Synthesis of (+)-N-(6-Ethoxy[2,4-^{3}H]phenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine ([^{3}H]BAY x 9228)

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Summary

The synthesis of tritium-labelled (+)-N-(6-Ethoxy[2,4-^{3}H]phenyl)-N-(1,2,2-trimethylpropyl-2-nitroethene-1,1-diamine ([^{3}H]BAY x 9228) a potassium channel opener, was achieved by dehalogenation of a dibrominated racemic precursor using palladium hydroxide on charcoal as catalyst. The separation into the enantiomers was achieved by an chiral HPLC-column. The specific activity was 47.9 Ci/mmol (1.8 TBq/mmol).

Key words: tritium labelling, potassium channel openers.

Introduction

Potassium channel openers offer a novel therapeutic approach to diseases like hypertension, angina pectoris, irritable bladder syndrome and asthma. The potassium channel openers hyperpolarize the cellular membrane and increase the efflux of K^+ (or Rb^+) from tissues [1, 2].

The enantiomerically pure diaminonitroethylene derivative BAY x 9228 is a novel potassium channel opener. For the search for specific binding sites in different cell types tritiated BAY x 9228 was required. BAY x 9227 (the other enantiomer) has no potassium channel activity. We report here the successful synthesis of tritium-labelled BAY x 9228.

Discussion

Aromatic compounds can usually be successfully labelled with tritium by catalytic dehalogenation of a halogen precursor. For that reason the parent compound (1) was successfully dibrominated using a fourfold excess of bromine in the presence of iron(III)-chloride (see fig. 1). But it is rather difficult to obtain the selective dehalogenation of a compound

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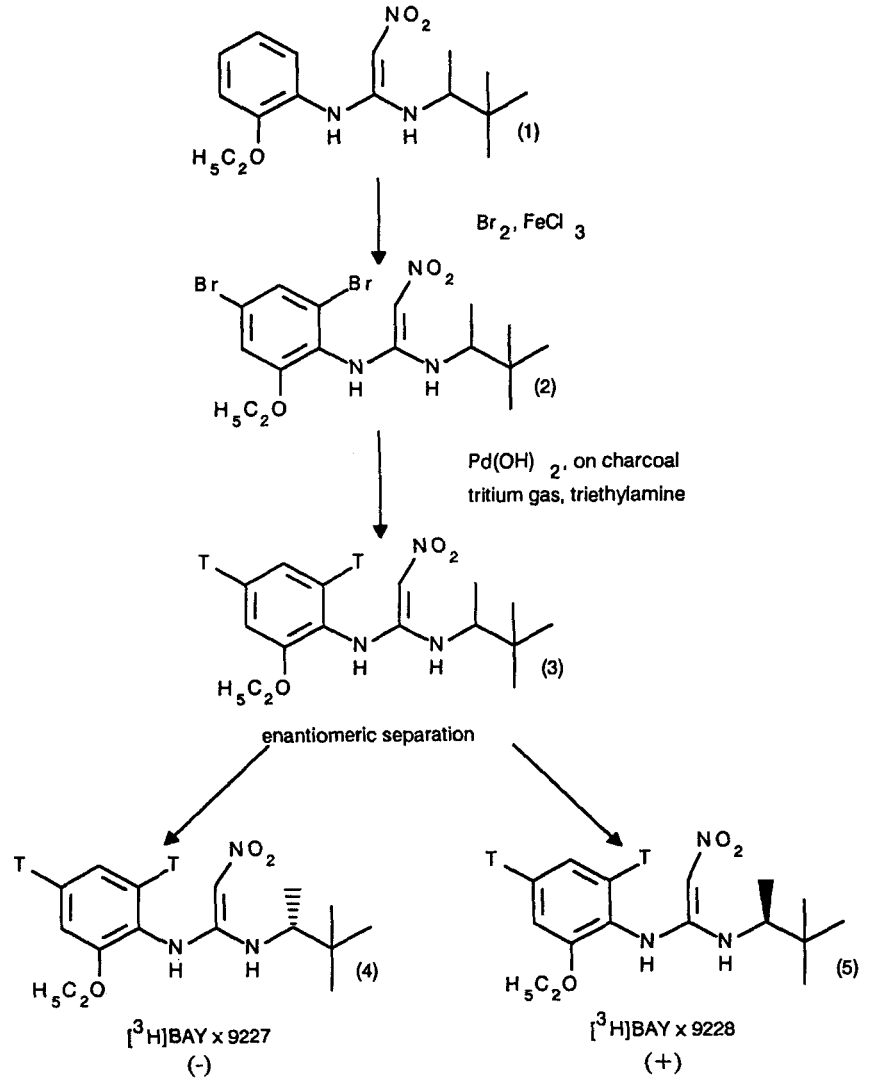
containing additionally a nitro group and an olefinic double bond. In many cases both of these functional groups are also hydrogenated under the conditions of a dehalogenation. In attempts to dehalogenate bromonitroaryldihydropyridines [3] Pearlman's catalyst [4] (palladium hydroxide on charcoal) proved to be an excellent dehalogenation catalyst, which dehalogenated more rapidly than it reduced the nitro group.

