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Syntheses of AZ12320927 labeled with H-3, C-14, and H-2

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In support of a program to develop a treatment for depression, three isotopically labeled forms of the 5-HT_{1B} antagonist AZ12320927 were synthesized. A tritium labeled version was synthesized for autoradiography using Ir-catalyzed hydrogen–tritium exchange. A C-14 labeled version was prepared for use in metabolism studies in four-steps from [*u*-^{14}C]*p*-nitrophenol. A stable isotope labeled version was synthesized for use as an internal standard for LC/MS/MS quantitation in two steps from chromenone 1.

Keywords: 5-HT_{1B} antagonist; Ir-catalyzed exchange; nucleophilic aromatic substitution

Introduction

Depression affects a large percentage of the population, yet its causes remain unclear. While grief, stress and many other environmental factors increase the risk of depression, everyone who is exposed to these factors does not develop the disease. There also appears to be a genetic predisposition towards depression as is evidenced by the fact that depression tends to run in families.[1]

Low levels of serotonin (5-HT) and norepinephrine are associated with depression, but it is not clear whether the association is causal or symptomatic.[2] Regardless of the association, drugs that enhance neurotransmission have been effective at alleviating the symptoms of depression in many cases.[2] There are four major classes of antidepressants currently in use: tricyclics, serotonin–norepinephrine reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors with the SSRIs being the primary treatment option for most patients.

SSRIs were designed to inhibit the re-uptake of serotonin by the presynaptic axion and thereby raise the concentration of 5-HT in the synapse. While administration of an SSRI results in rapid inhibition of serotonin re-uptake, antidepressant effects take several weeks to occur.[3] There are several hypotheses for this latency of action, but perhaps the most compelling proposal is that the SSRIs lead to down regulation of the 5-HT_{1B} receptors found on the presynaptic neuron.[3,4] 5-HT_{1B} receptors are proposed to participate in a feedback mechanism, which regulates release of serotonin into the synapse.[5] Therefore, a 5-HT_{1B} antagonist might avoid the latency period observed with SSRIs. AZ12320927 was identified as a potent and selective antagonist of the 5-HT_{1B} receptor and was required in labeled form.

Results and discussion

Stable isotope labeled AZ12320927 was required labeled in the chromane ring with a minimum mass increase of four to serve as an internal standard for quantitation of AZ12320927 in plasma samples by LC/MS/MS. Since this was to be used as an internal standard, racemic material was deemed acceptable, but no detectable amount of unlabeled AZ12320927 could be present. The medicinal chemistry synthesis utilized a reduction of chromenone **1** to afford the racemic **2**. This appeared to be an ideal step to incorporate an isotopic label by substituting deuterium for hydrogen. Deuteration of chromenone **1** in DO_2CCH_3 at 70°C over five days gave a 47% yield of the target chromane **rac-[$^2H_6$]-2** (Scheme 1). The main impurity – benzylic acetate **rac-[$^2H_5$]-3** – resulted from incomplete reduction and was removed by recrystallization of the HCl salt. **Rac-[$^2H_6$]-2** was coupled to *p*-morpholinoaniline using standard methodology to give rac-[$^2H_5$]AZ12320927 in 35% yield after three recrystallizations. The compound had a wide spread of isotopomers with the most prevalent being D_5; most importantly the unlabeled isotopomer was not detected, which permitted the use of this tracer as an internal standard.[6]

AZ12320927 was required labeled with tritium to support autoradiography studies. The anilide moiety appeared perfectly poised to undergo an Ir-catalyzed exchange reaction. Model reactions using deuterium gas and Crabtree's catalyst gave less than 5% incorporation of deuterium into AZ12320927; however, substituting catalyst **4**[7] for Crabtree's catalyst resulted in deuterium incorporation of 1.5 deuterium/molecule. Therefore, 2.2 equivalents of **4** and 3.5 Ci of tritium gas were reacted overnight with AZ12320927 to give 443 mCi of crude material (68% radiochemical purity) (Scheme 2). Purification of half the material by HPLC provided 56 mCi at 43 Ci/mmol and 99.2% radiochemical purity.

