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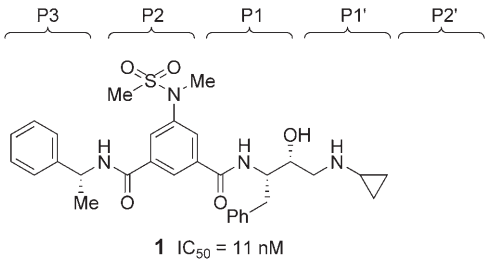
Design and Synthesis of 2,3,5-Substituted Imidazolidin-4-one Inhibitors of BACE-1

James C. Barrow,*[a] Kenneth E. Rittle,[a] Phung L. Ngo,[a] Harold G. Selnick,[a] Samuel L. Graham,[a] Steven M. Pitzenberger,[a] Georgia B. McGaughey,[b] Dennis Colussi,[c] Ming-Tain Lai,[c] Qian Huang,[c] Katherine Tugusheva,[c] Amy S. Espeseth,[c] Adam J. Simon,[c] Sanjeev K. Munshi,[d] and Joseph P. Vacca[a]

Alzheimer's disease is a slowly progressing neurodegenerative condition that is increasing in prevalence because of the ageing population and is a significant healthcare burden.[1] Although the pathophysiology of the disease has not been completely elucidated, abnormal production and/or clearance of a small peptide called Aβ has been implicated from genetic and other studies.[2] The Aβ peptide arises from proteolytic processing of the APP protein, first by β-secretase followed by γ-secretase. Based on these observations, β-secretase (BACE-1) has been identified as a promising drug target for disease-modifying therapy and has attracted significant attention from the medicinal chemistry community.[3]

BACE-1 is an aspartyl protease with a site of action inside the CNS and thus represents a challenging target. Hydroxyethylamine (HEA) transition state isosteres are well-known inhibitory motifs for aspartyl proteases, and prior work from these laboratories identified **1** (Figure 1) as a potent inhibitor of BACE-1.[4] Whereas this compound has good enzyme potency (IC_{50} = 11 nM) and cell activity (sAPPβ_NF = 29 nM),[5] molecular weight is still high (578 Daltons) and the compound is a P-glycoprotein transporter (PGP) substrate with poor brain penetration. To improve brain-penetration properties, truncation of the structure and removal of hydrogen bond donors and acceptors was deemed necessary.[6] One approach is to further optimize the P_3-P_1 portion and eliminate the hydroxyethyl amine.[7] The work described in this report instead focuses on prime side modifications with the goal of improving potency sufficiently to allow removal of the P_2P_3 portion of the inhibitor.



1 IC_{50} = 11 nM

Figure 1. Prototype BACE-1 inhibitor.

Closer examination of the X-ray crystal structure of **1** in the BACE-1 active site shows that the basic amine makes hydrogen-bond contacts to both Asp 228 and the Gly 34 carbonyl (Figure 2), and SAR studies have shown that primary and secondary amines are preferred over tertiary amines in this position. Our design then focused on incorporation of the amine functionality in a ring,[8] and preliminary modeling[9] of various heterocycles in the BACE-1 active site suggested an imidazolidin-4-one as an attractive scaffold because the carbonyl is poised to make a hydrogen bond contact with Thr 72 on the flap region. Additionally, substituents on the 2 and 3-positions on imidazolidin-4-one ring are poised to enter the S1′ and S2′ pockets of the enzyme, and the modular nature of the ring allows rapid modification of substituents for defining the SAR of the series.

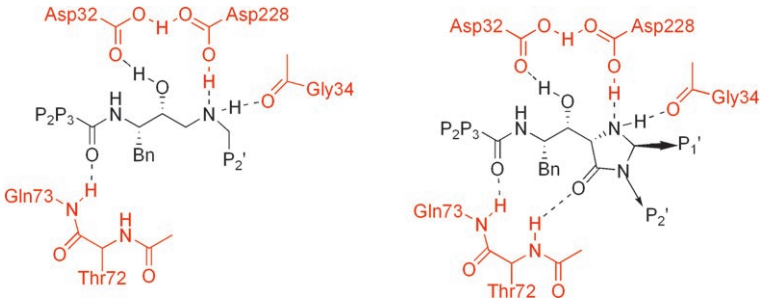


Figure 2. Design of prime-side HEA analogues.

