Synthesis of [8-^{14}C]-2,6-dichloro-9H-purine, a radiolabelled precursor for ^{14}C-nucleosides

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SUMMARY

The synthesis of [8-^{14}C]-2,6-dichloro-9H-purine (2), a radiolabelled precursor for preparing ^{14}C-labelled nucleosides, is described. Triethyl [^{14}C]orthoformate was reacted with 4,5-diamino-2,6-dichloropyrimidine (1) in acetonitrile at 90°C with methanesulfonic acid as catalyst to generate 2 in 84% radiochemical yield. Reaction of 2 with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose produced [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (3) in 86% yield. The radiochemical purity of 3 was higher than 98% with a specific activity of 36 mCi/mmol. This method has general application to ^{14}C-labelling of purines in drug development.

Key words: ^{14}C, triethyl [^{14}C]orthoformate, [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine, adenosine receptor agonist.

INTRODUCTION

Centrally acting adenosine receptor agonists or compounds which increase extracellular adenosine levels can exhibit what is termed neuromodulator activity. Such substances influence the release of neurotransmitters in regions of the central nervous system (CNS)[1,2], with particular inhibitory effects on the release of the excitatory amino acid glutamate[3,4].

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As part of the search for novel potent centrally acting adenosine receptor agonists[5], compounds with the basic structure given in Figure 1 have been identified. These compounds have a neuroprotective effect in animal models and limited side effects compared to previously investigated structures[6,7].

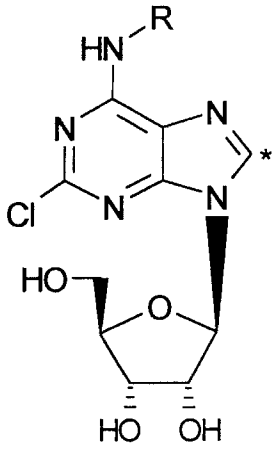


Figure 1 Structure of 2-chloro-*N*-(R)adenosine. * Indicates the position of labelling.

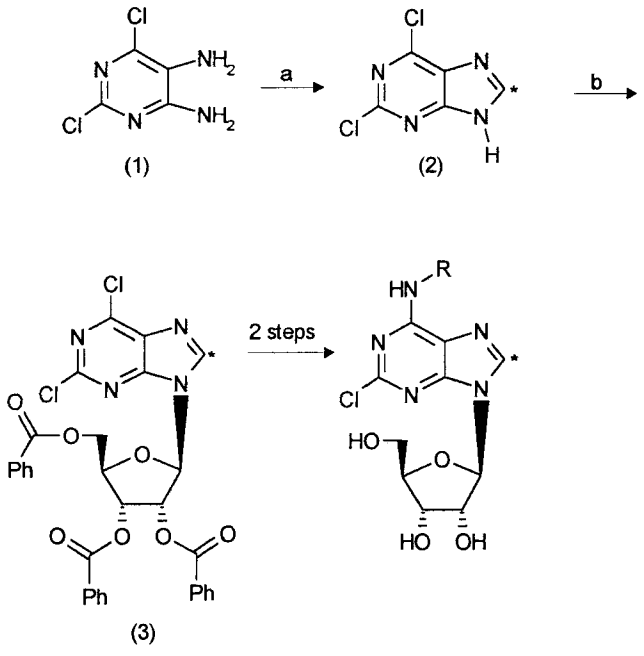
In order to study rationally the metabolic fate of these compounds a radiolabelled version of 3 was needed. The prefered isotope for such experiments is ^{14}C, sited in a stable location in the molecule; ^{14}C has a long half life (5730 years), a suitable energy of decay and ^{14}C can not be exchanged *in vivo*.

Our approach has been to use ^{14}C-labelled triethyl orthoformate as a radiolabelled starting material. Cyclisation of the diamino pyrimidine (1) with triethyl [^{14}C]orthoformate results in incorporation of radioactivity into the C-8 position in the purine.

This methodology has previously been used in the synthesis of [5-^{14}C]-pentostatin[8], [8-^{14}C]-carbovir[9] and [2-^{14}C]-griseolic acid 9'-(4-acetoxy-3-methoxybenzyl)ester[10].

RESULTS AND DISCUSSION

Scheme 1 shows the reaction sequence employed in the synthesis of [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (3).



a. Triethyl[^{14}C]orthoformate; MeCN; cat. CH_3SO_3H; 90°C

b. 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose; cat. I_2; 145°C

Scheme 1 Synthetic scheme for the preparation of 2-chloro-[8-purine-^{14}C] nucleosides.

^{14}C was introduced into the C-8 position of 2,6-dichloro-9H-purine (2) by reaction of 4,5-diamino-2,6-dichloropyrimidine (1) with triethyl [^{14}C]orthoformate. The diamine 1 was prepared in three steps by a modifided litterature procedure[11].

In previously described synthesis of purines utilizing triethyl orthoformate and diamines, we found that triethyl orthoformate has normally been used both as a reagent and a solvent. In synthesis using triethyl [^{14}C]orthoformate it was however important to scale down the amount of this reagent to one molar equivalent compared to 1 in order to achieve high radiochemical yields.

