^{14}C-Labelling of NNC 756, a new dopamine D_1 antagonist

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SUMMARY

A ^{14}C labelled form of (+)-8-chloro-5-(2,3-dihydrobenzofuran-7-yl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC 756) (8) was synthesized in 7 steps, including resolvation of the active (+)-form, starting from [^{14}C]methyl iodide and 7-benzofurancarbaldehyde. The overall radiochemical yield was 11%. The radiochemical purity of [4-^{14}C]NNC 756 was higher than 98% with a specific radioactivity of 24 mCi/mmol.

Key words: ^{14}C, NNC 756, dopamine D_1 receptor antagonist, benzazepine.

INTRODUCTION

(+)-8-Chloro-5-(2,3-dihydrobenzofuran-7-yl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC 756, Fig. 1) is a potent dopamin D_1 receptor antagonist, with high affinity (Ki = 0.17 nM)[1].

The ^{11}C-labelled form has been shown to be a useful PET (Positron Emission Tomografi) ligand for exploring the D_1 dopamine receptors in living primate brain[2,3]. NNC 756 is a potential new neuroleptic drug, which is about to enter clinical phase 2 studies.

To be able to do studies on the distribution and metabolic fate of NNC 756, a radiolabelled form of the compound was needed. The preferred isotope for such experiments is ^{14}C,

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located in a stable place in the molecule; ^{14}C has a long half life, a suitable energy of decay and ^{14}C can not be exchanged in vivo.

Previous ^{14}C-labelled 3-benzazepines have been synthesized in a six step sequence beginning with the appropriately labelled benzenes[4]. Such a synthetic route is unattractive due to the expensive starting material and, in this case, also due to the requirement of several additional steps in order to incorporate the benzofuran-structure.

An efficient incorporation af ^{14}C into the benzo ring via the Fujimoto-Belleau reaction has been reported previously[5], also starting with the inexpensive and readily available [^{14}C]methyl iodide. However, in order to incorporate the benzofuran-structure, this approach would also require several additional synthetic steps.

Other ^{14}C-labelled 3-benzazepines have been synthesized using different approaches, starting with specially designed ^{14}C-labelled starting materials[6]. Also tritium labelled analogs of benzazepines have been synthesized[6,7,8], but because of the labile character of tritium labelled products in vivo, this labelling approach was not considered suitable in our case.

A generally useful synthesis of 1-aryl-2,3,4,5-tetrahydro-1H-3-benzazepines is the reaction of a styrene oxide with a phenylethylamine, followed by an acid-catalyzed cyclization of the beta-aminoalcohol[9]. Using this approach, the synthetic pathway to obtain (8) allows ^{14}C to be incorporated in the C-4 position of the benzazepine ring via [^{14}C]methyl iodide, which is commercially available (Fig. 2).

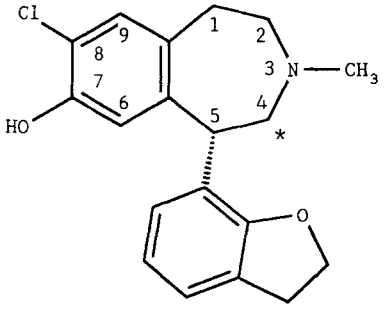

FIGURE 1

Structure of (+)-8-chloro-5-(2,3-dihydrobenzofuran-7-yl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC 756) with numbering of the positions in the benzazepine ring. * Indicates the position of labelling.

The ^{14}C-labelled 7-epoxyethylbenzofuran (2) could be prepared from [^{14}C]trimethyl sulphonium iodide or [^{14}C]trimethyl sulphoxonium iodide. This method has been used to prepare ^{14}C- labelled epoxides[10,11,12]. However, the disadvantage of this process is, that only one third of the ^{14}C can be incorporated into the epoxide. Some work has been done to

investigate the possibility of using other epoxidating agents such as diphenylmethyl sulphonium iodide and diphenylmethyl sulphoxonium iodide[12]. However, attempts to make these reagents using [^{14}C]methyl iodide have been unsuccessful.

Our approach was to use ^{14}C-labelled diphenylmethyl sulfonium tetrafluoroborate as the epoxidating agent. Such diphenyl sulfonium alkylides have been described as stable in their unlabelled form[13]. In the present paper, the preparation of [4-^{14}C]NNC 756 (8) in 7 steps, starting with [^{14}C]methyl iodide, is described.

