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Strategies toward improving the brain penetration of macrocyclic tertiary carbinamine BACE-1 inhibitors

Keith P. Moore,[a,*] Hong Zhu,[a] Hemaka A. Rajapakse,[a] Georgia B. McGaughey,[b] Dennis Colussi,[c] Eric A. Price,[c] Sethu Sankaranarayanan,[c] Adam J. Simon,[c] Nicole T. Pudvah,[d] Jerome H. Hochman,[d] Timothy Allison,[e] Sanjeev K. Munshi,[e] Samuel L. Graham,[a] Joseph P. Vacca[a] and Philippe G. Nantermet[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[b]*Department of Molecular Systems, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[c]*Department of Alzheimer's Research, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[d]*Department of Drug Metabolism, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[e]*Department of Structural Biology, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*

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Abstract—This letter describes replacements for the P3 amide moiety present in previously reported tertiary carbinamine macrolactones. Although P-gp efflux issues associated with these amide-macrolactones were solved and full brain penetration was measured in one case, potency was compromised in the process.

Alzheimer's Disease (AD) is a neurodegenerative disease of the brain that leads to a progressive decline in cognitive function and ultimately incapacitation and death.[1] AD is characterized by the presence of insoluble amyloid plaques and fibrillary tangles, which are key pathological features of this devastating disease. The major component of these plaques is $A\beta_{40-42}$, a neurotoxic peptide fragment of β-amyloid precursor protein (APP) generated by the proteolytic action of the β- and γ-secretase enzymes. Of these two enzymes, β-secretase (β-site APP Cleaving Enzyme or BACE-1), an aspartyl protease, is considered rate-limiting in this proteolytic cleavage process.[2] As such, BACE-1 inhibition is widely hypothesized to be one of the most promising therapeutic approaches for the treatment of AD.[3]

The development of low molecular weight, brain penetrant BACE-1 inhibitors has been extremely challenging.[3] Most efforts have relied on the use of transition state isosteres such as hydroxyethylamines (HEA), reduced amides, statines, and aminostatines to interact with the catalytic aspartates.[3] The incorporation of these motifs into high molecular weight inhibitors negatively impacts pharmacokinetics and penetration of the blood brain barrier (BBB). The discovery of non-transition state isostere tertiary carbinamine derived BACE-1 ligands was recently disclosed.[4] Inhibitors based on this novel 'warhead' motif displayed comparable potency

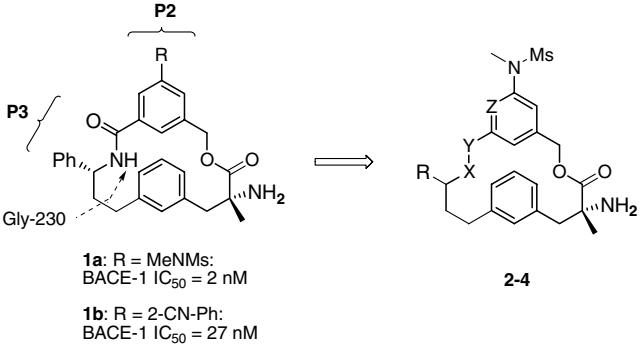

Figure 1. Macrolactone BACE inhibitor **1a** and proposed P3 amide replacements.

Keywords: Alzheimer's disease; BACE-1; Glycine 230; Tertiary carbinamine; P-gp efflux pump; Macrolactone; 10s loop; Macrocyclic N-arylation; Negishi coupling.
* Corresponding author. Tel.: +1 215 652 6976; e-mail: keith_moore2@merck.com

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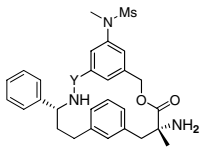
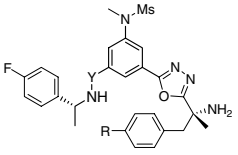
to classical HEA inhibitors although stability, pharmacokinetics and brain penetration remained an issue. Macrolactones such as **1a**,**b** (Fig. 1) derived from this new scaffold displayed a large increase in potency and stability but no significant improvement in pharmacokinetics and BBB penetration.[5] Additional work in this series indicated that the P_3 amide moiety, necessary for making a critical interaction with BACE-1 Gly230, could be altered to afford molecules with similar potency and reduced P-gp liabilities.[6,11] Herein is described our efforts to replace the P_3 amide in an attempt to improve BBB penetration.

