THE PREPARATION OF ^{14}C, ^{35}S AND ^{13}C LABELLED FORMS OF OMEPRAZOLE

A M Crowe*, R J Ife+, M B Mitchell* and D Saunders*

Smith Kline and French Research Limited

The Frythe, Welwyn, Hertfordshire, England, AL6 9AR

* Synthetic and Isotope Chemistry Department

\+ Medicinal Chemistry Department

SUMMARY

The syntheses of [benzimidazole-2-^{14}C]omeprazole, [benzimidazole-2-^{13}C]omeprazole, [^{35}S]omeprazole and [2-pyridyl-^{14}C-methyl]-omeprazole are described. The first three compounds were prepared from the appropriate isotopically labelled carbon disulphide. The key step in the synthesis of [2-pyridyl-^{14}C-methyl]omeprazole is nucleophilic alkylation of a 1-ethoxypyridinium salt using [^{14}C]methyl magnesium idodide. This is the first time that a reaction of this type has been used to prepare a 2-[methyl-^{14}C]methylpyridine.

INTRODUCTION

Omeprazole (6)[1] is one of a class of compounds which are H^+/K^+ ATPase inhibitors[2]. Inhibition of this enzyme leads to reduced levels of gastric secretion and compounds of this type are under investigation for the treatment of gastric ulcers[3]. In

0362-4803/86/010021–13$01.30

Received August 1, 1985

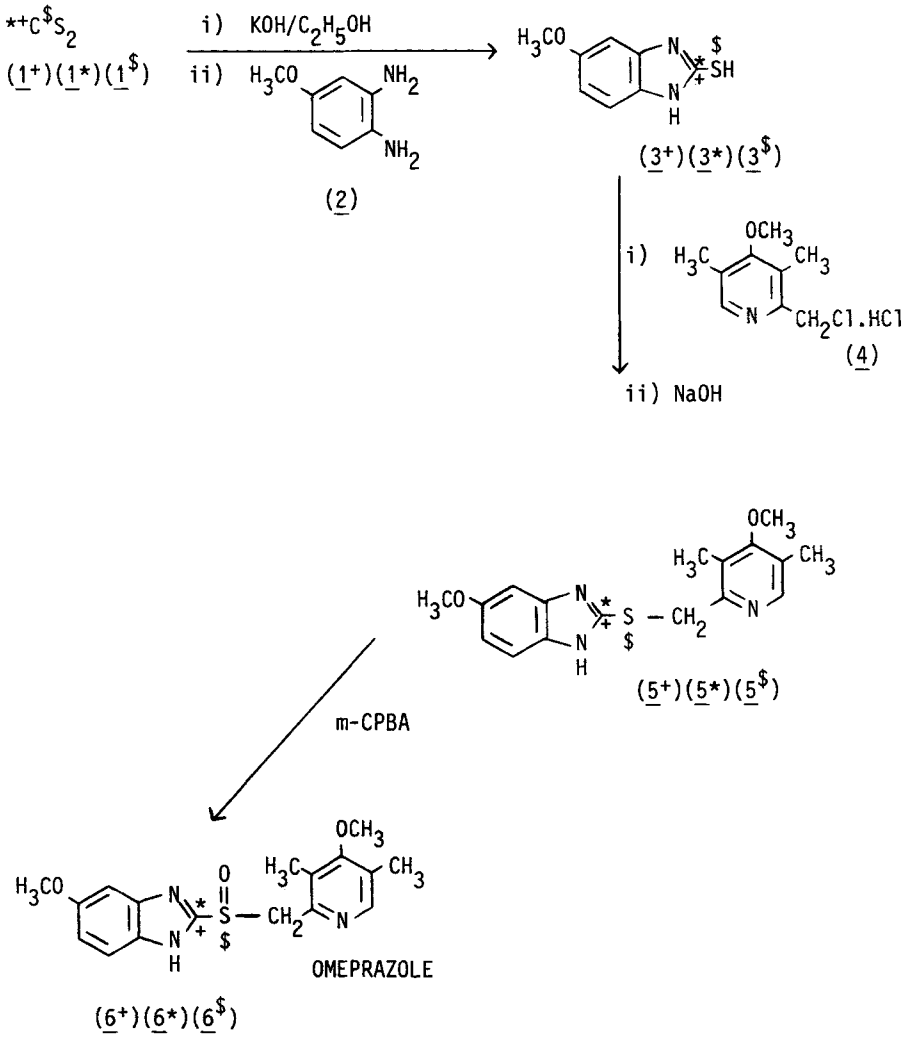
order to study the mode of action of this type of inhibitor[4] various isotopically labelled forms of omeprazole were required. Although there is one report of the use of radiolabelled omeprazole in the literature[5], no synthesis of isotopically labelled omeprazole has been published. The syntheses of these labelled compounds are described below.

