Design, Synthesis, and X-ray Structure of Potent Memapsin 2 (β-Secretase) Inhibitors with Isophthalamide Derivatives as the P_2–P_3-Ligands

Arun K. Ghosh,*[,†] Nagaswamy Kumaragurubaran,[†] Lin Hong,[‡,§] Sarang S. Kulkarni,[†] Xiaoming Xu,[†] Wanpin Chang,[§] Vajira Weerasena,[‡] Robert Turner,[‡] Gerald Koelsch,[‡,§] Geoffrey Bilcer,[‡] and Jordan Tang[§,⊥]

Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907, Athenagen Inc, Oklahoma City, Oklahoma 73104, Protein Studies Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104, and Department of Biochemistry and Molecular Biology, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma 73104

Received November 17, 2006

Structure-based design and synthesis of a number of potent and selective memapsin 2 inhibitors are described. These inhibitors were designed based upon the X-ray structure of memapsin 2-bound inhibitor **3** that incorporates methylsulfonyl alanine as the P_2-ligand and a substituted pyrazole as the P_3-ligand. Of particular importance, we examined the ability of the substituted isophthalic acid amide derivative to mimic the key interactions in the S_2-S_3 regions of the enzyme active sites of **3**-bound memapsin 2. We investigated various substituted phenylethyl, α-methylbenzyl, and oxazolylmethyl groups as the P_3-ligands. A number of inhibitors exhibited very potent inhibitory activity against mempasin 2 and good selectivity against memapsin 1. Inhibitor **5d** has shown low nanomolar enzyme inhibitory potency (K_i = 1.1 nM) and very good cellular inhibitory activity (IC_{50} = 39 nM). Furthermore, in a preliminary study, inhibitor **5d** has shown 30% reduction of $A\beta_{40}$ production in transgenic mice after a single intraperitoneal administration (8 mg/kg). A protein−ligand X-ray crystal structure of **5d**-bound memapsin 2 provided vital molecular insight that can serve as an important guide to further design of novel inhibitors.

Introduction

The deposition of amyloid β-peptide ($A\beta$) in the brain is one of the major events in the pathogenesis of Alzheimer's disease (AD).[1,2] $A\beta$ is formed by an initial cleavage of the membrane-anchored β-amyloid precursor protein (APP) by two proteases known as β- and γ-secretases. β-Secretase (BACE-1, memapsin 2) cleaves APP first in its luminal domain to form a membrane-bound C-terminal fragment of APP. This cleavage followed by hydrolysis by γ-secretase thus generates the 40−42 residue $A\beta$ peptides.[3] Since β-secretase catalyzes the first step in $A\beta$ production, it has become an attractive therapeutic target for Alzheimer's disease.[4]

On the basis of preliminary substrate specificity and kinetic information, we designed a number of potent peptidomemetic inhibitors incorporating a nonhydrolyzable Leu-Ala-based hydroxyethylene dipeptide isostere.[5,6] One of these inhibitors, OM99-2 (**1**), exhibited a K_i value of 1.6 nM against memapsin 2.[7] The X-ray crystal structure of **1**-bound memapsin 2 provided important molecular insight into the ligand binding interactions in the active site.[8] Our structure-based modifications led to the discovery of potent memapsin 2 inhibitors with reduced molecular weight.[9] However, these inhibitors lacked important selectivity against other aspartyl proteases. For example, inhibitor **2** showed K_i values of 1.6 nM and 1.2 nM against human memapsin 2 and memapsin 1, respectively.[10] The selectivity of memapsin 2 inhibitors over other human aspartic proteases is expected to be important, particularly against memapsin 1 and cathepsin D. Memapsin 1 has specificity similarity to memapsin 2,[11] and it is presumed to have important physiological functions. Cathepsin D is abundant in cells and plays an important role in cellular protein catabolism.[12] Therefore, development of selectivity against these other aspartyl proteases may be important due to the potential toxicity. We have recently designed and developed very potent and highly selective memapsin 2 inhibitors that incorporate methylsulfonyl alanine as the P_2-ligand along with pyrazole- and oxazole-derived heterocycles as the P_3-ligands. These inhibitors (**3** and **4)** exhibited enhanced potency against memapsin 2 and excellent selectivity over memapsin 1 and cathepsin D.[10] The protein−ligand X-ray structure of the pyrazole-bearing inhibitor **3** provided important molecular insight into the specific cooperative ligand-binding site interactions for selectivity design.[10] The inhibitor **3**-bound memapsin 2 X-ray structure indicated a number of important interactions in the S2 and S3 sites. The P_2-sulfone oxygens appear to interact with a tightly bound water molecule as well as involve in hydrogen bond with Arg-235 in the S_2 site. Also, one of the P_3-pyrazole nitrogens is within hydrogen-bonding distance to Thr-232, and the dimethyl groups on the pyrazole ring appear to effectively fill the S_3 pocket. In our continuing efforts toward development of novel memapsin 2 inhibitors, we now have investigated the suitability of substituted isophthalamide-based ligands to interact with various residues at the S2 and S3 regions of the enzyme active site. A number of reports incorporating isopthalamide-based P_2-ligand in memapsin 2 inhibitor design have appeared in literature.[13] Recently, Coburn and co-workers incorporated an *N*-sulfonyl derivative at the 3-position of the isopthalamide ring to interact with residues in the S_2 pocket.[14a] Herein, we report the results of our preliminary studies regarding the design and synthesis of a series of potent memapsin 2 inhibitors that incorporate substituted isopthalamides as P_2-P_3 ligands in combination with the Leu-Ala hydroxyethylene dipeptide isostere. A number of inhibitors have shown excellent memapsin 2 inhibitory potency. One of these inhibitors **5d** has shown good selectivity over memapsin 1 and cathepsin D as well as good cellular inhibitory activity of memapsin 2 in Chinese hamster ovary cells. Furthermore, the

* To whom correspondence should be addressed. Phone: (765)-494-5323; fax: (765)-496-1612; e-mail:akghosh@purdue.edu.
† Purdue University.
‡ Athenagen Inc.
§ Oklahoma Medical Research Foundation.
⊥ University of Oklahoma Health Science Center.

10.1021/jm061338s CCC: $37.00
Published on Web 04/14/2007

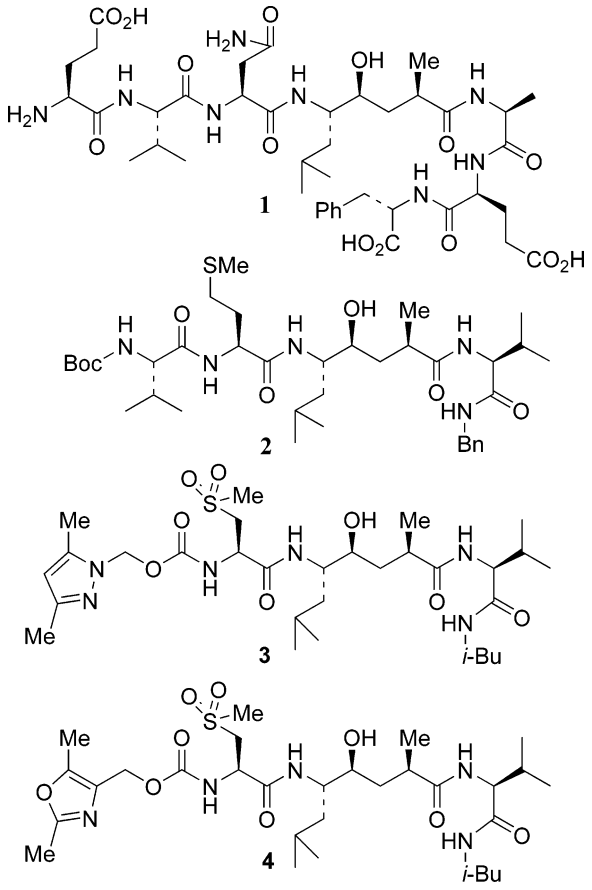
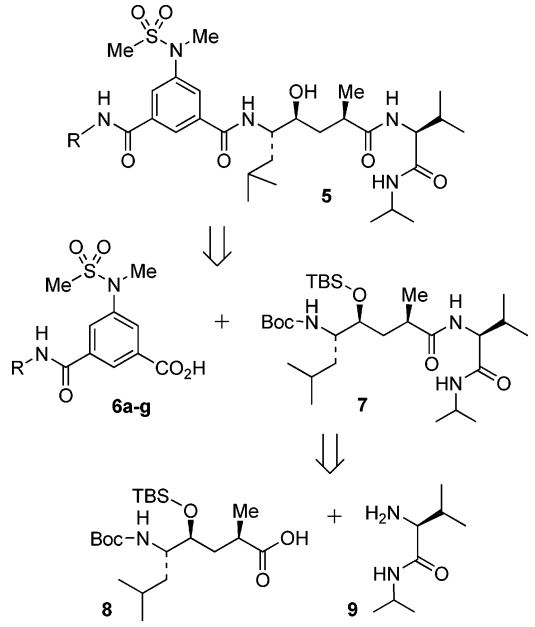


Figure 1. Structure of inhibitors **1**−**4**.

