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Rational design and synthesis of selective BACE-1 inhibitors☆

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Abstract—An effective approach for enhancing the selectivity of β-site amyloid precursor protein cleaving enzyme (BACE 1) inhibitors is developed based on the unique features of the S1′ pocket of the enzyme. A series of low molecular weight (<600) compounds were synthesized with different moieties at the P1′ position. The selectivity of BACE 1 inhibitors versus cathepsin D and renin was enhanced 120-fold by replacing the hydrophobic propyl group with a hydrophilic propionic acid group.

β-site amyloid precursor protein cleaving enzyme (BACE 1, also called memapsin 2, Asp 2), a type 1 membrane bound aspartyl protease, has been demonstrated to be the major β-secretase responsible for the β-site cleavage of amyloid precursor protein (APP) to generate a C-99 fragment.[1] The C-99 peptide is subsequently processed by γ-secretase to release Aβ40/42 peptides, which are the major components of amyloid plaques, a hallmark of Alzheimer's Disease (AD).[2] Tissue distribution studies have shown that BACE 1 is predominantly expressed in the brain and pancreas, where the protein in pancreas is an inactive BACE 1 spliced form.[3] BACE 1 deficient mice have been shown to be phenotypically normal and fertile.[4] More importantly, Aβ40 and 42 are not detectable in either BACE 1 deficient mouse brain or embryonic cortical neurons.[1,4] These unique features render BACE 1 an ideal therapeutic target for AD.

Consequently, since the discovery of BACE 1,[5] intensive efforts have focused on the synthesis of the enzyme inhibitors that possess appropriate properties for drug development, such as good potency, small size, and high selectivity.[6] A number of statine-containing compounds have shown good enzymatic[7] and cellular potency,[8] but the selectivity versus related aspartyl proteases has been poor.[7a] BACE 1 selectivity is important since the target enzyme resides in the brain, where closely related ubiquitous enzymes cathepsin D (Cat D) and renin can be found. BACE 1 inhibitors with poor selectivity against these enzymes are likely to cause undesired side effects.

To address this concern, we compared the X-ray structures of renin,[9] Cat D,[10] and BACE 1[11] and noticed significant differences in the length and sequence of the loop defining the S1′/S3′ pocket (Fig. 1). The BACE 1 loop is truncated (like the fungal aspartyl proteases)[12] and primarily hydrophilic, whereas the renin and Cat D loops are extended and primarily hydrophobic. In addition, Arg235 (numbering based on the mature form), which resides between the P1′ and P2 regions, is unique to BACE 1 (Val233 in Cat D and Ser222 in renin) and may contribute to the preference for a P1′ Asp in the BACE 1 substrate APP.[13] Based on this analysis, it seemed reasonable that the selectivity of a BACE 1 inhibitor could be greatly enhanced by increasing the polarity of the side chain at the P1′ position.

To test this hypothesis, side chains with different polarities were placed at the P1′ position of a BACE 1 inhibitor discovered via targeted screening of compounds similar to known BACE 1 inhibitors (Sta-Val[14] and

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OM99-2[7]), but different from Pepstatin A which does not inhibit BACE 1 at high concentration (>1 mM). As shown in Scheme 1, the protected intermediates **7**, **8**, and **10** were synthesized starting from lactones **1** and **2**.[7,15] Alkylation of lactones **1** or **2** with allyl iodide in the presence of LHMDS proceeded in good yield and afforded the predominantly kinetically controlled *trans* isomers, **3** or **4** in 72–79% yield. The other isomer (*cis*)

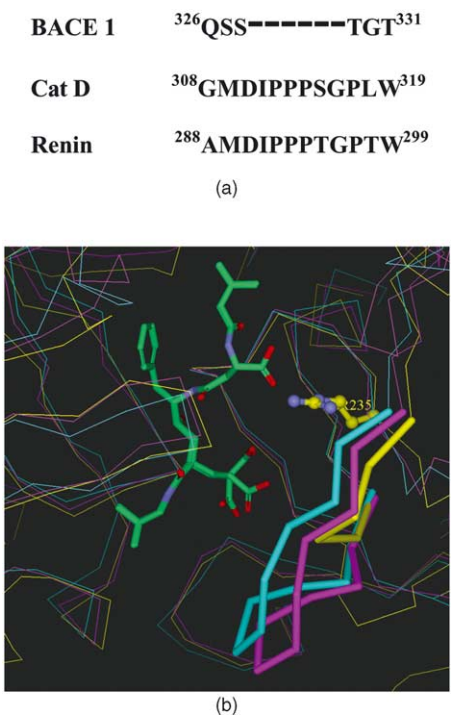
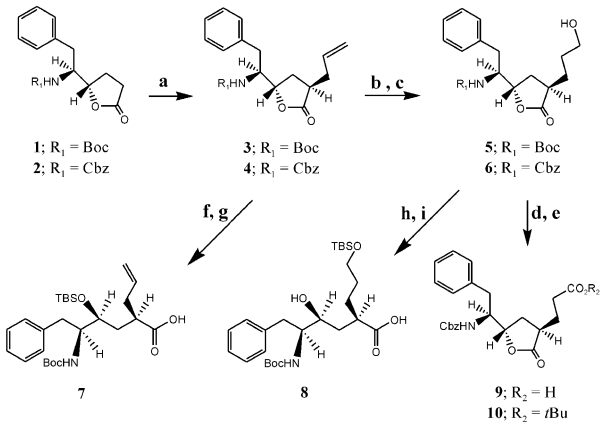


