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Design and synthesis of hydroxyethylamine (HEA) BACE-1 inhibitors: Prime side chromane-containing inhibitors

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ABSTRACT

The structure activity relationship of the prime region of conformationally restricted hydroxyethylamine (HEA) BACE inhibitors is described. Variation of the *P1′* region provided selectivity over Cat-D with a series of 2,2-dioxo-isothiochromanes and optimization of the *P2′* substituent of chromane–HEA(s) with polar substituents provided improvements in the compound's in vitro permeability. Significant potency gains were observed with small aliphatic substituents such as methyl, *n*-propyl, and cyclopropyl when placed at the C-2 position of the chromane.

It is estimated that by 2050, one in eighty five persons worldwide will be living with Alzheimer's disease (AD), a progressive neurodegenerative disorder.[1] This high global burden necessitates treatments that slow or halt AD progression. Alzheimer's disease is pathologically associated with the formation of extracellular insoluble amyloid plaques composed of β-amyloid peptides (Aβ).[2] The Aβ-peptides are in turn generated from sequential proteolytic cleavage of the β-amyloid precursor protein (β-APP) by the action of two aspartic acid proteases, β-secretase (BACE-1) and γ-secretase.[3,4] BACE-1 null mice were found to be unable to produce Aβ in the brain but were otherwise healthy and fertile.[5] Consequently, BACE-1 inhibitors have been intensely investigated by many laboratories as potential AD modifying therapeutics.[6]

We have recently disclosed a series of BACE-1 inhibitors based upon the hydroxyethylamine (HEA) scaffold[7] with cyclohexyl amine **1**[8] (Fig. 1) being a typical example of the class. The general binding mode of these compounds to BACE-1 is shown in Figure 1.[8] The hydroxyl and protonated amino group bind to Asp228 and Asp32 respectively while the difluoroaryl occupies the *S1* pocket. The cyclohexyl and *tert*-butyl substituents fill the *S1′* and *S2′* pockets respectively. Aryl cycloalkyl amines such as **1** (Fig. 1) possess excellent potency against BACE-1 but suffer from low permeability and consequently, poor brain exposure. In order to address this limitation, we decided to investigate conformational restriction around the cyclohexyl amine portion of the molecule in order to reduce the number of rotatable bonds in the inhibitor.[9] Cyclization of the *S1′* portion of the inhibitor with the aryl ring would lead to compounds such as **2** (Fig. 1) which is intriguing since the disposition of substitution into the *S1′* pocket would be different than that found in aryl cyclohexyl HEAs. Since there are sequence differences between BACE-1 and the closely related protein, Cathepsin-D (Cat-D), in the *S1′* pocket this provided an opportunity to gain selectivity and therefore avoid potential toxicity.[10] The poor pharmacokinetic properties of the HEAs could potentially be addressed by incorporating heteroatoms into the new bicyclic ring system.

The SAR of the conformationally restricted analogs was initially explored with a tetralin ring system and since the *P2′* substituent is crucial to providing potency, this region of the inhibitor was explored first. As in the cyclohexyl amine series, larger lipophilic substituents were more potent than smaller substituents (Table 1), but

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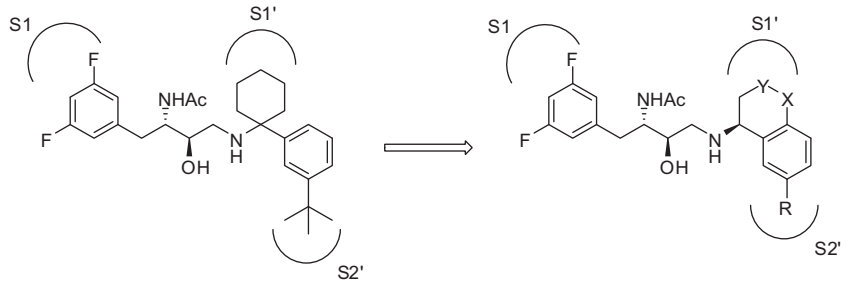
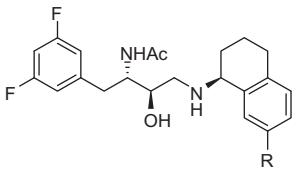


BACE-1 IC_{50} = 47 nM
Cat-D IC_{50} = 25 nM
P_{app} = 7 nm/s
P-gp efflux ratio = 19
pK_a = 8.1

Figure 1. Binding mode of the HEA's.