Key words: H^+/K^+ ATPase inhibitors, isotopically labelled carbon disulphide, omeprazole, high performance liquid chromatography, 2-[methyl-^{14}C]methylpyridine.

DISCUSSION

The strategy employed for preparation of [benzimidazole-2-^{14}C]omeprazole, [benzimidazole-2-^{13}C]omeprazole and [^{35}S]omeprazole is outlined in Scheme 1. In each case the starting material used was the appropriate isotopically labelled carbon disulphide. Preparation of the 2-mercaptobenzimidazole (3) was carried out by a modification of the published procedure[6] which avoided the use of excess carbon disulphide. Subsequent reaction with the 2-chloropyridine (4) followed by oxidation gave the crude product (6). Purification of the final compounds by semi-preparative normal phase hplc gave [benzimidazole-2-^{14}C]omeprazole (2.95mCi, 144μCi/mg, overall radiochemical yield 25%), [benzimidazole-2-^{13}C]omeprazole (overall 37% yield, 90% enriched in ^{13}C at C2), and [^{35}S]omeprazole (2.66mCi, 160μCi/mg, overall radiochemical yield 24%)[7]. The preparation of [2-pyridyl-^{14}C-methyl]omeprazole required introduction of a labelled methyl group in the 2-position of the pyridine ring. In an earlier preparation of 2,3-dimethyl-[2-methyl-^{14}C]pyridine[8], a 2-bromo-3-methylpyridine was converted to a 2-lithio derivative and alkylated with methyl iodide. This approach was inappropriate for

