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Synthesis of Isotopically Labelled WIN 33377, an Anticancer Agent related to Hycanthone

Gordon R. Duffin, George J. Ellames,* David I. Smith and Karen J. Wedge

Synthetic Chemistry and Radiochemistry Laboratories, Department of Chemical Development, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Research Centre, Alnwick, Northumberland, U. K. , NE66 2JH

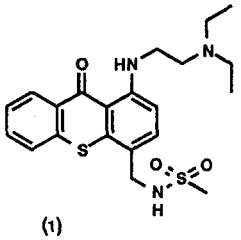
SUMMARY

^{14}C labelled and $^{13}C,^{15}N$ labelled WIN 33377, **1**, 1-(2-diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, have been prepared by related routes using the known 1-(2-diethyl-aminoethylamino)thioxanthen-9-one, **4**, as starting material and introducing the label with cyanide prior to further elaboration.

Key Words: 1-(2-Diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, ^{14}C-WIN 33377, $^{13}C,^{15}N$-WIN 33377, WIN 33377

INTRODUCTION

1-(2-Diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, **1**, WIN 33377,[1] is a novel antitumour agent related to Hycanthone, **2**,[2] and Lucanthone, **3**.[3] As part of a development programme, and to assist with various mode of action studies, WIN 33377 was required singly labelled with ^{14}C, and doubly labelled with ^{13}C and ^{15}N.



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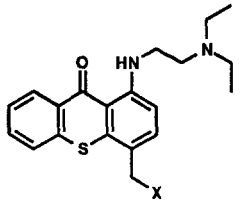

	X
(2)	OH
(3)	H

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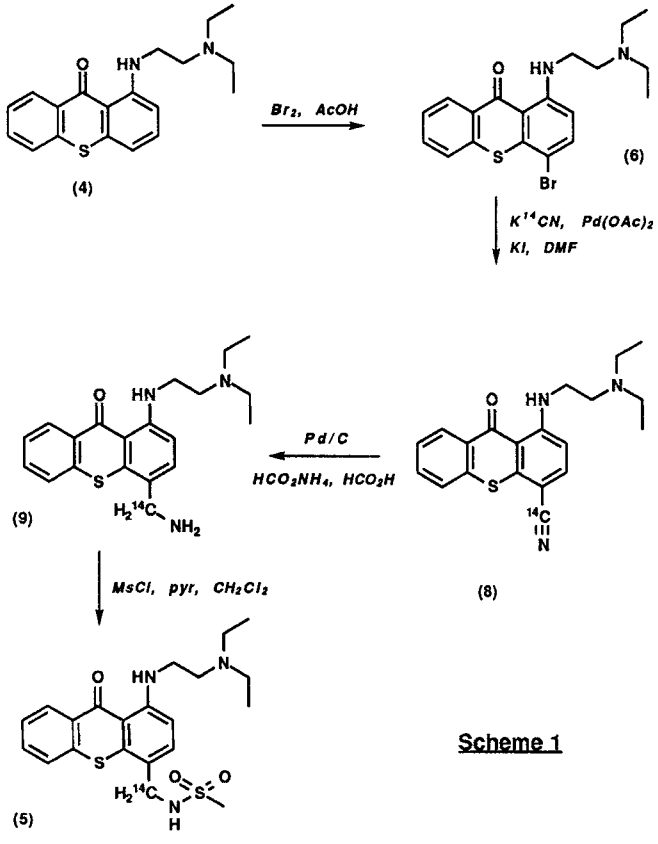
For metabolism studies it was considered essential that the label be either incorporated in the aromatic nucleus or the C-4 methylene substituent. Any sequence affording material labelled with ^{14}C within the aromatic nucleus would have been unacceptably lengthy. The route then currently employed in the development process used the known 1-(2-diethylaminoethyl-amino)thioxanthen-9-one, **4**,[4] as an intermediate and this material was therefore readily available to us. In that process, which was related to methodology reported[5] to prepare other analogues of Hycanthone, the C-4 methylene was attached as a one carbon unit using a considerable excess of formaldehyde, and was thus clearly inappropriate for use in a radiolabelled synthesis. It was therefore necessary to develop a novel method of attaching this one carbon unit that did not rely on use of an excess of what would be the labelled moiety.

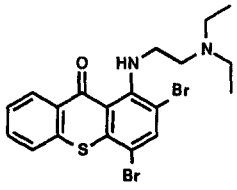
RESULTS AND DISCUSSION

^{14}C-WIN 33377, **5**, was prepared in four steps from the known precursor **4** by the route outlined in Scheme 1.