Figure 1. (a) Sequence comparison of the BACE 1, Cathepsin D, and renin S1′/S3′ loop derived from an overlay of the crystal structures 1FKN, 1LYB, and 1HRN, respectively. Numbering is taken from the crystal structure. Note the 6-residue deletion in BACE 1. (b) Structure comparison of S1′/S3′ region of BACE 1 (yellow), Cathepsin D (magenta), and human renin (cyan). Arg235 (BACE 1) is shown in ball-and-stick format. Models of 3 low-energy poses for **17** which vary in the orientation of the P1′ side-chain are illustrated bound in the BACE 1 active site.



Scheme 1. Synthesis of the intermediates of BACE 1 inhibitors. Reagents and conditions: (a) LHMDS, THF, −78 °C, allyl iodide, 30 min; (b) $Rh(PPh_3)_3$, THF, catechol borane, rt, 90 min; (c) 50 mM KH_2PO_4, THF/EtOH, pH 7.2, 30% H_2O_2, rt, 12 h; (d) MeCN, CCl_4, $NaIO_4$, $RuCl_3$, H_2O, 2 h; (e) H_2SO_4, isobutylene, CH_2Cl_2/dioxane, 48 h; (f) 1N LiOH, 16 h; (g) TBSCl, imidazole, DMF, rt, 24 h; (h) TBSCl, imidazole, DMF, rt, 12 h; (i) NaOH/dioxane/H_2O 30 min.

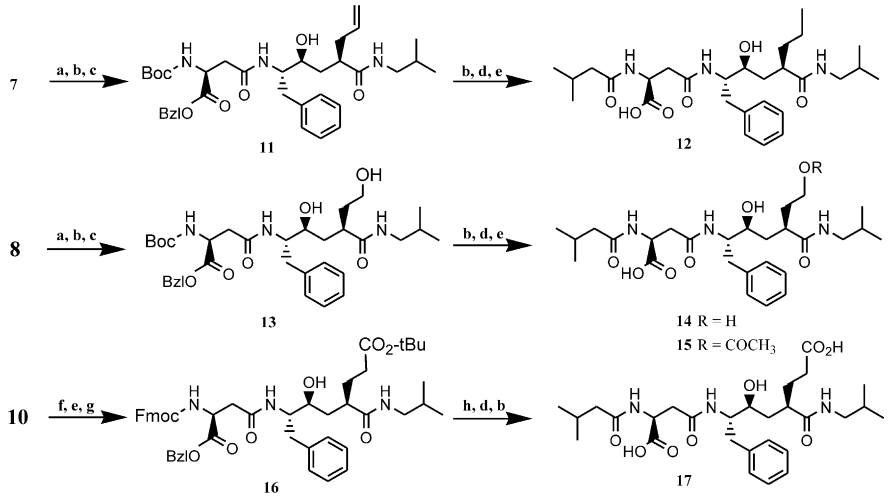
was also formed but only in <5% yield. The main side product in this reaction was, however, the dialkylated lactone, which presumably formed as a result of aggregation of anion.[16]

Lactones **3** and **4** were converted to primary alcohols **5** and **6**, respectively, in 70–73% yield via hydroboration with catecholborane in the presence of Wilkinson's catalyst and subsequent oxidation using H_2O_2 under neutral conditions.[17] Hydroboration in the absence of the rhodium catalyst gave poor yield due to competitive reduction of the γ-lactone to form a diol intermediate.[18] Lactone **3** was hydrolyzed with aq LiOH and silylated with TBSCl in the presence of imidazole to afford **7**, the synthetic precursor of compound **12**. Similarly, lactone **5** was silylated and then hydrolyzed to give compound **8**, the requisite intermediate for the synthesis of inhibitors **14**,**15**. Lactone **6** was subjected to a Sharpless oxidation in 76% yield to provide lactone **9**, which was selectively protected as a *tert*-Bu ester in 50% yield to furnish lactone **10**,[19,20] the intermediate required for the synthesis of **17** (Scheme 2).