Scheme 1



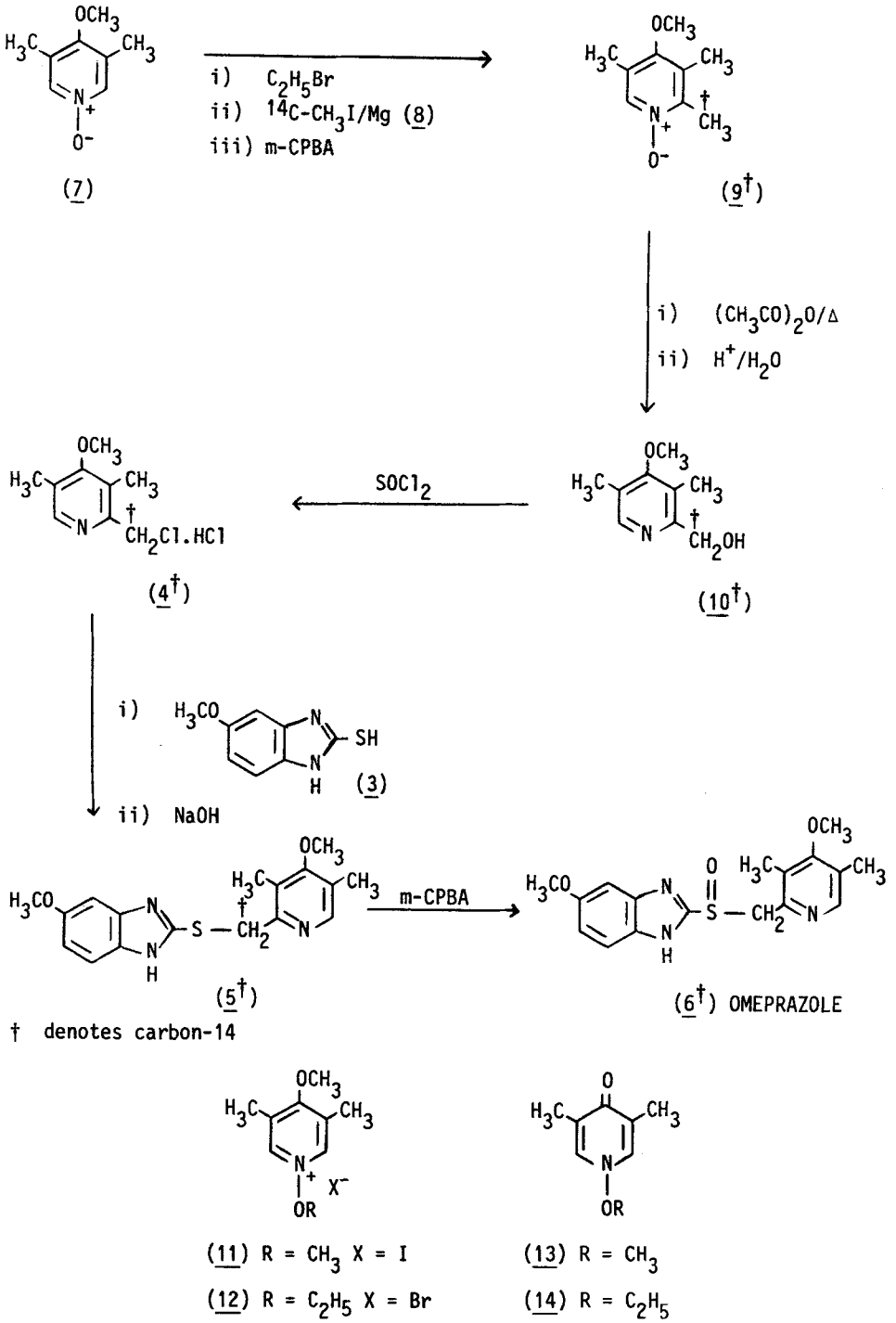
\+ Denotes carbon-14

* Denotes carbon-13

$ Denotes sulphur-35

this particular case, and the possibility of direct alkylation of a pyridine derivative with methyl magnesium iodide was investigated. Some activation of the pyridine ring towards nucleophilic attack is required for direct alkylation with a Grignard reagent. It is reported that pyridine-N-oxides[9] and quinoline-N-oxides[10] can be alkylated with Grignard reagents, although later reports[11, 12] indicated that the major pathway is via a ring opening reaction. In our hands attempted reaction of the N-oxide (7) with methyl magnesium iodide gave very low yields of the alkylated product, obtained either as the N-oxide (9) or the corresponding pyridine. It was considered that preparation of the 1-alkoxy derivatives by alkylation of the N-oxide (7) should further activate the pyridine ring to nucleophilic attack, and by subsequent elimination of the alcohol produce the mono-alkylated pyridine. In fact the reaction of 1-alkoxypyridine with Grignard reagents has been reported[13, 14], but the method has been little used[15]. Our initial investigations showed the reaction to proceed in moderate yield with either the 1-methoxy (11) or 1-ethoxypyridine (12). The major by-product in either case was the 1-alkoxy-4-pyridone ((13) (14), R = methyl, ethyl respectively). This ether cleavage is a well known reaction of Grignard reagents[16]. Thus the route shown in Scheme 2 was used for the synthesis of [2-pyridyl-^{14}C-methyl]omeprazole (6). Preparation of the Grignard reagent from [^{14}C]methyl iodide followed by reaction with the 1-ethoxypyridinium bromide (12) gave the labelled pyridine (9). Oxidation, Katada rearrangement[17] and hydrolysis gave the alcohol (10), which was converted to the chloromethyl hydrochloride (4). This was reacted as described above with the 2-mercaptobenzimidazole (3) and the resulting sulphide (5) was oxidised to give the crude product (6). Purification by normal phase semi-preparative hplc to gave [2-pyridyl-^{14}C-methyl]omeprazole (6) (2.94mCi, 55.3mCi/mmol)