Table 1
SAR of *P2′* perturbations in tetralin–HEA[12]



#	R	BACE-1 IC_{50}[a] (nM)	Cat-D IC_{50}[b] (nM)
3	Ethyl	538	126
4	*n*-Butyl	574	77
5	CH_2CHMe_2	93	Nd
6	*tert*-Butyl	378	74
7	CH_2*tert*-Butyl	45	8
8	CH_2Ph	4705	251

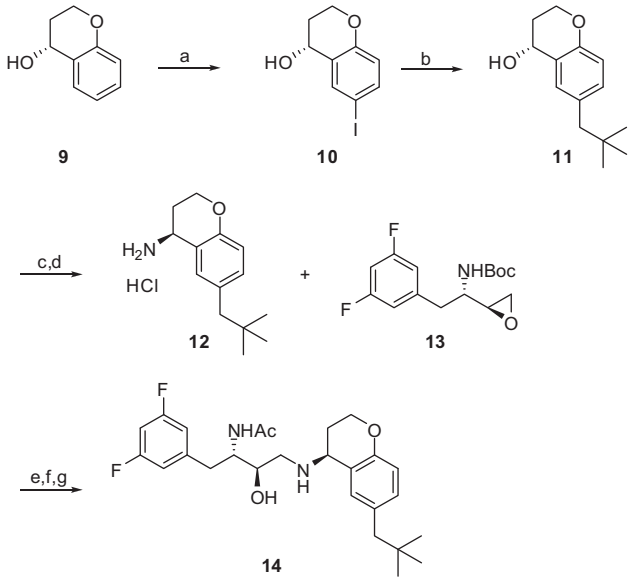
Nd = not determined.
[a] Ref. 13.
[b] Ref. 14.

interestingly, in contrast to the cyclohexyl amines, the neopentyl *P2′* substituent (**7**) appeared to provide the greatest potency against BACE-1. Molecular modeling showed a slight twisting of the tetralin ring compared to compound **1**, thereby placing the *tert*-butyl of the neopentyl group into *S2′*. Although tetralin **7** was equipotent to cyclohexyl-HEA **1** (IC_{50} = 45 nM) it still lacks sufficient passive permeability required for brain exposure (Papp = 3 nm/s).[11] Additionally, tetralin–HEAs **3**–**8** show greater potency in inhibiting Cat-D than BACE-1.

It is known that highly lipophilic molecules that possess a basic amine exhibit a affinity towards membranes.[15] Compound **7** has a measured log*P* of 4.9 and a pKa of 8.9, it is possible that the poor permeability of these analogs is due to the compound partitioning into the membrane[8] In order to overcome this liability, we envisioned introducing heteroatoms into the tetralin ring in order to lower lipophilicity and to enhance the permeability of the inhibitor.[16]

An exemplary synthetic route to prepare these analogs is shown in Scheme 1. Regioselective iodination of commercially available (*R*)—chromanol **9**, followed by Kumada coupling with neopentyl-MgCl provided chromanol **11**. Conversion of the (*R*)-alcohol to (*S*)-amine **12** was carried out by azide displacement followed by reduction. Alkylation of the amine with epoxide **13**[17] and subsequent deprotection and acetylation afforded **14**.

The SAR for this group of analogs is summarized in Table 2. Insertion of an N-methyl group into the tetralin ring decreased

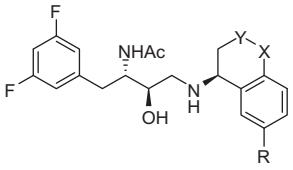


Scheme 1. Reagents and conditions: (a) I_2, HgO, CH_2Cl_2, rt, (59%); (b) neopentyl-MgCl, $Pd(dppf)_2Cl_2$, THF, 0 °C to rt (59%); (c) DPPA, DBU, PhMe, 0 °C to rt; (d) (1) $LiAlH_4$, THF, 0 °C, (2) HCl, dioxane (70% for two-steps); (e) (1) NaOH, (2) **13**, *i*PrOH, 85 °C (82%); (f) HCl, dioxane, 0 °C to rt; (g) NMO, AcOH, HOBt, CH_2Cl_2, rt (61% for two-steps).