Intermediates **7**, **8**, and **10** were subjected to standard deprotection and amide coupling procedures to yield compounds **12**–**17** with molecular weights less than 600. In the synthesis of analogue **12** the final hydrogenolytic cleavage of the benzyl ester after coupling of valeric acid to compound **11** proceeds with reduction of the side chain allyl double bond. In the route to analogue **14** the overall yield was significantly lowered by the formation of lactone in the amidation of intermediate **8** with isobutyl amine. In addition, the use of HCl in ethyl acetate in conversion of **13** resulted in formation about 50% of the *O*-acetyl by-product, which ultimately afforded analogue **15**. The problem of lactone formation was circumvented in the synthesis of **16** by direct aminolysis of the lactone precursor **10** with isobutyl amine, aspartic acid being incorporated as its Na-Fmoc derivative. The free amine generated upon treatment of **16** with piperidine could be coupled with valeric acid without the need for isolation, and treatment with TFA removed both the Boc and *t*-butyl ester moieties to afford analogue **17**. Compounds **14** and **17** also represent the two most functionalized hydroxyethylene isosteres at the P1′ position reported to date.

The ability of these four compounds to inhibit BACE 1, Cat D, and renin was evaluated (Table 1). A soluble truncated form of BACE 1 (1–460) was expressed in insect cells using Baculovirus and was then purified from a concanavalin A-Sepharose followed by a Sta-Val affinity column.[21] A fluorescence resonance energy transfer (FRET) substrate containing the Swedish sequence (KMDA→NLDA) was employed for monitoring the activity of the three enzymes.[22] The substrate appears to be cleaved efficiently by the enzymes at their optimum pH. K_i (app) values were calculated based on the initial rate of substrate cleavage in the absence or presence of inhibitors at different concentrations.

As shown in Table 1, compound **12** displays greater potency for inhibiting both Cat D and renin than BACE



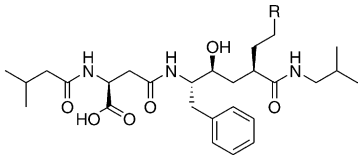
Scheme 2. Synthesis of BACE 1 inhibitors. Reagents and conditions: (a) isobutyl amine/DMF, EDT–HoBt, NMM, 20 h; (b) HCl/EtOAc, −20 °C to 0 °C, 20 min; (c) Na-Boc-L-aspartica-benzyl ester/DMF, EDT–HOBt, NMM, 20 h; (d) isovaleric acid/DMF, EDT–HOBt, NMM, 20 h; (e) H2-10% Pd(C)/EtOH, 2 h; (f) isobutylamine/THF, 2-hydroxypyridine, 72 h; (g) Na-Fmoc-L-aspartic a-*t*-butyl ester/DMF, EDT–HOBt, NMM, 20 h; (h) 30% piperidine/DMF, 10 min.

1. The addition of an ω-hydroxyl group to the P1′ side-chain of **12** yields inhibitor **14** and results in a 36-fold increase in potency against BACE 1 but only a 2-fold increase in potency versus Cat D and renin. Interestingly, the acetyl derivative (**15**) maintains BACE 1 potency but shows approximately 5-fold increase in the potency for Cat D and renin, thus diminishing its selectivity. Furthermore, replacement of the hydroxyl moiety with a carboxylic acid functional group (**17**) enhances Cat D selectivity from 6-fold to 47-fold as well as renin selectivity from 6-fold to 44-fold while maintaining BACE 1 potency. Overall, the selectivity of this series of BACE 1 inhibitors against Cat D and renin is enhanced approximately 120-fold by simply replacing the hydrophobic propyl group with a hydrophilic propionic acid group at the P1′ position. These results suggest that the selectivity of **17** is attributable to the carboxylic acid at P1′ and that it may have a favorable interaction with Arg235 or the more hydrophilic environment of the truncated S1′/S3′ loop of BACE 1 (Fig. 1).

Interestingly, these modifications did not offer any selectivity against BACE 2 (a homologue of BACE 1[23]). This is not surprising because BACE 2 also possesses the truncated S1′/S3′ loop as well as the Arg232 between P1′ and P2 pockets. On the other hand, we expect these compounds will display good selectivity against pepsin and cathepsin E because they are in the same family as Cat D and renin, which have the extended S1′/S3′ loop and Thr replacing the Arg235.[3]

This study demonstrates that selective BACE 1 inhibitors can be achieved based on the unique features of the BACE 1 S1′ pocket. This knowledge should facilitate the discovery of small, potent, and highly selective BACE 1 inhibitors.

Table 1. Selectivity and potency of BACE 1 inhibitors



	R_1	BACE 1 K_i (μM)	Cat D K_i (μM)	Renin K_i (μM)
12	$-CH_3-$	69.5±9.3	28.0±3.2	24.4±0.8
14	CH_2OH	1.9±0.1	12.5±0.2	10.9±0.8
15	CH_2OAc	2.7±0.4	2.2±0.2	1.9±0.1
17	CO_2H	1.7±0.1	79.7±6.7	73.6±8.2

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