Synthesis of Carbon-13 and Carbon-14 Labelled Triazolo-1,4-Benzodiazepines

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SUMMARY

An efficient two-step synthesis of 8-chloro-1-methyl-6-phenyl-[3H]-S-triazolo-[4,3-a][1,4]-benzodiazepine (alprazolam) and 8-chloro-6-(2-chlorophenyl)-1-methyl-[3H]-S-triazolo-[4,3-a][1,4]-benzodiazepine (triazolam) labelled with carbon-13 or carbon-14 from their corresponding hydrazines is reported. The method involves acylation of the appropriate hydrazine using the mixed carbonic anhydride of sodium [^{13}C] or [^{14}C] acetate and isobutylchloroformate under mild conditions. Thermolysis of the resulting acetylhydrazides gave the target carbon-14 and carbon-13 labelled compounds in good yields.

Key Words: Alprazolam, carbon-13, carbon-14, sodium acetate, triazolam, triazolobenzodiazepine.

INTRODUCTION

Alprazolam, 8-chloro-1-methyl-6-phenyl-[3H]-S-triazolo-[4,3-a][1,4]-benzodiazepine (4), and triazolam, 8-chloro-6-(2-chloro-phenyl)-1-methyl-[3H]-S-triazolo-[4,3-a][1,4]benzo-diazepine (5) are triazolobenzodiazepines which elicit anxiolytic and sedative-hypnotic effects respectively, and have been recently implicated as possible inhibitors of platelet activating factors (1,2).

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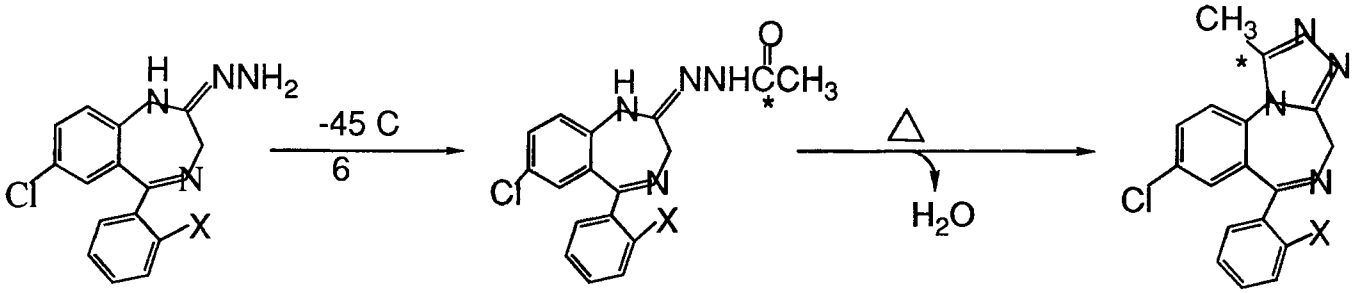
SCHEME 1

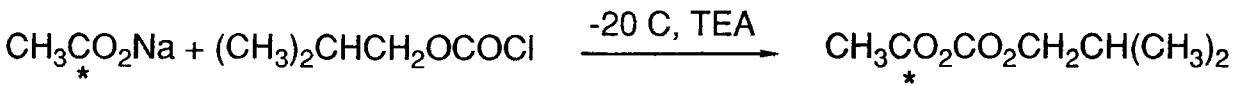


1a: X=H
1b: X=Cl

2a: X=H; ^{13}C
2b: X=H; ^{14}C
3a: X=Cl; ^{13}C
3b: X=Cl; ^{14}C

4a: X=H; ^{13}C
4b: X=H; ^{14}C
5a: X=Cl; ^{13}C
5b: X=Cl; ^{14}C

SCHEME 2



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In the course of investigating synthetic routes amenable to labelling alprazolam and triazolam with carbon-11 (3), a simple and efficient procedure for the preparation of carbon-14 and carbon-13 labelled triazolobenzodiazepine 4 and 5 was developed and is reported herein.