Our first strategy relied on the replacement of the P_3 amide with a trifluoroethylamine moiety, following precedent reported by Blacket al.[8] Previous crystallographic analyses demonstrated that the P_3 carbonyl does not interact directly with the enzyme,[4] which should allow for its replacement without consequences on inhibitory potency. The interaction of the amide NH with Gly230 could be maintained via an amide surrogate. The trifluoromethyl group has been shown to have an electronic effect similar to that of the carbonyl on the pK_a of the NH,[8] and should therefore not affect its critical H-bonding potential to Gly230. Furthermore, the amide carbonyl represents a classical recognition element to the P-gp efflux pump, its replacement with a trifluoromethyl group might decrease the compound's susceptibility to P-gp. Figure 2 illustrates the replacement of the P_3 amide carbonyl with a trifluoromethyl group and its transport by P-gp (**2a**: B − A/A − B = 1.3 vs. **1a**: B − A/A − B = 16);[9] unfortunately this is achieved at the cost of potency. The accompanying sp^2–sp^2 to sp^3–sp^3 hybridization switch in the context of a strained macrocyclic ring may have unfavorably altered the vector of the P_3 NH toward Gly230. In order to allow for more conformational flexibility, the acyclic analog **1d** was evaluated. Although **1d** displayed a significant reduction in P-gp susceptibility,[9,10] it was also considerably less potent than either carbonyl containing compounds **1a** or **1c**.



	Y	BACE-1 IC_{50} (nM)[7]	hu P-gp B-A/A-B[6]
1a	CO	2	16
2a	$CHCF_3$	3700	1.3



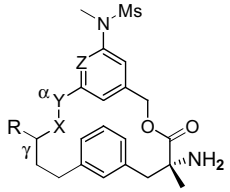
	Y	R	BACE-1 IC_{50} (nM)[7]	hu P-gp B-A/A-B[6]
1c	CO	H	9	>50
1d	$CHCF_3$	F	15000	3.2

Figure 2. CF_3 replacement of carbonyl in both acyclic and macrocyclic modes.

Replacement of the P_3–P_2 carboxamide-phenyl combination with a P_3–P_2 cyclopropylamino-pyridine scaffold has been shown to reduce P-gp susceptibility while maintaining adequate potency[11]and was considered as a second strategy. Indeed aminopyridine **3a** maintains modest potency at 400 nM with a concomitant lowering of P-gp susceptibility (B − A/A − B = 2.9, Table 1). *N*-Alkylation was then considered to simultaneously occupy S_3 and remove the NH that could serve as a recognition element for the P-gp efflux pump. However, *N*-substituted aminopyridine-macrolactones **3b**–**d** suffered a significant loss in potency, indicating that S_3 access directly from the α-position is suboptimal.

A third strategy involved the removal of any heteroatom from the P_2–P_3 linker and accessing S_3 from the more traditional γ-position.[5] The concept was initially applied in the P_2 phenyl mode with a P_3 methyl, providing macrolactone **4a**, as a non P-gp substrate (B − A/