Compound (2) was entirely dehalogenated in the presence of Pearlman's catalyst within a few minutes without side reactions. Using deuterium, incorporation rates of over 90 % in

Figure 1: Synthesis of (+)-N-(6-Ethoxy[2,4-^{3}H]phenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine ([^{3}H]BAY x 9228).



the positions 2 and 4 of the aromatic ring were achieved. Complete deuteration in fact cannot be expected, since the NH groups in the molecule dilute the deuterium at the catalyst surface by isotope exchange.

The reaction conditions of deuterium experiments were applied to the tritium labelling. After work-up and purification the specific activity was determined to be 47.9 Ci/mmol (1.8 TBq/mmol), corresponding to a labelling degree of about 80 %. The incorporation rate is somewhat lower than that after deuteration, but this can be explained by an isotope effect between deuterium and tritium.

For the interpretation of the tritium-NMR the exact assignment of the ^{1}H-signals to the proton positions in the unlabelled compound (1) was necessary. This was done by standard methods like ^{1}H, ^{1}H-COSY and ^{1}H, ^{13}C-COSY NMR-spectroscopy [6]. The ^{1}H-decoupled ^{3}H-NMR spectrum shows two singlets at 7.18 ppm and 7.34 ppm (see fig. 2). These signals correspond to the proton signals of the aromatic positions 2 and 4 (meta-positions to the ethoxy group). The ^{1}H-coupled ^{3}H-NMR spectrum gives a further confirmation of the labelling positions (see fig. 3). The doublet at 7.18 ppm proves one adjacent proton and the triplet at 7.34 ppm two adjacent protons.

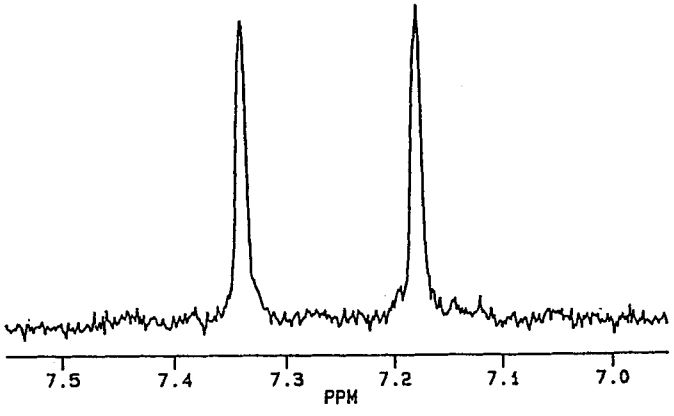


Figure 2: ^{1}H-decoupled ^{3}H-NMR spectrum of compound (3).

