24}N_4O_2$ $[M+H^+]$ 329.1972, found: 329.1971. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.43 – 6.95 (m, 5H), 4.65 (br s, 1H), 4.37 – 3.94 (m, 1H), 3.49 (br s, 1H), 3.30 (td, *J* = 13.3, 2.8 Hz, 1H), 3.16 (br s, 1H), 3.06 – 2.98 (m, 1H), 1.89 – 1.61 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.12, 149.78, 138.03, 132.74, 129.09, 128.62, 126.57, 68.82, 55.06, 42.43, 36.02, 30.31, 25.42, 18.83.

(2-Benzylpiperidin-1-yl)(4-(hydroxydi(pyridin-2-yl)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (11). The title compound was synthesized from 2-benzylpiperidine (59.6 mg, 0.340 mmol) and di(pyridin-2-yl)(1*H*-1,2,3-triazol-4-yl)methanol (95.0 mg, 0.374 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **11** (30 mg, 0.066 mmol, 19% yield). HRMS [ESI+] m/z: calculated for $C_{26}H_{26}N_6O_2$ $[M+H]^+$ 455.2190, found: 455.2186. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (br d, *J* = 4.4 Hz, 2H), 8.43 (br s, 2H), 8.02 – 7.90 (m, 4H), 7.48 – 7.45 (m, 2H), 7.24 – 6.95 (m, 4H), 4.70 – 3.80 (m, 2H), 3.23 (td, *J* = 13.5, 2.6 Hz, 1H), 3.07 (dd, *J* = 13.1, 6.3 Hz, 1H), 2.96 (br s, 1H), 1.79 – 1.61 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.82, 152.26, 149.47, 145.84, 140.26, 137.96, 135.16, 129.20, 128.68, 126.69, 124.42, 123.99, 76.10, 56.46, 45.37, 36.00, 25.57, 25.47, 18.79.

(2-Benzylpiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (12). The title compound was synthesized from 2-benzylpiperidine (100 mg, 0.571 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl)methanol (164 mg, 0.571 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **12** (71.0 mg, 0.145 mmol, 26% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{26}F_2N_4O_2$ $[M+H]^+$ 489.2097, found: 489.2097. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.34 – 7.24 (m, 5H), 7.18 (br, 3H), 7.02 – 6.97 (m, 5H), 4.47 – 4.26 (m, 1H), 3.80 (br s, 1H), 3.28 – 3.21 (m, 1H), 3.08 (dd, *J* = 13.2, 6.5 Hz, 1H), 2.94 (br s, 1H), 1.77 – 1.63 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.35 (d, *J* = 248.5 Hz), 155.29, 149.47, 140.84, 137.91, 134.89, 131.03, 129.08 (d, *J* = 8.1 Hz), 128.69, 126.75, 115.15 (d, *J* = 21.2 Hz), 76.50, 56.81, 43.26, 36.12, 25.56, 25.55, 18.88.

(2-Benzylpiperidin-1-yl)(4-(bis(4-fluorophenyl)(methoxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (13). The title compound was synthesized from 2-benzylpiperidine

(50.8 mg, 0.290 mmol) and 4-(bis(4-fluorophenyl)(methoxy)methyl)-1H-1,2,3-triazole (96.0 mg, 0.319 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **13** (13.0 mg, 0.025 mmol, 10% yield). HRMS [ESI+] m/z: calculated for $C_{29}H_{28}F_2N_4O_2$ $[M+Na]^+$ 525.2073, found: 525.2069. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.49 – 7.39 (m, 4H), 7.32 – 7.15 (m, 5H), 7.03 (td, *J* = 8.6, 1.5 Hz, 4H), 4.73 (br s, 1H), 4.39 (br s, 1H), 3.28 (td, *J* = 13.3, 2.9 Hz, 1H), 3.18 (s, 3H), 3.10 (dd, *J* = 13.2, 6.4 Hz, 1H), 3.04 – 2.94 (m, 1H), 1.85 – 1.56 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.14 (d, *J* = 247.2 Hz), 152.51, 149.48, 138.30, 137.87, 135.89, 129.68 (d, *J* = 13.0 Hz), 129.11, 128.59, 126.63, 115.07 (d, *J* = 21.4 Hz), 81.60, 54.56, 52.45, 43.32, 36.21, 26.40, 25.42, 18.72.

(4-(Hydroxydiphenylmethyl)-2*H*-1,2,3-triazol-2-yl)(2-(phenoxymethyl)piperidin-1-yl)methanone (14). The title compound was synthesized from 2-(phenoxymethyl)piperidine (60.0 mg, 0.314 mmol) and diphenyl(1*H*-1,2,3-triazol-4-yl)methanol (87.0 mg, 0.345 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **14** (36.7 mg, 0.078 mmol, 25 % yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{28}N_4O_3$ $[M+H]^+$ 469.2234, found: 469.2233. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.34 – 7.21 (m, 12H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.84 (br d, *J* = 8.0 Hz, 2H), 4.74 (br s, 1H), 4.26 – 4.22 (m, 1H), 4.06 (br s, 1H), 3.54 (br s, 1H), 3.15 (br s, 1H), 1.91 – 1.85 (m, 2H), 1.79 – 1.63 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.33, 155.75, 150.53, 144.98, 135.39, 129.62, 128.27, 127.91, 127.26, 121.27, 114.70, 77.33, 65.77, 53.87, 41.89, 25.45, 25.11, 19.46.

(4-(Bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)(2-(phenoxymethyl) piperidin-1-yl)methanone (15). The title compound was synthesized from 2-(phenoxymethyl)piperidine (60.0 mg, 0.314 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl)methanol (99.0 mg, 0.345 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **15** (71.0 mg, 0.141 mmol, 45% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{26}F_2N_4O_3$ $[M+H]^+$ 505.2046, found: 505.2046. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.30 – 7.21 (m, 6H), 6.98 – 6.92 (m, 5H), 6.82 (br d, *J* = 8.0 Hz, 2H), 4.70 (br s, 1H), 4.26 – 4.22 (m, 1H), 4.05 (br s, 1H), 3.76 (br s, 1H), 3.14 (br s, 1H), 1.93 – 1.59 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.32 (d, *J* = 248.5 Hz), 158.25, 155.52, 150.42, 140.78 (d, *J* = 10.1 Hz), 135.11, 129.57, 129.10 (d, *J* = 9.1 Hz), 121.35, 115.13 (d, *J* = 21.2 Hz), 114.63, 76.51, 65.65, 53.83, 41.99, 25.45, 25.07, 19.42.

(2-((4-Fluorophenoxy)methyl)piperidin-1-yl)(4-(hydroxydiphenylmethyl)-2*H*-1,2,3 -triazol-2-yl)methanone (16). The title compound was synthesized from 2-((4-fluorophenoxy)methyl)piperidine (60.0 mg, 0.287 mmol) and diphenyl(1*H*-1,2,3-triazol-4-yl) methanol (79.0 mg, 0.315 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by

silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **16** (27.9 mg, 0.057 mmol, 20% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{27}FN_4O_3$ $[M+H]^+$ 487.2140, found: 487.2140. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.32 – 7.27 (m, 11H), 6.90 (t, *J* = 8.4 Hz, 2H), 6.77 (br s, 1H), 4.71 (br s, 1H), 4.28 – 4.13 (m, 1H), 4.01 (br s, 2H), 3.13 (br s, 1H), 1.91 – 1.85 (m, 2H), 1.75 – 1.64 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.53 (d, *J* = 239.4 Hz), 155.83, 154.42, 150.56, 145.10, 135.41, 128.23, 127.87, 127.35 (d, *J* = 22.2 Hz), 115.99, 115.73 (d, *J* = 8.1 Hz), 77.28, 66.42, 53.87, 42.94, 25.36, 25.05, 19.42.

(4-(Bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)(2-((4-fluorophenoxy)methyl)piperidin-1-yl)methanone (17). The title compound was synthesized from 2-((4-fluorophenoxy)methyl)piperidine (60.0 mg, 0.287 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3- triazol-4-yl)methanol (82.0 mg, 0.287 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1→5:1) to afford 2,4-triazole urea **17** (30.3 mg, 0.058 mmol, 20% yield). HRMS [ESI+] m/z: calculated for $C_{28}H_{25}F_3N_4O_3$ $[M+H]^+$ 523.1952, found: 523.1952. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.30 – 7.26 (m, 5H), 6.99 – 6.89 (m, 6H), 6.76 (br s, 1H), 4.69 (br s, 1H), 4.23 – 4.18 (m, 1H), 4.01 (br s, 2H), 3.79 (br s, 1H), 3.13 (t, *J* = 11.8 Hz, 1H), 1.88 – 1.65 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.36 (d, *J* = 247.5 Hz), 157.59 (d, *J* = 240.4 Hz), 155.58, 154.41, 150.47, 140.8 (d, *J* = 9.0 Hz), 135.13, 129.08 (d, *J* = 8.1 Hz), 115.94 (d, *J* = 23.2 Hz), 115.71 (d, *J* = 8.1 Hz), 115.1 (d, *J* = 22.2 Hz), 76.54, 66.42, 53.07, 43.12, 25.39, 25.05, 19.42.

(4-(Bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)(2-(3-methoxybenzyl)piperidin-1-yl)methanone (18). The title compound was synthesized from 2-(3-methoxybenzyl)piperidine (130 mg, 0.634 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3- triazol-4-yl)methanol (200 mg, 0.697 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **18** (118 mg, 0.227 mmol, 36% yield). HRMS [ESI+] m/z: calculated for $C_{29}H_{28}F_2N_4O_3$ $[M+H]^+$ 519.2202, found: 519.2204. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.31 – 7.25 (m, 5H), 7.13 (br s, 1H), 7.04 – 6.98 (m, 5H), 6.72 (d, *J* = 8.0 Hz, 1H), 3.73 (s, 3H), 3.67 (br s, 1H), 3.49 (br s, 2H), 3.25 (t, *J* = 13.2 Hz, 1H), 3.07 (dd, *J* = 13.2, 6.7 Hz, 1H), 2.91 (br s, 1H), 1.82 – 1.57 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.42 (d, *J* = 248.2 Hz), 159.78, 155.32, 149.57, 140.85, 139.46, 134.95, 129.72, 129.11 (d, *J* = 7.6 Hz), 121.49, 115.21 (d, *J* = 21.4 Hz), 112.48, 111.80, 76.51, 57.52, 55.27, 41.10, 36.24, 28.99, 25.51, 18.83.

(4-(Bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)(2-(4-methoxybenzyl)piperidin-1-yl)methanone (19). The title compound was synthesized from 2-(4-methoxybenzyl)piperidine (92 mg, 0.448 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl) methanol (142 mg, 0.493 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **19**

(116 mg, 0.224 mmol, 30% yield). HRMS [ESI+] m/z: calculated for $C_{29}H_{28}F_2N_4O_3$ $[M+H]^+$ 519.2202, found: 519.2199. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.32 – 7.24 (m, 5H), 7.06 – 6.97 (m, 5H), 6.74 (br s, 2H), 4.37 (br s, 2H), 3.75 (s, 3H), 3.24 (td, *J* = 13.4, 2.6 Hz, 1H), 3.03 (dd, *J* = 13.5, 6.6 Hz, 1H), 2.89 (br s, 1H), 2.43 (br s, 1H), 1.86 – 1.57 (m, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 162.47 (d, *J* = 248.2 Hz), 158.45, 155.27, 149.53, 140.85, 134.87, 130.11, 129.93, 129.14 (d, *J* = 7.56 Hz), 115.27 (d, *J* = 21.42 Hz), 114.17, 76.58, 55.33, 51.04, 40.78, 35.33, 26.24, 25.55, 18.83.

(*S,E*)-N-(1-Phenylbut-3-en-2-yl)pent-3-en-1-amine (25a). Under an argon atmosphere, a flame dried three necked reaction flask was charged with a solution of 3-pentene nitrile **24** (285 mg, 3.34 mmol) in dry diethyl ether. At -78 °C a 1.0 M solution of DIBAL-H (12 mL, 12 mmol) in toluene was added drop wise. The reaction was warmed slowly on the cooling bath until 0 °C in circa 2h. After re-cooling to -90 °C, dry MeOH (10 mL) was added at once. After 5 min followed by a solution of (*S*)-1-phenylbut-3-en-2-amine[15,17] **23a** (1.80 g, 12.2 mmol, e.e. = 99%) in MeOH (10 mL). The cooling bath was removed and the mixture stirred at room temperature for 6h. Subsequently, an excess of $NaBH_4$ (870 mg, 22.9 mmol) was added at 0 °C in two portions with a five minute interval. The reaction was left stirring on the ice bath and slowly warmed up to room temperature overnight. The reaction mixture was poured into 0.8 M aqueous NaOH solution (80 mL) and extracted with diethyl ether (3 x 30 mL). The organic layers were combined, washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the crude product as an orange oil that was purified by silica gel column chromatography using pentane: EtOAc : TEA = 9 : 1 : 0 → 90 : 10 : 5 → 85 : 15 : 5 as the eluent to give the target compound (315 mg, 1.47 mmol, 44% yield). $[\alpha]_D^{23}$ = -9.2 (c = 1.0, $CHCl_3$). HRMS calculated for $C_{15}H_{21}N$ $[M+H]^+$ 202.1590; found: 202.1593. IR (film) 3063, 3026, 2967, 2918, 2818, 1495, 1554, 1111, 966, 918. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.25 (m, 2H), 7.20 (m, 3H), 5.65 (ddd, *J* = 16.9, 10.5, 8.1 Hz, 1H), 5.58 – 5.36 (m, 2H), 5.16 – 5.00 (m, 2H), 3.31 (m, 1H), 3.25 – 3.14 (m, 1H), 3.03 – 2.95 (m, 1H), 2.77 (d, *J* = 6.9 Hz, 2H), 1.64 (dd, *J* = 6.0, 1.2 Hz, 3H), 1.42 – 1.34 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.65, 138.51, 129.38, 129.29, 128.31, 127.09, 126.25, 116.14, 62.27, 49.09, 42.45, 17.77.

