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Synthesis of (+)-(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-u-^{14}C]carbazolepropanoic acid, [^{14}C]BAY u 3405

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Summary

The title compound [^{14}C]BAY u 3405 (1) was synthesized as part of 8-step sequence. Starting from [U-^{14}C]aniline hydrogensulfate the final product 1 was obtained with a specific activity of 741 MBq/mmol (20 mCi/mmol) and a radiochemical purity of > 98 % in an overall yield of 6 and 10 % depending on the method.

Key words

(+)-(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-U-^{14}C]carbazolepropanoic acid, [^{14}C]BAY u 3405

Introduction

(+)-(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-u-^{14}C]carbazolepropanoic acid (BAY u 3405, 1) is a highly potent and selective thromboxane A_2 receptor antagonist [1,2]. For studies of pharmacokinetics and biotransformation the carbon-14 labelled substance (1) was necessary. In principle the labelling synthesis followed the strategy used for the production of the drug [3]. This paper describes the synthesis and the conditions suitable for labelling and for purification of [^{14}C]BAY u 3405.

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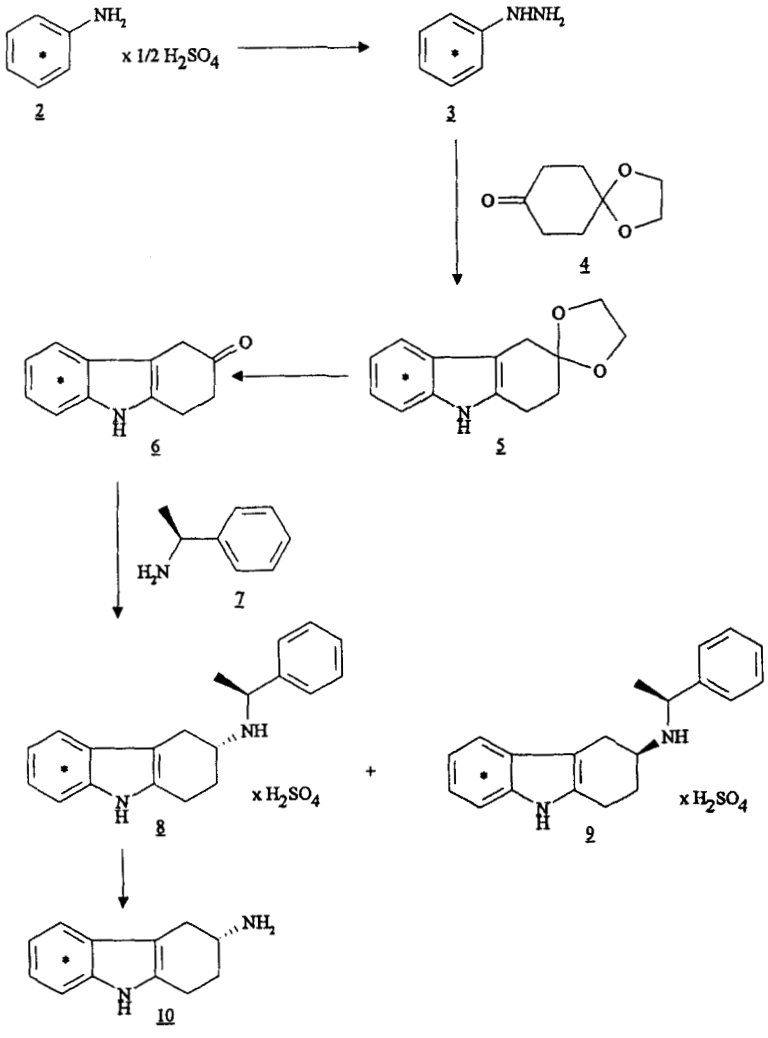
Results and discussion

Starting from commercially available [U-^{14}C]aniline hydrogensulfate (2) (see scheme 1) labelled phenylhydrazine (3) was prepared by diazotization and reduction with sodium sulfite in a yield of 73.9 % [2]. Following the conditions of Fischer's indole synthesis the [U-^{14}C]phenylhydrazine only reacts quantitatively with the monoketal of the hexane-1,4-dione (4) to the tetrahydrocarbazole (5) if one equivalent zinc chloride as catalyst is present. After refluxing for two hours a further 0.4 eqivalent of zinc chloride were added [4]. The best conditions for the hydrolysis of (5) involved stirring of the substance in a mixture of concentrated hydrochloric acid and tetrahydrofuran at room temperature.

