Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 8 September 2011, Revised 1 November 2011, Accepted 18 November 2011 Published online 23 January 2012 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1955

Tritium labeling of a γ-secretase inhibitor and two modulators as *in vitro* imaging agents

Jonas Malmquist,[a]* Alexandra Bernlind,[b] Johan Sandell,[a] Peter Ström,[a] and Magnus Waldman[b]

The γ-secretase inhibitor dibenzazepine (DBZ) and the γ-secretase modulators 1 and AZ8349 were prepared as tritium-labeled compounds with high specific activity and radiochemical purity. [^{3}H]DBZ was labeled via an iodinated precursor, [^{3}H]1 was labeled by [^{3}H]methylation of an *O*-desmethyl precursor, and [2-^{3}H]AZ8349 was labeled via a tribromoacetyl precursor by catalytic hydrogenation. [^{3}H]DBZ, [^{3}H]1, and [2-^{3}H]AZ8349 are promising *in vitro* imaging radioligands and have the potential to provide key information with regard to γ-secretase expression, function, stoichiometry, and pharmacology.

Keywords: isotopically labeled synthesis; H-3; ^{3}H; DBZ; AZ8349; GSI; GSM

Introduction

γ-Secretase is one of the enzymes that catalyze the cleavage of the amyloid precursor protein into shorter amyloid beta peptide fragments. Amyloid beta peptides aggregate into senile plaques in the brains of patients with Alzheimer's disease and are one of the neuropathological hallmarks of the disease. Two pharmacological classes of small molecules targeting γ-secretase have been described to date: γ-secretase inhibitors (GSIs) and γ-secretase modulators (GSMs).[1] GSIs and GSMs have attracted much attention as potential novel disease-modifying therapeutics for Alzheimer's disease, and several γ-secretase-based drug discovery programs are ongoing.[2,3]

Isotope-labeled γ-secretase binding molecules have been shown to be useful pharmacological tools that have provided valuable insights into the biology of γ-secretase and γ-secretase-based drug discovery efforts.[4,5,6] In this paper, we describe the radiolabeling of the two GSMs, **1** ((*E*)-1-(1-(4-fluorophenyl) ethyl)-3-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzylidene) piperidin-2-one[7]) and **AZ8349** (1-(4-benzyl-2-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)phenylamino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)ethanone[8]), and a GSI, dibenzazepine (**DBZ**) ((2*S*)-2-(2-(3,5-difluorophenyl)acetamido)-*N*-(5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b*,*d*]azepin-7-yl)propanamide,[9,10] (Figure 1).

A preliminary pharmacological characterization of [^{3}H]1, [^{3}H] **DBZ**, and [^{3}H]**AZ8349** including imaging with *in vitro* autoradiography has previously been reported by Borgegård *et al.*[11] By cross-competition and radioligand displacement studies, it was shown that the GSM and GSI radioligands have different γ-secretase binding sites and, thus, are valuable imaging tools in the study of target expression and drug–target interactions.

Results and discussions

Methyl groups provide an entry to a radioligand with high specific activity because of the opportunity to simultaneously introduce three tritium atoms. High specific activity increases the sensitivity of detection of the radioligand, which enables the radioligand to be used at a correspondingly lower concentration. This is of importance when imaging a protein that is expressed in a low density, or expressed only in very discrete brain areas. Accordingly, the labeling strategies of **1** and **AZ8349** took advantage of the presence of methyl groups in the compound structures.

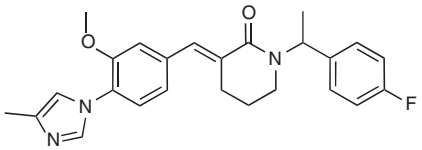
Borontribromide was used for the demethylation of 1. The *O*-desmethyl precursor **2** (Scheme 1) was prepared in high yield and purity after purification by flash chromatography. Methylation was accomplished by deprotonation of the phenol with sodium hydroxide in demethyl sulfoxide and subsequent addition of high specific activity, 2.8 TBq/mmol, [^{3}H]iodomethane at ambient temperature. The reaction was completed after 10 min. The high specific activity was retained, and [^{3}H]1 was isolated in 20%-radiochemical yield with no detectable impurities. The substance was characterized by co-elution on HPLC with 1 and by mass spectroscopy.

