Macrocyclic Inhibitors of β-Secretase: Functional Activity in an Animal Model

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Abstract: A macrocyclic inhibitor of β-secretase was designed by covalently cross-linking the P1 and P3 side chains of an isophthalamide-based inhibitor. Macrocyclization resulted in significantly improved potency and physical properties when compared to the initial lead structures. More importantly, these macrocyclic inhibitors also displayed in vivo amyloid lowering when dosed in a murine model.

Alzheimer's disease (AD[a]) is a debilitating neurodegenerative disease characterized by progressive cognitive decline, inevitably leading to incapacitation and death. Although researchers have uncovered a variety of genetic mutations that result in early onset AD over the past 20 years, our current understanding of genetic predisposition accounts for <10% of AD cases.[1] The majority of AD cases are sporadic in nature, and the risk factor increases in an age-dependent manner. It is generally recognized that β-amyloid plaques and neurofibrillary tangles are the key pathological features of the disease, although the specific roles of these agents in disease progression remain debatable. However, it is well-established that the principle component of amyloid plaque is a neurotoxic peptide fragment of the amyloid precursor protein (APP), namely, $A\beta_{40-42}$.[2] As such, many therapeutic strategies are founded on the ability to inhibit catabolic pathways of APP that result in Aβ formation.[3]

Proteolytic processing of APP is primarily accomplished through the activity of three discrete proteases (α-, β-, and γ-secretase).[4] The initial cleavage event is performed by either α-secretase or β-secretase (BACE-1) releasing the APP ectodomain. The remaining membrane-bound C-terminal domains are subsequently cleaved by γ-secretase, resulting in the nonamyloidogenic P3 peptide (α-cleavage product) or the amyloidogenic $A\beta_{40-42}$ (β-cleavage product). γ-Secretase inhibitors have been demonstrated to inhibit the production of Aβ in vivo, however, their development as therapeutic agents remains tenuous due to the small therapeutic window that results from untoward effects on notch processing.[5] In contrast, inhibitors of BACE-1 are predicted to be well-tolerated, as evidenced by viable knockout mice.[6] As such, BACE-1 inhibition is considered an attractive therapeutic target for the treatment and prevention of AD.[7] In this letter, we describe the development of a series of macrocyclic active site inhibitors of BACE-1. Most noteworthy, compound **19** was shown to reduce Aβ levels in a murine model following iv administration.

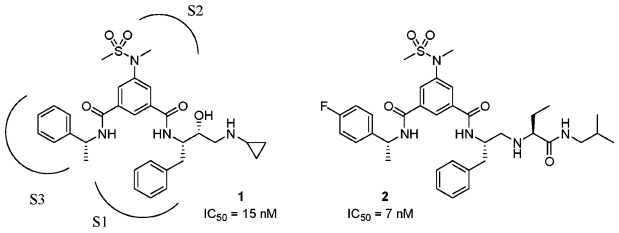

Figure 1. Representative potent BACE-1 inhibitors.

5-Substituted isophthalamides, as represented by structures **1** and **2** (Figure 1), emerged as early lead classes that were investigated for inhibition of BACE-1.[8] Both inhibitors possessed desirable properties of both good in vitro (IC_{50} = 15 and 7 nM, respectively) and good cell-based potencies (IC_{50} = 29 and 20 nM, respectively) against BACE-1. However, problems associated with their pharmacokinetic properties rendered them less than ideal candidates for advancement. A main concern was that **1** and **2** were found to be substrates of P-glycoprotein (P-gp) transport and both exhibited low apparent permeability in wild-type porcine LLC−PK1 cells. While iv administration of either **1** or **2** showed no effect on Aβ levels in a murine model, intracranial administration of **1** succeeded in reducing Aβ levels by 50−70%.[9] This result demonstrated that inhibition of BACE-1 can reduce Aβ levels in vivo, but to be clinically relevant, the compound must be CNS penetrant. To achieve greater CNS penetration, we sought to reduce the peptidic character of our inhibitors while maintaining potency. Macrocyclization is a well-precedented technique to enhance potency by conformational preorganization, and in many cases, the physical properties of the inhibitors were also improved.[10] We envisaged that, if macrocyclization were successful in enhancing potency, reduction of molecular weight and removal of some hydrogen bonding elements might be possible. Several research groups have recently reported similar macrocyclization strategies for BACE inhibitors, albeit with limited success.[11] In our case, examination of the X-ray crystallographic data of **1**[12] complexed in the active site of BACE-1 revealed the close spatial proximity of the lipophilic P1 and P3 groups and thus directed efforts toward the design of macrocycles that would link these groups in an isophthalamide-based inhibitor.

