

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

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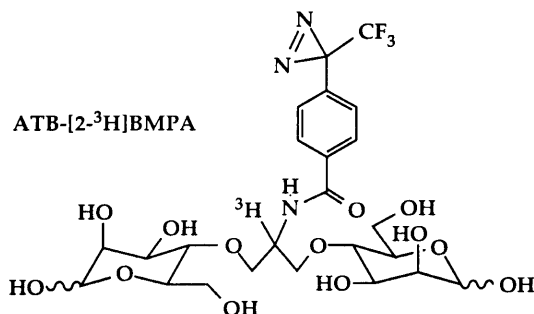
Summary

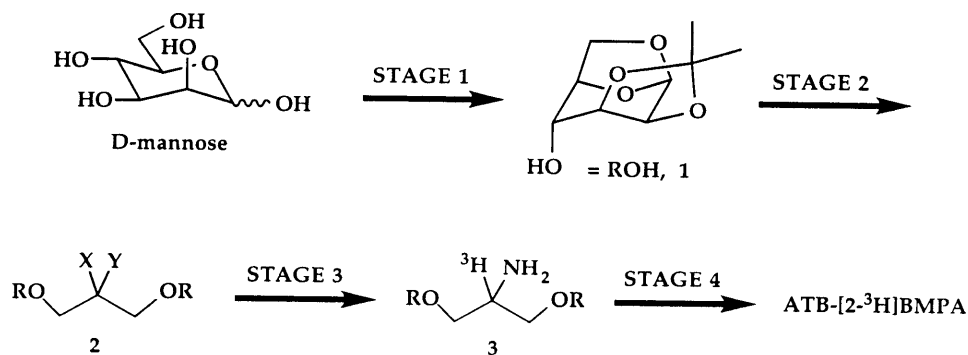
Major improvements in the efficiency of ATB-[2-³H]BMPA synthesis were achieved by using 1,3-dichloro-2-propanone O-benzyloxime (**6**) as the linking reagent.

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

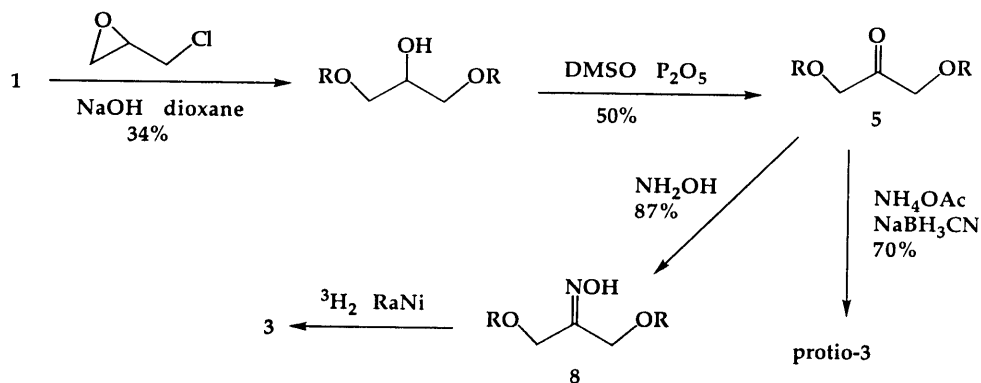
Introduction

ATB-[2-³H]BMPA (2-N-[4-(1-azitrifluoroethyl)benzoyl]-[2-³H]-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-[2-³H]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),



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-[2-³H]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-[2-³H]BMPA synthesis.

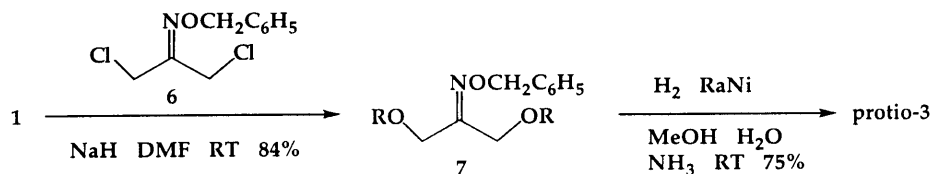
SCHEME 2

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.