The high density of stereogenic centers in these inhibitors poses synthetic challenges, especially as initial models suggested that the P1′ group originating on the 2-position of the imidazolidin-4-one ring needs to be *trans* across the ring for optimal interaction with the S1′ pocket of the enzyme. Aldol reactions of imidazolidin-4-ones have been explored extensively by Seebach,[10] and examination of Zimmerman–Traxler transition states between an imidazolidin-4-one enolate and the bis-benzyl protected alanal of Reetz **3**[11] predicts the desired stereochemical outcome (Scheme 1). An alternate approach that would allow for late-stage introduction of the P1′ and P2′

[a] *Dr. J. C. Barrow, K. E. Rittle, P. L. Ngo, Dr. H. G. Selnick, Dr. S. L. Graham, Dr. S. M. Pitzenberger, Dr. J. P. Vacca*
Department of Medicinal Chemistry
Merck Research Laboratories
P.O Box 4, WP14-1, West Point, PA 19486 (USA)
Fax: (+1) 215-652-6345
E-mail: james_barrow@merck.com

[b] *G. B. McGaughey*
Department of Molecular Systems
Merck Research Laboratories, West Point, PA (USA)

[c] *D. Colussi, Dr. M.-T. Lai, Q. Huang, K. Tugusheva, Dr. A. S. Espeseth, Dr. A. J. Simon*
Department of Alzheimer's Research
Merck Research Laboratories, West Point, PA (USA)

[d] *Dr. S. K. Munshi*
Department of Structural Biology
Merck Research Laboratories, West Point, PA (USA)

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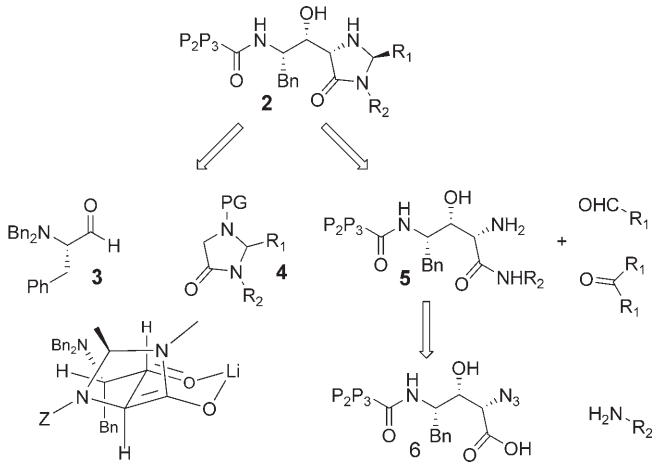
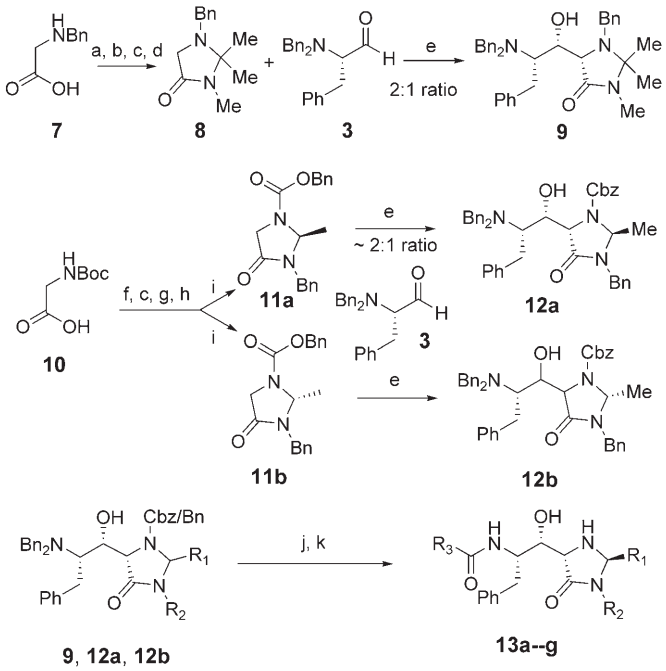


Scheme 1. Retrosynthesis of inhibitors.