***tert*-Butyl *(S,E)*-pent-3-en-1-yl(1-phenylbut-3-en-2-yl)carbamate (26a)**. The amine from above **25a** (315 mg, 1.47 mmol) was dissolved in a mixture of THF (20 mL) and Et_3N (1 mL). Boc_2O (450 mg, 2.06 mmol) was added and the reaction refluxed overnight. TLC analysis confirmed complete conversion of the amine and after evaporation of the solvents and silica gel column chromatography using pentane: EtOAc = 98 : 2 as the eluent afforded the Boc-protected amine (425 mg, 1.35 mmol, 92% yield). $[\alpha]_D^{22}$ = -58 (c =1.0, $CHCl_3$). HRMS calculated for $C_{20}H_{29}NO_2$ $[M+H]^+$: 316.2271; found: 316.2272. IR (film) 3029, 2980, 2936, 1690, 1456, 1396, 1371, 1308, 1256, 1211, 1169, 1117, 1069. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 – 6.77 (m, 5H), 5.96 (ddd, *J* = 16.6, 10.5, 6.0 Hz, 1H), 5.55 – 5.20 (m, *J* = 33.4 Hz, 2H), 5.17 – 5.07 (m, 2H), 4.48 (m, 1H), 3.41 – 2.69 (m, 4H), 2.38 – 1.92 (m, 2H), 1.62 (d, *J* = 6.1 Hz, 3H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.09, 138.59, 137.32, 129.26, 129.20,

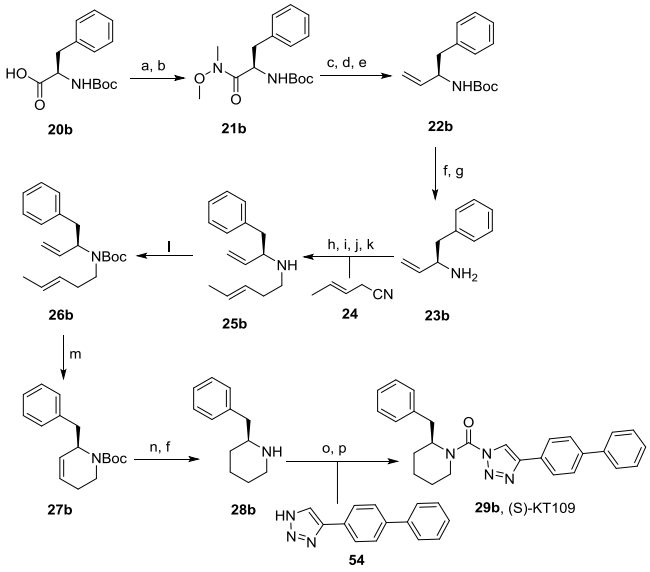
128.16, 128.11, 126.62, 126.13, 115.91, 79.16, 61.12, 45.76, 38.24, 33.12, 28.32, 17.93.

***tert*-Butyl *(S)*-6-benzyl-3,6-dihydropyridine-1(2H)-carboxylate (27a)**. The diene from above (425 mg, 1.35 mmol) was dissolved in DCM (10 mL) and purged with argon. After the addition of Grubb's 1st generation catalyst (42.0 mg, 0.050 mmol, 3.6 mol%) and refluxing overnight TLC analysis confirmed complete conversion. The solvent was evaporated and the crude product purified by silica gel column chromatography using pentane : EtOAc = 97 : 3 as the eluent to afford the title compound (252 mg, 0.920 mmol, 68% yield). $[\alpha]_D^{21}$ = +161 (c = 1.0, $CHCl_3$). HRMS calculated for $C_{17}H_{23}NO_2$ $[M+H]^+$: 274.1802; found: 274.1802. ^{1}H NMR (400 MHz, $CDCl_3$, 60 ^oC) δ 7.28 – 7.21 (m, 2H), 7.20 – 7.14 (m, 3H), 5.79 (dd, *J* = 10.3, 6.1 Hz, 1H), 5.54 (dt, *J* = 10.3, 3.3 Hz, 1H), 4.54 (s, 1H), 4.11 (s, 1H), 2.89 (dd, *J* = 13.0, 6.2 Hz, 1H), 2.83 – 2.67 (m, 2H), 2.15 (m, 1H), 1.88 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, 60 ^oC) δ 154.28, 138.23, 129.36, 128.10, 127.94, 126.06, 125.38, 79.17, 53.47, 40.20, 36.30, 28.29, 24.85.

(*R*)-2-Benzylpiperidine (28a). The compound from above **27a** (680 mg, 2.49 mmol) was dissolved in MeOH (10 mL) and an aqueous 6.0 M HCl solution (1 mL) and Pd/C-10% (24 mg) were added subsequently. The reaction was stirred overnight under a balloon of hydrogen. After filtering over a Whatman® filter and evaporation of the solvents, (*R*)-2-benzylpiperidine hydrochloride was obtained. The salt from above was dissolved in water (20 mL) and washed with EtOAc (2 x 10 mL). The water layer was basified with an aqueous 4.0 M NaOH solution (4 mL) and extracted with $CHCl_3$ (4 x 10 mL). The combined $CHCl_3$ layers were dried over Na_2SO_4, filtered and concentrated to give the crude material as a yellow oil that was purified by silica gel column chromatography (pentane : EtOAc : Et_3N = 80 : 15 : 5) to afford *(R)*-2-benzylpiperidine (306 mg, 1.75 mmol, 70%). $[\alpha]_D^{23}$ = -13 (c = 0.5, $CHCl_3$). HRMS calculated for $C_{12}H_{17}N$ $[M+H]^+$ 176.1432; found: 176.1434. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.23 (m, 2H), 7.23 – 7.16 (m, 3H), 2.98 (d, *J* = 11.5 Hz, 1H), 2.74 – 2.62 (m, 2H), 2.61 – 2.44 (m, 2H), 1.89 – 1.72 (m, 2H), 1.68 (d, *J* = 12.7 Hz, 1H), 1.57 (d, *J* = 12.5 Hz, 1H), 1.43 (m, 1H), 1.36 – 1.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.11, 129.12, 128.27, 126.06, 58.15, 47.05, 43.83, 32.83, 26.10, 24.77.

(*R*)-(4-([1,1'-Biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-benzylpiperidin-1-yl)methanone (29a, *(R)*-KT109**)**. A solution of *(R)*-2-benzylpiperidine (40.0 mg, 0.229 mmol) in THF was treated with DIPEA (0.120 mL, 0.685 mmol) and bis(trichloromethyl) carbonate (33.9 mg, 0.114 mmol) and the reaction mixture was stirred for 30 min at 0 ^oC. The mixture was poured into water and extracted with ethyl acetate (3 times). The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated under reduced pressure. The intermediate was dissolved in THF and DIPEA (0.120 mL, 0.685 mmol), DMAP (27.9 mg, 0.228 mmol) and triazole (55.5 mg, 0.251 mmol) were added to the solution. The mixture was stirred for 2h at 60 ^oC and poured into saturated aqueous NH_4Cl solution. The mixture was extracted with ethyl acetate, washed with water, brine, dried over $MgSO_4$, and concentrated under reduced

pressure. The residue was purified by flush column chromatography (pentane/EtOAc = 100 : 1 → 8 : 1) to afford 1,4-triazole urea *(R)*-KT109 (24.0 mg, 0.057 mmol, 30% yield) as top TLC spot. $[\alpha]_D^{22}$ = -14 (*c* = 0.1, $CHCl_3$). The enantiomeric purity was determined on a Daicel Chiralcel OD-H column (4.6 X 250 mm, 20:80 IPA/Hex, flow rate of 1 mL/min): 14.4 min, e.e.>95%. HRMS calculated for $C_{27}H_{26}N_4O$ $[M+H]^+$ 423.2179, found: 423.2179. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (br s, 1H), 7.75 – 7.67 (m, 4H), 7.53 – 7.48 (m, 2H), 7.43 – 7.04 (m, 6H), 4.90 (br s, 1H), 4.40 (br d, *J* = 16.0 Hz, 1H), 3.39 – 3.26 (m, 2H), 2.74 (br s, 1H), 2.07 – 1.69 (m, 6H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 146.14, 141.28, 140.49, 137.99, 129.20, 128.88, 128.74, 128.64, 127.61, 127.57, 127.03, 126.63, 126.18, 120.56, 57.20, 40.80, 36.41, 29.65, 25.44, 18.75.



Scheme 3. Enantioselective synthesis of (*S*)-KT109Reagents and conditions: (a) Me(OMe)NH·HCl; (b) EDCI, NMM; (c) $LiAlH_4$; (d) H_3O^+; (e) $(Ph)_3P{=}CH_2$, 79% (**22b**, based on **20b**); (f) MeOH, HCl, 81% (**28b**); (g) NaOH; (h) compound **24**, diethyl ether, DIBAL-H, -80 °C to 0 °C, 64% (**25b**, based on **23b**); (i) MeOH, -90 °C; (j) corresponding amine **23b** (3 equiv), r.t., 20h; (k) $NaBH_4$, 0 °C to r.t., 5h; (l) Boc_2O, Et_3N, THF, 50 °C, 20h; (m) Grubbs I cat. 4 mol %, DCM, reflux, 48h, 25%; (n) H_2, Pd/C, MeOH; (o) DIPEA, Triphosgene, THF, 0 °C; (p) DIPEA, DMAP, triazole , THF, 60 °C, 33%.

(*R*)-1-phenylbut-3-en-2-amine (23b). Compound **22b** was prepared according the procedure reported [17] on 48 mmol scale, affording 9.40 g of compound **22b** (79%, $[\alpha]_D^{23}$ = -34 (*c* = 1.0, $CHCl_3$); Lit [17]: $[\alpha]_D^{25}$ = -37 (*c* = 0.28, $CHCl_3$)). The Boc-protetected amine **22b** (9.40 g, 38.1 mmol) was dissolved in a mixture of MeOH (100 mL) and aqueous 6.0 M HCl (20 mL). After TLC confirmed total conversion of compound **22b**, evaporation of the solvents afforded a white solid that was dissolved in water (50 mL). After addition of aqueous 8.0 M NaOH (10 mL), extraction with chloroform (4 x 30 mL), drying ($MgSO_4$), filtering and evaporation of the solvent, amine **23b** was obtained as a pale brown liquid that was used without further purification. $[\alpha]_D^{20}$ = -14 (*c* = 1.0,

$CHCl_3$); Lit[24] $[\alpha]_D^{25}$ = -15 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (m, 5H), 5.88 (ddd, *J* = 17.2, 10.3, 6.3 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.03 (d, *J* = 10.3 Hz, 1H), 3.59 (q, *J* = 6.3 Hz, 1H), 2.82 (dd, *J* = 13.3, 5.3 Hz, 1H), 2.61 (dd, *J* = 13.3, 8.3 Hz, 1H), 1.28 (br s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.30, 138.65, 129.29, 128.28, 126.22, 113.52, 55.36, 44.23. For chiral HPLC analysis amine **23b** was derivatized as its benzoate, followed by analysis on a Daicel Chiralpak AD column (250 x 4.5 mm, 10µm particle size). Eluent hexane / 2-propanol = 90 /10, 1.0 mL / min., detection UV 254 nm. (*R*)-Enantiomer, R_t = 12.3 min (97.4%); (*S*)-enantiomer, R_t = 14.7 min (1.3%).

***tert*-Butyl *(R,E)*-pent-3-en-1-yl(1-phenylbut-3-en-2-yl)carbamate (26b)**. *(R,E)*-N-(1-Phenylbut -3-en-2-yl)pent-3-en-1-amine **25b** was prepared from 3-pentene nitrile **24** (586 mg, 7.23 mmol) and (*R*)-1-phenylbut-3-en-2-amine **23b** (e.e. = 97%, 3.50 g, 13.8 mmol) in 64% yield (1.24 g, ≈80% purity, 4.61 mmol) following the procedure as described for **25a**. This material was converted into Boc-protected compound **26b** as described for **26a**. The Boc-protected amine **26b** was obtained as colorless oil contaminated with circa 40 mol% Boc_2O (1.22 g). Analytical data from a pure sample: $[\alpha]_D^{22}$ = +66 (c =1.0 $CHCl_3$). HRMS calculated for $[C_{20}H_{29}NO_2+H]^+$: 316.2271; found: 316.2272. IR 2972, 2930, 1690, 1454, 1404, 1366, 1252, 1169, 1136, 966. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.15 (m, 5H), 5.96 (ddd, *J* = 16.7, 10.7, 6.0 Hz, 1H), 5.48 – 5.25 (m, 2H), 5.19 – 5.01 (m, 2H), 4.67 – 4.28 (m, 1H), 3.16 – 2.80 (m, 4H), 2.32 – 1.85 (m, 2H), 1.62 (d, *J* = 6.1 Hz, 3H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.09, 138.61, 137.41, 129.27, 129.21, 128.18, 128.13, 126.63, 126.14, 115.92, 79.31, 77.32, 77.00, 76.68, 60.18, 45.80, 38.25, 28.34, 17.93.

***tert*-Butyl *(R)*-6-benzyl-3,6-dihydropyridine-1(2*H*)-carboxylate (27b)**. Prepared as described for **27a**. The Boc-protected diene from above (1.22 g, 3.87 mmol) afforded the title compound in 25% overall yield (491 mg, 1.80 mmol). $[\alpha]_D^{21}$ = -172 (c =1.0 $CHCl_3$). HRMS calculated for $C_{17}H_{23}NO_2$ $[M+H]^+$: 274.1802; found: 274.1803. IR 2974, 2926, 1690, 1454, 1416, 1391, 1364, 1337, 1279, 1250, 1171, 1107. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.08 (m, 5H), 5.81 (s, 1H), 5.63 – 5.48 (m, 1H), 4.68 – 4.42 (m, 1H), 4.33 – 3.94 (m, 1H), 2.99 – 2.82 (m, 1H), 2.84 – 2.67 (m, 2H), 2.19 (m, 1H), 2.02 – 1.83 (m, 1H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.34, 138.25, 129.40, 128.22, 127.70, 126.15, 125.63, 79.33, 53.84, 40.27, 35.87, 28.29, 24.85.