Synthesis of carbon-14 and carbon-13 labelled alprazolam and triazolam via thermal cyclization of the appropriate acetylated hydrazine has been previously reported by Hsi, et al (4,5). Whereas quantitative cyclization of the labelled acetylhydrazides 2 and 3 was easily achieved in 15 minutes, their preparation from the corresponding hydrazine 1a and 1b and N-[-^{14}C]- or N-[1-^{13}C]acetyl imidazole was lengthy and tedious. For the above procedure to be effective, careful preparation of the acetylating species from [^{14}C]- or [^{13}C]- acetic acid and 1,1'-carbonyldiimidazole (CDI) and monitoring its purity by gas chromatography were required. Furthermore, the approach relied on the use of carbon-13 and carbon-14 labelled anhydrous acetic acid, a corrosive and hygroscopic liquid.

Therefore, efforts were focused on the development of an efficient and more economical synthesis of the acetylhydrazides using the cheaper, less hygroscopic and easier to handle sodium salt of acetic acid.

Initially, a modification of Hsi's procedure for the preparation of acetylhydrazide 2 was attempted whereby the acetylating moeity was prepared *in situ* from the sodium salt of acetic salt and CDI. Although the acetylhydrazide 2 was obtained in good yields as evidenced by TLC, its purification and separation from the sodium salt of imidazole which had formed during the reaction proved difficult. Attempts to cyclize the hydrazide 2 in the presence of the imidazole salt led to complex reaction mixtures and poor yields of the desired alprazolam 4.

An alternative approach, based on the acetylation of hydrazines

by the mixed anhydride method was adopted. Thus, reaction of hydrazine 1a with the mixed anhydride of acetic acid and isobutyl chloroformate (6, Scheme II) in THF/MeCN at -45°C, afforded the desired acylated compound 2 in high yields. Any reaction by-products, sodium chloride, triethylamine and isobutanol, were easily removed by extraction into water and during cyclization under vacuum. Thermal cyclization of compound 2 afforded pure alprazolam 4 in quantitative yield. Thus, the overall reaction sequence hydrazine to alprazolam could be achieved in under 6 hours with an overall yield of 80% (from the hydrazine) (Scheme I). Following the above procedure, alprazolam labelled with carbon-14 or carbon-13 in the C-1 position of the ring system was obtained in high specific activities.

The method was adapted to the synthesis of [^{13}C]- (5a) and [^{14}C]-triazolam (5b) with slight modifications due to the poor solubility of the starting hydrazine 1b in MeCN. Thus, treatment of hydrazine 1b with the mixed anhydride of acetic acid and isobutyl chloroformate in THF at 4°C, and allowing the reaction to proceed overnight afforded the desired acetylhydrazides 3 in moderate yields (70-80%). The lower yields obtained for triazolam as compared to alprazolam may be due to the instability of the mixed anhydride at the higher temperature employed. Futhermore MeCN appears to be a more suitable solvent than THF for the conversion of sodium acetate to the mixed anhydridge via the chloroformate, since in THF the chloroformate apparently did not react completely with the acetate.

In summary, an efficient two-step synthesis of ^{13}C- and ^{14}C-labelled alprazolam and triazolam has been developed. The method is based on the mixed anhydride of isobutyl chloroformate and the appropriately labelled sodium acetate salt. This approach may also be applicable to the labelling of amides and esters. Additionally, the mixed anhydride method could be amenable to Carbon-11 labelling of triazolobenzodiazepine provided sodium [C-11]acetate could be

obtained in an anhydrous form. This synthetic route is currently being investigated.

EXPERIMENTAL

Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. ^{1}H and ^{13}C-nmr spectra were recorded on either a Varian EM-360 (60 MHz) or VXR300 (300 MHz) spectrometer. Infrared spectra (IR) were recorded from KBr pellets using a Perkin-Elmer Model 1430 ratio recording spectrophotometer. Thin layer chromatography (TLC) was performed on Whatman silica gel glass plates (KF-6) or (PKF-6).

Sodium [1-^{13}C] acetate (99% atom enriched) was purchased from Aldrich Chemicals in a paraffin sealed screw top vial. Sodium [1-^{14}C] acetate was purchased from Amersham in septum sealed vials with specific activities of 30 mCi/mmol and 56 mCi/mmol.