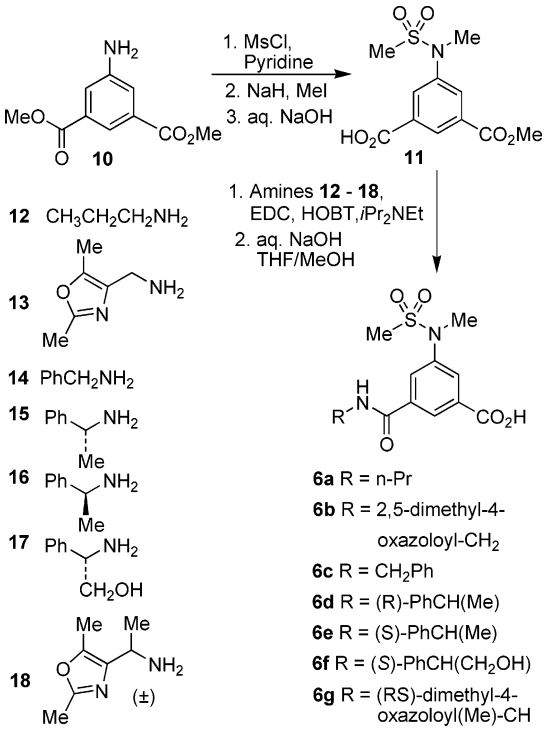
Scheme 1. General Synthetic Route to Inhibitors



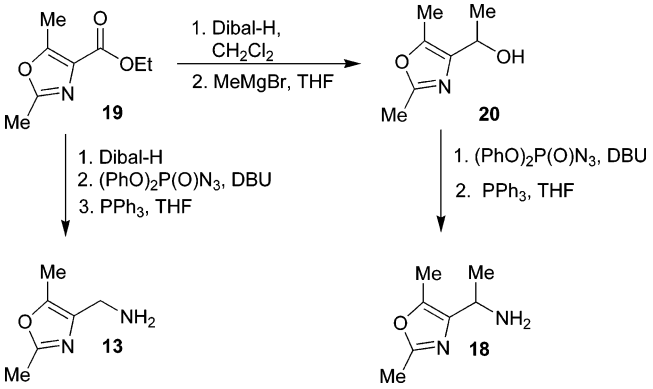
protein−ligand X-ray structure of this inhibitor provided important molecular insight into ligand-binding site interactions for further design.

Chemistry. Our general strategy for the synthesis of inhibitors containing the Leu-Ala hydroxyethylene isostere and isophthalamide derivatives is outlined in Scheme 1. Various inhibitors can be synthesized by coupling isophthalic acid derivatives **6** and the amine derived from BOC-derivative **7**. Amine **7** is obtained by coupling of Leu-Ala acid **8** and valine derivative

Scheme 2. Synthesis of Isophthalamide Ligands (**6a**−**g**)



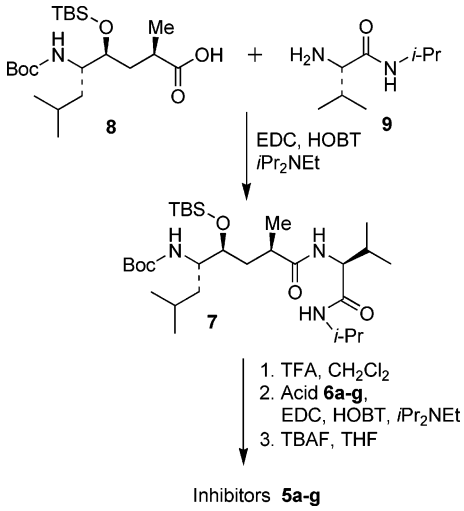
Scheme 3. Synthesis of Oxazolylamines **13** and **18**



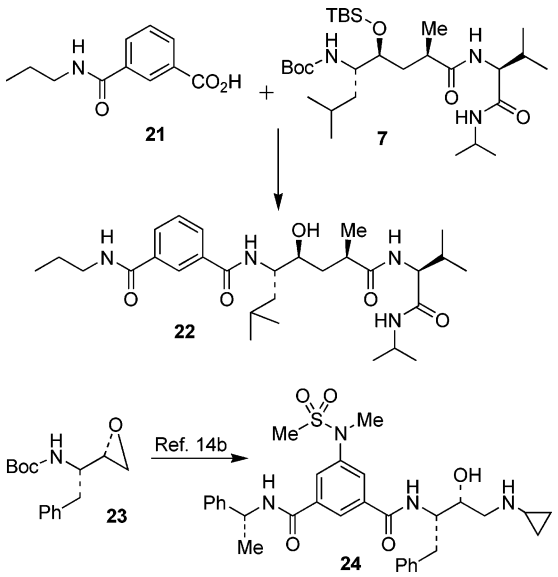
9. The requisite synthesis of isophthalamide derivatives from commercially available dimethyl 5-aminoisophthalate **10** is shown in Scheme 2. Mesylation of amine **10** with mesyl chloride in pyridine followed by alkylation of the resulting mesylate using sodium hydride and methyl iodide, as reported by Stachel and co-workers,[14b] provided the corresponding *N*-methylsulfonamide derivative. Selective hydrolysis of the bis-ester with aqueous sodium hydroxide afforded mono-carboxylic acid **11**. Coupling of acid **11** with various amines (**12**−**18**) using EDC/HOBT in the presence of *i*-Pr_2NEt afforded the respective amide derivatives in moderate to good yield (40−65%). Saponification of the resulting ester provided the required isophthalic acid derivatives **6a**−**g** in good yields.

Amines **12** and **14**−**17** were obtained from commercial sources and amines **13** and **18** were prepared as shown in Scheme 3. Reduction of the known[15] oxazole ester **19** with excess of Dibal-H in CH_2Cl_2 at −78 °C afforded the corresponding alcohol. Azidation of the resulting alcohol with diphenylphosphoryl azide in the presence of DBU provided the corresponding azide. Reduction of azide with triphenyl phosphine in THF afforded amine **13**. The synthesis of racemic amine **18** was also carried out from the same oxazole ester **19**. As shown, selectivereduction of ester **19** with 1.2 equiv of

Scheme 4. Synthesis of Inhibitors **5a**−**g**



Scheme 5. Synthesis of Inhibitors **22** and **24**



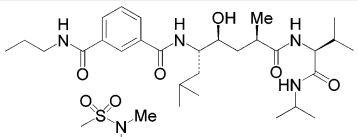
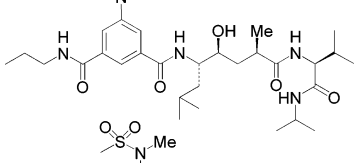
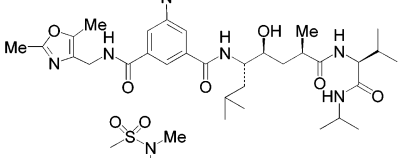
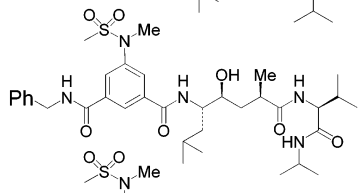
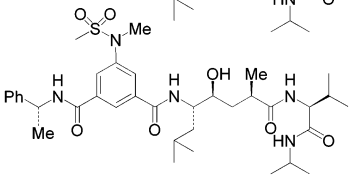
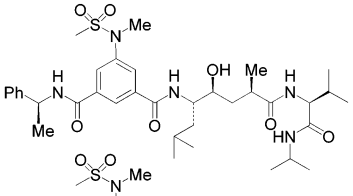
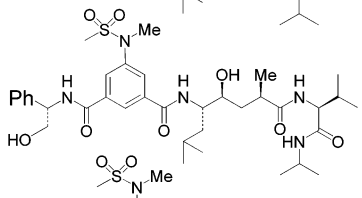
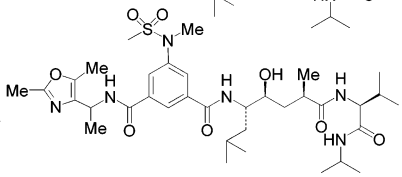
Dibal-H afforded the corresponding aldehyde. Reaction of the resulting aldehyde with methyl magnesium bromide in THF furnished racemic alcohol **20**. This alcohol was then converted to amine **18** by azidation followed by reduction as described for compound **13**.

