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Potent and selective isophthalamide S_2 hydroxyethylamine inhibitors of BACE1

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Abstract—The design and synthesis of a novel series of potent BACE1 hydroxyethylamine inhibitors. These inhibitors feature hydrogen bonding substituents at the C-5 position of the isophthalamide ring with improved selectivity over cathepsin D.

Alzheimer's disease (AD) is a debilitating and ultimately fatal form of dementia generally affecting people aged 60 and older. The disease progresses from mild cognitive impairment through profound dementia, loss of motor functions and finally death.[1] In addition to the devastating impact AD has on individuals and families there is also a societal impact. The financial cost to society, brought about by the prolonged nature of AD, is estimated at over $100 billion a year in the US alone.[2,3]

One of the major factors contributing to the onset of AD is the build-up of amyloid plaques in the brain. The primary component of the amyloid plaque is aggregated Aβ-peptide produced from the cleavage of Amyloid Precursor Protein (APP) by beta- and gamma-secretase.[4–6] β-Secretase (BACE1) cleaves APP, releasing the soluble portion of APP (sAPP) and leaving behind an APP fragment still anchored to the membrane. Gamma-secretase cleaves the anchored APP fragment a second time producing two forms of peptide, Aβ(1–40) and Aβ(1–42), with Aβ(1–42) being the major component in amyloid plaques.[7]

Inhibition of BACE1 appears to be a viable therapeutic target for the reduction of Aβ(1–40) and Aβ(1–42). It has been shown that BACE1 knockout mice, transgenic for human APP, do not have amyloid plaque build-up in the brain.[6] This data helps validate BACE1 as a therapeutic target for AD.

Hydroxyethylamine (HEA), **1** (Fig. 1) was found to be a potent inhibitor of BACE1 containing the optimized transition state insert (TSI) 3,5-difluoro Phe and C-terminal *m*-ethyl benzyl.[8] Although highly potent, this compound suffered from poor metabolic stability.

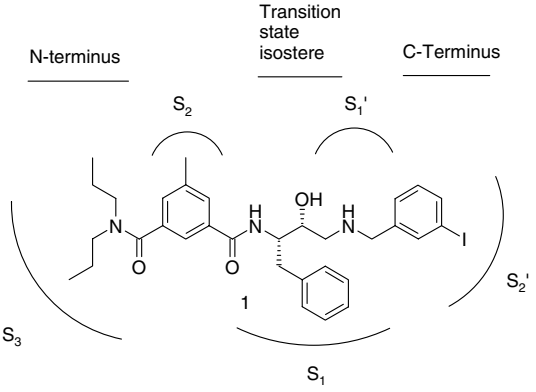

Figure 1. Isophthalamide hydroxyethyl amine.

Keywords: BACE1; Isophthalamide; HEA; Alzheimer's disease; Hydroxyethylamine; Inhibitor; S2 pocket; β-Secretase.
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X-ray crystal structures of **1** bound to BACE1, along with modeling, suggested a hydrogen bonding substituent at the C-5 position of the isophthalamide ring (S_2 pocket of BACE1) might result in improved binding with BACE1. Subsequent to our work on the isophthalamide HEA series, other groups have described work on related HEA series of inhibitors.[9]

Although **1** exhibits high affinity for BACE1 (2 nM) and reasonable selectivity over cathepsin D (Cat D) (75-fold), it was rapidly metabolized by rat and human microsomes. The main pathways for metabolism are *N*-debenzylation at the C-terminus and *N*-depropylation at the N-terminus (data not shown). Unfortunately, removal of the dipropyl amide results in a significant loss of affinity for BACE1.[11] Due to this loss we needed to improve BACE1 affinity before addressing the metabolic stability issues surrounding the HEA template.