Radiochemical Assessment

Radioactivity determinations were executed using a refrigerated Packard Tricarb Liquid Scintillation Spectrometer in Scintiverse II scintillation cocktail. The concentrations of the stock solution used for specific activity measurements were determined by the standard curve method utilizing a Varian-Cary 2200 UV-VIS Spectrophotometer. Radiochemical purity of the products was assessed autoradiographically (TLC solvent 10% MeOH-$CHCl_3$, v/v) and by thin layer scanning using a Berthoid LB1760 TLC Scanner.

HPLC evaluation of radiochemical purity was achieved using a system consisting of an Altex 110A pump, a Varian Varichrom Analytical (223nm) or Waters 440 (280nm) UV detector, a Radiomatic Flo-One HP flow through radioactivity detector, and a Linear Model 500 dual channel recorder. The injection volume was 20 µl of a solution made from a stock solution of alprazolam or triazolam (200 µl, 0.1mg/ml) spiked with [^{14}C]alprazolam or [^{14}C]triazolam (10 µl).

The analytes were introduced via a Rhepdyne 7125 injector fitted with a 50 µl loop onto a Zorbax Silica column (25 cm x 4.6 mm). The mobile phase, consisted of MeCN: i-PrOH:H_2O:$HClO_4$ (840:100:65:0.31, v/v), and was delivered at a flow rate of 1.0 mL/min. The effluent passed through the UV detector and then into the flow-through radioactivity detector. The programmed detector mixed scintillation cocktail with effluent in a 3:1 (v/v) ratio. Both the UV and the radioactivity signal were recorded simultaneously, on separate channels.

1-[1-^{13}C]Acetyl-2-(7-chloro-5-phenyl-[3H]-1,4-benzodiazepin-2-yl) hydrazine (2a).

A suspension of sodium [1-^{13}C]acetate (64.3 mg, 0.8 mmol) and triethylamine (TEA) (0.078 mL, 0.56 mmol) in MeCN/THF (10 mL, 7:3, v/v) was stirred for 5 min at room temperature. The flask was then cooled to -25°C (dry ice-acetone), isobutylchloroformate (0.101 ml, 0.8 mmol) was added, and stirring continued for an additional 15 min at -20°C. The reaction mixture was further cooled to -35°C and a solution of 7-chloro-5-phenyl-[3H]-1,4-benzodiazepine-2-yl hydrazine (1a) (4) (200 mg 0.704 mmol) in THF (2mL) was added. It was then allowed to slowly warm to room temperature, and stirring continued for 4-6 h. $CHCl_3$ (25 mL) was then added and the solution washed with 5% aqueous $NaHCO_3$ (1x15 mL) and H_2O (2x20mL), dried over K_2CO_3, and the solvent evaporated to give the desired compound 2a (223.8 mg, 97.2% yield); m.p. 199-202°C (Lit[4]. m.p. 199-200°C); IR (KBr) 3220, 1635, 1555, 1480, 1390, and 1360 cm^{-1}; ^{1}H-nmr (60 MHz) ($CDCl_3$) δ 2.0 (m, 3H, $COCH_3$), 4.4 (br s, 2H, CH_2), 7.0-7.6 (m, 8H, aromatic).

8-Chloro-1-methyl-6-phenyl-[3H]-S-[1-^{13}C]triazolo-[4,3-a][1,4] benzodiazepine (4a).

Compound 2a (223 mg, 0.71 mmol) was heated in an oil bath at 205°C in vacuo (2 mm Hg) for 15-20 min. After cooling to room

