A PRACTICAL SYNTHESIS OF ATB-[2-^{3}H]BMPA, A PHOTOLABELLING REAGENT FOR EXOFACIAL GLUCOSE TRANSPORTERS

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Summary

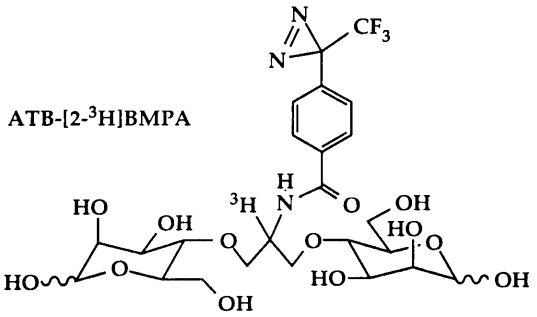
Major improvements in the efficiency of ATB-[2-^{3}H]BMPA synthesis were achieved by using 1,3-dichloro-2-propanone O-benzyloxime (**6**) as the linking reagent.

Key Words: ATB-BMPA, ATB-[2-^{3}H]BMPA, 1,3-dichloro-2-propanone O-benzyloxime, photolabel, GLUT (glucose transporter), linker

Introduction

ATB-[2-^{3}H]BMPA (2-N-[4-(1-azitrifluoroethyl)benzoyl]-[2-^{3}H]-1,3-bis-(D-mannos-4-yloxy)-2-propylamine) is a symmetrical bis-D-mannose derivative employed as a photoaffinity labelling reagent for exofacial glucose transporters (1). The synthesis of ATB-[2-^{3}H]BMPA is a four-stage process as outlined in Scheme 1. The first stage is protection of D-mannose as **1** (1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose),

ATB-[2-^{3}H]BMPA



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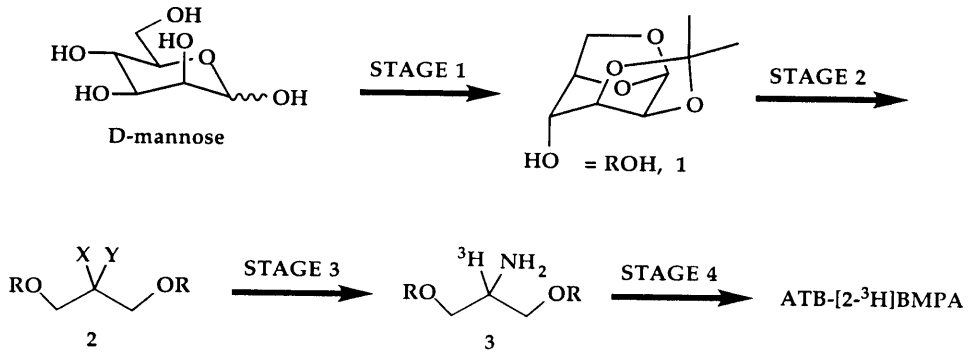
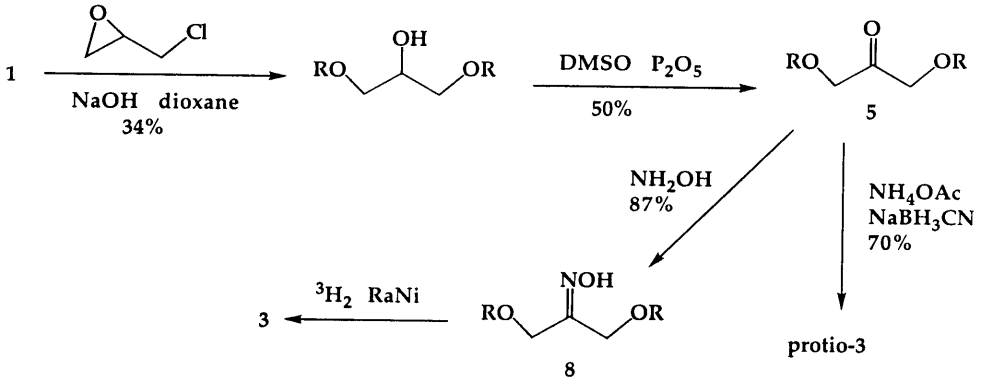
SCHEME 1



the second stage constructs an appropriately linked bis-mannose intermediate **2**, the third yields the tritiated primary amine **3**, and the fourth furnishes ATB-[2-^{3}H]BMPA with its photolabelling diazirine group and deprotected mannose moieties. Stage 1 has been accomplished in low overall yield (11%) relying on pyrolysis to form the anhydro linkage (2). More recently, formation of the anhydro linkage via the C-6 tosylate has provided **1** in 60% overall yield on a small scale and 29% overall yield on a large scale (3). The subject of this paper is the improvement of the second and third stages of the synthesis. The reported methods for these transformations (4) are shown in Scheme 2. Length and low overall yields make these methods unattractive. In particular, the linking reaction, in which **1** is treated with epichlorohydrin, proceeds in poor yield (5). We report here exploration of other linking reagents and chemistry for the second and third stages of ATB-[2-^{3}H]BMPA synthesis.

