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Research Article

Synthesis of [^{14}C]-imexon

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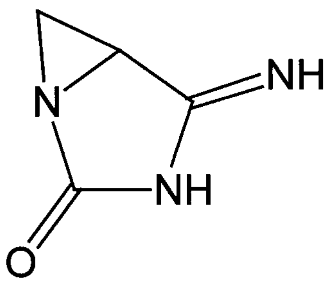
Summary

A four-step synthesis of [^{14}C]-imexon is described, starting from [^{14}C]-phosgene. The overall yield is 27% and the specific activity is 55 mCi/mmol. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: imexon; aziridine; sulfhydryl-binding; anticancer agent

Introduction

Imexon (4-imino-l,3-diazabicyclo[3.1.0]hexane-2-one, **1**) is entering clinical trials as a prospective anticancer agent. In a previous clinical trial based on its immunomodulatory properties, imexon showed positive responses against a variety of tumors.[1] More recently, its activity in immunodeficient SCID mice and in cell cultures suggests that immunomodulation is not responsible for cytotoxicity.[2]



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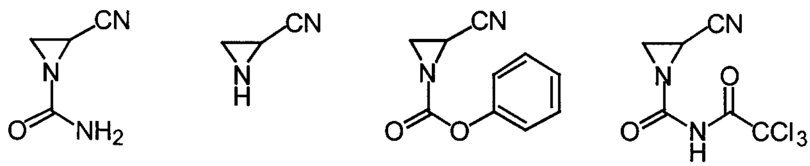
Although imexon has an aziridine ring, it does not alkylate DNA nor does it react with lysine, a model compound for the free amino groups of proteins.[3] It does react readily with cellular thiols, including glutathione and cysteine,[3] and it depletes them in a dose- and time-dependent manner.[4] This depletion results in an increase in the level of reactive oxygen species in cells, which damage mitochondrial but not nuclear DNA.[4] The mitochondrial membrane potential of imexon-sensitive cells undergoes a significant decrease, and cytochrome C is released from the mitochondria. This effect induces apoptotic cell death.[5]

The mode of action described above accounts for many of the properties of imexon, but it leaves unanswered a number of important questions, including (1) What is the distribution of imexon among important tissues such as liver and kidney? (2) What is the metabolic fate of imexon? (3) How strongly does imexon bind to serum proteins? (4) Is the depletion of glutathione and other thiols the result of direct interaction, or is it controlled by reaction of imexon with enzymes such as glutathione S-transferase 2 and glutathione peroxidase 1 that are essential to the maintenance of glutathione levels? Many such questions are best addressed by experiments based on the use of radiolabeled imexon.

Imexon **1** was first prepared by Bicker *et al.*[6] by cyclization of the carboxamide **2** with KOH in anhydrous methanol. Two methods have been described for the synthesis of **2**. In the first method 2-cyanoaziridine **3** is reacted with isocyanic acid.[7] In the second method aziridine **3** is reacted with phenyl chloroformate and the resulting carbamate **4** is treated with ammonia to give **2**.[8]



2 **3** **4** **5**

Recently, Remers *et al.*[9] reported a new synthesis of imexon. Trichloroacetylisocyanate was reacted with **3** to give **5**. Compound **5** was converted to **2** by treatment with ammonia in ethanol. Cyclization was achieved by treatment of **2** with benzyltrimethylammoniurn hydroxide in absolute ethanol.

Results and discussion

Keeping in mind the availability of 2-cyanoaziridine **3** and the relative efficiencies of the reaction pathways described above, we planned to incorporate the ^{14}C label at the carbonyl carbon atom. Radiolabeled isocyanic acid and trichloroacetyl isocyanate are not commercially available. Isocyanic acid is unstable above 0°C, and preparation of radiolabeled trichloroacetyl isocyanate presented an undesirable synthetic challenge. On the other hand, the ^{14}C-labeled carbamate **4** might be prepared by treating **3** with ^{14}C-labeled phenyl chloroformate, which in turn could be obtained from phenol and [^{14}C]-phosgene.