Table 1. Macrolactone optimization



Compound	R	–X–Y–	Z	BACE-1 IC_{50}[a] (nM)	hu P-gp[d]	
					P_{App}	Ratio
3a	H	$–CH_2–NH–$	N	400[b]	11	2.9
3b	H	$–CH_2–NBn–$	N	13000[b]	20	5.3
3c	H	$–CH_2–N$*i*Pr–	N	22000[b]	ND	ND
3d	H	$–CH_2–NCH_2$*cy*Pr–	N	6000[b]	8	0.8
4a	Me	$–CH_2–CH_2–$	C	1700[c]	16	1.4
4b	Me	$–CH_2–CH_2–$	N	1800	14	0.4
4c	Ph	$–CH_2–CH_2–$	N	7000[c]	9	0.4
4d	H	$–CH_2–CH_2–$	N	15000[b]	29	0.8

[a] IC_{50} is an average of at least three measurements.[7]
[b] Calculated from a mixture of 2 enantiomers.[12]
[c] Calculated from a mixture of 4 diastereoisomers.[12]
[d] P_{App} in 10^{-6} cm/s; Efflux ratio: B − A/A − B.[6]

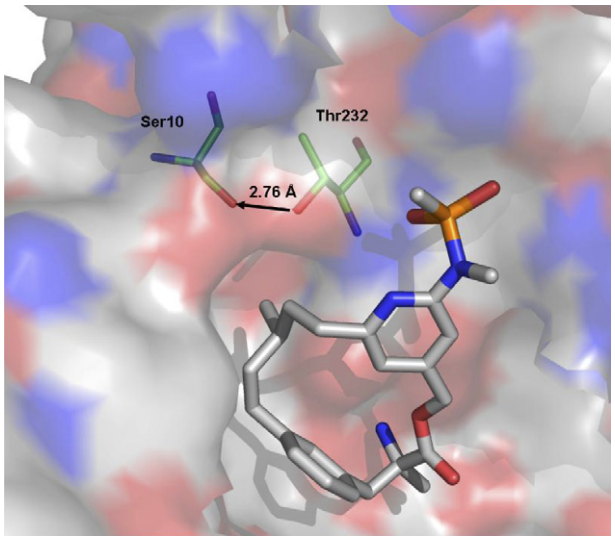


Figure 3a. X-ray of inhibitor **4b** complexed with BACE-1. Flap was removed for clarity. PDB ID code: 2PH8.

A – B = 1.4) displaying moderate potency against BACE-1 (IC_{50} = 1700 nM). Encouraged by this result, we applied this strategy to the P_2 pyridyl analog and resolved all four diastereoisomers. Enantiomer **4b** was evaluated as a 1800 nM inhibitor. X-ray crystallographic analysis of **4b** bound to BACE-1 (Fig. 3a) confirmed[4,5] the *R* configuration at the tertiary carbinamine center; however, the absolute configuration at the γ-position was unexpectedly found to be *S*.[13] Gratifyingly, **4b** was found to effectively penetrate the BBB (at 2 h, $[\mathbf{4b}]_{brain}$ = 637 nM, [brain]/[plasma] = 130%).[14] Guided by earlier SAR in the P_3 amide mode,[5] the previously optimal P_3 phenyl was incorporated (**4c**) resulting in a surprising decrease in potency. Complete removal of the P_3 group (**4d**) resulted in further loss of potency.