potency by 15-fold (compound **14**) but, interestingly, chromane **15** proved to be equipotent to tetralin **7** while isochromane **16** lost significant potency. Since the *S1′* pocket in BACE-1 is primary hydrophilic while that of Cat-D is primarily hydrophobic[18] the placement of polar substituents into the *P1′* region of the molecule may improve the selectivity of the inhibitor. Molecular modeling suggested that a sulfone placed in the *S1′* pocket would be able to make close contact with Lys227, Arg235, and Thr329 (Fig. 2) and therefore a series of sulfones were prepared (**17**–**19**, Table 2). As observed with the tetralin series, larger *P2′* substituents were more potent than smaller alkyl groups but while the most potent analog **19**, had similar potency to tetralin **7**, the sulfone analogs did possess improved selectivity over Cat-D.

Although 25-fold selectivity for BACE-1 over Cat-D was realized with sulfones **17**, the addition of heteroatoms into the tetralin ring was not sufficient for imparting enough passive permeability for adequate CNS exposure. For example, although compound **17** possessed a more desirable basicity and lipophilicity (p*K*a = 5.8 and log*P* = 3.2), it still showed poor permeability, (Papp = 3 nM/s)[11], compared to **7**.

Table 2
Introduction of heteroatoms into tetralin-ring[12]



#	X	Y	R	BACE-1 IC_{50}[a] (nM)	Cat-D IC_{50}[b] (nM)
7	CH_2	CH_2	CH_2*tert*-Butyl	45	8
14	NMe	CH_2	CH_2*tert*-Butyl	643	68
15	O	CH_2	CH_2*tert*-Butyl	46	12
16	CH_2	O	CH_2*tert*-Butyl	19,700	Nd
17	CH_2	SO_2	Ethyl	116	2844
18	CH_2	SO_2	Isopropyl	127	940
19	CH_2	SO_2	CH_2*tert*-Butyl	42	362

Nd = not determined.
[a] Ref. 13.
[b] Ref. 14.

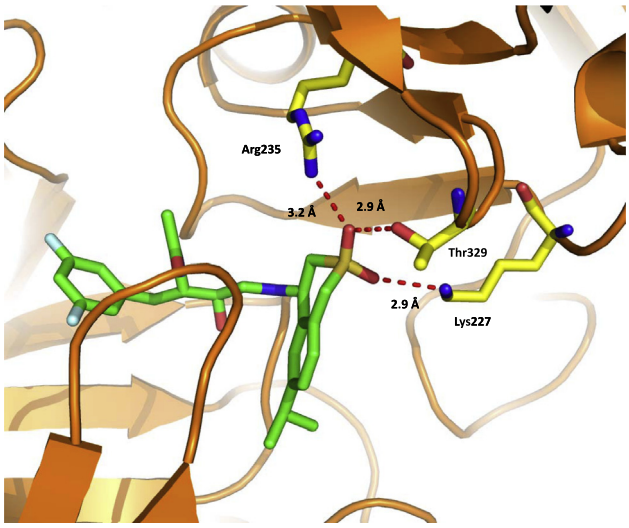
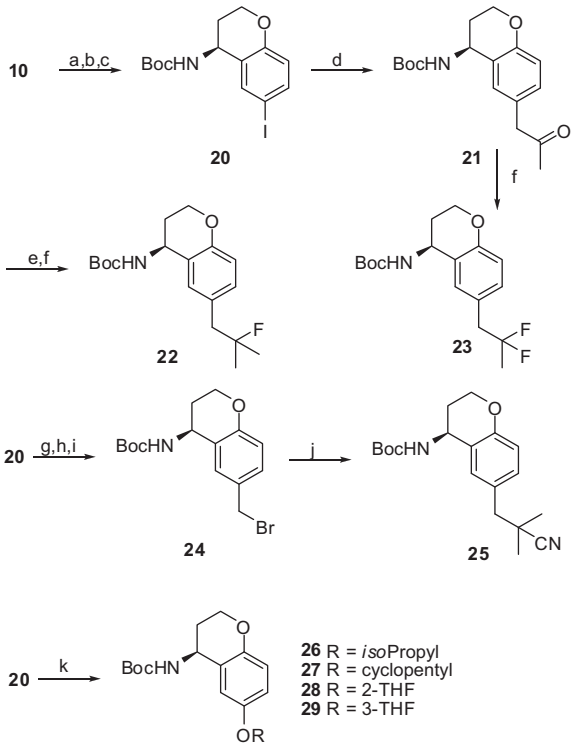