RESULTS AND DISCUSSION

Figure 2 shows the reaction sequence employed in the synthesis of [4-^{14}C]NNC 756 (8).

^{14}C-Labelled diphenylmethyl sulfonium tetrafluoroborate (1) was prepared from [^{14}C]methyl iodide using silver tetrafluoroborate and diphenyl sulfide as reagents. The raw product was used without purification. Reaction of the labelled epoxidating agent with 7-benzofurancarbaldehyde resulted in a >95% radiochemically pure raw material after removal of all volatiles. The raw material contained considerable amounts of non-radioactive byproducts, mostly diphenyl sulfide. These non-radioactive byproducts could easily be separated from the product by preparative chromatography, resulting in the pure 7-epoxy[2-^{14}C]ethyl-benzofuran (2) as a yellow oil in a radiochemical purity >98%. The radiochemical yield was 53% from [^{14}C]methyl iodide. The specific radioactivity was 55 mCi/mmol.

N-Methyl-N-(2-(benzofuran-7-yl)-2-hydroxy-[2-^{14}C]ethyl)-3-chloro-4-methoxy-phenethylamine (3) could be synthesized from the purified epoxide (2). However, the best overall yield of (3) from [^{14}C]methyl iodide was achieved, when the raw product of the epoxide (2) was used directly and reacted with N-methyl-3-chloro-4-methoxy-phenethylamine. The resulting β-aminoalcohol (3) could be isolated by preparative C-18 HPLC in 34% of the overall radiochemical yield from [^{14}C]methyl iodide as a yellow oil, with the same high specific radioactivity as the starting material (55 mCi/mmol).

Opening of the epoxide (2) by the nucleophile did not exclusively take place at the labelled C-2 ethyl position, resulting in (3). Further, an opening at the C-1 ethyl position occurred, resulting in the byproduct (4) (Fig. 2). The ratio between (3) and (4) was 9:1. The by-product (4) could also be isolated by preparative C-18 HPLC.

Cyclization of (3) with sulfuric acid in trifluoroacetic acid gave [4-^{14}C]-(+/-)-5-(benzofuran-7-yl)-8-chloro-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (5), which was resolved as the dibenzoyl-D-tartrate (DBDT) salt in a radiochemical purity >96% (Fig. 3).

