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Structure-Based Design of Highly Selective β-Secretase Inhibitors: Synthesis, Biological Evaluation, and Protein-Ligand X-ray Crystal Structure

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Abstract

Structure-based design, synthesis and X-ray structure of protein-ligand complexes of exceptionally potent and selective β-secretase inhibitors are described. The inhibitors are designed specifically to interact with S_1' active site residues to provide selectivity over memapsin 1 and cathepsin D. Inhibitor **5** has exhibited exceedingly potent inhibitory activity (K_i = 17 pM) and high selectivity over BACE 2 (>7,000-fold) and cathepsin D (>250,000-fold). A protein-ligand crystal structure revealed important molecular insight into these selectivities. These interactions may serve as an important guide to design selectivity over the physiologically important aspartic acid proteases.

Introduction

Alzheimer's disease is a progressive, degenerative brain disorder with no effective treatment to date and the development of new drugs is an urgent priority in medicine.[1] The hallmark of Alzheimer's disease (AD) is the formation of neutritic plaques containing 40/42 residue amyloid-β (Aβ) peptides and neurofibrillary tangles in the brain.[2] β-Secretase (memapsin 2, BACE 1) is one of two proteases which cleaves β-amyloid precursor protein (APP) and generates Aβ and its aggregation product.[3] There is considerable evidence that excess Aβ leads to brain inflammation, neuronal death, and AD.[4] Consequently, β-secretase has become a major therapeutic target for drug development.[5,6] Since our design of initial transition-state inhibitor (**1**, Figure 1) and subsequent determination of inhibitor-bound memapsin 2 X-ray structure, nearly a decade ago, steady progress has been made towards the evolution of small molecule potent and brain-penetrable inhibitor drugs.[7,8] Recently, we have shown that administration of β-secretase inhibitor **2** rescued cognitive decline in transgenic AD mice, validating β-secretase as an important drug design target.[9,10] However, the development of clinical β-secretase inhibitor drug is faced with numerous formidable challenges, including lack of selectivity against other physiologically important aspartic acid proteases and issues of poor pharmacological profiles including blood-brain penetration.[7,8] In our continuing work towards the design of small molecule potent and selective inhibitors,

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[†]The PDB accession code for **5**-bound β-secretase X-ray structure is 4GID.

Supporting Information: HPLC, and HRMS data of inhibitors **4**–**9** and **18**–**22**; crystallographic data collection and refinement statistics for inhibitor **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

we have been particularly interested in developing tools for selectivity against relevant physiologically important aspartic acid proteases especially cathepsin D and BACE 2. BACE 2 has specificity similarity to BACE 1 and this is known to have important physiological functions.[11] Cathepsin D plays a key role in important biological functions like protein catabolism.[12] The abundance of cathepsin D in various cells, especially in CNS tissue cells, is very high. Furthermore, cathepsin D gene knock-out studies in mice showed marked phenotypic response including high mortality rate.[13] Therefore, the selective inhibition of β-secretase over cathepsin D and BACE 2 is very critical to reduce toxicity and other side effects of β-secretase inhibitor drugs.

As described by us previously, the X-ray crystal structure of inhibitor **1**-bound β-secretase showed an interesting hydrogen bonding between P_2'-carbonyl and the hydroxyl of Tyr-198, forming a rare kink at the P_2' site.[8] We have exploited this interaction in the design and synthesis of very potent and highly selective β-secretase inhibitors such as **3** by incorporating hydroxyethylene isosteres.[14] However, cellular β-secretase inhibitory activity of this class of inhibitors was only in micromolar range. In an attempt to design small molecule inhibitors with improved selectivity and cellular activity exploiting this unique interaction, we have further explored β-secretase inhibitors with a reduced amide isostere and incorporated functionality to improve potency and selectivity. The basic amine functionality in the reduced amide isostere may also improve cell permeability.[15] Herein, we report our structure-based design and synthesis of very potent and exceptionally selective inhibitors with excellent cellular inhibitory properties. A protein-ligand X-ray structure provided important molecular insight into the specific cooperative ligand-binding site interactions for selectivity.

The inhibitors containing reduced amide isostere have been reported however, they exhibited only marginal selectivity against memapsin 1 (BACE 2).[6,16] A reduced amide β–secretase inhibitor **4** was synthesized by us and this compound has exhibited a BACE 1 K_i of 27 nM and marginal selectivity against BACE 2 and cathepsin D in our in-house enzyme inhibitory assays. An energy-minimized model of **4** was created based upon the protein-ligand X-ray structure of **2**-bound β-secretase.[9] Our preliminary model suggested that an introduction of a hydroxyl group with *S*-configuration on the ethyl group of homo-alanine moiety would make enhanced interactions in the active site and possibly enhance selectivity. Based upon this molecular insight, we have designed, synthesized and evaluated inhibitors **5** and **6** (Figure 2) to investigate the influence of the hydroxyl group and also the role of stereochemistry on the potency and selectivity. We have also synthesized and evaluated inhibitors **7–9** to examine the importance of various functional groups at the P_1' and P_2' sites on the potency.

Chemistry

The synthesis of inhibitors **4–6** is shown in scheme 1. Amino acids **10a–c** were coupled with isobutyl amine in the presence of EDC, HOBt and $^{i}Pr_2NEt$ to afford the corresponding amides in 71–91% yield. Removal of the Boc group with trifluoroacetic acid followed by reductive amination of the resulting amine with aldehyde **11**[17] provided compounds **12a-c** in 49–76% yields. Treatment of **12a–c** with trifluoroacetic acid followed by coupling with acid **13**[18] provided the inhibitors **4–6** in 68–75% yields.

Synthesis of inhibitors **7–9** are shown in Schemes 2 and 3. Coupling of Boc protected allothreonine derivative **10b** with *N*-isobutyl-*N*-methyl amine using EDC, HOBt, and $^{i}Pr_2NEt$ followed by Boc removal using trifluoroacetic acid afforded (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-isobutyl-*N*-methyl-butanamide in 49 % yield over two steps. Reductive amination of this amine with known aldehyde **11**, provided **12d** in 30% yields. Inhibitor **7**

was synthesized in 61% yields from **12d** by Boc removal using trifluoroacetic acid followed by coupling of the resulting amine with the known acid **13** (Scheme 2). As shown in scheme 2, inhibitor **8** was prepared from **15** following the similar reaction sequence utilized for the synthesis of inhibitors **4–7** (schemes 1 and 2). Compound **15**, in turn, has been synthesized by O-methylation of **14** using MeI and Ag_2O[19] followed by hydrolysis using aqueous LiOH.

Synthesis of inhibitor **9** has been carried-out as shown in scheme 3. Treatment of **14** with TBSOTf in the presence of Et_3N followed by reaction with DiBALH provided the corresponding aldehyde. Reductive amination of this crude aldehyde with isobutyl amine afforded **16** in 66% yields over three steps. Removal of the Boc group using trifluoroacetic acid followed by reductive amination of the resulting amine with the aldehyde **11** afforded compound **17**.

Reaction of compound **17** with trifluoroacetic acid followed by coupling with acid **13** using EDC, HOBt, and $^{i}Pr_2NEt$ provided TBS protected inhibitor which was upon treating with TBAF gave the inhibitor **9** in 46 % yields over three steps.

Based on the results obtained from these inhibitors, we have directed our attention to reduce the peptidic nature of **5** by keeping all the key hydrogen bonding interactions of the prime region intact. In this direction, we have designed and synthesized the inhibitors **18–22** (Fig. 3) containing 7,6,5-tricyclic indole moieties as P_2 ligands.

For synthesis of inhibitor **18**, known indolecarboxylic acid **23** was prepared as described in the literature.[20] The synthesis of α,α-dimethyl tricyclic indole derivative **25**, present in inhibitor **20**, was synthesized as shown in scheme 4. Reaction of the known tricyclic indole derivative **24**[21] with MeI in the presence of NaH provided the corresponding α,α-dimethyl derivative. Saponification of the resulting ester with aqueous NaOH afforded α,α-dimethylindole carboxylic acid **25** (37% yields over 2 steps). Inhibitor **18** was synthesized by treatment of Boc-derivative **12b** with trifluoroacetic acid followed by coupling of the resulting amine with acid **23**[20] (68% yield). Similarly, coupling of the above amine with cyclopropyl indole derivative **30** provided inhibitor **21**.

Synthesis of tricyclic indole derivative in inhibitors **21** and **22** has been carried out from butyl 3-chloropropanesulfonate **26** as shown in Scheme 5. In a one-pot two step reaction, **26** was treated first with BuLi followed by treatment again with BuLi and BOMCl provided the corresponding butyl 1-(benzyloxymethyl)cyclopropanesulfonate in 80% yield. Hydrolysis of this sulfonate with KSCN followed by refluxing of the resulting potassium sulfonate with $SOCl_2$ afforded sulfonyl chloride **27** in 91% yield over two steps. Reaction of indole derivative **28**[22] with **27** in the presence of pyridine and catalytic amount of DMAP furnished sulfonamide **29** in 74% yields. Hydrogenolysis of **29**, followed by mesylation of the resulting alcohol using mesyl chloride and Et_3N afforded the corresponding mesylate. This mesylate was subjected to a one pot cyclization and N-methylation using NaH and MeI followed by hydrolysis in the presence of aqueous NaOH to obtain the desired carboxylic acid **30** in 65% yields over two steps.

Synthesis of (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-*iso*butyl (or *iso*propyl)hexanamide moiety present in the prime region of the inhibitors **19**, **20** and **22** has been carried out as shown in Scheme 6. Asymmetric dihydroxylation of **31** using AD-mix α followed by reaction of the resulting dihydroxy compound with *p*-nitrobenzenesulfonyl chloride in the presence of Et_3N afforded nosylate **32** in 32% yields over two steps.[23] Treatment of **32** with NaN_3 followed by hydrogenation of the corresponding azide using 10% Pd-C in the presence of $(Boc)_2O$ afforded **33** in 80% yields. Hydrolysis using aqueous LiOH followed by coupling with isobutyl amine and isopropyl amine using EDC, HOBt and $^{i}Pr_2NEt$ afforded **34a** and **34b**

respectively. Boc removal using trifluoroacetic acid followed by reductive amination of the resulting amine with aldehyde **11** gave **35a** and **35b** in 49% and 74% yields respectively. Inhibitors **19**, **20** and **22** were synthesized by coupling of the amine, obtained by Boc removal of **35a** and **35b** using trifluoroacetic acid, with tricyclic indole derivatives **23** or **25** or **30**.

Results and Discussions

The BACE 1 inhibitory activity of synthetic inhibitors **4–9** was determined against recombinant β-secretase using our previously reported assay protocols.[24] The results are shown in Table 1. As can be seen, inhibitor **4** with a homoalanine P_1'-side chain has shown a K_i of 27 nM (entry 1). Inhibitor **5** with an allothreonine P_1'-side chain has exhibited remarkable BACE 1 inhibitory activity with a K_i of 17 *p*M (entry 2). Inhibitor **6** with a threonine P_1'-side chain has shown significant reduction of BACE 1 activity over the deoxyderivative **4** or the inhibitor **5** with an allothreonine P_1'-side chain (entries 1–3). We have also evaluated the cellular inhibition of β-secretase in neuroblastoma cells.[25] Consistent with potent BACE 1 inhibitory activity, inhibitor **5** exhibited an average cellular IC_{50} value of 1 nM. The corresponding inhibitor **6** with a threonine P_1'-side chain has shown a cellular IC_{50} value of >1 μM in the same assay. Inhibitor **7** with a *N*-methyl amide abolished all BACE 1 inhibitory activity. Inhibitor **8** with an 'OMe' group in the P_1'-region also showed substantial reduction in inhibitory potency (entry 5) compared to inhibitor **5** with hydroxyl group. Inhibitor **9** with a reduced amide in the P_2'-region resulted in total loss in potency. These results clearly demonstrate the significance of hydrogen bonding interactions of inhibitor **5** with the prime region of the BACE 1 active site.

To gain further molecular insight, we have determined the X-ray structure of **5**-bond to β-secretase at a 2.2 Å resolution. As shown in Figure 4, the amine functionality of the reduced amide isostere forms two tight hydrogen bonds (2.4 and 2.7 Å bond distances) with active site aspartic acid Asp228.[7] Interestingly, the other active site Asp32 is not directly interacting with the reduced amide isostere. Asp32 is extensively hydrogen bonded to three groups: the amide nitrogen of Gly34 (2.8 Å), the hydroxyl group of Ser35 (2.5 Å to 3 Å with rotations of the involved groups), and the amide nitrogen of Gly230 (3.3 Å). These interactions appear to lock Asp32 in a rigid conformation thus its hydrogen bonding to Asp228 produced a network of hydrogen bond interactions to include the interaction of the inhibitor and the protease. A similar inhibitor-enzyme interacting pattern has been reported for the crystal structure of reduced amide isosteres.[16b] The fact that inhibitors **2** and **5** are highly potent suggests that the interaction of both active site carboxyls with the transition-state isostere is not a necessary feature for the design of potent inhibitors. The P_3-phenyl ring occupies a unique position which spans S_3 and S_4 subsites and causes a significant positional shift of a protein loop containing residues from 8 to 13 (the 10's loop)[26] located in the S_3/S_4 pocket similar to inhibitor **2**. This flexible part of the active site cleft can be further exploited for ligand design. The P_2'-carbonyl as well as P_2'-NH are within proximity to form hydrogen bonds with Thr72 and Gly34 respectively. Most significantly, the allothreonine hydroxyl group is oriented toward Tyr-198 hydroxyl group. This interaction is presumably absent in inhibitor **4**. Also, the P_1'-hydroxyl group stereochemistry is optimal for critical hydrogen bonding with Tyr-198. The combinations of active site interactions are responsible for potency and selectivity of inhibitor **5**.