temperature, the glassy residue was dissolved in CH_2Cl_2 (12 mL), filtered to remove trace solids, the volume reduced to approximately 3 mL in vacuo and C_6H_6 (10 mL) added. Reduction of the volume to 5 ml followed by the addition of hexane (20 mL) caused precipitation of fine crystals which were allowed to stand overnight. Filtration and recrystallization from CH_2Cl_2/hexane afforded 4a (192.1 mg 88.8% yield from compound 2a) m.p. 222-224°C (lit.[4] m.p. 225-227°C); IR (KBr) 1610, 1480, 1425, 1360, 820, and 680, cm^{-1}; ^{1}H-nmr (300 MHz) ($CDCl_3$) δ 2.64 (d, 3H, $J_{^{13}C-H}$=7.3 Hz, CH_3), 4.09 (d, 1H J=12.9 Hz, C_4-H) 5.50 (d, 1H J=12.9 Hz, C_4-H); ^{13}C-nmr (300 MHz) ($CDCl_3$) δ 167.79 (C_6), 154.94 (C_{3a}), 149.9 (C_1), 138.8 (C_1'), 133.10 (C_8), 132.11 (C_{10a}), 131.60 (C_9), 131.56 (C_7), 130.80 (C_4'), 130.53 (C_{6a}), 129.17 (C_2', C_6'), 128.32 (C_3', C_5'), 124.49 (C_{10}), 46.34 (C_4), 12.75 (CH_3), J_{C-C}=57.6 Hz). Enrichment at C_1: 97.26%; none seen at the exocyclic methyl group. Percent [^{13}C] enrichment at C_1 was assessed by comparison of the ^{13}C-nmr integrals at 46.34 δ (C_4) and 149.9 δ (C_1) of a solution comprised of a known ratio of [^{13}C] enriched and unenriched 4 (5). MS (EI, 70keV) 309 (M+).

8-Chloro-1-methyl-6-phenyl-[3H]-S-[1-^{14}C]triazolo[4,3-a][1,4]-benzodiazepine (4b).

Compound 4b was synthesized in a similar manner as described for 4a; however, the acetylhydrazide 2b was not isolated. Thus sodium [1-^{14}C]acetate (30 mCi/mmol, 23.17 mCi) 64.9 mg., 0.77 mmol) and TEA (0.115 ml, 0.7 mmol) were combined in MeCN/THF (10 mL, 7:3, v/v). The mixture was sonicated for 10 min and stirred at room temperature for 5 min. The turbid mixture was cooled to -20°C, isobutylchloroformate (0.108 ml, 0.833 mmol) added, and stirring continued for 45 min at this temperature. At the end of the period, the mixture was further cooled to -45°C, and the 7-chloro-5-phenyl-[3H]-1,4-benzodiazepine-2-yl hydrazine (1a) (6) (225.4 mg., 0.79 mmol) in THF (3mL) was introduced. Stirring was continued for

4h while the mixture was allowed to slowly warm to room temperature. Work up of the reaction mixture, as described for 2a, afforded the acetylhydrazide 2b which was used in the next step without further purification. Thus, heating 3b at 205° in vacuo (2 mm Hg) for 15 min afforded 5b (215.9 mg, 90.1% chemical yield, 20.7 mCi, 89.4% radiochemical yield, specific activity 29.74 mCi/mmol) m.p. 221-224°C (Lit.[4] m.p. 225-227°C). The radiochemical purity was found to be greater than 97% as determined by HPLC, and autoradiography (MeOH-$CHCl_3$, 10% v/v). The compound was further identified by comparison of its spectral data to that of an authentic sample.

8-Chloro-6-(2-chlorophenyl)-1-methyl-[3H]-S-[1-^{13}C]triazolo-[4,3-a]-[1,4]-benzodiazepine (5a).

Sodium [1-^{13}C]acetate (24.9 mg, 0.299 mmol) was suspended in anhydrous THF (4 ml) followed by the addition of TEA (0.042ml, 0.307 mmol). Isobutylchloroformate (0.0396 ml, 0.305 mmol) was added and the mixture stirred for 1 h. At the end of this period, the reaction mixture was cooled in an ice bath to 4°C, and a solution of 7-chloro-5-(2-chlorophenyl)-[3H]-1,4-benzodiazepine-2-yl hydrazine (1b) (6). (100 mg, 0.318 mmol) in THF (7 mL) was added. The suspension was stirred for 30 min at 4°C and allowed to warm to room temperature. Stirring was stopped after 4 h, and precipitation of 3a was allowed to proceed overnight. The precipitate was filtered and washed with Et_2O (20 mL) and THF (20 mL). The resulting solid was then cyclized by heating at 205° for 15 min in vacuo (2 mm Hg). TLC (MeOH-$CHCl_3$, 10% v/v) showed complete conversion to the desired compound 5a. The residue was dissolved in CH_2Cl_2 (20 mL), filtered to remove the insoluble salts, and the volume reduced to 3 mL in vacuo. C_6H_6 (20 mL) was added, and the resulting solution was again concentrated to 5 ml in vacuo. Addition of hexane (10 mL) caused the rapid precipitation of 5a. Filtration followed by