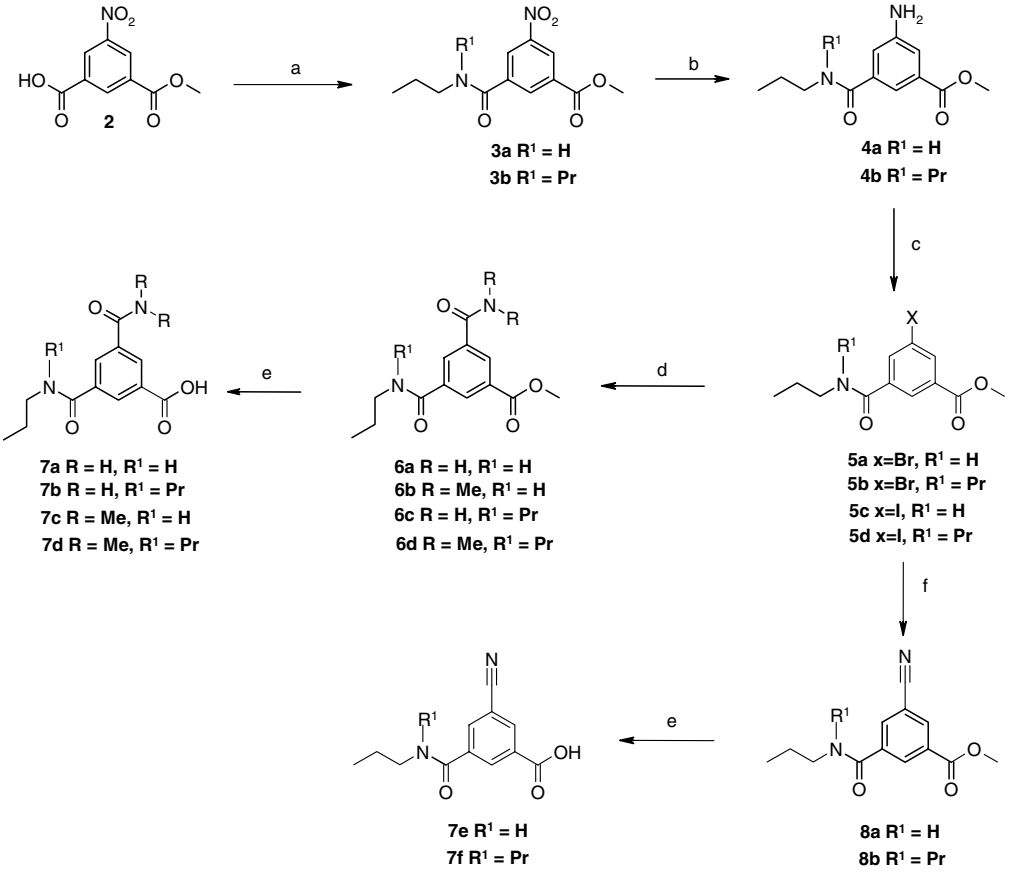
The S_2 pocket of Cat D is large and contains a lipophilic valine residue whereas the S_2 pocket of BACE1 contains an arganine residue.[12] Substituting the C-5 position of the isophthalamide ring with hydrogen bonding substituents may create an additional binding interaction with hydrogen bonding substituents in BACE1 and improve selectivity over Cat D. Towards these goals, we have prepared a series of C-5 isophthalamide HEA analogs in order to increase affinity for BACE1 and selectivity over Cat D.

The synthetic route used to prepare the C-3 amide and nitrile isophthalamide rings is outlined in Scheme 1.