Figure 2. Model of compound **18** with BACE-1. The compound forms three H-bond between the sulfone and *S*1′ pocket of BACE-1. The H-bonds are: Lys227 (2.9 Å), Arg235 (3.2 Å), and Thr329 (2.9 Å).



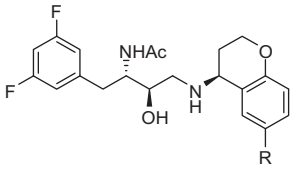
Scheme 2. Reagents and conditions: (a) DPPA, DBU, 0 °C to rt (%); (b) $LiAlH_4$, Et_2O, 0 °C; (c) Boc_2O, Et_3N, CH_2Cl_2 (99%); (d) isopropenylacetate, ((o-tolyl)$_3$P)$PdCl_2$, CH_3OSnBu_3, PhMe, 100 °C (26%); (e) MeMgBr, THF, 0 °C; (f) DAST, PhMe, −78 °C (78% for two-steps); (g) *n*BuLi, PhMe, DMF, rt (50%); (h) $NaBH_4$, MeOH, 0 °C; (i) PBr_3, CH_2Cl_2, rt (86% for two-steps); (j) isobutyronitrile, LDA, THF (63%); (k) R'OH, CuI, Cs_2CO_3, 1,10-phenanthroline, 90 °C, 2–4 days (40–45%).

Chromane analog **15** displayed good potency and improved permeability (Papp = 30 nm/s)[11] compared to tetralin **7** and was deemed to be a good starting point to further enhance permeability and potency. We have previously reported the effect of polar *P*2′ substituents on the permeability of cyclohexyl amine series of BACE-1 inhibitors[8] and therefore decided to investigate if similar substituents would aid in the permeability of the chromanes. These analogs were prepared as shown in Scheme 2. (*R*)-6-Iodochroman-4-ol **10** was converted to key intermediate **20** by azide displacement, reduction, and protection as the Boc-carbamate. The 6-position of the chromane was diversified by homologation of **20** with isopropenylacetate to afford ketone **21**. Addition of MeMgBr to ketone **21** generated the corresponding tertiary alcohol. This alcohol and ketone **21** were treated with DAST and converted to the corresponding fluorohydrocarbons, which were later coupled with **19** to prepare **30** and **31**. Iodide **20** was also converted to the benzyl bromide **24** by formylation, reduction, and bromination. Alkylation of isobutyronitrile with benzyl bromide **24** afforded **25**. Ethers **26**–**29** were accessed by Ullmann coupling of the versatile intermediate **20** with alcohols. With the appropriate chromanes in hand, removal of the Boc group and coupling with epoxide **13** as previously discussed (Scheme 1) led to the preparation of the corresponding chromane-HEAs (Table 3).

The SAR for the *P*2′ modifications are shown in Table 3. As observed with our cycloalkyl HEA series of BACE inhibitors [8] replacing one or two methyls of the neopentyl group with fluorine (**30** and **31**) incrementally improved the in vitro membrane permeability, albeit potency dropped 3–4 fold. Incorporating a cyano group (**32**) onto the *P*2′ side chain also provided an increase in permeability while maintaining potency (BACE-1 IC_{50} = 60 nM). Replacement of the neopentyl group with an isopropyloxy (**35**) group improved permeability fourfold (Papp = 112 nm/s) but also caused a 10-fold loss in potency. In an attempt to further increase the potency of the ethers, larger substituents were incorporated (**36**–**38**) but unfortunately these modifications lead to disappointing decreases in potency. Since improvement on ligand permeability was possible, albeit with potency loss, modification of the chromane to improve potency was next explored.