The general synthesis of inhibitors containing the Leu-Ala hydroxyethylene isostere and isophthalamide derivatives is shown in Scheme 4. Coupling of previously described[7,9] Leu-Ala isostere **8** with valine derivative **9** using EDC and HOBt in the presence of *i*-Pr_2NEt afforded amide derivative **7**. Removal of the BOC group by exposure of **7** to trifluoroacetic acid in CH_2Cl_2 at room temperature provided the corresponding amine derivative. Reaction of the resulting amine with various acids **6a**−**g** provided the respective coupling product. Removal of the TBS-group with tetrabutylammonium fluoride in THF furnished inhibitors **5a**−**g** in good yields. Inhibitor **22** was prepared by analogous procedure using **7**-derived amine and the propyl amide of isophthalic acid **21** (Scheme 5). The known *β*-secretase inhibitor **24** was prepared from commercially available BOC-oxirane **23** as described in the literature.[14b]

Results and Discussion

Structure and inhibitory potency of various inhibitors against recombinant memapsin 2 are shown in Table 1. We initially examined the ability of an unsubstituted isophthalimide derivative to function as the P_2-ligand with a Leu-Ala isostere. As shown, incorporation of propyl isophthalamide as the P_2- and P_3-ligands in place of P_2-methylsulfonyl alanine and P_3-oxazolylmethyl ligands provided inhibitor **22** with a K_i value of 788 nM. This inhibitor is considerably less potent than inhibitors with amino acid-derived ligands described previously.[9a] In an effort to interact with the residues in the S_2-site, we examined the potential of the *N*-methylsulfonamide functionality on the P_2-isophthalamide ligand. The corresponding inhibitor **5a** exhibited a significant improvement in memapsin 2 inhibitory potency compared to **22**. This is consistent with our energy-minimized model structure of inhibitor **4** and a prototype inhibitor **5b** where P_2-methylsulfonyl alanine is replaced with a *N*-methylsulfonyl-substituted isophthalimide P_2-ligand, and P_3-oxazolyl urethane is replaced with an oxazolylmethyl amide as shown in Figure 2. The model of inhibitor **4** was created based upon the crystal structure of inhibitor **3**-bound memapsin 2 reported by us recently.[10] Compound **5b** exhibited a K_i value of 1.8 nM, a 28-fold potency enhancement over propylamide

Table 1. Structure and Inhibitory Potency of Inhibitors

entry	compound structure	compound number	K_i (nM)
1.		**22**	788
2.		**5a**	57.8
3.		**5b**	1.8
4.		**5c**	136
5.		**5d (GRL-7234)**	1.1
6.		**5e**	315
7.		**5f**	438
8.		**5g**	27

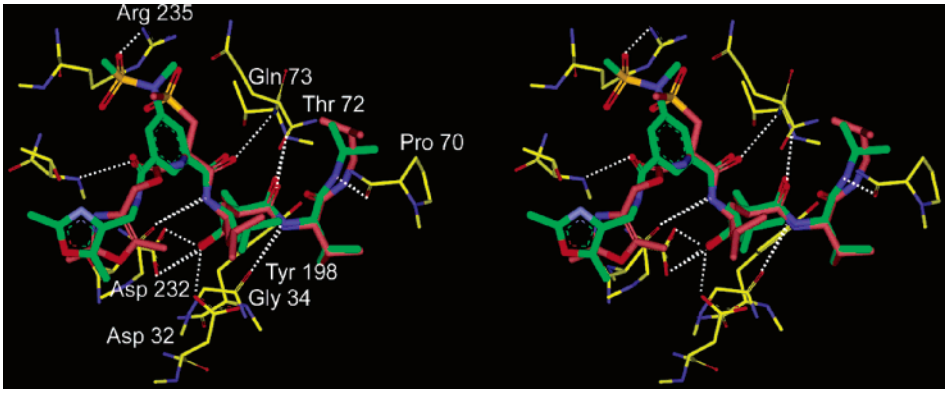


Figure 2. Inhibitors **4** (red) and **5b** (green) modeled into the active site of memapsin 2. Inhibitor model **4** was created based upon X-ray structure of **3**-bound memapsin 2.

derivative **5a**. The molecular model in Figure 2 suggests that the *N*-methylsulfonamide derivative in **5b** may interact with active site residues similar to the P_2-sulfone of inhibitor **4**. In addition, the model suggests an additional interaction which may contribute to the increased potency of compound **5b**. The P_3-dimethyloxazole in **5b** appears to effectively fill in the hydrophobic pocket in the S_3-subsite. On the basis of this speculation, we explored the suitability of other hydrophobic groups as P_3-ligands. As can be seen, inhibitor **5c** containing a P_3-benzyl amide is significantly less potent than **5b**. Incorporation of an α-methylbenzyl amide (*R*-configuration) resulted in a very potent memapsin 2 inhibitor **5d**. This inhibitor exhibited a memapsin 2 K_i of 1.1 nM, a 120-fold improvement over the benzylamide derivative **5c**. The inhibitor with *S*-configuration (**5e**), however, proved to be significantly less potent, thus indicating a strong stereochemical preference at this position. This is consistent with the observation reported by Stachel and co-workers in their studies with hydroxyethylamine isostere.[14b] Also, the polar α-hydroxymethyl substituent with *S*-configuration (inhibitor **5f**) was not very effective. Since the α-methyl group of the P_3-ligand in inhibitor **5d** is preferred by the S_3-subsite of memapsin 2, we decided to incorporate an α-methyl group in the P_3-oxazolylmethyl derivative of inhibitor **5b**. For preliminary evaluation, we prepared inhibitor **5g** as a diastereomeric mixture (1:1) at the P_3-stereocenter, and we have determined the inhibitory potency with this mixture of diastereomers. While still a potent inhibitor (K_i = 27 nM), this compound displayed reduced potency compared to unsubstituted inhibitor **5b**. However, actual contribution of individual diastereomers has not been determined.

We then evaluated the potencies of selected inhibitors against recombinant memapsin 1 and human cathepsin D. Furthermore, we determined the cellular memapsin 2 inhibitory potency of these compounds in Chinese hamster ovary cells.[16] The results are shown in Table 2. Inhibitor **5a** showed a modest reduction of potency against memapsin 1 and cathepsin D. Compound **5b**, however, is a very potent and selective memapsin 2 inhibitor. It is over 308-fold selective against memapsin 1 and over 136-fold selective against cathepsin D. Its cellular IC_{50} value is similar to inhibitor **4**.[10] Inhibitor **5d** is the most potent memapsin 2 inhibitor in this series. It exhibited a modest selectivity against memapsin 1 (28-fold) and cathepsin D (37-fold). Of particular note, **5d** displayed excellent cellular inhibition of memapsin 2 (39 nM). In comparison, an in-house prepared inhibitor **24**[14b] with a hydroxyethylamine isostere showed little to no selectivity against memapsin 1 and cathespin D.[17] This inhibitor is also less potent in our cellular assay.[16]

In a preliminary investigation, we have also examined the *in vivo* inhibitory effect on brain Aβ-production in transgenic mice by inhibitor **5d**. Intraperitoneal administration of inhibitor **5d** to Tg2576 mice[18] effected a 30% reduction of $A\beta_{40}$ production at 4 h after a single administration at 8 mg/kg (Figure 3). The inhibition of memapsin 2 activity *in vivo* was significant relative to the vehicle-treated control animals ($p = 0.001$) and relative to the baseline $A\beta_{40}$ levels for the treated group ($p = 0.01$). The exhibition of $A\beta_{40}$ inhibition at 4 h after a single injection of **5d** follows the established $A\beta_{40}$ inhibition profile by the prototypic conjugated pseudopeptide inhibitor OM003-DR_9 designed in our laboratories.[16] Since Aβ production in this mouse model is brain specific at the age used in the study[18] and the efflux of Aβ from brain to plasma occurs rapidly,[16] there is a good possibility that the observed plasma $A\beta_{40}$ reduction is mainly due to an inhibition of $A\beta_{40}$ production in the brain. However, further studies will be needed to verify the pharmacokinetic aspects of **5d** including the brain penetration.

In order to obtain molecular insight into the enzyme−inhibitor interactions of inhibitor **5d**, we determined the crystal structure of inhibitor-bound memapsin 2 at 2.5 Å resolution. A stereoview of **5d**-bound memapsin 2 structure is shown in Figure 4. The transition-state hydroxyl group of the Leu-Ala isostere forms two tight hydrogen bonds (2.4 and 2.7 Å bond distances) with both active site aspartic acid residues (Asp32 and Asp228).[7] 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)[19] located in the S_3/S_4 pocket. This conformational change to accommodate the bound inhibitor identifies a flexible part of the active site cleft which can be further exploited for ligand design. This binding pocket consists of residues 11−14, 229−232, 307, and 335 which make direct contact with the P_3-phenyl ring group.