Concentrated hydrochloric acid has often been used as a catalyst in these types of reactions[12]. In our laboratory preliminary studies showed that when the amount of triethyl orthoformate was limited, the use of aqueous hydrochloric acid as catalyst proved ineffevtive. Due to the water in the hydrochloric acid solution triethyl

orthoformate partly decomposed to formic acid ethyl ester. This observation has also been described by Gopinathan *et al.*[9]. In view of this fact strictly anhydrous conditions were adopted for the ring closure, leading to the use of dry methanesulfonic acid as catalyst.

In order to obtain the best possible radiochemical yield several other parameters also had to be optimized. These included the temperature (50-130°C), the solvent (dimethyl acetamide (DMA), ethanol and acetonitrile) and the amount of precursor (1) (1-2 molar eqv.). Optimization studies using low specific radioactivity showed that the highest radiochemical yield was obtained using a temperature of 90°C, 0.14 molar eqv. of methanesulfonic acid as catalyst and 1.5 molar eqv. of the precursor (1). The labelling yields determined by radio-HPLC as a function of reaction time for three different reaction conditions are shown in Figure 2. The results shown are from the optimization studies.

The optimized reaction conditions were used in the synthesis of 2 with high specific radioactivity, resulting in 84% radiochemical yield with a radiochemical purity >98% and a specific radioactivity of 36 mCi/mmol.

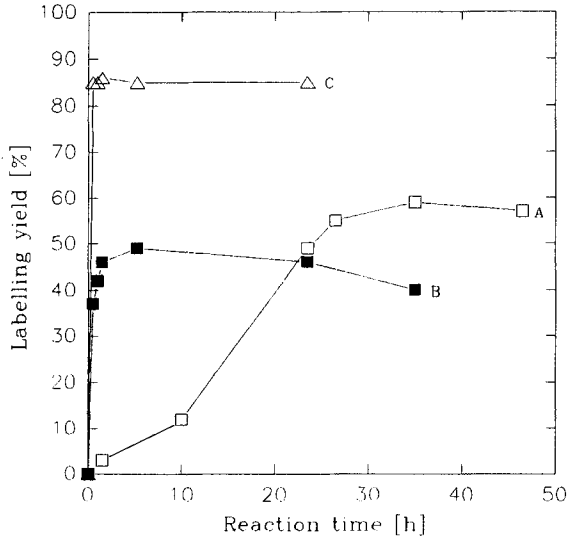


Figure 2 Optimization studies using triethyl [^{14}C]orthoformate (21 µCi, 0.10 mmol). ***A***: 200 µl DMA, 90°C, 1.0 molar eqv. 1 (no catalyst). ***B***: 200 µl DMA, 90°C, 0.14 molar eqv. CH_3SO_3H, 1.0 molar eqv. 1. ***C***: 1000 µl MeCN, 90°C, 0.14 molar eqv. CH_3SO_3H, 1.5 molar eqv. 1.

Fusion at 145°C of [8-^{14}C]-2,6-dichloro-9H-purine (2) and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose in the presence of catalytic amounts of iodine yielded [8-^{14}C]-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (3) in 86% radiochemical yield with a radiochemical purity >98% and a specific radioactivity of 36 mCi/mmol. A small impurity seen on HPLC was isolated and identified as the N-7-β-nucleoside by MS and by HPLC using a reference standard.

Owing to the benzoyl group in the 2-position - which can participate in a neighbouring group effect - this synthetic method provides only the β-isomer. Assignment of the anomeric configuration of 3 can be determined on the basis of a Nuclear Overhauser Effect (NOE) experiment which make use of the known ribo-configuration at C-3' and C-4'. Thus, for 3 H-1' and H-4' are located on the same face of the furan ring, and for the α-anomer H-1' and H-3' are located on the same face[13,14].

In conclusion, we have described a generally useful method for ^{14}C-labelling of purine derivatives at the 8-position. The synthesis of the final compound shown in Figure 1 will be the subject of a separate publication, where a full structure will be given.

EXPERIMENTAL

Optimization studies.

Material:

To an ampoule containing triethyl [^{14}C]orthoformate (5 mCi, 0.09 mmol, specific activity given by Amersham 59 mCi/mmol) was added 2.37 ml of non-radioactive triethyl orthoformate. The seal of the ampoule was then broken and the radioactive triethyl [^{14}C]orthoformate was mixed with the non-radioactive triethyl orthoformate. The specific radioactivity of this "spiked" solution was shown to be 0.21 mCi/mmol.

General procedure using low specific activity:

4,5-Diamino-2,6-dichloropyrimidine (1) was placed in a reaction vial with the chosen solvent (200-1000 µl). Triethyl [^{14}C]orthoformate (21 µCi, 0.10 mmol) from the above "spiked" solution and catalyst (0.91 µl, 0.014 mmol) were added. The vial was sealed and heated to the chosen temperature. The reaction was then followed by taking samples (approximately 1 µCi) at different intervals. The labelling yields were determined using radio-HPLC.

