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A route to the γ-secretase inhibitor [2,3,4-^{3}H]BMS299897 via an α-phenylselenide derivative

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A method for the preparation of [2,3,4-^{3}H]BMS299897 has been developed. The methyl ester of BMS299897 was oxidized to its double bond derivative, via a phenyl selenide. The resulting double bond was reduced with tritium using Wilkinson's catalyst. The tritiated BMS299897 was isolated, after basic hydrolysis, with a specific activity of 1.6 TBq/mmol.

Keywords: [^{3}H]BMS299897; ^{3}H NMR; Wilkinson's catalyst; secretase

Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder and is the most common form of dementia. Pathological lesions and plaques, consisting of the amyloid β-peptide (Aβ), are found in the brains of the AD patients. Aβ is heterogeneously produced by the sequential cleavages of amyloid precursor protein (APP) by the two aspartic proteases, β- and γ-secretase.

In this work, a method for tritiation of the γ-secretase inhibitor **BMS299897**[1] (Scheme 1) has been developed for the evaluation in autoradiographic studies. Initial trials showed that halogenation of **BMS299897** was difficult to accomplish using multiple reaction conditions. The working method for the preparation of [2,3,4-^{3}H]BMS299897 **1** was via reduction of a newly created double bond in the aliphatic chain[2].

Results and discussions

BMS299897 was esterified with trimethylsilyldiazomethane in methanol and toluene at slightly elevated temperature to give the methyl ester **2** (Scheme 1) in nearly quantitative yield. The α-carbon was deprotonated with LHMDS and alkylated with phenylselenyl chloride at a low temperature and the selenide **3** was isolated in a moderate yield. Oxidation of the selenium with peroxide, with subsequent elimination, created the α,β-unsaturated ester **4** in a moderate yield. Saturation of the double bond was performed using Wilkinson's catalyst and tritium-gas to give ester **5** in a good yield. The final deprotection of **5** was performed by hydrolysis using lithium hydroxide. The target compound **1** was isolated in good yield with a specific activity of 1.6 TBq/mmol. Analysis with ^{1}H/^{3}H-NMR of the labeled material shows incorporation in all CH_2 positions. The proportions of ^{3}H incorporation were about 1:3:1, in α-, β- and γ-position, respectively (Figure 1).

Experimental

General methods

All solvents used were 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 mannifold system from RC TRITEC AG, Teufen.

^{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 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 TMS in a ^{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.*[3] ^{1}H spectra were referenced to TMS that was set to 0 ppm.

Mass spectra were recorded on a Waters LCMS consisting of an Waters 1525 micro (LC), Waters PDA 2996, and ELS detector (Sedex 75) and a ZMD single quadrupole mass spectrometer.

HPLC analyses were performed on a Agilent 1100, HPLC-system with a binary pump, auto-injector, 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 Zorbax SB (C18, 2.1 × 100 mm, 3.5 μm); the column temperature was set to 40°C and the flow rate to 0.5 ml/min. A linear gradient was applied, starting at 100% 10 mM ammonium acetate in 5% acetonitrile and ending at 70% acetonitrile, in 13.5 min.

Preparative chromatography was run on a Gilson 305 with a Gilson UV/VIS-151 and a RAYTEST Ramona equipped with a Kromasil column (C18, 5 μm, 10 × 250 mm or C8, 7 μm, 10 × 250 mM) using acetonitrile/50 mM ammonium acetate in MilliQ Water.