The P_2-sulfonamide functionality fits well into the S_2 pocket and makes extensive interactions with memapsin 2. One of the sulfone oxygen atoms is within hydrogen-bonding distance with the Asn233 amide nitrogen and the Ser325 hydroxyl oxygen (both at 3.0 Å bond distance). The same sulfone oxygen also makes ionic interactions with the guanidine side chain of Arg235. This side chain appeared to be quite flexible and adopted a different conformation than our previously reported structures.[7, 20] The other sulfonamide oxygen makes hydrogen bonds to Thr232 and Asn233 main chain nitrogen atoms (3.2 and 2.8 Å bond distances, respectively). Significant Van der Waal interactions with the nonpolar atoms of Thr231, 232, Asn233, and Arg235 are also evident. The $P_{3'}$-isopropyl group makes Van der Waal contacts with the flap residues Pro70,

Table 2. Inhibitory and Cellular Potency of Inhibitors

compd	M2[a] (nM)	M1[b] (nM)	CD[c] (nM)	M1/M2	CD/M2	IC_{50} (μM)
5a	52.5 ± 7.4	340 ± 37	483 ± 96	6.5	9.2	3.6 ± 3.2
5b	1.8 ± 1.1	556 ± 16	245 ± 17	308	136	1.8 ± 0.34
5c	136 ± 8.5	523 ± 302	nd	3.8	-	5.6 ± 1.85
5d	1.1 ± 0.37	31 ± 10.7	41 ± 12.4	28	37	0.039 ± 0.013
5f	438 ± 52	397 ± 123	633 ± 116	1	1.4	190 ± 309
5g	27 ± 1.8	408 ± 97	490 ± 103	15	18	10.3 ± 5.7
24	233 ± 23	240 ± 47	448 ± 368	1	2	0.078 ± 0.009

[a] M2: Memapsin-2/BACE-1. [b] M1: Memapsin-1/BACE-2. [c] CD: Cathepsin D.

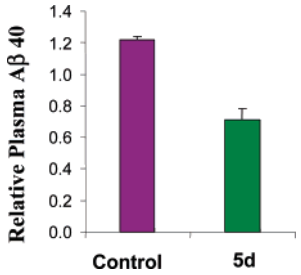


Figure 3. Inhibitor **5d** reduces Aβ_{40} production following a single intraperitoneal administration at 8 mg/kg. Plasma Aβ_{40} assessed at 4 h postinjection was expressed relative to baseline levels of individual mice and was significantly lower than the vehicle-treated control group ($p = 0.001$).

Thr72, and Arg128. It appears that the isopropyl/flap interactions facilitate two directional hydrogen bonds with the $P_{2'}$-carbonyl to Tyr198 hydroxyl group and the $P_{3'}$-NH to carbonyl of Pro70. The protein/ligand interactions at P_1, $P_{1'}$, and $P_{2'}$ are very similar to protein−ligand structures reported by us previously.[7,20,21]

Conclusion

In conclusion, our structure-based design strategies led to design of novel memapsin 2 inhibitors incorporating various substituted isophthalamides as P_2−P_3 ligands in inhibitors with a hydroxyethylene dipeptide isostere. The inhibitors were designed to interact with various residues at the S_2- and S_3-sites of the memapsin 2 active site. Of particular interest, we examined the structural requirements for selectivity over other aspartyl proteases and improvement of cellular inhibitory potency. Inhibitor **5b** exhibited excellent potency against memapsin 2 and selectivity over memapsin 1 and Cathepsin D. However, its cellular memapsin 2 inhibitory potency was not improved. Inhibitor **5d**, on the other hand showed very good enzyme inhibitory activity as well as cellular inhibitory potency in Chinese hamster ovary cells (46-fold improvement over **5b**). Intraperitoneal administration of inhibitor **5d** to Tg2576 mice resulted in a 30% reduction of Aβ_{40} production at 4 h after a single dose of 8 mg/kg. An inhibitor-bound X-ray crystal structure of **5d**-bound memapsin 2 (2.5 Å) indicated extensive critical interactions in the enzyme active site. Particularly notable are the interactions with the P_2-sulfonamide functionality at the S_2-region of memapsin 2 and an interesting conformational change in the 10's loop upon inhibitor binding to accommodate the P_3-phenyl ring. This molecular insight will be utilized to design more potent and selective inhibitors. Further design and synthesis of inhibitors based upon the current molecular insight are in progress.

Experimental Section

General. All moisture sensitive reactions were carried out under nitrogen or argon atmosphere. Anhydrous solvents were obtained as follows: THF, diethyl ether, and benzene, distilled from sodium and benzophenone; dichloromethane, pyridine, triethylamine, and diisopropylethylamine, distilled from CaH_2. All other solvents were HPLC grade. Column chromatography was performed with Whatman 240−400 mesh silica gel under low pressure of 5−10 psi. TLC was carried out with E. Merck silica gel 60-F-254 plates. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and Bruker Avance 400 and 500 spectrometers. Infrared spectra were recorded on a Mattson Genesis II FTIR instrument. Optical rotations were measured using a Perkin-Elmer 341 polarimeter.

Leu-Ala Derivative 7. The dipeptide isostere **8**[7] (209 mg, 0.5 mmol) and the amine **9**[10] (82 mg, 0.52 mmol) were dissolved in CH_2Cl_2 (5 mL). To this solution EDC (115 mg, 0.6 mmol) and HOBT (81 mg, 0.6 mmol) followed by diisopropylethylamine (0.5 mL) were successively added and stirred overnight at room temperature. After this period, water was added and extracted with ethyl acetate. The combined organic layers were washed with aqueous $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. The solvents were evaporated under reduced pressure and purified by silica gel flash column chromatography (30% EtOAc in hexanes) to provide **7** in 94% yield (0.26 g) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.86−0.96 (21H, m), 1.07−1.22 (10H, m), 1.31−1.50 (11H, m), 1.54−1.62 (1H, m), 1.71−1.80 (1H, m), 1.99−2.06 (1H, m), 2.47−2.54 (1H, m), 3.60−3.72 (2H, m), 3.99−4.09 (2H, m), 4.49 (1H, br d J = 9.9 Hz), 6.17 (1H, br d J = 7.5 Hz), 6.33 (1H, br d J = 8.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.4, 170.2, 156.2, 79.2, 72.0, 59.0, 50.1, 42.0, 41.2, 38.3, 37.24, 30.7, 28.5, 25.9, 24.9, 23.1, 22.7, 22.5, 22.2, 19.3, 18.7, 18.1, 16.6, −4.0, −4.4.

3-Methoxycarbonyl-5-(*N*-methylmethan-5-ylsulfonamido)-benzoic Acid 11. To a stirred solution of dimethyl 5-aminoisophthalate (2g, 10 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added pyridine (2.4 mL, 30 mmol) followed by methanesulfonyl chloride (0.9 mL, 11 mmol). The resulting solution was allowed to warm to 23 °C and stirred for 14 h. The solvent was removed under reduced pressure. Ethyl acetate was added to the residue which resulted in the formation of a precipitate. The precipitate was filtered and washed with hexane to give the corresponding sulfonamide as an amorphous solid (2.7 g, 95%).

To a stirred suspension of NaH (0.4 g, 10 mmol, 60% oil dispersion) in DMF (10 mL) at 23 °C, the above sulfonamide (1.4 g, 5 mmol) followed by methyl iodide (0.6 mL, 10 mmol) was added. The reaction mixture was stirred at 23 °C for 5 h. After this period, the reaction was carefully quenched with water, and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to provide the corresponding methyl sulfonamide (1.4 g, 91%).

To a stirred solution of above diester (1.4 g, 4.5 mmol) in a mixture (1:1:1) of THF, MeOH, and water (15 mL) at 0 °C, solid NaOH (180 mg, 4.5 mmol) was added and the mixture was stirred for 14 h at 23 °C. After this period, the solvent was evaporated and saturated $NaHCO_3$ (10 mL) was added. The aqueous layer was extracted with toluene (2×) to remove unreacted diester. Aqueous layer was then acidified with 1 N HCl to pH 3 and extracted with EtOAc (3×). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to provide mono acid **11** as an amorphous solid (1.1 g, 82%). ^{1}H NMR (500 MHz, CD_3OD): δ 2.97 (s, 3H), 3.42 (s, 3H), 3.99 (s, 3H), 8.29−8.31 (m, 2H), 8.57−8.59 (m, 1H).

2,5-Dimethyloxazol-4-ylmethanamine 13. To a stirred solution of (2,5-dimethyloxazol-4-yl)methanol (127 mg, 1.0 mmol) in toluene (5 mL) were added DPPA (216 μL, 1.0 mmol) and DBU (180 μL, 1.2 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred. After 17 h, the reaction mixture was quenched with 10% aqueous dil HCl and extracted with EtOAc. The combined layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product thus obtained was purified by silica gel flash column chromatography (3% MeOH/$CHCl_3$) to give the corresponding azide in 77% yield (117.5 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (3H, s), 2.37 (3H, s), 4.12 (2H, s).