Scheme 2


OCH3
H3C
CH3
N+
O-
(7)
i) C2H5Br
ii) 14C-CH3I/Mg (8)
iii) m-CPBA
(9†)
i) (CH3CO)2O/Δ
ii) H+/H2O
(10†)
SOCl2
CH2Cl.HCl
(4†)
i)
H3CO
SH
N
H
(3)
ii) NaOH
S
CH2
(5†)
m-CPBA
O
(6†) OMEPRAZOLE
† denotes carbon-14
X-
OR
(11) R = CH3 X = I
(12) R = C2H5 X = Br
(13) R = CH3
(14) R = C2H5

in an overall radiochemical yield of 3% from [^{14}C]methyl iodide. The key step in the above synthesis, namely introduction of a labelled methyl group at the 2-position of the pyridine ring *via* nucleophilic attack on an activated pyridine, complements a previously reported method[8] for the preparation of a 2-methyl labelled pyridine *via* a nucleophilic 2-lithiopyridine and labelled methyl iodide.

EXPERIMENTAL

[^{14}C]Carbon disulphide and [^{14}C]methyl iodide were obtained from ICI, Physics and Radioisotope Services, [^{35}S]carbon disulphide was obtained from Amersham International plc, and [^{13}C]carbon disulphide was obtained from Stohler Isotope Chemicals, Switzerland. HPLC purifications were performed on Arcksil 10μm silica columns of length 250mm and i.ds of 22mm or 8mm using a Perkin Elmer Series 2 pump with a Perkin Elmer LC55 UV/VIS Spectrophotometer. The solvent systems used were i) chloroform: (methanol/conc. ammonium hydroxide 95/5), (98.5:1.5, v/v); ii) ethyl acetate: methanol: conc. ammonium hydroxide, (100:4:2, by volume). Radiochemical purities were determined by tlc on Analtec 02511 silica gel plates followed by radiochromatogram scanning using a Berthold Linear Analyser. The tlc systems used were i) dichloromethane:methanol:conc. ammonium hydroxide, (90:10:1, by volume); ii) ethyl acetate:methanol:conc. ammonium hydroxide, (20:2:1, by volume); iii) ethyl acetate: methanol:conc. ammonium hydroxide, (10:1:1, by volume); iv) chloroform:methanol, (9:1, v/v).

2-Mercapto-5-methoxy[2-^{14}C]benzimidazole (3+)

4-Methoxyphenylenediamine monohydrochloride (174mg, 1.00mmol) was dissolved in sodium hydroxide solution (1M, 5ml) and extracted with dichloromethane (3 x 5ml). The combined organic extracts were dried

(MgSO4), filtered, evaporated to dryness and the residue was dried under high vacuum to give 4-methoxyphenylenediamine (2) (110mg, 0.80mmol). This was dissolved in ethanol (690µl) to give a total volume of ca 800µl. Potassium hydroxide (90%, 1.143g, 18.4mmol) was dissolved in ethanol containing water (250µl) and made up to 5ml with ethanol. An aliquot (62µl, 0.229mmol) of the potassium hydroxide solution was cooled to 0°C and [^{14}C]carbon disulphide (1+) (11.8mCi, 51.5mCi/mmol, 0.229mmol) in ethanol (100µl) was added. The vial containing the [^{14}C]carbon disulphide was washed out with ethanol (2 x 250µl) and these washings were added to the reaction mixture. The reaction mixture was stirred at 0°C for 15min, an aliquot (252µl, 0.252mmol) of 4-methoxyphenylenediamine solution was added, followed by water (50µl) and the mixture was stirred under reflux for 3h. The solvent was removed by rotary evaporation under reduced pressure, water was added to the residue and the mixture was acidified with glacial acetic acid (27µl, 0.47mmol) and extracted with ethyl acetate (3 x 5ml). The ethyl acetate extracts were combined, washed with water (1 x 5ml) and dried (MgSO4). The solution was filtered and evaporated to dryness to give the crude 2-mercapto-5-methoxy[2-^{14}C]benzimidazole (3+) (39mg, 0.217mmol, 72% radiochemical purity in tlc solvent system (iv)). The material was used without further purification.