recrystallization (CH_3COCH_3/hexane) afforded 5a (79.9 mg, 78% yield), m.p. 235-236°C (Lit.[4] 239-241°C). IR (KBr) 1625, 1553, 1490, 1430, and 1310 cm^{-1}; H^1-nmr (60 MHz) ($CDCl_3$) δ 2.6 (d, 3H, J=7.3 Hz, CH_3), 4.1 (d, 1H, J=11 Hz, CH_2), 5.4 (d, 1H, J=11 Hz, CH_2), 7.0 - 7.6 (m, 7H, aromatic); ^{13}C-nmr (300 MHz) ($CDCl_3$) δ 167.62 (C_6), 154.64 (C_{3a}), 150.30 (C_1), 138.02 (C_1'), 133.57 (C_8), 132.49 (C_2'), 131.53 (C_{10a}, C_9), 131.29 (C_7), 130.83 (C_4'), 130.17 (C_6), 129.63 (C_3'), 127.23 (C_5'), 124.44 (C_{10}), 46.38 (C_4), 12.21 (CH_3, J_{C-C} = 57.3 Hz). Enrichment at C_1: 97.32%; none seen at the exocyclic methyl group. Percent [^{13}C] enrichment at C_1 was assessed by comparison of the ^{13}C-nmr integral at 46.38 δ (C_4) and 150.30 δ (C_1) of a solution comprised of a known ratio of ^{13}C enriched and unenriched 5 (5). MS(EI, 70 keV) 343(M^+).

8-Chloro-6-(2-chlorophenyl)-1-methyl-[3H]-S-[1-^{14}C]triazolo-[4,3-9]-[1,4]-benzodiazepine,(5b)

A procedure analogous to that presented above for 5a was used for the synthesis of 5b. Sodium [1-^{14}C]acetate (75 mg, 0.893 mmol, 50 mCi, 56 mCi/mmol) was carefully transferred to an oven dried flask, the ampoule rinsed with anhydrous THF (12x1 mL), and the washings added to the reaction vessel. TEA (0.128 mL, 0.920 mmol) was added and the suspension stirred for 5 min. Isobutylchloroformate (0.118 ml, 0.920 mmol) was next added and stirring continued for 1 h. The mixture was cooled to 4°C and hydrazine 1b (298.1 mg, 0.937 mmol) added as a solution in anhydrous THF (12 mL). The resulting mixture was allowed to warm to ambient temperature while stirring for an additional 4h. Workup of the reaction mixture as described for 5a, afforded 5b (150 mg, 48% chemical yield, 20.84 mCi, 41.7% radiochemical yield, specific activity, 47.65 mCi/mmol) m.p. 235-237°C (Lit.[4] m.p. 239-241°C). Spectral data of 5b were identical to an authentic sample. HPLC and autoradiography showed the product 5b to be 99.9% radiochemically

pure. Workup of the organic filtrate afforded an additional amount of 5b. TLC of the remaining organic filtrate showed the presence of the desired intermediate acetylhydrazide 4b along with a large excess hydrazine 1b, indicating an incomplete reaction. Thus, the filtrate was diluted with CH_2Cl_2 (30 mL), washed with 4% citric acid (2x10mL) and H_2O (1x15mL), to remove excess hydrazine (2b). The organic layer was dried over anhydrous K_2CO_3, filtered, concentrated in vacuo, and the oily residue was then heated at 205° for 15 min under vacuum. The resulting residue was purified by preparative TLC by successive elution of the plate with MeOH-$CHCl_3$ (5% v/v) then MeOH-$CHCl_3$ (10% v/v). Extraction of the appropriate band yielded approximately 78 mg of the desired compound (5b) (overall yield 5b; 228 mg, 70.9% chemical yield; 31.64 mCi, 63.2% radiochemical yield).

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