Liquid scintillation analysis was performed on a PACKARD TRI-CARB 2900TR. Thin layer chromatography (TLC) was performed on Merck TLC-plates (Silica gel 60 F_{254}) and UV-light (254 nm)

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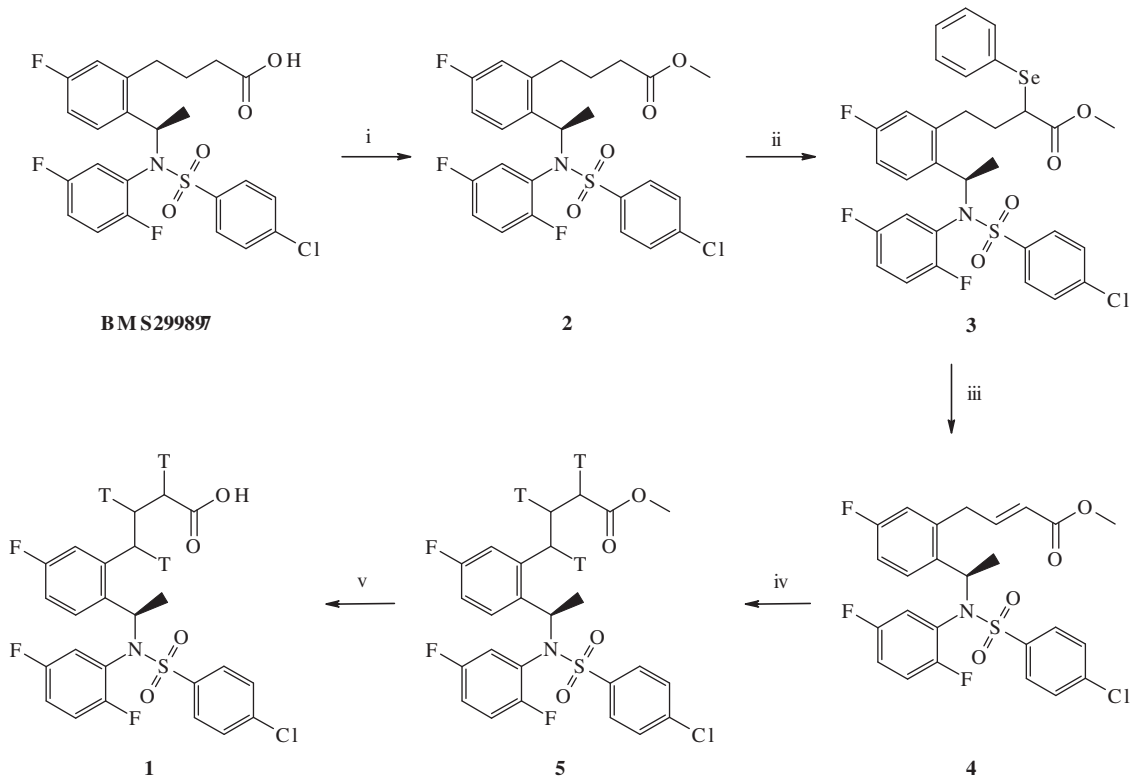

Scheme 1. (i) TMS-CH_2N_2 (ii) LHMDS, PhSe–Cl, −78°C (iii) $KHCO_3$, H_2O_2 (iv) $^3H_2$, Wilkinson's catalyst, (v) LiOH.

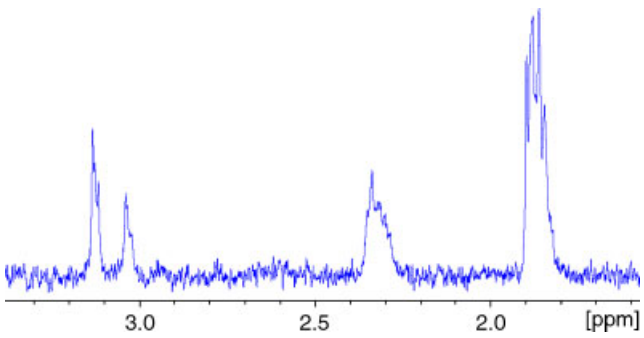

Figure 1. ^{3}H-NMR on 30 MBq [2,3,4-^{3}H]-BMS299897.

visualized the spots. Flash column chromatography was performed on a Redisep™ prepacked column.

