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Design of potent inhibitors of human β-secretase. Part 2

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Abstract—We describe an optimized series of acyclic hydroxyethylamine transition state isosteres of β-secretase that incorporates a variety of P_2 side chains that yield potent inhibitors with excellent cellular activity. A 2.2 Å crystal structure of compound **13** is shown.

In our previous communication,[1a] and from earlier work,[1e] we described the discovery of a potent series of BACE inhibitors that utilize a hydroxyethyl amine transition state isostere which imparts a much better cell to enzyme IC_{50} ratio relative to other transition state isosteres previously described as potent BACE enzyme inhibitors (hydroxyethylene and statine BACE inhibitors).[2] Moreover, we also described how we were able to increase potency by occupying the S_2 site from our earlier leads which only occupied S_3, S_1, and $S_{2'}$ in the active site of the BACE enzyme. This communication will outline the synthesis of acyclic sulfones that incorporate 3,5-difluorophenylalanine at P_1. It has already been described how this key P_1 residue imparted increased potency to a series of N-terminal isophthalate hydroxyethyl amine BACE inhibitors.[1b,1c,1d] We will also describe P_2 replacements for the benzyloxy carbonyl (Cbz) group described in the previous paper that retain good potency against BACE and, more importantly, give improved selectivity for BACE versus Cathepsin-D.

Incorporation of the 3,5-difluorophenylalanine into the chiral α-Cbz heptyl sulfone yielded an inhibitor, **9**, that had an $IC_{50} = 3$ nM against BACE and an $IC_{50} = 20$ nM for Cathepsin D (vs 112 nM IC_{50} for BACE and 67 nM IC_{50} for Cat-D with racemic heptyl sulfone that incorporated Phe at P_1).[1a] The synthesis of the racemic Cbz acid was described in the previous paper. Chiral separation afforded the desired *S*-isomer that was employed exclusively in this communication.[3] As mentioned in the previous paper the *S*-isomer of the racemic Cbz compound was the only observed isomer that co-crystallized in the presence of BACE. Also noteworthy was the replacement of the *m*-iodo group with the *m*-ethyl with no loss in enzyme or cell potency. This was particularly useful in keeping the molecular weight of the desired inhibitors as low as possible.

In Figure 1, we show the general route to a variety of amide and carbamate analogs that were rapidly prepared utilizing standard transformations. These amide and carbamate side chains project into the S_2 subsite of BACE. Tables 1 and 2 illustrate the IC_{50}s against BACE and Cat-D as well as the BACE cellular activity in a HEK293[4] assay for a select group of analogs. In general the SAR was fairly flat with good cell to enzyme ratios and, with the exception of entries **4**, **6**, and **9**, good selectivity for Cathepsin-D.

Keywords: β-Secretase; Hydroxyethylamine (HEA) isostere.
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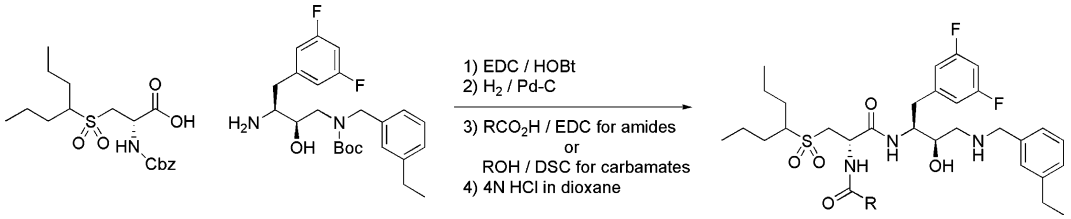
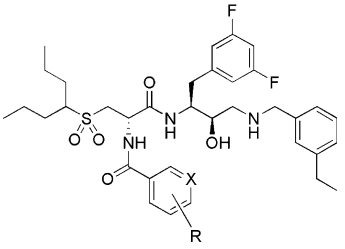


Figure 1. Synthesis of desired inhibitors.

Table 1. Data for selected P-2 amides



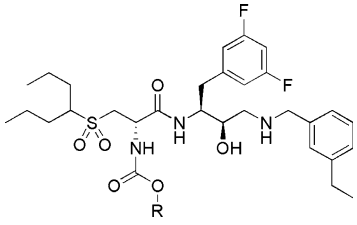
Compound	X	R	Bace IC_{50} (nM)	Cat-D IC_{50} (nM)	HEK EC_{50} (nM)
1	C	H	2	495	14
2	C	*p*-Me	4	396	19
3	C	*p*-OMe	2	320	11
4	C	*p*-CF_3	53	316	76
5	C	*m*-Cl	5	271	48
6	C	*o*-Me	15	404	59
7	C	*m*-OH	2	150	9
8	N	H	1	186	1

From modeling and crystallographic data we knew that the P_2 pocket of Cathepsin-D was more lipophilic than BACE. Therefore, analogs were prepared with increasing polar functionality in the P_2 region to try and increase selectivity against Cat-D over the original Cbz lead molecule. We also investigated the introduction of ureas and sulfonamides into P_2 and observed poor cell to enzyme ratios for urea containing analogs and poor selectivity against Cat-D for sulfonamides (data not shown).