Scheme 1

Bromination of **4** with bromine in glacial acetic acid afforded a mixture of the desired monobromide, **6**, in 45% yield, and the dibromide, **7**, which was readily separated by chromatography.

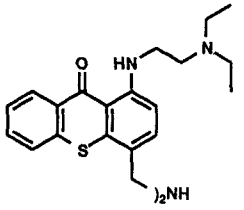


(7)

The introduction of cyanide was achieved by treatment of a continuously stirred solution of **6** in dimethylformamide with a mixture of potassium cyanide, palladium(II) acetate and potassium iodide under an atmosphere of nitrogen, and maintaining this at 135°C overnight.[6] The desired nitrile, **8**, was obtained in a moderate yield of 21%. Earlier attempts at displacing the bromine with copper(I) cyanide had only led to complex mixtures.

Reduction of the nitrile , **8**, to the amine, **9**, was carried out using ammonium formate and formic acid over 10% palladium on charcoal. The amine was produced in ~50% yield and was mesylated directly to afford the desired ^{14}C-WIN 33377, **5**, in 25% yield for the two steps combined.

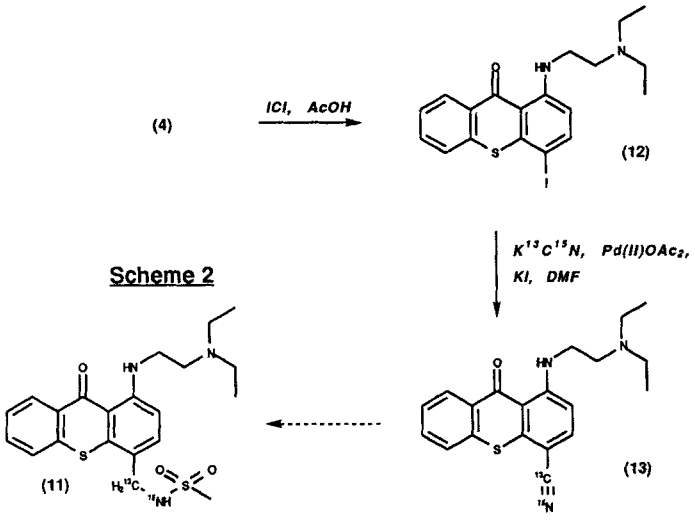
In the absence of ammonium formate the product mixture was more complex and contained primarily the dimeric secondary amine, **10** - a common product of nitrile hydrogenations.[7] The major by-product of the ammonium formate / formic acid reduction appeared to result from loss of the ketone - a transformation previously reported for aryl ketones using ammonium formate in boiling acetic acid.[8] However neither over-reduction of the nitrile to a methyl group, as previously reported using ammonium formate in methanol,[9] nor reduction of the aromatic rings, as previously described using formic acid in methanol,[10] were observed.



(10)

Using the above methodology, ^{14}C-WIN 33377, **5**, (specific activity 15 mCi/mmol) of radiochemical purity >97% was prepared from ^{14}C-potassium cyanide (specific activity 57.6 mCi/mmol)[11] in 5.2% overall radiochemical yield.

With a minor modification, the same methodology was used to prepare ^{13}C,^{15}N-WIN 33377, **11**, and this is outlined in Scheme 2. In this instance the starting material, **4**, was iodinated with iodine monochloride in acetic acid to afford the monoiodide, **12**, with no detectable dihalogenated material. The iodide , **12**, was isolated in 44% yield. This underwent conversion to the corresponding nitrile, **13**, in 37% yield, using the same methodology as applied to the bromide in the ^{14}C synthesis. Completion of the synthesis by analogy with Scheme 1 afforded the desired ^{13}C,^{15}N-WIN 33377, **11**, in 5.8% overall yield based on ^{13}C,^{15}N-potassium cyanide (>99 atom % ^{13}C, >99 atom % ^{15}N).[12]



Scheme 2

The ^{1}H nmr of **11** clearly revealed the splitting of the methylene signal at 4.4 ppm in the unlabelled material, with this acquiring an extra coupling of ~96 Hz due to the introduction of the ^{13}C atom.

It is noteworthy that there was no detectable loss of ^{15}N label in the reduction using ammonium formate in formic acid. Mass spectroscopy could detect no ^{13}C-4-(aminomethyl)-1-(2-diethylaminoethylamino)thioxanthen-9-one in the intermediate amine, and the mass spectrum of the final product, **11**, indicates that this is >99% isotopically pure. This suggests that secondary amine formation is suppressed by the presence of formate anion rather than by involvement of ammonia in the imine to aminal equilibration postulated to take place during gaseous hydrogenations.[13] Mass spectral studies indicate the rapid formation of an intermediate, presumably the imine, followed by slow build up of the fully reduced amine. We assume that it is a protonated imine that is the reactive electrophile involved in the formation of the secondary amine and that the formate anion acts as a buffer and reduces the concentration of this iminium ion to a negligible extent.