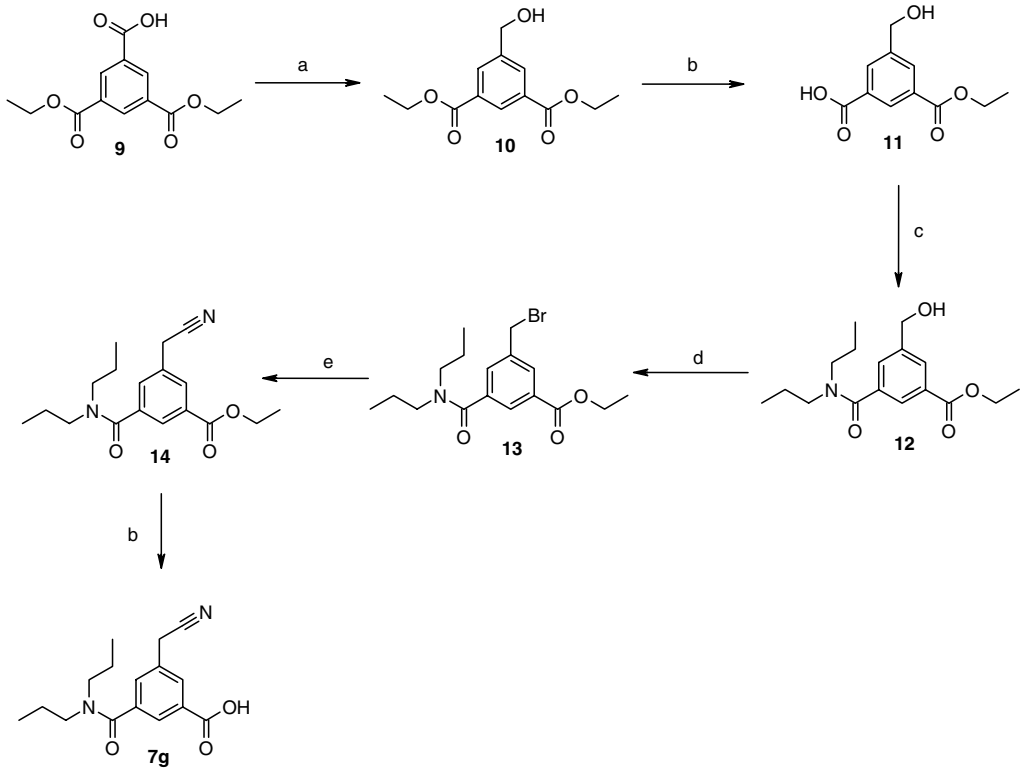
Mono methyl 5-nitroisophthalate was converted to isophthalamide **3a**–**b** using standard CDI amide coupling conditions. Nitro **3a**–**b** was reduced to aniline **4a**–**b** under catalytic hydrogenation conditions with palladium on carbon. Diazotization of anilines **4a**–**b** and displacement with either copper (II) bromide or potassium iodide gave halides **5a**–**d**. Under palladium acetate/dppp catalyzed carbonylation conditions halides **5a**–**d** gave amides **6a**–**d**.[15] Cyanide displacement of halides **5a**–**d** using copper cyanide gave nitriles **8a**–**b**. The esters of amides **6a**–**d** and **8a**–**b** were hydrolyzed using lithium hydroxide to give acids **7a**–**f**.

The C-3 acetonitrile was prepared using the synthesis outlined in Scheme 2.

Acid **9** was reduced to alcohol **10** using borane dimethyl sulfide complex. Selective hydrolysis of one ester of alcohol **10** was accomplished using 0.9 equiv of lithium hydroxide to give acid **11**. Acid **11** was coupled with dipropylamine using EDC/HOBt to give amide **12**.



Scheme 1. Synthesis of 3-amide-5-[(dipropylamino)carbonyl]benzoic acids **7a**–**7f**. Reagents and conditions: (a) CDI/CH_2Cl_2/dipropyl or propylamine/rt/5 h (75%); (b) 5%Pd on carbon/50PSI H_2/18 h (70%); (c) butyl nitrite/$CuBr_2$/CH_3CN/rt/3 h (**5a**,**b**) (75%); H_2SO_4/sodium nitrite/KI; 0 °C/1 h (**5c**,**d**) (80%); (d) $Pd(OAc)_2$/dppp/CO/DIEA/dimethylamine or hexamethyldisilazane/NMP/rt/18 h (70%); (e) LiOH/methanol/rt/24 h then HCl (70–80%); (f) CuCN/NMP/160 °C/6 h (70–80%).



Scheme 2. Synthesis of 3-(cyanomethyl)-5-[(dipropylamino)carbonyl]benzoic acid **7g**. Reagents and conditions: (a) $(CH_3)_2S$ BH_3/THF/rt/18 h/MeOH (71%); (b) LiOH–H_2O/H_2O/EtOH/rt/30 min (75%); (c) EDC/HOBt/CH_2Cl_2/dipropylamine/rt/18 h (50%); (d) PBr_3/50 °C/4 h (85%); (e) NaCN/DMSO/rt/3.5 h (95%).

The alcohol of amide **12** was converted to bromide **13** using PBr_3 at 50 °C. Bromide **13** was displaced with sodium cyanide to give nitrile **14**. Hydrolysis of the ester of nitrile **14** gave acid **7g**.

The $P_1 - P_2'$ fragments synthesis and final coupling are outlined in Scheme 3.

Epoxides **15a–c** were refluxed with benzyl amines **16a–b** to form $P_1 - P_2'$ fragments **17a–c**. BOC deprotection of $P_1 - P_2'$ fragments **17a–c** using TFA gave amines **18a–c**. HATU coupling of amines **18a–c** to acids **7a–g** gave HEA analogs **19a–k** and **19m–n**. The lower yields associated with this coupling reaction was due to unwanted coupling of the acid with the benzyl amine portion of **18a–c**.