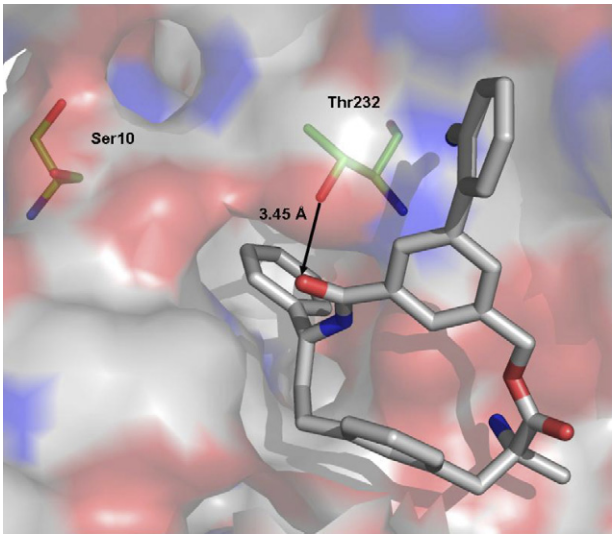
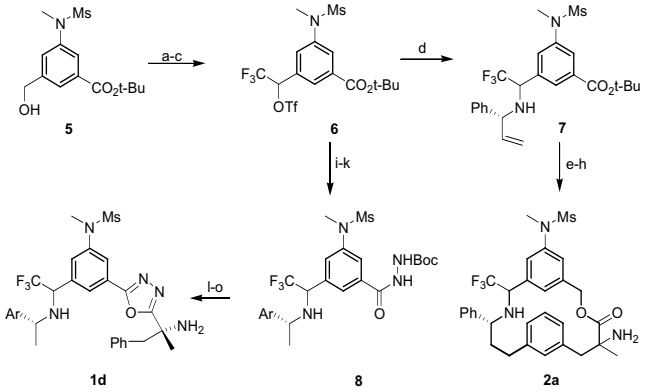
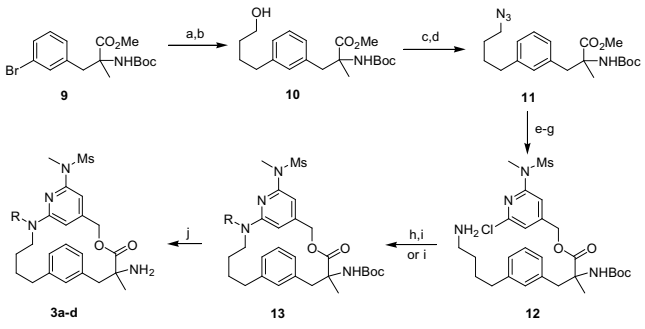


Figure 3b. X-ray of inhibitor **1b**[5] complexed with BACE-1. Flap was removed for clarity. PDB ID code: 2QZK.

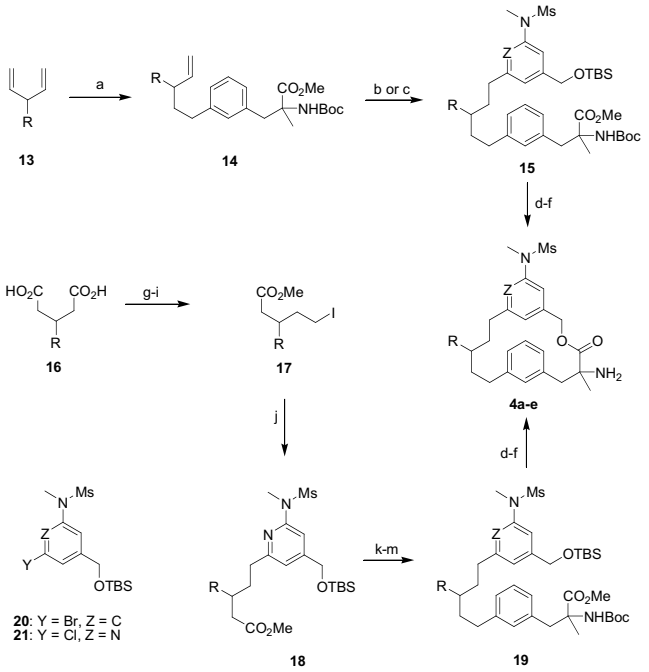
X-ray crystallography was used to compare the binding of ligands **4b** and **1b** and to help rationalize these results. In the absence of heteroatoms in the P_2–P_3 linker of ligands **4b–d**, there can be no hydrogen bonding interaction with BACE-1 Thr232, giving Thr232 the opportunity to hydrogen bond to Ser10 and thus inducing a 10s loop "down" configuration (Fig. 3a). As predicted[15] and as observed earlier,[11] a small hydrophobic P_3 group such as the methyl exemplified in **4a**,**b** is the best fit for the small S_3 pocket in this 10s loop "down" configuration. The larger P_3 phenyl (**4d**) likely maintains the 10s loop in an "up" configuration that is non-optimal in such cases where the ligand cannot H-bond with Thr232. In the case of P_3 amide inhibitors such as **1b**,[5] Thr232 is engaged by the amide carbonyl resulting in a 10s loop "up" configuration and a larger S_3 pocket which is best occupied by a P_3 phenyl (Fig. 3b).[5,15]