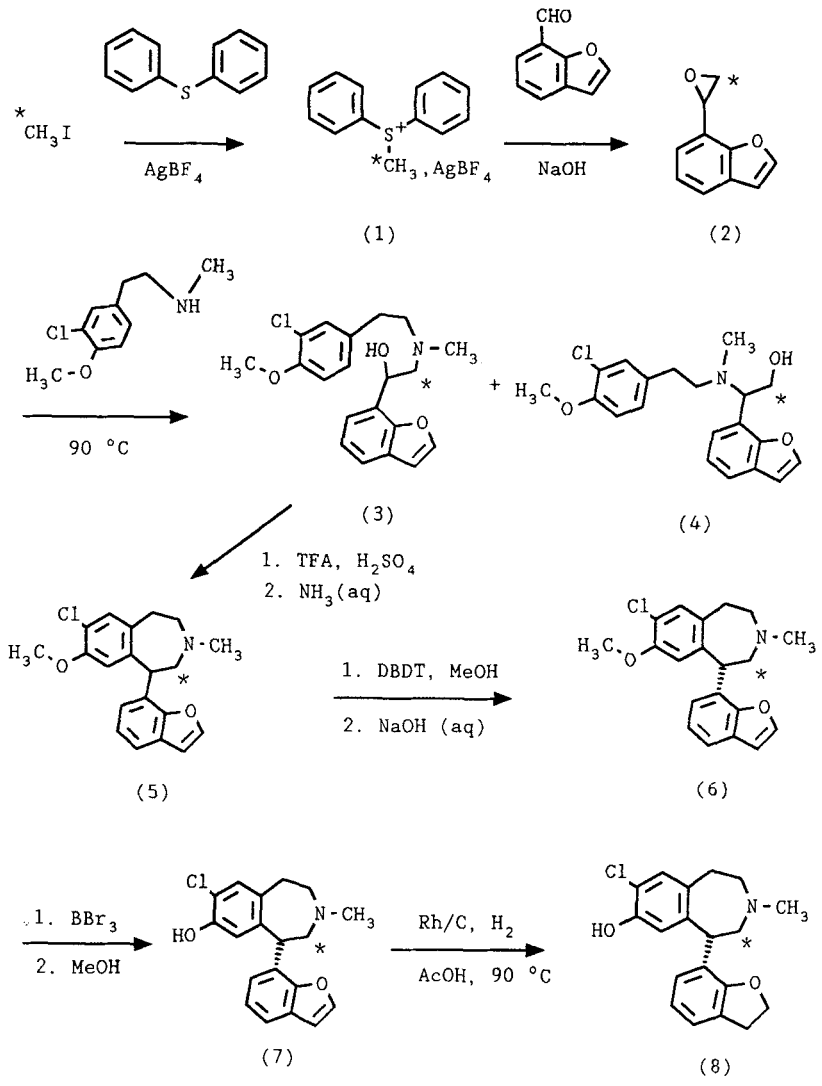

FIGURE 2

Synthetic scheme for the preparation of [4-^{14}C]NNC 756 (8), starting from [^{14}C]methyl iodide.

Addition of "cold" material in the precipitating procedure increased the radiochemical yield by 30%. The addition of "cold" material was limited to such an amount, that the specific radioactivity of the resulting product would be sufficient for the studies planned. The specific radioactivity of the product [4-^{14}C]-(+)-5-(benzofuran-7-yl)-8-chloro-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (6) was found to be 24 mCi/mmol, after recrystallization. The radiochemical yield of the cyclization and resolution step was 59% and the enantiomeric purity >97% (Fig 4).

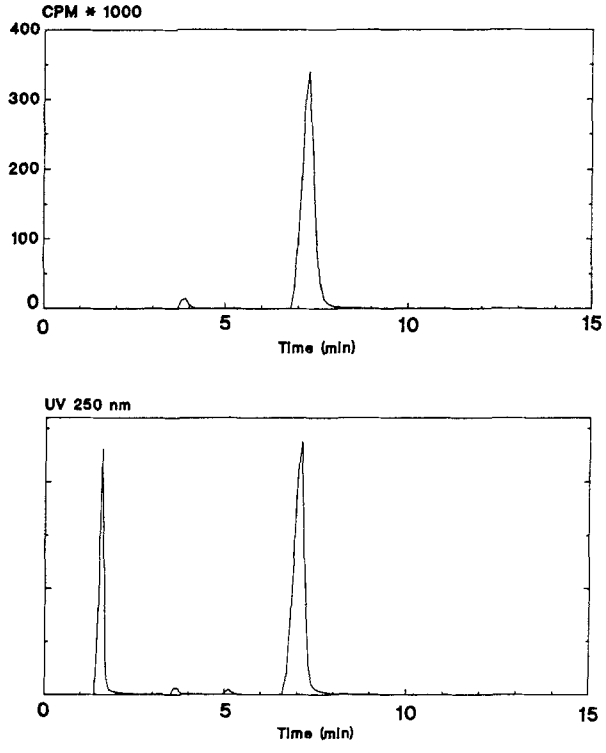

FIGURE 3

HPLC radiochromatogram and UV-chromatogram of the DBDT salt of [4-^{14}C]-(+)-5-(benzofuran-7-yl)-8-chloro-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (6), using a C-18 column.

Synthesis, using non-radioactive material, showed that the cyclization did not proceed well, when the dihydrobenzofuran-analog of (3) was used. Consequently, it was decided that 7-(2,3-dihydrobenzofuran)carbaldehyde was not to be used as starting material, although this would have saved the last hydrogenation step.

Ether cleavage of (6) with boron tribromide gave [4-^{14}C]-(+)-5-(benzofuran-7-yl)-8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (7) in 86% radiochemical yield.

The de-protected ^{14}C-labelled benzazepine (7) was converted to the product (8) by hydrogenation over rhodium on carbon. The radiochemical yield was 63% after HPLC purification. Using rhodium on carbon as catalyst resulted in selective reduction of the benzofuran in (7). Only very small amounts of the dehalogenated benzazepine was observed after the reaction.

The purified product (8) had a specific radioactivity of 24 mCi/mmol. The radiochemical purity was >98% (Fig. 5) and the enantiomeric purity >97%.