The X-ray crystal structure of **5** bound memapsin 2 demonstrates the importance of allothreonine moiety as it forms key hydrogen bonding interactions with the prime region of memapsin 2. Therefore, inhibitors **18–22**, with 7,6,5 tricyclicindole moiety as P_2 ligand, were designed with a view to reduce labile amide bonds in isophthalic acid amide-derived ligand. Synthetic inhibitors **18–22** were evaluated against recombinant BACE 1 and the

results are summarized in Table 2. Inhibitor **18**, containing a known 7,6,5 tricyclic moiety as P_2 ligand showed a low nanomolar activity towards BACE 1 (K_i = 7.3 nM). This inhibitor is substantially less potent than inhibitor **5** however, the ratio of cell inhibitory to enzyme inhibitory efficacy was improved significantly (3 *vs* >58), indicating better cell permeability for compound **18**. Inhibitor **19** with a sterically more demanding propyl group in the P_1'-region has shown around 18-fold improvement in the potency (entries 1 and 2). Inhibitor **20** with a dimethyl substituted indole derivative as the P_2 ligand resulted in >10-fold potency enhancement over unsubstituted inhibitor **19**. This inhibitor exhibited a cellular IC_{50} value of 15 nM. The ligand was also designed especially to halt the possibility of retro-Michael reaction of the P_2-α,α-unsubstituted sultam functionality in inhibitors **18** and **19**.[27] Inhibitors **21** and **22**, designed by replacing the two methyl groups of P_2 ligand in **20** with cyclopropyl group, were also exhibited impressive potency.

We then evaluated the potencies of selected inhibitors against recombinant BACE 2 and human cathepsin D and the results are shown in Table 3. Interestingly, inhibitor **5** displayed very impressive selectivity against BACE 2 (BACE 2, K_i = 120 nM, selectivity >7000 fold) and cathepsin D (K_i = 4.3 PM, selectivity >250,000 fold) as well. In comparison, deoxyinhibitor **4** has shown a BACE 2 K_i of 1450 nM (selectivity >50 fold) and cathepsin D K_i of 8264 nM (selectivity >300 fold). This result suggested that the allothreonine hydroxyl group on the P_1'-side chain is critical to selectivity and potency of inhibitor **5**. Inhibitor **18** has also exhibited good selectivity (over 970 fold selective) towards BACE 1 over cathepsin D (entry 3). Inhibitor **19** with a butyl side chain has shown improvement in cathepsin D selectivity (entry 4). Inhibitor **20** displayed good selectivity against BACE 2 and excellent selectivity against cathepsin D (entry 5). Inhibitor **22** has also shown a >4200-fold selectivity over cathepsin D (entry 6).

Conclusion

In conclusion, we have designed, synthesized and examined the biological activity of isophthalamide based BACE 1 inhibitors containing various functional groups in the prime region. Selectivity of inhibitors **4** and **5** against BACE 2 and cathepsin D were also examined. Inhibitor **5** with an allothreonine moiety exhibited superior potency and exceptionally high selectivity when compared to inhibitors with a threonine moiety or an ethylglycine (homoalanine) moiety. Inhibitors with N-isobutyl-N-methylamide and P_2' reduced amide groups on the prime side lost their efficacy. These results clearly demonstrate the significance of prime region of the inhibitors on the potency and selectivity. The X-ray structure of **5**-bond β-secretase also showed the presence of effective hydrogen bond between the prime side of inhibitor and Thr72, Gly34, and Tyr 198 residues of BACE 1. Based upon this molecular insight, we have further designed and synthesized inhibitors by replacing the isophthalamide moiety with conformationally constrained 7,6,5-tricyclicindole moieties and by keeping the allothreonine moiety intact. These inhibitors have also shown very good potency and selectivity against cathepsin D. These results further support the significance of hydrogen bonding interactions in the prime region for the potency and selectivity. The combination of active site interactions along with the Tyr-198 may be responsible for the observed selectivity. This molecular insight may aid further design of selectivity against other aspartic acid proteases. Further investigations into the origin of selectivity are in progress.

Experimental Section

General

All anhydrous solvents were obtained according to the following procedures: diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon;

dichloromethane from calcium hydride. Other solvents were used without purification. All moisture-sensitive reactions were carried out in flame-dried flasks under argon atmosphere. Reactions were monitored by thin layer chromatography (TLC) using Silicycle 60A-F254 silica gel pre-coated plates. Flash column chromatography was performed using Silicycle 230–400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-300 (300 MHz and 75 MHz, respectively), Bruker Avance ARX- 400 (400 MHz and 100 MHz), and Bruker Avance DRX-500 (500 MHz and 125 MHz). High and low resolution mass spectra were carried out by the Mass Spectroscopy Center at Purdue University. The purity of all test compounds was determined by HRMS and HPLC analysis. All test compounds showed ≥95% purity.

Synthesis of compound 12a—To a mixture of (*S*)-2-[(*tert*-butoxycarbonyl)amino]butanoic acid **10a** (2.3 mmol, 0.47 g) and ^{i}Pr$_2$NEt (2.76 mmol, 0.48 mL) in CH_2Cl_2 (12 mL), HOBt.H_2O (2.76 mmol, 0.37 g), isobutyl amine (2.76 mmol, 0.27 mL) and EDC.HCl (2.76 mmol, 0.53 g) were added simultaneously at 23 °C and the resulting mixture was stirred for 17 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (1–3% MeOH/CH_2Cl_2) to furnish (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate in 86% yields (0.51 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (brs, 1H), 5.08 (br, 1H), 4.07-3.89 (m, 1H), 3.18-2.96 (m, 2H), 1.92-1.70 (m, 2H), 1.62 (hept, *J* = 7.3 Hz, 1H), 1.43 (s, 9H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 6H).

To a solution of (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate (1.97 mmol, 0.51 g) in CH_2Cl_2 (9 mL), trifluoroacetic acid (3 mL) was added at 0 °C and the resulting mixture was stirred for 1.5 h at 23 °C. Excess trifluoroacetic acid and CH_2Cl_2 were removed under reduced pressure and the resulting residue was purified by silica gel column chromatography [2–5% (5%NH_3/MeOH)/CH_2Cl_2] to furnish (*S*)-2-amino-*N*-*iso*butylbutanamide in 96% yield (0.298 g).

To a solution of the above (*S*)-2-amino-*N*-*iso*butylbutanamide (1.87 mmol, 0.296 g) and (*S*)-*tert*-butyl (1-oxo-3-phenylpropan-2-yl)carbamate **11** (prepared from the corresponding Weinreb amide (2 mmol) following a similar literature procedure)[7] in CH_2Cl_2 (20 mL), $Na(OAc)_3BH$ (2.62 mmol, 0.55 g) was added at 0 °C. The resulting mixture was stirred for 1 h at 0 °C and 15 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na$_2$SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1–3% MeOH/CH_2Cl_2) to furnish the corresponding adduct **12a** in 79% yields (0.58 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.26 (m, 2H), 7.25-7.11 (m, 4H), 4.50 (br, 1H), 3.95 (brs, 1H), 3.10-2.96 (m, 2H), 2.92 (dd, *J* = 7.3, 5.0 Hz, 1H), 2.86-2.69 (m, 2H), 2.62 (dd, *J* = 11.9, 4.3 Hz, 1H), 2.52 (dd, *J* = 12.0, 7.9 Hz, 1H), 1.80-1.63 (m, 2H), 1.63-1.50 (m, 1H), 1.41 (s, 9H), 0.92 (t, *J* = 7.5 Hz, 3H), 0.85 (d, *J* = 6.5 Hz, 6H).

Synthesis of inhibitor 4—To a solution of **12a** (0.72 mmol, 0.282 g) in CH_2Cl_2 (9 mL), trifluoroacetic acid (3 mL) was added at 0 °C. After stirring the reaction mixture for 1 h at 23 °C, CH_2Cl_2 and trifluoroacetic acid were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography [2–6% (5%NH_3/MeOH)/CH_2Cl_2] to furnish the corresponding amine in 93% yield (0.195 g).

To a solution of the above amine (0.125 mmol, 36.4 mg) in CH_2Cl_2 (10 mL), $^{i}Pr_2NEt$ (0.03 mL), HOBt.H_2O (0.16 mmol, 21.6 mg), (*R*)-3-(*N*-methylmethylsulfonamido)-5-[(1-phenylethyl)-carbamoyl]benzoic acid **13** (0.16 mmol, 60.2 mg) and EDC.HCl (0.16 mmol, 30.7 mg) were added simultaneously at 23 °C and the resulting mixture was stirred for 14 h at the same temperature. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (1–3% MeOH/CH_2Cl_2) to furnish the inhibitor **4** in 73% yields (59.6 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.03 (s, 1H), 7.98 (s, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.39-7.34 (m, 2H), 7.33-7.24 (m, 5H), 7.24-7.18 (m, 3H), 6.71 (t, *J* = 6.2 Hz, 1H), 5.31 (p, *J* = 7.5 Hz, 1H), 4.40-4.26 (m, 1H), 3.29 (s, 3H), 3.04-2.91 (m, 3H), 2.88-2.83 (m, 1H), 2.81 (s, 3H), 2.81-2.73 (m, 2H), 2.55 (dd, *J* = 12.3, 4.1 Hz, 1H), 1.75-1.58 (m, 2H), 1.57 (d, *J* = 7.0 Hz, 3H), 1.55-1.48 (m, 1H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.77 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.15, 165.23, 164.53, 143.06, 142.23, 137.64, 135.65, 129.1, 128.62, 128.58, 127.93, 127.87, 127.33, 126.64, 126.23, 123.39, 65.20, 51.68, 50.24, 49.56, 46.41, 38.55, 37.84, 35.52, 28.36, 26.96, 21.60, 19.98, 19.95, 10.33. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{48}N_5O_5S$ 650.3376, found 650.3370.

Synthesis of compound 12b—*Tert*-butyl [(2*S*, 3*S*)-3-hydroxy-1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate was synthesized in 71% yield by coupling of (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoic acid **10b** with isobutyl amine in the presence of EDC, HOBt and $^{i}Pr_2NEt$ as described for (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (brs, 1H), 5.53 (brs, 1H), 4.10-3.75 (m, 3H), 3.23-3.09 (m, 1H), 3.06-2.92 (m, 1H), 1.78 (hept, *J* = 6.7 Hz, 1H), 1.45 (s, 9H), 1.29 (d, *J* = 6.1 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 6H).

Compound **12b** was synthesized in 52% yield (for two steps) from *tert*-butyl [(2*S*, 3*S*)-3-hydroxy-1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate following the procedure described for the synthesis of compound **12a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.27 (m, 3H), 7.26-7.19 (m, 1H), 7.17 (d, *J* = 7.4 Hz, 2H), 4.53 (d, *J* = 8.9 Hz, 1H), 4.05-3.86 (m, 2H), 3.39 (brs, 1H), 3.13-3.00 (m, 2H), 2.99 (d, *J* = 5.7 Hz, 1H), 2.87-2.71 (m, 2H), 2.67 (dd, *J* = 12.1, 4.4 Hz, 1H), 2.58 (dd, *J* = 12.1, 7.6 Hz, 1H), 1.73 (hept, *J* = 6.7 Hz, 1H), 1.41 (s, 9H), 1.18 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 7.1 Hz, 6H).

Synthesis of inhibitor 5—Inhibitor **5** has been prepared (yield 71%, over 2 steps) from **12b** by boc deprotection followed by coupling with (*R*)-3-(*N*-methylmethylsulfonamido)-5-[(1-phenylethyl)carbamoyl]benzoic acid **13** following the similar reaction procedure described for the inhibitor **4**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.93 (s, 1H), 7.88 (s, 1H), 7.39-7.27 (m, 6H), 7.26-7.14 (m, 6H), 5.27 (p, *J* = 7.3 Hz, 1H), 4.48-4.30 (m, 1H), 4.04-3.94 (m, 1H), 3.24 (s, 3H), 3.09 (d, *J* = 4.3 Hz, 1H), 3.02-2.88 (m, 3H), 2.88-2.77 (m, 1H), 2.80 (s, 3H), 2.73 (dd, *J* = 12.4, 3.7 Hz, 1H) 2.64 (dd, *J* = 12.3, 7.7 Hz, 1H), 1.64 (hept, *J* = 6.7 Hz 1H), 1.56 (d, *J* = 6.9 Hz, 3H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.37, 166.01, 164.75, 142.91, 142.03, 137.54, 135.90, 135.77, 129.16, 128.65, 128.59, 127.89, 127.62, 127.43, 126.65, 126.23, 123.87, 68.21, 68.06, 52.16, 51.60, 49.66, 46.40, 39.00, 37.83, 35.56, 28.34, 21.65, 20.06, 18.21. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{48}N_5O_6S$ 666.3325, found 666.3321.

Synthesis of compound 12c—*Tert*-butyl [(2*S*, 3*R*)-3-hydroxy-1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate has been synthesized (yield 91%) by coupling of (2*S,* 3*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoic acid **10c** with isobutyl amine in the presence

of $^{i}Pr_2NEt$, HOBt and EDC as described for (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (brs, 1H), 5.52 (d, *J* = 7.6 Hz, 1H), 4.45-4.30 (m, 1H), 3.97 (dd, *J* = 8.2, 1.9 Hz, 1H), 3.22-3.08 (m, 1H), 3.07-2.92 (m, 1H), 1.77 (heptet, *J* = 6.7 Hz, 1H), 1.45 (s, 9H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 6H).

Compound **12c** was prepared from *tert*-butyl [(2*S*, 3*R*)-3-hydroxy-1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate following the procedure described for the synthesis of compound **12a** (yield 49% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.26 (m, 2H), 7.25-7.13 (m, 4H), 4.59 (d, *J* = 8.5 Hz, 1H), 4.04-3.75 (m, 2H), 3.15-2.96 (m, 2H), 2.94 (d, *J* = 5.2 Hz, 1H), 2.80 (d, *J* = 6.2 Hz, 2H), 2.70-2.55 (m, 2H), 1.92 (br, 1H), 1.74 (hept, *J* = 6.7 Hz, 1H), 1.40 (s, 9H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 6H).

Synthesis of inhibitor 6—Inhibitor **6** has been prepared (yield 75%, over 2 steps) from **12c** by boc deprotection followed by coupling with (*R*)-3-(*N*-methylmethylsulfonamido)-5-[(1-phenylethyl)carbamoyl]benzoic acid **13** following the similar reaction procedure described for the inhibitor **4**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.92 (s, 1H), 7.87 (s, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.38-7.27 (m, 5H), 7.26-7.15 (m, 5H), 7.08 (t, *J* = 5.9 Hz, 1H), 5.27 (p, *J* = 7.1 Hz, 1H), 4.42-4.27 (m, 1H), 3.79 (p, *J* = 6.3 Hz, 1H), 3.22 (s, 3H), 3.01-2.87 (m, 4H), 2.84 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.78 (s, 3H), 2.71-2.59 (m, 2H), 1.69-1.59 (m, 1H), 1.56 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 6.2 Hz, 3H), 0.79 (d, *J* = 6.4 Hz, 3H), 0.78 (d, *J* = 6.6 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 172.78, 165.82, 164.81, 143.03, 142.05, 137.58, 135.88, 135.77, 129.14, 128.61, 127.78, 127.64, 127.39, 126.64, 126.23, 123.80, 69.13, 68.38, 52.04, 50.90, 49.68, 46.54, 38.59, 37.79, 35.56, 28.33, 21.68, 20.03, 19.68. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{48}N_5O_6S$ 666.3325, found 666.3335.