EXPERIMENTAL

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. N.m.r. spectra were recorded on a Bruker AC 80 and are reported relative to internal tetramethylsilane. Mass spectra were obtained on a Finnigan TSQ-700 mass spectrometer and accurate mass measurements (Dr T Dransfield, York University) obtained on a VG Analytical Autospec mass spectrometer..

4-Bromo-1-(2-diethylaminoethylamino)thioxanthen-9-one, 6: To a solution of 1-(2-diethylaminoethylamino)thioxanthen-9-one, **4**, (10.18 g, 31.2 mmole) in glacial acetic acid (400 ml)

was added dropwise bromine (6 g, 37.5 mmole) and the reaction mixture was stirred overnight. Solid was removed by filtration and washed with ethyl acetate. The solid was then dissolved in aqueous sodium hydroxide and the solution extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulphate to afford, following removal of solvent, material (12 g) which was chromatographed on silica gel eluting with increasing proportions of ethyl acetate in dichloromethane (up to 1:1) first the by-product 2,4-dibromo-1-(2-diethyl-aminoethylamino)thioxanthen-9-one, **7**, as an orange solid, (2.03 g, 13%), m.pt. 57°C (Found: C, 47.30; H, 4.18; N, 5.75: $C_{19}H_{20}Br_2N_2OS$ requires C, 47.13; H, 4.16; N, 5.78%. NMR ($CDCl_3$) δ_H 1.05 (6H, m), 2.65 (6H, m), 3.6 (2H, q J 7 Hz), 7.6 (3H, m), 7.85 (1H, s), 8.45 (1H, m) and 9.5 (1H, br. s), and then the desired 4-bromo-1-(2-diethylamino-ethylamino)thioxanthen-9-one, **6**, (5.74 g, 45%) as an orange solid, m.pt. 105°C (m/e 404, Found: 404.055048. $C_{19}H_{21}BrN_2OS$ requires 404.055796, NMR ($CDCl_3$) δ_H 1.3 (6H, m), 3.3 (4H, m), 3.75 (4H, m), 6.9 (1H, d, J 10 Hz), 7.4-7.9 (4H, m) and 8.4 (1H, m, J 7 and 3 Hz).

^{14}C-4-Cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, 8: To a solution of 4-bromo-1-(2-diethylaminoethylamino)thioxanthen-9-one, **6**, (2.56 g, 6.3 mmole) in dimethyl-formamide (20 ml) under an atmosphere of nitrogen was added ^{14}C-potassium cyanide (95 mCi, 1.65 mmole), unlabelled potassium cyanide (303 mg, 4.7 mmole), palladium(II) acetate (713 mg, 3.2 mmole) and potassium iodide (524 mg, 3.2 mmole) and the mixture heated to 135°C overnight. Distilled water (50 ml) and dichloromethane (100 ml) were added and the palladium(0) was removed by filtration through celite. The filtrate was washed with dichloro-methane (250 ml) containing triethylamine (5 ml). The organic extracts were dried over anhydrous magnesium sulphate and the solvents removed under vacuum to afford crude material which was chromatographed on silica gel in hexane : ethyl acetate : triethylamine (40:9:1) to afford the desired ^{14}C-4-cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, **8**, (19.7 mCi, 15 mCi/mmole, 21% radiochemical yield).

^{14}C-4-(Aminomethyl)-1-(2-diethylaminoethylamino)thioxanthen-9-one , 9: To a mixture of ^{14}C-4-cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, **8**, (19.7 mCi, 1.3 mmole), 10% palladium on charcoal (1 g) and ammonium formate (689 mg, 10.9 mmole) under an atmosphere of nitrogen was added formic acid (50 ml) and the mixture was stirred overnight. The formic acid was then removed under vacuum at room temperature. Ethyl acetate was added and the residual formic acid was neutralised by the addition of saturated aqueous sodium hydrogen carbonate. The organic phase was decanted and the aqueous phase extracted with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulphate and filtered to remove palladium. It was evident that significant amounts of radiolabelled material remained on the catalyst and attempts were made to free as much of this as possible by sequential washings of the catalyst with dichloromethane : isopropylamine (9:1) and dichloromethane : methanol (9:1). The combined organic washings were removed under vacuum to leave a residue which was chromatographed on silica gel eluting with ethyl acetate : methanol : isopropylamine (48:1:1) to afford material (12.0 mCi) which by TLC radioanalysis was predominantly (~84%) the desired ^{14}C-4-(aminomethyl)-1-(2-diethylaminoethylamino)thioxanthen-9-one, **9**, which was used directly in the following step.