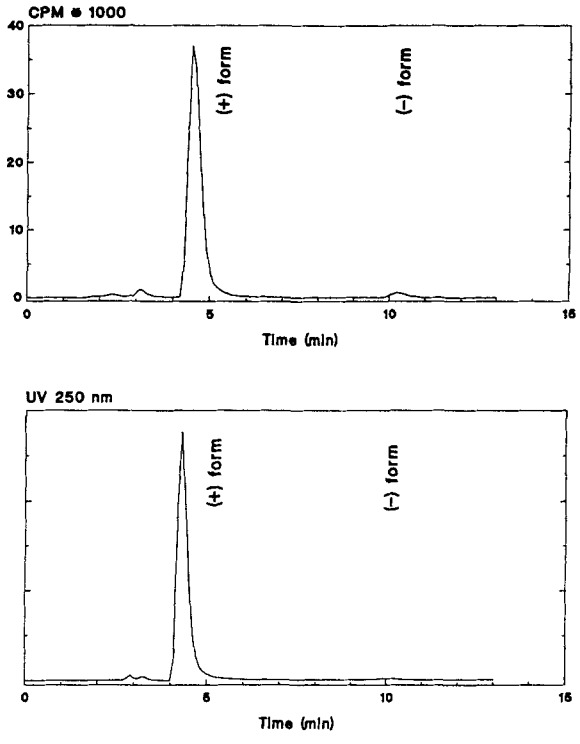

FIGURE 4

Chiral HPLC radiochromatogram and UV-chromatogram of [4-^{14}C]-(+)-5-(Benzofuran-7-yl)-8-chloro-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (6). using a chiral ovomucoid column.

In conclusion, [4-^{14}C]NNC 756 was synthesized in its enantiomeric pure form in 7 steps, starting from [^{14}C]methyl iodide. The overall radiochemical yield was 11%, the radiochemical purity was >98% and the specific radioactivity was 24 mCi/mmol.

EXPERIMENTAL

7-Epoxy[2-^{14}C]ethylbenzofuran (2).

To a suspension of silver tetrafluoroborate (243 mg, 1.25 mmol) in 5 ml of dry dichloromethane, was added diphenyl sulfide (0.200 ml, 224 mg, 1.20 mmol). To this suspension was added [^{14}C]methyl iodide (40 mCi, 53 mCi/mmol, 0.75 mmol) in 1.0 ml of toluene and the mixture was stirred for 4 hours. The yellow/orange suspension became black. The suspension was filtered and the volatiles removed by nitrogen flow. To the

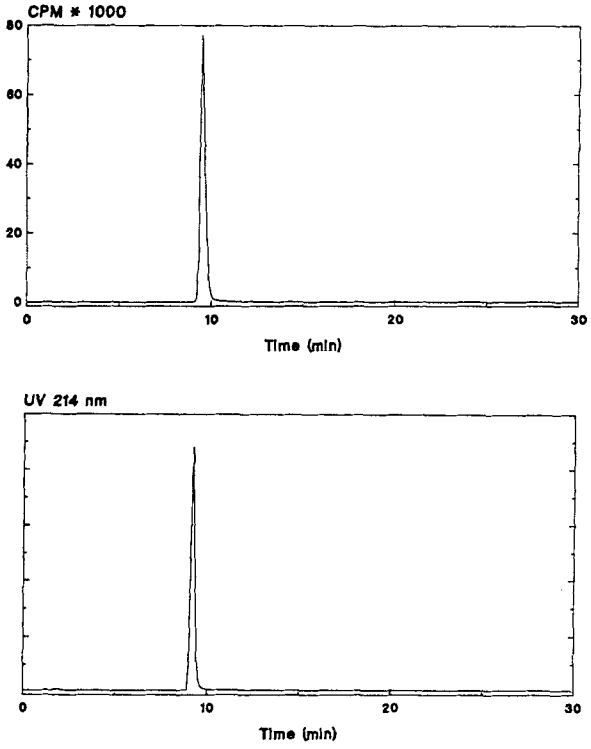

FIGURE 5

HPLC radiochromatogram and UV-chromatogram of [4-^{14}C]NNC 756 (8), using a C-18 column.

resulting oil, dissolved in 2 ml of acetonitrile, were added 7-benzofurancarbaldehyde (146 mg, 1.0 mmol) and powdered sodium hydroxide (80 mg). A black solid was precipitating. The mixture was stirred for 4 hours. The volatiles were removed by nitrogen flow and 10 ml of dichloromethane was added. The mixture was stirred for 30 minutes and then centrifuged. The black solid was extracted with 10 ml of dichloromethane. The combined liquid layers were concentrated by evaporation and the resulting oil was chromatographed on 100 g silica (Merck 60).