SCHEME 2

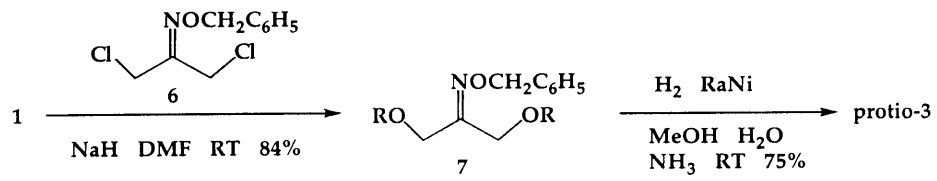


Results and Discussion

In order to avoid the problems associated with the original methods for carrying out synthetic stages 2 and 3 (Scheme 2), we proposed to replace epichlorohydrin as the linking reagent. Our first attempts were directed at using 1,3-dichloroacetone to produce **5** directly from **1**. However, under a variety of reaction conditions (potassium carbonate, acetone, RT; pyridine, chloroform, RT to 55°; diisopropylethylamine, chloroform, RT; sodium hydride, DMF (N,N-dimethylformamide), -78° to RT) 1,3-dichloroacetone was consumed, but **1** remained unreacted. Apparently, even under mildly basic conditions, 1,3-dichloroacetone was acidic enough to undergo self-condensation.

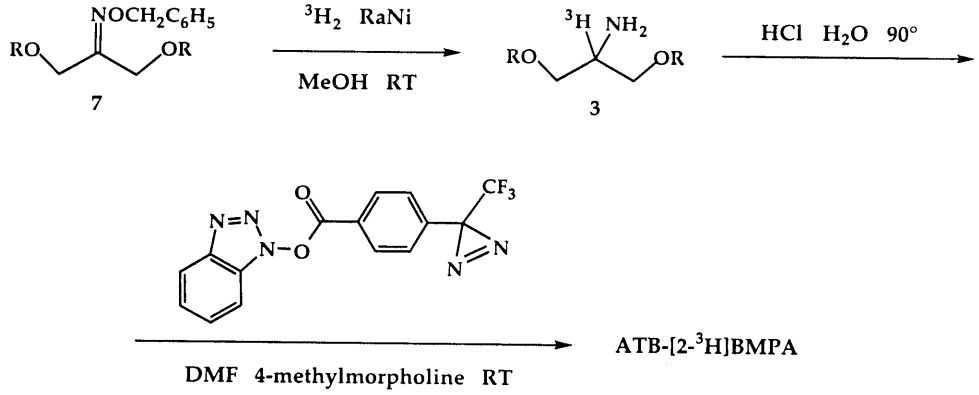
We imagined that an oxime ether of 1,3-dichloroacetone would be less acidic, and therefore, less prone to self-condensation. Accordingly, 1,3-dichloro-2-propanone O-benzyloxime **6** (Scheme 3), a previously unknown reagent, was prepared from 1,3-dichloroacetone and O-benzylhydroxylamine hydrochloride (ethanol, RT, 98%). The 2:1 coupling of **1** with **6** to form **7** was efficient (84%) and set the stage for catalytic hydrogenation to liberate the amino group and introduce tritium. In experiments with nonradioactive hydrogen, hydrogenation of **7** over Raney® nickel (RaNi) catalyst in dry methanol produced an inseparable mixture of protio-**3** with its N-methyl and N,N-dimethyl analogues (5-10% total). Reasoning that N-methylation comes from reductive amination with formaldehyde, itself available by catalytic dehydrogenation of methanol, we experimented with additives that would compete for formaldehyde. Simply using wet Raney® nickel improved the situation somewhat (6). However, inclusion of concentrated aqueous ammonia completely suppressed N-methylation and allowed isolation of a 75% yield of pure protio-**3** (7). This material was identical (^{1}H NMR, MS) to that reported (4a). The overall yield for our two-step conversion of **1** to protio-**3** was 63%. This was vastly superior to the three-step, 12% overall yield process previously reported (Scheme 2).