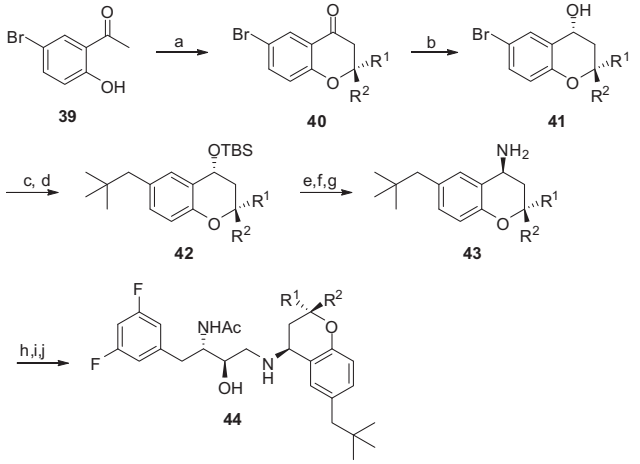
Molecular modeling suggested that there would be sufficient space in the *S*1′ pocket to accommodate up to a 3-atom substituent when placed on the C-2 position of the chromane ring and this additional hydrophobic contact could possibly increase the compound's potency. Chromanes with substituents at C-2 could be conveniently prepared by reacting acetophenone **39** with an appropriate aldehyde or ketone to give chromanone **40** (Scheme 3). Corey–Bakshi–Shibata reduction of the chromanone provided alcohol **41** in 95% ee. Alcohol protection followed by Negishi coupling with neopentyl-ZnBr gave **42**. Deprotection of the alcohol followed treatment with DPPA and azide reduction gave aminochromane intermediate **43**. Alkylation with epoxide **13** followed by deprotection and acetylation gave the desired analogs.

Table 3
SAR of P2′ perturbations in chromane–HEA



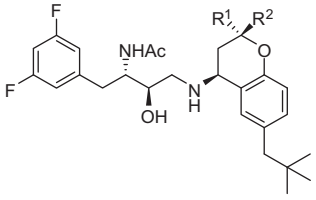
#	R	BACE-1 IC_{50}[a] (nM)	Cat-D IC_{50}[b] (nM)	Papp[c] (nm/s)
14	CH_2*tert*-Butyl	46	12	30
30	CH_2CMe_2F	148	80	83
31	CH_2CMeF_2	186	144	146
32	CH_2CMe_2CN	60	70	64
33	$CH_2CMe_2(CHF_2)$	1300	478	Nd
35	O-*iso*-Propyl	414	429	112
36	O-cyclopentyl	221	145	Nd
37[d]	O-(2-tetrahydrofuranyl)	1325	286	110
38[d]	O-(3-tetrahydrofuranyl)	858	255	71

Nd = not determined.
[a] Ref. 13.
[b] Ref. 14.
[c] Ref. 11.
[d] 1:1 Mixture of epimers at THF stereocenter.



Scheme 3. Reagents and conditions: (a) $R^1C(O)R^2$, pyrrolidine, CH_3CN (65%); (b) (*S*)-Me-CBS–oxazaborolidine, BH_3–DMS, PhMe, 0 °C (64%); (c) TBSCl, imidazole, DMF (86%); (d) neopentyl–ZnBr, $(dppf)_2PdCl_2$, THF, 60 °C; (e) TBAF, THF, rt (72% for two-steps); (f) DPPA, DBU, 0 °C to rt (70%); (g) $LiAlH_4$, Et_2O, 0 °C; (h) **13**, iPr_2NEt, *i*PrOH, 85 °C; (i) HCl, dioxane, 0 °C to rt; (j) $(Ac)_2NOMe$, iPr_2NEt, CH_2Cl_2, rt (10% for four-steps).

Table 4
SAR of P2′ perturbations of chromane–HEA



#	R^1	R^2	BACE-1 IC_{50}[a] (nM)	Cat-D IC_{50}[b] (nM)
45	Me	Me	8	18
46	*n*-Propyl	Me	6	1
47	Me	*n*-Propyl	15	2
48[c]	CH_2OMe	Me	9	5
49	CH_2OCH_2	—	27,105	22,506
50[c]	*cyclo*Propyl	H	22	6

[a] Ref. 13.
[b] Ref. 14.
[c] 1:1 Mixture of epimers.