In our previous paper we described, based on the crystal structure of the corresponding P_1 phenylalanine analog how the Cbz group extends into the S_2 subsite past Arg 235 toward Gln73, and thus explaining the lack of selectivity toward Cat-D. Interestingly small changes within the distal portion of the Cbz (i.e., from benzyl, to *m*-CH_2 and *p*-CH_2-pyridyl, **9**–**11**) yielded inhibitors with improved Cat-D selectivity. This helped confirm our hypothesis that the S_2 pocket of Cat-D is more lipophilic and less tolerant of the introduction of polarity into that particular subsite relative to BACE. See Table 2.

Additional crystal structures of a variety of amide and carbamate inhibitors bound to BACE showed the amide/carbamate side chain oriented into P_2 as expected.[5] It was felt that this additional interaction with the protein might allow for truncation of the P_3 heptyl moiety and could provide a means to reduce molecular weight and alter physiochemical properties without negatively impacting potency and selectivity.

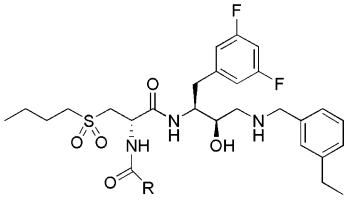
Table 2. Data for selected P_2 heptyl carbamates



Compound	R	Bace IC_{50} (nM)	Cat-D IC_{50} (nM)	HEK EC_{50} (nM)
9	Benzyl	3	20	ND
10	CH_2-3-pyridyl	4	369	5
11	CH_2-4-pyridyl	3	158	5
12	Me	3	126	2

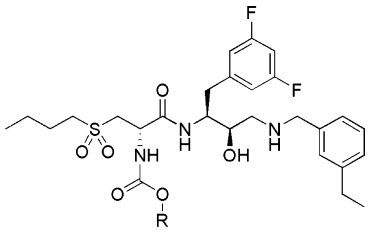
Tables 3 and 4 contain a series of truncated butyl amides and carbamates that were prepared via an analogs sequence to the heptyl analogs shown in Figures 1 and 2. Chiral chromatography yielded the preferred *S*-isomer which has been utilized in all of the reported inhibitors in this communication.

Table 3. Butyl sulfone amides



Compound	R	BACE IC_{50} (nM)	Cat-D IC_{50} (nM)	HEKcell (nM)
13	3-Pyridyl	2	474	1
14	Me	13	360	6
15	CH_2OMe	22	1535	4
16	*c*-Propyl	10	446	4
17	4-Pyridyl	5	1260	1
18	5-Me-pyrazole-3-yl	4	341	4
19	2-Pyrazinyl	4	133	0.2
20	2-Imidazoyl	4	606	4
21	4-OH-3-pyridyl	5	770	24

Table 4. Butyl sulfone carbamates



Compound	R	BACE IC_{50} (nM)	Cat-D IC_{50} (nM)	HEKcell (nM)
22	Me	4	171	2
23	CH_2CH_2CN	8	205	5
24	CH_2CF_3	4	19	ND
25	*S*-3-THF	15	436	4
26	CH_2CH_2NAc	27	935	12
27	CH_2-3-pyridyl	10	444	0.6
28	CH_2-4-pyridyl	11	383	2

As in the heptyl series, the butyl analogs are potent and exhibit varying degrees of selectivity against Cat-D with good BACE cell activity. A variety of P_2 side chains are tolerated, and as expected, an increase in the polarity of the P_2 substituent leads to an increase in selectivity against Cat-D (i.e., compounds **13**, **17**, **20**, and **21** and the *N*-acetyl substituted carbamate **26**). However, the results of compound **21** suggest that if the P_2 substituent becomes too polar, cell activity may decrease. Conversely if one puts in very lipophilic P_2 side chains Cat-D activity can be easily restored (i.e., **24**) thus eliminating the desired selectivity.