The ester and acid HEA analogs **19l** and **19n**, respectively were made from acid **9** utilizing standard EDC/HOBt coupling to form the desired amide and selective hydrolysis as described above gave the desired isophthalamide. The acid of **19l** was formed by saponification of ester **19n** using lithium hydroxide.[10]

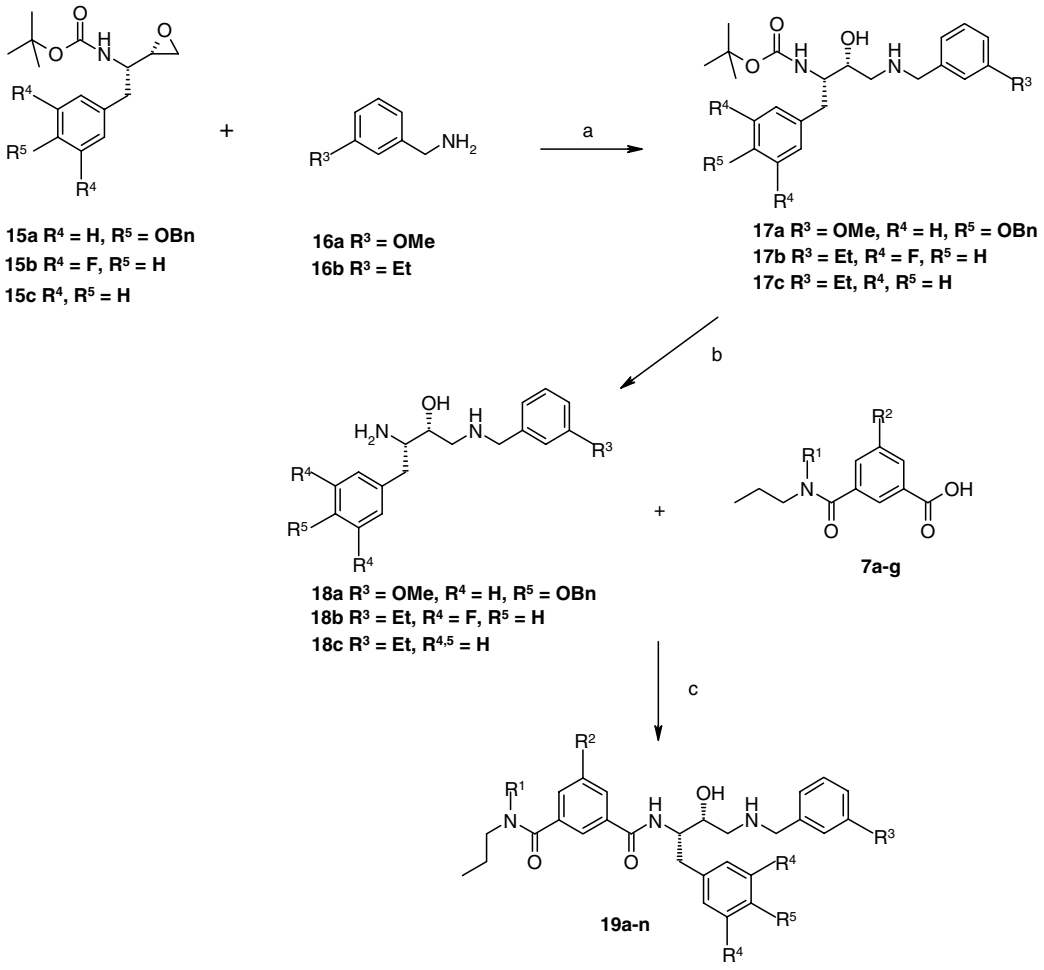
BACE1 and Cat D IC_{50} values are contained in Table 1.[14] Compounds **19a** and **19b** are included for comparison.[8]

Direct comparison of the parent compound **19b** with the corresponding carboxamide substituted analog **19c** showed comparable values for BACE1 but a 6-fold decrease in Cat D affinity. The dimethyl amide **19m** exhibited an 8-fold decrease in Cat D affinity when compared with the parent compound. Increasing the alkyl chain length of the C-5 amide to propyl as in **19i** resulted in little change in Cat D affinity. BACE1 affinity was reduced by a factor of 2.5 as compared with the parent compound. Acid **19l** was 3-fold more potent and 4-fold more selective than amide **19c** while ester **19n** lost selectivity 4-fold compared to **19c**.