AZ12320927 was required labeled with C-14 for use in detailed metabolism studies. To meet the requirements of the end user, two regions of the molecule were deemed

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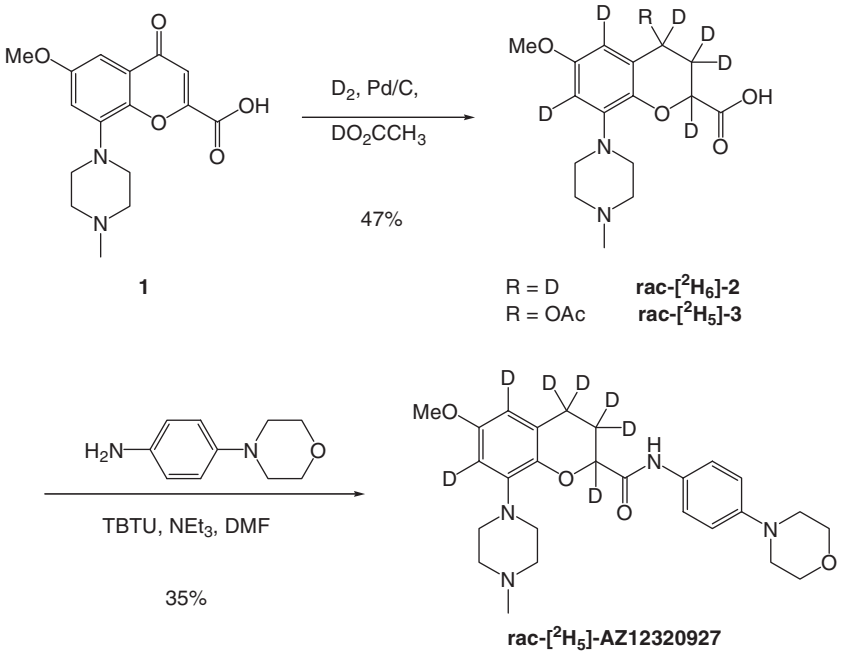
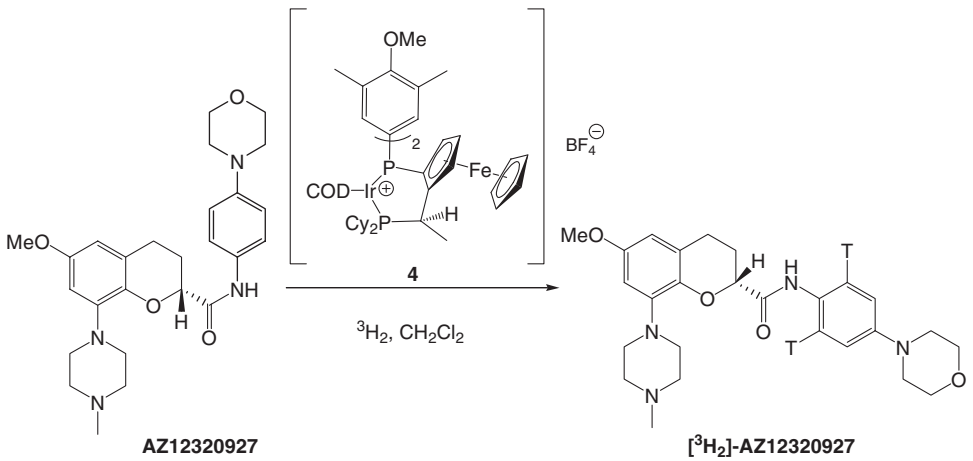


Scheme 1. Synthesis of rac-[$^2H_5$]-AZ12320927.