We first optimized the synthesis of non-radioactive imexon **1** on a micromolar scale. Phenyl chloroformate was prepared *in situ* from phenol and phosgene (20% in toluene) according to an analogous procedure for making phenyl chlorothionoformate.[10] Thus, reaction of **3** with phenyl chloroformate gave the carbamate **4** in 67% yield. Carboxamide **2** was obtained in 80% yield by treatment of an ether solution of **4** with liquid ammonia. Cyclization of **2** with benzyltrimethylammonium hydroxide (40% in methanol, Triton B) in absolute ethanol produced imexon **1** in 71% yield.

[^{14}C]-Imexon was prepared in a similar manner from [^{14}C]-phosgene as described in the experimental section and depicted in Scheme 1. The yield of [^{14}C]-imexon was 7 mg (63 μmol, 3.5 mCi, 27% over four chemical steps starting from [^{14}C]-phosgene). The product [^{14}C]-imexon was shown to be chemically and radiochemically pure (Figure 1).

Experimental

Materials

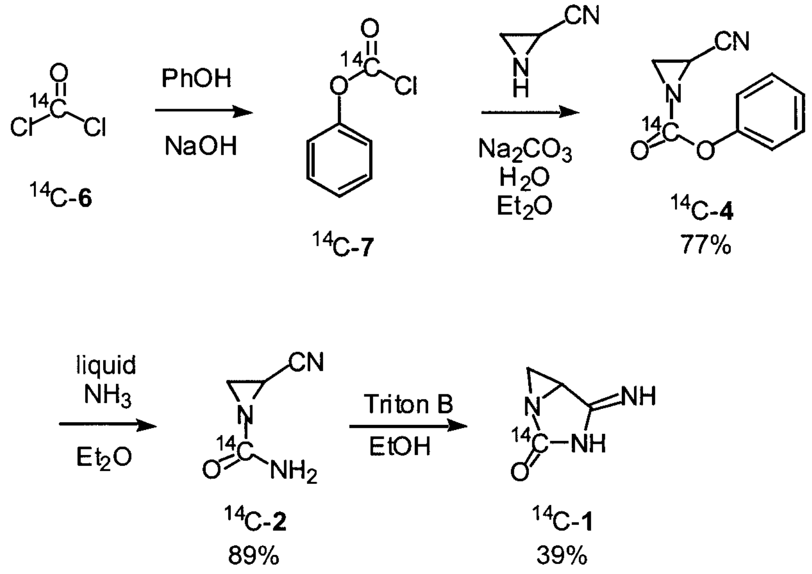
2-Cyanoaziridine (**2**) was prepared by a literature procedure.[11] [^{14}C]-Phosgene, which is commercially available, was prepared from [^{14}C]-carbon monoxide and chlorine.[12, †]

Methods

^{1}H-NMR and ^{13}C-NMR spectra were obtained at 300 and 75 MHz, respectively, using a Varian Unity-300 spectrometer. Proton NMR spectra were referenced to tetramethylsilane (0 ppm) or to the residual HOD in D_2O solvent (4.63 ppm). Carbon-13 NMR spectra were referenced to the $CDCl_3$ signal (77.0 ppm) or to the DMSO-d_6 signal (39.5 ppm). Mass spectra were obtained from the Mass Spectrometry Lab in the Department of Chemistry at The University of Arizona, Tucson, Arizona.