Synthesis of compound 12d—To a mixture of (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoic acid (**10b**) (1 mmol, 0.22 g) and $^{i}Pr_2NEt$ (1.2 mmol, 0.21 mL) in CH_2Cl_2 (5 mL), were added HOBt.H_2O (1.2 mmol, 0.162 g), *N*-isobutyl-*N*-methylamine (1.2 mmol, 0.14 mL) and EDC.HCl (1.2 mmol, 0.23 g) at 23 °C and the resulting reaction mixture was stirred for 15 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1-3%MeOH/CH_2Cl_2) to obtain *tert*-butyl {(2*S*, 3*S*)-3-hydroxy-1-[*iso*butyl(methyl)amino]-1-oxobutan-2-yl}carbamate.

To a solution of the above *tert*-butyl {(2*S*, 3*S*)-3-hydroxy-1-*iso*butyl(methyl)amino]-1-oxobutan-2-yl}carbamate in CH_2Cl_2 (6 mL), was added trifluoroacetic acid (2 mL) at 0 °C. The resulting reaction mixture was warmed to 23 °C and stirred for 1.5 h at 23 °C. Reaction mixture was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography [2–5% (5%NH_3/MeOH)/CH_2Cl_2] to furnish (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-*iso*butyl-*N*-methyl-butanamide in 49% (93.2 mg) yields over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89-3.76 (m, 1H), 3.67 & 3.64 (two doublets, *J* = 4.7 Hz, 4.6 Hz, 1H), 3.36 (dd, *J* = 13.2, 8.0 Hz, 0.5H), 3.19 (dd, *J* = 14.4, 8.3 Hz, 0.5H), 3.10 (dd, *J* = 14.3, 7.0 Hz, 0.5H), 3.05 (s, 1.7H), 3.03-2.98 (m, 0.5H), 2.91 (s, 1.3H), 2.51 (brs, 3H), 2.00-1.87 (m, 1H), 1.10 (two doublets, *J* = 6.3, 6.3 Hz, 3H), 0.93-0.81 (m, 6H).

Compound **12d** was synthesized from (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-*iso*butyl-*N*-methyl-butanamide by reductive amination with aldehyde **11** following a similar procedure described for the synthesis of compound **12a** (yield 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.10 (m, 5H), 4.80-4.64 (m, 1H), 3.95-3.78 (m, 2H), 3.43 (dd, *J* = 11.8, 4.4 Hz, 1H), 3.35 – 3.09 (m, 2H), 3.06-2.96 (m, 2.5H), 2.91 (s, 1.5H), 2.84 (br, 2H), 2.72 – 2.59 (m, 1H),

2.48-2.33 (m, 1H), 2.01-1.87 (m, 1H), 1.39 (s, 9H), 1.10 & 1.06 (two doublets, *J* = 6.4, 6.4 Hz, 3H), 0.90 & 0.86 (two doublets, *J* = 6.7, 6.3 Hz, 6H).

Synthesis of inhibitor 7—Inhibitor **7** was synthesized from compound **12d** by boc removal using trifluoroacetic acid followed by coupling of the resulting amine with the known acid **13** using EDC, HOBt, and $^{i}Pr_2NEt$ following the similar procedure described for the synthesis of inhibitor **4** (yield 61%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 & 8.17 (two singlets, 1H), 8.07 – 7.92 (m, 2H), 7.45 – 7.30 (m, 4H), 7.30 – 7.15 (m, 6H), 7.07 (t, *J* = 7.8 Hz, 1H), 5.40-5.26 (m, 1H), 4.34 – 4.19 (m, 1H), 3.97-3.82 (m, 1H), 3.55 & 3.53 (two doublets, *J* = 4.2, 4.2 Hz, 1H), 3.33 (s, 3H), 3.23 – 3.03 (m, 2.5H), 3.01 (s, 2H), 2.95 – 2.87 (m, 1H), 2.85 & 2.84 (two singlets, 3H), 2.77 (s, 1H), 2.73 – 2.57 (m, 2.5H), 1.96-1.78 (m, 1H), 1.60 (d, *J* = 6.9 Hz, 3H), 1.13 & 1.10 (two doublets, *J* = 6.4, 6.5 Hz, 3H), 0.89 & 0.84 (two doublets, *J* = 6.6, 6.5 Hz, 2.7H), 0.79 & 0.75 (two doublets, *J* = 6.7, 6.7 Hz, 3.3H). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{36}H_{50}N_5O_6S$ 680.3482, found 680.3478.

Synthesis of (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxybutanoic acid (15)—To a solution of (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoate (**14**) (2.14 mmol, 0.5 g) in CH_3CN (21 mL), were added Ag_2O (10.7 mmol, 2.48 g) and MeI (21.4 mmol, 1.3 mL) at 23 °C and the resulting reaction mixture was stirred for 8 days at 23 °C. Reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20–25% EtOAc/hexanes) to furnish (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-methoxybutanoate in 55% yields (0.29 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (d, *J* = 9.3 Hz, 1H), 4.41 (dd, *J* = 8.6, 3.6 Hz, 1H), 3.74 (s, 3H), 3.66-3.55 (m, 1H), 3.34 (s, 3H), 1.43 (s, 9H), 1.18 (d, *J* = 6.4 Hz, 3H).

To a solution of (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-methoxybutanoate (0.86 mmol, 0.213 g) in THF (4 mL) and H_2O (2 mL), was added $LiOH.H_2O$ (5.16 mmol, 0.216 g) at 23 °C. The resulting reaction mixture was stirred for 5 h at 23 °C. Reaction mixture was diluted with H_2O and diethyl ether. Organic layer was separated and the aqueous layer was carefully acidified with 2N HCl and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide crude (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxybutanoic acid (**15**). This crude acid was used in the coupling reaction with-out any further purification.

Synthesis of compound 12e—Synthesis of *tert*-butyl [(2*S*, 3*S*)-1-(*iso*butylamino)-3-methoxy-1-oxobutan-2-yl]carbamate has been carried out by coupling of the above crude acid **15** with isobutyl amine using EDC, HOBt, $^{i}Pr_2NEt$ following the procedure described for the synthesis of (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate (yield 74%, over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.22 (brs, 1H), 5.15 (brs, 1H), 4.10 (dd, *J* = 7.7, 6.8 Hz, 1H), 3.59 (p, *J* = 6.4 Hz, 1H), 3.34 (s, 3H), 3.21-3.10 (m, 1H), 3.09 – 2.98 (m, 1H), 1.77 (hept, *J* = 6.7 Hz, 1H), 1.44 (s, 9H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 6H).

To a solution of *tert*-butyl [(2*S*, 3*S*)-1-(*iso*butylamino)-3-methoxy-1-oxobutan-2-yl]-carbamate (0.624 mmol, 0.18 g) in CH_2Cl_2 (6 mL), was added trifluoroacetic acid (2 mL) at 0 °C and the resulting mixture was stirred for 1.5 h at 23 °C. Trifluoroacetic acid and CH_2Cl_2 were removed under reduced pressure and the resulting residue was diluted with EtOAc and basified with saturated aqueous $NaHCO_3$ solution. Organic layer was separated and the aqueous layer was extracted several times with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain crude (2*S*, 3*S*)-2-amino-N-isobutyl-3-methoxybutanamide in 88% yield.

To a solution of the above crude amine (0.55 mmol, 0.104 g) and (*S*)-*tert*-butyl (1-oxo-3-phenylpropan-2-yl)carbamate **11** [prepared from the corresponding Weinreb amide (0.55 mmol) following the similar literature procedure][7] in CH_2Cl_2 (6 mL), $Na(OAc)_3BH$ (0.825 mmol, 0.175 g) was added at 0 °C. The resulting mixture was stirred for 1 h at 0 °C and 15 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20–45% EtOAc/hexanes) to furnish the corresponding adduct **12e** in 67% yield (0.155 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (brs, 1H), 7.29 – 7.21 (m, 2H), 7.21-7.10 (m, 3H), 4.64 (d, *J* = 8.2 Hz, 1H), 3.94 (brs, 1H), 3.77 – 3.68 (m, 1H), 3.29 (s, 3H), 3.27 (d, *J* = 4.0 Hz, 1H), 3.00 (t, *J* = 6.4 Hz, 2H), 2.82 (dd, *J* = 12.9, 5.7 Hz, 1H), 2.69 (dd, *J* = 13.6, 7.7 Hz, 1H), 2.58 – 2.46 (m, 2H), 1.68 (hept, *J* = 6.7 Hz, 1H), 1.39 (s, 9H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 6H).

Synthesis of inhibitor 8—To a solution of **12e** (0.073 mmol, 30.8 mg) in CH_2Cl_2 (3 mL), was added trifluoroacetic acid (1 mL) at 0 °C. The resulting reaction mixture was warmed to 23 °C and stirred for 1.5 h at 23 °C. Reaction mixture was concentrated under reduced pressure and the resulting residue was used in the next step without any further purification.

To a solution of the above residue in CH_2Cl_2 (5 mL), were added ^{i}Pr$_2$NEt (0.1 mL), HOBt.H_2O (0.14 mmol, 18.9 mg), acid **13** (0.07 mmol, 26.3 mg), EDC.HCl (0.14 mmol, 26.8 mg) simultaneously at 23 °C. The resulting reaction mixture was stirred for 17 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (2%MeOH/CH_2Cl_2) to obtain the inhibitor **8** in 67% yields (32.1 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 8.02 (s, 1H), 7.96 (s, 1H), 7.42 – 7.29 (m, 5H), 7.28-7.25 (m, 2H), 7.22 (d, *J* = 7.4 Hz, 3H), 7.14-7.02 (m, 3H), 5.32 (p, *J* = 7.2 Hz, 1H), 4.48-4.36 (m, 1H), 3.73-3.61 (m, 1H), 3.33 (s, 3H), 3.30 (s, 3H), 3.27 (d, *J* = 4.2 Hz, 1H), 3.04-2.94 (m, 3H), 2.90-2.80 (m, 4H), 2.76 – 2.65 (m, 2H), 1.69-1.61 (m, 1H), 1.61 (d, *J* = 6.9 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.84, 165.41, 164.41, 142.87, 142.20, 137.42, 135.67, 135.64, 129.11, 128.60, 128.57, 127.80, 127.73, 127.38, 126.63, 126.18, 123.41, 65.95, 56.52, 51.99, 51.19, 49.53, 46.33, 38.73, 37.83, 35.43, 28.32, 21.63, 20.00, 14.62. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{36}H_{50}N_5O_6S$ 680.3482, found 680.3488.

Synthesis of *tert*-butyl {(2*R*, 3*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-(*iso*butylamino)butan-2-yl}carbamate (16)—To a solution of (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoate (**14**) (2.2 mmol, 0.513 g) in CH_2Cl_2 (11 mL), were added Et_3N (3.3 mmol, 0.46 mL), and TBSOTf (2.86 mmol, 0.66 mL) at 0 °C. The resulting reaction mixture was warmed to 23 °C and stirred for 2 h at the same temperature. Reaction mixture was diluted with H_2O and CH_2Cl_2. Organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% EtOAc/hexanes) to obtain (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)-amino]-3-[(*tert*-butyldimethylsilyl)oxy]butanoate in 88% yields (0.67 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (d, *J* = 8.4 Hz, 1H), 4.16 (dd, *J* = 8.3, 3.7 Hz, 1H), 4.05 – 3.96 (m, 1H), 3.66 (s, 3H), 1.36 (s, 9H), 1.16 (d, *J* = 6.3 Hz, 3H), 0.79 (s, 9H), −0.03 (s, 6H).

To a solution of (2*S*, 3*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-[(*tert*-butyldimethyl-silyl)oxy]butanoate (0.288 mmol, 0.1 g) in Et_2O (3 mL), was added DiBAL-H (0.63 mmol,

0.63 mL, 1M solution in CH_2Cl_2) at −78 °C and the resulting mixture was stirred for 1.5 h at −78 °C. Reaction mixture was quenched with 0.5 mL EtOAc and diluted with Et_2O and saturated aqueous sodium potassium tartrate. After stirring for 0.5 h, organic layer was separated and the aqueous layer was extracted with Et_2O. The combine extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was used in the next step without any further purification.

To a solution of the above aldehyde in CH_2Cl_2 (6 mL), were added isobutyl amine (0.864 mmol, 0.086 mL) and $Na(OAc)_3BH$ (0.576 mmol, 0.122 g) at 0 °C and the resulting reaction mixture was stirred for 1.5 h at 0 °C & 15 h at 23 °C. Reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography [3% (5%NH_3/MeOH)/CH_2Cl_2] to obtain (*tert*-butyl {(2*R*, 3*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-(isobutylamino)butan-2-yl}-carbamate (**16**) in 75% yields (80.7 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (d, *J* = 7.9 Hz, 1H), 4.11-3.98 (m, 1H), 3.57-3.42 (m, 1H), 2.90 (dd, *J* = 12.2, 5.3 Hz, 1H), 2.62 (dd, *J* = 12.2, 4.3 Hz, 1H), 2.44-2.30 (m, 2H), 1.72 (hept, *J* = 6.7 Hz, 1H), 1.43 (s, 9H), 1.14 (d, *J* = 6.4 Hz, 3H), 0.92-0.84 (m, 15H), 0.04 (s, 3H), 0.03 (s, 3H).

Synthesis of compound 17—Compound **17** was synthesized by Boc removal of *tert*-butyl {(2*R*, 3*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-(*iso*butylamino)butan-2-yl}carbamate (**16**) using trifluoroacetic acid followed by reductive amination with known aldehyde **11** following the procedure described for the synthesis of **12e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.23 (m, 2H), 7.23-7.15 (m, 3H), 5.20 (br, 1H), 3.94 – 3.75 (m, 2H), 2.95-2.81 (m, 1H), 2.79 – 2.67 (m, 2H), 2.58 (dd, *J* = 12.4, 5.0 Hz, 2H), 2.50-2.43 (m, 2H), 2.38 (qd, *J* = 11.5, 6.9 Hz, 2H), 1.74 (hept, *J* = 6.7 Hz, 1H), 1.40 (s, 9H), 1.10 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 6H), 0.87 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H).