Compounds **19d** and **19j** illustrate the contributions the difluoro Phe and *m*-ethyl benzyl amine moieties make toward BACE1 affinity compared to the amide **19c**. Replacing the P_1 Phe with 3,5 difluoro Phe increased BACE1 affinity 10-fold whereas Cat D affinity was unchanged compared to **19c**. Replacing the P_2' *m*-methoxy **19d** with *m*-ethyl **19j** results in a two-fold increase in BACE1 affinity.

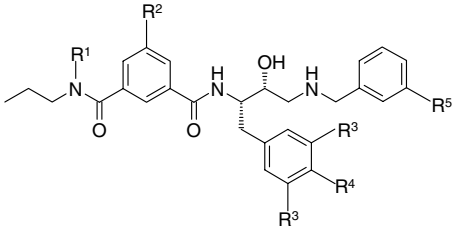
The X-ray crystal structure of **19d** in the BACE1 enzyme shows a stacking interaction between the amide carbonyl group and Arg 235 as shown by Figure 2.[13] The distance between the amide carbonyl group and Arg 235 is between 2.8 and 3.1 Å. In Cat D the amide carbonyl likely interacts with a more lipophilic Val 238, resulting in a less desirable interaction which is reflected in the affinities for Cat D. This same interaction may be responsible for the results obtained for **19e**.

In an effort to increase the interaction between the amide carbonyl and Arg 235, a methylene spacer was



Scheme 3. Synthesis of (2*R*,3*S*)-3-amino-1-(benzylamino)-4-phenylbutan-2-ols **18a**–**c** and final coupling. Reagents and conditions: (a) isopropanol/reflux/5 h (70–80%); (b) TFA/CH_2Cl_2/rt/1 h/sodium bicarbonate (90%); (c) HATU/TEA/CH_2Cl_2;/rt/18 h (30–50%).

Table 1. Table of BACE1 and Cat D IC_{50} values



Compound	R^1	R^2	R^3	R^4	R^5	BACE IC_{50} (nM)	Cat D IC_{50} (nM)
19a	Pr	Me	F	H	Et	2	150
19b	Pr	Me	H	H	OMe	100	450
19c	Pr	$CONH_2$	H	H	OMe	100	2800
19d	Pr	$CONH_2$	F	H	OMe	11	2400
19e	Pr	CN	H	H	OMe	90	660
19f	Pr	CH_2CN	H	H	OMe	360	570
19g	H	CN	H	H	OMe	770	>20,000
19h	H	$CONH_2$	H	H	OMe	1900	>20,000
19i	Pr	$CON(Pr)_2$	H	H	OMe	250	3600
19j	Pr	$CONH_2$	F	H	Et	5	1600
19k	Pr	$CONH_2$	H	OH	OMe	300	>20,000
19l	Pr	COOH	H	H	OMe	30	3600
19m	Pr	$CON(Me)_2$	H	H	OMe	70	3800
19n	Pr	COOEt	H	H	OMe	132	866

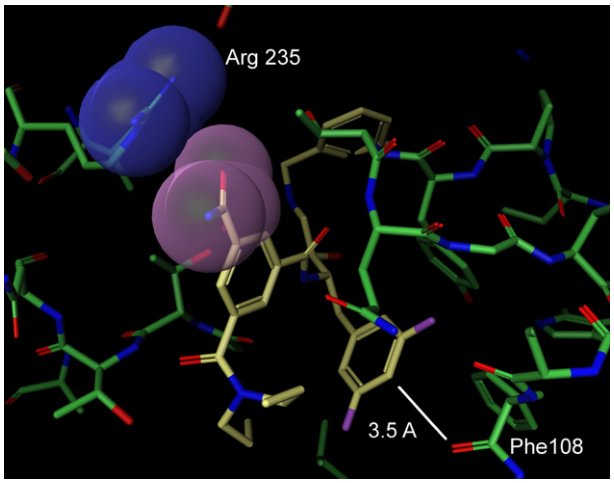


Figure 2. A 3D view of the 2.5 Å structure of **19d** bound to BACE showing the stacking of the amide to Arg235 using van der Waals surfaces (pink and blue). The carbonyl oxygen of Phe108 which is 3.5 Å away from the para position of the P1 phenyl is also shown. Waters have been omitted for clarity.

placed between the isophthalamide ring and the nitrile substituent to give **19f**. Unfortunately this brought about a decrease in BACE1 affinity with a slight increase in the Cat D affinity. Attempts to convert **19f** to the primary carboxamide were unsuccessful.