Phenyl 2-cyano-1-aziridine-carboxylate ([^{14}C]-**4**). [^{14}C]-Phosgene (see Scheme 1, 22 mCi, specific activity 55 mCi/mmol, 0.40 mmol) was condensed into 350 μl of toluene using vacuum line techniques and the solution kept under

† [^{14}C]-Calcium carbonate was prepared by absorption of [^{14}C]-carbon dioxide into aqueous calcium chloride solution. Pyrolysis of a mixture of [^{14}C]-calcium carbonate and zinc powder in a ratio of 1:2 at 700°C for 30 min gave [^{14}C]-carbon monoxide. [^{14}C]-Phosgene was synthesized by the photolysis of [^{14}C]-carbon monoxide with a slight excess of chlorine gas under incandescent light over 8 h. The overall yield of [^{14}C]-phosgene from [^{14}C]-carbon dioxide is about 85%.



Scheme 1.

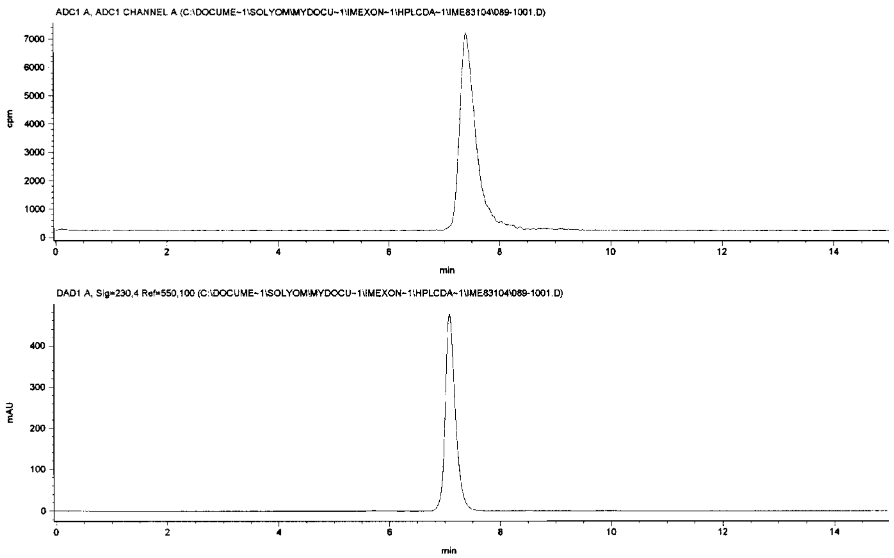


Figure 1. HPLC UV trace (lower panel) and radiochromatogram (upper panel) of a mixture of pharmaceutical grade imexon and synthetic [^{14}C]-imexon (concentrations 20.0 and 22.6 ppm, respectively). Area under the curve for the UV peak 6055.7 mAU (100%). Area under the curve for the ^{14}C peak 138 082 cpm (97.2%)

nitrogen. This solution was added slowly via syringe to a solution of phenol (37.6 mg, 0.40 mmol) in 380 µl of 1N NaOH at −20°C. The mixture was allowed to attain room temperature and stirring was continued for 2 h. The intermediate phenyl chloroformate, [^{14}C]-**7**, was not isolated. A solution of 2-cyanoaziridine (**3**, 27 mg, 0.40 mmol) in 450 µl of 2N Na_2CO_3 and 800 µl of ether were added to the solution of [^{14}C]-**7** and the resulting mixture was stirred for 2 h. The reaction mixture was then diluted with ether (30 ml) and water (15 ml), the phases were separated, and the aqueous phase extracted with ether (2 × 15 ml). The organic phases were combined, washed with brine (20 ml), and dried over $MgSO_4$. The mixture was filtered and volatiles removed by evaporation. The light yellow residual oil was fractionated by gravity column chromatography on silica gel. Elution with 10% ether/pentane gave 1.8 mCi (8%) of the by-product [^{14}C]-diphenyl carbonate as a white solid. Further elution with 40% ether/pentane gave 17 mCi (77%) of [^{14}C]-**4** as a viscous oil which solidified upon storage at −20°C. The thin layer chromatographic mobility of this material was identical to that of an authentic standard (R_f = 0.49 on 0.25 mm silica gel plates eluted with 1:2 ether:pentane).