The separation into the enantiomers was achieved by chiral chromatography. Because of the small difference of the retention times of BAY x 9227 and BAY x 9228 no base line separation was achievable. To get an enantiomerically pure compound the eluates were generously fractioned accepting a loss of over 50 %.

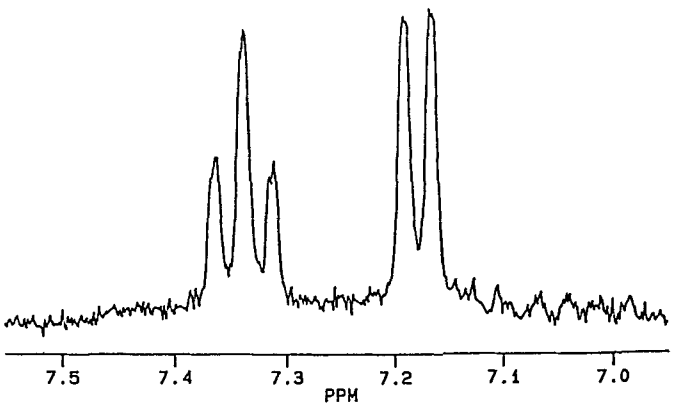


Figure 3: ^{1}H-coupled ^{3}H-NMR spectrum of compound (3).

Experimental

The tritiation reaction was carried out in our tritium-labelling apparatus [5]. NMR spectra were recorded on a Bruker AM 300 spectrometer operated at 320.136 MHz for [^{3}H], 300.13 MHz for [^{1}H] and 75.48 MHz for [^{13}C]. All the measurements were done in the FT mode, chemical shifts are reported in ppm (d), using TMS as internal standard. Mass spectra were recorded on a Finnigan Mat 8239.

For the analytical HPLC a Varian-Chromatograph model 5060 equipped with a Raytest radioactivity detector Ramona® 4 was used. Radioactivity of liquid samples was measured on a Philips LS-counter PW 4700.

(±)-N-(2,4-Dibromo-6-ethoxyphenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine (2)

To a solution of (±)-N-(6-Ethoxyphenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine (1) (220 mg, 0.72 mmol) in a mixture of 10 ml ethanol and 2 ml water, $FeCl_3 \cdot 6H_2O$ (220 mg, 0.72 mmol) and a 1 molar solution of bromine in tetrachloromethane (2.86 ml) were added. The reaction mixture was stirred for 5 hours. The reaction was stopped by a solution of sodium thiosulphate (10 %). After evaporation of the ethanol, dichloromethane (30 ml) was added. The organic phase was washed with a saturated solution of sodium thiosulphate, a saturated solution of sodium hydrogencarbonate and finally with water. After drying over magnesium sulphate the organic phase was evaporated under reduced pressure and purified by column chromatography on silica gel (dichloromethane/methanol 200:1), giving the colorless product (2), 168 mg ≙ 0.36 mmol ≙ 50 % yield.

$C_{16}H_{23}Br_2N_3O_3$, MM = 465

^{1}H-NMR, [D_6]DMSO: d = 0.98 (s, t-Bu), 1.13 - 1.31 (m, -OCH_2-$\underline{CH_3}$, 1-$\underline{CH_3}$), 3.84 (mc, $\underline{CH}$-CH_3), 4.10 (mc, -$O\underline{CH_2}$-CH_3), 5.65 (bs, = $CHNO_2$), 7.41 (s, Ar-H), 7.61 (s, Ar-H), 8.84 (bs, N-H), 10.6 (d, N-H)

MS (EI): 467 (M^+), 465, 463, 421, 419, 417.