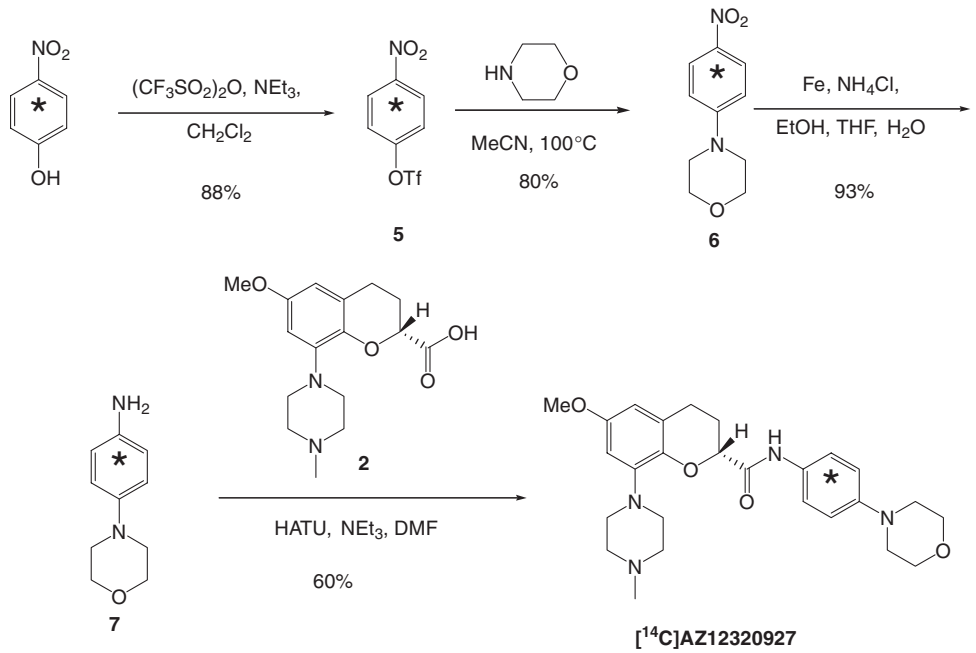


Scheme 2. Synthesis of [$^3H_2$]AZ12320927.

acceptable for the C-14 label: the chromane ring and the morpholino-substituted aromatic ring. Placement of the label in the chromane ring would have required a lengthy synthesis and a chiral separation. Alternatively, placement of the label in the morpholino-substituted aromatic ring gave a relatively short synthesis and avoided the chiral purification; therefore, the latter route was selected. [*u*-^{14}C]-*p*-nitrophenol (80 mCi/mmol) was converted to triflate **5** in high yield, and the crude reaction mixture was then reacted with morpholine to give nitrobenzene **6** in 70% yield over two steps (Scheme 3).[8] Iron-catalyzed reduction gave aniline **7,** which was reacted with acid **2** (>99.5% ee) to give [^{14}C]AZ12320927 in 60% yield (71% radiochemical purity). A large impurity resulting from direct coupling of **7** with the coupling agent was observed. Use of TBTU gave this adduct as a 60% impurity while HATU gave it as a 21% impurity. Since the impurity was readily removed by recrystallization, the results with HATU were deemed acceptable. The final product was purified by recrystallization to give 5.8 mCi in 99% radiochemical purity with a specific activity of 89 mCi/mmol. The enantiomeric purity of the final compound was not determined on the radioactive batch, but was performed on unlabeled batches prepared in the same manner. These analyses showed that there was no loss of enantio enrichment during the peptide coupling.

Experimental

General: [***u*-^{14}C]-*p*-nitrophenol was obtained from American Radiolabeled Chemical (80 mCi/mmol) as a solution in CH_2Cl_2, and chromenone **1** and chromane **2** were obtained from AstraZeneca Medicinal Chemistry. Anhydrous solvents were



Scheme 3. Synthesis of [^{14}C]AZ12320927.

obtained from Aldrich. Tritiation reactions were performed on a Tritec tritiation manifold system. Analytical HPLC was performed using a Agilent 1100 series HPLC system. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 300, Avance 500 and Avance 600 with Dual Cryoprobe spectrometers and were referenced to the residual solvent peak (7.26 and 77.00 ppm for $CDCl_3$, 3.30 and 49.2 ppm for CD_3OD, and 2.49 and 39.5 ppm for $^{2}H_6$-DMSO). LC/MS analyses were performed on an HP MSD-1100 using a Luna-C18(2) column, with 10–100% gradient over 10 min with MeCN-0.1% formic acid and electrospray ionization. The reaction products were identified by HPLC comparison with commercially available materials or AstraZeneca Medicinal Chemistry intermediates using either method A (10/90–100/0 MeCN/0.1% aqueous HCO2 H gradient elution over 10 min, Phenomenex Luna C-18(2)); method B (0/100–40/60 MeCN/0.1% TFA over 20 min, Luna C-18(2); or method C (20/80–25/75 MeCN/0.1% TFA over 20 min, Luna C-18(2). All HPLC analyses were conducted using a flow rate of 1 mL/min on 4.6 mm × 100 mm columns heated to 30°C and concluded with a 5 min wash of 100% MeCN.