Synthesis of inhibitor 9—Boc removal of compound **17** using trifluoroacetic acid followed by coupling of the resulting amine with known acid **13** using EDC, HOBt and ^{i}Pr$_2$NEt following the procedure described for the synthesis of inhibitor **8** afforded the corresponding amide in 70% yields. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.00 (s, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.92 (s, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.29 – 7.19 (m, 5H), 7.20-7.12 (m, 1H), 5.29 (p, *J* = 7.1 Hz, 1H), 4.20 – 4.06 (m, 1H), 4.06 – 3.93 (m, 1H), 3.28 (s, 3H), 3.20 (dd, *J* = 12.4, 8.2 Hz, 1H), 3.10 – 2.98 (m, 2H), 2.86 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.82-2.76 (m, 4H), 2.76 – 2.67 (m, 3H), 2.60 (dd, *J* = 12.4, 7.1 Hz, 1H), 1.81 (hept, *J* = 6.8 Hz, 1H), 1.59 (d, *J* = 7.0 Hz, 3H), 1.11 (d, *J* = 6.4 Hz, 3H), 0.86 (s, 9H), 0.75 (d, *J* = 6.7 Hz, 3H), 0.75 (d, *J* = 6.7 Hz, 3H), 0.07 (s, 3H), 0.05 (s, 3H).

To a solution of the above amide (0.104 mmol, 79.7 mg) in THF (10 mL), was added TBAF (1.25 mmol, 1.25 mL, 1M solution in THF) at 0 °C and the resulting reaction mixture was stirred for 18 h at 23 °C. Solvent was removed under reduced pressure and the resulting residue was purified by silica gel column chromatography [3–5% (5%NH_3/MeOH)/CH_2Cl_2] to obtain inhibitor **9** in 65% (43.9 mg) yields. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.01 – 7.93 (m, 2H), 7.40 (d, *J* = 7.3 Hz, 2H), 7.37 – 7.24 (m, 7H), 7.24 – 7.16 (m, 3H), 5.31 (p, *J* = 7.3 Hz, 1H), 4.37-4.25 (m, 1H), 3.93-3.83 (m, 1H), 3.31 (s, 3H), 3.04 (dd, *J* = 13.5, 5.8 Hz, 1H), 2.81 (s, 3H), 2.80 – 2.67 (m, 5H), 2.44 – 2.27 (m, 3H), 1.70-1.54 (m, 4H), 1.13 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.78, 164.64, 143.13, 142.13, 137.87, 135.80, 129.24, 128.63, 128.53, 128.16, 127.46, 127.37, 126.53, 126.34, 123.78, 68.48, 60.61, 57.62, 51.94, 49.74, 49.48, 49.30, 38.67, 37.88, 35.46, 27.73, 21.78, 20.41, 20.38, 20.34. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{50}N_5O_5S$ 652.3533, found 652.3543.

Synthesis of inhibitor 18—Inhibitor **18** was synthesized from compound **12b** by Boc removal using trifluoroacetic acid followed by coupling of the resulting amine with known acid **23** using EDC, HOBt, and $^{i}Pr_2NEt$ following the similar procedure described for the synthesis of inhibitor **4** (yield 68%, over two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.47 (s, 1H), 7.38-7.28 (m, 3H), 7.24-7.19 (m, 2H), 6.84 (s, 1H), 6.47 (d, *J* = 8.0 Hz, 1H), 4.62-4.36 (m, 3H), 4.02 (p, *J* = 6.0 Hz, 1H), 3.90-3.81 (m, 2H), 3.47 (s, 3H), 3.12 (d, *J* = 5.0 Hz, 1H), 3.10-2.93 (m, 4H), 2.84 (dd, *J* = 12.2, 4.7 Hz, 1H), 2.77 (dd, *J* = 11.1, 6.8 Hz, 1H), 2.72 (q, *J* = 7.5 Hz, 2H), 1.76-1.65 (m, 1H), 1.30 (t, *J* = 7.5 Hz, 3H), 1.15 (d, *J* = 6.4 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.75, 167.90, 137.52, 133.84, 130.72, 129.28, 128.65, 127.72, 127.23, 126.75, 125.78, 120.26, 118.03, 117.60, 68.32, 67.48, 56.77, 52.30, 51.39, 46.41, 43.60, 39.66, 39.03, 28.42, 20.11, 18.95, 17.93, 14.25. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{44}N_5O_5S$ 598.3063, found 598.3060.

Synthesis of compound 25—To a solution of **24** (0.43 mmol, 0.145 g) in DMF (4 mL), was added NaH (1.72 mmol, 68.8 mg, 60% NaH in mineral oil) at 23 °C and the resulting mixture was stirred for 15 min at the same temperature. Iodo methane (1.72 mmol, 0.11 mL) was added to the reaction mixture and stirring was continued for further 2.5 h at 23 °C. Reaction mixture was quenched with methanol and then diluted with EtOAc. The resulting solution was washed with H_2O & brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (30% EtOAc/hexanes) to obtain the corresponding α,α-dimethylated adduct in 51% (76.8 mg) yields. ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 1.0 Hz, 1H), 7.74 (s, 1H), 6.81 (s, 1H), 4.14 (s, 2H), 3.93 (s, 3H), 3.55 (s, 3H), 2.76 (q, *J* = 7.5 Hz, 2H), 1.57 (s, 6H), 1.32 (t, *J* = 7.5 Hz, 3H).

A mixture of the above ester (0.219 mmol, 76.7 mg) and NaOH (20 mmol, 10 mL, 2N NaOH) in EtOH (10 mL) and THF (10 mL) was stirred for 2.5 days at 23 °C. Solvent was removed under reduced pressure and the resulting mixture was diluted with H_2O and diethyl ether. Organic layer was separated, aqueous layer was acidified with aqueous 1N HCl and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to furnish the corresponding crude acid (**25**) in 72% yields (53.2 mg) which was used directly in the coupling reaction without any further purification. LRMS-ESI (*m/z*): $[M+Na]^+$ 359.19

Synthesis of 1-(benzyloxymethyl)cyclopropanesulfonyl chloride (27)—Solutions of BuLi [17.2 mmol, 6.9 mL (2.5 M in hexanes) in 15 mL THF] and butyl 3-chloro-1-propanesulfonate (**26**) (16.3 mmol, 3.5 g in 15 mL THF) were added at the same time via cannula to an oven dried flask containing THF (100 mL) at −78 °C and the resulting mixture was stirred for 5–10 min at −78 °C and 30 min at 0 °C. Reaction mixture was cooled back to −78 °C and a solution of BuLi [19.6 mmol, 7.8 mL (2.5 M in hexanes)] was added to this mixture. After stirring for 15 min at −78 °C, BOMCl (19.6 mmol, 2.7 mL) was added to the reaction flask and stirring was continued for further 2 h at −78 °C and 3 h at 23 °C. Reaction mixture was quenched with H_2O and THF was removed under reduced pressure. The resulting mixture was diluted with CH_2Cl_2 and H_2O. Organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (8–12% EtOAc/hexanes) to furnish butyl 1-(benzyloxymethyl)cyclopropanesulfonate in 80% yields (3.9 g). ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.25 (m, 5H), 4.55 (s, 2H), 4.23 (t, *J* = 6.6 Hz, 2H), 3.79 (s, 2H), 1.73-1.58 (m, 2H), 1.48 (ABq, *J* = 6.9, 5.0 Hz, 2H), 1.44-1.30 (m, 2H), 1.11 (ABq, *J* = 7.0, 5.1 Hz,

2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.41, 128.33, 127.78, 127.61, 73.09, 70.66, 69.84, 37.81, 31.04, 18.55, 13.41, 10.72.

To a mixture of butyl 1-(benzyloxymethyl)cyclopropanesulfonate (13 mmol, 3.88 g) in DME (40 mL) and H_2O (40 mL), KSCN (13.65 mmol, 1.33 g) was added at 23 °C and the resulting reaction mixture was refluxed for 15 h. Reaction mixture was cooled to 23 °C and diluted with H_2O and ethyl acetate. Organic layer was separated and the aqueous layer was concentrated under reduced pressure to provide the crude potassium 1-(benzyloxymethyl)cyclopropanesulfonate which was used directly in the next step without additional purification.

A mixture of potassium 1-(benzyloxymethyl)cyclopropanesulfonate in $SOCl_2$ (35 mL) and DMF (3.5 mL) was refluxed for 1.5 h and excess $SOCl_2$ was removed under reduced pressure. Water was added carefully to the resulting mixture and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5–10% EtOAc/hexanes) to obtain 1-(benzyloxymethyl)cyclopropanesulfonyl chloride **27** in 91% yields (3.1 g) (over 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.28 (m, 5H), 4.63 (s, 2H), 4.01 (s, 2H), 1.85-1.77 (m, 2H), 1.46-1.37 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.99, 128.41, 127.89, 127.61, 73.29, 68.38, 52.39, 14.03.

Synthesis of sulfonamide 29—To a mixture of amine **28** (3.66 mmol, 0.8 g), pyridine (11 mmol, 0.89 mL) and DMAP (0.73 mmol, 89.2 mg) in CH_2Cl_2 at 0°C, was added a solution of 1-(benzyloxymethyl)-cyclopropanesulfonyl chloride (**27**) (3.84 mmol, 1 g in 5 mL CH_2Cl_2) and the resulting mixture was stirred for 44 h at 23 °C. Reaction mixture was diluted with CH_2Cl_2, washed with aqueous 1N HCl, brine and dried over anhydrous Na_2SO_4. Dichloromethane solution was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20–30% EtOAc/hexanes) to afford the corresponding sulfonamide **29** in 74% yields (1.2 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.38 (s, 1H), 8.25 (s, 1H), 7.87 (s, 1H), 7.49 (d, *J* = 7.1 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.39-7.33 (m, 1H), 6.79 (s, 1H), 6.69 (s, 1H), 4.73 (s, 2H), 3.91 (s, 3H), 3.90 (s, 2H), 2.75 (q, *J* = 7.5 Hz, 2H), 1.30 (t, *J* = 7.5 Hz, 3H), 1.11-0.99 (m, 2H), 0.77-0.65 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.62, 136.35, 135.01, 128.91, 128.63, 128.44, 122.14, 121.20, 120.85, 120.76, 120.61, 120.36, 74.15, 73.10, 51.84, 38.91, 18.09, 14.25, 10.25.

Synthesis of 7,6,5-tricyclic indole derivative 30—To a solution of sulfonamide **29** (2.7 mmol, 1.19 g) in MeOH (75 mL) and AcOH (25 mL), 10% Pd/C (0.2 g) was added under argon. Argon ballon was now replaced with H_2 ballon and the resulting mixture was stirred for 16 h at 23 °C. Reaction mixture was filtered through celite and washed with MeOH. Solvent was removed under reduced pressure and the resulting residue was diluted with toluene and concentrated under reduced pressure to furnish the corresponding alcohol in 95% yield.

To a mixture of the above alcohol (0.6 mmol, 0.21 g) and Et_3N (0.9 mmol, 0.12 mL) in CH_2Cl_2 (25 mL) at 0°C, was added methanesulfonyl chloride (0.63 mmol, 0.049 mL) and the resulting mixture was stirred for 2 h at 23 °C. Reaction mixture was diluted with CH_2Cl_2, washed with aqueous 1N HCl, brine and dried over anhydrous Na_2SO_4. Dichloromethane solution was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5–15% diethyl ether/CH_2Cl_2) to afford the corresponding mesylate in 46% (70% BRSM) yields (0.12 g, 69 mg of alcohol was recovered). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.26 (s, 1H), 7.87 (d, *J* = 1.0 Hz,

1H), 7.41 (s, 1H), 7.07 (s, 1H), 4.58 (s, 2H), 3.93 (s, 3H), 3.16 (s, 3H), 2.79 (q, *J* = 7.5 Hz, 2H), 1.38-1.28 (m, 5H), 1.06-0.97 (m, 2H).

To a solution of the above mesylate (0.69 mmol, 0.297g) in DMF (30 mL), was added NaH (2.76 mmol, 0.11 g, 60% dispersion in mineral oil) at 23 °C. After stirring the mixture for 3 h at 23 °C, iodo methane (3.5 mmol, 0.22 mL) was added to the flask and stirring was continued for further 1 h at 23 °C. Reaction mixture was carefully quenched with MeOH, diluted with ethyl acetate and washed with aqueous 1N HCl. Ethyl acetate solution was filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (35–40% EtOAc/hexanes) to afford the corresponding 7, 6, 5-tricyclic indole derivative in 87% yields (0.21 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 1.1 Hz, 1H), 7.83 (d, *J* = 1.0 Hz, 1H), 6.78 (s, 1H), 4.32 (s, 2H), 3.93 (s, 3H), 3.48 (s, 3H), 2.77 (q, *J* = 7.5 Hz, 2H), 1.53 (t, *J* = 6.6 Hz, 2H), 1.31 (t, *J* = 7.5 Hz, 3H), 1.03-0.95 (m, 2H).

A mixture of the above 7, 6, 5-tricyclic indole derivative (0.2 mmol, 69.7 mg) and NaOH (20 mmol, 0.8 g in 10 mL H_2O) in EtOH (5 mL) and THF (10 mL) was stirred for 4 days at 23 °C. Solvent was removed under reduced pressure and the resulting mixture was diluted with H_2O and diethyl ether. Organic layer was separated, aqueous layer was acidified with aqueous 1N HCl and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to furnish the corresponding crude acid (**30**) in 75% yields (50 mg) which was used directly in the coupling reaction without any further purification. LRMS-ESI (*m/z*): $[M+Na]^+$ 357.26

Synthesis of (2*R*, 3*S*)-ethyl 3-hydroxy-2-{[(4-nitrophenyl)sulfonyl]oxy}hexanoate (32)—To a solution of *(E)*-ethyl hex-2-enoate (**31**) (12.6 mmol, 1.79 g) in ^{t}BuOH (30 mL) and H_2O (30 mL), were added AD-mixα (17.6 g) and $MeSO_2NH_2$ (15.1 mmol, 1.44 g) at −1 °C and the resulting reaction mixture was stirred for 7 days at −1 °C. Reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ solution and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20–35% EtOAc/hexanes) to furnish (2*R*, 3*S*)-ethyl 2,3-dihydroxyhexanoate in 60% yields (1.34 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.29 (q, *J* = 7.2 Hz, 2H), 4.10-4.04 (m, 1H), 3.96-3.84 (m, 1H), 3.07 (d, *J* = 5.2 Hz, 1H), 1.90 (d, *J* = 7.7 Hz, 1H), 1.68-1.38 (m, 4H), 1.32 (t, *J* = 7.1 Hz, 3H), 0.96 (t, *J* = 7.2 Hz, 3H).