(*S*)-2-Benzylpiperidine(28b). A solution of *tert*- butyl *(R)*-6-benzyl-3,6-dihydropyridine-1(2*H*) -carboxylate **27b** (277 mg, 1.01 mmol) in methanol (10 mL) and 6.0 M HCl (1 mL) was purged with argon and Pd/C 10% (100 mg) was added subsequently. The flask was sealed with a septum, placed under a balloon of hydrogen, and stirred vigorously overnight. The mixture was filtered over a Whatman® filter and the solvents evaporated to afford the hydrochloride as a white foam that was dissolved in methanol (1 mL) and loaded onto a flash silica gel column that was eluted subsequently with pentane/EtOAc/Et_3N = 97:3:0 → 9:1:0 → 85:10:5 → 75:20:5 to yield the target compound as a pale yellow oil (142 mg, 81%). $[\alpha]_D^{27}$ = +14 (c = 1.0, $CHCl_3$). HRMS calculated for $[C_{12}H_{17}N+H]^+$: 176.1434; found: 176.1433. IR

3026, 2928, 2851, 2799, 1495, 1452, 1441, 1331, 1319, 1119, 1053. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.25 (m, 2H), 7.20 (dt, *J* = 7.1, 2.9 Hz, 3H), 2.99 (ddt, *J* = 11.5, 3.9, 2.1 Hz, 1H), 2.76 – 2.63 (m, 2H), 2.63 – 2.46 (m, 1H), 2.04 (s, 1H), 1.83 – 1.74 (m, 1H), 1.68 (d, *J* = 12.7 Hz, 1H), 1.62 – 1.52 (m, 1H), 1.44 (qt, *J* = 12.2, 3.7 Hz, 1H), 1.37 – 1.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.10, 129.14, 128.30, 126.08, 58.17, 47.03, 43.80, 32.79, 26.08, 24.76.

***(S)*-(4-([1,1'-Biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-benzylpiperidin-1-yl)methano ne (29b**, (*S*)-KT109). The title compound was synthesized from (*S*)-2-benzylpiperidine **28b** (40.0 mg, 0.229 mmol) according to the procedures described for *(R)*-KT109. This yielded (*S*)-(4-([1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)(2-benzylpiperidin-1-yl) methanone (*S*)-KT109 (66.0 mg, 0.156 mmol, 33 % yield). $[\alpha]_D^{22}$ = 13 (*c* = 1.0, $CHCl_3$). The enantiomeric purity was determined on a Daicel Chiralcel OD-H column (4.6 X 250 mm, 20:80 IPA/Hex, flow rate of 1 mL/min): 18.4 min, e.e.>95%. HRMS calculated for $C_{27}H_{27}N_4O$ $[M+H]^+$ 423.2179, found: 423.2179. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.86 (s, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 4.0 Hz, 2H), 7.49 – 7.41 (m, 3H), 7.39 – 7.33 (m, 1H), 7.21 – 7.01 (m, 5H), 4.85 (s, 1H), 4.35 (d, *J* = 12.0 Hz, 1H), 3.35 (br d, *J* = 12.0 Hz, 1H), 3.21 (s, 1H), 2.69 (s, 1H), 1.93 – 1.69 (m, 6H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 146.13, 142.13, 141.37, 140.55, 138.05, 129.25, 128.94, 128.79, 128.74, 127.67, 127.62, 127.08, 126.68, 126.24, 120.61, 50.75, 40.28, 36.47, 28.67, 25.50, 19.08.

((*3R,6S*)-6-Benzyl-3-hydroxy-3,6-dihydropyridin-1(2H)-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (30). The title compound was synthesized from (*3R,6S*)-6-benzyl-3-((tert-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (120 mg, 0.281 mmol) and bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl) methanol (89.0 mg, 0.309 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 8:1). HF-pyridine (1.55 mL, 1.70 mmol) was subsequently added to a solution of N2-carbamoyl triazole urea in THF and pyridine (1:1) with ice cooling, and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with ethyl acetate (40 eq.), and then washed with $NaHCO_3$, brine, dried with $MgSO_4$, and concentrated under reduced pressure. Purified by flash chromatography to furnish the title compound **30** (34.2 mg, 0.068 mmol, 24% yield for four steps). HRMS [ESI+] m/z: calculated for $C_{28}H_{24}F_2N_4O_3$ $[M+H]^+$ 503.1889, found: 503.1889. $[\alpha]_D^{20}$ = 245 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.32 – 7.24 (m, 9H), 7.05 – 6.98 (m, 4H), 5.84 (br d, *J* = 10.4 Hz, 1H), 5.64 (br s, 1H), 4.79 (br s, 1H), 4.49 (br s, 1H), 3.25 (s, 4H), 2.95 (t, *J* = 8.0 Hz, 1H), 2.88 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.51 (d, *J* = 248.5 Hz), 156.03, 149.01, 140.63, 136.67, 135.68, 130.76, 129.54, 129.12 (d, *J* = 8.1 Hz), 128.77, 128.20, 127.10, 115.38 (d, *J* = 22.2 Hz), 76.65, 63.99, 55.62, 47.98, 38.94.

((*2R,5R*)-2-Benzyl-5-hydroxypiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)meth yl)-2*H*-1,2,3-triazol-2-yl)methanone (31). The title compound was synthesized *(2R,5R)*-2-benzyl-5-((tert-butyldiphenylsilyl)oxy)piperidine (80.0 mg, 0.186 mmol),

bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl)methanol (58.8 mg, 0.205 mmol) and HF-pyridine (0.612 mL, 0.673 mmol) according to the procedures described for compound **30**. This furnished N2-carbamoyl triazole urea ((*2R,5R)*-2-benzyl-5-hydroxypiperidin-1-yl)(4-(bis(4-fluorophenyl) (hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone **31** (13.6 mg, 0.027 mmol, 16% yield). $[\alpha]_D^{20}$ = 191 (*c* = 0.1, $CHCl_3$). HRMS [ESI+] m/z: calculated for $C_{28}H_{26}F_2N_4O_3$ $[M+H]^+$ 505.2046, found: 505.2046. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.34 – 7.16 (m, 9H), 7.07 – 6.98 (m, 4H), 4.57 (br s, 1H), 4.21 (br s, 1H), 3.88 – 3.80 (m, 1H), 3.16 – 3.02 (m, 2H), 2.98 – 2.89 (m, 1H), 2.35 (br s, 2H), 2.03 – 2.00 (m, 1H), 1.79 – 1.72 (m, 3H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 162.50 (d, *J* = 248.2 Hz), 155.67, 149.43, 140.68, 137.62, 135.21, 131.07, 129.16 (d, *J* = 8.6 Hz), 128.86, 126.78, 115.34 (d, *J* = 21.4 Hz), 76.62, 67.08, 53.68, 45.66, 35.89, 28.59, 27.80.

((*3S,6S*)-6-Benzyl-3-hydroxy-3,6-dihydropyridin-1(2*H*)-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (32). The title compound was synthesized from *(3S,6S)*-6-benzyl-3-((tert-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (80.0 mg, 0.187 mmol), bis(4-fluorophenyl)(2*H*-1,2,3-triazol-4-yl) methanol (59.1 mg, 0.206 mmol) and HF-pyridine (0.613 mL, 0.675 mmol) according to the procedures described for compound **30**. This furnished N2-carbamoyl triazole urea ((*3S,6S*)-6-benzyl-3-hydroxy-3,6-dihydropyridin-1(2*H*)-yl)(4- (bis(4-fluorophenyl) (hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone **32** (13.9 mg, 0.028 mmol, 15% yield). $[\alpha]_D^{20}$ = 142 (*c* = 0.5, $CHCl_3$). HRMS [ESI+] m/z: calculated for $C_{28}H_{24}F_2N_4O_3$ $[M+H]^+$ 503.1889, found: 503.1888. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.45 – 7.19 (m, 9H), 7.04 – 7.00 (m, 4H), 6.03 – 5.99 (m, 1H), 5.82 (br s, 1H), 4.96 – 4.88 (m, 1H), 4.14 – 4.03 (m, 2H), 3.25 – 3.17 (m, 2H), 3.03 – 2.90 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.50 (d, *J* = 248.5 Hz), 156.00, 149.43, 140.64, 136.46, 135.48, 130.05, 129.67, 129.13 (d, *J* = 8.1 Hz), 128.77, 127.12, 124.90, 115.38 (d, *J* = 22.2 Hz), 76.58, 62.51, 55.90, 48.00, 38.19.

((*2R,5S*)-2-Benzyl-5-hydroxypiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)meth yl)-2*H*-1,2,3-triazol-2-yl)methanone (33). The title compound was synthesized (*2R,5S*)-2-benzyl-5- ((*tert*-butyldiphenylsilyl)oxy)piperidine (100 mg, 0.233 mmol), bis(4-fluorophenyl)(1*H*-1,2,3- triazol-4-yl)methanol (73.5 mg, 0.256 mmol) and HF-pyridine (2.45 mL, 2.69 mmol) according to the procedures described for compound **30**. This furnished N2-carbamoyl triazole urea **33** (18.3 mg, 0.036 mmol, 16% yield). $[\alpha]_D^{20}$ = 48 (*c* = 0.5, $CHCl_3$). HRMS [ESI+] m/z: calculated for $C_{28}H_{26}F_2N_4O_3$ $[M+H]^+$ 505.2046, found: 505.2045. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.33 – 7.31 (m, 6H), 7.03 – 6.99 (m, 7H), 4.65 (br s, 1H), 4.30 (br s, 1H), 3.54 (br s, 3H), 3.02 (br s, 2H), 1.57 – 1.32 (m, 5H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.41 (d, *J* = 247.6 Hz), 152.82, 148.40, 140.91, 137.26, 133.37, 130.98, 129.13, 126.88, 124.21, 115. 22 (d, *J* = 21.1 Hz), 76.02, 51.09, 45.71, 41.81, 38.56, 37.57, 30.18, 27.42.

((*2R,5R*)-2-Benzyl-5-methoxypiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)meth yl)-2*H*-1,2,3-triazol-2-yl)methanone (34). The title compound was synthesized

(2R,5R)-2-benzyl- 5-methoxypiperidine (100 mg, 0.487 mmol), bis(4-fluorophenyl)(1*H*-1,2,3- triazol-4-yl)methanol (154 mg, 0.536 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1 → 5:1) to afford 2,4-triazole urea **34** (70.7 mg, 0.136 mmol, 28% yield). $[\alpha]_D^{22}$ = -3.1 (*c* = 0.5, $CHCl_3$). HRMS [ESI+] m/z: calculated for $C_{29}H_{28}F_2N_4O_3$ $[M+H]^+$ 519.2202, found: 519.2203. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.32 – 7.19 (m, 9H), 7.04 – 6.92 (m, 4H), 4.84 – 3.93(m, 2H), 3.57 (br s, 1H), 3.50 – 3.33 (m, 3H), 3.25 (br s, 1H), 3.07 (br s, 1H), 3.03 – 2.92 (m, 2H), 2.05 (br s, 1H), 1.67 (br s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.45 (d, *J* = 246.4 Hz), 155.75, 149.25, 140.68, 137.63, 135.27, 130.84, 129.05 (d, *J* = 8.1 Hz), 128.82, 126.93, 115.31 (d, *J* = 21.2 Hz), 76.58, 75.46, 56.35, 56.07, 46.52, 35.82, 25.59, 25.42.

((*2R,5R*)-2-Benzyl-5-(cyclopropylmethoxy)piperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (35). The title compound was synthesized from *(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidine (50.0 mg, 0.204 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1→5:1) to afford 2,4-triazole urea (*(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone **35** (25.0 mg, 0.045 mmol, 22% yield). $[\alpha]_D^{20}$ = 21.4 (*c* = 0.5, $CHCl_3$). HRMS calculated for $C_{32}H_{33}F_2N_4O_3$ $[M+H]^+$ 559.2515, found: 559.2516. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (br s, 1H), 7.32 – 7.27 (m, 6H), 7.17 (br s, 2H), 7.05 – 6.99 (m, 4H), 6.90 (br s, 1H), 4.65 (br s, 1H), 4.38 – 4.02 (m, 1H), 3.50 – 3.42 (m, 3H), 3.19 – 2.90 (m, 3H), 2.02 (br s, 1H), 1.80 – 1.62 (m, 3H), 1.12 – 0.90 (m, 1H), 0.57 (br s, 2H), 0.28 – 0.08 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.48 (d, *J* = 251.5 Hz), 155.54, 149.15, 140.73, 137.68, 135.15, 130.92, 129.43, 129.09 (d, *J* = 8.1 Hz), 128.82, 126.91, 115.30 (d, *J* = 21.2 Hz), 76.60, 73.80, 56.49, 46.94, 44.55, 35.63, 26.10, 24.65, 11.05, 3.24.

((*2R,5S*)-2-Benzyl-5-methoxypiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (36). The title compound was synthesized from *(2R,5S)*-2-benzyl-5-methoxypiperidine (20.0 mg, 0.097 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1→5:1) to afford 2,4-triazole urea (*(2R,5S)*-2-benzyl-5-methoxypiperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone **36** (13.1 mg, 0.025 mmol, 26% yield). $[\alpha]_D^{22}$ = -8.1 (*c* = 0.4, $CHCl_3$). HRMS [ESI+] m/z: calculated for $C_{29}H_{28}F_2N_4O_3$ $[M+H]^+$ 519.2202, found: 519.2202. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.33 – 7.19 (m, 8H), 7.05 – 6.94 (m, 5H), 4.87 – 4.28 (m, 2H), 3.65 – 2.87 (m, 6H), 2.72 (br s, 4H), 2.09 – 1.83 (m, 2H), 1.54 – 1.39 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.44 (d, *J* = 251.5 Hz), 155.37, 149.24, 140.83, 137.80, 134.91, 130.01, 129.18 (d, *J* = 9.1 Hz), 128.79, 126.89, 115.18 (d, *J* = 21.2 Hz), 76.57, 73.10, 56.63, 56.46, 45.70, 35.95, 23.09, 21.09.