Scheme 1. Reagents: (a) TPAP, NMO, 74%; (b) CF_3–TMS, cat. TBAF, 87%; (c) Tf_2O, lutidine, 81%; (d) K_2CO_3, (*R*)-1-phenylprop-2-en-1-amine, 84%; (e) 9-BBN, NaOH, $(Ph_3P)_4Pd$, **9**, 71%; (f) 1—TFA, DCM, 99%; 2—Boc_2O, hunig's base, 92%; 3—BH_3–THF, 69%; (g) LiOH, MeOH, 99%; (h) 1—DIAD, PPh_3, THF, 85%; 2—TFA, DCM, 99%; (i) K_2CO_3, (*R*)-1-(4-fluorophenyl)ethanamine, 88%; (j) TFA, DCM, 99%; (k) Boc-hydrazine, EDC, HOAt, 66%; (l) TFA, DCM, 99%; (m) Boc-α-methyl-D-Phe, EDC, HOAt, 73%; (n) CBr_4, PPh_3, DCM, 88%; (o) TFA, DCM, 99%.



Scheme 2. Reagents: (a) 9-BBN, NaOH, $(Ph_3P)_4Pd$, but-3-en-1-yloxy-*t*-butyldimethyl silane, 68%; (b) TBAF, THF, 99%; (c) CBr_4, PPh_3, imid., 85%; (d) NaN_3, DMF, 83%; (e) LiOH, MeOH, 99%; (f) Cs_2CO_3, **22**, DMF, 91%; (g) PPh_3, H_2O, THF, 70%; (h) for **3b-d**: RCHO, $NaBH(OAc)_3$, 60–80%; (i) for **3a–d**: $[(t\text{-}Bu)_3P]_2Pd$, K_3PO_4, DMA, 65–82%; (j) TFA, DCM, 99%.



Scheme 3. Reagents: (a) 9-BBN, NaOH, $(Ph_3P)_4Pd$, **9**, 72%; (b) for **4a**: 9-BBN, NaOH, $(Ph_3P)_4Pd$, **20**, 78%; (c) for **4b**, **4d**: 9-BBN, NaOH, $(Ph_3P)_4Pd$, **21**, 73%; (d) TBAF, THF, 99%; (e) LiOH, MeOH, 99%; (f) DIAD, PPh_3, THF, 88%; (g) MeI, Cs_2CO_3, DMF, 55%; (h) BH_3–THF, 69%; (i) I_2, PPh_3, imid., 77%; (j) Reike Zinc, $[(t\text{-Bu})_3P]_2Pd$, **21**, 82%; (k) $LiBH_4$, THF, 86%; (l) I_2, PPh_3, imid., 91%; (m) Reike Zinc, $[(t\text{-Bu})_3P]_2Pd$, **9**, 78%.

The synthetic routes[16] toward trifluoromethyl derivatives **2a** and **1d** are outlined in Scheme 1. Benzyl alcohol **5** was oxidized to the corresponding aldehyde, which then underwent nucleophilic addition of CF_3–TMS in the presence of catalytic TBAF.[17] The resulting alcohol was then converted to triflate **6**, and amine displacement afforded **7**. Hydroboration of **7** followed by Suzuki-Miyaura coupling to aryl bromide **9** (from Scheme 2) sets the key molecular framework. The synthesis of **2a**culminated in a macrolactonization under Mitsunobu conditions. For **1d**, (*R*)-1-(4-fluorophenyl)ethanamine was employed to displace triflate **6**; formation of the oxadiazole ring was accomplished under dehydrative conditions using carbon tetrabromide and triphenylphosphine. Boc deprotection afforded inhibitor **1d**.