To a solution of (2*R*, 3*S*)-ethyl 2,3-dihydroxyhexanoate (6.7 mmol, 1.18 g) in CH_2Cl_2 (30 mL), were added Et_3N (10 mmol, 1.39 mL) and 4-nitrobenzenesulfonyl chloride (6.7 mmol, 1.48 g) at 0 °C. The resulting reaction mixture was stirred for 24 h at 23 °C. Reaction mixture was diluted with H_2O and CH_2Cl_2. Organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10–25% EtOAc/hexanes) to obtain (2*R*, 3*S*)-ethyl 3-hydroxy-2-{[(4-nitrophenyl)sulfonyl]oxy}hexanoate (**32**) in 54 % yields (1.3 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 8.8 Hz, 2H), 8.16 (d, *J* = 8.9 Hz, 2H), 4.96 (d, *J* = 2.9 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.12-4.04 (m, 1H), 2.07 (brs, 1H), 1.62 – 1.44 (m, 3H), 1.43 – 1.30 (m, 1H), 1.21 (t, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.74, 150.75, 141.85, 129.45, 124.18, 80.97, 71.23, 62.30, 34.98, 18.47, 13.89, 13.63.

Synthesis of (2*S*, 3*S*)-ethyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxyhexanoate (33)—To a solution of (2*R*, 3*S*)-ethyl 3-hydroxy-2-{[(4-nitrophenyl)sulfonyl]oxy}hexanoate (**32**) (3.6 mmol, 1.3 g) in DMF (10 mL), NaN_3 (5.76 mmol, 0.374 g) was added at 23 °C and the resulting mixture was heated at 55 °C for 15 h.

Reaction mixture was cooled to 23 °C, diluted with ethyl acetate and the resulting solution was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (15% EtOAc/hexanes) to obtain (2*S*, 3*S*)-ethyl 2-azido-3-hydroxyhexanoate in 83% yields (0.6 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (qd, *J* = 7.2, 1.3 Hz, 2H), 3.94 (s, 2H), 2.32 (brs, 1H), 1.59-1.45 (m, 3H), 1.44-1.37 (m, 1H), 1.33 (t, *J* = 7.2 Hz, 3H), 0.94 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.92, 71.56, 66.15, 61.99, 35.03, 18.52, 14.06, 13.74.

To a mixture of (2*S*, 3*S*)-ethyl 2-azido-3-hydroxyhexanoate (2.98 mmol, 0.6 g), and $(Boc)_2O$ (4.47 mmol, 0.975 g) in EtOH (15 mL) was added 10% Pd-C (0.15 g) at 23 °C under argon. Argon balloon was replaced with H_2 balloon and the reaction mixture was stirred for 15 h under H_2 atmosphere. Reaction mixture was filtered through celite, washed with ethyl acetate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10–25% EtOAc/hexanes) to obtain (2*S*, 3*S*)-ethyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxyhexanoate (**33**) in 96% yields (0.79 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.52 (d, *J* = 7.2 Hz, 1H), 4.42 – 4.28 (m, 1H), 4.27 - 4.13 (m, 2H), 3.94 – 3.80 (m, 1H), 3.03 (brs, 1H), 1.59 – 1.30 (m, 13H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.68, 155.98, 80.20, 72.67, 61.48, 58.34, 35.29, 28.18, 18.86, 14.06, 13.82. LRMS-ESI (m/z): $[M+Na]^+$ 298.26

Synthesis of *tert*-butyl [(2*S*, 3*S*)-3-hydroxy-1-(*iso*butylamino)-1-oxohexan-2-yl]carbamate (34a)—To a solution of (2*S*, 3*S*)-ethyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxyhexanoate (**33**) (0.67 mmol, 0.18 g) in THF (6 mL) and H_2O (3 mL), was added $LiOH.H_2O$ (3.45 mmol, 0.145 g). The resulting reaction mixture was stirred for 12 h at 23 °C. Reaction mixture was diluted with H_2O and ethyl acetate. Organic layer was separated and the aqueous layer was acidified with 1N HCl and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used in the coupling reaction with-out any further purification.

Synthesis of *tert*-butyl [(2*S*, 3*S*)-3-hydroxy-1-(*iso*butylamino)-1-oxohexan-2-yl]carbamate (**34a**) has been carried out by coupling of (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxyhexanoic acid with isobutyl amine using EDC, HOBt and ^{i}Pr$_2$NEt following the similar procedure described for the synthesis of (*S*)-*tert*-butyl [1-(*iso*butylamino)-1-oxobutan-2-yl]carbamate (yield 60%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (brs, 1H), 5.62 (d, *J* = 7.2 Hz, 1H), 3.98-3.82 (m, 2H), 3.69 (brs, 1H), 3.21-3.08 (m, 1H), 3.06-2.92 (m, 1H), 1.77 (hept, *J* = 6.7 Hz, 1H), 1.63-1.46 (m, 3H), 1.44 (s, 9H), 1.41 – 1.32 (m, 1H), 0.97-0.83 (m, 9H).

Synthesis of compound 35a—Compound **35a** was synthesized from *tert*-butyl [(2*S*, 3*S*)-3-hydroxy-1-(*iso*butylamino)-1-oxohexan-2-yl]carbamate (**34a**) by Boc removal using trifluoroacetic acid followed by reductive amination of the resulting amine with aldehyde **11** following the procedure described for the synthesis of compound **12a** (yield 49%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.2 Hz, 2H), 7.22 (d, *J* = 7.0 Hz, 1H), 7.19-7.13 (m, 2H), 4.63 (d, *J* = 7.4 Hz, 1H), 3.92 (brs, 1H), 3.84 – 3.74 (m, 1H), 3.12-2.94 (m, 3H), 2.86-2.71 (m, 2H), 2.64 (dd, *J* = 12.1, 4.6 Hz, 1H), 2.55 (dd, *J* = 12.0, 7.4 Hz, 1H), 1.71 (hept, *J* = 6.7 Hz, 1H), 1.58-1.21 (m, 13H), 0.90 (t, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 6H).

Synthesis of inhibitor 19—Inhibitor **19** has been synthesized from compound **35a** by Boc removal using trifluoroacetic acid followed by coupling of the resulting amine with known acid **23** using EDC, HOBt, and ^{i}Pr$_2$NEt following the similar procedure described for

the synthesis of inhibitor **8** (yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 1.0 Hz, 1H), 7.46 (s, 1H), 7.35 – 7.28 (m, 2H), 7.26-7.19 (m, 3H), 6.84 (s, 1H), 6.41 (d, *J* = 8.2 Hz, 1H), 4.54 – 4.44 (m, 3H), 3.89 – 3.76 (m, 3H), 3.47 (s, 3H), 3.13 (d, *J* = 4.9 Hz, 1H), 3.11 – 2.99 (m, 2H), 2.97 (d, *J* = 6.8 Hz, 2H), 2.83 (dd, *J* = 12.3, 4.8 Hz, 1H), 2.79-2.68 (m, 3H), 1.71 (hept, *J* = 6.7 Hz, 1H), 1.54 – 1.33 (m, 4H), 1.30 (t, *J* = 7.5 Hz, 3H), 0.91 – 0.80 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.89, 167.85, 137.51, 133.83, 130.72, 129.30, 128.67, 127.72, 127.26, 126.78, 125.83, 120.25, 117.93, 117.56, 72.00, 66.43, 56.81, 51.98, 51.20, 46.44, 43.62, 39.61, 38.94, 35.26, 28.42, 20.10, 18.98, 17.94, 14.26, 13.94. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{33}H_{48}N_5O_5S$ 626.3376, found 626.3369.

Synthesis of inhibitor 20—Inhibitor **20** has been synthesized from compound **35a** by Boc removal using trifluoroacetic acid followed by coupling of the resulting amine with acid **25** using EDC, HOBt, and ^{i}Pr$_2$NEt following the similar procedure described for the synthesis of inhibitor **8** (yield 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 0.8 Hz, 1H), 7.40 (s, 1H), 7.34 – 7.28 (m, 2H), 7.26-7.18 (m, 3H), 6.80 (s, 1H), 6.39 (d, *J* = 8.2 Hz, 1H), 4.54-4.40 (m, 1H), 4.11 (s, 2H), 3.80 (p, *J* = 4.2 Hz, 1H), 3.50 (s, 3H), 3.13 (d, *J* = 4.8 Hz, 1H), 3.11 – 2.99 (m, 2H), 2.97 (d, *J* = 6.8 Hz, 2H), 2.82 (dd, *J* = 12.3, 5.0 Hz, 1H), 2.79 – 2.64 (m, 3H), 1.70 (hept, *J* = 6.7 Hz, 1H), 1.56 (s, 6H), 1.53 – 1.33 (m, 4H), 1.30 (t, *J* = 7.5 Hz, 3H), 0.93 – 0.79 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.59, 167.99, 137.38, 134.28, 130.53, 129.22, 128.81, 128.59, 127.37, 126.83, 126.71, 120.29, 116.36, 114.86, 71.83, 66.29, 65.68, 56.50, 51.89, 51.03, 46.38, 39.40, 38.81, 35.15, 28.33, 22.49, 22.46, 20.03, 19.71, 18.90, 17.88, 14.02, 13.87. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{52}N_5O_5S$ 654.3689, found 654.3696.

Synthesis of Inhibitor 21—Inhibitor **21** was synthesized (yield 65%, over 2 steps) from **12b** by Boc deprotection followed by coupling with 7,6,5 tricyclic indole derivative **30** as described for the inhibitor **4**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.47 (s, 1H), 7.36 (t, *J* = 6.0 Hz, 1H), 7.33-7.27 (m, 2H), 7.26-7.17 (m, 3H), 6.77 (s, 1H), 6.55 (d, *J* = 8.2 Hz, 1H), 4.56-4.43 (m, 1H), 4.27 (s, 2H), 4.03 (p, *J* = 6.2 Hz, 1H), 3.41 (s, 3H), 3.12 (d, *J* = 5.1 Hz, 1H), 3.08-2.99 (m, 2H), 2.95 (dd, *J* = 6.5, 4.4 Hz, 2H), 2.82 (dd, *J* = 12.2, 4.7 Hz, 1H), 2.78-2.65 (m, 3H), 1.69 (hept, *J* = 6.6 Hz, 1H), 1.52 (t, *J* = 6.3 Hz, 2H), 1.28 (t, *J* = 7.5 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.00 (t, *J* = 6.4 Hz, 2H), 0.83 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.75, 167.89, 137.56, 134.44, 130.80, 129.26, 128.62, 128.14, 127.09, 126.71, 126.13, 120.33, 118.15, 117.90, 68.26, 67.53, 52.30, 52.23, 51.39, 46.39, 43.05, 39.77, 39.02, 28.40, 20.09, 18.86, 17.94, 14.19, 12.67. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{33}H_{46}N_5O_5S$ 624.3220, found 624.3223.

Synthesis of compound 35b—(2*S*, 3*S*)-2-Amino-3-hydroxy-*N*-*iso*propylhexanamide was synthesized from (2*S*, 3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxyhexanoic acid by coupling with isopropyl amine using EDC, HOBt, and ^{i}Pr$_2$NEt followed by boc removal using trifluoroacetic acid following the procedure described for the synthesis of (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-*iso*butyl-*N*-methylbutanamide (yield 79% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (br, 1H), 4.14-3.92 (m, 1H), 3.81 – 3.67 (m, 1H), 3.22 (d, *J* = 6.2 Hz, 1H), 2.40 (brs, 2H), 1.62 – 1.24 (m, 4H), 1.18 – 1.08 (m, 6H), 0.91 (t, *J* = 6.9 Hz, 3H).

Compound **35b** was prepared from (2*S*, 3*S*)-2-amino-3-hydroxy-*N*-isopropylhexanamide by reductive amination with aldehyde **11** following the procedure described for the synthesis of compound **12a** (yield 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.4 Hz, 2H), 7.22 (d, *J* = 7.3 Hz, 1H), 7.20-7.13 (m, 2H), 7.00 (br, 1H), 4.62 (d, *J* = 8.1 Hz, 1H), 4.08 – 3.85 (m, 2H), 3.83 – 3.72 (m, 1H), 2.98 (d, *J* = 5.3 Hz, 1H), 2.82 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.75 (dd, *J* = 13.3, 7.4 Hz, 1H), 2.60 (dd, *J* = 12.2, 4.7 Hz, 1H), 2.54 (dd, *J* = 12.1, 7.3 Hz, 1H), 1.59 – 1.20 (m, 13H), 1.11 (d, *J* = 6.5 Hz, 3H), 1.06 (d, *J* = 6.6 Hz, 3H), 0.90 (t, *J* = 7.0 Hz, 3H).

Synthesis of inhibitor 22—Inhibitor **22** was synthesized by Boc removal of **35b** using trifluoroacetic acid followed by coupling of the resulting amine with the acid **30** using EDC, HOBt, and $^{i}Pr_2NEt$ following the similar procedure described for the synthesis of inhibitor **4** (yield 65% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 1.1 Hz, 1H), 7.46 (d, *J* = 1.0 Hz, 1H), 7.36 – 7.28 (m, 2H), 7.28 – 7.20 (m, 3H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.78 (s, 1H), 6.38 (d, *J* = 8.2 Hz, 1H), 4.54 – 4.42 (m, 1H), 4.28 (ABq, *J* = 20.6, 14.9 Hz, 2H), 4.08 – 3.95 (m, 1H), 3.84-3.75 (m, 1H), 3.44 (s, 3H), 3.09 (d, *J* = 4.9 Hz, 1H), 2.98 (d, *J* = 6.8 Hz, 2H), 2.81 (dd, *J* = 12.3, 4.9 Hz, 1H), 2.77 – 2.69 (m, 3H), 1.56-1.33 (m, 5H), 1.33-1.24 (m, 4H), 1.07 (t, *J* = 6.9 Hz, 6H), 1.01 – 0.96 (m, 2H), 0.87 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.67, 167.77, 137.47, 134.36, 130.68, 129.20, 128.58, 128.06, 126.97, 126.69, 126.05, 120.23, 118.05, 117.83, 71.81, 66.05, 52.15, 51.63, 50.99, 43.01, 40.92, 39.60, 38.81, 35.05, 22.46, 18.90, 17.89, 14.15, 13.86, 12.64. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{48}N_5O_5S$ 638.3376, found 638.3371.