((*2R,5S*)-2-Benzyl-5-(cyclopropylmethoxy)piperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (37). The title compound was

synthesized from *(2R,5S)*-2-benzyl-5-(cyclopropylmethoxy)piperidine (50.0 mg, 0.204 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1→5:1) to afford 2,4-triazole urea **37** (23.9 mg, 0.043 mmol, 21% yield). $[\alpha]_D^{22}$ = -16 (*c* = 0.3, $CHCl_3$). HRMS calculated for $C_{32}H_{33}F_2N_4O_3$ $[M+H]^+$ 559.2515, found: 559.2516. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.34 – 7.24 (m, 5H), 7.19 (s, 3H), 7.04 – 6.99 (m, 5H), 4.56 (br s, 2H), 3.65 (br s, 1H), 3.31 (br d, *J* = 18.0 Hz, 1H), 3.11 (dd, *J* = 13.4, 6.9 Hz, 2H), 2.99 – 2.88 (m, 1H), 2.80 (br s, 2H), 2.24 – 2.03 (m, 1H), 1.96 – 1.77 (m, 2H), 1.47 –137 (m, 1H), 0.88 – 0.83 (m, 1H), 0.44 (br s, 2H), 0.12 (br s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.46 (d, *J* = 248.5 Hz), 155.27, 150.05, 140.72, 137.85, 134.83, 129.22, 129.18 (d, *J* = 8.1 Hz), 129.04, 128.77, 126.85, 115.29 (d, *J* = 21.2 Hz), 76.57, 72.97, 55.84, 45.20, 42.84, 36.01, 24.09, 21.31, 10.73, 3.23.

((2*R*,5*R*)-2-benzyl-5-(prop-2-yn-1-yloxy)piperidin-1-yl)(4-(bis(4-fluorophenyl)(hydroxy)methyl)-2*H*-1,2,3-triazol-2-yl)methanone (38, DH376). The title compound was synthesized from (2*R*,5*R*)-2-benzyl-5-(prop-2-yn-1-yloxy)piperidine (80 mg, 0.349 mmol) according to the procedures described for compound **3**. The N2-carbamoyl triazole urea was isolated by silica gel chromatography (pentane/EtOAc 100:1→5:1) to afford 2,4-triazole urea **38** (47.3 mg, 0.087 mmol, 25% yield) as lower TLC spot. $[\alpha]_D^{22}$ = -5.16 (*c* = 1.00, $CHCl_3$); HRMS(m/z):$[M+H]^+$ calcd. for $C_{31}H_{28}F_2N_4O_3$, 543.21575; found 543.21552. ^{1}H NMR (400 MHz, $(CD_3)_2SO$, 110 ^oC) δ 7.90 (s, 1H), 7.39 – 7.35 (m, 4H), 7.23 – 7.16 (m, 3H), 7.13 – 7.07 (m, 5H), 6.64 (br d, *J* = 4.0 Hz, 1H), 4.38 (br s, 1H), 4.16 (d, *J* = 2.4 Hz, 2H), 4.05 (br d, *J* = 12.0 Hz, 1H), 3.62 – 3.55 (m, 1H), 3.15 (br d, *J* = 4.0 Hz, 1H), 3.08 – 2.97 (m, 3H), 2.01 – 1.96 (m, 1H), 1.76 – 1.63 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 60 ^oC) δ 162.57 (d, *J* = 247 Hz), 155.76, 149.37, 140.95 (d, *J* = 3.0 Hz), 137.67, 135.22, 129.96, 129.20 (d, *J* = 8.1 Hz), 128.83, 126.94, 115.28 (d, *J* = 22 Hz), 79.88, 76.69, 74.77, 73.61, 56.26, 55.17, 45.94, 36.10, 25.99, 25.88; IR (film)：3425, 2925, 1709, 1602, 1506, 1429, 1225, 1159, 1093 cm^{-1}.

(*3R,6S*)-6-Benzyl-3-((*tert*-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (45). To a solution of *tert*-butyl (*3R,6S*)-6-benzyl-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridine-1(2*H*)-carboxylate **44** [15] (120 mg, 0.227 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$, and concentrated under reduced pressure. Filtering and concentration under reduced pressure afforded the crude product that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.59 (m, 4H), 7.45 – 7.29 (m, 8H), 7.28 – 7.18 (m, 3H), 5.72 – 5.61 (m, 2H), 4.18 (br s, 1H), 4.09 (br s, 1H), 3.29 (br d, *J* = 31.9 Hz, 2H), 3.09 (br d, *J* = 48.0 Hz, 2H), 1.02 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.86, 135.72, 134.10, 132.91, 132.42, 130.36, 130.24, 129.69, 129.12, 128.39, 128.11, 127.91, 127.74, 126.24, 61.00, 54.53, 48.13, 38.18, 26.75, 19.01.

***tert*-Butyl *(2R,5R)*-2-benzyl-5-methoxypiperidine-1-carboxylate (51)**. To a solution of *tert*-butyl *(2R,5R)*-2-benzyl-5-hydroxypiperidine-1-carboxylate **50**[15]. (100 mg, 0.343 mmol) and NaH (60%, 34.3 mg, 0.858 mmol) in DMF (3 mL) at 0 °C, MeI (0.064 mL, 1.03 mmol) was added dropwise with continuous stirring, and the mixture was allowed to stand at room temperature for 24h. The mixture was diluted with water (10 mL), and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (pentane/ethyl acetate = 20:1 → 5:1) to furnish *tert*-butyl (*2R,5R*)-2-benzyl-5-methoxypiperidine-1- carboxylate (87.0 mg, 0.285 mmol, 83% yield). $[\alpha]_D^{22}$ = -31 (*c* = 0.8, $CHCl_3$). LC-MS m/z: calculated for $C_{18}H_{27}NO_3$ $[M+H]^+$ 306.20, found: 306.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 2H), 7.18 – 7.12 (m, 3H), 4.39 (br s, 2H), 3.41 (s, 3H), 3.18 (t, *J* = 4.0 Hz, 1H), 2.97 – 2.87 (m, 1H), 2.74 – 2.58 (m, 2H), 1.96 (br s, 1H), 1.66 – 1.49 (m, 3H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.82, 139.12, 129.22, 128.47, 126.29, 79.61, 75.89, 56.33, 52.24, 42.06, 35.94, 26.25, 26.21, 25.84.

***(2R,5R)*-2-Benzyl-5-methoxypiperidine.** To a solution of compound *tert*-butyl *(2R,5R)*-2-benzyl- 5-methoxypiperidine-1-carboxylate **51** (44.0 mg, 0.144 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$, and concentrated under reduced pressure. Filtering and concentration under reduced pressure afforded the crude product *(2R,5R)*-2-benzyl-5-methoxypiperidine that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.22 (m, 3H), 7.18 (d, *J* = 6.5 Hz, 2H), 3.57 (s, 1H), 3.46 (br s, 1H), 3.33 (s, 4H), 3.17 (br d, *J* = 11.6 Hz, 1H), 3.03 (br s, 1H), 2.84 (br s, 1H), 2.07 (d, *J* = 15.0 Hz, 1H), 1.92 – 1.73 (m, 1H), 1.61 (d, *J* = 13.4 Hz, 1H), 1.53 – 1.41 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.33, 129.47, 128.91, 127.35, 70.16, 58.07, 56.12, 47.01, 39.64, 25.73, 22.63.

***tert*-Butyl *(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidine-1-carboxylate (52)**. To a solution of *tert*-butyl *(2R,5R)*-2-benzyl-5-hydroxypiperidine-1-carboxylate (100 mg, 0.343 mmol) and NaH (60%, 24.7 mg, 1.03 mmol) in DMF (3 mL) at 0 °C, (bromomethyl)cyclopropane (139 mg, 1.03 mmol) was added dropwise with continuous stirring, and the mixture was allowed to stand at room temperature for 24h. The mixture was diluted with water (10 mL), and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (pentane/ethyl acetate = 20:1 → 5:1) to furnish *tert*-butyl *(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidine- 1-carboxylate (96.0 mg, 0.277 mmol, 81% yield). $[\alpha]_D^{22}$ = -32 (*c* = 0.9, $CHCl_3$). LC-MS m/z: calculated for $C_{21}H_{31}NO_3$ $[M+H]^+$ 346.23, found: 346.40. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.25 (m, 2H), 7.20 – 7.15 (m, 3H), 4.58 – 4.11 (m, 2H), 3.37 (d, *J* = 4.0 Hz, 2H), 3.30 – 3.20 (m, 1H), 2.96 – 2.85 (m, 1H), 2.71 (t, *J* = 8.0 Hz, 2H), 1.93 (br s, 1H), 1.62 (br s, 2H), 1.39 (br s, 1H), 1.28 (s, 9H), 1.12 – 1.03 (m, 1H), 0.54 (br d, *J* = 8.0 Hz, 2H), 0.21 (br d, *J* = 4.0 Hz, 2H). ^{13}C NMR

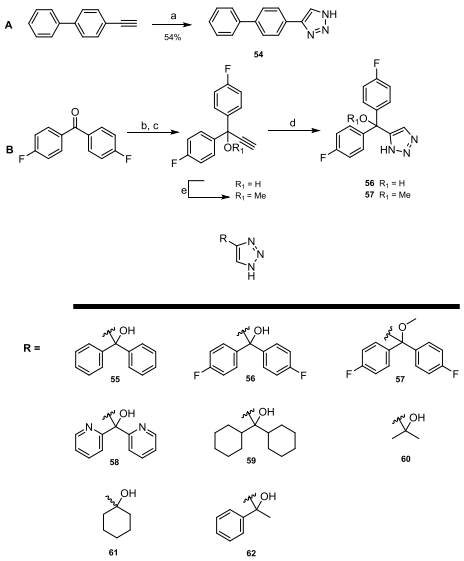
(101 MHz, $CDCl_3$) δ 154.79, 138.71, 129.23, 128.46, 126.27, 79.56, 74.18, 73.72, 52.25, 42.57, 36.01, 28.26, 26.44, 26.11, 11.02, 3.21.

***(2R,5R)*-2-Benzyl-5-(cyclopropylmethoxy)piperidine** To a solution of compound *tert*-butyl *(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidine-1-carboxylate (30.0 mg, 0.087 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$. Filtering and concentration under reduced pressure afforded the crude product that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 5H), 3.75 (s, 1H), 3.47 (s, 1H), 3.41 – 3.17 (m, 2H), 3.17 (d, *J* = 10.1 Hz, 2H), 3.06 (d, *J* = 12.1 Hz, 1H), 2.88 (d, *J* = 7.7 Hz, 1H), 2.03 (d, *J* = 11.7 Hz, 1H), 1.85 (d, *J* = 11.5 Hz, 1H), 1.62 (d, *J* = 14.2 Hz, 1H), 1.26 (br s, 1H), 1.02 (br s, 1H), 0.52 (d, *J* = 8.0 Hz, 2H), 0.16 (d, *J* = 4.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.43, 129.50, 128.89, 127.30, 73.27, 68.20, 57.92, 47.49, 39.39, 26.29, 22.70, 10.49, 3.08, 3.04.

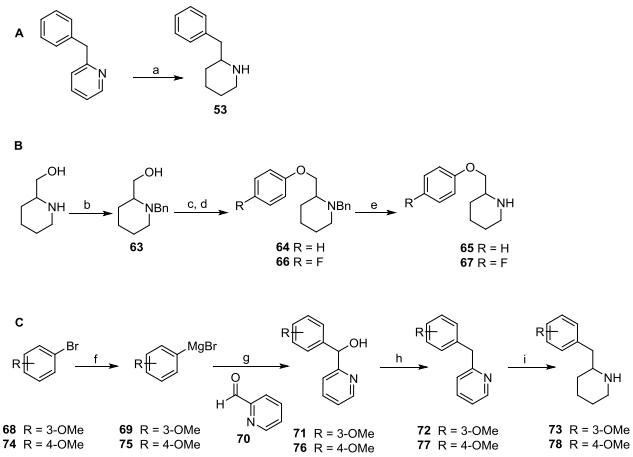
***tert*-Butyl (2*R*,5*R*)-2-benzyl-5-(prop-2-yn-1-yloxy)piperidine-1-carboxylate**: To a solution of *tert*-butyl *(2R,5R)*-2-benzyl-5-hydroxypiperidine-1-carboxylate (130 mg, 0.446 mmol) in DMF (3 mL) at 0 ^{o}C, was added NaH (44.6 mg, 1.12 mmol). After 5 minutes followed by drop wise addition of 3-bromoprop-1-yne (0.144 mL, 1.34 mmol). The reaction was allowed to warm to room temperature and stirred for 24 h. The mixture was diluted with water (10 mL), and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (5-20% ethyl acetate/pentane) to furnish title compound (128 mg, 0.389 mmol, 87 %) as a yellow oil. $[\alpha]_D^{22}$ = -24.9 (*c* = 1.00, $CHCl_3$); HRMS(m/z):$[M+H]^+$ calcd. for $C_{20}H_{27}NO_3$, 330.20637; found 330.20643. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.25 (m, 2H), 7.20 – 7.16 (m, 3H), 4.42 (br s, 1H), 4.32 (br s, 1H), 4.22 (s, 2H), 3.50 – 3.45 (m, 1H), 2.88 (dd, *J* = 13.5, 7.9 Hz, 1H), 2.76 – 2.70 (m, 2H), 2.46 (t, *J* = 4.0 Hz, 1H), 2.00 – 1.93 (m, 1H), 1.66 – 1.57 (m, 3H), 1.28 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.71, 138.96, 129.24, 128.51, 126.34, 80.03, 79.71, 74.43, 74.07, 56.02, 52.14, 42.15, 35.90, 28.31, 25.85;

(2*R*,5*R*)-2-benzyl-5-(prop-2-yn-1-yloxy)piperidine: To a solution of *tert*-Butyl (2*R*,5*R*)-2-benzyl-5-(prop-2-yn-1-yloxy)piperidine-1-carboxylate (140 mg, 0.425 mmol) in DCM (2 mL) was added 20% TFA/DCM (5 mL), the reaction mixture was stirred at room temperature for 2.5 hours, after which TLC showed complete conversion of the starting material. Toluene (20 mL) was added and the mixture concentrated. The mixture was dissolved in toluene (2 x 20 mL) two times more and concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed subsequently with aqueous 10% Na_2CO_3 solution, water, brine and dried over $MgSO_4$. Filtering and concentration under reduced pressure afforded the crude product **21** that was used without further purification. LC-MS (m/z) :$[M+H]^+$ calcd. for $C_{15}H_{19}NO$, 230.32; found 230.10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.26 (m, 2H), 7.23 (d, *J* =

6.9 Hz, 1H), 7.18 (d, *J* = 6.9 Hz, 2H), 4.21 (s, 2H), 4.03 (s, 1H), 3.45 (d, *J* = 12.7 Hz, 1H), 3.29 (br s, 1H), 3.17 (dd, *J* = 12.9, 4.1 Hz, 2H), 3.06 (d, *J* = 11.5 Hz, 1H), 2.85 (dd, *J* = 12.9, 9.7 Hz, 1H), 2.41 (t, *J* = 2.1 Hz, 1H), 2.05 (d, *J* = 13.3 Hz, 1H), 1.92 – 1.77 (m, 1H), 1.62 (d, *J* = 11.9 Hz, 1H), 1.51 (t, *J* = 13.3 Hz, 1H), 1.26 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ135.40, 129.45, 128.87, 127.28, 78.87, 75.31, 66.94, 57.89, 55.32, 47.04, 39.54, 25.91, 22.66.