Treatment of **DBZ** with *N*-iodosuccinimide in trifluoroacetic acid afforded the iodinated precursor **3** (Scheme 2). The position of iodination was confirmed with NMR spectroscopy. Catalytic deiodohydrogenation was performed in the presence of palladium (II) oxide with an excess of tritium at 803 mbar. A specific activity of 0.6 TBq/mmol was obtained for **[^{3}H]DBZ** in 32% yield and a radiochemical purity of 99.7%. The tritium position was confirmed by ^{3}H NMR, and because proton atoms were decoupled in the ^{3}H NMR experiment, 3-bond as well as 5-bond *J*-couplings between ^{3}H and ^{19}F could be measured in the spectrum (Figure 2).

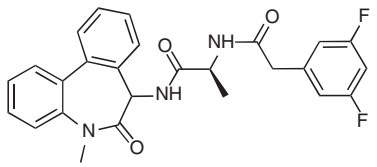
[a]*Isotope Chemistry, Screening and Profiling Global DMPK IM, AstraZeneca Research and Development Innovative Medicines, Södertälje SE-151 85, Sweden*

[b]*Medicinal Chemistry, CNSP iMed Science Södertälje, AstraZeneca Research and Development Innovative Medicines, Södertälje SE-151 85, Sweden*

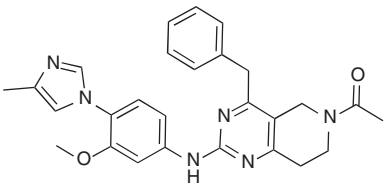
**Corresponndence to: Jonas Malmquist, Isotope Chemistry, Screening and Profiling Global DMPK IM, AstraZeneca Research and Development Innovative Medicines, Södertälje SE-151 85, Sweden.*
E-mail: jonas.malmquist@astrazeneca.com



1



DBZ



AZ8349

Figure 1. Structure of substances labeled.

Several routes were investigated for tritium incorporation into **AZ8349** and found unsuccessful:

- H/^{3}H exhange with an iridium (I) complex for catalytic hydrogen isotope exchange:[12] No reaction was observed.
- Methylation with iodomethane of an *O*-desmethyl precursor: Only *N*-alkylation was observed.
- Methylation with iodomethane of an *O*-desmethyl precursor with *N*-BOC or *N*-SEM protection: The desired product could not be identified.
- Acylation of **4** with [^{3}H]acetic anhydride:[13] Unfruitful, although acylation worked on a larger scale with acetic anhydride.[8]

To circumvent this unexpected problem, another substrate was prepared by the introduction of a tribromoacetyl moiety. The objective was to have a fair option of a decent specific activity. To our knowledge, this method of labeling an acetyl group has not been reported in the literature before. The *N*-acylation was

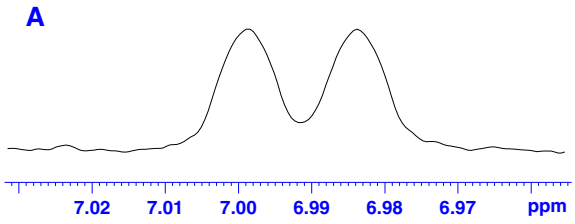


Figure 2. ^{3}H spectra of **[^{3}H]DBZ**, expanded to show the ^{3}H signal. A: ^{3}H spectrum. B: ^{3}H spectrum with ^{1}H decoupling, which allows the 3-bond and 5-bond ^{3}H, ^{19}F couplings to be identified.

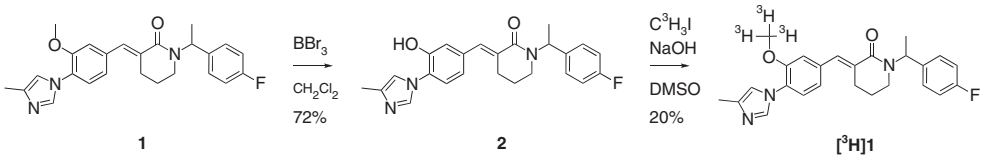
achieved by reacting tribromoacetyl chloride with **4**[8] to provide **5** in moderate yield (Scheme 3). Tritium was introduced at 621 mbar with palladium catalysis to perform a debromohydrogenation of **5**. The reaction was run overnight in ethanol. The unoptimized yield of **[^{3}H]AZ8349** was 1.3%, with a radiochemical purity of 97% and a specific activity of 1.2 TBq/mmol. The substance was characterized by co-elution on HPLC with **AZ8349** and by mass spectroscopy. ^{3}H NMR was not performed because of the small amount isolated. The yield and specific activity would possibly improve with a shorter exposure time to tritium gas.