Determination of X-ray Structure of β-secretase-Inhibitor 5 Complex—Expression and purification of recombinant human beta-secretase, crystal growing, inhibitor soaking of the crystal and diffraction data collection were as previously described.[8] The structure was determined by molecular replacement implemented with the program AMoRe using the the C molecule of previously determined memapsin 2 structure (PDB ID: 1FKN) as a search model[8] with removed inhibitor and water molecules. Rotation and translation functions followed by the rigid-body refinement with data from 15 Å to 3.5 Å resolution in space group P21 gave unambiguous solutions for the four memapsin 2 molecules in the asymmetric unit. A random selection of 7% of reflections (9028 reflections) was set aside as the test set for cross-validation during the refinement. The refined model has well-defined electron density for the inhibitor and its corresponding structure was built into the active site. The four molecules in the crystallographic asymmetric unit have essentially identical structures. The crystal form was determined to be monoclinic with a resolution of 2.0 Å. The unit cell parameters are a = 86.4Å, b = 130.3 Å, c = 88.4 Å, β = 97.5. The coordinates and structure factors of the β-secretase and **5** complex have been deposited in Protein Data Bank[28] with pdb id: 4GID.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AD	Alzheimer's Disease
BACE 1	Beta-site APP Cleaving Enzyme 1
BACE 2	Beta-site APP Cleaving Enzyme 2
APP	β-Amyloid Precursor Protein
CD	Cathepsin D

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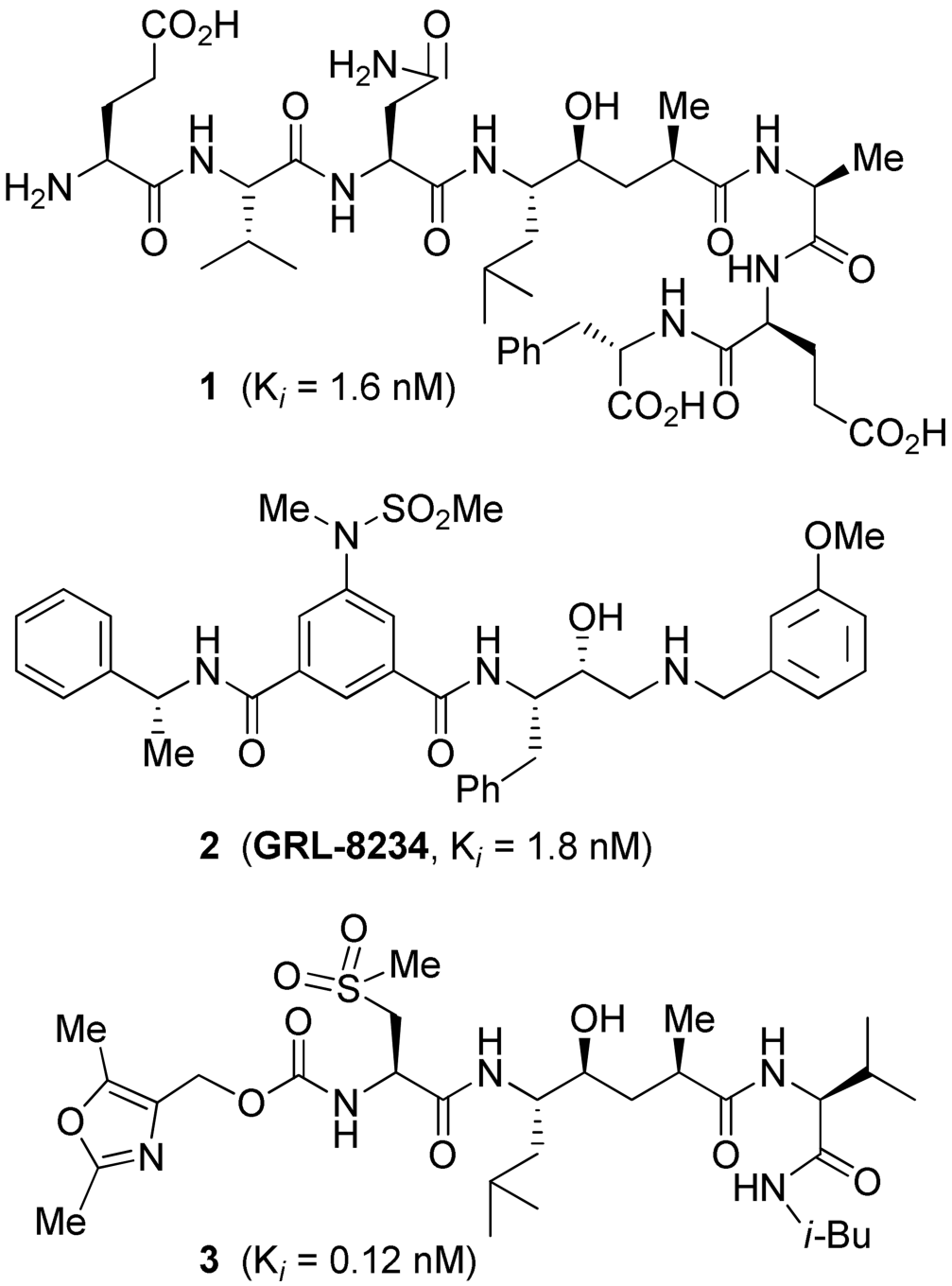
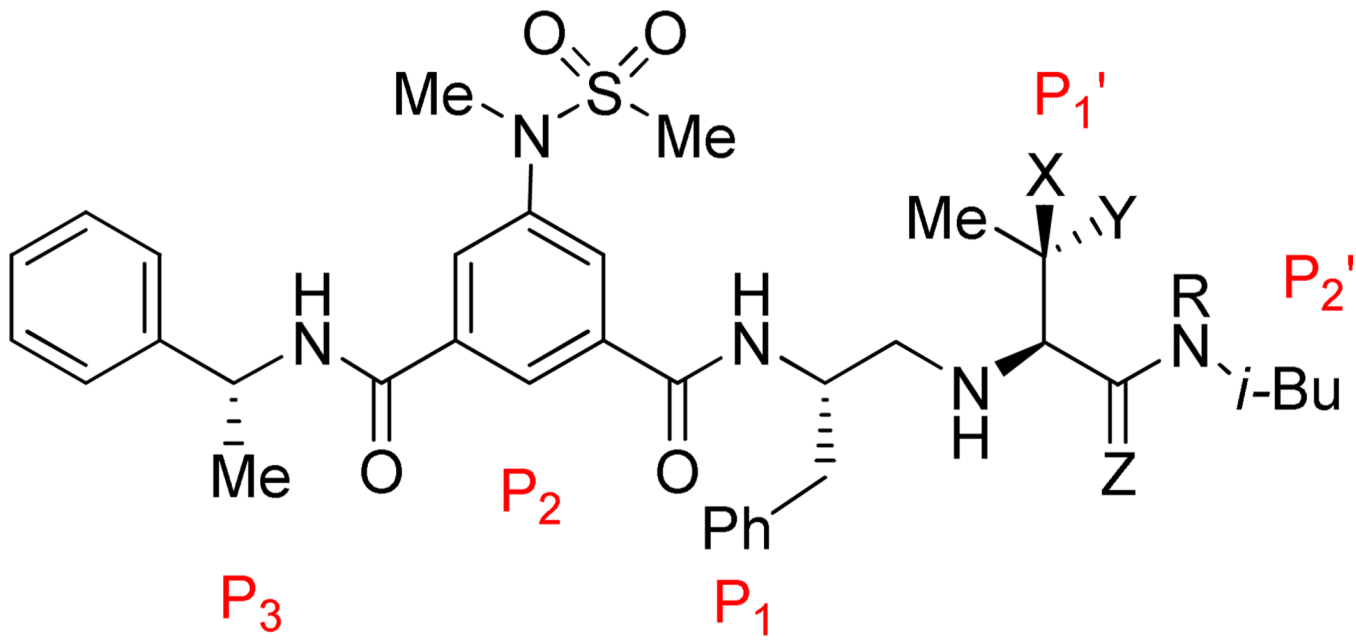


Figure 1.
Structures of β-secretase inhibitors **1-3**.



4 (X = Y = R = H, Z = O)

5 (X = R = H, Y = OH, Z = O)

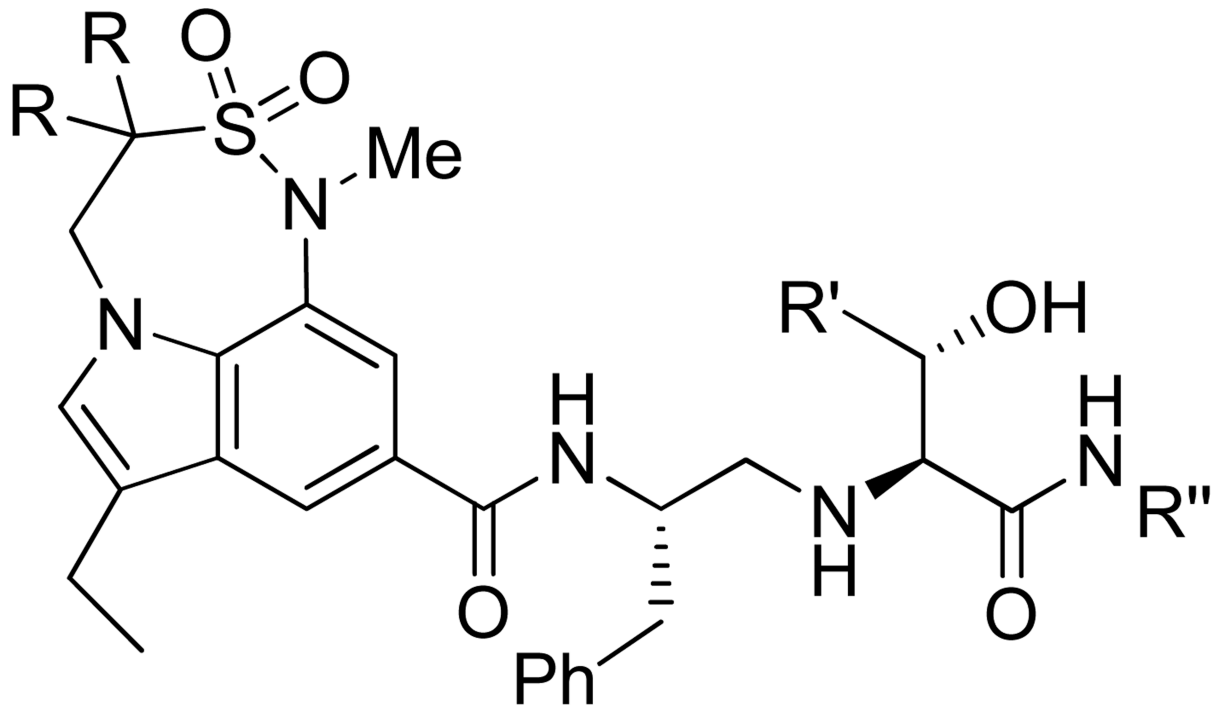
6 (X = OH, Y = R = H, Z = O)

7 (X = H, Y = OH, Z = O, R = Me)

8 (X = R = H, Y = OMe, Z = O)

9 (X = R = H, Y = OH, Z = H, H)

Figure 2.
Structure of isophthalamide-derived β-secretase inhibitors **4–9**



18 (R = H, R' = Me, R" = ^{i}Bu)

19 (R = H, R' = Pr, R" = ^{i}Bu)

20 (R = Me, R' = Pr, R" = ^{i}Bu)

21 (R-R = -$(CH_2)_2$-, R' = Me, R" = ^{i}Bu)

22 (R-R = -$(CH_2)_2$-, R' = Pr, R" = ^{i}Pr)

