[^{14}C]-Labelling of Benzisothiazolone based Inhibitors of Human Leukocyte Elastase

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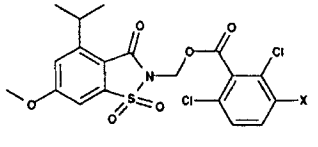
SUMMARY

The [^{14}C] labelled synthesis of two benzisothiazolone based inhibitors of Human Leukocyte Elastase is described. These are [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [$^{14}C_2$]-WIN 63394, **2**, and [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733, **3**. The synthesis of the intermediate [3-^{14}C]-2-(chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **8**, common to both these syntheses, was achieved in 8 steps in 37% radiochemical yield.

Key Words: [3-^{14}C]-[6-Methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [$^{14}C_2$]-WIN 63394, [3-^{14}C]-[6-Methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733

INTRODUCTION

It has been proposed that Human Leukocyte Elastase is the primary mediator of pulmonary emphysema.[1] Compounds described by the generic structure **1** have been disclosed as potent, selective mechanism-based inhibitors of Human Leukocyte Elastase.[2]



(1)

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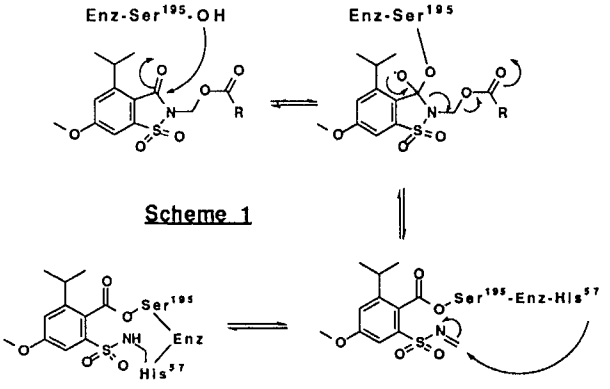
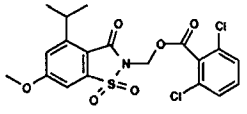
The rationale for the design of agents related to **1** has previously been described.[3,4] In simplified terms the proposed mechanism of action can be outlined as in Scheme 1. These inhibitors have been designed to inactivate the enzyme by cross-linking the active site Ser^{195} and His^{57}.



It is clear from the above mechanism that, by their mode of action involving expulsion of the carboxylate anion, it should be anticipated that such molecules separate into two parts *in vivo*, and thus for any metabolic study it would be desirable to label each portion. Therefore in the cases of [6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl 2,6-dichlorobenzoate, WIN 63394, **2**,[5] and [6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benz-isothiazol-2(2H)-yl]methyl 2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, WIN 64733, **3**, it would be desirable to label both the common benzisothiazolone portion ,**4**, and each leaving group, **5** and **6**.



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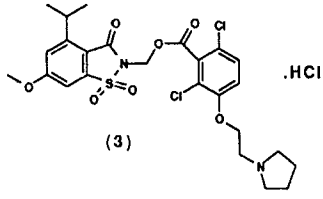

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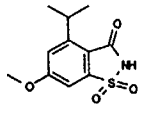

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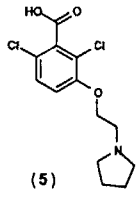

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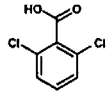
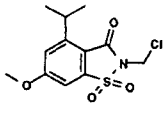


(6)

The common synthetic intermediate in the synthesis of both WIN 63394, **2**, and WIN 64733, **3**, was 2-(chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **7**. Given that the possible metabolic fate of the methoxy group was unknown, it was considered that this might be most conveniently labelled at C-3, as in **8**, by a modification to related unlabelled syntheses.[6]



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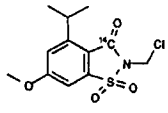
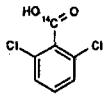


(8)

It was considered that the carboxylic acid leaving groups **5** and **6** could be labelled at the carboxyl functions as in [*carboxy*-^{14}C]-2,6-dichlorobenzoic acid, **9**, and **10**.