Apparently the additional protein interaction afforded by occupying S_2 and the concurrent potency increase allow for the heptyl sulfone to be truncated to a butyl sulfone without being detrimental to BACE potency and Cat-D selectivity. As an example, the butyl counterpart, **13**, of the 3-pyridyl heptylamide, **8**, had an $IC_{50} = 2$ nM for BACE, an $IC_{50} = 474$ nM for Cat-D, and an $EC_{50} = 1$ nM in the HEK cell assay versus 1, 186, and 1 nM, respectively. It is also interesting to note that both the amide and carbamate series gain significant potency, typically ~100×, versus the butyl sulfone with no P_2 side chain which has an $IC_{50} = 215$ nM for BACE and 6174 nM for Cat-D, underscoring the importance of occupying the S_2 site in BACE (structure not shown).

Along with a reduction in molecular weight, truncation to the butyl sulfone lowered both clog *D* and clog *P* significantly. For example, comparison of amide **8** (clog *D* = 5.85, clog *P* = 5.52) to amide **13** (clog *D* = 4.04, clog *P* = 4.16) as well as carbamate **10** (clog *D* = 5.76, clog *P* = 5.43) to carbamate **27** (clog *D* = 4.3, clog *P* = 4.06) illustrates this trend. Another advantage in utilizing a P_3 butyl in place of heptyl was an observed increase in selectivity against BACE-2. The heptyl carbamate **12** was very similar to its butyl counterpart **22** in terms of BACE-1 potency, Cat-D selectivity, and cell activity. However when these compounds were tested against BACE-2 **12** had an $IC_{50} = 570$ nM while **22** had an $IC_{50} = 2530$ nM.[6]

From earlier crystal structures[1a–1d] and modeling we knew that the potential to interact with the Arg235 in the S_2 subsite of BACE may afford very potent BACE inhibitors with good selectivity against Cat-D. This hypothesis was based on the presence of two methionine residues in Cat-D (Met307 and Met309) that help form the S_2 pocket in contrast to Arg 235 found in BACE.[7] As shown in Tables 1–4 we were able to fine-tune selectivity based on the physical properties and size of the S_2 side chain. To further confirm our original hypothesis, a 2.2 Å X-ray crystal structure of recombinant human BACE expressed and purified from *Escherichia coli*[8,9] and co-crystallized in the presence of **13** was obtained. This structure reveals a close association between the 3-pyridyl nitrogen and Arg235 in S_2 (orientation of the pyridine nitrogen is confirmed by the presence of a water molecule hydrogen bonded to the pyridine nitrogen). Although the interaction geometry does not permit a hydrogen bond, the partial negative charge of the pyridine is complemented by the positively charged arginine providing a basis for the specificity of this compound for BACE over Cat-D. In addition, there is a key water molecule that is hydrogen bonded between the hydroxyl side chains of Thr231 (2.8 Å) and Thr72 (3.2 Å) which is observed within hydrogen bonding distance to the carbonyl oxygen of the amide (2.6 Å). This water mediated interaction likely accounts for the significant gain in potency of the amide versus the butyl sulfone with no P_2 side chain.

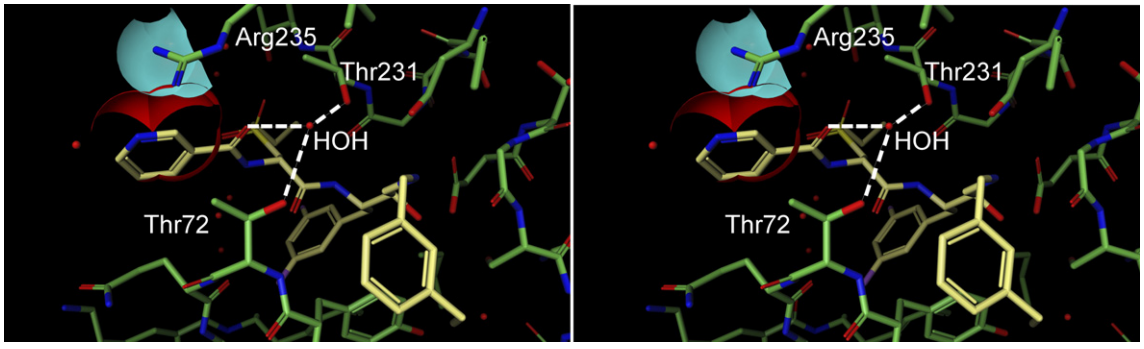


Figure 2. A stereoview of the 2.2 Å structure of **13** bound to BACE showing the close approach of the pyridine to Arg235 using van der Waals surfaces (red and cyan). The water molecule involved in the water mediated hydrogen bond to the amide carbonyl is also shown.

In summary, we have described a series of very potent and selective inhibitors of BACE that show excellent selectivity against Cat-D with good cellular potency. By occupying the S_2 subsite and introducing a water mediated hydrogen bond we achieve a ~100× increase in potency both in the enzyme assay as well as in the HEK cell assay.[1e] Further aspects of the development of several of these inhibitors will be reported in the future.

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5. Unpublished results of Dr. T.E.B.
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