The reductive amination of the labelled 3-oxo-1,2,3,4-tetrahydrocarbazole (6) with S-phenethylamine (7) and tetra-n-butylammonium boron hydride gives a mixture of the

Scheme 1

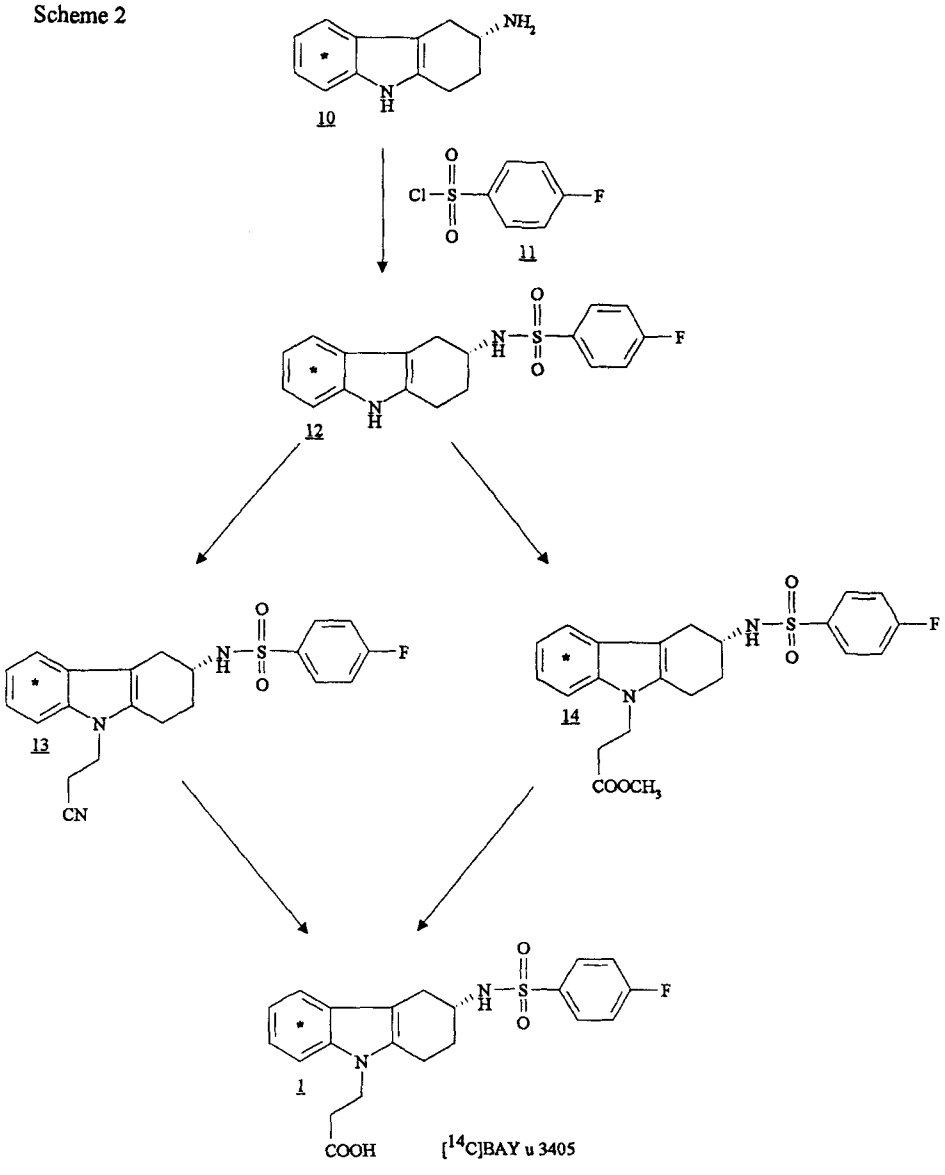


diastereomeric amine hydrogensulfates (8 and 9) showing a diastereomeric ratio of 3:1 in favour of the desired amine (8). The raw material is precipitated as the hydrogensulfate . After recrystallization from a mixture of methanol/ dichloromethane diastereomerically pure amine hydrogensulfate (8) was obtained in a yield of 43 %.

The cleavage of the phenethyl group is accomplished by a transfer hydrogenation with ammonium formiate as a hydrogen donor and palladium on charcoal as a catalyst to give the enantiomerically pure (3R)-3-amino-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazole (10) in a yield of 79.5%.