Since a major metabolite of compounds such as **19a** is depropylation of the dipropyl amide on the N-terminus, compounds such as **19g** and **19h** were prepared to eliminate this mode of metabolism and improve overall metabolic stability. Unfortunately the added interaction at the C-5 position of the isophthalamide ring did not compensate for the loss in affinity resulting from the removal of one of the propyl groups in the dipropyl amide. BACE1 affinity decreased ~9 to 19-fold for the mono propyl amide analogs.

Due to the loss in affinity for BACE1 in compounds such as **19g** and **19h** we needed to further improve upon the affinity for BACE1. Based on the X-ray crystal structure of **19d** in the BACE1 enzyme, Phe-108, which resides in the bottom of the S_3 pocket of BACE1, may offer an additional hydrogen bonding opportunity. In an attempt to gain hydrogen bonding between the BACE1 inhibitor and the carbonyl oxygen of Phe-108, we replaced the difluoro phenyl portion of **19d** with a *para*-hydroxy group; however **19k** showed an approximately 30-fold decrease in BACE1 affinity. As a result, further analogs with the tyrosine HEA insert were not pursued.

A carboxamide HEA with optimized P_1 and P'_2 groups **19j** resulted in a 5 nM BACE1 inhibitor with 320-fold selectivity over Cat D compared with 75-fold selectivity for **19a**. The added interaction with BACE1 at S_2 was not enough to overcome the affinity loss associated with the removal of one of the propyl substituents of the N-terminal dipropyl amide. Several other HEAs with various substituents in the C-5 position of the isophthalamide ring were prepared but did not improve upon **19d**.

Acknowledgments

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13. Coordinates for the complex of BACE with inhibitor **36d** have been deposited in the Protein Data Bank (www.rcsb.org) under PDB ID 2P83.
14. The BACE1 and cathepsin D assays were used to measure enzymatic activities in a fluorescence polarization format and in an endpoint mode. Both assays were performed in non-binding 384-well plates and in a volume of 30 μL/well. Enzyme, substrate, and stop solutions were added to the assay plates with a LabSystems Multidrop 384. The reactions in the BACE1 assay were run at 37 °C for 3 h under the following conditions: 100 μM sodium acetate (pH 4.5), 150 nM substrate 1, 0.1 nM BACE1, 2% DMSO, and 0.001% (v/v) Tween-20. The cathepsin D assay reactions were run at 37 °C for 90 min under the following conditions: 100 μM sodium acetate (pH 4.5), 150 nM substrate 2, 0.5 nM cathepsin D, 2% DMSO, and 0.001% (v/v) Tween-20. Reactions were terminated by the addition of stop solution (30 μL/well). Following a 15-min incubation at room temperature, sample fluorescence polarization was measured on a LJL Biosystems Acquest (Sunnyvale, CA) using an excitation 485 nm filter, a 530 nm emission, and G factor settings of 0.872 and 0.864 for the BACE1 and the cathepsin D assays, respectively.
15. *Carbonylation procedure for compound* **6d**: To a mixture of methyl 3-[(dipropylamino)carbonyl]-5-iodobenzoate (0.42 g, 1.08 mmol) in dry NMP (6.0 mL) bubbled with carbon monoxide for 10 min was added diisopropyl ethylamine (0.14 g, 1.08 mmol), palladium (II) acetate (0.025 g, 0.108 mmol), 2 M dimethylamine in THF (1.0 mL, 2.0 mmol) and diphenylphosphino propane (0.067 g, 0.162 mmol). The mixture was stirred at room temperature under a carbon monoxide atmosphere overnight. The mixture was partitioned between water and ethyl acetate. The layers were separated and the organic layer washed twice with water, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (100 mL) using 3% CH_3OH in CH_2Cl_2 to give 0.249 g (69%) of the title compound: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.745–0.765 (m, 3 H), 0.972–0.998 (m, 3H), 1.51–1.59 (m, 2H), 1.65–1.73 (m, 2H), 2.99 (s, 3H), 3.09–3.17 (m, 5H), 3.41–3.53 (m, 2H), 3.94 (s, 3H), 7.62 (d, J = 1.2 Hz, 1H), 8.07 (d, J = 1.2 Hz, 1H), 8.12 (d, J = 1.2 Hz, 1H).