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SYNTHESES OF ^{14}C AND ^{3}H LABELLED FORMS OF DONETIDINE - A HISTAMINE H_2-ANTAGONIST

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SUMMARY

Three syntheses of radiolabelled 2-[2-(2-N,N-Dimethylaminomethyl-5-furanyl-methylthio)ethylamino]-5-(6-hydroxy-4-picolyl)-4-pyrimidone trihydrochloride (donetidine trihydrochloride) are described. One describes the preparation of the free base, and two of its trihydrochloride.

1. A five stage synthesis (Scheme 1) which gave ^{14}C-donetidine (8) labelled in the C_2 position of the pyrimidone ring starting from barium [^{14}C]cyanamide. The overall radiochemical yield for the synthesis was 9% to give (8) at a specific activity of 57.8mCi/mmol.
2. A three stage synthesis (Scheme 2) which gave $^{14}C_2$-donetidine trihydrochloride (14) labelled in both methylenes of the aminoethylthio moiety starting from [1,2-$^{14}C_2$]-cysteamine hydrochloride. The overall radiochemical yield for the synthesis was 18% to give (14) at a specific activity of 15.4mCi/mmol.

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3. A five stage synthesis (Scheme 3) which gave ^{3}H-donetidine trihydrochloride (22) labelled in the methylene of the furanylmethylthio moiety starting from sodium boro[^{3}H]-hydride. The overall radiochemical yield for the synthesis was 1% to give (22) at a specific activity of 33.7mCi/mmol.

Keywords: Reductive tritiation, ^{14}C-labelling, histamine H_2 antagonist, sodium boro[^{3}H]hydride, barium [^{14}C]cyanamide, [1,2-$^{14}C_2$]cysteamine hydrochloride

INTRODUCTION

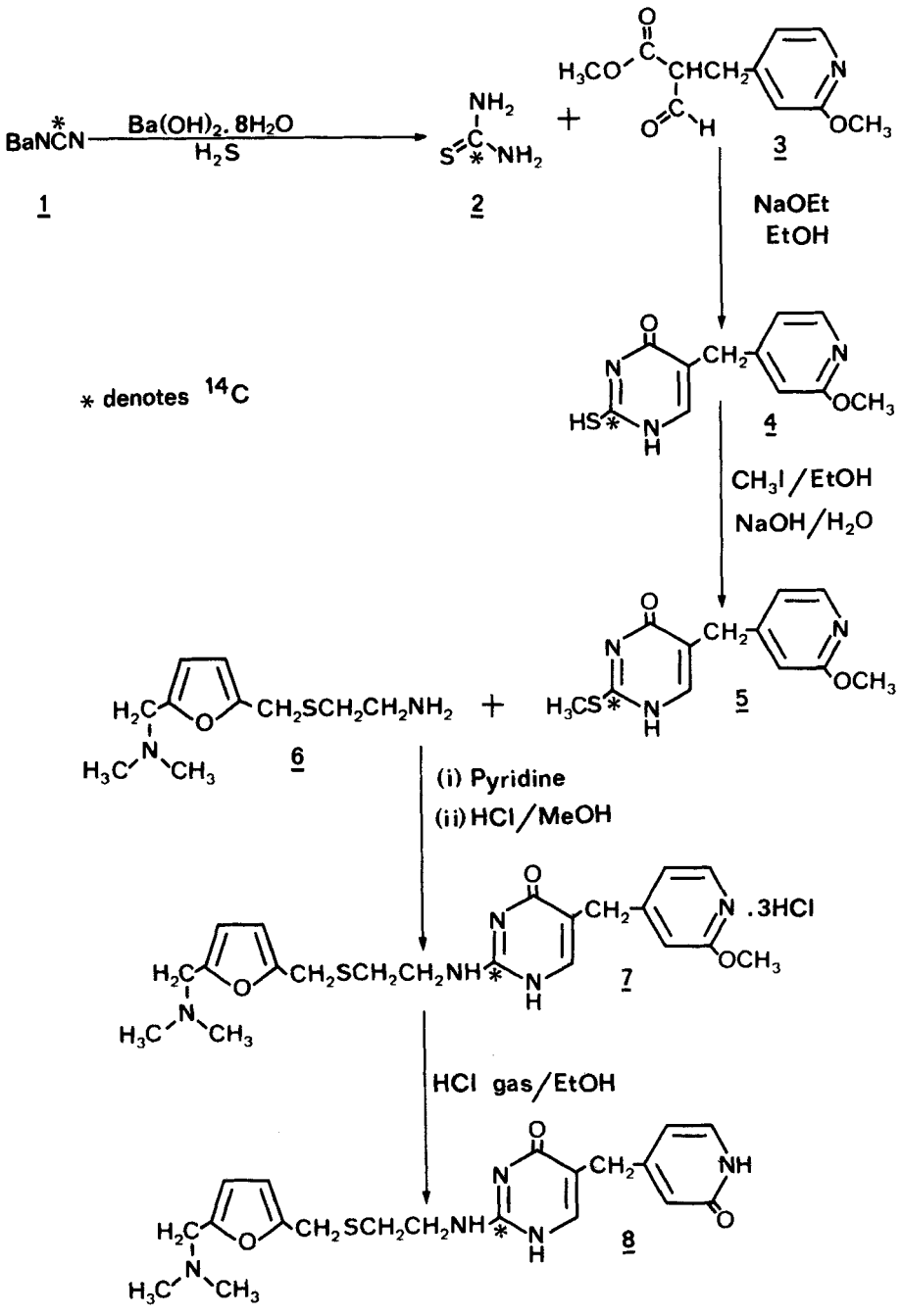
Donetidine trihydrochloride[1] is a highly potent inhibitor of histamine stimulated acid secretion in both animals[2] and man[3]. It exhibits a high degree of selectivity for histamine H_2 receptors having 30 times the potency of cimetidine in vitro and has little or no activity at histamine H_1 receptors or ß-adrenoceptors.