The sulfonamide (12) is prepared by reaction of (10) with 4-fluorobenzenesulfonylchloride (11) (see Scheme 2). The following alkylation in the presence of sodium hydride can be carried

Scheme 2



out in two different ways, either by reaction with acrylonitrile or by reaction with methyl acrylic acid. The reaction with methyl acrylic acid yielding (14) is more suitable because of the smaller amounts of by-products and the resulting mixture is easy to separate by column chromatography. The subsequent saponification of both products, the nitrile (13) or the ester (14) respectively, provided the crude [^{14}C]BAY u 3405 (1), which was purified by HPLC.

Two syntheses were carried out starting from different amounts of [u-14C]aniline hydrogensulfate. In the first synthesis using method A 432 mg [^{14}C]BAY u 3405 (1) were obtained corresponding to an overall radiochemical yield of 6.0 % after purification. The second synthesis using method B generated one batch with 1015 mg and another batch with 242 mg [^{14}C]BAY u 3405 (1). This total amount corresponds to an overall radiochemical yield of 10.2 %.

Experimental

1. [u-^{14}C]Phenylhydrazine (3)

A mixture of 939.4 mg (6.62 mmol) of [u-^{14}C]aniline hydrogensulfate (2) with a specific activity of 1919.8 MBq/mmol (51.9 mCi/mmol) purchased from ICI, Cambridge Research Biochemicals, UK, and 128 mg (0.90 mmol) of (2) delivered from MOBAY, USA, with a specific activity of 707.2 MBq/mmol (19.1 mCi/mmol) were diluted with 1224 mg (8.62 mmol) non-labelled aniline hydrogensulfate. This batch of (2) containing a total activity of 13336 MBq (360.4 mCi) and a specific activity of 826.4 MBq/mmol (22.3 mCi/mmol) as starting material was dissolved in water and 4.2 ml concentrated hydrochloric acid. To this acidic mixture a solution of 1227 mg (17.8 mmol) sodium nitrite was added dropwise at 0 °C. After stirring for 15 minutes at 0 °C this yellow-brown mixture was transferred into a dropping funnel. The diazotization mixture was added at 0 °C within 10 minutes to a sodium sulfite solution which was freshly prepared by absorption of sulfur dioxide in a solution of 3.2 g sodium hydroxide in 40 ml water. The reaction mixture was stirred for 2 hours at 0 °C and then heated to 65 °C within 30 minutes. After 20 minutes at 65 °C the colour changed from red to yellow. 8.1 ml concentrated hydrochloric acid were added and the mixture was stirred at 70 °C for 5.5 hours.

After keeping overnight at room temperature the mixture was made alkaline by addition of 12.7 ml concentrated sodium hydroxide solution and subsequently extracted six times with 10 ml dichloromethane each time. The combined extracts were dried over anhydrous sodium sulfate and evaporated in vacuo. The residue was dissolved in 25 ml toluene and the proportion of [u-^{14}C]phenylhydrazine (3) was determined by GC. Yield of (3): 1288 mg (11.93 mmol) = 73.9 %.

2. 3,3-Ethylenedioxy-1,2,3,4-tetrahydro[5,6,7,8,12,13,-u-^{14}C]carbazole (5)

1850 mg (11.93 mmol) 4,4-ethylenedioxycyclohexanone (4) were dissolved in 6.5 ml toluene and 25 ml of a toluene solution containing 1288 mg (511.93 mmol) of (3) were added. The

mixture was refluxed for 1 hour using a Dean-Stark apparatus. After cooling 1.5 g (11.1 mmol) powdered zinc chloride were added and the mixture was refluxed for 2 hours. After addition of a further 0.5 g (3.7 mmol) zinc chloride the mixture was refluxed again for 1 hour and then decanted. The residue was extracted with toluene and the toluene solutions were combined and triturated with 23 ml of 2 N sodium hydroxide solution. The aqueous layer was extracted three times with 10 ml toluene and twice with 10 ml ethyl acetate each time. The different organic extracts were combined, dried over anhydrous sodium sulfate and evaporated to dryness.
Yield of (5): 2.26 g (9.8 mmol), radiochemical purity: 95 % (TLC, silica gel Si60, eluent: ethyl acetate/ dichloromethane 1:1).

3. *1,2,3,4-Tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazol-3-one (6)*

A solution of 2.26 g of the crude ethylene ketal (5) in 12 ml tetrahydrofuran was treated dropwise with 12 ml concentrated hydrochloric acid at 0 °C. After stirring for 30 minutes at room temperature 80 ml water were added and the precipitate was filtered off 15 minutes later. The white solid was washed with water and dried in vacuo.
Yield of (6): 1.73g (9.34 mmol) = 95 %, radiochemical purity: 95 % (TLC, silica gel Si60, eluent: toluene/ ethyl acetate 6: 4).