Scheme 4. Synthesis of triazole building blocks (A and B). Reagents and conditions: (a) CuI, azidotrimethylsilane, DMF:MeOH = 5:1, 100 °C; (b) butyllithium, ethynyltrimethylsilane, THF, -10 °C; (c) NaOH, MeOH; (d) MeI, K_2CO_3, DMF, 60 °C, 48% (**55**), 48% (**56**), 35% (**57**), 44% (**58**), 49% (**59**), 84% (**60**), 90% (**61**), 80% (**62**).



Scheme 5 Synthesis of 2-substituent piperidine building blocks. Reagents and conditions: (a) PtO_2, H_2 (1.6 bar), HCl/EtOH (1:25), r.t., 1.5h, 57%; (b) $NaBH_4$, AcOH, benzylaldehyde, THF, r.t., 90%; (c) tosylchloride, Et_3N, DMAP, DCM, 0 °C; (d) NaH, corresponding phenol, THF, 0 °C (30 min), reflux (16h), 77%; (e) Pd/C (10 mol%), H_2, DCM/MeOH=1:2, r.t., 58% (**65**), 35% (**67**); (f) Mg, I_2, dry THF, 60 °C, 15 min; (g) pyridine-2-aldehyde, dry THF, r.t., overnight, 97% (**71**), 94% (**76**); (h) H_2 (1 bar), Pd/C, H_2SO_4 (98%)/MeOH (1:5), r.t., 3.5 – 5.5h, 57% (**72**), 55% (**77**); (i) H_2 (1.6 bar), PtO_2, HCl/EtOH (1:25), r.t., 1.5h.

2-Benzylpiperidine (**53**)**.** To a solution of 2-benzylpyridine (3.00 mL, 18.7 mmol) in EtOH (25 mL) was added HCl ((37% in H_2O), 1 mL) and PtO_2 (170 mg, 0.750 mmol, 0.04%). The suspension was purged with hydrogen three times and was put under 1.2 bar of H_2. The reaction was shaken vigorously for 1 hour after which the hydrogen had been absorbed. Repeating this step twice led to the desired hydrogenated adduct. The mixture was filtered and washed with EtOH (2 x 5 mL) and solvents were removed under reduced pressure. The residue was further washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$, and concentrated under reduced pressure to yield the crude product **53** (1.93 g, 11.0 mmol, 57%), which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.19 (m, 5H), 3.20 – 3.09 (m, 1H), 2.71 – 2.59 (m, 2H), 2.59 – 2.47 (m, 2H), 1.98 (br s, 1H), 1.81 – 1.22 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.60, 128.51, 128.12, 127.48, 58.12, 46.44, 42.23, 32.97, 25.82, 24.93.

4-([1,1'-Biphenyl]-4-yl)-2*H*-1,2,3-triazole (**54**)**.** In a 50 mL 2-neck round-bottom flask, a solution of 4-ethynyl-1,1'-biphenyl (415 mg, 2.30 mmol) in DMF (20 mL) and MeOH (6 mL) was purged with argon three times. To this solution was added N_3-TMS (0.772 mL, 5.82 mmol) and CuI (100 mg, 0.538 mmol). The reaction mixture was stirred for 2 days at 120 °C. The reaction was allowed to cool to room temperature and H_2O (30 mL) was added after removal of the solvents. Products were extracted with DCM (3 x 75 mL). Organic fractions were combined, washed with brine (30 mL), and dried over $MgSO_4$. After filtering and removal of the solvents, the crude material was purified over silica gel using pentane/ethyl acetate (1:1, 1% Et_3N) and yielded **54** (277 mg,

1.25 mmol, 54%). ^{1}H NMR (400 MHz, DMSO) δ: 8.42 (s, 1H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 139.73, 139.46, 129.38, 129.02, 127.58, 127.12, 126.48, 126.14.

1,1-Diphenylprop-2-yn-1-ol. To a solution of ethynyltrimethylsilane (0.430 mL, 3.10 mmol) in anhydrous THF (10 mL) under nitrogen atmosphere was added slowly butyllithium (1.92 mL, 3.02 mmol) (1.6 M in hexane) at -10 °C and the solution was stirred 1h at – 10 °C. A solution of benzophenone (500 mg, 2.74 mmol) in dry THF 5 mL was then added at -10 °C. After 3h stirring at -10 °C, the temperature was raised to 0°C and a solution of NaOH (143 mg, 3.57 mmol) in MeOH (2.80 mL) was added. After warming the solution to room temperature, the solution was neutralized to pH 7 with acetic acid and the resulting solution poured into water (38 mL). The organic layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography over silica gel using pentane/ethyl acetate and finished the title compound (1.08 g, 4.42 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.0 Hz, 4H), 7.25 – 7.11 (m, 6H), 3.16 (s, 1H), 2.70 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.38, 128.22, 127.74, 126.00, 86.36, 75.70, 74.22.

Diphenyl(1*H*-1,2,3-triazol-4-yl)methanol (55). 1,1-Diphenylprop-2-yn-1-ol (260 mg, 1.25 mmol) and CuI (12.0 mg, 0.062 mmol) were dissolved in DMF/MeOH (5:1, 36 mL), azidotrimethylsilane (0.250 mL, 1.87 mmol) was added and the reaction mixture was stirred at 100 °C over the weekend. The reaction mixture was quenched with H_2O, and the organic layer was extracted with DCM. The combined organic layers were washed with H_2O and brine, dried on $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel using pentane/ethyl acetate (1:1) with 1% Et_3N, yielding **55** (151 mg, 0.599 mmol, 48% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.54 (s, 1H), 7.36 – 7.26 (m, 10H).

Bis(4-fluorophenyl)(1*H*-1,2,3-triazol-4-yl)methanol (56). To a solution of ethynyltrimethylsilane (0.712 mL, 5.04 mmol) in anhydrous THF (20 mL) under a nitrogen atmosphere was slowly added n-butyllithium (3.15 mL, 5.04 mmol) (1.6 M in hexane) at -10°C. After stirring for one hour at -10 °C a solution of bis(4-fluorophenyl)methanone (1.00 g, 4.58 mmol) in dry THF (10 mL) was added. After stirring for three hours at -10 °C, the temperature was raised to 0 °C and a solution of NaOH (238 mg, 5.95 mmol) in MeOH (4.60 mL) was added. The solution was warmed to room temperature, neutralized to pH 7 with acetic acid and poured into water. Subsequent extraction with ethyl acetate (3x10 mL), drying over $MgSO_4$, filtering and concentration *in vacuo* afforded a crude product that was purified by flash chromatography over silica gel using pentane/ethyl acetate, yielding 1,1-bis(4-fluorophenyl)prop-2-yn-1-ol (1.08 g, 4.42 mmol, 96 %) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.51 (m, 4H), 7.03 – 6.98 (m, 4H), 2.89 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.50 (d, *J* = 248 Hz), 140.24 (d, *J* = 3.1 Hz), 127.98 (d, *J*

= 9.1 Hz), 115.32 (d, *J* = 21 Hz), 86.08, 76.09, 73.52; HRMS(m/z):$[M+H]^+$ calcd. for $C_{15}H_{10}F_2O$, 245.07782; found: 245.07735. 1,1-bis(4-fluorophenyl)prop-2-yn-1-ol (1.00 g, 4.09 mmol) and CuI (0.153 g, 0.819 mmol) were dissolved in DMF/MeOH (5:1, 36 mL). Azidotri- methylsilane (0.815 mL, 6.14 mmol) was added and the mixture was stirred at 100 °C over the weekend. The reaction mixture was quenched with H_2O (90 mL), the organic layer extracted with DCM (3 x 100 mL). The combined organic layers were washed with H_2O and brine and dried on $MgSO_4$. Filtering and concentration under reduced pressure gave a residue that was purified by flash chromatography over silica gel using pentane/ethyl acetate with 1% Et_3N (30–100% pentane/ethyl acetate) yielding **56** (0.694 g, 2.42 mmol, 59 %) as a white solid. ^{1}H NMR (400 MHz, MeOD) δ 7.58 (s, 1H), 7.38 – 7.31 (m, 4H), 7.06 – 7.01 (m, 4H); ^{13}C NMR (101 MHz, MeOD) δ 163.45 (d, *J* = 246 Hz), 143.57 (d, *J* = 2.0 Hz), 130.62, 128.59, 130.31 (d, *J* = 9.1 Hz), 115.52 (d, *J* = 22 Hz), 76.99; HRMS(m/z):$[M+H]^+$ calcd. for $C_{15}H_{11}F_2N_3O$, 288.09429; found 288.09473.

4-(Bis(4-fluorophenyl)(methoxy)methyl)-1H-1,2,3-triazole (57). (1,1'-Bis(4-fluorophenyl)prop- 2-yn-1-ol) (50.0 mg, 0.205 mmol) was dissolved in anhydrous THF (5 mL) and purged with argon 3 times. NaH (60%, 10 mg, 0.25 mmol;) was added and the reaction mixture was stirred for 30 min at 0°C. Subsequently, MeI (16.0 µL, 0.250 mmol) was added and the mixture was stirred for 3h at 0 °C. The mixture was washed with NH_4Cl solution (10 mL), H_2O (5 mL) and extracted with DCM (3 x 20 mL). Organic fractions were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified with pentane/ethyl acetate (1:1, 1% Et_3N) and obtained the methylated compound (45.0 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.48 (m, 4H), 7.11 – 6.98 (m, 4H), 3.37 (s, 3H), 2.94 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.32 (d, *J* = 246.9 Hz), 138.63 (d, *J* = 3.2), 128.38 (d, *J* = 7.7 Hz), 115.12 (d, *J* = 21.6 Hz), 82.03, 79.75, 78.01, 52.42. The obtained intermediate (46.0 mg, 0.178 mmol) was converted into 1,2,3-triazole according to the same procedures described for compound **55** (20.0 mg, 35%). LC-MS m/z: calculated for $C_{16}H_{13}N_3O$ $[M+H]^+$ 302.10, found 302.52. ^{1}H NMR (400 MHz, MeOD) δ 7.56 (s, 1H), 7.53 – 7.42 (m, 4H), 7.14 – 7.01 (m, 4H), 3.17 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 163.42 (d, *J* = 245.4 Hz), 144.20, 140.83, 130.91 (d, *J* = 8.2 Hz), 123.55, 115.62 (d, *J* = 21.7 Hz), 93.33, 52.63.

1,1-Di(pyridin-2-yl)prop-2-yn-1-ol. To a solution of ethynyltrimethylsilane (0.850 mL, 6.00 mmol) in anhydrous THF (20 mL) under nitrogen atmosphere was added slowly butyllithium (3.69 mL, 5.90 mmol) (1.6 M in hexane) at -10 °C and the solution was stirred 1h at -10 °C. A solution of di(pyridin-2-yl)methanone (1.00 g, 5.43 mmol) in dry THF (10 mL) was then added at -10 °C. After 3h stirring at -10 °C, the temperature was raised to 0 °C and a solution of NaOH (238 mg, 5.95 mmol) in MeOH (4.60 mL) was added. After the solution was warmed to room temperature, the solution was neutralized to pH 7 with acetic acid and the resulting solution poured into water (38 mL). The organic layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography over silica gel using pentane/ethyl acetate (1:1) with 1% Et_3N.

Yielding 1,1-di(pyridin-2-yl)prop-2-yn-1-ol (690 mg, 3.27 mmol, 60% yield). LC-MS m/z: calculated for $C_{13}H_{10}N_2O$ $[M+H]^+$ 211.08, found: 211.11. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 4.8 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.72 (td, *J* = 7.8, 4.0 Hz, 2H), 7.25 – 7.21 (m, 2H), 6.79 (br s, 1H), 2.75 (s, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 160.44, 148.06, 137.49, 123.22, 121.37, 85.85, 75.73, 73.66.

Di(pyridin-2-yl)(1*H*-1,2,3-triazol-4-yl)methanol (58). The title compound was synthesized from 1,1-di(pyridin-2-yl)prop-2-yn-1-ol (1.10 g, 5.23 mmol) according to the same procedures described for compound **55**. This furnished di(pyridin-2-yl)(1*H*-1,2,3-triazol-4-yl)methanol **58** (580 mg, 2.30 mmol, 44% yield). LC-MS m/z: calculated for $C_{13}H_{11}N_5O$ $[M+H]^+$ 254.10, found: 254.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 – 8.51 (m, 2H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.72 (s, 1H), 7.67 (td, *J* = 7.8, 1.8 Hz, 2H), 7.20 – 7.17 (m, 2H), 4.99 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.30, 159.55, 147.58, 136.98, 130.82, 122.75, 121.98, 75.87.