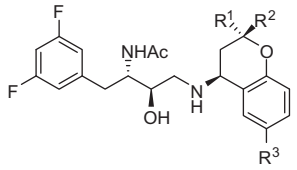
As shown in Table 4, 2,2-dimethyl substitution (**45**) on the chromane boosted the potency fivefold over the des-methyl analog **15** and also provided slight selectivity for BACE-1 over Cat-D. *n*-Propyl analogs **46** and **47** were also tolerated but offered no advantage over gem-dimethyl analog **45**. The *n*-propyl group was replaced with methoxymethyl ether (**48**) in order to lower the *c*log*P* and increase selectivity over Cat-D. Molecular modeling showed a putative electrostatic interaction between the ether oxygen of **48** with Arg235 of BACE-1. Gratifyingly, the potency of **48** (IC_{50} = 9 - nM; 1:1 mixture of diastereomers) rivaled the potency of **44** although the ether oxygen of **48** did not impart selectivity for BACE-1. Spiro-oxetane **49** is inactive in both BACE-1 and Cat-D indicating a poor fit in the *S1′* pocket, while cyclopropyl analog **50** lost potency compared to gem-dimethyl analog **45**.

With the potency of the analogs increased, it remained to combine the more potent C-2-substituted chromanes with the *P2′* substituents that provided an increase in permeability. Compounds **51**–**52** (Table 5) were prepared using methods outlined in Scheme 2 while **53** was prepared starting from 2-hydroxy-5-trifluoromethoxy-acetophenone by employing a similar sequence as Scheme 3. Compound **54** was prepared via alkylation of 6-hydroxy-2,2-dimethyl-4-chromanone with Boc_2O in the presence of $Sc(OTf)_2$,[19] followed by elaboration to **54** as in Scheme 3.

Replacement of one or two of the methyl groups of the neopentyl substituent with either fluoro (**51**) or cyano (**52**) maintained the potency of **45** and also maintained a twofold selectivity over Cat-D, but the increase in the permeability was modest (**52** Papp = 25 nm/s compared to **45** Papp = 3 nm/s). The addition of ethers (**52**–**54**) caused a significant decrease in potency against BACE-1.

In summary, using structure based design, we have demonstrated that selectivity for BACE-1 over Cat-D is possible with 2,2-dioxo-isothiochromanes. Furthermore, the addition of polar substituents into the *P2′* region of the inhibitor provided substantial increases in the permeability of the inhibitors. The addition of alkyl substituents to the C-2 position of the chromane resulted in

Table 5
SAR of *P1′*/*P2′* variation



#	R^1	R^2	R^3	BACE-1 IC_{50}[a] (nM)	Cat-D IC_{50}[b] (nM)	Papp[c] (nm/s)
45	Me	Me	CH_2*tert*-Butyl	8	18	3
51	Me	Me	CH_2CMeF_2	9	16	17
52	Me	Me	CH_2CMe_2CN	6	8	25
53	Me	Me	OCF_3	89	59	12
54	Me	Me	O-*tert*-Butyl	690	374	11

[a] Ref. 13.
[b] Ref. 14.
[c] Ref. 11.

single-digit nanomolar inhibitors with moderate permeability and moderate selectivity over Cat-D. Our progress in balancing potency, good membrane permeability, Cat-D selectivity within our HEA scaffold will be reported in due course.

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14. The Cathepsin-D was obtained from Sigma (cat. #C8696). Cathepsin-D was first dissolved with water to a 2 μM concentration and then subsequently with 100 mM sodium acetate buffer pH 4.5 to a 1.8 nM working concentration. A biotinylated peptide Cathepsin-D substrate was labeled at the C-terminus of the peptide with an Oregon Green fluorophore. Compounds were serially diluted threefold in DMSO at a 100× concentration and then subsequently diluted 33× with 100 mM sodium acetate buffer pH 4.5. Compound was added to Cathepsin-D (0.6 nM final concentration) for 30 min before the addition of peptide substrate to initiate the reaction. The reaction was allowed to proceed for 110 min at 37 °C and then quenched by the addition of streptavidin in 200 mM sodium phosphate buffer pH 7.5. The amount of fluorescence polarization in the well was measured using a LJL Analyst (Perkin Elmer). If Cathepsin-D cleaves the peptide substrate then polarization will be reduced.
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