^{14}C-labelled donetidine (8) was synthesised for pharmacokinetic studies in healthy human volunteers, and both $^{14}C_2$-donetidine trihydrochloride (14) and ^{3}H-donetidine trihydrochloride (22) for pharmacokinetic and drug metabolism studies.

DISCUSSION

The strategy employed for preparation of ^{14}C-donetidine (8) labelled in the C_2 position of the pyrimidone ring is shown in Scheme 1. ^{14}C-Thiourea (2) was prepared in quantitative yield from barium[^{14}C]cyanamide (1) by the methods of Murray[4] and Bills[5]. Methoxide catalysed cyclisation of the thiourea with the α-formyl ester[1] (3) yielded the thiouracil (4) in 65% yield. Sulphur methylation with methyl iodide proceeded cleanly

SCHEME ONE


Ba(OH)2. 8H2O
H2S
1
2
3
NaOEt
EtOH
4
* denotes 14C
CH3I / EtOH
NaOH / H2O
5
6
(i) Pyridine
(ii) HCl / MeOH
7
.3HCl
HCl gas / EtOH
8

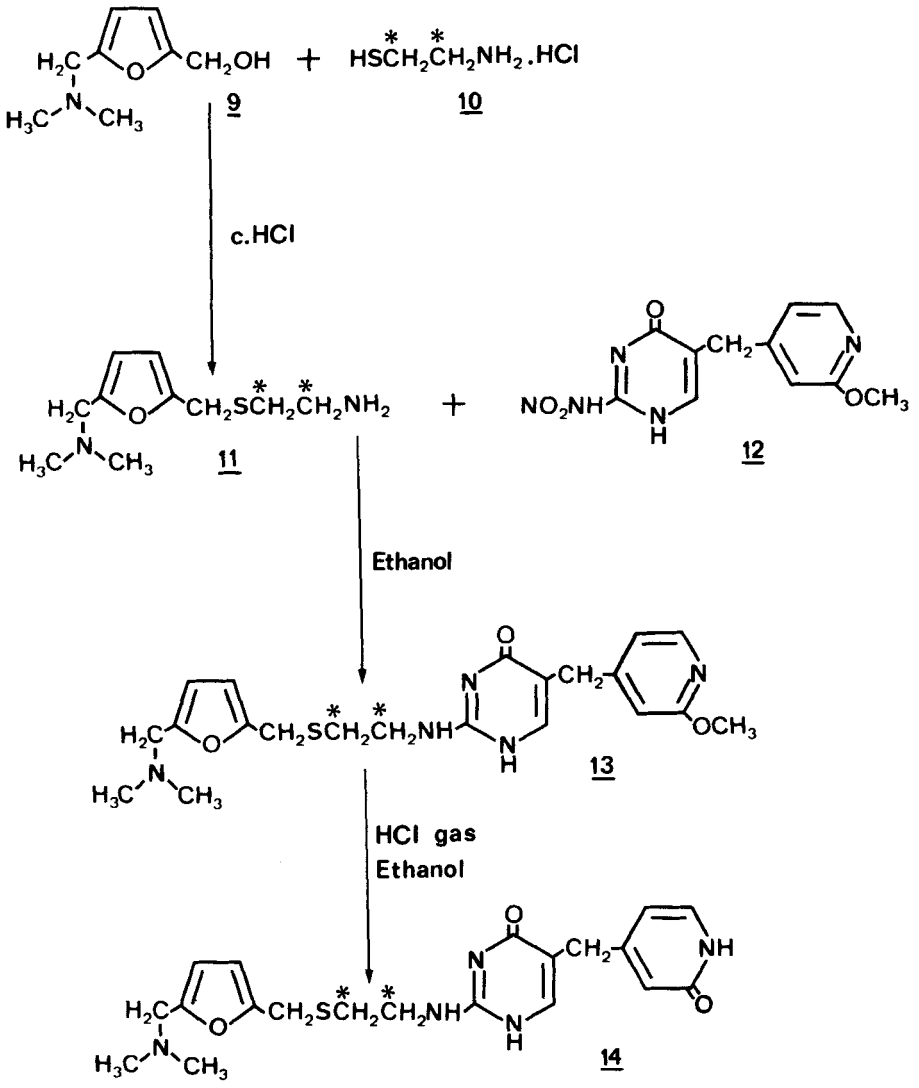
with little N-methylation to give the S-methyl pyrimidone (5) as a crystalline solid in 80% yield. Displacement of the methylthio group by the amine[1] (6) in refluxing pyridine yielded the O-methylated derivative of donetidine, isolated as the trihydrochloride (7) in 85% yield after preparative hplc purification.

The demethylation has been effected in a number of ways and the optimum condition appears to be a reflux in ethanol saturated with HCl for 24 hours. Extraction of the product into dichloromethane is pH dependent as donetidine has one strongly basic centre with a pKa of 8.19 due to the deprotonation of the protonated dimethylamino and two acidic centres with estimated pKa's of 10.2 and 11.6 due to proton loss from the pyrimidone and pyridone groups respectively, hence a pH of 8.5 is maintained for optimum yield.

Thus ^{14}C-donetidine (8) was prepared in 20% yield after preparative hplc purification (324mg, 45.1mCi, 139.2μCi/mg).