Experimental

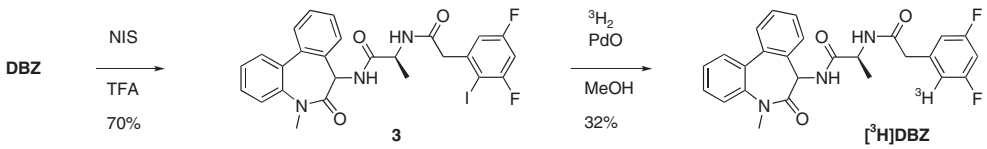
General methods

All solvents used were of analytical grade and commercially available. Anhydrous solvents were routinely used for reactions. Reactions were typically run under an inert atmosphere of nitrogen or argon. Tritium gas was handled in a tritium gas manifold system (RC TRITEC AG, Teufen). [^{3}H]Iodomethane was purchased from Larodan Fine Chemicals AB.

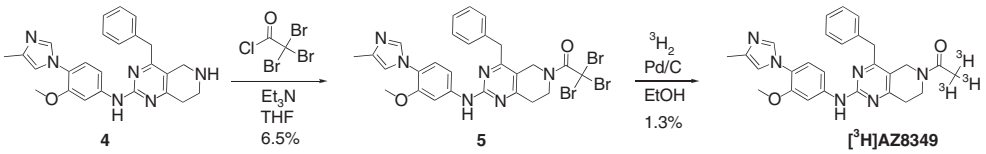
^{3}H and ^{1}H spectra were recorded on a Bruker DRX600 NMR Spectrometer, operating at 640 MHz for tritium and at 600 MHz for proton, equipped with a 5-mm ^{3}H/^{1}H SEX probehead with



Scheme 1.



Scheme 2.



Scheme 3.

Z-gradients. ^{1}H decoupled ^{3}H spectra were recorded on samples dissolved in CD_3OD. For ^{3}H NMR spectra referencing, a ghost reference frequency was used, as calculated by multiplying the frequency of internal tetramethylsilane in an ^{1}H spectrum with the Larmor frequency ratio between ^{3}H and ^{1}H (1.06663975), according to the description by Al-Rawi *et al.*[14] ^{1}H spectra were referenced to external tetramethylsilane, which was set to 0 ppm.

Mass spectra were recorded on a Waters liquid chromatography coupled mass spectroscopy (LC-MS) consisting of a Waters 1525 micro (LC), Waters photodiode array 2996, and evaporative light scattering detector (Sedex 75) and a z-spray mass detector (ZMD) single quadrupole mass spectrometer.

HPLC analyses were performed on an Agilent 1100, HPLC system with a binary pump, auto-injector, diode array detector (DAD) and column oven, coupled in series with a Packard Radiomatic Flow Scintillator 525TR, equipped with a solid scintillator (SolarScint) cell with a volume of 33 µl. A Gemini column (C18, 3 × 100 mm, 5 µm) with the column temperature set to 40 °C use a gradient of 5%–95% acetonitrile in 10 mmol/L ammonium acetate in MilliQ water at 1 ml/min over 13.5 min. Purities were reported as UV area% or radioactive area% by HPLC.

Preparative chromatography was run on an XBridge™ column (C8, 10 µm 19 × 250 mm) using a gradient of 55%–90% methanol/ 0.2% ammonia in MilliQ Water at 20 ml/min or a Kromasil column (C8, 5 µm, 10 × 250 mm) using acetonitrile/ 50 mmol/L ammonium acetate in MilliQ Water at 2 ml/min.

Liquid scintillation analysis was performed on a PACKARD TRICARB 2900TR. Specific activities were determined with mass spectrometry and confirmed with ^{3}H NMR. Thin layer chromatography was performed on Merck TLC-plates (Silica gel 60 F_{254}), and UV light (254 nm) visualized the spots. Flash column chromatography was performed on Redisep™ prepacked columns.