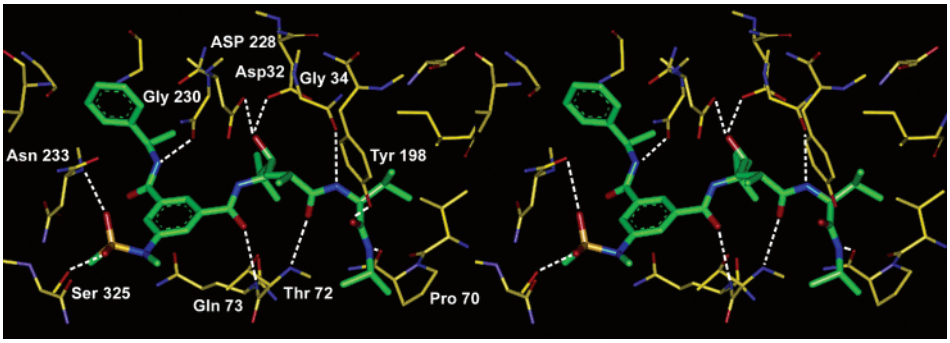


Figure 4. Stereoview of inhibitor **5d**-bound memapsin 2 X-ray structure. The residues contacting the inhibitor (green) are shown in yellow, and the protein/ligand hydrogen bonds are shown in white dotted lines.

The above azide (117 mg, 0.7 mmol) was taken up in THF, and PPh_3 (183.6 mg, 0.7 mmol) was added. The reaction mixture was stirred at room temperature for 7 h. Then, a few drops of water were added and refluxed at 55 °C for overnight. Evaporation of solvent followed by flash column chromatography over silica gel (10−15% MeOH/$CHCl_3$ and 2% Et_3N) afforded amine **13** as a yellow liquid (52 mg, 41%) after two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (3H, s), 2.27 (3H, s), 2.54 (2H, br), 3.54 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5, 143.2, 134.6, 36.9, 13.9, 10.0.

2,5-Dimethyloxazol-4-yl-1-ethanamide 18. To a stirred solution of ethyl 2,5-dimethyloxazole-4-carboxylate (350 mg, 2 mmol) in CH_2Cl_2 (5 mL) at −78 °C was was added DIBAL-H (3 mL, 4.5 mmol, 1.5 M soln in toluene). The mixture was stirred for 5 min at −78 °C, and the reaction was quenched with saturated aqueous potassium sodium tartrate solution. The solution was diluted with CH_2Cl_2 and vigorously stirred at 23 °C for 30 min. The aqueous layer was extracted with CH_2Cl_2 (2×). The combined layers were dried on anhydrous Na_2SO_4. Evaporation of solvent under reduced pressure provided the corresponding aldehyde as a colorless liquid (200 mg). This aldehyde was used for the next step without further purification.

To a stirred solution of the above aldehyde in THF (5 mL) at −78 °C was added methylmagnesium bromide (640 μL, 1.9 mmol, 3 M soln in ether). The reaction mixture was warmed to 23 °C over a period of 3 h. The reaction was quenched with saturated aqueous NH_4Cl and was extracted with EtOAc (3×). The combined organic layers were dried on anhydrous Na_2SO_4. Evaporation of solvent under reduced pressure gave secondary alcohol **20** as a colorless liquid (143 mg).

To a stirred solution of above alcohol and diphenyl phosphoryl azide (260 μL, 1.2 mmol) in toluene (3 mL) at 0 °C was added DBU (180 μL, 1.2 mmol). The reaction was warmed to 23 °C and stirred for 17 h. After this time, the reaction mixture was quenched with 10% aqueous HCl and extracted with EtOAc (3×). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product thus obtained was purified by silica gel column chromatography (3% MeOH/$CHCl_3$) to provide the corresponding azide (159 mg).

The above azide was dissolved in THF (5 mL) at 23 °C, and PPh_3 (262 mg, 1 mmol) was added. The reaction mixture was stirred at 23 °C for 7 h. After this period, a few drops of water were added, and the reaction mixture was heated at 55 °C for 12 h. After this period, the mixture was concentrated and the residue was purified by silica gel chromatography (10−25% MeOH/$CHCl_3$ and 2% Et_3N) to provide amine **18** as a yellow liquid (57.5 mg, 20%, three steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (d, J = 6.5 Hz, 3H), 2.20 (s, 3H), 2.32 (s, 3H), 4.13 (q, J = 6.5 Hz, 2H), 5.69 (br, 2H).

3-Propylcarbamoylbenzoic Acid 21. To a stirring solution of methyl 3-(propylcarbamoyl)benzoate (167 mg, 0.75 mmol) in THF:H_2O mixture (1 mL:1 mL) at 0 °C was added solid LiOH (47 mg, 1.1 mmol), and the resulting solution was stirred at 23 °C for 4 h. After this period, the reaction mixture was extracted with toluene to remove organic impurities. The aqueous layer was cooled to 0 °C, acidified with 1 N HCl, extracted with EtOAc, and dried over anhydrous Na_2SO_4. The solvent was evaporated and dried under reduced pressure to give pure acid **21** as a white solid in 69% yield (105 mg). ^{1}H NMR (CD_3OD, 300 MHz): δ 0.9 (t, J = 6.5 Hz, 3H), 1.58 (q, J = 6.5 Hz, 2H), 3.3 (m, 2H), 7.5 (t, J = 8.4 Hz, 1H), 7.93 (d, J = 8 Hz, 1H), 8.1 (d, J = 8 Hz, 1H), 8.4 (s, 1H), 8.6 (bs, 1H). MS-CI (m/z): $[M + H]^+$ 208.15.

3-(*N*-Methylmethan-5-ylsulfonamido)-5-(propylcarbomoyl)-benzoic Acid 6a. To a solution of the mono acid **11** (143.5 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:3 mL), EDC (114.6 mg, 0.6 mmol) and HOBT (81 mg, 0.6 mmol) were added followed by *n*-propylamine (**12**) (82 μL, 1 mmol) and diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 13 h. After this time, water was poured into reaction mixture, and the mixture was extracted with EtOAc and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and purification by silica gel flash column chromatography yielded the *n*-propylamide derivative which further underwent hydrolysis with 1 N NaOH in THF/MeOH to provide the acid **6a** in 71% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): 0.88 (3H, t, J = 7.5 Hz), 1.55 (2H, m), 2.83 (3H, s), 3.27 (3H, s), 3.30 (2H, m), 7.82 (1H, s), 7.91 (1H, s), 8.05 (1H, m), 8.27 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD): δ 166.9, 141.3, 135.5, 129.8, 128.1, 127.0, 41.8, 37.7, 35.2, 22.4, 11.2.

3-{[(2,5-Dimethyloxazol-4-yl)methyl]carbamoyl}-5-(*N*-methylmethan-5-ylsulfonamido)benzoic Acid 6b. To a stirred solution of the mono acid **11** (172 mg, 0.6 mmol) in DMF/CH_2Cl_2 (1:5 mL) were added EDC (143.2 mg, 0.75 mmol) and HOBT (101.2 mg, 0.75 mmol) followed by (2,5-dimethyloxazol-4-yl)methanamine (**13**) (76 mg, 0.6 mmol) and diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 13 h. Then workup similar to that for **6a** followed by silica gel flash column chromatography gave the corresponding amide derivative which was further hydrolyzed in presence of 1 N NaOH in THF/MeOH to give the benzoic acid **6b** in 33% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 2.37 (3H, s), 2.39 (3H, s), 2.84 (3H, s), 3.32 (3H, s), 4.39 (2H, s), 8.13 (1H, s), 8.18 (1H, m), 8.73 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD): δ 166.6, 166.0, 159.7, 145.45, 141.70, 135.18, 132.0, 129.9, 128.7, 126.6, 37.1, 34.7, 34.3, 12.5, 9.0. MS-ESI (m/z): $[M + Na]^+$ 403.96.

3-(Benzylcarbamoyl)-5-(*N*-methylmethan-5-ylsulfonamido)-benzoic Acid 6c. The acid **6c** was prepared by following a procedure similar to that described above. To the mono acid **11** (144.8 mg, 0.4 mmol), EDC (96 mg, 0.5 mmol), and HOBT (67.5 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:4 mL) at room temperature was added benzylamine (**14**) (57 μL, 0.5 mmol) followed by diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 20 h. Then workup similar to that for **6a**, followed by silica gel flash column chromatography, provided the corresponding amide which was further hydrolyzed by using 1 N NaOH in presence of THF/MeOH to give the benzoic acid **6c** in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 2.84 (3H, s),

3.08 (3H, s), 4.56 (2H, d, $J = 5.4$ Hz), 7.28 (5H, m), 7.86 (1H, bs), 7.97 (1H, s), 8.09 (1H, m), 8.32 (1H, m).

(*R*)-3-(*N*-Methylmethan-5-ylsulfonamido)-5-((1-phenylethyl)-carbomoyl)benzoic Acid 6d. Acid **6d** was prepared by following a similar procedure to that described for acid **6b**. To mono acid **11** (215 mg, 0.75 mmol), EDC (172 mg, 0.9 mmol), and HOBT (122 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:5 mL) at room temperature was added (*R*)-α-methylbezylamine (**15**) (100 μL, 0.75 mmol) followed by diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 20 h. Then workup similar to **6a**, followed by silica gel flash column chromatography, provided the corresponding amide which was further hydrolyzed by using 1 N NaOH in presence of THF/MeOH to give the benzoic acid **6d** (198 mg, 60%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3 + CD_3OD$): δ 1.56 (3H, d, $J = 7.5$ Hz), 2.87 (3H, s), 3.32 (3H, s), 5.24 (1H, m), 7.19−7.36 (5H, m), 7.97 (1H, m), 8.11 (1H, m), 8.35 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3 + CD_3OD$): δ 168.6, 166.0, 143.0, 141.3, 135.3, 134.9, 129.3, 128.3, 127.9, 126.61, 125.64, 49.1, 37.2, 34.6, 20.8.