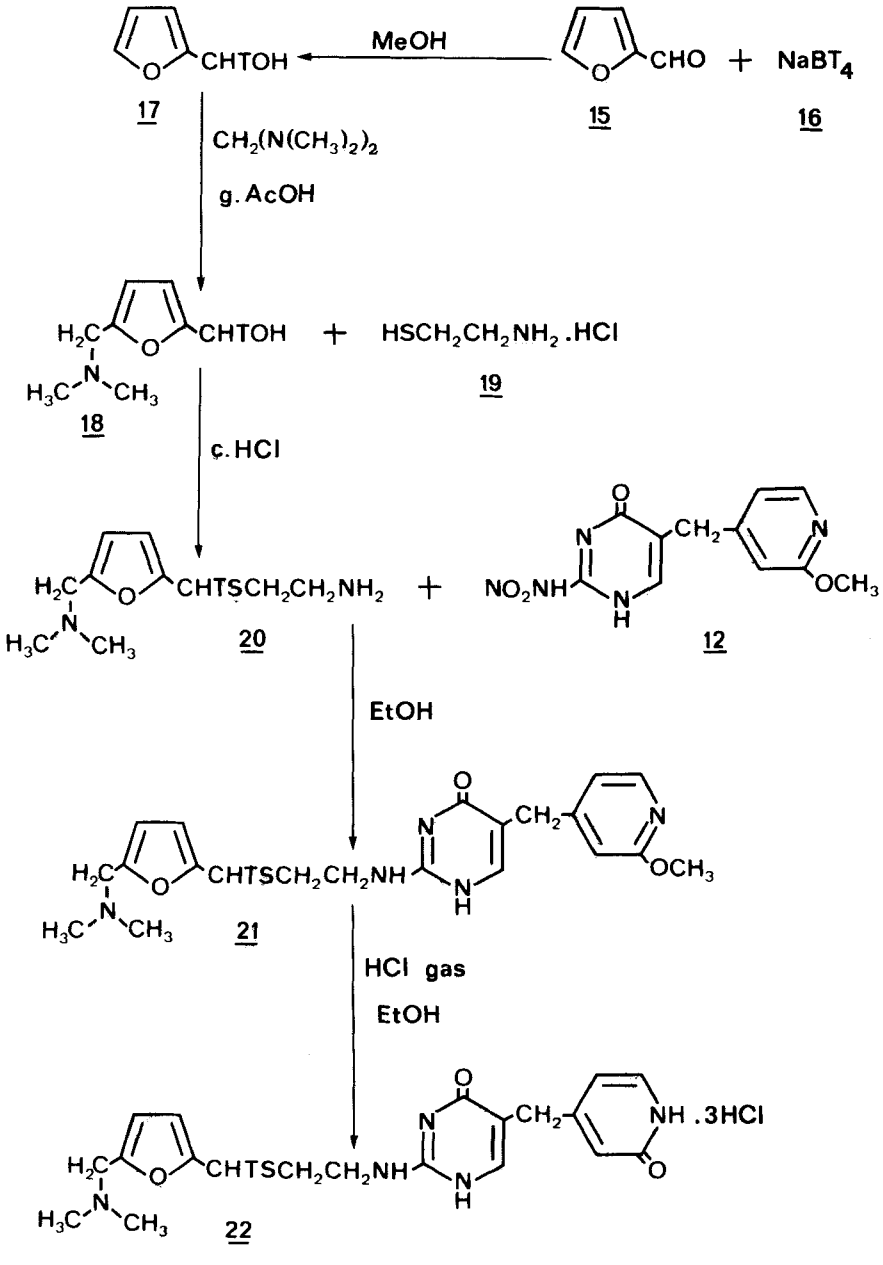
$^{14}C_2$-donetidine trihydrochloride (14) was prepared by the route shown in Scheme 2. [1,2-$^{14}C_2$]cysteamine hydrochloride (10) was condensed with the furan alcohol[1] (9) under strong acid conditions to give the amine (11) in 53% yield after preparative hplc purification. The amine (11) was reacted with the nitroamino pyrimidone[1] (12) in refluxing ethanol to give the O-methylated derivative of $^{14}C_2$-donetidine (13). The nitroamino pyrimidone[1] (12) is the reagent of choice for non labelled syntheses as it involves one less step in the synthesis and is more stable than the S-methyl pyrimidone[1] (5). Couplings with the S-methyl pyrimidone (5) were carried out in refluxing pyridine due to its poor solubility in ethanol and the necessity of a higher temperature to effect reaction. Deprotection was performed as before to yield $^{14}C_2$-donetidine trihydrochloride (14) after preparative hplc in 55% yield (238mg, 6.97mCi, 29.3μCi/mg).

SCHEME TWO



* denotes ^{14}C

SCHEME THREE



T denotes tritium

Scheme 3 illustrates the synthetic route to tritiated donetidine trihydrochloride (22). A key intermediate in this synthesis is the tritiated 2-furanmethanol (17). Non labelled 2-furanmethanol has been prepared previously by reduction of furfuraldehyde (15) with sodium amalgam[6], platinum oxide[7] and copper chromite[8].
In order to avoid the use of tritium gas we adapted the method of Chaikin and Brown[9]. Thus reduction with sodium borotritide (16) in methanol afforded the monotritiated 2-furanmethanol (17) in 96% yield. Reaction of this with the preformed Mannich reagent bis-(N,N-dimethylamino)methane in acetic acid gave (18) in 50% yield. The use of this reagent gave significantly better yields than that using dimethylamine and paraformaldehyde.
The alcohol (18) was coupled with cysteamine hydrochloride (19) as previously described to give (20) in 72% yield. This was reacted with the nitroamino pyrimidone[1] (12) using ethanol as the solvent. The protected ^{3}H-donetidine (21) was demethylated as before to yield, after preparative tlc, tritiated donetidine trihydrochloride (22) in 17% yield (121mg, 7.77mCi, 64.2μCi/mg).