[Benzimidazole-2-^{14}C]omeprazole (6+)

2-Mercapto-5-methoxy[2-^{14}C]benzimidazole (39mg, 0.217mmol) was dissolved in ethanol (1.7ml) and 2-chloromethyl-4-methoxy-3,5-dimethylpyridine hydrochloride (4) (48mg, 0.217mmol) was added, followed by sodium hydroxide solution (1.01M, 430µl, 0.434mmol). The reaction mixture was stirred at room temperature for 8h, and evaporated to dryness. Dichloromethane (4ml) was added and the mixture was filtered. The filtrate was cooled to -30°C, and

m-chloroperbenzoic acid (37.4mg, 0.217mmol) was added. The reaction mixture was stirred at -30°C for 2h, allowed to warm to room temperature and stirred under an atmosphere of ammonia gas for 5min. The precipitate was separated by filtration, washed with dichloromethane and the filtrate evaporated to dryness and dried under high vacuum to give the crude [benzimidazole-2-^{14}C]omeprazole (6+) (67mg, 54% RCP, tlc system (ii)). Purification of this crude product by normal phase semi-preparative hplc in hplc solvent systems (i) and (ii) gave [benzimidazole-2-^{14}C]omeprazole (6+) (20.5mg, 0.059mmol, 2.95mCi, 144μCi/mg, 49.7mCi/mmol, RCP 98.8% in tlc solvent system (i), 97.7% in tlc solvent system (ii), overall radiochemical yield 25% from [^{14}C]carbon disulphide).

2-[^{35}S]Mercapto-5-methoxybenzimidazole (6$)

This was prepared in an analogous way to the ^{14}C-labelled intermediate described above, starting with [^{35}S]carbon disulphide (1$) (12.1mg, 0.168mmol, 22mCi, 1.8mCi/mg, 131mCi/mmol). This provided the crude 2-[^{35}S]mercapto-5-methoxybenzimidazole (3$) (27mg, 0.150mmol, 73% RCP in tlc solvent system (i)). This was used in the next step without further purification.

[^{35}S]Omeprazole (6$)

This preparation from 2-[^{35}S]mercapto-5-methoxybenzimidazole (27mg, 0.15mmol) prepared above, was carried out analogously to that described for [benzimidazole-2-^{14}C]omeprazole. The crude product (46mg) was purified by semi-preparative normal phase hplc in two systems (see above) to give [^{35}S]omeprazole (6$) (16.6mg, 0.045mmol, 2.66mCi, 160μCi/mg, 55.4mCi/mmol, RCP 99.5% in tlc solvent systems (i) and (ii), overall radiochemical yield 24% from [^{35}S]carbon disulphide).

[Benzimidazole-2-^{13}C]omeprazole (6*)

This compound was prepared in an analogous way to the [benzimidazole-2-^{14}C]omeprazole, starting from 90% enriched [^{13}C]carbon disulphide (1*) (1.00g 13.0mmol), except that the intermediate 2-mercapto[2-^{13}C]benzimidazole was isolated from the crude reaction mixture to give the purified 2-mercapto-5-methoxy [2-^{13}C]benzimidazole (3*) (1.71g, 9.45mmol, 73% yield). The final crude product (6*) was purified by gravity column chromatography on silica (ethyl acetate:methanol, 10:1, v/v) and normal phase semi-preparative hplc, (hplc solvent system (i)) to give [benzimidazole-2-^{13}C]omeprazole (6*) (1.68g, 4.87mmol, 37%, degree of enrichment 90% by ^{13}C-nmr spectroscopy, found; C, 58.91; H, 5.56; N, 12.14%; theory: C, 58.96; H, 5.53; N, 12.13%).