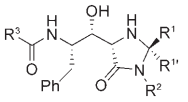
Scheme 2. Reagents: a) Boc_2O; b) $MeNH_2$, EDC; c) HCl; d) acetone, MeOH; e) LDA, THF, −78 °C; f) $BnNH_2$, EDC; g) CH_3CHO; h) Cbz-Cl; i) Chiral HPLC; j) H_2, $Pd(OH)_2$, MeOH; k) R_3CO_2H, BOP, iPr_2NEt or R_3COCl, Et_3N.

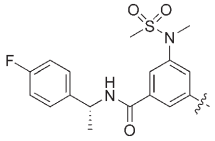
groups is cyclization of amino-amide precursor **5**, although control of stereochemistry across the ring during cyclization is not precedented to be high.[12] With this strategy, the key stereochemical relationships of the 4-position of the imidazolidin-4-one can be established using well-precedented Sharpless asymmetric dihydroxylation conditions.[13]

We initially chose the Seebach aldol approach and began with synthesis of the simplified imidazolidin-4-one **8** as shown in Scheme 2. Aldol coupling with aldehyde **3** proceeded in modest yield (~20%) to give two of the possible 4 diastereomers in a 2:1 ratio. Imidazolidin-4-ones **11a** and **11b** were separated by chiral HPLC and subjected to aldol conditions to give adducts **12a** and **12b**. Diastereoselectivity was difficult to determine because of the modest yield (10–20%) and the propensity of the hydroxyl group to cyclize on the benzyl carbamate. The stereochemistry of the major adduct **9** was determined by X-ray crystallography of **13a** bound in the BACE-1 active site (vide infra), whereas **12a** was assigned by correlation with **22**. No assignment was made for **12b** and derivatives made from **12b** were inactive. Hydrogenation of the aldol adducts and coupling the resulting primary amine with various P_2-P_3 acids[14] or acid chlorides afforded the inhibitors **13a–g** shown in Table 1.

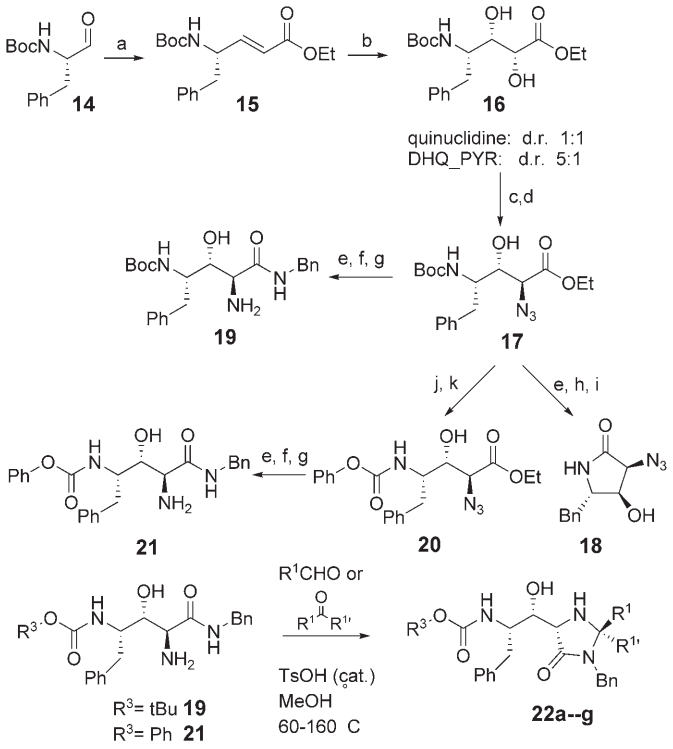
Late stage introduction of the P1′ and P2′ groups was accomplished as outlined in Scheme 3. Sharpless asymmetric dihydroxylation of **15** with the DHQ_PYR ligand[15] provided diol **16** in a 5:1 ratio of diastereomers. The resident stereocenter in **15** did not impart any bias to the dihydroxylation event as determined by catalysis with the achiral quinuclidine ligand. Selective conversion of the hydroxyl to an azide[16] gave key compound **17** whose stereochemistry was confirmed by nOe analysis of lactam **18**. Further manipulation of **17** provided cyclization precursors **19** and **21**. Optimization of imidazolidin-4-one ring closure upon treatment of **19** with an aldehyde or ketone and catalytic acid showed that methanol was superior to THF, toluene, or CH_2Cl_2, and *p*-toluene sulfonic acid (TsOH) gave the most consistent results. *Cis*/*trans* selectivity appears to be under kinetic control with the *cis* product favored, especially with small aldehydes.[17] Separation of the *cis*/*trans* isomers and resubmitting the minor *trans* product to the aldehyde and TsOH in methanol at 60 °C overnight