Dicyclohexyl(2*H*-1,2,3-triazol-4-yl)methanol (59). Following the procedure that was described for 1,1-diphenylprop-2-yn-1-ol, dicyclohexylmethanone (0.990 mL, 5.20 mmol) was reacted with ethynyltrimethylsilane (0.710 mL, 5.66 mmol), n-BuLi (3.33 mL, 5.66 mmol) and NaOH (206 mg, 5.15 mmol, in 5 mL MeOH) to obtain 1,1-dicyclohexylprop-2-yn-1-ol (1.06 g, 94%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 1H), 1.93 – 1.25 (m, 22H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 85.8, 73.43, 43.4, 27.7, 26.5, 26.5, 26.3, 26.0. The obtained 1,1-dicyclohexylprop-2-yn-1-ol (804 mg, 3.65 mmol) was converted into its 1,2,3-triazole according to the same procedures described for compound **55**. This furnished **59** (469 mg, 49%). LC-MS m/z: calculated for $C_{15}H_{25}N_3O$ $[M+H]^+$ 264.20, found: 264.24. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.37 (m, 1H), 1.98 – 0.68 (m, 22H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.53, 139.29, 44.11, 29.72, 27.13, 26.54, 26.38, 26.22

2-(2*H*-1,2,3-Triazol-4-yl)propan-2-ol (60). The title compound was synthesized from 2-methyl-3-butyn-2-ol (581 µL, 5.94 mmol) according to the same procedures described for compound **55**. This furnished **60** (635 mg, 84%). LC-MS m/z: calculated for $C_5H_9N_3O$ $[M+Na]^+$ 150.06, found: 150.21. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.52, 131.53, 68.48, 30.12.

1-(2*H*-1,2,3-Triazol-4-yl)cyclohexan-1-ol (61). The title compound was synthesized from 1-ethynyl-1-cyclohexanol (642 µL, 5.00 mmol) according to the same procedures described for compound **55**. This furnished **61** (843 mg, 90%). LC-MS m/z: calculated for $C_8H_{13}N_3O$ $[M+Na]^+$ 190.10, found: 190.41. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 1.91 (dt, *J* = 9.2, 4.7 Hz, 4H), 1.81 – 1.71 (m, 2H), 1.68 – 1.52 (m, 3H), 1.42 – 1.23 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 131.42, 123.03, 76.33, 69.82, 38.24, 38.15, 25.28, 21.90.

1-Phenyl-1-(1*H*-1,2,3-triazol-4-yl)ethan-1-ol (62). The title compound was synthesized from 2-phenylbut-3-yn-2-ol (500 mg, 3.42 mmol) according to the same procedures described for compound **55**. This furnished 1-phenyl-1-(1*H*-1,2,3

-triazol-4-yl)ethan-1-ol **62** (453 mg, 2.40 mmol, 80% yield). LC-MS m/z: calculated for $C_{10}H_{11}N_3O$ $[M+H]^+$ 212.08, found 212.10. ^{1}H NMR (400 MHz, MeOD) δ 7.67 (d, *J* = 16.0 Hz, 1H), 7.53 (m, 2H), 7.39 – 7.15 (m, 3H), 2.00 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 156.22, 146.91, 132.63, 127.89, 126.92, 125.10, 75.92, 29.69.

(1-Benzylpiperidin-2-yl)methanol (63). $NaBH_4$ (1.97 g, 52.1 mmol) was treated with AcOH (10.4 mL, 174 mmol) in anhydrous THF (50 mL) for 30 min at 0 °C. To this solution was added 2-hydroxymethyl piperidine (2.00 g, 17.4 mmol) in anhydrous THF (10 mL), and benzaldehyde (5.30 mL, 51.9 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was filtered and concentrated *in vacuo*. Purification by column chromatography over silica gel (pentane/EtOAc = 10:1 → 1:1) yielded **63** (1-benzyl)2-hydroxymethyl piperidine as yellow oil (3.19 g, 15.5 mmol, 90%). LC-MS *m/z*: calculated for $C_{13}H_{19}NO$ $[M+H]^+$ 206.15, found: 206.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.21 (m, 5H), 4.11 (d, *J* = 13.4 Hz, 1H), 3.86 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.61 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.35 (d, *J* = 13.4 Hz, 1H), 3.21 (s, 1H), 2.89 (d, *J* = 11.5 Hz, 1H), 2.47 (s, 1H), 2.14 (t, *J* = 10.5 Hz, 1H), 1.83 – 1.24 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 139.02, 129.01, 128.45, 127.12, 62.48, 61.32, 57.91, 51.13, 27.6, 24.29, 23.52.

1-Benzyl-2-(phenoxymethyl)piperidine (64). ((1-Benzyl)2-hydroxymethyl piperidine) (200 mg, 0.975 mmol) was dissolved in DCM (2 mL) and cooled to 0 °C. To this were added Et_3N (215 µL, 2.93 mmol), DMAP (24.0 mg, 0.195 mmol) and *p*-toluenesulfonyl chloride (558 mg, 2.93 mmol). The reaction mixture was stirred at 0°C for 16h, warming up to room temperature 20 °C overnight. The mixture was concentrated *in vacuo* and dissolved in DCM (50 mL). The organic layer was washed with H_2O (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was obtained. Phenol (175 mg, 1.86 mmol) was reacted with NaH (60% dispersion, 74.3 mg, 1.86 mmol) in anhydrous THF (2 mL) at 0 °C for 30 min. After which the crude product from above (139 mg, 0.62 mmol) was added and the mixture heated to reflux for 16h. The reaction mixture was concentrated *in vacuo* and dissolved in DCM (25 mL). Organic layer was washed with 1M NaOH (2 x 10 mL), brine (10 mL), and dried over $MgSO_4$, filtered,. concentrated and purified over silica gel (pentane/EtOAc 10:1 → 1:1), yielding **64** (134 mg, 0.477 mmol, 77%). LC-MS m/z: calculated for $C_{19}H_{23}NO$ $[M+H]^+$ 282.18, found: 282.34. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.22 (m, 7H), 7.03 – 6.86 (m, 3H), 4.23 (dd, *J* = 9.8, 4.9 Hz, 1H), 4.14 (d, *J* = 13.7 Hz, 1H), 4.04 (dd, *J* = 9.8, 4.7 Hz, 1H), 3.44 (d, *J* = 13.7 Hz, 1H), 2.90 – 2.74 (m, 2H), 2.20 – 2.09 (m, 1H), 1.92 – 1.83 (m, 1H), 1.79 – 1.37 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.89, 129.43, 128.92, 128.13, 126.75, 120.67, 118.90, 114.64, 70.32, 60.45, 59.03, 52.04, 29.45, 25.42, 23.46.

2-(Phenoxymethyl)piperidine (65). 1-Benzyl-2-(phenoxymethyl)piperidine (118 mg, 0.420 mmol) was treated with 10% palladium on carbon (44.0 mg, 0.042 mmol) in MeOH/DCM (2:1, 1.5 mL) under hydrogen atmosphere for 16h at room temperature. After reaction was completed, solvents were removed under reduced pressure and mixture was dissolved in EtOAc (30 mL). Organic layer was washed with H_2O (10 mL),

sat. $NaHCO_3$ (10 mL), and brine (10 mL) before dried over $MgSO_4$, concentrated and purified over silica gel (pentane/EtOAc 10:1 → 1:1) yielding **65** (47.0 mg, 0.246 mmol, 58%). LC-MS *m/z*: calculated for $C_{12}H_{17}NO$ $[M+H]^+$ 192.13, found: 192.15. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.22 (m, 2H), 6.97 – 6.85 (m, 3H), 3.89 (dd, *J* = 9.0, 3.6 Hz, 1H), 3.80 (t, *J* = 9.0 Hz, 1H), 3.15 – 3.06 (m, 1H), 3.00 – 2.90 (m, 1H), 2.69 (td, *J* = 11.6, 2.7 Hz, 1H), 1.88 – 1.80 (m, 1H), 1.71 – 1.59 (m, 2H), 1.55 – 1.19 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 129.4, 120.8, 114.9, 72.54, 53.1, 46.6, 28.7, 26.3, 24.4.

2-((4-Fluorophenoxy)methyl)piperidine (67). ((1-Benzyl)2-hydroxymethyl piperidine) (200 mg, 0.974 mmol) was dissolved in DCM (2 mL) and cooled to 0 °C. To this were added Et_3N (407 µL, 2.92 mmol), DMAP (24.0 mg, 0.196 mmol) and *p*-toluenesulfonyl chloride (558 mg, 2.90 mmol). The reaction mixture was stirred at 0 °C for 16h, slowly warming up to room temperature. The mixture was concentrated *in vacuo* and dissolved in DCM (50 mL). The organic layer was washed with H_2O, brine, dried over $MgSO_4$, filtrated and the crude tosylate product was obtained. 4-Fluorophenol (752 mg, 6.71 mmol) was reacted with NaH (60% dispersion, 179 mg, 4.47 mmol) over 30 min at 0 °C in anhydrous DMF (5 mL). The crude tosylate product from above in anhydrous DMF (2 mL) was added to the reaction mixture and stirred at 70 °C for 18h. The mixture was cooled to room temperature, diluted with H_2O (50 mL) and extracted with DCM (3 x 75 mL). Organic fractions were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude **66** was then dissolved in MeOH/DCM (6 mL, 2:1), 10% Pd/C (200 mg) was added and the mixture was flushed with hydrogen gas three times. The reaction mixture was stirred under hydrogen atmosphere for 16h. Purification over silica gel using pentane : EtOAc = 1:1 as the eluent with 1% Et_3N to yield product **67** (71.0 mg, 0.340 mmol, 35%). LC-MS m/z: calculated for $C_{12}H_{16}FNO$ $[M+H]^+$ 210.12, found: 210.41. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (t, *J* = 8.7 Hz, 2H), 6.85 – 6.79 (m, 2H), 3.84 (dd, *J* = 8.9, 3.6 Hz, 1H), 3.74 (t, *J* = 8.6 Hz, 1H), 3.09 (d, *J* = 11.8 Hz, 1H), 2.98 – 2.88 (m, 1H), 2.68 (td, *J* = 11.7, 2.7 Hz, 1H), 1.88 – 1.78 (m, 1H), 1.68 – 1.59 (m, 2H), 1.55 – 1.31 (m, 2H), 1.30 – 1.17 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.32 (d, *J* = 238.2 Hz), 155.01 (d, *J* = 2.1 Hz), 115.68 (d, *J* = 23.0 Hz), 115.52 (d, *J* = 7.9 Hz), 73.31, 55.82, 46.53, 28.62, 26.28, 24.32.

(3-Methoxyphenyl)(pyridin-2-yl)methanol (71). After flame-drying and flushing with argon, the flask containing magnesium turnings (156 mg, 6.50 mmol) was charged, dry THF (2.5 mL) and a catalytic amount of iodine were added under argon atmosphere. Then, 1/10 of a argon flushed solution of 1-bromo-3-methoxybenzene **68** (0.675 mL, 5.33 mmol) in dry THF (2.5 mL) was added at room temperature. The mixture was heated with a heating gun until initiation of the reaction was observed. The rest of the 1-bromo-3-methoxybenzene solution was then added dropwise, and the reaction mixture was stirred under reflux for 15 min, after which it was allowed to cool to room temperature. The solution containing Grignard reagent **69** was cooled to 0 °C and pyridine-2-aldehyde **70** (0.430 mL, 4.52 mmol) was added dropwise under argon atmosphere. Extra dry THF was added (2.5 mL) and the reaction mixture was allowed to warm to room temperature while stirring overnight. After quenching with

aqueous saturated NH_4Cl (35 mL), the aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc = 10:1→2:1) afforded compound **71** (940 mg, 4.37 mmol, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 4.8 Hz, 1H), 7.52 (td, *J* = 7.7, 1.7 Hz, 1H), 7.21 – 7.17 (m, 2H), 7.10 – 7.05 (m, 1H), 6.95 – 6.93 (m, 2H), 6.78 – 6.74 (m, 1H), 5.72 (s, 1H), 5.56 (br s, 1H), 3.69 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.16, 159.64, 147.75, 144.76, 136.81, 129.40, 122.31, 121.11, 119.20, 113.19, 112.20, 75.03, 55.02. LC-MS m/z: calculated for $C_{13}H_{13}NO_2$ $[M+H]^+$ 216.10, found 215.73.

2-(3-Methoxybenzyl)pyridine (72). A hydrogenation flask containing compound **71** (100 mg, 0.465 mmol) in a mixture of H_2SO_4 (98%)/MeOH (1:5, 5.6 mL) was flushed with argon. Pd/C (10%, 250 mg) was added and the mixture was hydrogenated at 1 bar at room temperature for 4h. The reaction mixture was then filtered, concentrated *in vacuo* and neutralize with aqueous NaOH solution (1.0 M). The aqueous layer was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc = 10:1→4:1) afforded compound **72** (51.0 mg, 0.256 mmol, 57%). LC-MS m/z: calculated for $C_{13}H_{13}NO$ $[M+H]^+$ 200.10, found 200.41. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 7.3 Hz, 1H), 7.56 (td, *J* = 7.7, 1.8 Hz, 1H), 7.27 – 7.18 (m, 1H), 7.14 – 7.06 (m, 2H), 6.87 – 6.80 (m, 2H), 6.76 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.13 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.88, 159.83, 149.36, 141.08, 136.66, 129.62, 123.22, 121.57, 121.36, 114.87, 111.87, 55.22, 44.79.