SCHEME 3



When 7 was reduced with tritium and the resulting 3 was converted to ATB-[2-^{3}H]BMPA (Scheme 4) by the methods reported for the conversion of oxime 8 to 3 to ATB-[2-^{3}H]BMPA (1, 4b), the overall yield and product specific activity were essentially identical to those reported (8).

SCHEME 4



In summary, we have found 1,3-dichloro-2-propanone O-benzyloxime 6 to be a superior linking reagent for the execution of stages 2 and 3 of the synthesis of ATB-[2-^{3}H]BMPA. We expect this finding will make ATB-[2-^{3}H]BMPA a more accessible research tool.

Experimental

General: All reagents were purchased from commercial sources and used without further purification unless otherwise indicated. Analytical TLC (thin layer chromatography) was performed on Merck Kieselgel 60 F_{254} plates (0.25 mm). TLC visualization was by dip-staining with PMA (phosphomolybdic acid) and/or short wave UV illumination. Solvent evaporations were performed under reduced pressure with a rotary evaporator. Flash chromatography was performed as reported (9) using EM Science Silica Gel 60 (230-400 mesh ASTM). ^{1}H NMR and ^{13}C NMR spectra were measured at the indicated frequencies on Jeol CPF 270 or GX 270 instruments. NMR chemical shifts are reported as δ (ppm downfield) values relative to internal $Si(CH_3)_4$, with $CHCl_3$ assigned at δ 7.24 (^{1}H NMR) or δ 77.0 (^{13}C NMR).

1,3-Dichloro-2-propanone O-benzyloxime (6): A slurry of O-benzylhydroxylamine hydrochloride (6.42 g, 40.2 mmol) in absolute ethanol (50 mL) was stirred at room temperature as 1,3-dichloroacetone (5.11 g, 40.2 mmol) was added. The mixture became homogeneous after several hours. After stirring overnight TLC indicated complete consumption of O-benzylhydroxylamine. The solvent was evaporated, and the mixture was redissolved in ethanol and again evaporated. The resultant oil contained a trace of suspended solid. After dilution with hexane, drying over sodium sulfate, and filtration, the solvent was evaporated under high vacuum. This procedure gave **6** (free base, 9.14 g, 98% yield) as a clear, colorless oil (density = 1.23 g/mL). This material could be stored for two months or more at room temperature without any appreciable decomposition. TLC (50% EtOAc in hexane, PMA, UV) R_f O-benzylhydroxylamine hydrochloride, 0.46; 1,3-dichloroacetone, 0.76; **6**, 0.90. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.27-7.40 (m, 5H), 5.15 (s, 2H), 4.35 (s, 2H), 4.24 (s, 2H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 151.4, 136.7, 128.4, 128.1, 128.1, 76.9, 42.1, 32.8. IR (neat, cm^{-1}) 3090, 3067, 3034, 2940, 2884, 1497, 1454, 1431, 1368, 1250, 1018, 851, 752. MS (CI) 232, 234, 236 ($M+H^+$); 249, 251, 253 ($M+NH_4^+$); 266, 268, 270, 272 ($M+Cl^-$). Elemental analysis calculated for $C_{10}H_{11}NCl_2O$: C, 51.75; H, 4.78; N, 6.03; Cl, 30.55. Found: C, 51.28; H, 4.68; N, 6.21; Cl, 30.58.

1,3-Bis(1,6-anhydro-4-deoxy-2,3-O-isopropylidene-β-D-mannopyranose-4-yloxy)-2-propanone O-benzyloxime (7): In an oven-dried flask **1** (1.54 g, 7.62 mmol) was dissolved in DMF (15 mL) under argon. To this solution stirring at room temperature, was added unwashed 60% NaH mineral oil dispersion (0.34 g, 0.20 g net NaH, 8.5 mmol). The mixture exothermed slightly, bubbled, and became amber in color. After bubbling had ceased, **6** (0.72 mL, 0.88 g, 3.81 mmol) was added. A mild exotherm ensued, and the reaction mixture darkened. TLC showed progress to desired product with an intermediate forming and then disappearing. Some **1** and no **6** remained. Additional unwashed 60% NaH mineral oil dispersion (0.06 g, 0.04 g net NaH, 1.5 mmol) and **6** (0.10 mL, 0.12 g, 0.53 mmol) were introduced. Only a trace of **1** remained subsequent to stirring at room temperature overnight and then at 45° for 4 h. After addition of AcOH (0.3 mL) and solvent evaporation (under vacuum, 45-75° bath), water was added, and the mixture was extracted four times with CH_2Cl_2. The combined extracts were dried over sodium sulfate and evaporated. Flash chromatography (silica gel, 10% to 50% EtOAc in hexane