Table 1. BACE-1 inhibition of compounds **13a–g** and **22a**.



Compd	R^3	R^2	R^1	$R^{1'}$	BACE-1 IC_{50}[a] [nM]	sAPPβ NF IC_{50}[b] [nM]
13a		Me	Me	Me	71	18000
13b		Bn	Me	Me	4	30
13c		Bn	Me	H	2.1	28
13d	*t*BuO-	Bn	Me	H	19000	NT
22a	*t*BuO-	Bn	H	Me	>50000[c]	NT
13e	PhO-	Bn	Me	Me	880	NT
13f	PhO-	Bn	Me	H	143	>20000
13g	$PhCH_2$-	Bn	Me	Me	8400	NT

[a] IC_{50} determinations were performed as described in [5] and were run $n>2$ with a $CV<0.3$ except where noted. [b] Cell based assay as described in [5], NT = not tested. [c] $n=1$.



Scheme 3. Reagents: a) $Ph_3P{=}CHCO_2Et$, toluene; b) $K_3Fe(CN)_6$, K_2CO_3, $K_2OsO_2(OH)_4$, DHQ-PYR, $CH_3SO_2NH_2$; c) Nos-Cl, Et_3N; d) NaN_3; e) LiOH, 0 °C, THF; f) $BnNH_2$, EDC, HOAt; g) H_2, Pd/C; h) HCl; i) EDC; j) HCl; k) PhOCOCl.

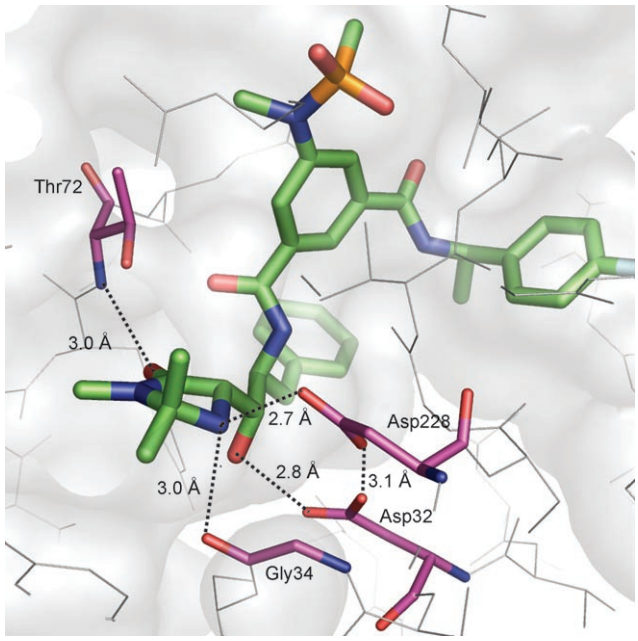


Figure 3. A view of the X-ray crystal structure of **13a** in the BACE-1 active site showing key hydrogen bond contacts between inhibitor and enzyme. The heavy atoms on the ligand/protein pair were used to calculate the hydrogen bonding distances as indicated by the dashed lines.