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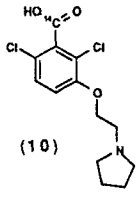

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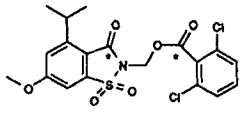
Coupling of each of **9** and **10** would produce the desired doubly labelled compounds **11** and **12**.[7]



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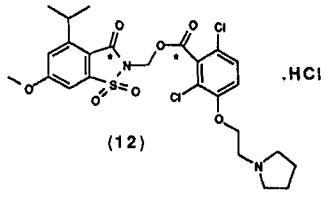
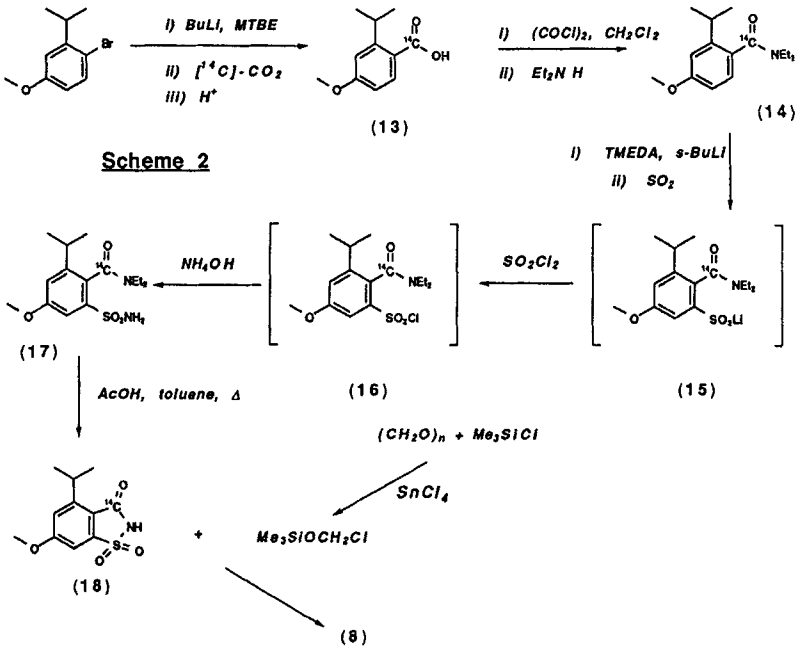


.HCl

(12)

RESULTS AND DISCUSSION

The synthesis of the common intermediate **8** was carried out by the route outlined in Scheme 2.

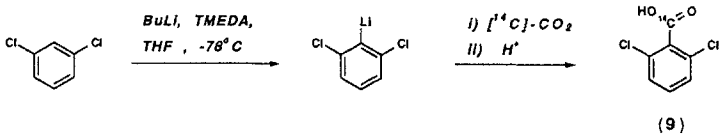


The substituted benzoic acid, **13**, was prepared by treatment of 4-bromo-2-(1-methylethyl)-anisole with butyllithium in *tert*-butyl methyl ether and quenching the resulting anion with [^{14}C]-carbon dioxide generated from [^{14}C]-barium carbonate. This was accomplished in 95% radiochemical yield. Conversion of the acid, **13**, to the diethylamide, **14**, was accomplished via formation of the acid chloride using oxalyl chloride and subsequent treatment with diethylamine. This afforded the diethylamide, **14**, in 96% radiochemical yield.

The diethylamide, **14**, in diethyl ether, was treated with *sec*-butyllithium at -78^{o}C and then quenched with sulphur dioxide. The resultant lithium sulphinate, **15**, was then treated with sulphuryl chloride and the crude sulphonyl chloride, **16**, treated with aqueous ammonium hydroxide to afford the sulphonamide, **17**. The sulphonamide, **17**, was isolated in 57% radiochemical yield. The sulphonamide, **17**, was cyclised to the desired benzisothiazolone, **18**, by heating the former under reflux in a mixture of glacial acetic acid and toluene. The cyclised product, **18**, was isolated in 79% radiochemical yield.