3,4-dihydro-6-methoxy-8-(4-methylpiperazin-1-yl)-[2,3,3,4,4,5,7-$^{2}H_7$]-2H-chromene-2-carboxylic acid (***rac-$^{2}H_6$-2***): A slurry of 2.13 g (6.70 mmol) of 6-methoxy-8-(4-methylpiperazin-1-yl)-4-oxo-4H-chromene-2-carboxylic acid and 1.21 g of 10% Pd/C in 75 mL of $^{2}HO_2CCH_3$ was placed in a stainless steel pressure vessel, which was pressurized to 60 psi with $^{2}H_2$ gas. The reaction was mechanically stirred for five days at 70°C. The Pd/C was removed by filtration through Celite and the solids were rinsed with 2 × 50 mL of EtOAc and 2 × 50 mL MeCN and the combined filtrates were concentrated to dryness to give 2.115 g. The residue was dissolved in 50 mL of MeCN and 10 mL of 4 M HCl in dioxane. The solution was stirred for 1 h during which time a precipitate formed. The crystals were isolated to give 1.21 g and were recrystallized from 80 mL of MeCN to give 1.10 g (47%) of an off-white solid. LC/MS M+H (relative intensity): 309 (1.5%), 310 (9%), 311 (33%), 312 (75%), 313 (100%), 314 (59%), 315 (11%), 316 (5%). ^{1}H NMR (500 MHz, D_6-DMSO): 6.36 (s, 0.1H), 6.34 (s, 0.2H), 4.77 (d, J = 6.72 Hz, 0.2H), 3.90 (d, J = 12.8 Hz, 1H), 3.67 (s, 3H), 3.43 (m, 3H), 3.21 (m, 3H), 2.97 (m, 1H), 2.77 (s, 3H), 2.05 (m, 1.2H). ^{13}C NMR (150 MHz, D_6-DMSO, using a cryo probe) 172.6, 153.2, 140.1, 139.6, 122.6, 107.2 (br), 103.9 (br), 72.7 (br), 55.7, 52.9, 52.7, 47.4, 47.1 (br) 46.8, 46.4 (br), 23.5 (br), 22.8 (q).

3,4-dihydro-6-methoxy-8-(4-methylpiperazin-1-yl)-N-(4-morpholino-phenyl)-[2,,3,3,4,4-,5,7-$^{2}H_7$]-2H-chromene-2-carboxamide (***rac-[$^{2}H_5$]AZ12320927***): A solution of 368 mg (1.05 mmol) of **1**, 1.97 g (6.14 mmol) of TBTU, and 2 mL (14.3 mmol) of NEt_3 in 5 mL of DMF was stirred as 615 mg (3.45 mmol) of *p*-morpholinoaniline was added. The reaction was stirred for 2 h at which time HPLC showed the reaction to be complete. The solution was diluted with 500 mL of water and the resulting solution was extracted 3 × 100 mL CH_2Cl_2. The combined organic layers were washed sequentially with 2 × 100 mL water, 100 mL sat aq $NaHCO_3$, and 100 mL water. The organic layer was concentrated to dryness and the residue was dissolved in 70 mL of hot EtOAc. After cooling to room temperature, the solution was filtered to give 250 mg of a tan solid. This was recrystallized twice from 10 mL of EtOAc to give 120 mg. A second crop of crystals was obtained from the 70 mL EtOAc supernatant to give 73 mg after two recrystallizations. The two batches were combined to give 193 mg. HPLC (method B) showed the product to have a UV area % > 99% at 254 and 210 nm. LC/MS M+H (relative abundance): 469(6.3%), 470 (28%), 471 (71.5%), 472 (100%), 473 (67.4%), 474 (31.1%), 475 (12.7%). ^{1}H NMR (300 MHz, $CDCl_3$): 8.68 (br s, 1H), 7.49 (d, 2H, J = 8.5 Hz), 6.90 (d, 2H, J = 8.5 Hz), 6.44 (s, 0.05H), 6.29 (s, 0.09H), 4.65 (d, 0.22 H), 3.86 (m, 4H), 3.75 (s, 3 H), 3.29 (m, 2H), 3.13 (m, 4H), 2.97 (m, 2H), 2.65 (m, 4H), 2.37 (s, 3H), 2.0 (m, 1.02H). ^{13}C NMR (75 mHz, $CDCl_3$): 168.9, 154.1, 148.4, 141.8, 140.0, 130.1, 123.8 (m), 121.1, 116.3, 106.5 (m), 75.4 (m), 66.9, 55.7, 55.6, 50.9, 49.8, 46.2, 25.0 (m), 24.7 (m).