Figure 3.
Structure of indole-derived β-secretase inhibitors **18–22**

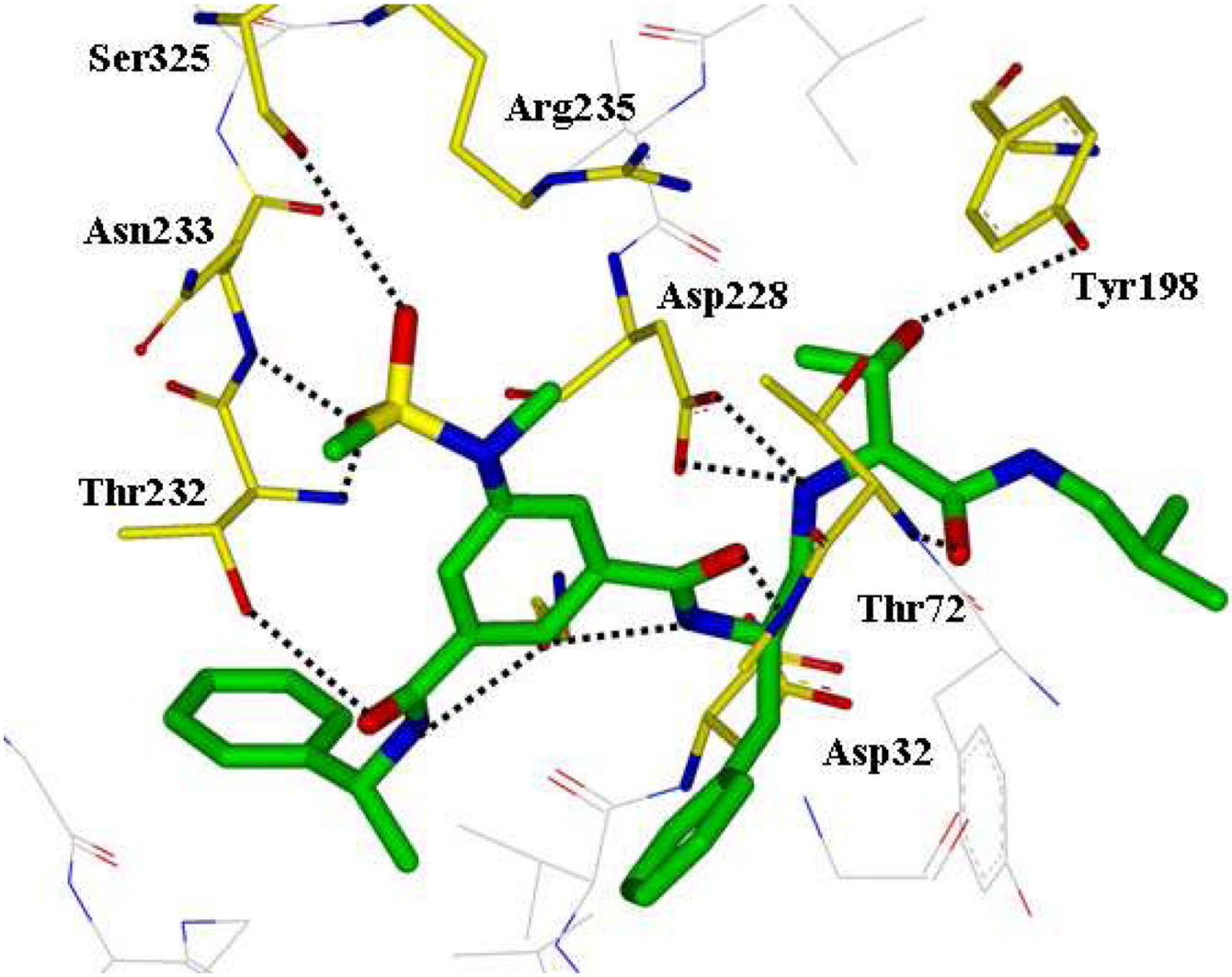
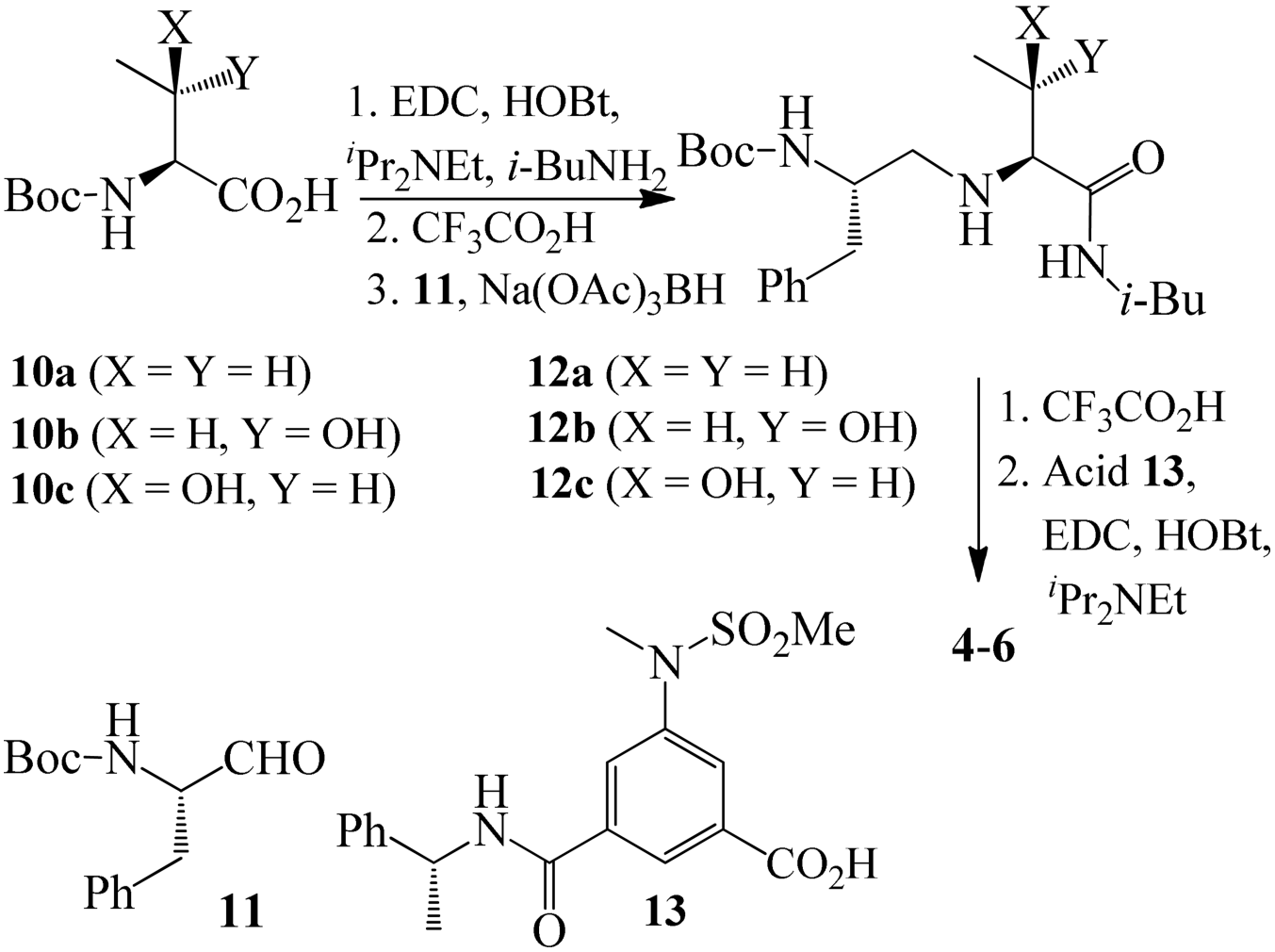
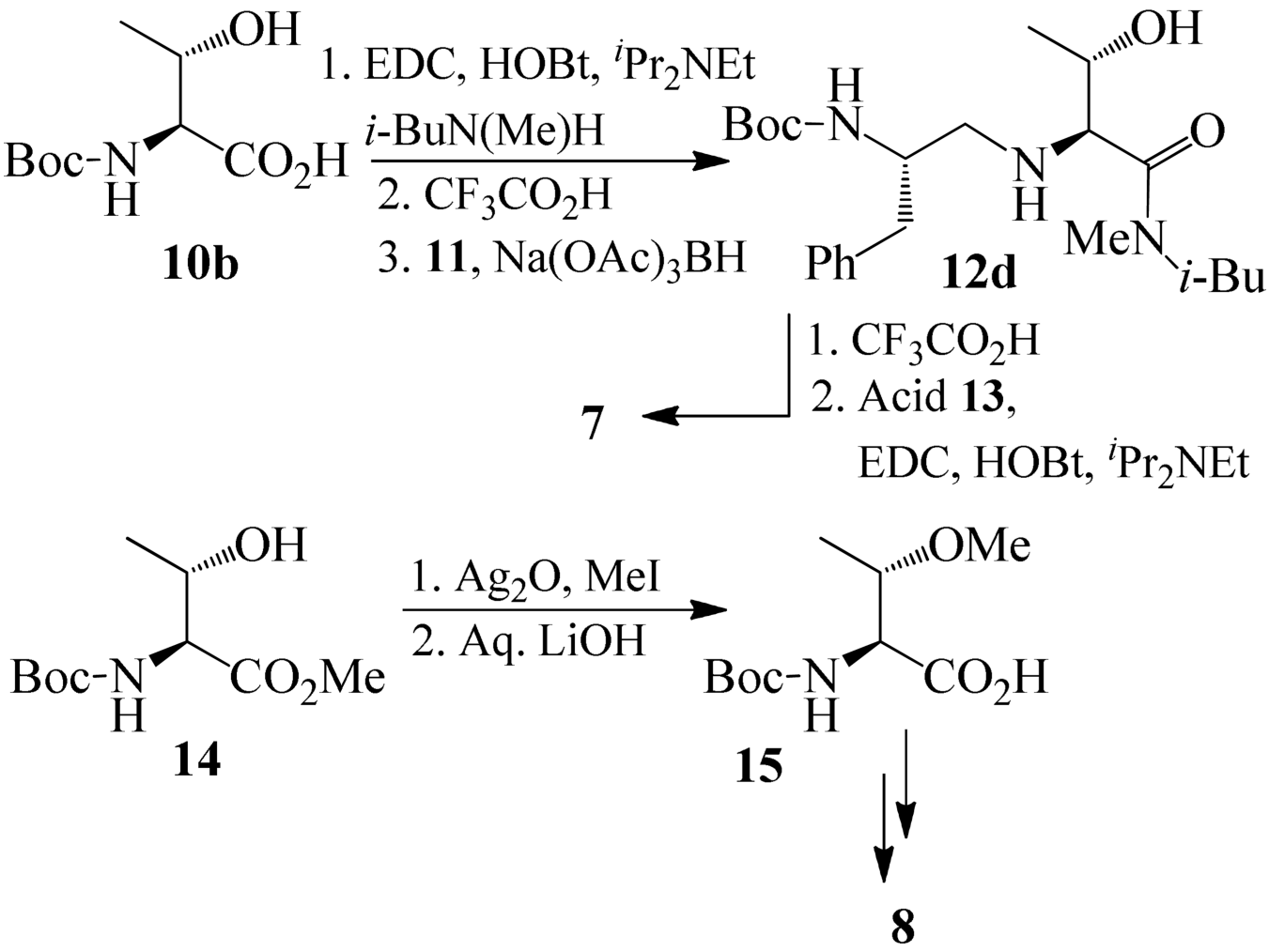


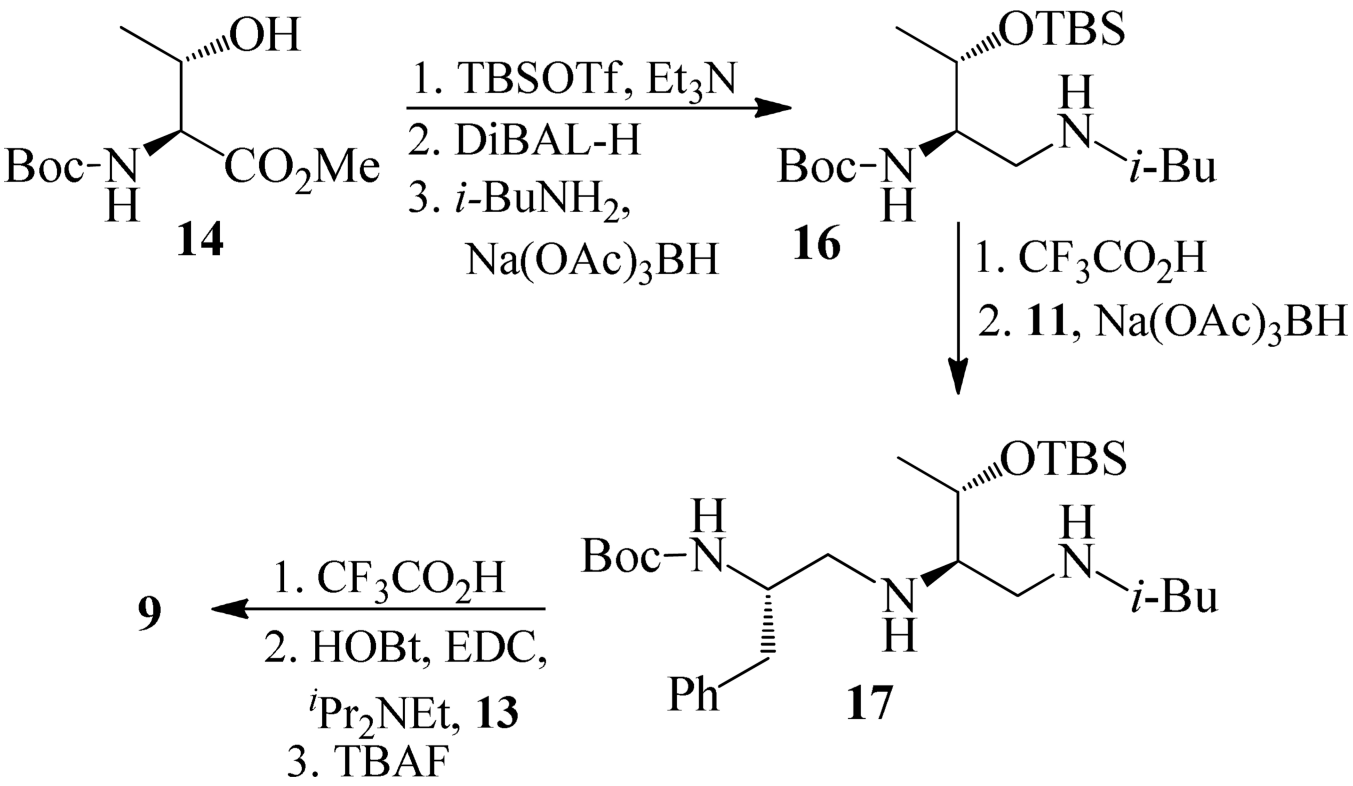
Figure 4.
The X-ray structure of **5** (green)-bound-β-secretase complex. Hydrogen bonds are shown in dotted line (pdb id: 4GID).



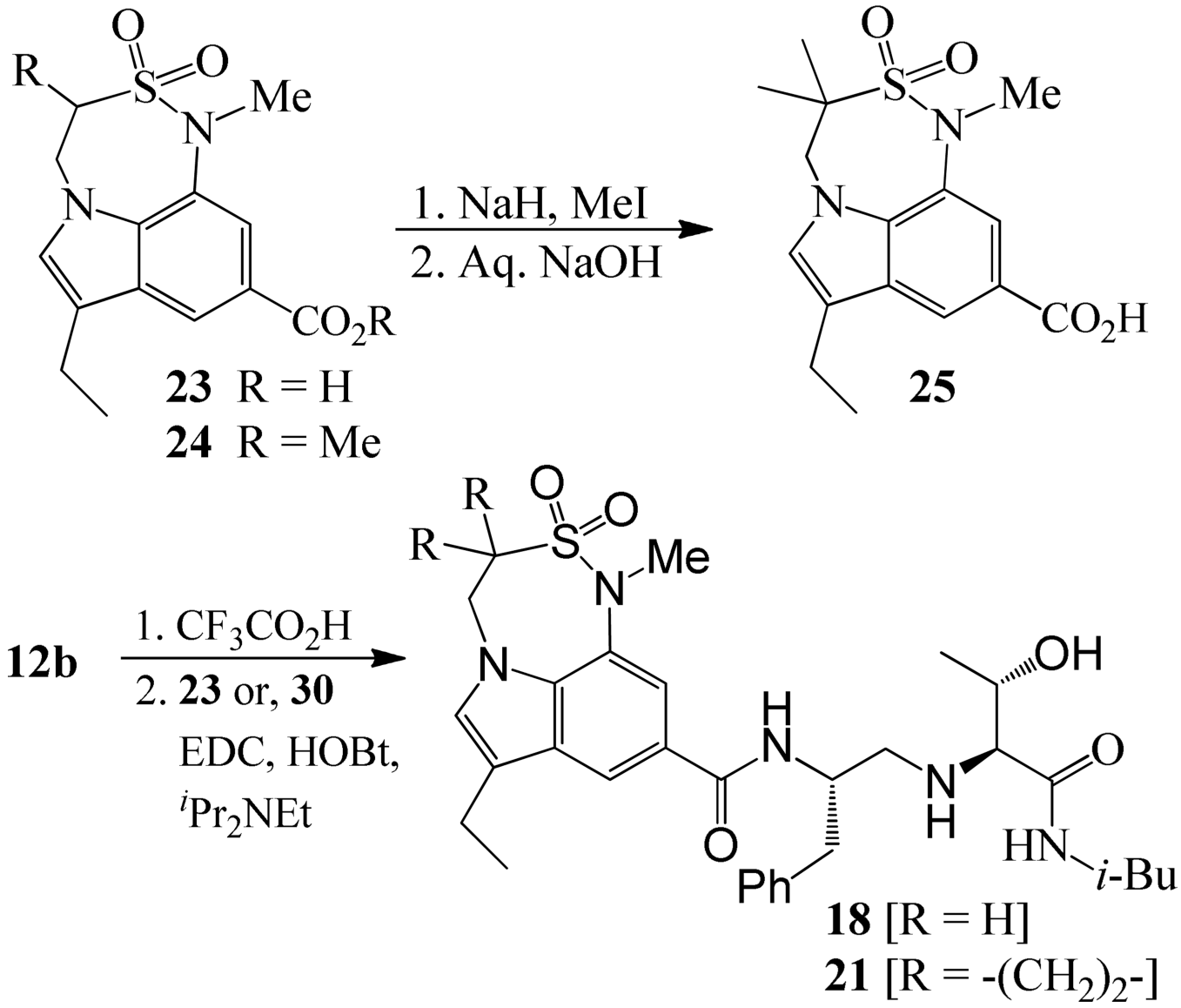
Scheme 1.
Synthesis of inhibitors **4–6**.