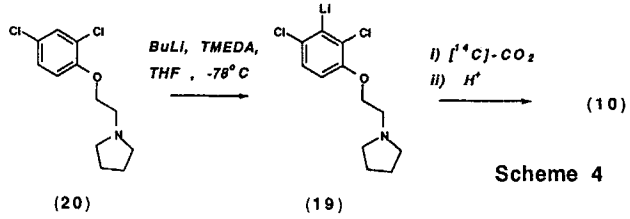
The benzisothiazolone, **18**, was chloromethylated using chloromethyl trimethylsilyl ether, generated from reaction of paraformaldehyde with chloromethylsilane and tin(IV) chloride in 1,2-dichloro-methane, to afford the methylchloride, **8**, in 89% radiochemical yield. The overall radiochemical yield for the synthesis of the [3-^{14}C]-2-(chloromethyl)-6-methoxy-4-(1-methyl-ethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **8**, from [^{14}C]-barium carbonate was 37%.

The synthesis of [*carboxy*-^{14}C]-2,6-dichlorobenzoic acid was carried out by carboxylation of 2,6-dichlorophenyllithium[8] as outlined in Scheme 3.



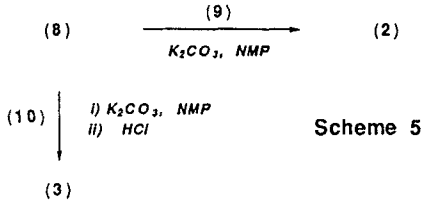
Scheme 3

[*carboxy*-^{14}C]-2,6-Dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, **10**, was prepared by quenching, with [^{14}C]-carbon dioxide, the lithium anion, **19**, formed on treatment of 1-[2-(2,4-dichlorophenoxy)ethyl]pyrrolidine, **20**, with *n*-butyllithium in tetrahydrofuran in the presence of *N,N,N',N'*-tetramethylethylenediamine, as outlined in Scheme 4. The desired carboxylic acid, **10**, was isolated in 73% radiochemical yield. The position of carboxylation was determined by comparison with authentic unlabelled material and by ^{1}H nmr. It is noteworthy that no trace of any other carboxylation product was observed.



Scheme 4

Coupling of **8** with each of **9** and **10**, as outlined in Scheme 5, afforded the desired [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [$^{14}C_2$]-WIN 63394, **2**, and [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)-ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733, **3**, in 65% and 38% radiochemical yield respectively.



Scheme 5

The overall radiochemical yield (calculated from [^{14}C]-barium carbonate used in the synthesis of **13**) for the synthesis of [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [$^{14}C_2$]-WIN 63394, **2**, was 20%, and the material was analysed by radioTLC to have 97% radiochemical purity. The similarly calculated overall radiochemical yield for the synthesis of [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733, **3**, was 14% and the material was analysed by radioTLC to have 98% radiochemical purity.