Radiochemical yield: 21 mCi (53% from [^{14}C]methyl iodide) as a yellow oil. Radiochemical purity was > 98%, determined by radio-HPLC analysis.

N-Methyl-N-(2-(benzofuran-7-yl)-2-hydroxy-[2-^{14}C]ethyl)-3-chloro-4-methoxy-phenethylamine (3).

To a suspension of silver tetrafluoroborate (243 mg, 1.25 mmol) in 5 ml of dry dichloromethane, was added diphenyl sulfide (0.200 ml, 224 mg, 1.20 mmol). To this

suspension was added [^{14}C]methyl iodide (50 mCi, 55.4 mCi/mmol, 0.90 mol) in 1.0 ml of toluene and the mixture was stirred for 4 hours. The yellow/orange suspension became black. The suspension was filtered and the volatiles removed by nitrogen flow. To the resulting oil, dissolved in 2 ml of acetonitrile, were added 7-benzofurancarbaldehyde (155 mg, 1.06 mmol) and powdered sodium hydroxide (80 mg). A black solid was precipitating. The mixture was stirred for 4 hours. The volatiles were removed by nitrogen flow and 10 ml of dichloromethane was added. The mixture was stirred for 30 minutes and then centrifuged. The black solid was extracted with 10 ml of dichloromethane. The combined liquid layers were concentrated by evaporation and the resulting oil dissolved in 5 ml of acetonitrile. N-methyl-3-chloro-4-methoxy-phenethylamine (240 mg, 1.2 mmol) was added and the mixture was heated to 90°C for 20 hours. The solvent was removed in vacuo and the orange oil purified on a preparative C-18 HPLC column (16 x 250 mm, 7 μm) using acetonitrile/triethylamine-buffer (0.2%, pH 6.5) as eluent; gradient 50/50 to 70/30 over 40 minutes, flow 5 ml/min. The collected fractions of the product (3) were concentrated and extracted with dichloromethane. The organic layer was dried (magnesium sulfate), filtered and evaporated in vacuo to a yellow oil.

Radiochemical yield: 17 mCi (34% from [^{14}C]methyl iodide) as a yellow oil. Radiochemical purity was >98%, determined by radio-HPLC analysis.

N-Methyl-N-(1-(benzofuran-7-yl)-2-hydroxy-[2-^{14}C]ethyl)-3-chloro-4-methoxy-phenethylamine (4).

The byproduct (4) from the ring-opening of the epoxide (2), could be isolated on a preperative C-18 column from the mixture of (3) and (4) as described above.

Radiochemical yield: 2.3 mCi as a yellow oil. Radiochemical purity >95%, determined by radio-HPLC analysis.

[4-^{14}C]-(+)-5-(Benzofuran-7-yl)-8-chloro-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (6).

^{14}C-Labelled phenethylamine (3) (17 mCi) was dissolved in 3.0 ml trifluoroacetic acid. 0.100 ml concentrated sulfuric acid was added, and the mixture was stirred for 20 minutes. Then the mixture was added to 5.0 ml of 25% aqueous ammonia in 20 g ice. The aqueous phase was extracted with dichloromethane and the organic layer was dried (magnesium sulfate), filtered and evaporated in vacuo to a yellow oil. To the oil, dissolved in 3.0 ml of methanol, was added 80 mg of dibenzoyl-D-tartaric acid. The mixture was heated until all material was dissolved and then cooled (5°C). A crystal of the dibenzoyl-D-tartrate salt of

the "cold" benzazepine (6) was added and the mixture was cooled (5 °C) for two days. White crystals were precipitating. The mixture was centrifuged and the crystals washed with cold methanol. To the combined organic layers was added 65 mg of "cold" benzazepine (6), dibenzoyl-D-tartrate, and the mixture was heated to obtain a clear solution.
The crystallizing procedure was repeated twice and the resulting crystals were combined. These crystals were recrystallized in methanol to obtain the product (6) as the dibenzoyl-D-tartrate. The crystals were dissolved in dichloromethane and the product was isolated as the free base by addition of 1 N aqueous sodium hydroxide. The organic layer was washed with water, dried (magnesium sulfate), filtered and concentrated in vacuo to a white solid of (6).
Radiochemical yield: 5.0 mCi (59%). Radiochemical purity >96% and enantiomeric purity >97%, determined by chiral radio-HPLC analysis.