4-Methoxy-2,3,5-trimethyl-[2-methyl-^{14}C]pyridine-N-oxide (9†)

4-Methoxy-3,5-dimethylpyridine-N-oxide (7) (256mg, 1.67mmol) was stirred in ethyl bromide (2ml) at room temperature for 24h, evaporated and dried under high vacuum. Magnesium turnings (50mg, 2.08mmol) were added to a solution of [^{14}C]methyl iodide (100mCi, 60mCi/mmol, 1.67mmol) in diethyl ether (1.7ml). After the initial reaction had subsided, the mixture was refluxed for 1h and cooled. 1-Ethoxy-4-methoxy-3,5-dimethylpyridinium bromide (12), from above reaction, (1.67mmol) was suspended in diethyl ether (1.7ml) and the solution of the Grignard reagent prepared above was added dropwise to the vigorously stirred suspension. The mixture was refluxed for 2h, cooled and quenched with ammonium chloride (424mg, 8.0mmol) in water (10ml). The resulting aqueous solution was extracted with diethyl ether (3 x 10ml), the combined extracts were dried (MgSO4), filtered and the solvent was removed by rotary evaporation *in vacuo* to give 4-methoxy-2,3,5-trimethyl-[2-methyl-^{14}C]pyridine (96mg,

0.63mmol, 27mCi). This was dissolved in dichloromethane (2ml), cooled to 0°C and m-chloroperbenzoic acid (130mg, 0.76mmol) was added. After 1.25h at room temperature, ammonia gas was passed over the stirred solution for 5min and the precipitated solid removed by filtration. The filtrate was evaporated to give the crude N-oxide ($\underline{9}^{\dagger}$) (RCP 75% in tlc solvent system (iii)).

2-[^{14}C]Hydroxymethyl-4-methoxy-3,5-dimethylpyridine ($\underline{10}^{\dagger}$)

The crude N-oxide ($\underline{9}^{\dagger}$) (0.63mmol, assumed quantitative from the pyridine) was heated at 100°C in acetic anhydride (2ml) for 1h, cooled and evaporated to dryness. Water (2ml) and conc. hydrochloric acid (1ml) were added to the residue and the solution was stirred at room temperature for 6h. The solution was added to water (10ml) and conc. ammonium hydroxide (2ml) was added. The mixture was extracted with dichloromethane (3 x 10ml), the extracts were combined, dried (MgSO4), filtered and evaporated to dryness to give the crude product ($\underline{10}^{\dagger}$) (20.2mCi, RCP 65% in tlc solvent system (ii)). This material was purified by preparative layer chromatography (tlc solvent system (ii)). The band corresponding to the required product ($\underline{10}^{\dagger}$) was extracted with dichloromethane (3 x 20ml) followed by ethyl acetate (2 x 20ml). The extracts were combined, evaporated to dryness and the residue was dissolved in dichloromethane (20ml), dried (MgSO4), filtered and evaporated to dryness to give 2-[^{14}C]hydroxymethyl-4-methoxy-3,5-dimethyl pyridine ($10^{\dagger}$) (41mg, 0.25mmol, 12.2mCi, RCP 92% in tlc solvent system (ii)).

2-[^{14}C]Chloromethyl-4-methoxy-3,5-dimethylpyridine hydrochloride ($\underline{4}^{\dagger}$)

The purified 2-[^{14}C]hydroxymethylpyridine ($10^{\dagger}$) (41mg, 0.25mmol, 12.2mCi) was stirred in chloroform (1.5ml) with thionyl chloride

(0.5ml) at room temperature for 2h. The solution was evaporated to dryness, and the residue was dried under high vacuum and used directly without further purification.