Spectral data for non-radioactive **4**: mp 56–58°C; IR cm^{-1} 3108, 3060, 2371, 2255, 1742, 1591, 1489, 1308, 1201; ^{1}H NMR ($CDCl_3$) δ 2.78 (1, d, J = 5.7 Hz), 2.82 (1, d, J = 3 Hz), 3.21 (1, dd, J = 6, 3.3 Hz), 7.16 (2, d, J = 7.8 Hz), 7.27 (1, t, J = 7.2 Hz), 7.40 (2, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 22.4, 31.8, 115.7, 120.9, 126.4, 129.5, 150.4, 158.5; HRMS (FAB^+) calculated for $C_{10}H_9N_2O_2$ 189.0664, found 189.0663.

2-Cyano-1-aziridine-carboxamide-([^{14}C]-**2**). To A solution of phenyl carbamate [^{14}C]-**4** (34.2 mg, 0.18 mmol, 10 mCi) in diethyl ether (5 ml) was added liquid ammonia (0.5 ml) and the reaction mixture stirred for 2 h at the boiling temperature of ammonia and then allowed to come to ambient temperature overnight. The solvent was removed at reduced pressure and the residue washed with 40% diethyl ether/pentane. The carboxamide [^{14}C]-**2** was obtained as a white solid (18 mg, 0.162 mmol, 8.9 mCi) in 89% yield.

Spectral data for non-radioactive **2**: mp 70–72°C; IR cm^{-1} 3388, 3203, 2362, 2259, 1690, 1625, 1399, 1004; ^{1}H NMR ($CDCl_3$) δ 2.55 (1, d, J = 3.9 Hz), 2.58 (1, d, J = 6.3 Hz), 3.06 (1, dd, J = 6.6, 3.6 Hz), 5.31 (2, br s); ^{13}C NMR (DMSO-d_6) δ 21.4, 30.8, 118.3, 162.0; HRMS (FAB^+) calculated for $C_4H_6N_3O$ 112.0511, found 112.05114.

4-Imino-1,3-diazabicyclo[3.1.0]hexan-2-one ([^{14}C]-imexon, [^{14}C]-**1**). To a solution of carboxamide [^{14}C]-**2** (18 mg, 162 µmol, 8.9 mCi) in absolute ethanol (200 µl) in a 2 ml vial fitted with a Teflon lined screw cap was added benzyltrimethylammonium hydroxide (40% solution in methanol, Triton B, 7.36 µl, 16 µmol). The reaction mixture was agitated using a vortex mixer at

30 min intervals for 3 h and then left overnight. Ethanol (200 μl) was then added, the reaction mixture was agitated, centrifuged, and the solvent decanted from an off-white precipitate. This process was repeated three times and the remaining solid dried under vacuum. Analysis of this solid by HPLC-MS confirmed its chemical identity as imexon. The yield of [^{14}C]-**1** was 7 mg (63 μmol, 3.5 mCi, 39%). The chemical and radiochemical purity of this product were >97%.

Spectral data for non-radioactive **1**: mp >250°C; IR cm^{-1} 3300-2500 (b), 1800-1400 (b), 1296, 12343, 1181, 1013, 820; ^{1}H NMR (D_2O) δ 2.39 (1, d, J=3 Hz), 2.55 (1, d, J=5.4 Hz), 3.59 (1, dd, J=5.4, 3.3 Hz); HRMS (FAB$^+$) calculated for $C_4H_6N_3O$ 112.0511, found 112.0511.

Conclusion

In summary, we have synthesized [^{14}C]-imexon starting from [^{14}C]-phosgene and **2**-cyanoaziridine in an overall yield of 27% with a specific activity of 55 mCi/mmol. The experimental procedures are simple and efficient. To the best of our knowledge this is the first reported synthesis of [^{14}C]-imexon.

Acknowledgements

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