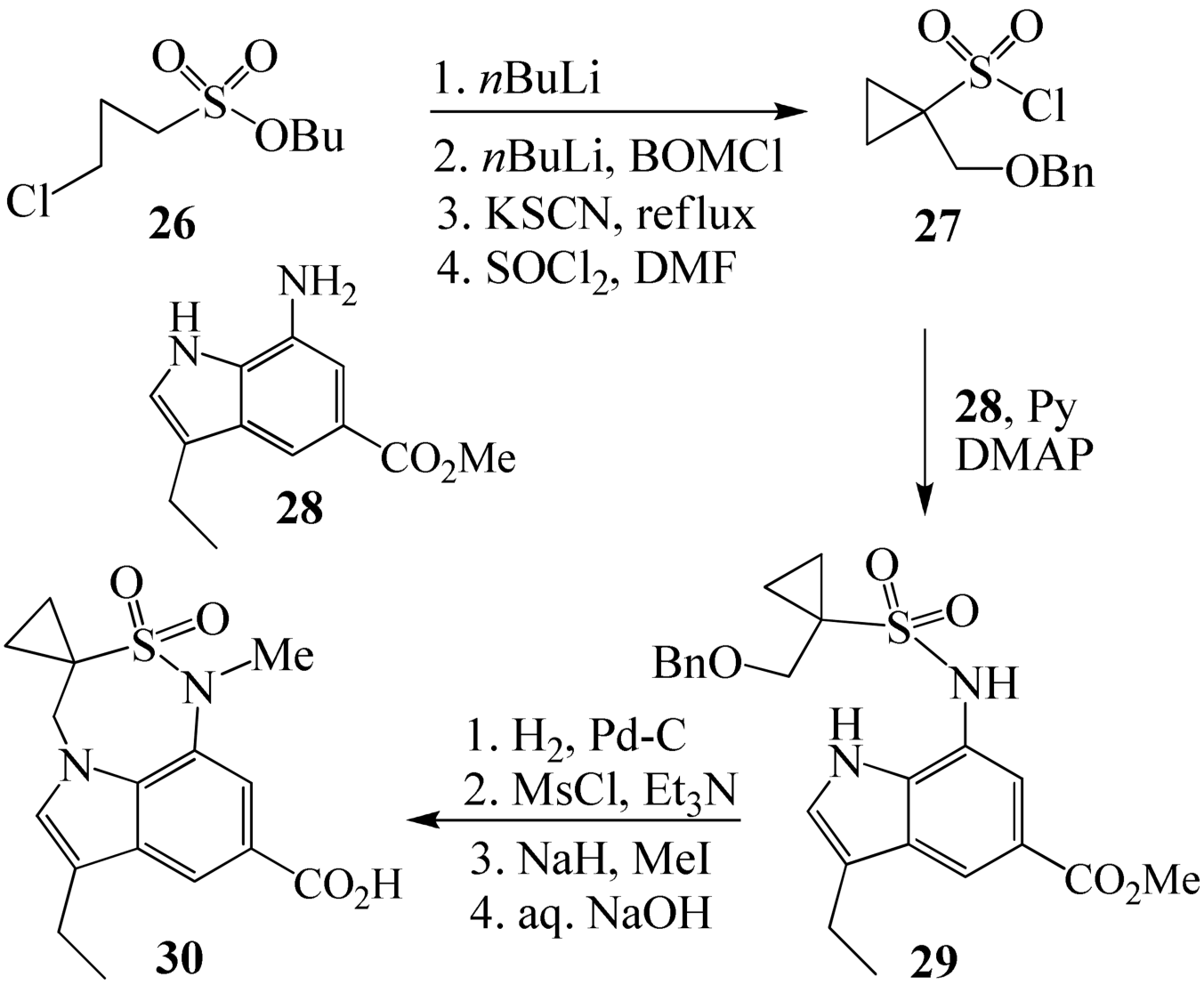
Scheme 2.
Synthesis of inhibitors **7** and **8**



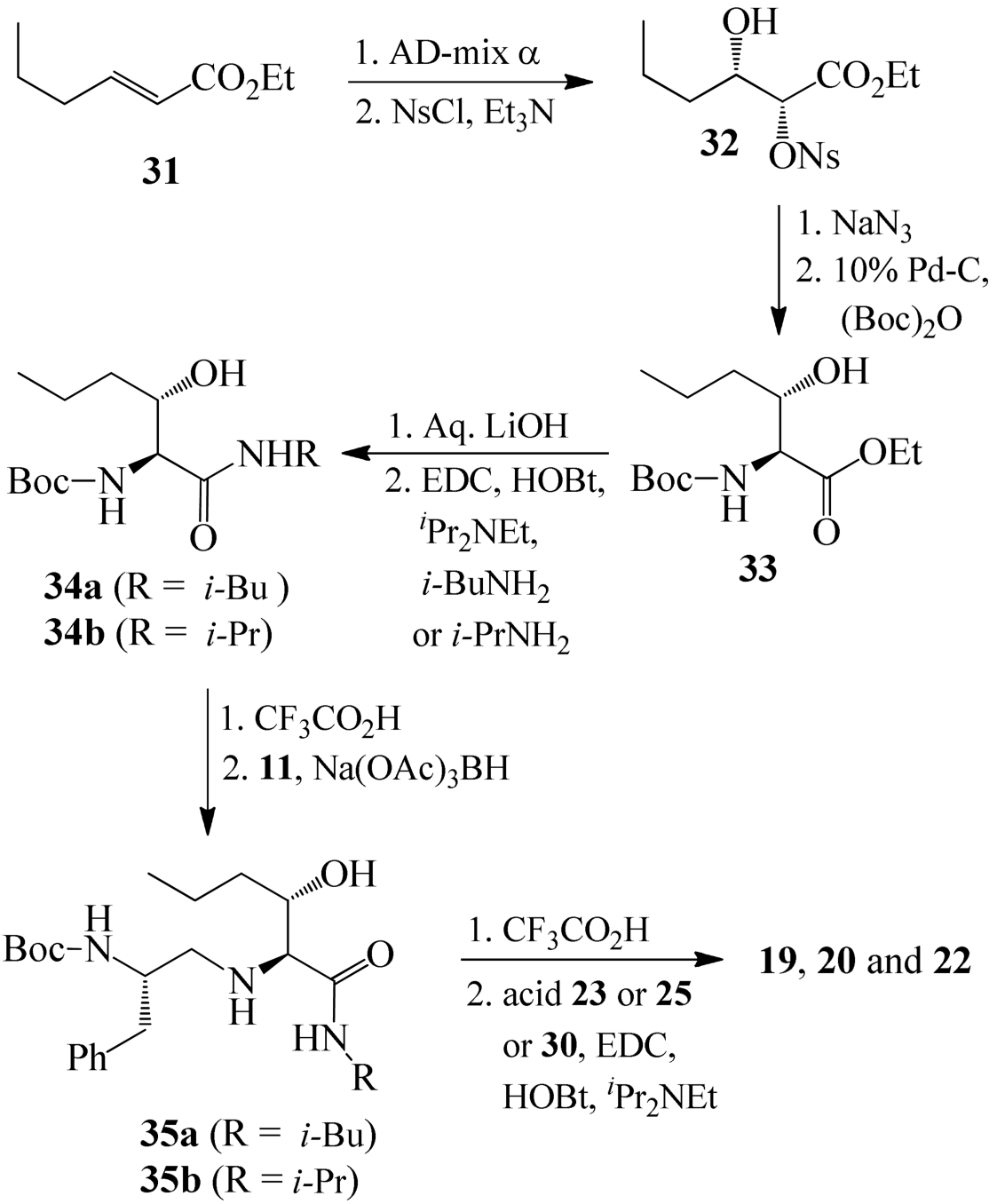
Scheme 3.
Synthesis of inhibitor **9**



Scheme 4.
Synthesis of carboxylic acid **25** and inhibitors **18** and **21**.



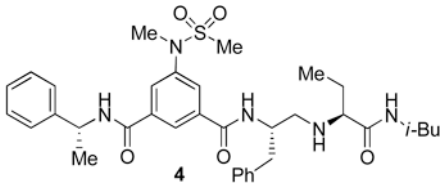
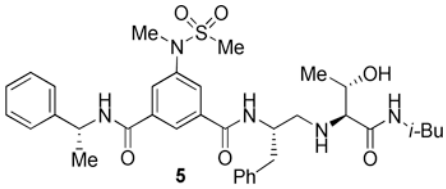
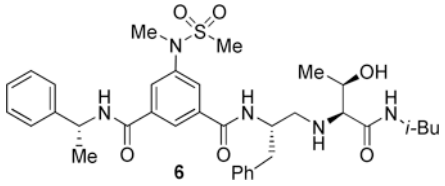
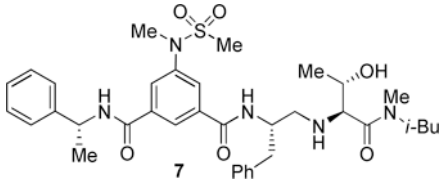
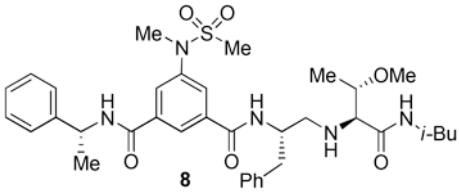
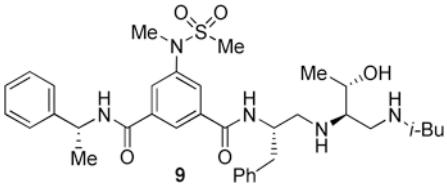
Scheme 5.
Synthesis of carboxylic acid **30**.



Scheme 6.
Synthesis of inhibitors **19, 20** and **22**

Table 1

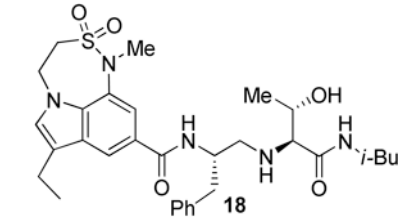
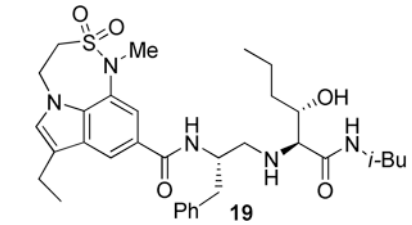
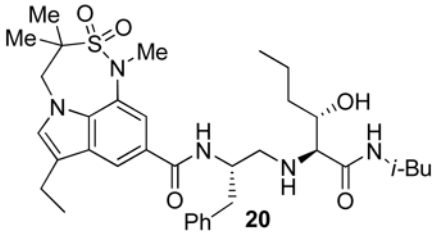
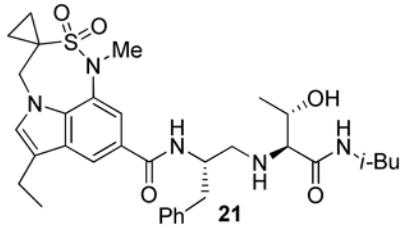
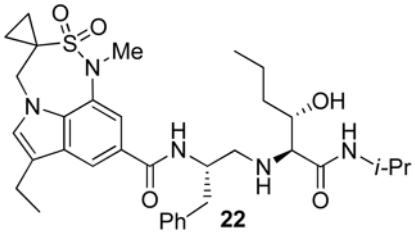
BACE 1 inhibitory and cellular activity of inhibitors **4–9**.

Entry	Inhibitor	K_i (nM)	IC_{50} (nM)[a]
1	 **4**	27.12	9.5
2	 **5**	0.017	1
3.	 **6**	98.8	>1000
4.	 **7**	>1000	---
5.	 **8**	25	---
6.	 **9**	>1000	---

[a] IC_{50} was determined in neuroblastoma cells. **GRL-8234** exhibited K_i = 1.8 nM, IC_{50} = 2.5 nM in this assay.

Table 2

BACE 1 inhibitory and cellular activity of inhibitors **18–22**.

Entry	Inhibitor	K_i (nM)	IC_{50} (nM)[a]
1	 **18**	7.3	22
2	 **19**	0.4	---
3	 **20**	0.036	15
4	 **21**	7.2	---
5	 **22**	0.47	14

[a] IC_{50} was determined in neuroblastoma cells. **GRL-8234** exhibited K_i = 1.8 nM, IC_{50} = 2.5 nM in this assay.

Table 3

Selectivity studies of BACE 1 inhibitors against BACE 2 & cathepsin D

Entry	Inhibitor	BACE 1 K_i(nM)	BACE 2 K_i(nM	CD K_i(nM)	BACE 2/BACE 1 (Selectivity)	CD/BACE1 (Selectivity)
1	**4**	27.12	1450	8264	>55	>300
2	**5**	0.017	120	4300	>7000	>250,000
3	**18**	7.3	---	7100	---	>970
4	**19**	0.4	---	690	---	>1700
5	**20**	0.036	11	530	>300	>14,700
6	**22**	0.47	---	2000	---	>4,200