(*E*)-1-(1-(4-Fluorophenyl)ethyl)-3-(3-hydroxy-4-(4-methyl-1*H*-imidazol-1-yl)benzylidene)piperidin-2-one (2)

(*E*)-1-(1-(4-Fluorophenyl)ethyl)-3-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzylidene)piperidin-2-one, **1**,[7] (20 mg, 0.05 mmol) was dissolved in dichloromethane (1 ml) under an argon atmosphere and was cooled to approximately −78 °C. Borontribromide (1 mol/L in dichloromethane, 100 µl, 0.10 mmol) was added, and the mixture was warmed to ambient temperature over a couple of hours and was stirred overnight. The reaction was neutralized with sat. sodium bicarbonate and was extracted with ethyl acetate (4 × 1 ml). The organic phase was dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography on silica gel (4 g) using a gradient of dichloromethane to dichloromethane/methanol 25:1 to give **2** (14 mg, 0.03 mmol) in 72% yield and 98% UV purity. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.48 (d, *J* = 6.94 Hz, 3 H), 1.55–1.65 (m, 2 H), 1.68–1.80 (m, 1 H), 2.69 (dq, *J* = 20.77, 10.36 Hz, 2 H), 2.82–2.91 (m, 1 H), 3.17 (ddd, *J* = 12.30, 8.67, 3.47 Hz, 2 H), 6.07 (q, *J* = 6.99 Hz, 2 H), 6.91 (d, *J* = 8.35 Hz, 1 H), 6.94–7.00 (m, 4 H), 7.08 (s, 2 H), 7.64 (s, 2 H), 8.38 (br s, 1 H). LC-MS *m/z* 406.0 ($[M + H]^+$).

[3-$^{3}H_3$]-(*E*)-1-(1-(4-Fluorophenyl)ethyl)-3-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzylidene)piperidin-2-one ([^{3}H]1)

(*E*)-1-(1-(4-Fluorophenyl)ethyl)-3-(3-hydroxy-4-(4-methyl-1*H*-imidazol-1-yl)benzylidene)piperidin-2-one, **2**, (1.2 mg, 3.0 µmol) was dissolved in dimethyl sulfoxide (0.5 ml). Sodium hydroxide (10%, 5 µl) was added. The mixture was stirred for 5 min at ambient temperature. [^{3}H]Iodomethane (2.8 TBq/mmol, 1850 MBq in 0.05 ml toluene, 0.66 µmol) was added to this mixture. After 10 min of stirring at ambient temperature, the mixture was filtered through silica (1 cm in a Pasteur pipette) and was eluted with dichloromethane. The concentrated filtrate was purified on HPLC using the Kromasil column and 60% acetonitrile. Pooled fractions were concentrated and dissolved in ethanol (abs. 12 ml) to give **[^{3}H]1** (371 MBq, 2.8 TBq/mmol) in a 20% yield and >99.9% radiochemical purity. LC-MS *m/z* 424, 426 ($[M + H]^+$).

(2*S*)-2-(2-(3,5-Difluoro-2-iodophenyl)acetamido)-*N*-(5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b,d*]azepin-7-yl) propanamide (3)

To (2*S*)-2-(2-(3,5-difluorophenyl)acetamido)-*N*-(5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b,d*]azepin-7-yl) propanamide, **DBZ** (10 mg, 0.02 mmol) in trifluoroacetic acid (1 ml) was added *N*-iodosuccinimide (5 mg, 0.02 mmol), and the reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated. The residue was purified on an HPLC with the Kromasil column and 60% acetonitrile. Pooled fractions were concentrated to give **3** (8 mg, 0.014 mmol) in a 63% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.46 (d, *J* = 6.94 Hz, 3 H), 3.37 (s, 3 H), 3.77 (s, 2 H), 4.74 (quin, *J* = 7.01 Hz, 1 H), 5.25 (d, *J* = 6.62 Hz, 1 H), 6.32 (br. s., 1 H), 6.79 (td, *J* = 8.12, 2.68 Hz, 1 H), 7.00 (d, *J* = 8.83 Hz, 1 H), 7.27–7.31 (m, 1 H), 7.34–7.39 (m, 2 H), 7.40–7.46 (m, 2 H), 7.46–7.51 (m, 1 H), 7.53 (d, *J* = 5.36 Hz, 1 H), 7.55–7.58 (m, 1 H), 7.61 (d, *J* = 7.57 Hz, 1 H). LC-MS *m/z* 590 ($[M + H]^+$).