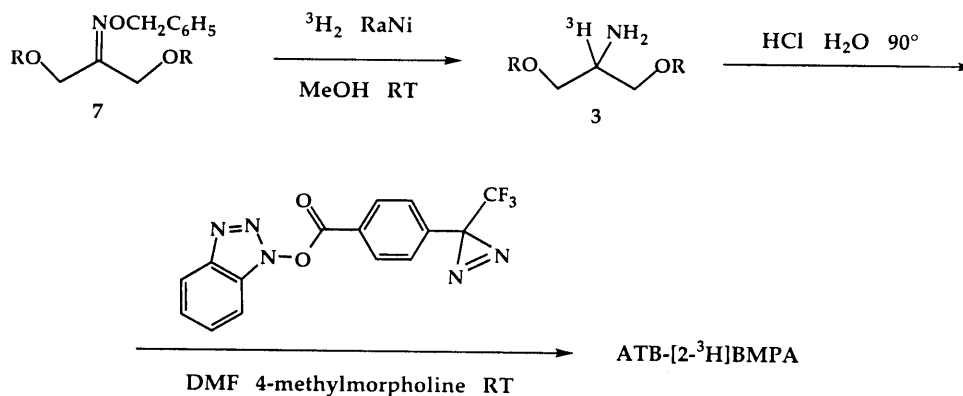
We imagined that an oxime ether of 1,3-dichloroacetone would be less acidic, and therefore, less prone to self-condensation. Accordingly, 1,3-dichloro-2-propanone O-benzyloxime **6** (Scheme 3), a previously unknown reagent, was prepared from 1,3-dichloroacetone and O-benzylhydroxylamine hydrochloride (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 (¹H 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-[2-³H]BMPA (Scheme 4) by the methods reported for the conversion of oxime **8** to **3** to ATB-[2-³H]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-benzylloxime **6** to be a superior linking reagent for the execution of stages 2 and 3 of the synthesis of ATB-[2-³H]BMPA. We expect this finding will make ATB-[2-³H]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 F₂₅₄ 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). ¹H NMR and ¹³C 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(CH₃)₄, with CHCl₃ assigned at δ 7.24 (¹H NMR) or δ 77.0 (¹³C NMR).

1,3-Dichloro-2-propanone O-benzoyloxime (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. ¹H NMR (270 MHz, CDCl₃) δ 7.27-7.40 (m, 5H), 5.15 (s, 2H), 4.35 (s, 2H), 4.24 (s, 2H). ¹³C NMR (67.8 MHz, CDCl₃) 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+NH₄⁺); 266, 268, 270, 272 (M+Cl⁻); Elemental analysis calculated for C₁₀H₁₁NCl₂O: 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-benzoyloxime (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 CH₂Cl₂. The combined extracts were dried over sodium sulfate and evaporated. Flash chromatography (silica gel, 10% to 50% EtOAc in hexane

step-wise gradient) gave **Z** (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; **Z**, 0.58. ^1H 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 ($\text{M}+\text{H}^+$), calculated for $\text{C}_{28}\text{H}_{38}\text{NO}_{11}$ 564.2445. Elemental analysis calculated for $\text{C}_{28}\text{H}_{37}\text{NO}_{11}$: 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 **Z** (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 **Z**, 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.

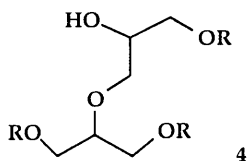
ATB-[2- ^3H]BMPA: Compound **Z** (12.9 mg, 0.023 mmol) was converted by Amersham International plc. (Buckinghamshire, England) to ATB-[2- ^3H]BMPA (33 mCi, 13.4 Ci/mmol) as outlined in Scheme 4 by the methods reported (1, 4b) for the conversion of **8** to ATB-[2- ^3H]BMPA.

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References and Notes

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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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