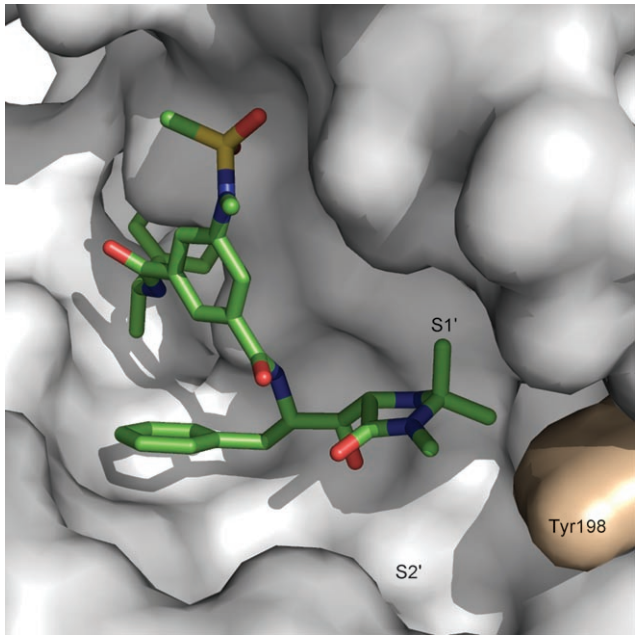


Figure 4. A view of the X-ray crystal structure of **13a** showing the surface of BACE-1 (without the flap residues). The S1′ and S2′ regions are indicated, as is the surface of Tyr 198 which is in close contact with the inhibitor.

showed no equilibration. The amount of the desired *trans* product can be increased by quickly superheating the reaction to 160 °C in a sealed vessel using a microwave reactor. The stereochemical outcome of the cyclization was determined by nOe analysis for **22**, and correlation between the two synthetic approaches was confirmed by comparison of **13d** from Scheme 2 with **22** from Scheme 3. The phenyl carbamate **21** could also be used in the cyclization reaction with similar selectivity results.

BACE-1 inhibition of the initial compounds prepared using Scheme 2 are shown in Table 1.[5] Compound **13a** contains a similar P_2P_3 region to lead compound **1** with only a small potency loss, confirming the imidazolidin-4-one as a reasonable scaffold. X-ray crystallographic analysis of **13a** in the BACE-1 active site[18] shows that the inhibitor binds as designed with the imidazolidin-4-one carbonyl making a contact with the flap and the protonated nitrogen of the heterocycle engaging in hydrogen bonds with Asp 28 and Gly 34 (Figure 3).[19] Further examination of the complex suggests improvements can be made as the *cis* methyl group $R^{1\prime}$ appears to be in a slightly unfavorable steric clash with the Tyr 198 residue, whereas the *trans* methyl group could be extended into S1′ (Figure 4). The S2′ pocket is not completely filled by the R^2 methyl group suggesting larger groups at this position of the imidazolidin-4-one ring. Accordingly, increasing the size of the P2′ group from methyl to benzyl (compound **13b**) results in more than tenfold improvement in potency, and removal of the undesired $R^{1\prime}$ group results in a further twofold enhancement in BACE-1 enzyme inhibitory potency to 2.1 nM for compound **13c**.

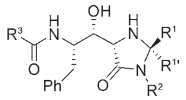
With the improved prime site substituents from **13c**, truncation of the structure to compounds of more reasonable molecular weight was attempted. Replacement of the entire P_2P_3 portion of the inhibitor with a *tert* butyl carbamate resulted in a potency loss of approximately five orders of magnitude (**13c**

versus **13 d**), but this could be used to screen new P1′ and P2′ groups (vide infra). Examination of a variety of other amides and carbamates revealed that inhibitors containing a phenyl carbamate were submicromolar.

Without the refined P_2P_3 "anchor" of compound **1**, stereochemistry at the 2-position of the imidazolidin-4-one was also important as seen in the potency difference between **22 a** and **13 d.** The most active combination **13 f** is only 13-fold worse than the original compound **1** with a molecular weight reduction of 119 Daltons.

With these results in hand, the chemistry outlined in Scheme 3 was used to probe the P1′ and P2′ portions of the inhibitor. As seen in Table 2, extension into the P1′ group led

Table 2. BACE-1 inhibition of compounds **13d** and **22b–i**.