3,4-dihydro-6-methoxy-8-(4-methylpiperazin-1-yl)-N-(4-morpholino-[2,6-$^3H_2$]phenyl)-2H-chromene-2-carboxamide (**[$^3H_2$]AZ12320927**): A solution of 2.5 mg (5.8 μmol) of AZ12320927 and 12 mg of [(1R)-1-[bis(4-methoxy-3,5-dimethylphenyl)phosphino-κP]-2-[(1R)-1-(dicyclohexylphosphino-κP)ethyl]ferrocene][(1,2,5,6-η)-1,5-cyclooctadiene]-iridium tetrafluoroborate (**4**, 12 μmol) in 600 μL of CH_2Cl_2 was degassed by freeze-thaw three times and the frozen solution was charged with 400 mbar of H_2. The solution was warmed to rt and after it stopped taking up H_2, the solution was again frozen, evacuated and degassed via one freeze thaw iteration. The vessel was then filled with 216 mbar of $^3H_2$ (3.5 Ci) and the solution stirred overnight at rt. Residual $^3H_2$ gas was removed to a waste uranium-bed and the volatiles transferred to a waste flask. The residue was taken up in 1 mL of EtOH to give 443 mCi (67% radiochemical purity (method B)). HPLC purification (10 × 250 mm Phenomenex Luna C-18(2), 0–40% MeCN-0.1% TFA over 20 min, 3 mL/min) of 215 mCi of the crude reaction mixture gave 56 mCi (99.2% radiochemical purity using method B, 43 Ci/mmol). LC/MS (M+H): 467 (8%), 469 (70%), 470 (20%), 471 (100%), 472 (43%).

[*u*-^{14}C]-*p-nitrobenzenetrifluoromethylsulfonate* (***5***): A solution of 64.5 mCi (0.81 mmol, 80 mCi/mmol) of *p*-nitrophenol and 0.45 mL (3.2 mmol) of NEt_3 in 22 mL of CH_2Cl_2 at −40°C was stirred as 0.30 mL (1.8 mmol) of triflic anhydride was added. After 30 min, 20 mL of CH_2Cl_2 and 20 mL of sat aq $NaHCO_3$ were added and the layers separated. The organic layer was then washed with 20 mL of sat aq $NaHCO_3$ and 20 mL of sat aq NaCl and was then dried ($MgSO_4$). The drying agent was removed by filtration to give 36 mL of CH_2Cl_2 containing 55 mCi (85%). HPLC analysis (method A) showed 98% radiochemical purity of the triflate with 2% of the starting phenol remaining.

[*u*-^{14}C]-*p-nitromorpholinobenzene* (***6***): A solution of 55 mCi (0.69 mmol) of **5** in 1 mL of CH_2Cl_2 was diluted with a solution of 3 mL of morpholine (34.5 mmol) in 30 mL of MeCN and the solution was heated at reflux overnight. The resulting precipitate was removed by filtration and the solution concentrated to near dryness at reduced pressure. The residue was dissolved in 50 mL of CH_2Cl_2 and washed with 3 × 20 mL 1 M HCl, 2 × 20 mL NaHCO3 and 1 × 20 mL sat aq NaCl. The solvent was removed to afford 49 mCi of **7** (80%). HPLC analysis (method A) showed 90% radiochemical purity with 7% of *p*-nitrophenol **5** remaining.

p-morpoholino[*u*-^{14}C]*aniline* (***7***): A solution of 70 mCi (90% radiochemical purity, 0.79 mmol) of nitrobenzene **6** in 40 mL of EtOH, 10 mL THF, 10 mL of water and 10 mL of sat aq NH_4Cl was stirred as 650 mg (11.6 mmol) of Fe powder was added. The solution was stirred under N_2 at 100°C for 2 h at which time HPLC showed the reaction to be complete. The solution was basified with 40 mL of sat aq $NaHCO_3$ and extracted 4 × 40 mL CH_2Cl_2. The combined organic layers were washed with 20 mL of sat aq NaCl and dried ($MgSO_4$). The drying agent was removed by filtration and the solution counted at 65 mCi (93%). HPLC showed 90% radiochemical purity with the major impurity being *p*-aminophenol.