We chose **4** as a simplified core to test whether macrocyclization would provide an increase in potency due to a reduction in conformational freedom (Figure 2). We were gratified to discover that while **4** was not a high affinity ligand for BACE-1 (IC_{50} = 2.9 μM) it was vastly superior to the corresponding seco variant **3** (IC_{50} > 100 μM). It should be noted that compound **4** was designed as a model system and thus lacks several potency enhancing features paramount for high binding affinity.

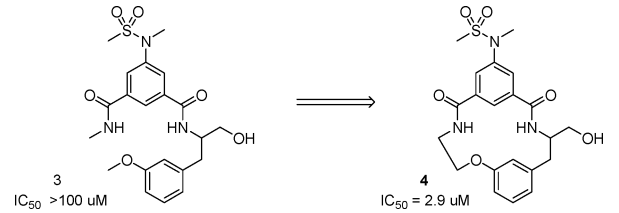

Figure 2. Macrocyclization results in increased potency.

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[a] Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; BACE-1, beta-site APP cleaving enzyme 1; APP−YAC, amyloid precursor protein−yeast artificial chromosome.

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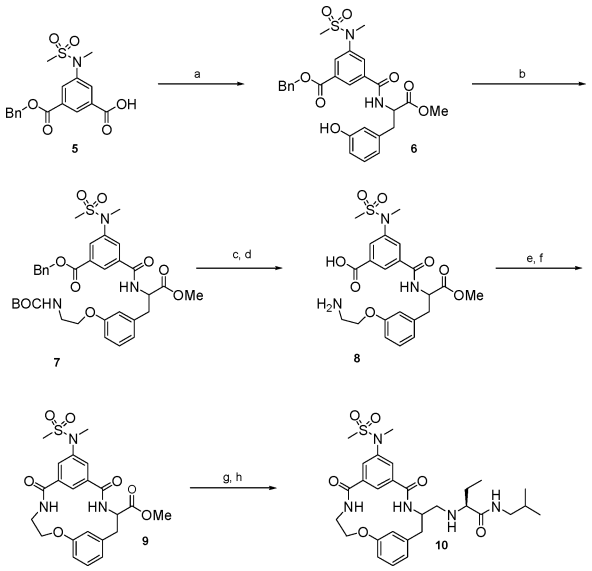
Scheme 1[a]



[a] Reaction conditions: (a) *m*-tyrosine ethyl ester BOP, DIPEA, CH_2Cl_2, 86%; (b) *tert*-butyl(2-iodoethyl)carbamate, K_2CO_3, DMF, 75%; (c) Pearlman's catalyst, MeOH, 100%; (d)TFA, CH_2Cl_2, 100%; (e) BOP, DIPEA, CH_2Cl_2, 37%; (f) $LiBH_4$, THF, 51%; (g) SO_3-pyridine, TEA, DMSO; (h) (*S*)-2-amino-*N*-isobutylbutanamide, $NaBH_3CN$, MeOH, 60%.

Based on the encouraging results of the model system, we focused on incorporating a prime side element to enhance binding. We chose to incorporate a ψ[CH_2NH]-reduced amide isostere as present in **2**, because we have previously shown that such isosteres are not only highly active pharmacophores for BACE inhibition, but also improved cell permeability in similar series.[8b]

The synthesis of macrocycle **10** began with a coupling reaction between isophthalamide **5** and *m*-tyrosine methyl ester to produce phenol **6** (Scheme 1). Alkylation of the phenol using *tert*-butyl(2-iodoethyl)carbamate afforded the differentially protected macrocyclic precursor **7** in 75% yield. Deprotection of the benzyl ester, followed by removal of the amino BOC protecting group, afforded *seco*-acid **8** in near quantitative yield. *seco*-Acid **8** was subjected to macrocyclization mediated by BOP reagent under high dilution conditions to provide **9** in modest yield. The ester was reduced to the alcohol **4** with lithium borohydride and subsequently oxidized to the corresponding aldehyde using Parikh−Doering conditions. Reductive amination of the corresponding aldehyde with (*S*)-2-amino-*N*-isobutylbutanamide afforded macrocyclic inhibitor **10**.

Macrocycle **10**, as a 1:1 mixture of diastereomers at the tyrosinyl center, was assayed for BACE-1 activity and exhibited an $IC_{50} = 32$ nM. This represents a 90-fold increase in potency due to addition of the prime-side binding domain. It should be noted that macrocycle **10** is only 4-fold less-potent than **2** and lacks the P3 phenyl substituent that has been shown to contribute upward of 90-fold in potency. Unfortunately, when **10** was evaluated in the cell-based assay, there was a significant loss in potency ($IC_{50} = 5400$ nM) when compared with **2**. It was also suspected that **10** was still a severe P-gp substrate, but due to a low apparent permeability (0.5×10^{-6} cm/s), its susceptibility could not be accurately determined. Despite the ability to concomitantly increase potency and trim the molecular weight of the inhibitor, it was apparent that to succeed in lowering Aβ in vivo it would be necessary to improve the cell permeability of our inhibitors.