(+)-N-(6-Ethoxy[2,4-^{3}H]phenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine (3)

The precursor (2) (5.3 mg, 11.4 μmol) was stirred for 30 min with 6.5 mg palladium hydroxide on charcoal [4] in a mixture of 0.5 ml tetrahydrofuran and 0.1 ml triethylamine with 350 GBq tritium gas at a pressure of 67 kPa using the tritium-labelling apparatus. After readsorption of non-incorporated tritium, the reaction mixture was freeze-dried, taken up in 1 ml of a 2:1 mixture of tetrahydrofuran and ethanol and the catalyst filtered off. The clear solution was freeze-dried. To remove labile tritium the crude product was dissolved in 1 ml of a 2:1 mixture of tetrahydrofuran/ethanol and freeze-dried. This procedure was repeated three times.

The residue was dissolved in a mixture of 500 μl ethanol, 300 μl acetonitrile and 200 μl water and purified in 10 equal portions using the following HPLC conditions: column Nucleosil® C18, 7 μm, 250 x 10 mm (Macherey & Nagel, Düren, FRG), eluent acetonitrile/water 60:40, flow rate 2 ml/min, UV detection at 206 nm, retention time for (3) = 13.1 min. The eluate containing (3) was fractionated on the basis of the UV signal. The fractions were combined (total volume was 25.6 ml) and the substance content of the solution was determined by UV extinction absorbance. According to this method the total quantity of (3) corresponds to 1.62 mg ≙ 3.5 μmol ≙ 31 % of theory. The total radioactivity was 250 mCi (9.25 GBq). The specific activity was calculated to be 47.9 Ci/mmol (1.8 TBq/mmol). The radiochemical purity detected by HPLC was 99.3 %. The labelling positions were confirmed by NMR spectroscopy (see fig. 2 and fig. 3).

^{13}C- and ^{1}H-NMR of the unlabelled compound (1) for comparison:

^{13}C-NMR (75 MHz, [D_6]DMSO): d = 14.46 (q, $OCH_2\underline{CH_3}$), 15.75 (q, CH-$\underline{CH_3}$), 25.63 (q, t-Bu), 34.32 (s, t-Bu), 54.90 (d, $\underline{CH}$-CH_3), 63.77 (t, O-CH_2), 98.35 (d, = $CHNO_2$), 113.32 (d, CH[5]), 120.65 (d, CH[3]), 124.51 (s, C[1]), 128.96 (d, CH[2]), 129.23 (d, CH[4]), 154.09 (s, C[6]), 155.59 (s, $\underline{C}$ = $CHNO_2$);

^{1}H-NMR (300 MHz, [D_6]DMSO): d = 0.98 (t-Bu), 1.19 (d, CH-$\underline{CH_3}$), 1.28 (t, CH_2-$\underline{CH_3}$), 3.85 (m, $\underline{CH}$-CH_3), 4.05 (q, $O\underline{CH_2}$-CH_3), 5.83 (s, = $CHNO_2$), 7.01 (t, H[3]), 7.14 (d, H[5]), **7.18 (d, H[2])**, **7.34 (t, H[4])**, 8.82 (s, NH), 10.57 (s, NH).

(+)-N-(6-Ethoxy[2,4-^{3}H]phenyl)-N-(1,2,2-trimethylpropyl)-2-nitroethene-1,1-diamine (5)

An aliquot of the solution of (3) (10 ml containing 650 μg racemate) was evaporated in vacuo at room temperature. The residue was dissolved in a mixture of 240 μl 2-propanol and 360 μl heptane and separated in 5 equal portions under the following HPLC-conditions: column Chiralcel® OD, 250 x 4.6 mm (Baker Chemikalien, Groß-Gerau, FRG), eluent heptane/2-propanol 90:10, flow rate 1.0 ml/min, UV detection at 365 nm, retention time for (5) = 27.4 min, retention time for (4) 31.6 min. The eluates containing (5) were fractionated on the basis of the UV signal. After separation the corresponding fractions were combined and evaporated under reduced pressure. For storage the residues were dissolved in ethanol giving a radiochemical concentration of 1 mCi/ml (37 MBq/ml). The total activity for (5) was 32.5 mCi (1.2 GBq) showing the same specific activity as of (3). The enantiomeric purity for (5) was 98.3 % detected by HPLC under the same conditions described above.

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