(*R*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)butanoate (2)

(*R*)-4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)butanoic acid, **BMS299897**, (49.7 mg, 0.10 mmol) was dissolved in methanol (0.2 mL) and toluene (1 mL). Trimethylsilyldiazomethane (0.2 mL, 0.40 mmol) was added. The mixture was heated to 50°C under N_2-atmosphere for 45 min. The solvents were evaporated and the residue was dissolved in ethyl acetate (2 mL), washed with sodium carbonate (sat., 2 × 1 mL), dried over sodium sulfate, filtered and concentrated to give **2** in almost quantitative yield. ^{1}H NMR δ ppm 1.29–1.34 (m, 1 H) 1.49 (d, *J* = 5.67 Hz, 1 H) 1.54 (d, *J* = 6.94 Hz, 2 H) 1.89–2.00 (m, 3 H) 2.48 (t, *J* = 6.86 Hz, 3 H) 2.64–2.75 (m, 1 H) 3.08–3.16 (m, 1 H) 3.73 (s, 4 H) 5.83–5.90 (m, 1 H) 6.32–6.41 (m, 1 H) 6.53–6.60 (m, 1 H) 6.61–6.69 (m, 1 H) 6.86–6.87 (m, 1 H) 6.90–7.01 (m, 1 H) 7.02–7.09 (m, 1 H) 7.09–7.19 (m, 2 H) 7.53 (d, *J* = 7.57 Hz, 1 H) 7.58 (d, *J* = 8.35 Hz, 2 H) 7.62–7.68 (m, 1 H) 7.70–7.77 (m, 2 H). LC-MS *m/z* 543 ($[M+Na]^+$).

(*R*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)-2-(phenylselanyl)-butanoate (3)

(*R*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)butanoate (52 mg, 0.10 mmol) was dissolved in tetrahydrofuran (1 mL) under argon-atmosphere. The mixture was cooled to −78°C. Lithium *bis*(trimethylsilyl)amide (0.13 mL, 0.13 mmol) was added. After 10 min phenylselenyl chloride (18.93 mg, 0.10 mmol) dissolved in tetrahydrofuran (0.3 mL) was added. Room temperature was reached in 15 min. The reaction was quenched with ammonium chloride (sat., 1 mL), extracted with ethyl acetate (3 × 1 mL), dried over sodium sulfate, filtered and concentrated. The crude product was chromatographed on silica (12 g) using a gradient of petroleum ether to petroleum ether/diethyl ether 4:1 to give **3** (40 mg, 0.059 mmol) in 59% yield. ^{1}H NMR ($CDCl_3$) δ ppm 1.49 (d, 3 H) 1.91–2.06 (m, 1 H) 2.17–2.30 (m, 1 H) 2.63–2.93 (m, 2 H) 3.00–3.26 (m, 2 H) 3.73–3.80 (m, 1 H) 3.82 (s, 1 H) 3.86 (s, 3 H) 3.87–3.94 (m, 1 H) 5.76–5.96 (m, 1 H) 6.53–6.60 (m, 2 H) 6.66–6.81 (m, 1 H) 6.85–6.95 (m, 2 H) 6.95–7.05 (m, 2 H) 7.40–7.47 (m, 3 H) 7.54–7.75 (m, 4 H). LC-MS *m/z* 704 ($[M+Na]^+$).

(*R,E*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)but-2-enoate (4)

(*R*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)-2-(phenylselanyl)butanoate, **3**,