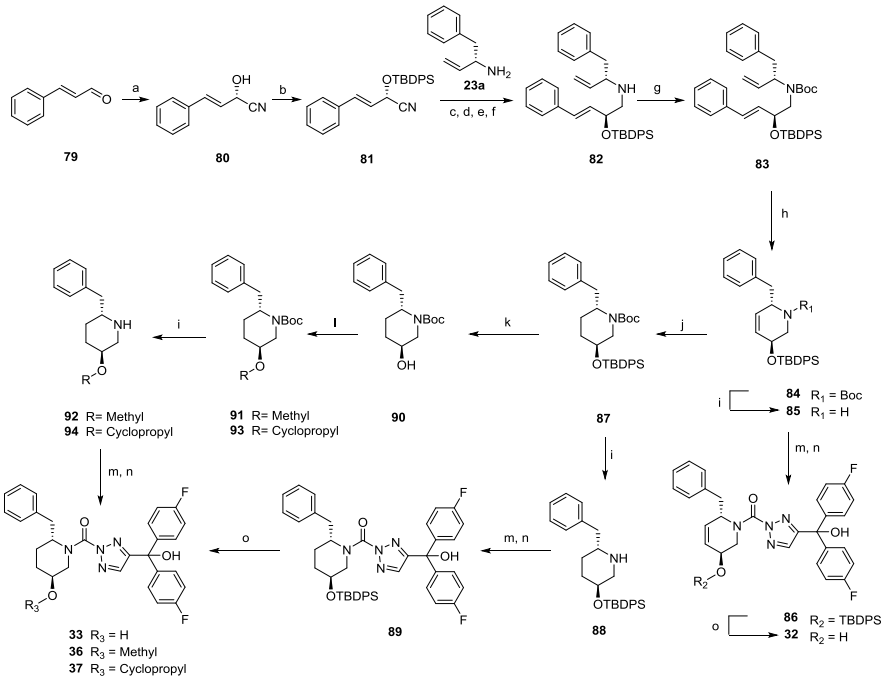
2-(3-Methoxybenzyl)piperidine (**73**). A hydrogenation flask containing compound **72** (51.0 mg, 0.256 mmol) in a mixture of HCl/EtOH (1:25, 6.7 mL) was flushed with argon. PtO_2 (4.60 mg, 20.3 µmol) was added and the mixture was hydrogenated at 1 bar at room temperature for 2h. The reaction mixture was filtered, concentrated *in vacuo* and coevaporated with EtOH (3 x 10 mL) and with toluene (3 x 10 mL) to give crude compound **73**, which was used in the following step without any further purification. LC-MS m/z: calculated for $C_{13}H_{19}NO$ $[M+H]^+$ 206.10, found 206.21. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.08 (m, 1H), 6.96 – 6.58 (m, 3H), 3.79 (s, 3H), 3.57 (d, *J* = 7.0 Hz, 2H), 3.28 (br s, 1H), 2.97 (d, *J* = 5.3 Hz, 2H), 2.08 – 1.59 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.46, 137.00, 129.48, 121.67, 114.95, 112.34, 58.50, 55.38, 49.03, 39.91, 29.32, 27.39, 22.25.

(4-Methoxyphenyl)(pyridin-2-yl)methanol (**76**). After flame-drying and flushing with argon, the flask containing magnesium turnings (156 mg, 6.50 mmol) was charged dry THF (2.5 mL) and a catalytic amount of iodine were added. Then, 1/10 of a argon flushed solution of 1-bromo-4-methoxybenzene **74** (0.675 mL, 5.33 mmol) in dry THF (2.5 mL) was added at room temperature. The mixture was heated with a heating gun until initiation of the reaction was observed. The rest of the 1-bromo-4-methoxybenzene solution was added dropwise, and the reaction mixture was stirred under reflux for 15 min, after which it was allowed to cool to room temperature. The solution containing Grignard reagent **75** was then further cooled to 0

°C and pyridine-2-aldehyde **70** (0.430 mL, 4.52 mmol) was added dropwise under argon atmosphere. Extra dry THF(2.5 mL) was added and the reaction mixture was allowed to warm to room temperature while stirring overnight. After quenching with aqueous saturated NH_4Cl (35 mL), the aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc = 10:1→2:1) afforded compound **76** (910 mg, 4.23 mmol, 94%). LC-MS m/z: calculated for $C_{13}H_{13}NO_2$ $[M+H]^+$ 216.10, found: 215.73. ^{1}H NMR (400 MHz, MeOD) δ 8.45 – 8.37 (m, 1H), 7.82 (td, *J* = 7.7, 1.7 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.30 – 7.25 (m, 3H), 6.89 – 6.81 (m, 2H), 5.76 (s, 1H), 4.91 (s, 1H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 164.75, 160.65, 149.06, 138.84, 136.64, 129.19, 123.73, 122.06, 114.76, 77.06, 55.66.

2-(4-Methoxybenzyl)pyridine (77). The title compound was prepared from compound **76** (100 mg, 0.465 mmol) and Pd/C (10%, 250 mg) in H_2SO_4/MeOH (1:5, 8.4 mL) according to the same procedures described for compound **72**. Purification by column chromatography (pentane/EtOAc = 10:1 → 4:1) afforded compound **77** (80.0 mg, 0.404 mmol, 55%) as a yellow oil. LC-MS m/z: calculated for $C_{13}H_{13}NO$ $[M+H]^+$ 200.11, found: 199.87. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 3.8 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 7.7 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.09 (s, 2H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.39, 158.19, 149.27, 136.55, 131.60, 130.07, 122.99, 121.17, 114.01, 55.22, 43.81.

2-(4-Methoxybenzyl)piperidine (78). The title compound was prepared from compound **77** (68.0 mg, 0.342 mmol) and PtO_2 (6.20 mg, 27.3 µmol) in HCl/EtOH (1:25, 10 mL) according to the same procedures described for compound **72**. This furnished crude compound **78**, which was used in the following steps without any further purification. LC-MS m/z: calculated for $C_{13}H_{19}NO$ $[M+H]^+$ 206.16, found 205.93. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (s, 2H), 6.82 (s, 2H), 3.77 (s, 3H), 3.50 (br s, 2H), 3.17 (br s, 1H), 2.93 (br s, 2H), 2.03 – 1.66 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.59, 130.67, 127.72, 114.14, 58.91, 55.37, 45.58, 39.03, 29.64, 27.53, 22.45.



Scheme 6 Enantioselective synthesis of chiral compounds. Reagents and conditions: (a) HCN, EtOAc, 0.1M aq. citrate buffer, pH 5.4, Hydroxynitrile lyase, 55%; (b) TBDPS-Cl, imidazole, DMF, 0 °C, 98%; (c) diethyl ether, DIBAL-H, -80 °C to 0 °C; (d) MeOH, -90 °C; (e) *(S)*-amine (**23a**) (3 equiv), r.t., 20h; (f) $NaBH_4$, 93%; (g) Boc_2O, TEA, THF, 50 °C, 20h; (h) Grubbs I cat. 4 mol %, DCM, reflux, 48h, 87% (**84**, based on **82**); (i) 25% TFA, DCM, r.t.; (j) Hydrazine, $CuSO_4$, EtOH, 0 °C to 70 °C, 80%; (k) TBAF, THF, r.t., 80%; (l) NaH, corresponding bromide, 83% (**91**), 89% (**93**); (m) DIPEA, Triphosgene, THF, 0 °C; (n) DIPEA, DMAP, triazole, THF, 60 °C, 26% (**36**, based on **91**), 21% (**37**, based on **93**); (o) HF-pyridine, THF : pyridine = 1:1 (v/v), 15% (**32**, based on **84**), 16% (**33**, based on **87**).

(*S,E*)-2-((*tert*-Butyldiphenylsilyl)oxy)-4-phenyl-*N*-((*S*)-1-phenylbut-3-en-2-yl)but-3-en-1-amine (82). Under an argon atmosphere cyanohydrin **81**[25,26] (2.80 g, 7.05 mmol) was dissolved in dry diethyl ether (40 mL) and at -78 °C a 1.0 M solution of DIBAL-H in toluene (11.0 mL, 11.0 mmol) was added dropwise in 15 min. The mixture was slowly warmed on the cooling bath to 5 °C. After re-cooling to -90 °C methanol (10 mL) was added at once, followed by a solution of *(S)*-1-phenylbut-3-en-2-amine **23a** (3.00 g, 20.4 mmol) in methanol (10 mL). The cooling bath was removed and the remaining mixture stirred at room temperature, under a light flow of argon, for 28h. The remaining mixture was cooled on an ice-bath and $NaBH_4$ (880 mg, 23.0 mmol) was added in three portions with five minute intervals. The mixture was stirred overnight while slowly warming to room temperature. The reaction was quenched with a 0.8 M NaOH solution (150 mL) and the resulting mixture extracted with diethyl ether (3 x 60 mL). The combined organic layers were washed subsequently with 1.0 M HCl solution (2 x 30 mL) and 0.8 M NaOH solution, dried ($MgSO_4$), filtered and concentrated *in*

vacuo to afford the crude product that was purified by silica gel column chromatography (pentane/EtOAc = 97 : 3 → 9 : 1) to give the target amine **82** as a colorless oil (3.50 g, 93%). $[\alpha]^{23}_{D}$ = +116 (*c* = 1.0, $CHCl_3$). HRMS calculated for $C_{36}H_{41}NOSi$ $[M+H]^+$: 532.3030 found: 532.3021. IR: 3071, 3026, 2930, 2893, 2857, 1495, 1454, 1427, 1362, 1109, 1059, 962, 918. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.61 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.42 – 7.05 (m, 16H), 6.09 – 5.91 (m, 2H), 5.59 (ddd, *J* = 17.0, 10.3, 8.0 Hz, 1H), 5.07 – 4.92 (m, 2H), 4.37 (app. q, *J* = 6.1 Hz, 1H), 3.23 (app q, *J* = 7.7 Hz, 1H), 2.76 – 2.61 (m, 4H), 1.59 (br s, 1H), 1.00 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.67, 138.36, 136.66, 135.89, 135.88, 134.05, 133.95, 131.00, 130.58, 129.54, 129.41, 129.28, 128.30, 128.22, 127.47, 127.34, 127.30, 126.38, 126.21, 116.00, 74.22, 62.69, 53.55, 42.40, 26.99, 19.25.

The acidic water layer was basified with 8.0 M NaOH (12 mL) and extracted with $CHCl_3$ (4 x 30 mL). After drying ($MgSO_4$), filtering and evaporation of the solvent, excess (*S*)-1-phenylbut-3-en-2-amine (2.02 g, 13.7 mmol) was recovered.

***tert*-Butyl ((*S,E*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-yl)((*S*)-1-phenylbut-3-en- 2-yl)carbamate (83)**. The amine from above (3.30 g, 6.21 mmol) was dissolved in THF (50 mL), Boc_2O (2.80 g, 12.8 mmol) and Et_3N (2 mL) were added subsequently. The mixture was refluxed overnight upon which TLC confirmed complete conversion. Concentration *in vacuo* and purification by silica gel column chromatography (pentane/EtOAc = 99 : 1 → 97 : 3) afforded the title compound (5.10 g) as a mixture with unseparated Boc_2O. Analytical data are from a pure fraction. $[\alpha]_D^{21}$ = -4.6 (c =1.0, $CHCl_3$). HRMS calculated for $C_{41}H_{49}NO_3Si$ $[M+H]^+$: 632.3555; found: 632.3555. IR 3069, 3028, 2963, 2930, 2859, 1694, 1454, 1427, 1410, 1366, 1250, 1167, 1113, 964. ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers (2:1 ratio): δ 7.75 (d, *J* = 6.8 Hz, 2H),7.68 (d, *J* = 6.8 Hz, 2H), 7.47 – 7.29 (m, 6H), 7.29 – 7.11 (m, 8H), 7.08 – 6.95 (m, 2H), 6.10 (s, 0.35H), 6.06 (s, 0.65H), 5.92 – 5.63 (m, 2H), 4.93 (d, *J* = 10.5 Hz, 1H), 4.88 (d, *J* = 17.4 Hz, 1H), 4.33 (br s, 0.4H), 4.12 (br s, 1H), 3.91 (br s, 0.6H), 3.53 – 3.15 (m, 1H), 3.13 – 2.64 (m, 3H), 1.23 (s, 3H), 1.19 (s, 6H), 1.07 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) mixture of rotamers (2:1 ratio): δ 154.77, 138.63, 137.93, 137.23, 137.01, 135.98, 135.88, 134.16, 133.59, 130.73, 130.41, 129.66, 129.30, 128.22, 128.16, 127.59, 127.47, 127.11, 126.35, 126.17, 115.87, 79.34, 73.59 (minor CHO), 72.58 (major CHO), 62.70 (major CHN), 61.71 (minor CHN), 53.02 (major CH_2N), 50.99 (minor CH_2N), 38.64 (minor CH_2Ph), 38.12 (major CH_2Ph), 28.13, 27.03, 19.27.

***tert*-Butyl *(3S,6S)*-6-benzyl-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridine-1(2H)-carboxylate (84)**. The Boc-protected diene **83** (max 6.21 mmol) from above was dissolved in DCM under argon. Grubbs I catalyst (309 mg, 0.375 mmol) was added and the mixture refluxed overnight after which TLC confirmed complete conversion. The solvent was evaporated and the crude product purified by silica gel column chromatography (pentane/EtOAc = 99 : 1 → 96 : 4) to afford the target cyclic alkene as a colorless oil (2.84 g, 5.39 mmol, 76% over two steps). $[\alpha]_D^{21}$ = +159 (c =1.0,

$CHCl_3$). HRMS calculated for $C_{33}H_{41}NO_3Si$ $[M+H]^+$: 528.2929; found: 528.2922. IR 3028, 2971, 2931, 2858, 1810, 1756, 1692, 1454, 1448, 1417, 1364, 1112, 1072. ^1H NMR (400 MHz, $CDCl_3$, 60 ^oC) δ 7.70 (d, *J* = 7.4 Hz, 2H), 7.64 (d, *J* = 7.7 Hz, 2H), 7.41 – 7.27 (m, 6H), 7.24 – 7.09 (m, 5H), 5.62 (dd, *J* = 10.1, 3.7 Hz, 1H), 5.59 – 5.52 (m, 1H), 4.69 (br s, 1H), 4.23 (br s, 1H), 4.07 – 3.98 (m, 1H), 2.82 (dd, *J* = 12.9, 5.9 Hz, 1H), 2.77 – 2.67 (m, 2H), 1.44 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, 60 ^oC) δ 154.73, 138.04, 135.80, 134.65, 134.22, 131.16, 129.60, 129.46, 128.22, 127.61, 127.42, 126.53, 126.25, 79.35, 64.08, 38.95, 28.44, 26.95, 19.20.