Compd	R^3	R^2	R^1	$R^{1'}$	BACE IC_{50}[a] [nM]
13d	*t*BuO-	Bn	Me	H	19000
22b	*t*BuO	Bn	*i*Pr	H	11000
22c	*t*BuO-	Bn	Bu	H	2600
22d	PhO-	Bn	*i*Pr	H	480
22e	PhO-	Bn	Ph	H	880
22f	PhO-	Bn	Bu	Bu	>20000[b]
22 g	PhO-	Bn	cyclobutyl		6300
22 h	*t*BuO-	CH_2CH_2Ph	*i*Pr	H	>20000[b]
22i	*t*BuO-	pentyl	*i*Pr	H	15000

[a] IC_{50} determinations were performed as described in [5] and were run $n>2$ with a CV < 0.3 except where noted. [b] $n=1$.

to modest increases in potency with either *tert*-butyl or phenyl carbamates (entries **22 b**–**e**). Compounds derived from ketone cyclizations were less active (entries **22 f**–**g**). A small screen of diverse P2′ groups revealed benzyl to be favored along with medium-sized alkyl chains (for example, pentyl, entry **22 i**).

Whereas many of the imidazolidin-4-one compounds prepared in this study had good intrinsic enzyme inhibition as measured in cell-free systems, most had weak activity in the cell-based assay (all compounds in Table 2 > 10 μM) except for the very potent compounds **13 b** and **13 c**. Whereas ring-opening of the compounds in the aqueous buffer of the cell-based assay cannot be discounted,[12a] the compounds were stable in dilute acid (1:1 CH_3CN:H_2O with 0.1% TFA) and dilute base (1:1 MeOH:H_2O with 0.05% NH_4OH) for more than three days. To predict blood-brain barrier penetration, several were examined for directional transport in PGP expressing preparations;[20] however, permeability was low (Papp $< 5\times10^{-6}$ cm s)$^{-1}$ thereby precluding conclusive determination. The calculated polar surface area of these compounds is high (> 85 Å^2) which may be partly responsible for their lack of efficient cell penetration.

In summary, structure-based drug design was applied to lead compound **1** resulting in the preparation of a series of imidazolidin-4-ones that were shown to be potent BACE-1 inhibitors. The large P_2P_3 group of **1** could be replaced with smaller carbamates resulting in good inhibitors with molecular weight less than 500 Daltons. X-ray crystallographic analysis of **13 a** indicates that the imidazolidinone ring indeed serves as a good scaffold that makes several hydrogen bonds as well as orienting substituents toward the important S1′ and S2′ pockets of the enzyme. Efforts to further improve the potency as well as cell penetration are ongoing.