EXPERIMENTAL

[^{14}C]Thiourea (2)

To barium[^{14}C]cyanamide (1) (Amersham, Lot CJL/4/207, 500mCi, 58mCi/mmol, 8.62mmol) was added a solution of barium hydroxide (5.175g, 16.4mmol) in distilled water (200ml). Hydrogen sulphide gas was bubbled through the resulting solution for 5h.
After being refluxed for 16h the mixture was cooled. Carbon dioxide was bubbled through the solution for 5h and the precipitated barium carbonate removed by filtration. The filter cake was washed with distilled water (25ml) followed by ethanol (25ml). The combined filtrates were evaporated to dryness under reduced pressure to give the required product (2) as a white solid (670mg, 100%).

5-(2-Methoxypyrid-4-ylmethyl)-[2-^{14}C]-2-thiouracil (4)

Sodium metal (205mg, 8.92mmol) was dissolved in ethanol (10ml) and to the resulting solution was added methyl 2-formyl-3-(2-methoxypyrid-4-ylmethyl)-propionate (3) (1.97g, 8.85mmol) in ethanol (10ml). The yellow solution was stirred at room temperature for 0.5h prior to the dropwise addition of a solution of (2) (670mg, 8.61mmol) in ethanol (20ml) over a period of 10min. The reaction mixture was refluxed for 20h.
After cooling the ethanol was removed under reduced pressure. The yellow residue was dissolved in distilled water (10ml) and the solution acidified to pH 5.0 with glacial acetic acid. The precipitated solid was filtered, washed with distilled water and dried under high vacuum to yield (4) as a pale yellow solid (1.404g, 65%).

5-(2-Methoxypyrid-4-ylmethyl)-2-methylthio-[2-^{14}C]-4-pyrimidone (5)

To a flask containing (4) (1.404g, 5.63mmol) was added sodium hydroxide (450mg, 11.25mmol), methyl iodide (800mg, 5.64mmol), distilled water (18ml) and ethanol (18ml). The flask was stoppered and heated at 50°C for 3h.
After cooling the solvent was removed under reduced pressure. The solid residue was dissolved in distilled water (15ml) and the solution acidified to pH 5.0 with glacial acetic acid. The precipitated solid was filtered, washed with distilled water and dried under high vacuum to yield (5) as a pale yellow solid (1.194g, 80%).

2-[2-(2-N,N-Dimethylaminomethyl-5-furanylmethylthio)ethylamino]-5-(2-methoxypyrid-4-ylmethyl)-[2-^{14}C]-4-pyrimidone trihydrochloride (7)

2-(2-Aminoethylthiomethyl)-5-(N,N-dimethylaminomethyl)furan (6)

(1.055g, 4.98mmol) and dry pyridine (5ml) were added to a flask containing (5) (1.194g, 4.52mmol) and the resulting mixture refluxed for 24h.

After cooling the pyridine was removed under reduced pressure and the resultant oil purified by preparative hplc on an Arcksil 10 Silica 250mm x 22.5mm i.d column with dichloromethane:methanol: ammonia (SG 0.880) (90:10:1, by vol) as the mobile phase at a flow rate of 15ml/min and u.v detection at 330nm.

The appropriate fractions were combined and concentrated under reduced pressure to give a yellow oil which was dissolved in HCl saturated methanol (10ml). Removal of the solvent under reduced pressure afforded (7) as an off-white solid (2.071g, 85%).

2-[2-(2-N,N-Dimethylaminomethyl-5-furanylmethylthio)ethylamino]-5-(6-hydroxy-4-picolyl)-[2-^{14}C]-4-pyrimidone (8)

HCl saturated ethanol (15ml) was added to (7) (2.071g, 3.84mmol) and the resulting solution refluxed for 24h.

After cooling the solvent was removed under reduced pressure and the oily residue dissolved in distilled water (15ml). The solution was basified to pH 8.50 with solid sodium bicarbonate and the precipitated oil extracted into dichloromethane (10 x 10ml). The extracts were combined and dried ($MgSO_4$) before removal of the solvent under reduced pressure to yield the title compound (8) as a red oil (945mg, 59%). Tlc on silica gel GF developed in ethyl acetate:methanol:ammonia (SG 0.880) (5:1:1 by vol) indicated a radiochemical purity of 55% by radiochromatogram scanning.

The oil was purified by preparative hplc on an Arcksil 10 Silica 250mm x 22.5mm i.d column using acetronitrile:methanol:ammonia (SG 0.880) (70:30:6, by vol) as the mobile phase at a flow rate of 15ml/min and uv detection at 280nm.