***tert*-Butyl *(2R,5S)*-2-benzyl-5-((*tert*-butyldiphenylsilyl)oxy)piperidine-1-carboxylate (87)**. A suspension of substrate *tert*-butyl *(3S,6S)*-6-benzyl-3-((tert-butyldiphenylsilyl)oxy)-3,6-dihydropyridine-1(2H)-carboxylate (500 mg, 0.947 mmol) and copper (II) sulfate (1.50 g, 9.47 mmol) in ethanol (2 mL) was cooled on an ice bath. Hydrazine (3.01 mL, 96.0 mmol) was added drop wise and the reaction was subsequently stirred for 15 min. After that, the reaction mixture was stirred at 70 °C for 24h until TLC showed the reaction was completed. The mixture was filtered over celite and concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with water, brine and dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography (pentane/EtOAc = 99 : 1 → 90 : 10) to furnish *tert*-butyl *(2R,5S)*-2-benzyl-5-((*tert*-butyldiphenylsilyl)oxy)piperidine-1-carboxylate **87** (400 mg, 0.756 mmol, 80 % yield) as a colorless oil. LC-MS m/z: calculated for $C_{33}H_{43}NO_3Si$ $[M+H]^+$ 530.30, found 530.81. ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.65 (m, 4H), 7.42 – 7.33 (m, 6H), 7.27 – 7.20 (m, 2H), 7.18 – 7.14 (m, 3H), 4.56 (br s, 1H), 4.09 (br d, *J* = 7.0 Hz, 1H), 3.91 (br s, 1H), 2.94 – 2.78 (m, 2H), 2.72 – 2.61 (m, 1H), 2.19 (br, 1H), 1.72 – 1.47 (m, 3H), 1.35 (s, 9H), 1.06 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.20, 139.35, 135.97, 135.79, 134.52, 133.99, 129.72, 129.66, 129.27, 128.36, 127.69, 127.64, 126.16, 79.04, 65.79, 50.37, 44.02, 35.99, 28.41, 27.04, 26.24, 20.28, 19.38.

(*2R,5S*)-2-Benzyl-5-((*tert*-butyldiphenylsilyl)oxy)piperidine (88). To a solution of *tert*-butyl *(2R,5S)*-2-benzyl-5-((*tert*-butyldiphenylsilyl)oxy)piperidine-1-carboxylate **87** (120 mg, 0.226 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$, and concentrated under reduced pressure. Filtering and concentration under reduced pressure afforded the crude product that was used without further purification. $[\alpha]_D^{20}$ = -13 (c =1.0, $CHCl_3$). HRMS calculated for $C_{28}H_{35}NOSi$ $[M+H]^+$: 430.2554; found: 430.2555. IR 3069, 3026, 2930, 2855, 2805, 1495, 1454, 1427, 1288, 1103, 1082, 1030, 993. ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.9 Hz, 4H), 7.42 – 7.29 (m, 6H), 7.26 – 7.18 (m, 3H), 7.08 – 7.03 (m, 2H), 4.00 (br s, 1H), 3.19 (br d, *J* = 10.0 Hz, 1H), 3.14 – 2.90 (m, 2H), 2.73 (br d, *J* = 10.2 Hz, 1H), 2.63 – 2.49 (m, 1H), 1.86 (br s, 1H), 1.69 (br s, 1H), 1.38 (br t, *J* = 11.3 Hz, 2H), 1.02 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.63, 135.57, 135.46, 133.41, 133.00, 130.17, 130.07, 129.29, 128.89, 127.96, 127.84, 127.36, 66.03, 57.45, 49.28, 39.12, 32.21, 26.91, 26.04, 19.20.

***tert*-Butyl *(2R,5S)*-2-benzyl-5-hydroxypiperidine-1-carboxylate (90)**. A solution of TBAF (2.83 mL, 2.83 mmol) was added to a solution of *tert*-butyl *(2R,5S)*-2-benzyl-5-((*tert*-butyldiphenylsilyl)oxy)piperidine-1-carboxylate **87** (1.00 g, 1.89 mmol) in THF (15 mL) with ice cooling and the mixture was stirred at r.t. for 2.5h, After being diluted with water, the mixture was extracted with ethyl acetate (3 times), the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated under *in vacuo*. The residue was purified by flash chromatography (pentane/EtOAc = 99 : 1→80 : 20) to furnish *tert*-butyl *(2R,5S)*-2-benzyl-5- hydroxypiperidine-1-carboxylate (440 mg, 1.51 mmol, 80% yield) as a colorless oil. $[\alpha]_D^{22}$ = -33 (*c* = 1.0, $CHCl_3$). LC-MS m/z: calculated for $C_{17}H_{25}NO_3$ $[M+H]^+$ 292.18, found: 292.71. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.25 (m, 2H), 7.21 – 7.15 (m, 3H), 4.35 (br s, 1H), 4.25 (br s, 1H), 3.64 – 3.57 (m, 1H), 2.91 (dd, *J* = 13.1, 8.1 Hz, 1H), 2.75 (dd, *J* = 12.8, 10.8 Hz, 2H), 1.90 (br s, 1H), 1.66 -1.59 (m, 3H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.78, 138.98, 129.20, 128.44, 126.26, 79.72, 67.03, 53.90, 45.69, 35.81, 28.26, 28.12, 26.28.

***tert*-Butyl (*2R,5S*)-2-benzyl-5-methoxypiperidine-1-carboxylate (91)**. To a solution of *tert*-butyl *(2R,5S)*-2-benzyl-5-hydroxypiperidine-1-carboxylate (70.0 mg, 0.240 mmol) and NaH (60%, 17.3 mg, 0.721 mmol) in DMF (3 mL) at 0 °C, MeI (0.045 mL, 0.721 mmol) was added dropwise with continuous stirring, and the mixture was allowed to stand at room temperature for 24h. The mixture was diluted with water (10 mL), and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (pentane/EtOAc = 99 : 1→90 : 10) to furnish *tert*-butyl *(2R,5S)*-2-benzyl-5-methoxypiperidine-1-carboxylate (61.0 mg, 0.200 mmol, 83% yield). $[\alpha]_D^{22}$ = -36 (*c* = 0.6, $CHCl_3$). LC-MS m/z: calculated for $C_{18}H_{27}NO_3$ $[M+H]^+$ 306.20, found: 306.82. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.24 (m, 2H), 7.19 – 7.14 (m, 3H), 4.43 (br s, 1H), 4.33 (br d, *J* = 13.5 Hz, 1H), 3.41 (br s, 1H), 3.34 (s, 3H), 2.97 – 2.82 (m, 2H), 2.74 (dd, *J* = 13.1, 8.3 Hz, 1H), 1.94 – 1.85 (m, 1H), 1.88 – 1.72 (m, 2H), 1.34 (s, 9H), 1.31 – 1.28 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.27, 139.22, 129.28, 128.48, 126.28, 79.35, 73.19, 56.08, 52.47, 40.38, 36.00, 28.42, 23.84, 21.47.

***(2R,5S)*-2-Benzyl-5-methoxypiperidine (92).** To a solution of compound *tert*-butyl *(2R,5S)*-2-benzyl-5-methoxypiperidine-1-carboxylate (30.0 mg, 0.098 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine and dried over $MgSO_4$, and concentrated under reduced pressure. Filtering and concentration under reduced pressure afforded the crude product *(2R,5S)*-2-benzyl-5-methoxypiperidine **92** that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 3H), 7.19 – 7.13 (m, 2H), 3.69 – 3.46 (m, 2H), 3.36 (s, 3H), 3.25 – 2.95 (m, 2H), 2.77 – 2.71 (m, 1H), 2.66 – 2.62 (m, 1H), 2.21 (br s, 1H), 1.98 – 1.83 (m, 1H), 1.71 – 1.52 (m, 1H), 1.34 – 1.16 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.46, 129.34, 128.99, 127.47, 72.84, 57.81, 56.77, 47.10, 39.13, 28.58, 25.86.

***tert*-Butyl *(2R,5S)*-2-benzyl-5-(cyclopropylmethoxy)piperidine-1-carboxylate (93).** To a solution of *tert*-butyl *(2R,5S)*-2-benzyl-5-hydroxypiperidine-1-carboxylate **90** (100 mg, 0.344 mmol) and NaH (60%, 24.7 mg, 1.03 mmol) in DMF (3 mL) at 0 °C, (bromomethyl)cyclopropane (139 mg, 1.03 mmol) was added dropwise with continuous stirring, and the mixture was allowed to stand at room temperature for 24h. The mixture was diluted with water (10 mL), and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (pentane/EtOAc = 99 : 1→90 : 10) to furnish *tert*-butyl *(2R,5S)*-2-benzyl-5-(cyclopropylmethoxy) piperidine-1- carboxylate **93** (110 mg, 0.318 mmol, 89% yield). $[\alpha]_D^{22}$ = -38 (*c* = 1.0, $CHCl_3$). LC-MS m/z: calculated for $C_{21}H_{31}NO_3$ $[M+H]^+$ 346.18, found 346.40. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.25 (m, 2H), 7.22 – 7.15 (m, 3H), 4.44 (br s, 1H), 4.28 (d, *J* = 13.9 Hz, 1H), 3.54 (s, 1H), 3.40 – 3.20 (m, 2H), 2.96 – 2.84 (m, 2H), 2.74 (dd, *J* = 13.2, 8.1 Hz, 1H), 2.02 – 1.93 (m, 1H), 1.89 – 1.67 (m, 2H), 1.34 (s, 9H), 1.29 – 1.24 (m, 1H), 1.08 – 0.98 (m, 1H), 0.50 – 0.47 (m, 2H), 0.30 – 0.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.17, 139.27, 129.27, 128.33, 126.23, 79.20, 72.66, 71.08, 51.90, 40.42, 36.08, 28.40, 24.50, 21.74, 10.83, 3.18, 2.92.

***(2R,5S)*-2-Benzyl-5-(cyclopropylmethoxy)piperidine (94)**. To a solution of solution of *tert*-butyl *(2R,5R)*-2-benzyl-5-(cyclopropylmethoxy)piperidine-1-carboxylate **93** (50.0 mg, 0.145 mmol) in DCM was added 20% TFA, the reaction mixture was stirred at r.t. for 2.5h until TLC showed the reaction was completed. When the reaction is finished, the mixture was co-evaporated with toluene (3 times), the residue was diluted with ethyl acetate and washed with 10% Na_2CO_3, water, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure afforded the crude product that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.23 (m, 3H), 7.20 – 7.10 (m, 2H), 3.70 (s, 1H), 3.51 (s, 1H), 3.31 (p, *J* = 9.9 Hz, 2H), 3.16 (s, 2H), 2.74 (br s, 2H), 2.17 (br d, *J* = 11.6 Hz, 1H), 1.87 (br d, *J* = 13.4 Hz, 1H), 1.62 (br d, *J* = 11.5 Hz, 1H), 1.32 (br d, *J* = 11.7 Hz, 1H), 0.99 (br s, 1H), 0.51 (d, *J* = 7.7 Hz, 2H), 0.16 (d, *J* = 4.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.38, 129.33, 128.98, 127.46, 74.17, 71.12, 57.86, 47.58, 39.23, 29.26, 26.09, 10.82, 3.12, 3.07.

Supporting Figures

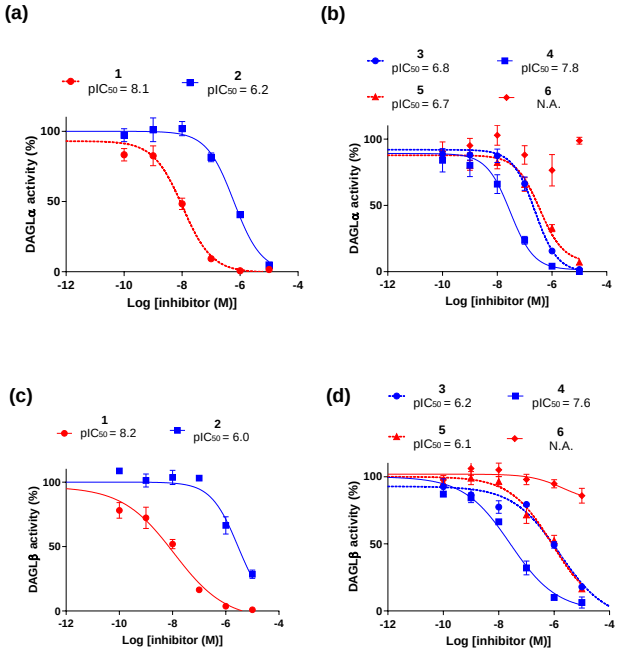


Figure S.1. Concentration-dependent inhibition curves of recombinant human DAGLα (a, b) and human DAGLβ (c, d) as determined with competitive ABPP labeled by DH379 (representative gels in Figure 1f and g). Data represent average values ± SEM; n = 3 per group .

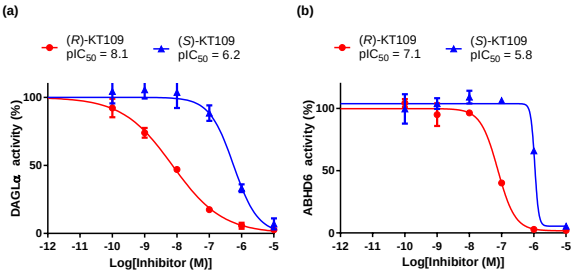


Figure S.2. *In vitro* characterization of *(R)*-KT109 and *(S)*-KT109 in mouse brain membrane proteome. (a-b) Dose response curves of DAGLα (a) and ABHD6 (b) inhibition as determined with competitive ABPP labeled by MB064 (Figure 2d and e). Data represent average values ± SEM; n = 3 per group.

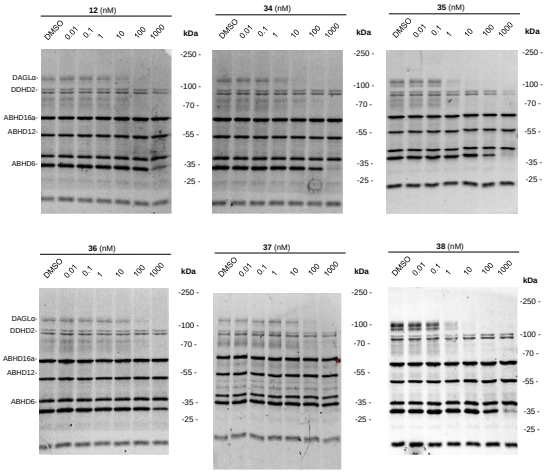


Figure S.3., related to Figure 3b. Full gels of *in vitro* competitive ABPP for compounds **34-38**, and **12** in mouse brain membrane proteome using probe MB064 (0.25 µM, 20 min).

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