[4-^{14}C]-(+)-5-(Benzofuran-7-yl)-8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (7).

The ^{14}C-labelled benzazepine (6) (4.0 mCi) was dissolved in 5 ml of dry dichloromethane. 0.30 ml of 20% (volumen) boron tribromide in dichloromethane was added during 2 minutes. The orange mixture was stirred for 3 hours at room temperature. 10 ml of methanol was added, and the mixture was stirred at 50 °C overnight. The solvents were evaporated and the mixture dissolved in 5 ml of methanol. 5.0 ml of water was added and the mixture was heated to 50 °C for 1 hour. Aqueous sodium hydroxide was added until pH 7. A white solid was precipitating. The methanol was removed by evaporation, and the aqueous phase extracted with dichloromethane. The organic layer was dried (magnesium sulfate), filtered and evaporated in vacuo to a yellow foam of (7).
Radiochemical yield: 3.5 mCi (88%). Radiochemical purity 95%, determined by radio-HPLC analysis.

[4-^{14}C]-(+)-8-Chloro-5-(2,3-dihydrobenzofuran-7-yl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (8).

The de-protected ^{14}C-labelled benzazepine (7) (3.5 mCi) was dissolved in 5.0 ml of acetic acid. 10% Rh/C catalyst (50 mg) was added. The mixture was hydrogenated at 90 °C for 4 hours. The mixture was filtered and the acetic acid evaporated at reduced pressure. The raw product was dissolved in 4 ml of dimethyl sulfoxide/water : 50/50 and purified on a preparative C-18 HPLC column (250 x 16 mm, 7 μm) using triethylamine (0.2%, pH 6.5)/acetonitrile: 1/2 as eluent. The collected fractions were concentrated by evaporation and extracted with dichloromethane. The organic layer was dried (magnesium sulfate), filtered and evaporated in vacuo to give a white solid.

Radiochemical yield: 2.1 mCi (60%). Radiochemical purity >98% and enantiomeric purity >97%, determined by radio-HPLC analysis. The specific radioactivity was 24 mCi/mmol, determined by MS using reference standard.

MATERIALS

[^{14}C]Methyl iodide was obtained from Amersham (55 mCi/mmol) and was used without further purification. Diphenyl sulfide and silver tetrafluoroborate were obtained from Aldrich-Chemie, West-Germany. 7-Benzofurancarbaldehyde and N-methyl-3-chloro-4-methoxy-phenethylamine were synthesized at Novo Nordisk A/S, Denmark.
All other reagents and solvents used were of analytical grade. Anhydrous solvents were dried over 4-Å molecular sieves prior to use.

RADIOACTIVITY COUNTING

Determination of total radioactivity was done on a Packard 2000 CA tri-carb liquid scintillation analyzer, using 20 ml counting vials and Pico-aqua™ Packard liquid scintillator.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC analysis were performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 μl loop) and a Merck UV-detector L-4000 (operating at 220 or 250 nm). Separations were accomplished at RT with a C-18 column (250 x 4,6 mm, 5 μm) from NOVO NORDISK A/S, using an eluent of triethylamine (0.2%, pH was adjusted to 6.5, using phosphoric acid) and acetonitrile. The flow rate was 1.0 ml/min.
Chiral HPLC analysis was accomplished with an ovomucoid column ULTRON ES OVM (150 x 4.6 mm, 5 μm, 120 Å) from Shinwa Chemical Industries Ltd. The guard column was an ES-OVM.G column.
The mobile phase was 20 mM potassium dihydrogen phosphate (pH 4.6) and acetonitrile. Radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One beta detector A-200, using a 500 μl liquid flow cell. The ratio of column effluent to liquid scintillator (pico-aqua™, Packard) was 1:2. Data collection was done by Flo-One data software on a PC-XT computer.

MS

The mass spectrometer was a Finnigan 5100. The solution was injected on-column to a 25 m x 0.32 mm x 0.25 μm fused silica CP-Sil-5CB (Chrompack). Carrier gas: Helium. The oven was programmed from 60°C after 0.1 min to 280°C with 25°C/min. The temperature was held at 280°C for 30 min. The column was directly interfaced to the ion source of the GC/MS system. The mass spectrometer was operated in the EI 70 eV mode and scanned m/e = 40 to 600 within 1.0 sec. Ion source temperature = 150°C.

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