[2-^{3}H]-(2*S*)-2-(2-(3,5-Difluorophenyl)acetamido)-*N*-(5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b,d*]azepin-7-yl) propanamide ([^{3}H]DBZ)

(2*S*)-2-(2-(3,5-Difluoro-2-iodophenyl)acetamido)-*N*-(5-methyl-6-oxo-6,7-dihydro-5*H*dibenzo[*b,d*]azepin-7-yl)propanamide, **3**, (1.6 mg, 2.7 µmol) and palladium (II) oxide (1.4 mg, 0.01 mmol) were dissolved in methanol (0.3 ml). The mixture was stirred for 1 h in 803 mbar tritium atmosphere at ambient temperature. The mixture was filtered, and the residue was lyophilized with methanol (1 ml) twice. The product was isolated by purification on HPLC using the Kromasil column and 60% acetonitrile. Pooled fractions were concentrated to give **[^{3}H]DBZ**, (540 MBq, 0.60 TBq/mmol) in 32% yield and a radiochemical purity of 99.7%. ^{1}H NMR (600 MHz, CD_3OD) δ ppm 1.43 (d, *J* = 7.12 Hz, 3 H), 1.92 (s, 3 H), 3.62 (d, *J* = 1.57 Hz, 2 H), 4.59 (q, *J* = 7.12 Hz, 1 H), 5.20 (s, 1 H), 6.78–6.84 (m, 1 H), 6.95 (d, *J* = 8.26 Hz, 1 H), 7.33–7.36 (m, 1 H), 7.37–7.40 (m, 1 H), 7.41–7.46 (m, 2 H), 7.50–7.57 (m, 2 H),

7.62 (d, $J = 7.54$ Hz, 1 H), 7.67 (dd, $J = 7.76$, 1.21 Hz, 1 H). ^{3}H NMR (640 MHz, CD_3OD) δ ppm 6.99 (dd, $J = 9.8$, 0.7 Hz). The ^{3}H signal is split into a double doublet by $^3J_{TF}$ and $^5J_{TF}$. LC-MS *m/z* 464, 466 ($[M + H]^+$).

1-(4-Benzyl-2-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl) phenylamino)-7,8-dihydropyrido[4,3-d]pyrimidin-6(5*H*)-yl)-2,2,2-tribromoethanone (5)

4-Benzyl-*N*-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-2-amine, **4**, (56 mg, 0.13 mmol) was mixed with triethylamine (27 μl, 0.20 mmol) and 2,2,2-tribromoacetyl chloride (25 μl, 0.13 mmol) in tetrahydrofuran (2 ml).The mixture was stirred at ambient temperature for 2½ h. The mixture was then concentrated, and the residue was dissolved in dimethyl sulfoxide (1 ml) and was purified on a preparative HPLC twice using the XBridge column. Pooled fractions were concentrated to give **5** (6 mg, 8.5 μmol) in 6.5% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.09 (br. s., 3 H), 2.14 (s, 3 H), 2.94 (br. s., 1 H), 3.73 (s, 2 H), 4.03 (s, 2 H), 4.14 (s, 1 H), 4.81 (br. s., 1 H), 7.01 (s, 1 H), 7.16 (d, $J = 8.40$ Hz, 1 H), 7.21–7.27 (m, 1 H), 7.28–7.38 (m, 6 H), 7.64 (s, 1 H), 7.89 (s, 1 H), 9.81 (br. s., 1 H). LC-MS *m/z* 703, 705, 707, 709 ($[M + H]^+$).

[2-^{3}H]1-(4-Benzyl-2-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl) phenylamino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) ethanone ([^{3}H]AZ8349)

1-(4-Benzyl-2-(3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)phenylamino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-2,2,2-tribromoethanone, **5**, (0.89 mg, 1.26 μmol) and palladium (10% on carbon) (1.3 mg) were mixed in ethanol (0.4 ml). The mixture was connected to the tritium manifold and placed under tritium atmosphere (excess) at 621 mbar. The reaction mixture was stirred at ambient temperature overnight. The reaction mixture was filtered and lyophilized with ethanol (1 ml) thrice. The residue was dissolved in acetonitrile (0.4 ml) and purified by preparative HPLC with the Kromasil column and 47% acetonitrile. Pooled fractions were concentrated and dissolved in ethanol (2.00 ml) to give **[^{3}H]AZ8349** (20 MBq, 1.2 TBq/mmol) in 1.3% yield and a radiochemical purity of 97%. LC-MS *m/z* 469, 471, 473, 475, 477 ($[M + H]^+$).

Conclusions

Methods for the preparation of **[^{3}H]1**, **[^{3}H]DBZ**, and **[^{3}H]AZ8349** have been developed providing a specific activity sufficient for *in vitro* imaging of brain tissue γ-secretase. To overcome the unexpected problems with *N*-acylation of **4**, the tribromoacyl motif was introduced as a precursor for tritium-labeled acetyl groups and derivatives thereof.

Conflict of interest

The authors did not report any conflict of interest.

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