The appropriate fractions were combined and concentrated under reduced pressure to yield a yellow oil which was dissolved in

ethanol (2ml). Addition of diethyl ether (20ml) precipitated a buff solid which was filtered and dried under high vacuum to yield the title compound (8) (324mg, 45.1mCi, 20%). The specific activity was determined by liquid scintillation counting and found to be 139.2μCi/mg, 57.8mCi/mmol with a radiochemical purity of 99%.

2-(2-Amino-[ethyl-1,2-$^{14}C_2$]ethylthiomethyl)-5-(N,N-dimethylamino-methyl)furan (11)

[1,2-$^{14}C_2$]Cysteamine hydrochloride (10) (Physics and Radioisotope Services Lot 056435, 40mCi, 17.3 mCi/mmol, 2.31mmol) was dissolved in concentrated hydrochloric acid (0.65ml) and the resulting solution cooled to 0°C.

5-(N,N-Dimethylaminomethyl)-2-hydroxymethyl furan (9) (392mg, 2.53mmol) was added and the mixture stirred at room temperature for 24h. Sodium hydroxide solution (40% w/v) (0.82ml) was added to neutralise the solution before removal of the solvent under reduced pressure.

The residue was purified by preparative hplc on a Partisil 10 Silica 250mm x 10mm i.d column using dichloromethane:methanol: ammonia (SG 0.880) (90:10:1, by vol) as eluant at a flow rate of 3ml/min and uv detection at 254nm.

The appropriate fractions were combined and concentrated to dryness under reduced pressure to give the required product (11) (260mg, 53%).

2-[2-(2-N,N-Dimethylaminomethyl-5-furanylmethylthio)-[ethyl-1,2-$^{14}C_2$]ethylamino]-5-(2-methoxypyrid-4-ylmethyl)-4-pyrimidone (13)

To a flask containing (11) (260mg, 1.22mmol) in dry ethanol (10ml) was added 5-(2-methoxypyrid-4-ylmethyl)-2-nitroamino-4-pyrimidone (12) (325mg, 1.22mmol). The resulting mixture was refluxed for 24h, cooled to room temperature and evaporated to dryness under reduced pressure.

The residue was purified by preparative hplc on a Partisil 10 Silica 250mm x 10mm i.d column using dichloromethane:methanol: ammonia (SG 0.880) (90:10:1, by vol) as the mobile phase at a flow rate of 4ml/min and uv detection at 254nm.
The appropriate fractions were combined and concentrated under reduced pressure to give the required product (13) (355mg, 69%).

2-[2-(2-N,N-Dimethylaminomethyl-5-furanylmethylthio)-[ethyl-1,2-$^{14}C_2$]ethylamino]-5-(6-hydroxy-4-picolyl)-4-pyrimidone trihydrochloride (14)

HCl saturated ethanol (5ml) was added to (13) (355mg, 0.826mmol) and the resulting solution refluxed for 20h.
After cooling the solvent was removed under reduced pressure and the residue purified by preparative hplc on a Partisil 10 Silica 250mm x 10mm i.d column using dichloromethane:methanol:ammonia (SG 0.880) (90:20:1, by vol) as the mobile phase at a flow rate of 4ml/min and u.v detection at 254nm. The appropriate fractions were collected, combined and concentrated under reduced pressure to yield a yellow oil which was dissolved in HCl saturated ethanol (2ml). The solvent was removed and the oily residue triturated with diethyl ether (20ml). The white solid which resulted was collected by filtration and dried under high vacuum to yield the title compound (14) (238mg, 6.97mCi, 55%). The specific activity was determined by liquid scintillation counting and found to be 29.3μCi/mg, 15.4mCi/mmol with a radiochemical purity of 97.3%.

2-([Methyl-^{3}H]hydroxymethyl)furan (17)

Sodium boro[^{3}H]hydride (16) (Amersham Lot C2, 750mCi, 272mCi/mmol, 2.75mmol) was added portionwise to a solution of furfuraldehyde (15) (501.7mg, 5.22mmol) in methanol (3ml) and maintained under an inert atmosphere of nitrogen at 0°C. After effervesence had subsided the reaction was allowed to stand at

room temperature for 2h before removal of the methanol under reduced pressure. The solid residue was suspended in brine (2ml) and the insoluble oil extracted with ethyl acetate (3 x 3ml). The extracts were combined and dried ($MgSO_4$) before removal of the solvent under reduced pressure to yield the required product (17) (490mg, 96%).