^{14}C-1-(2-Diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, ^{14}C-WIN 33377, 5: The crude ^{14}C-4-(aminomethyl)-1-(2-diethylaminoethylamino)thioxanthen-9-one, **9**, (12.0 mCi with radiochemical purity 84% = ~10.1 mCi, 0.67 mmole) was azeotroped with benzene (20 ml) before being dissolved in dichloromethane (11 ml). This solution, under an atmosphere of nitrogen, was treated with pyridine (80 µl, 0.99 mmole) and methanesulphonyl chloride (75 µl, 1.0 mmole) and the reaction mixture left for 48 hours. The crude reaction mixture was then filtered and the solvent was removed under vacuum. The residue was chromatographed on silica gel eluting with dichloromethane : methanol (up to 24:1) to afford partially purified material. This was then chromatographed on HPLC using a Mega Bond C-18 reverse phase column eluting with methanol : dichloromethane (7:3) to afford the desired ^{14}C-1-(2-diethylaminoethylamino)-4-methanesulphonamidomethyl)thioxanthen-9-one, ^{14}C-WIN 33377, **5**, (5.0 mCi, 143 mg, Specific Activity 15 mCi/mmole, 49% yield).

1-(2-Diethylaminoethylamino)-4-iodothioxanthen-9-one, 12: To a solution of 1-(2-diethylaminoethylamino)thioxanthen-9-one, **4**, (652 mg, 2.0 mmole) in glacial acetic acid (3 ml) was added a solution of iodine monochloride (495 mg, 3.1 mmole) in glacial acetic acid (1 ml) and the reaction mixture stirred for 48 hours at room temperature. After this time the reaction mixture was carefully added to an aqueous solution (100 ml) of sodium hydrogen carbonate (5 g) and sodium metabisulphite (300 mg). This was then extracted with diethyl ether and the organic phase washed with distilled water before being dried over anhydrous magnesium sulphate. The solvent was removed under vacuum to afford the desired 1-(2-diethylaminoethylamino)-4-iodothioxanthen-9-one, **12**, (400 mg, 0.9 mmole, 44% yield), as an orange solid, m.pt. 103.5^{o}C (m/e 452 Found: 452.041611. $C_{19}H_{21}IN_2OS$ requires 452.041937. NMR ($CDCl_3$) δ_H 1.1 (6H, t, J 7 Hz), 2.8 (6H, m), 3.3 (2H, q, J 7 Hz), 6.4 (1H, d, J 9Hz), 7.4 (3H, m), 7.7 (1H, d, J 9 Hz), 8.5 (1H, d, J 7 Hz) and 10.5 (1H, br. s)

^{13}C,^{15}N-4-Cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, 13: ^{13}C,^{15}N- 4-Cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, **13**, (960 mg, 2.7 mmole, 37 % yield) was prepared from 1-(2-diethylaminoethylamino)-4-iodothioxanthen-9-one, **12**, and ^{13}C,^{15}N-potassium cyanide (500 mg, 7.5 mmole, >99 atom % ^{13}C, >99 atom % ^{15}N) by a method analogous to that used in the synthesis of ^{14}C-4-cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, **8**, from ^{14}C-4-bromo-1-(2-diethylaminoethylamino)thioxanthen-9-one, **6**, described above.

^{13}C,^{15}N-1-(2-Diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, ^{13}C,^{15}N-WIN 33377, 11: ^{13}C,^{15}N-1-(2-Diethylaminoethylamino)-4-(methanesulphonamidomethyl)thioxanthen-9-one, ^{13}C,^{15}N-WIN 33377, **11**, (133 mg, 0.4 mmole, 16% yield, NMR ($CDCl_3$) δ_H 1.1 (6H, t, J 7 Hz), 2.8 (6H, m), 3.3 (2H, q, J 7 Hz), 4.4 (2H, dd, J 96 and 7 Hz), 6.6 (1H, d, J 9 Hz), 7.4 (4H, m), 8.5 (1H, d, J 7 Hz) and 10.4 (1H, br. s). was prepared from ^{13}C,^{15}N-4-cyano-1-(2-diethylaminoethylamino)thioxanthen-9-one, **13**, (852 mg, 2.4 mmole) by a method analogous to that used in the synthesis of ^{14}C-WIN 33377, **5**, from ^{14}C-4-cyano-1-(2-diethylaminoethyl-amino)thioxanthen-9-one, **8**, described above.

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