EXPERIMENTAL

[*carboxy*-^{14}C]-4-Methoxy-2-(1-methylethyl)benzoic acid, 13: The following sequence was carried out on a vacuum manifold. Under an atmosphere of nitrogen, a mixture of 4-bromo-2-(1-methylethyl)anisole (690 mg, 3.0 mmole) and *tert*-butyl methyl ether (5 ml) was cooled to 0°C and butyllithium (1.6 ml, 2,5 mmole of a 1.6 M solution in hexane) added. The reaction mixture warmed to room temperature over six hours before being frozen in a liquid nitrogen trap. Conc. sulphuric acid (1.5 ml) was frozen to -80°C in a separate vessel and the manifold evacuated. The sulphuric acid was warmed to room temperature while [^{14}C]-barium carbonate (53.6 mCi, 0.91 mmole)[9] was added. The [^{14}C]-carbon dioxide thus liberated was trapped in the vessel containing the other reactants. The reaction vessel containing the lithiated substrate and the [^{14}C]-carbon dioxide was then removed from the liquid nitrogen trap and warmed to -78°C and stirred for one hour. The reaction mixture was then quenched with saturated aqueous ammonium chloride (1 ml) and warmed to room temperature before being poured into distilled water and extracted with ethyl acetate. The aqueous phase was washed with further ethyl acetate before being acidified with hydrochloric acid and then extracted with ethyl acetate. This organic phase was washed with saturated aqueous sodium chloride before being dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to afford the desired [*carboxy*-^{14}C]-4-methoxy-2-(1-methylethyl)-benzoic acid, **13** (50.9 mCi, radiochemical yield 95%).

[*carboxy*-^{14}C]-*N,N*-Diethyl 4-methoxy-2-(1-methylethyl)benzamide, 14: To a solution of [*carboxy*-^{14}C]-4-methoxy-2-(1-methylethyl)benzoic acid, **13** (50.9 mCi, 0.86 mmole), in

dichloromethane (5 ml) was added oxalyl chloride (150 µl, 1.72 mmole) and the reaction mixture left overnight. The reaction mixture was then heated under reflux for 15 minutes before the solvents were removed under reduced pressure. Dichloromethane (15 ml) and diethylamine (440 µl, 4.3 mmole) were added and the reaction mixture was left at room temperature for 24 hours. After this time, ethyl acetate (100 ml) was added and the organic phase washed sequentially with water, aqueous sodium hydrogen carbonate, water, dilute hydrochloric acid and finally saturated aqueous sodium chloride, before being dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to afford the desired [*carboxy*-^{14}C]-*N,N*-diethyl 4-methoxy-2-(1-methylethyl)benzamide, **14** (48.8 mCi, radiochemical yield 96%).

[*carboxy*-^{14}C]-2-[(*N,N*-Diethyl)carboxamido]-5-methoxy-3-(1-methylethyl)benzenesulphonamide, 17: The following sequence was carried out on a vacuum manifold. [*carboxy*-^{14}C]-*N,N*-Diethyl 4-methoxy-2-(1-methylethyl)benzamide, **14** (48.8 mCi, 0.83 mmole) and *N,N*-diethyl 4-methoxy-2-(1-methylethyl)benzamide (604 mg, 2.4 mmole) were frozen to liquid nitrogen temperature and the atmosphere above the mixture evacuated and replaced by nitrogen gas. To this was added diethyl ether (10 ml) and *N,N,N',N'*-tetramethylethylenediamine (570 µl, 3.8 mmole) and the mixture warmed to -78°C and *sec*-butyllithium (2.9 ml of 1.3 M solution in cyclohexane, 3.77 mmole) added dropwise. The reaction was stirred for 1.5 hours and formation of an orange precipitate was observed. Elsewhere on the manifold, a flask was filled with sulfur dioxide which had been dried by passage through anhydrous calcium sulphate. The nitrogen atmosphere above the reaction mixture was evacuated and the reaction frozen to liquid nitrogen temperature. The sulfur dioxide was then transferred to the reaction mixture which was warmed to -78°C. After a further 30 minutes the reaction was warmed to room temperature. The reaction mixture was then cooled to 0°C and sulfuryl chloride (310 µl, 3.9 mmole) added dropwise. The reaction mixture was stirred for 30 minutes before the atmosphere above was evacuated. With the temperature maintained at 0°C, tetrahydrofuran (5 ml) and aqueous ammonium hydroxide (1ml of 25% solution, 14.7 mmole) were added. The reaction mixture was then warmed to room temperature over 15 minutes before being acidified with dilute hydrochloric acid and extracted with ethyl acetate. The organic phase was washed with saturated aqueous sodium chloride before being dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure and the residue chromatographed on silica gel eluting with dichloromethane : ethyl acetate (17:3) to afford the desired [^{14}C]-2-[(*N,N*-diethyl)carboxamido]-5-methoxy-3-(1-methylethyl)benzenesulphonamide, **17** (27.8 mCi, Sp. Ac. 15 mCi/mmole, 57% radiochemical yield).