In a previous communication, we demonstrated that the P2/P3 amide is noncritical for BACE-1 activity.[13] The amide

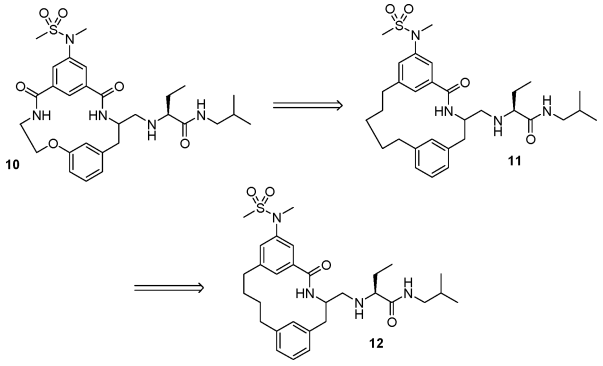


Figure 3.

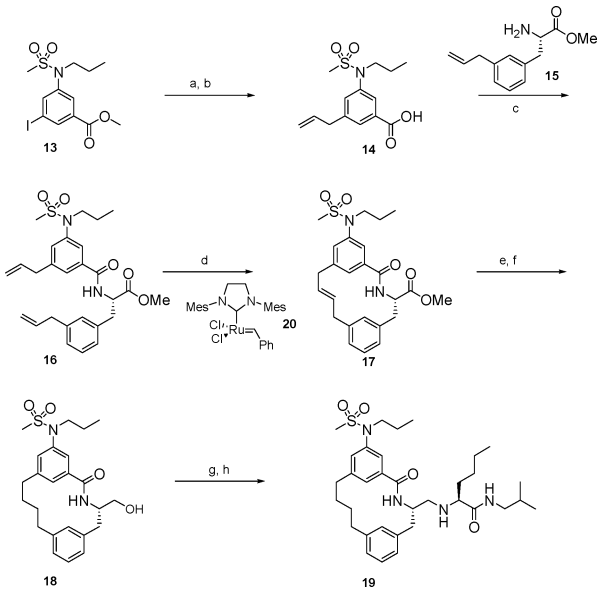
functionality acts mainly to orient the P3 ligand, and the amide hydrogen bonding contacts do not significantly contribute to the binding energy. With this in mind, we posited that the conformational constraint induced by the macrocycle would allow for excision of the amide bond without abrogating potency. It was also hoped that removal of the amide bond would result in improved physical properties, namely, cell-permeability. To test this hypothesis, we synthesized the corresponding alkyl-linked 15-membered macrocycle **11**, but were disappointed by a greater than 34-fold loss in intrinsic potency, $IC_{50} = 1100$ nM (Figure 3).

Recognizing that the 15-membered macrocyclic ring size for the bisamide-containing macrocycle **10** may not be optimal for the carbon analogue, we synthesized the ring-contracted 14-membered macrocycle **12** for comparison. We were gratified to see that potency was recovered and **12** exhibited a potency of 90 nM when assayed against BACE-1. Even more importantly, activity for **12** in the cell-based assay ($IC_{50} = 1139$ nM) as well as apparent permeability ($P_{app} = 19 \times 10^{-6}$ cm/s) were also improved when compared with **10**. With these encouraging results, we employed some potency enhancing modifications to the sulfonamide and P1′ regions of the inhibitor learned from previous studies culminating in the synthesis of macrocycle **19** as a single diastereomer.[14]

An efficient synthesis of the alkyl macrocycle **19** was devised as depicted in Scheme 2. The synthesis began with the iodoarene **13** that we recently reported as an intermediate in alternative series of inhibitors.[13] Allylation of **13** under Stille conditions and subsequent hydrolylsis of the methyl ester provide benzoic acid **14**. Carboxylic acid **14** was coupled with (*S*)-*m*-allyl phenylalanine **15** using standard conditions to provide the macrocyclic precursor **16**. Macrocyclization of **16** was accomplished through the use of second-generation Grubbs catalyst[15] **20** to provide macrocycle **17** as a single regioisomer. The metathesis reaction was high yielding and resulted in exclusive formation of the *E*-isomer, as confirmed by ^{1}H NMR. Hydrogenation of the olefin followed by further elaboration of the macrocycle was done in a manner similar to **10**. Reduction of the ester using $LiBH_4$ provided alcohol **18**, and Parik−Doering oxidation followed by reductive amination with *N*-isobutyl-L-norleucinamide afforded the desired alkyl-linked macrocycle **19**.