3,4-dihydro-6-methoxy-8-(4-methylpiperazin-1-yl)-N-(4-morpholino-[u-^{14}C]phenyl)-2H-chromene-2-carboxamide (***[^{14}C]AZ12320927***): A solution of 194 mg (0.63 mmol) of acid **2** (>99.5% ee), 1.5 g (3.94 mmol) of *O*-(7-Azabenzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium hexafluorophosphate (HATU), and 1 mL (7.1 mmol) of NEt_3 in 2 mL of DMF was stirred for 30 min under N_2 and then 30 mCi (90% radiochemical purity, 0.38 mmol) of aniline **7** was added in 2 mL of DMF. HPLC analysis showed the reaction to be complete after 2 h; therefore, 40 mL of water was added and the solution extracted with 50 mL of CH_2Cl_2. The organic layer was washed with 2 × 40 mL water and 2 × 35 mL sat aq $NaHCO_3$ and then extracted with 2 × 25 mL 1 M HCl. The aqueous layer was basified with $NaHCO_3$(s) and extracted 3 × 50 mL CH_2Cl_2 to give a solution containing 30 mCi. HPLC showed a 71% radiochemical purity with a 21% impurity, which arose from a direct coupling between HATU and the aniline. The solution was concentrated to dryness and 10 mL of CH_3CN was added. The slurry was heated to 50°C and the supernatant removed. The solid was treated with 15 mL of EtOAc and the slurry heated to reflux. The supernatant was removed to give 14.2 mCi, which was concentrated to 1/3 volume. The solution was cooled to −20°C and the crystals collected to give 5.8 mCi of a white solid (21% yield, 99.0% radiochemical purity (method C), 89 mCi/mmol) and 9 mCi in the supernatant (28% yield, 85% radiochemical purity (method C)). LC/MS (M+1) 467 (51%) 468 (16%), 469 (100%) 470 (28%), 471 (84%) 472 (22), 473 (37%) 474 (9%), 475 (10%) 476 (2%). ^1H NMR (500 mHz, CD3OD): 7.50 (d, 2H, J = 8.9), 6.96 (d, 2H, J = 8.9), 6.50 (d, 1H, J = 2.8), 6.42 (d, 1H, J = 2.8), 4.79 (s, 3H), 4.63 (dd, 1H, J = 3.2, 10.1), 3.82 (m, 4H), 3.11 (m, 4H), 2.92 (m, 3H), 2.78 (m, 6H), 2.38 (s, 3H), 2.03 (m, 1H). ^{13}C NMR (125 mHz, CD3OD): 171.5, 155.7, 150.2, 142.1, 141.5, 131.6, 125.2, 123.1, 117.38, 117.35, 108.9, 105.7, 77.1, 68.03, 56.1, 55.9, 51.7, 51.0, 46.0, 26.7, 25.8.

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References

[1] M. Maes, H. Y. Meltzer in *Psychopharmacology: The Fourth Generation of Progress* (Eds.: F. E. Bloom, D. J. Kepfer), Raven Press Ltd., New York, **1995**, pp. 933–945.
[2] P. Blier, C. Montigny, *Trends Pharmacol. Sci.* **1994**, *15*, 220–226.
[3] S. Halazy, M. Lamothe, C. Jorand-Lebrun, *Expert Opin. Ther. Patents* **1997**, *7*, 339–352.
[4] M. Briley, C. Moret, *Clin. Neuropharmacol.* **1993**, *16*, 387–400.
[5] S. Hjorth, C. S. Suchowski, M. P. Galloway, *Synapse* **1995**, *19*, 170–176D. Hoyer, D. N. Middlemiss, *Trends Pharmcol. Sci.* **1989**, *10*, 130–132.
[6] A slight degradation in isotopic incorporation was observed during the peptide coupling as the predominant isotopic species changed from D_6 to D_5 during the reaction.
[7] R. Salter, M. Chappelle, A. Morgan, T. Moenius, P. Ackermann, M. Studer, F. Spindler, in *Synthesis and Applications of Isotopically Labelled Compounds*, Vol. 7 (Eds.: U. Pleiss, R. Voges), **2001**, pp. 63–67.
[8] H. Kotsuki, S. Kobayashi, H. Suenaga, H. Nishizawa, *Synthesis* **1990**, 1145–1148.