4. *(3R,3S)-3-(1S-Phenylethylamino)-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazole (8)*

1.73 g (9.3 mmol) 1,2,3,4 tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazol-3-one (6) and 1.34 ml (10.4 mmol) S-phenethylamine were refluxed for 1 h in 30 ml toluene at a Dean-Stark trap. The toluene was evaporated, the flask was flushed with nitrogen and the residue was dissolved in 12 ml dichloromethane. This solution was treated with 1215 mg (4.72 mmol) tetra-butylammonium borohydride in 3 ml dichloromethane. After stirring for 1 h at room temperature the reaction mixture was cooled to 0 °C and 2.2 ml methanol were added followed by 31 ml 2 N sulfuric acid (foaming). After stirring for 1 h at room temperature the dichloromethane was removed, the precipitate was filtered off, washed with water and dried in vacuo. The raw material was dissolved in 20 ml methanol and after addition of 115 ml dichloromethane it was recrystallized at room temperature overnight. 865 mg of precipitate were filtered off and the mother liquor was treated with an additional 130 ml dichloromethane. After filtration the precipitates were combined to obtain 1569 mg of hydrogen sulfate (8). The diastereomeric purity was checked by HPLC.
yield of (8): 1569 mg (4.06 mmol) = 43. 4 %
diastereomeric excess: 99 %
analyzed by HPLC under the following conditions:
column: Lichrosorb® Si60, (Merck), 5 µm, 250 x 4.6 mm
flow rate: 2.0 ml/min
solvent: dichloromethane/methanol 95 : 5
detection 235 nm

5. (3R)-3-Amino-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazole (10)

To a solution of 1567 mg (4.06 mmol) (8) in 27 ml ethanol 1480 mg 10 % palladium on charcoal, 1560 mg (24.7 mmol) ammonium formiate and 3446 mg (42.0 mmol) anhydrous sodium acetate were added. The reaction mixture was heated for 20 min to 80 °C. After cooling and treatment with 4.69 ml 2 N sulfuric acid the catalyst was filtered off and washed three times with 14 ml methanol and two times with 20 ml water. The combined filtrate was concentrated in vacuo and the resulting aqueous solution was washed three times with ethyl acetate. The aqueous layer was made alkaline with 3.5 ml 2 N sodium hydroxide and extracted six times with 10 ml ethyl acetate. This last ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated to dryness.
yield of (10): 600 mg (3.23 mmol) = 79.5 %

6. (3R)-3-(4-Fluorosulfonamido)-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazole (12)

To a suspension of 600 mg (3.23 mmol) aminocarbazole (10) in 6 ml dichloromethane were added at 0 °C 475 µl (3.41 mmol) triethylamine and 600 mg (3.1 mmol) 4-fluorobenzene-sulfonylchloride (11). After stirring for 1 h at room temperature the reaction mixture was extracted twice with 10 ml 2 N sulfuric acid and then twice with 2 N sodium hydroxide. The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. Yield of crude (12): 1.22 g.

8. (3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-u-^{14}C] carbazolepropanoic acid ,[^{14}C]BAY u 3405, (1)

Method A

7a. (3R)-3-(4-Fluorophenylsulfonamido)-9-(2-cyanoethyl)-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazole (13)

To a stirred solution of 1.22 g raw material (12) in 48 ml absolute dimethyl formamide 130 mg (4.3 mmol) 80 % sodium hydride suspension in mineral oil were added under nitrogen. After the hydrogen evolution had ceased, 0.47 ml (7.0 mmol) acrylonitrile were added and the reaction mixture was stirred for 1 h at room temperature. 125 ml ethyl acetate were added and the mixture was extracted twice with 7.5 ml 2 N sulfuric acid and twice with 10 ml water. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo to dryness. The residue was chromatographed on silica gel using dichloromethane as eluent. The suitable fractions were combined and evaporated. This residue was dissolved in 30 ml acetonitrile and continously extracted with 100 ml n-hexane for 120 h. After evaporation of the extract 670 mg were obtained.
yield of (13): 670 mg (1.70 mmol) = 53 %

8a. (3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro[5,6,7,8,12,13-u-^{14}C]carbazolepropanoic acid, [^{14}C]BAY u 3405, (1)

The saponification of the nitrile was performed by addition of 30 ml 10 % sodium hydroxide solution to 670 mg (1.70 mmol) of (13) and refluxing the mixture for 4 h. After cooling the aqueous phase was washed twice with 10 ml dichloromethane and acidified by addition of 40 ml 2 N sulfuric acid at 0 °C. This acidic mixture was extracted six times with 8 ml dichloromethane, the extracts were combined, dried over anhydrous sodium sulfate and concentrated. The resulting solid was further dried in vacuo to yield 640 mg raw material.