The syntheses[16] of inhibitors **3a**–**d** are outlined in Scheme 2. Hydrolysis of methyl ester **11** was followed by carboxylate alkylation with **22**, and reduction of the azide moiety with triphenylphosphine afforded amine **12**. With the ester bond intact, macrocyclization could be executed using a palladium mediated macrocyclic *N*-arylation[18] was followed by protecting group removal to give inhibitor **3a**. The macrocyclization could also be achieved using secondary amines, resulting from reductive aminations with the necessary aldehydes to give inhibitors **3b**–**d**.

Macrolactones **4a**,**b**,**d** were prepared[16] as outlined in Scheme 3. A fully saturated carbon linker could be constructed from the hydroboration of alkene **14** followed by Suzuki-Miyaura coupling to halides **20** or **21**. After silyl deprotection and ester hydrolysis, macrocyclization was executed using an intramolecular Mitsunobu reaction which provided macrolactones **4a**,**b**,**d** in excellent yields.

For compound **4c**, bis-carboxylic acid **16** was used in place of the corresponding diene to avoid double bond migration that would generate styrenes. Mono-esterification of **16** followed by borane reduction, and conversion to the alkyl iodide provided the corresponding ester-iodide. The resulting iodide was activated using Rieke zinc and utilized in a Negishi coupling with pyridyl chloride **21** to give ester **18**. Reduction of the ester, conversion to the alkyl iodide and Rieke zinc activation enabled a second Negishi coupling with aryl bromide **9** to yield **19**, which underwent macrocyclization using the established protocol to afford macrolactone **4c**.

In conclusion, various replacements for the P3 amide functionality embedded in tertiary carbinamine macrolactones were discussed. Although P-gp efflux issues were solved in most cases and brain penetration was attained in vivo for **4b**, potency was compromised in the process. Optimization of S3 access from the aminopyridine scaffold **3a** will be the subject of future work, to be described in due time.

Acknowledgments

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Supplementary data

Experimental procedures for the synthesis of compounds **2**–**4**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.040.

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9. Compounds **2a** and **1d** were evaluated as 1:1 diastereomeric mixtures. $P_{App} > 20 \times 10^{-6}$ cm/s for both Ref. 6.
10. The 4-fluoro substitution on the P_1 aryl group is known to improve inhibitory potency against BACE-1 by 2-fold at best (unpublished results) and is expected to have no significant impact on P-gp susceptibility.
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12. The stereochemistry of the active diastereoisomer is known to always be *R* at the tertiary carbinamine center, based on earlier X-ray crystallographic analysis of tertiary carbinamine/P3-amide based acyclic and macrocyclic inhibitors Refs. 4,5. Additionally, chiral separations performed earlier on similar mixture of diastereoisomers indicated that the diastereoisomers derived from the *S* tertiary carbinamine are all devoid of significant activity against BACE-1, thus justifying IC_{50} calculations from mixtures Refs. 5,12.
13. The *R* configuration at the γ-position for inhibitor **4b** is opposite to what was previously observed with P_3 amide tertiary carbinamine inhibitors (*S*) Ref. 5. This inversion of configuration is likely allowed by the increased conformational flexibility introduced in the chain when switching from an amide to ethylene linker. Only one of the other three diastereoisomers displayed inhibitory potency at 17 μM. This isomer most likely has the correct *R* configuration at the tertiary carbinamine center and the *S* configuration at the γ-position.
14. Brain penetration was assessed from mice brain homogenates, two hours post i.p. dosing in 25% DMSO/75% PEG300, at 30 mpk.
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