[3-^{14}C]-6-Methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, 18: [*carboxy*-^{14}C]-2-[(*N,N*-Diethyl)carboxamido]-5-methoxy-3-(1-methylethyl)benzenesulphonamide, **17** (27.8 mCi, 1.85 mmole) was dissolved in a mixture of toluene : glacial acetic acid (20 ml at 9:1) and heated under reflux for 54 hours. The solvents were then removed under reduced pressure and the residue chromatographed on silica gel eluting with chloroform : methanol : glacial acetic acid (97:2:1) to afford material was further chromatographed on

silica gel eluting with ethyl acetate : methanol (9:1) to afford [3-^{14}C]-6-methoxy-4-(1-methyl-ethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **18** (24.3 mCi, 90% pure, 79% radiochemical yield) which was considered sufficiently pure for being taken forward to the next step.

[3-^{14}C]-2-(Chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, 8: Paraformaldehyde (240 mg, 8 mmole) was suspended in 1,2-dichloroethane (20 ml) under an atmosphere of nitrogen and chloromethylsilane (2 ml, 15.9 mmole) was added followed by tin(IV) chloride (150 µl, 0.82 mmole) in 1,2-dichloroethane (10 ml). The mixture was warmed to 50-60^{o}C until the suspended solid dissolved. The solution was then cooled to room temperature and a portion (11 ml) was removed and added to [3-^{14}C]-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **18** (15 mCi, 1 mmole). The reaction mixture was stirred overnight at room temperature. Chromatography on silica gel eluting with hexane : ethyl acetate (9:1) afforded the desired [3-^{14}C]-2-(chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **8** (13.4 mCi, 89% radiochemical yield).

[*carboxy*-^{14}C]-2,6-Dichlorobenzoic acid, 9: The following sequence was carried out on a vacuum manifold. To a mixture of 1,3-dichlorobenzene (60µl, 0.52 mmole) and tetrahydrofuran (8 ml) at -78^{o}C was added butyllithium (192 µl, 0.48 mmole of 2.5 M solution in hexane) and the reaction mixture was stirred for one hour before being frozen in a liquid nitrogen trap. Conc. sulphuric acid (2 ml) was frozen to -80^{o}C in a separate vessel and the manifold evacuated. The sulphuric acid was then warmed to room temperature while [^{14}C]-barium carbonate (21.3 mCi, 0.35 mmole)[9] was added. The [^{14}C]-carbon dioxide thus liberated was trapped in the vessel containing the other reactants. The reaction vessel containing the lithiated dichlorobenzene and the [^{14}C]-carbon dioxide was then removed from the liquid nitrogen trap, warmed to -78^{o}C and stirred for one hour. The reaction mixture was then quenched with saturated aqueous ammonium chloride (1ml) and warmed to room temperature before being poured into distilled water and extracted with ethyl acetate. The aqueous phase was then acidified with hydrochloric acid and reextracted with ethyl acetate. This organic phase was washed with water and then dried over anhydrous magnesium sulphate to afford the desired [*carboxy*-^{14}C]-2,6-dichlorobenzoic acid, **9** (21.3 mCi, 100% radiochemical yield).