(61.3 mg, 0.09 mmol) was dissolved in ethyl acetate (1 mL) and tetrahydrofuran (0.5 mL). The mixture was cooled to 0°C. Potassium hydrogen carbonate (50 mg, 0.50 mmol) and hydrogen peroxide (100 μL, 0.98 mmol) was added. The mixture was stirred overnight at 21°C. Ethyl acetate (1 mL) was added and the organic phase was washed with sodium bisulfite (1 M, 2 × 1 mL), sodium bicarbonate (sat.) and was then dried over sodium sulfate. The organic phase was filtered and concentrated. The crude product was chromatographed on silica (4 g) using a gradient of dichloromethane to dichloromethane/methanol 50:1. Final purification was performed by preparative HPLC using a gradient of 65–85% acetonitrile. **4** (4 mg, 7.63 μmol) was isolated in 8% yield. ^{1}H NMR δ ppm 1.45 (d, *J* = 5.20 Hz, 1 H) 1.51 (d, *J* = 6.78 Hz, 1 H) 3.63 (dd, *J* = 17.10, 4.97 Hz, 1 H) 4.06–4.14 (m, 1 H) 5.65–5.91 (m, 1 H) 6.35–6.47 (m, 1 H) 6.65 (t, *J* = 8.12 Hz, 1 H) 6.69–6.78 (m, 1 H) 6.82–6.94 (m, 1 H) 6.98 (d, *J* = 9.30 Hz, 1 H) 7.01–7.06 (m, 1 H) 7.14 (ddd, *J* = 12.77, 3.70, 3.39 Hz, 1 H) 7.53 (d, *J* = 8.04 Hz, 1 H) 7.59 (d, *J* = 8.35 Hz, 1 H) 7.65 (d, *J* = 7.88 Hz, 1 H) 7.74 (d, *J* = 8.51 Hz, 1 H). ^{13}C NMR δ ppm 19.45, 35.92, 52.27, 55.82, 111.20, 117.73, 117.88, 121.37, 123.35, 130.54, 130.65, 130.73, 130.76, 130.79, 132.74, 136.57, 138.79, 142.03, 145.92, 148.76, 154.33, 154.54, 158.31, 163.33, 164.99, 166.09. LC-MS *m/z* 546 ($[M+Na]^+$).

(*R*)-[2,3,4-^{3}H]-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)-phenylsulfonamido)ethyl)-5-fluorophenyl)butanoate (5)

(*R,Z*)-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)but-2-enoate (0.8 mg, 1.53 ìmol) was mixed with chlorotris(triphenylphosphine)rhodium(I) (3 mg, 3.24 μmol) in *N,N*-dimethyl formamide (0.4 mL) and toluene (0.400 mL). The mixture was connected to tritium (1666 mbar) and stirred for 17 h at 21°C. The reaction was quenched by filtration through silica (1/2 cm) and was eluted with absolute ethanol (1 mL). The eluate was lyophilized with absolute ethanol (3 × 1 mL). Chromatography on silica (4 g) using a gradient of petroleum ether to diethyl ether. Fractions containing product were pooled and concentrated to give ~500 MBq of **5**. LC-MS *m/z* centered around 552 ($[M+Na]^+$).

(*R*)-[2,3,4-^{3}H]-4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)butanoic acid (1)

(*R*)-[2,3,4-^{3}H]-Methyl 4-(2-(1-(4-Chloro-*N*-(2,5-difluorophenyl)phenylsulfonamido)ethyl)-5-fluorophenyl)butanoate (0.809 mg, 1.53 μmol) was dissolved in acetonitrile (0.7 mL) and water (0.2 mL).

Lithium hydroxide (4 mg, 0.17 mmol) was added. The solution was stirred at 21°C overnight. The mixture was acidified with hydrochloric acid (2 M, 4 drops) and was then concentrated. The residue was dissolved in dimethyl sulfoxide (250 μL) and was purified on preparative HPLC using 62% acetonitrile. An amount of 354 MBq was isolated in a specific activity of 1.6 TBq/mmol and a 99.9% purity. The product was stored in absolute ethanol at 44 MBq/mL. ^{3}H NMR (200 MBq/mL) δ ppm 1.87 (m, CHT), 2.34 (m, –CHT–C = O), 3.08 (m, CHT-Ar). LC-MS *m/z* centered around 514 ($[M-H]^-$).

Conclusions

A working method for the preparation of [2,3,4-^{3}H]-BMS299897 has been developed with good specific activity.

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