(*S*)-3-(*N*-Methylmethylsulfonamido)-5-(1-phenylethylcarbamoyl)benzoic Acid 6e. Acid **6e** was prepared by following a procedure similar to that described for acid **6b**. To mono acid **11** (100 mg, 0.35 mmol), EDC (77 mg, 0.4 mmol), and HOBT (54 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:3 mL) at room temperature was added (*S*)-α-methylbezylamine (**16**) (49 μL, 0.4 mmol) followed by diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 20 h. Then workup similar to that for **6a**, followed by silica gel flash column chromatography, provided the corresponding amide which was further hydrolyzed by using 1 N NaOH in presence of THF/MeOH to give the benzoic acid **6e** as a white solid in 45% yield (60 mg). ^{1}H NMR (500 MHz, CD_3OD): 1.61 (d, $J = 7$ Hz, 3H), 2.98 (s, 3H), 3.40 (s, 3H), 5.28 (m, 1H), 7.27−7.45 (m, 5H), 8.11 (s, 1H), 8.11 (s, 1H), 8.11 (s, 1H). MS-ESI (*m/z*): $[M + Na]^+$ 398.90.

(*R*)-3-{[2-Hydroxy-1-phenylethyl]carbamoyl}-5-(*N*-methylmethan-5-ylsulfonamido)benzoic Acid (6f). Acid **6f** was prepared by following a procedure similar to that described for acid **6b**. To mono acid **11** (115 mg, 0.4 mmol), EDC (96 mg, 0.5 mmol), and HOBT (66 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:4 mL) at room temperature was added (*R*)-(2)-phenylglycinol (**17**) (69 mg, 0.4 mmol) followed by diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 20 h. Then workup similar to that for **6a**, followed by silica gel flash column chromatography, provided the corresponding amide which was further hydrolyzed by using 1 N NaOH in presence of THF/MeOH to give the benzoic acid **6f.**

3-{[1-(2,5-Dimethyloxazol-4-yl)ethyl]carbamoyl}-5-(*N*-methylmethan-5-ylsulfonamido)benzoic Acid 6g. Acid **6g** was prepared by following a procedure similar to that described for acid **6b**. To mono acid **11** (115 mg, 0.4 mmol), EDC (96 mg, 0.5 mmol), and HOBT (66 mg, 0.5 mmol) in DMF/CH_2Cl_2 (1:4 mL) at room temperature was added (2,5-dimethyloxazol-4yl)ethanamine (**18**) (56 mg, 0.4 mmol) followed by diisopropylethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 20 h. Then workup similar to that for **6a**, followed by silica gel flash column chromatography, provided the corresponding amide which was further hydrolyzed by using 1 N NaOH in presence of THF/MeOH to give the benzoic acid **6g** as a white solid in 40% (63 mg) yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (3H, d, $J = 7.5$ Hz), 2.43 (3H, s), 2.46 (3H, s), 2.86 (3H, s), 3.37 (3H, s), 5.45 (1H, m), 8.27 (1H, s), 8.32 (1H, m), 9.10 (1H, m), 9.33 (1H, br). MS-ESI (*m/z*): $[M + Na]^+$ 418.04.

Inhibitor 5a. To a stirred solution of Boc-protected hydroxyethyl dipeptide isostere (**7**) (25 mg, 0.045 mmol) in dichloromethane (1.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure to give the corresponding amine.

The amine was dissolved in dichloromethane (1 mL), and diisopropylethylamine (0.2 mL) was added. The combined mixture was added to a stirred solution of acid **6a** (16 mg, 0.05 mmol), HOBT (8 mg, 0.054 mmol) and EDC (10.3 mg, 0.054 mmol) in DMF/dichloromethane (1:2 mL) and stirred at room temperature for 44 h. Then water (5 mL) was added to the reaction mixture and extracted with ethyl acetate (2 × 10 mL). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The TBS-protected inhibitor thus obtained was used for the next step.

To the stirred solution of TBS-protected inhibitor in THF (3 mL) was added TBAF (1 M THF, 0.23 mL) at room temperature, and the resulting mixture was stirred overnight. The mixture was concentrated under reduced pressure and purified by silica gel flash column chromatography (4% methanol in chloroform) to give the inhibitor **5a** as a white solid in 61% yield (17.5 mg). ^{1}H NMR (500 MHz, $CDCl_3 + CD_3OD$): δ 0.75 (3H, d, $J = 6.5$ Hz), 0.79 (3H, d, $J = 7.0$ Hz), 0.90 (6H, d, $J = 6.5$ Hz), 0.94 (3H, t, $J = 7.0$ Hz), 1.09−1.11 (9H, m), 1.38 (1H, m), 1.51 (1H, m), 1.59−1.70 (5H, m), 1.79−1.85 (1H, m), 2.68 (1H, m), 2.88 (3H, s), 3.33−3.39 (5H, m), 3.62 (1H, m), 3.90 (1H, d, $J = 8.5$ Hz), 3.97 (1H, m), 4.17 (1H, m), 7.96 (2H, s), 8.29 (1H, s). MS-ESI (*m/z*): $[M + Na]^+$ 662.17. HRMS $[M + Na]^+$ calcd for $C_{31}H_{53}N_5NaO_7S$ 662.3558, found 662.3560.

Inhibitor 5b. To a stirred solution of Boc-protected hydroxyethyl dipeptide isostere (**7**) (41.8 mg, 0.075 mmol) in dichloromethane (1.5 mL) was added TFA (0.5 mL), and the mixture was stirred at room temperature. The reaction mixture was stirred for 30 min and then concentrated under reduced pressure to provide the corresponding amine.

The amine was dissolved in dichloromethane (1 mL), and diisopropylethyl amine (0.2 mL) was added. The combined mixture was added to a stirred solution of acid **6b** (27.2 mg, 0.075 mmol), HOBT (13.5 mg, 0.1 mmol), and EDC (19.2 mg, 0.1 mmol) in DMF/dichloromethane (1:2 mL) and stirred at room temperature for 28 h. Then water (5 mL) was added to the reaction mixture and extracted with ethyl acetate (2 × 10 mL). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude TBS-protected inhibitor thus obtained was used as such for the next step.

The stirred solution of TBS-protected inhibitor in THF (3 mL), TBAF (1 M THF, 0.38 mL) was added at room temperature, and the resulting mixture was stirred overnight. The mixture was concentrated under reduced pressure and purified by column chromatography (2% methanol in chloroform) to give the inhibitor **5b** as a white solid in 28% yield (15 mg). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (3H, d, $J = 6.5$ Hz), 0.87 (3H, d, $J = 6.5$ Hz), 0.93 (3H, d, $J = 6.5$ Hz), 0.95 (3H, d, $J = 6.5$ Hz), 1.08 (3H, d, $J = 6.5$ Hz), 1.13 (3H, d, $J = 6.5$ Hz), 1.19 (3H, d, $J = 7.0$ Hz), 1.43−1.47 (1H, m), 1.62−1.72 (3 H, m), 1.82−1.84 (1H, m), 1.96−2.02 (1H, m), 2.40 (3H, s), 2,45 (3H, s), 2.71−2.75 (1H, m), 2.86 (3H, s), 3.33 (3H, s), 3.76 (1H, m), 3.98−4.02 (1H, m), 4.10−4.19 (2H, m), 4.33 (1H, dd, $J = 5.0$, 15.0 Hz), 4.44 (1H, dd, $J = 5.5$, 15.0 Hz), 6.60 (1H, b), 6.81 (1H, b), 6.96 (1H, b), 8.02 (2H, d, $J = 5.5$ Hz), 8.09 (1H, b), 8.33 (1H, s). MS-ESI (*m/z*): $[M + Na]^+$ 729.15. HRMS $[M + Na]^+$ calcd for $C_{34}H_{54}N_6NaO_8S$ 729.3616, found 729.3646.