[3-^{14}C]-[6-Methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl)methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [^{14}C$_2$]-WIN 63394, 2: To [*carboxy*-^{14}C]-2,6-dichlorobenzoic acid, **9** (5.47 mCi, 0.36 mmole) and potassium carbonate (56 mg, 0.41 mmole) in 1-methyl-2-pyrrolidinone (2 ml) at room temperature under an atmosphere of nitrogen was added [3-^{14}C]-2-(chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide, **8** (4.22 mCi, 0.28 mmole) in 1-methyl-2-pyrrolidinone (5 ml) and the mixture stirred for 20 hours. The reaction was then quenched by the addition of water (20 ml) and extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulphate and the solvent then removed under reduced pressure. The residue was chromatographed on silica gel eluting with hexane : ethyl acetate (8:2) to afford the desired

[3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl)methyl [*carboxy*-^{14}C]-2,6-dichlorobenzoate, [$^{14}C_2$]-WIN 63394, **2** (5.3 mCi, 82 mg, Specific Activity μCi/mg, 55% radiochemical yield), with a radiochemical purity by radioTLC of >97% on silica gel eluting with either chloroform : acetic acid (98:2) or chloroform : isopropylamine (96:4).

[*carboxy*-^{14}C]-2,6-Dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoic acid, 10: The following sequence was carried out on a vacuum manifold. To a solution of 1-[2-(2,4-dichloro-phenoxy)pyrrolidine, **20** (315 mg, 1.2 mmole), in tetrahydrofuran (2 ml) at -78°Cwas added *N,N,N',N'*-tetramethylethylenediamine (182 μl, 1.2 mmole) and butyllithium (0.68 ml, 1.1 mmole of a 1.6 M solution in hexane) and the reaction mixture stirred for one hour before being frozen in a liquid nitrogen trap. Conc. sulphuric acid (1.5 ml) was frozen to -80°C in a separate vessel and the manifold evacuated. The sulphuric acid was then warmed to room temperature while [^{14}C]-barium carbonate (22.5 mCi, 0.38 mmole)[9] was added. The [^{14}C]-carbon dioxide thus liberated was trapped in the vessel containing the other reactants. The reaction vessel containing the lithiated substrate and the [^{14}C]-carbon dioxide was then removed from the liquid nitrogen trap, warmed to -78°C and stirred for one hour. The reaction mixture was then quenched with saturated aqueous ammonium chloride (0.5 ml) and warmed to room temperature before being poured into distilled water and extracted with ethyl acetate. The aqueous phase was then evaporated under reduced pressure to afford a white solid which on washing with water gave the desired [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoic acid,**10** (16.4 mCi, radiochemical yield 72%).

[3-^{14}C]-[6-Methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-di-chloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733, 3: To [3-^{14}C]-2-(chloromethyl)-6-methoxy-4-(1-methylethyl)-1,2-benziso-thiazol-3(2H)-one 1,1-dioxide, **8** (13.4 mCi, 0.89 mmole) was added [*carboxy*-^{14}C]-2,6-di-chloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoic acid, **10** (13.4 mCi, 0.53 mmole), 2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoic acid, (124 mg, 0.37 mmole), 1-methyl-2-pyrrolidinone (20 ml) and potassium carbonate (210 mg, 1.52 mmole). The reaction mixture was stirred at room temperature for 36 hours. After this time the solvents were removed under reduced pressure and the residue redissolved in ethyl acetate. The solution was washed sequentially with water, dilute aqueous sodium hydrogen carbonate and finally with saturated aqueous sodium chloride before being dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to give a residue which was chromatographed on reverse phase (C_8) silica gel eluting with methanol : water : c. hydrochloric acid (80:20:0.1) to afford the desired [3-^{14}C]-[6-methoxy-4-(1-methylethyl)-1,1,3-trioxo-1,2-benzisothiazol-2(2H)-yl]methyl [*carboxy*-^{14}C]-2,6-dichloro-3-[(2-pyrrolidin-1-yl)ethoxy]benzoate hydrochloride, [$^{14}C_2$]-WIN 64733, **3** (10.3 mCi, 214 mg, Specific Activity 48.1 μCi/mg, 38% radiochemical yield) with a radiochemical purity by radioTLC of 98% on reverse phase (C_{18}) silica gel eluting with methanol : water : conc. hydrochloric acid (80:20:0.1).

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