Experimental Section

Preparation of **13 a**: Butyllithium (2.5 M) in THF solution (330 μL, 0.825 mmol) was added to 4 mL anhydrous THF at −78 °C, followed by diisopropylamine (116 μL, 0.825 mmol) and a solution of **8** (150 mg, 0.687 mmol) in THF (2 mL). After stirring 20 min, a solution of **3** (249 mg, 0.756 mmol) in THF (1.2 mL) was added. The mixture continued to stir for 1 h at −78 °C and then was poured into saturated aqueous $NaHCO_3$ solution and extracted 3×EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a yellow oil. Flash column chromatography on silica gel using a gradient of 0–60% EtOAc/hexanes gave a mixture of two products. Further separation by reverse phase chromatography on a C18 column using a gradient elution of 95–5% H_2O (0.1% TFA)/CH_3CN (0.1% TFA) afforded the individual products as single diastereomers. The trifluoroacetate salts were treated with saturated aqueous $NaHCO_3$, extracted with EtOAc, dried with Na_2SO_4, filtered, and concentrated in vacuo to give their respective free base forms as yellow oils. The earlier eluting diastereomer **9** (30 mg, 8% yield) was determined to have the *S*,*S*,*S* configuration (by X-ray crystallography of **13 a**) and the configuration of the later eluting diastereomer (11 mg, 3% yield) was not determined. 20 wt% $Pd(OH)_2$ catalyst (8 mg) was added to a solution of **9** (5.9 mg, 0.11 mmol) in MeOH (1.5 mL) under a nitrogen atmosphere. The mixture was purged with an atmosphere of hydrogen and stirred over night at RT. A second portion of 20 wt% $Pd(OH)_2$ (5 mg) was necessary to drive the reaction to completion. The mixture was filtered through a pad of celite and concentrated in vacuo to give the trifluoroacetate salt of 5(5*S*)-[(1*S*,2*S*)-2-amino-1-hydroxy-3-phenylpropyl]-3,2,2-trimethylimidazolidin-4-one as a crude light brown foam (36 mg). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.31$–7.24 (m, 5H), 4.14 (brs, 1H), 4.07 (brs, 1H), 3.93 (brs, 1H), 3.39 (brs, 1H), 3.14 (brs, 1H), 2.76 (s, 3H), 1.42 (s, 3H), 1.31 ppm (s, 3H); ES MS $[M+1]=278$. To a solution of this amine (20 mg, 0.072 mmol) in DMF (200 μL) was added 3-({[(1*R*)-1-(4-fluorophenyl)ethyl]amino}carbonyl)-5-[methyl (methylsulfonyl) amino] benzoic acid[14] (19 mg, 0.048 mmol) in DMF (200 μL), Hunig's base (26 μL, 0.145 mmol), and BOP reagent (23 mg, 0.053 mmol) in DMF (250 μL). The reaction was stirred 1 h at RT, then purified directly by reverse phase chromatography on a C18 column using a gradient elution of 95–5% H_2O (0.1% TFA)/CH_3CN (0.1% TFA). Lyophilisation of the product fractions gave the trifluoroacetate salt of **13 a** (9 mg, 24% yield). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=8.99$ (d, $J=$ 7.9 Hz, 1H), 8.84 (br s, 1H), 8.18 (s, 1H), 7.98 (s, 1H), 7.88 (s, 1H), 7.45–7.41 (m, 2H), 7.23 (d, $J=4.2$ Hz, 4H), 7.17 (t, d, $J=8.6$ Hz, 3H), 5.18 (m, 1H), 4.58 (brs, 1H), 4.16 (br s, 1H), 4.01 (br s, 1H), 3.29 (s, 3H), 3.04 (s, 3H), 2.91(m, 1H), 2.66 (s, 3H), 1.49 ppm (m, 8H); HRMS (FT-ICR) $C_{33}H_{40}FN_5O_6S+H=654.2730$; calculated 654.2756.

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ments, H. Rajapakse and S. R. Stauffer for careful review of the manuscript, and D. Richards for assistance with Figures 3 and 4.

Keywords: alzheimer's disease · β-secretase · drug design · nitrogen heterocycles · structure–activity relationships

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[8] After completion of this work, several patent applications describing a similar approach appeared. See WO2005014540 and WO2005108358 which are summarized in reference [3a].

[9] For details of the docking and scoring of potential BACE-1 inhibitors, see M. K Holloway, G. B. McGaughey, C. A. Coburn, S. J. Stachel, K. G. Jones, E. L. Stanton, A. R. Gregro, M.-T. Lai, M.-C. Crouthamel, B. L. Pietrak, S. K. Munshi, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 823 – 827.

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[17] Cyclization of **19** with CH_3CHO at 60 °C affords > 10:1 *cis*:*trans*, at 160 °C affords 2:1 *cis*:*trans*. Cyclization of **19** with *i*PrCHO at 60 °C affords approx 2:1 *cis*:*trans*.

[18] A PDB file for the BACE-1/inhibitor **13a** complex (PDB identifier 2P8H) has been deposited with the Protein Data Bank (http://www.rcsb.pdb). Crystallization of BACE-1 is described in the following: V. Sardana, B. Xu, J. Zugay-Murphy, Z. Chen, M. Sardana, P. Darke, S. Munshi, L. C. Kuo, *Protein Expression Purif.* **2004**, ***34***, 190 – 196.

[19] PyMol, v.99 distributed by DeLano Scientific LLC was used to generate the images in Figures 3 and 4.

[20] For a description of the PGP assay, see M. Yamazaki, W. E. Neway, T. Ohe, I.-W. Chen, J. F. Rowe, J. H. Hochman, M. Chiba, J. H. Lin, *J. Pharmacol. Exp. Ther.* **2001**, *296*, 723 – 735.

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