[8-^{14}C]-2,6-dichloro-9H-purine (2)

An ampoule containing triethyl [^{14}C]orthoformate (10 mCi, 0.169 mmol, specific radioactivity given by Amersham 59 mCi/mmol) was dried in a dessicator under reduced pressure (oil pump) at room temperature overnight. To the ampoule was added 16.9 µl (0.102 mmol) non-radioactive triethyl orthoformate and 1300 µl acetonitrile. The seal of the ampoule was then broken and the radioactive triethyl [^{14}C]orthoformate was mixed with acetonitrile and non-radioactive triethyl orthoformate. The total radioactivity transferred from the ampoule was shown to be to 8.43 mCi. The liquid was transferred to the reaction vial containing 4,5-diamino-2,6-dichloropyrimidine (1) (54.01 mg, 0.302 mmol) and methanesulfonic acid (1.85 µl, 0.03 mmol). The reaction vial was sealed and heated to 90°C. After 30 min the reaction vial was cooled. The volatiles were removed by nitrogen flow and the raw product was dissolved in a mixture of dimethyl sulfoxide and water (25/75, 1 ml) and purified on a semi-preparative C-18 HPLC column (250 x 16 mm, 7 µm) using a mixture of water and acetonitrile (90/10) as eluent. The collected fractions were concentrated by evaporation to give a colorless solid.

Radiochemical yield: 7.08 mCi (84%). Radiochemical purity >98%, determined by radio-HPLC analysis (system A). The specific radioactivity was 36 mCi/mmol, determined by MS using a reference standard.

[8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (3)

The ^{14}C-labelled purine 2 (3.26 mCi, 0.091 mmol) was placed in a reaction vial with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (100.1 mg, 0.198 mmol) and iodine (1.07 mg, 0.004 mmol) and the vial was sealed. The reaction vial was heated to 145°C. On stirring the solids gradually melted. After 60 min at 145°C the reaction vial was cooled. The melt was dissolved in a mixture of acetonitrile and water (80/20, 1 ml) and purified on a semi-preparative C-18 HPLC column (250 x 25 mm, 10 µm) using a mixture of water and acetonitrile (30/70) as eluent. The collected fractions were concentrated by evaporation to give a colorless solid.

Radiochemical yield: 2.80 mCi (86%). Radiochemical purity >98%, determined by radio-HPLC analysis (system B) and radio-TLC with a retention time identical to a standard reference sample. The specific radioactivity was the same as 2 (36 mCi/mmol) determined by HPLC using a reference standard.

MATERIALS

Triethyl [^{14}C]orthoformate was obtained from Amersham (59 mCi/mmol) and was used without further purification. All solvents used were of analytical grade. 1-*O*-acetyl-

2,3,5-tri-*O*-benzoyl-D-ribofuranose was obtained from Phanstiehl Laboratories, Waukegan, Illinois. 4,5-Diamino-2,6-dichloropyrimidine was synthezed in our laboratory in three steps by a modifided litterature procedure[11]. Dry triethyl orthoformate for the optimizing experiments was obtained from Aldrich and was delivered under nitrogen in a Sure/Seal™ bottle.

RADIOACTIVITY COUNTING

Determination of total radioactivity was carried out on a Packard 2000 CA tri-carb liquid scintillation analyzer, using 20 ml counting vials and Opti-fluor™ Packard liquid scintillator.

MASS SPECTROSCOPY

The mass spectrometer was a VG Autospec Ultima. The solution was injected on-column to a 25 m * 0.25 mm * 0.40 µm fused silica CP-Sil-8CB (Chrompack), with helium as carrier gas. The oven was programmed from 60°C after 0.1 min to 280°C with 25 °C/min. The temperature was held at 280 °C for 30 min. The column was directly interfaced to the ion source of the GC/MS system. The mass spectrometer was operated in the EI 70 eV mode and scanned m/e = 40 to 700 within 1.0 sec. Ion source temperature was 220°C.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC analyses were performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 µl loop) and a Merck UV-detector L-4000 (operating at 274 nm). Separations were accomplished at RT with a C-18 column (250 x 4,6 mm, 5 µm) from Novo Nordisk A/S, using a mixture of water and acetonitrile. The flow rate was 1.0 ml/min. Radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One beta detector A-500, using a 500 µl liquid flow cell. The ratio of column effluent to liquid scintillator (Opti-fluor™, Packard) was 1:2. Data collection was done by Flo-One data software on a PC-80386 computer. Two different analytical HPLC systems were used. System A: Isocratic, 90/10 : water/acetonitrile. System B: Isocratic, 30/70 : water/acetonitrile.

TLC

TLC was performed on glass plates (5x20 cm) coated with 0.25 mm silica gel 60 F_{254} (Merck). The mobile phase was ethyl acetate:heptane (50:50). TLC analysis were performed using a Bioscan Imaging Scanner System 200-IBM with Autochanger 1000.

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