Inhibitor 5c. The procedure described for synthesis of inhibitor **5a** was used for the synthesis of **5c**. Accordingly, Boc-protected hydroxyethyl dipeptide isostere **7** (42 mg, 0.075 mmol), HOBT (14 mg, 0.1 mmol), EDC (20 mg, 0.1 mmol), and acid **6c** (27 mg, 0.075) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (5% methanol in chloroform) yielded inhibitor **5c** as a white solid in 64% yield (33 mg). ^{1}H NMR (500 MHz, $CDCl_3 + CD_3OD$): δ 0.75 (3H, d, $J = 6.5$ Hz), 0.78 (3H, d, $J = 7.0$ Hz), 0.91 (6H, d, $J = 6.5$ Hz), 1.08 (6H, t, $J = 6.5$ Hz), 1.11 (3H, d, $J = 7.0$ Hz), 1.36−1.41 (1H, m), 1.51−1.57 (1H, m), 1.60−1.72 (3H, m), 1.80−1.85 (1H, m), 2.72 (1H, m), 2.88 (3H, s), 3.34 (3H, s), 3.66 (1H, m), 3.93 (2H, m), 4.19 (1H, m), 4.59 (2H, ABq, $\upsilon = 17.5$ Hz, $J = 15$ Hz, 20 Hz,), 7.22−7.33 (5H, m), 7.98 (1H, d, $J = 1.5$ Hz), 8.03 (1H, t, $J = 1.5$ Hz), 8.41 (1H, s). MS-ESI (*m/z*): $[M + Na]^+ = 710.17$. HRMS $[M + Na]^+$ calcd for $C_{35}H_{53}N_5NaO_7S$ 710.3558, found 710.3557.

Inhibitor 5d. The procedure described for synthesis of inhibitor **5a** was used for the synthesis of **5d**. Accordingly, Boc-protected

hydroxyethyl dipeptide isostere **7** (62 mg, 0.11 mmol), HOBT (17 mg, 0.125 mmol), EDC (24 mg, 0.125 mmol) and acid **6d** (41 mg, 0.11) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (5% methanol in chloroform) yielded inhibitor **5d** as a white solid in 26% yield (20 mg). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80 (3H, d, J = 6.5 Hz), 0.85 (3H, d, J = 6.5 Hz), 0.90 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.5 Hz), 1.10 (6H, d, J = 6.5 Hz), 1.13 (3H, d, J = 7.0 Hz), 1.39−1.43 (1H, m), 1.51 (3H, d, J = 7.0 Hz), 1.59−1.64 (2H, m), 1.66−1.72 (1 H, m), 1.73−1.79 (1H, m), 1.87−1.93 (1H, m), 2.77 (1H, m), 2.84 (3H, s), 3.32 (3H, s), 3.72 (1H, m), 3.98 (1H, m), 4.10 (1H, t, J = 9.0 Hz), 4.25 (2H, m), 5.26 (1H, m), 6.38 (1H, d, J = 7.0 Hz), 6.99 (1H, d, J = 9.5 Hz), 7.17 (1H, d, J = 9.0 Hz), 7.23 (1H, t, J = 7.0 Hz), 7.30 (2H, t, J = 7.0 Hz), 7.36 (2H, d, J = 7.5 Hz), 7.50 (1H, d, J = 7.5 Hz), 8.02 (2H, d, J = 1.5 Hz), 8.42 (1H, s). MS-ESI (*m/z*): $[M + Na]^+$ = 724.18. HRMS $[M + Na]^+$ calcd for $C_{36}H_{55}N_5NaO_7S$ 724.3714, found 724.3715.

Inhibitor 5e. The procedure described for synthesis of inhibitor **5a** was used for the synthesis of **5e**. Accordingly, Boc-protected hydroxyethyl dipeptide isostere **7** (62 mg, 0.11 mmol), HOBT (17 mg, 0.125 mmol), EDC (24 mg, 0.125 mmol), and acid **6e** (42 mg, 0.11) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (5% methanol in chloroform) yielded inhibitor **5e** as a white solid in 44% yield (34 mg). ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ 0.83 (d, J = 7 Hz, 3H), 0.86 (d, J = 7 Hz, 3H), 1.13−1.19 (m, 9H), 1.42−1.46 (m, 1H), 1.63 (d, J = 7 Hz, 6H), 1.65−1.81 (m, 5H), 1.92−1.95 (m, 1H), 2.00−2.10 (m, 1H), 1.83−1.87 (m, 1H), 2.76 (q, J = 7 Hz, 1H), 2.88 (s, 3H), 3.38 (s, 3H), 3.78 (t, J = 6 Hz, 1H), 4.02−4.10 (m, 2H), 4.26−4.27 (m, 1H), 5.32 (t, J = 7 Hz, 1H), 6.18 (bs, 1H), 6.93 (d, J = 9 Hz, 1H), 9.97 (d, J = 8 Hz, 1H), 7.33−7.39 (m, 5H) 8.05 (s, 1H), 8.34 (s, 1H). MS-ESI (*m/z*): $[M + Na]^+$ = 724.16. HRMS $[M + H]^+$ calcd for $C_{36}H_{56}N_5O_7S$ 702.3900, found 702.3907.

Inhibitor 5f. The procedure described for synthesis of inhibitor **5a** was used for the synthesis of **5f**. Accordingly, Boc-protected hydroxyethyl dipeptide isostere **7** (32 mg, 0.057 mmol), HOBT (10 mg, 0.07 mmol), EDC (14 mg, 0.07 mmol), and acid **6f** (22 mg, 0.057) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (3−5% methanol in chloroform) yielded inhibitor **5f** as a white solid in 48% yield (20 mg). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.76 (3H, d, J = 7.0 Hz), 0.82 (3H, d, J = 7.0 Hz), 0.89−0.93 (6H, m), 1.11 (6H, d, J = 6.5 Hz), 1.16 (3H, d, J = 7.0 Hz), 1.33−1.45 (2H, m), 1.57−1.69 (2 H, m), 1.78−1.93 (2H, m), 2.75 (1H, m), 2.85 (3H, s), 2.95 (1H, m), 3.31 (3H, s), 3.71 (1H, m), 3.85−3.92 (2H, m), 3.97−4.02 (1H, m), 4.10 (1H, m), 4.25 (1H, m), 5.20 (1H, m), 6.63 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 8.5 Hz), 7.12 (1H, d, J = 8.5 Hz), 7.24−7.35 (5H, m), 8.00 (1H, s), 8.05 (1H, s), 8.21 (1H, d, J = 7.0 Hz), 8.38 (1H, s). MS-ESI (*m/z*): $[M + Na]^+$ 740.26. HRMS $[M + Na]^+$ calcd for $C_{36}H_{55}N_5NaO_8S$ 740.3669, found 740.3672.

Inhibitor 5g. The procedure described for synthesis of inhibitor **5a** was used for the synthesis of **5g**. Accordingly, Boc-protected hydroxyethyl dipeptide isostere **7** (14 mg, 0.025 mmol), HOBT (4 mg, 0.03 mmol), EDC (6 mg, 0.03 mmol), and acid **6g** (10 mg, 0.025) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (10% methanol in chloroform) yielded inhibitor **5g** as a white solid in 36% yield (6 mg). The ^{1}H NMR showed a mixture of diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78−0.88 (6H, m), 0.91−0.98 (6H, m1.07−1.09 (3H, m), 1.13−1.16 (3H, m), 1.18 (3H, d, J = 7.0 Hz), 1.48−1.52 (3H, m), 1.54−1.65 (2H, m), 1.76 (2H, m), 1.83−1.99 (2H, m), 2.32 and 2.38 (6H, 2s, diastereomers), 2.69 (1H, m), 2.86 (3H, s), 2.98 (1H, m), 3.33−3.54 (3H, m), 3.68−3.83 (1H, m), 3.96−4.06 (1H, m), 4.22−4.25 (2H, m), 5.12−5.18 (1H, m), 6.19 and 6.58 (1H, d, J = 8.0 Hz, diastereomers), 6.75 (1H, t, J = 8.0 Hz), 6.85 and 6.94 (1H, d, J = 8.0 Hz, diasteromers), 7.98−8.06 (2H, m), 8.22 and 8.29 (1H, 2s, diastereomers). MS-ESI (*m/z*): $[M + Na]^+$ 743.30. HRMS $[M + Na]^+$ calcd for $C_{35}H_{56}N_6NaO_8S$ 743.3778, found 743.3785.

Inhibitor 22. The procedure described for the synthesis of inhibitor **5a** was used for the synthesis of **22**. Accordingly, Boc-protected hydroxyethyl dipeptide isostere **7** (25 mg, 0.045 mmol), HOBT (8 mg, 0.054 mmol), EDC (10 mg, 0.054 mmol), and acid **21** (23 mg, 0.05 mmol) gave the TBS-protected inhibitor. Exposure to TBAF followed by column chromatography (5% methanol in chloroform) yielded inhibitor **22** as a white solid in 41% yield (11 mg). ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ 0.76 (d, J = 7 Hz, 3H), 0.81 (d, J = 7 Hz, 3H), 0.91−0.97 (m, 9H), 1.11 (d, J = 7 Hz, 12H), 1.36−1.41 (m, 1H), 1.49−1.56 (m, 1H), 1.59−1.73 (m, 5H), 1.83−1.87 (m, 1H), 2.68 (q, J = 7 Hz, 1H), 3.32−3.40 (m, 4H), 3.63−3.66(m, 1H), 3.91 (d, J = 8 Hz, 1H), 3.94−4.00 (m, 1H), 4.16−4.18 (m, 1H), 7.50 (t, J = 8 Hz, 1H), 7.92 (d, J = 8 Hz, 1H), 8.01 (d, J = 8 Hz, 1H), 8.28 (s, 1H). MS-ESI (*m/z*): $[M + Na]^+$ 555.20. HRMS $[M + Na]^+$ calcd for $C_{29}H_{48}N_4NaO_5$ 555.3522, found 555.3539.