When assayed against BACE-1 enzyme, macrocycle **19** displayed an $IC_{50} = 4$ nM. This represents a greater than 8-fold increase in intrinsic activity when compared to compound **10**. Even more impressive, in the cell-based assay, **19** displayed an $IC_{50} = 76$ nM, representing a 70-fold improvement over **10** in functional activity. Most gratifying, the apparent permeability of **19** compared with **10** was significantly improved ($P_{app} = 13 \times 10^{-6}$ cm/s). With the improved permeability and reduced P-gp

Scheme 2[a]



[a] Reaction conditions: (a) $Bu_3SnAllyl$, $Pd(PPh_3)_4$, 82%; (b) NaOH, MeOH/THF, 83%; (c) **15**, BOP, DIPEA, 88%; (d) Grubbs2, CH_2Cl_2, 50 °C, 84%; (e) Pd/C, H_2, MeOH, 93%; (f) $LiBH_4$, THF, 99%; (g) SO_3-pryidine, TEA, DMSO; (h) *N*-isobutyl-L-norleucinamide, $NaBH_3CN$, MeOH, 71%, 2 steps.

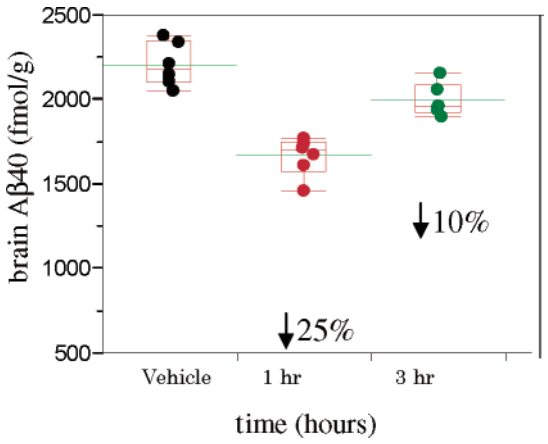


Figure 4. In vivo Aβ40 lowering of 100 mpk iv does of **19** at 1 and 3 h.

susceptibility (BA/AB = 5.5 mdr1a), we dosed macrocycle **19** at 100 mpk in the APP−YAC[16] mouse model to obtain a more relevant physiological determination of compound levels in the brain versus plasma. The ratio of compound concentration between the brain and the plasma was determined to be 20−30%, and compound concentrations of **19** in the brain were measured at ~1100 nM. With these encouraging results, we undertook a more robust study to evaluate efficacy. APP−YAC mice were administered a 100 mpk iv bolus of **19** and euthanized after 1 and 3 h, respectively, to determine functional efficacy. Analysis of the DEA brain extracts demonstrated a robust decrease in Aβ(40)[17,18] levels of 25% when compared to vehicle. To our knowledge, this is the first reported BACE-1 inhibitor to demonstrate efficacy from an iv administration.[19] In the study, the Aβ reduction was statistically significant in the 1 h grouping compared to the vehicle (Figure 4). However, by the 3 h timepoint, reduction of Aβ had begun to abate and only a 10% reduction was measurable in the group.[20] Analysis of the concentration of **19** in the brain correlated well with reduction

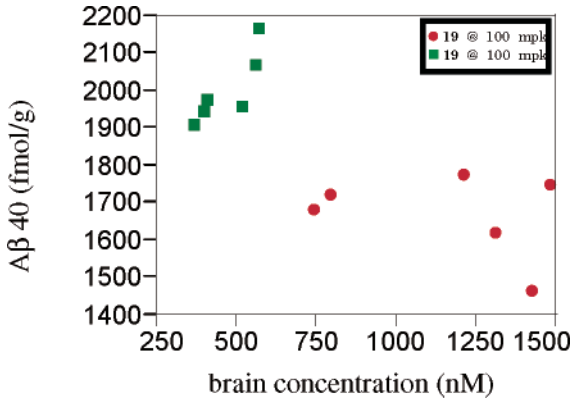


Figure 5. Brain compound levels versus Aβ levels.

in Aβ levels, and a statistically significant reduction was observed when brain concentrations of >700 nM were achieved (Figure 5).

In summary, we have developed a series of macrocyclic inhibitors of BACE by covalently cross-linking the P1 and P3 side-chains of an isophthalamide-based inhibitor. Macrocyclization resulted in significantly improved physical properties when compared to the initial lead structure. Macrocyclic inhibitors such as **19** also displayed in vivo amyloid lowering when dosed in a murine model.

Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) The turnover of Aβ is reported to be approximately 2 h. This is in accord with the rapid recovery when compound levels fall to subefficacious levels.

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