step-wise gradient) gave 7 (1.79 g, 84% yield) as an oil. TLC (50% EtOAc in hexane, PMA, UV) R_f 1, 0.27; 6, 0.85; intermediate, 0.76; 7, 0.58. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.27-7.40 (m, 5H), 5.31-5.33 (m, 2H), 5.11 (s, 2H), 4.59-4.61 (m, 2H), 4.57 (d, J= 14.7 Hz, 1H), 4.51 (d, J= 14.7 Hz, 1H), 4.34 (d, J= 11.7 Hz, 1H), 4.29 (d, J= 11.7 Hz, 1H), 4.19-4.22 (m, 2H), 4.02-4.06 (m, 2H), 3.85-3.89 (m, 2H), 3.69-3.72 (m, 2H), 3.66 (s, 1H), 3.62 (s, 1H), 1.53 (s, 6H), 1.32 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$, DEPT) δ C 154.5, 137.1, 109.8, 109.7; CH 128.4, 128.1, 128.1, 99.1, 77.6, 76.9, 73.7, 73.4, 72.9, 72.8, 72.2; CH_2 76.5, 67.2, 64.4, 62.6; CH_3 25.9, 25.8. IR (neat, cm^{-1}) 2982, 2936, 2901, 1456, 1381, 1370, 1242, 1219, 1152, 1117, 1096, 1074, 1028, 933. HRMS (FAB) found 564.2455 ($M+H^+$), calculated for $C_{28}H_{38}NO_{11}$ 564.2445. Elemental analysis calculated for $C_{28}H_{37}NO_{11}$: C, 59.67; H, 6.62; N, 2.49. Found: C, 59.78; H, 6.54; N, 2.58.

1,3-Bis(1,6-anhydro-4-deoxy-2,3-O-isopropylidene-β-D-mannopyranose-4-yloxy)-2-propylamine (protio-3): Compound 7 (195 mg, 0.35 mmol) was dissolved in MeOH (10 mL), and to the solution was added concentrated aqueous NH_3 (1.0 mL, 15 M, 15 mmol) and 50% Raney® nickel catalyst in water slurry (Aldrich Chemical Co., about 2 mL, about 3.5 g of slurry). The resulting mixture was stirred at room temperature under a hydrogen balloon. TLC indicated complete consumption of starting material after 1 h. In addition to product, TLC revealed formation of benzyl alcohol. No other TLC spots were evident. After 2 d the mixture was filtered through Celite, and the solvent was evaporated. Flash chromatography (silica gel, 5% to 10% {10% conc. aq. NH_3 in MeOH} in CH_2Cl_2 step-wise gradient) gave pure protio-3 (0.12 g, 75% yield) as an oil. Under these reaction conditions no N-methylation was observed. TLC (10% {10% conc. aq. NH_3 in MeOH} in CH_2Cl_2, PMA, UV) R_f 7, 0.85; benzyl alcohol, 0.55; protio-3, 0.36. ^{13}C NMR (67.8 MHz, $CDCl_3$, DEPT) δ C, 109.9; CH 99.2, 77.7, 73.7, 72.9, 72.2, 50.8; CH_2 71.2, 64.5; CH_3 26.0, 25.9. See reference 4a for other physical data.

ATB-[2-^{3}H]BMPA: Compound 7 (12.9 mg, 0.023 mmol) was converted by Amersham International plc. (Buckinghamshire, England) to ATB-[2-^{3}H]BMPA (33 mCi, 13.4 Ci/mmol) as outlined in Scheme 4 by the methods reported (1, 4b) for the conversion of 8 to ATB-[2-^{3}H]BMPA.

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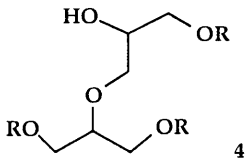
5. Our experiments suggested that formation of the 3:2 adduct **4** is a major problem.



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7. Hydrogenation produced benzyl alcohol as well. This is consistent with N-O bond reduction being faster than O-benzyl bond reduction and suggests that the identity of the oxime's O-substituent is not critical.
8. This work was performed by Amersham International plc. In their conversion of **7** to **3**, aqueous ammonia was not included.
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