The first step of the purification was a chromatography on silica gel using ratios of dichloromethane/methanol varying stepwise from 99 : 1 to 95 : 5. After purity check by HPLC the product containing fractions were combined and evaporated to dryness.
The residue was further purified by preparative HPLC using the following conditions:

column:	Hibar Lichrosorb® RP18, (Merck), 7 µm, 250 x 10 mm
flow rate:	6.5 ml/min
solvent:	acetonitrile / 0.1 % acetic acid 50 : 50
detection:	226 nm

The solvent of the fractions containing [^{14}C]BAY u 3405 (1) was removed by lyophilization and 432 mg of a white solid was obtained.

yield:	432 mg (1.04 mmol), 6 % overall radiochemical yield
chemical purity:	≥ 99 %
radiochemical purity:	≥ 98 %
specific activity:	741.5 MBq/mmol (20.1 mCi/mmol)
total activity:	772.2 MBq (20.9 mCi)

Method B:

7b. Methyl (3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-u-^{14}C]-carbazolepropanoate (14)

3194 mg (9.28 mmol) sulfonamide (12) were synthesized following the above described procedures starting with 35779 MBq (967 mCi) [u-^{14}C]aniline hydrogensulfate (2).

To a stirred solution of 3194 mg (9.28 mmol) of (12) in 110 ml absolute dimethylformamide 378 mg (12.5 mmol) 80 % sodium hydride suspension in mineral oil were added portionwise. After the hydrogen evolution had ceased, 0.92 ml (10.4 mmol) methyl acrylate were added and the mixture was stirred for 1 h at room temperatue. The reaction was stopped by addition of 16.5 ml 2 N sulfuric acid, water and 165 ml ethyl acetate. The organic layer was washed with 15 ml 2 N sulfuric acid, twice with water and dried over anhydrous sodium sulfate. After evaporation by vacuum to dryness the residue was used as starting material for the saponification without further purification.

8b. (3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[5,6,7,8,12,13-u-^{14}C]carbazolepropanoic acid, [^{14}C]BAY u 3405, (1)

To the raw material (14), obtained from *7b*, dissolved in 65 ml ethanol 11.5 ml 2 N sodium hydroxide solution was added and the mixture was stirred for two hours at room temperature. After evaporation of the solvent the oily residue was triturated with dichloromethane and 2 N sulphuric acid. The organic layer was extracted with 2 N sodium hydroxide solution. The alkaline phase was acidified with 2 N sulfuric acid and the precipitate was dissolved by extracting three times with dichloromethane. The solvent was evaporated yielding a residue of 2.78 g.

This crude material was purified in 40 mg portions by HPLC under the following conditions:

column: Hibar Lichrosorb® RP 18, (Merck), 7 µm, 250 x 10 mm
flow rate: 3.0 ml/min
solvent: acetonitrile / 0.1 % acetic acid 50 : 50
detection: 226 nm

The fractions containing sufficiently pure [^{14}C]BAY u 3405 were combined and subsequently lyophilized.

yield: 1015 mg (2.44 mmol)
chemical purity: ≥ 98 %
radiochemical purity: ≥ 98 %
specific activity: 1308.3 MBq/mmol (35.4 mCi/mmol)
total activity: 3185.7 MBq (86.1 mCi)

Another batch with a lower specific activity but the same chemical and radiochemical purity was obtained by adding non-labelled material (8) to the mixture of the hydrogensulfates (8) and (9). Yield: 242 mg.

In total starting with 35799 MBq (967 mCi) [u-^{14}C]aniline hydrogensulfate (2) a radiochemical yield of 10.2 % was obtained.

9. Determination of the chemical and radiochemical purity

[^{14}C]BAY u 3405 (1) batches were analyzed by HPLC using a radioactivity detector Ramona® 5 (Raytest GmbH) with one of the following procedures:

a. column: Lichrosorb® RP 18 (Merck), 7 µm, 250 x 4.6 mm
flow rate: 2 ml/min
solvent: A: 0.1 % acetic acid, B: acetonitrile
detection: 226 nm

b. column: Nucleosil® 100 RP 18 (Macherey & Nagel), 7 µm, 250 x 4.6 mm
flow rate: 1.5 ml/minute
solvent: A: KH_2PO_4 - buffer (10 mmol/L, pH=3), B: acetonitrile

gradient: 0 - 15 min 33 % B
35 min 50 % B
40 min60 % B
50 min 60 % B
52 min 33 % B
57 min 33 % B
detection: 228 nm

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