Preliminary in Vivo Inhibition of Aβ Production by Inhibitor 5d. Inhibitor **5d** was dissolved in DMSO to 4 mg/mL and diluted into an equal volume of Solutol HS-15/propylene glycol (1:2). The resulting solution was diluted further with an equal volume of H_2O, to a final concentration of 1 mg/mL **5d**. Tg2576[17] female mice age 3 months were injected intraperitoneally with 8 mL/kg of **5d** formulated as above (8 mg/kg/bw dose). Animals were housed at the Oklahoma Medical Research Foundation, and procedures adhered to IACUC guidelines to minimize stress to experimental subjects. Blood was sampled from the saphenous vein prior to injection in lithium-heparin Microvette tubes (Sarstedt). Mice in two groups of three each were sampled at 4 h postinjection. Three control mice, receiving vehicle alone (8 mL/kg), were likewise injected and their blood sampled. Plasma was separated by centrifugation at 1000*g* and stored at −70 °C. Aβ_{40} was analyzed by sandwich ELISA (Invitrogen). Aβ_{40} was expressed relative to baseline level for each individual animal to allow utilization of smaller cohorts of animals. Data is reported as average ± SEM (standard error of the mean) and analyzed by Student's *t*-test using a critical value of 0.05.

Acknowledgment. Financial support by the National Institutes of Health (AG 18933) is gratefully acknowledged.

Supporting Information Available: HPLC and HRMS data for compounds **5a**−**g** and Figure 5. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Scarpini, E.; Scheltens, P.; Feldman, H. Treatment of Alzheimer's Disease: Current Status and New Perspectives. *Lancet Neurol.* **2003**, *2*, 539−47.

(2) (a) Vassar, R.; Martin, C. Aβ-generating enzymes: recent advances in β- and γ-secretase research. *Neuron* **2000**, *27*, 419−22. (b) Selkoe, D. J. Translating Cell Biology into Therapeutic Advances in Alzheimer's Disease. *Nature* **1999**, *399A*, 23−31.

(3) (a) Sinha, S.; Lieberburg, I. Cellular Mechanisms of β-Amyloid Production. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049−53. (b) Potter, H.; Dressler, D. The Potential of BACE Inhibitors for Alzheimer's Therapy. *Nature* **2000**, *18*, 125−26.

(4) (a) Ghosh, A. K.; Hong, L.; Tang, J. β-Secretase as a Therapeutic Target for Inhibitor Drugs. *Curr. Med. Chem.* **2002**, *9*, 1135−44. (b) John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Heinrikson, R. L. Human β-Secretase (BACE) and BACE inhibitors. *J. Med. Chem.* **2003**, *46*, 4625−30. (c) Cumming, J. N.; Iserloh, U.; Kennedy, M. E. Design and Development of BACE-1 Inhibitors. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 536−56.

(5) (a) Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. Human Aspartic Protease Memapsin 2 Cleaves the β-Secretase Site of β-Amyloid Precursor Protein. *Proc. Natl. Acad. Sci, U. S. A.* **2000**, *97*, 1456−60. (b) Vassar, R.; Bennettt, B. D.; Babu-Khan, S.; Khan, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J. Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. β-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science* **1999**, *286*, 735−41 and references cited therein.

(6) (a) Ermolieff, J.; Loy, J. A.; Koelsch, G.; Tang, J. Proteolytic Activation of Recombinant Pro-memapsin 2 (Pro-β-secretase) Studied with new Fluorogenic Substrates. *Biochemistry* **2000**, *39*, 12450−

56. (b) Turner, R. T., III; Koelsch, G.; Hong, L.; Castanheira, P.; Ghosh, A. K.; Tang, J. Subsite Specificity of Memapsin 2 (β-Secretase): Implications for Inhibitor Design. *Biochemistry* **2001**, *40*, 10002−06.

(7) Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. Design of Potent Inhibitor for Human Brain Memapsin 2 (β-Secretase). *J. Am. Chem. Soc.* **2000**, *122*, 3522−3523.

(8) Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. Structure of the Protease Domain of Memapsin 2 (β-Secretase) Complexed with Inhibitor. *Science* **2000**, *290*, 150−53.

(9) (a) Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. Structure-Based Design: Potent Inhibitors of Human Brain Memapsin 2 (β-Secretase). *J. Med. Chem.* **2001**, *44*, 2865−68. (b) Ghosh, A. K.; Devasamudram, T.; Hong, L.; DeZutter, C.; Xu, X.; Weerasena, V.; Koelsch, G.; Bilcer, G.; Tang, J. Structure-Based Design of Cycloamide-Urethane-Derived Novel Inhibitors of Human Brain Memapsin 2 (β-Secretase). *Bioorg. Med. Chem. Lett.* **2005**, *15*, 15−20.

(10) Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Lei, H.; Hussain, K. A.; Liu, C.; Devasamudram, T.; Weerasena, V.; Turner, R.; Koelsch, G.; Bilcer, G.; Tang, J. Design Synthesis and X-ray Structure of Protein-Ligand Complexes: Important Insight into Selectivity ofMemapsin 2 (β-Secretase) Inhibitors. *J. Am. Chem. Soc.* **2006**, *128*, 5310−11.

(11) Turner, R. T.; Loy, J. A; Nguyen, C.; Devasamudram, T; Ghosh, A. K; Koelsch, G.; Tang, J. Specificity of Memapsin 1 and Its Implications on the Design of Memapsin 2 (β-Secretase) Inhibitor Selectivity. *Biochemistry* **2002**, *41*, 8742−46.

(12) Diment, S.; Leech, M. S.; Stahl, P. D. Cathepsin D is membrane-associated in macrophage endosomes. *J. Biol. Chem.* **1988**, *263*, 6901−07.

(13) Horn, R. K.; Gailunas, M.; Fang, L. Y.; Tung, J. S.; Walker, D. D.; Thorsett, E. D.; Jewette, N. E.; Moon, J. B.; Varghese, J. Design and Synthesis of Hydroxyethylene-Based Peptidomimetic Inihibitors of Human β-Secretase. *J. Med. Chem.* **2004**, *47*, 158−164.

(14) (a) Coburn, C. A.; Stachel, S. J.; Li, Y. M.; Rush, D. M.; Steele, T. G.; Jones, Chen-Dodson, E.; Holloway, M. K.; Xu, M.; Huang, Q.; Lai, Ming-Tian; DiMuzio, J.; Crouthamel, M. C.; Shi, X. P.; Sardana, V.; Chen, Z.; Munshi, S.; Kuo, L.; Makara, G. M.; Annis, D. A.; Tadikonda, P. K.; Nash, H. M.; Vacca, J. P.; Wang, T. Identification of a Small Molecule Nonpeptide Active Site β-Secretase Inhibitor That Displays a Nontraditonal Binding Mode for Aspartyl Proteases. *J. Med. Chem.* **2004**, *47*, 6117−6119. (b) Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, Ming-Tain; Crouthamel, M. C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X. P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. Structure-Based Design of Potent and Selective -Secretase (BACE-1). β-Cell-Permeable Inhibitors of Human *J. Med. Chem.* **2004**, *47*, 6447−50.

(15) Kukla, M. J.; Fortunato, J. M. Oxygenated analogs of 4-[(1H-imidazol-4-yl)methyl]-2,5-dimethyloxazole. *J. Org. Chem.* **1984**, *49*, 5003−06.

(16) Chang, W. P.; Koelsch, G.; Wong, S.; Downs, D.; Da, H.; Weerasena, V.; Gordon, B.; Devasamudram, T.; Bilcer, G.; Ghosh, A. K.; Tang, J. *J. Neurochem.* **2004**, *89*, 1409−16.

(17) Compound **24** is reported[14b] to have following profile: BACE-1 IC_{50} 15 nM; BACE-2 (memapsin 1) IC_{50} 230 nM and cellular IC_{50} 29 nM.

(18) Hsiao, K.; Chapman, P.; Nilsen, S.; Eckman, C.; Harigaya, Y.; Younkin, S.; Yang, F.; Cole, G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **1996**, *274*, 99−102.

(19) Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. Apo and inhibitor complex structures of BACE (β-secretase). *J. Mol. Biol.* **2004**, *343*, 407−16.

(20) Hong, L.; Turner, R. T., 3rd; Koelsch, G.; Shin, D.; Ghosh, A. K.; Tang, J. Crystal structure of memapsin 2 (beta-secretase) in complex with an inhibitor OM00-3. *Biochemistry* **2002**, *41*, 10963−67.

(21) Coordinates and structure factors for the memapsin2-inhibitor **5d** complex were deposited in PDB with the accession code 2P4J.

JM061338S