5-(N,N-Dimethylaminomethyl)-2-[methyl-^{3}H]hydroxymethyl-furan (18)

To a flask containing (17) (490mg, 5.00mmol) was added bis-(N,N-dimethylamino)methane (720mg, 7.05mmol) and glacial acetic acid (4ml). The resultant mixture was allowed to stand at room temperature for 24h before removal of the glacial acetic acid under pressure. The oily residue was dissolved in ethyl acetate (20ml) and dried ($MgSO_4$) before removal of the solvent under reduced pressure to yield the required product (18) (390mg, 50%).

2-(2-Aminoethylthio-[methyl-^{3}H]methyl)-5-(N,N-dimethylamino methyl)furan (20)

To a solution of (18) (390mg, 2.51mmol) in hydrochloric acid (36% w/w) (1ml) at -20°C was added cysteamine (19) (428mg, 3.77mmol) portion wise over a period of 20min. The mixture was stirred at room temperature for 24hr.

Sodium hydroxide solution (40% w/v) (1.5ml) was added and the resulting mixture extracted with ethyl acetate (3 x 5ml). The extracts were combined and dried ($MgSO_4$) before removal of the solvent under reduced pressure to yield the required product (20) (435mg, 72%).

2-[2-(2-N,N-Dimethylaminomethyl-5-furanyl-[methyl-^{3}H]methylthio) ethylamino]-5-(2-methoxypyrid-4-ylmethyl)-4-pyrimidone (21)

5-(2-Methoxypyrid-4-ylmethyl)-2-nitroamino-4-pyrimidone (12) (688mg, 2.48mmol) and dry ethanol (5ml) were added to a flask

containing (20) (435mg, 1.80mmol). The resultant mixture was refluxed for 17h after which time tlc on silica gel GF developed in ethyl acetate:methanol:ammonia (SG 0.880) (5:1:1, by vol) indicated completion of reaction. The ethanol was removed under reduced pressure and the oily residue purified by preparative tlc on 4 x 1000μm silica gel GF plates developed in chloroform: methanol (9:1, v/v). The required band was removed from the silica plate and eluted with the same solvent mixture to give the required product (21) (580mg, 75%). Tlc on silica gel GF developed in chloroform:methanol (9:1, v/v) indicated a radiochemical purity of 93.5% by radiochromatogram scanning.

2-[2-(2-N,N-Dimethylaminomethyl-5-furanyl-[methyl-^{3}H]methylthio) ethylamino]-5-(6-hydroxy-4-picolyl)-4-pyrimidone trihydrochloride (22)

HCl saturated ethanol (10ml) was added to a flask containing (21) (580mg, 1.35mmol) and the resulting mixture refluxed for 24h. After cooling, the volume of the reaction mixture was reduced to 10ml under reduced pressure. Isopropyl alcohol (10ml) was added and the precipitated solid collected by filtration. Tlc on silica gel GF developed in ethyl acetate:methanol:ammonia (SG 0.880) (5:1:1, by vol) indicated a radiochemical purity of 83% by radiochromatogram scanning.

Purification was effected by preparative tlc on a single 1000μm silica gel GF plate developed in the above solvent system. The required band was removed from the silica and eluted with methanol to give a pale yellow oil which was dissolved in HCl saturated ethanol (2ml). Removal of the solvent under reduced pressure yielded the title compound (22) (121mg, 7.8mCi, 17%). The specific activity was determined by liquid scintillation counting and found to be 64.2μCi/mg, 33.7mCi/mmol with a radiochemical purity of 98.3%.

ANALYSIS

Two tlc systems were employed to determine the purity of the final products using Analtec 250μm silica gel GF plates with subsequent scanning on a Berthold LB2832 tlc linear analyser. The Rf's of the final products in both systems were compared to samples of authentic unlabelled donetidine base and trihydrochloride and found to be identical.

Results of the analyses are tabulated below:

Solvent System	^{14}C-Donetidine Base (8)	$^{14}C_2$-Donetidine Trihydrochloride (14)	^{3}H-Donetidine Trihydrochloride (22)
A	99.0%	97.3%	98.3%
B	99.4%	97.1%	98.0%

A: Ethyl acetate:methanol:ammonia (SG 0.880) (5:1:1, by vol)

B: Propan-1-ol:ammonia (SG 0.880) (7:3, v/v)

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