[2-Pyridyl-^{14}C-methyl]omeprazole (6†)

The crude chloromethylpyridine hydrochloride (4†) (0.25mmol) prepared above and 2-mercapto-5-methoxybenzimidazole (3) (50mg, 0.275mmol) were suspended in ethanol (2ml) and sodium hydroxide solution (525µl, 1.0N) was added. After 3.5h the solution was evaporated to dryness and the residue triturated with dichloromethane (6ml) and filtered. The filtrate was cooled to -40°C and m-chloroperbenzoic acid (47mg, 0.275mmol) was added. The mixture was stirred for 1h at -40°C prior to the addition of a further portion of m-chloroperbenzoic acid (9mg, 0.05mmol). After a further 1h at -40°C ammonia gas was passed over the surface of the solution for 5min. The mixture was allowed to warm to room temperature, filtered, dichloromethane (15ml) was added and the total solution washed with potassium bicarbonate solution (0.5M, 10ml), followed by water (10ml). The organic layer was dried (MgSO4) filtered and evaporated to give the crude product (6†) (40mg) as an oil. This material was purified by normal phase semi-preparative hplc as described above for [benzimidazole-2-^{14}C] omeprazole to give [2-pyridyl-^{14}C-methyl]omeprazole (6†) (18.3mg, 0.053mmol, 2.94mCi, 160µCi/mg, 55.3mCi/mmol overall radiochemical yield 3%, RCP 99.5% in both tlc systems (i) and (ii)).

REFERENCES

1. Junggren U.K. and Sjostrand S.E. - European Patent Application No 79850022.9, 3 April, (1979).
2. Im W.B., Blakeman D.P. and Davis J.P. - Biochem. Biophys. Res. Comm. 126:78, (1985); Wallmark B., Brandstrom A. and Larsson H.

- Biochem. Biophys. Acta. 778:549, (1984); Beil W., Hanneman H., Sewing K.F., Klemm K. and Senn-Bilfinger J. - Gastroenterology. 86:1023, (1984).

3. Wallmark B., Larrson H., Carlsson F. and Helander H. - Hepatogastroenterol. 31:98, (1984); Pritchard P.J., Yeomans N.D., Mihaly G.W., Jones D.B., Smallwood R.A. and Louis W.J. - Aust. NZ. J. Med. 13, 432, (1983); Konturek S.J., Cieszkowski M. and Kwiecien N. - Hepatogastroenterol. 30, 74, (1983).
4. Keeling D.J., Fallowfield C., Milliner K.J., Tingley S.K., Ife R.J. and Underwood A.H. - Biochem. Pharmacol. (Accepted for publication). In press.
5. Helander H.F. and Ramsay C.H. - Gastroenterology. 86:1109, (1984).
6. Van Allan J.A. and Deacon B.D. - Organic Syntheses, Coll. Vol 4,569, (1963).
7. This assumes that a maximum of 50% of the ^{35}S-label contained in the [^{35}S]carbon disulphide can be incorporated into the molecule.
8. Cashyap M.M., Mitchell M.B., Osborne D.C. and Saunders D. - J. Labelled Compounds and Radiopharms. Accepted for publication.
9. Otroshchenko O.S., Sadykov A.S., Utebaev M.V. and Isametova A.I. - ZI. Obschch. Khim. 33:(3) 1038, (1963); Chem. Abstracts, 59:10142d, (1963).
10. Kato T., Yamanaka H. and Hikichi M. - Yakugaku Zasshi. 85:(4) 331, (1965); Chem. Abstracts. 63:4250a, (1965).

11. Schiess P., Monnier C., Ringele P. and Sendi E. - Helv. Chim. Acta. 57:1676, (1974).

12. Van Bergen T.J. and Kellogg R.M. - J. Org. Chem. 36:1705, (1962).

13. Cervinka O. - Chem. Ind. 1482, (1960).

14. Cervinka O. - Coll. Czech. Chem. Commun. 27:567, (1962).

15. Bosch J., Canals J. and Granados R. - Annales de Química. 71:835, (1975).

16. Hurd C.D. and Winberg H.E. - J. Amer. Chem. Soc. 64:2085, (1942); Schonberg A. and Moubasher R. - J. Chem. Soc. 462, (1944).

17. For a general discussion, see Katritzky A.R. and Lagowski J.M. - "Chemistry of the Heterocyclic N-oxides", Academic Press, New York, pp352-365, (1971).