SYNTHESIS OF CARBON-14 LABELLED ROPIVACAIN, A LOCAL ANAESTHETIC AGENT

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SUMMARY
Ropivacaine hydrochloride monohydrate (LEA 103) is a new local anaesthetic agent which currently is under preclinical and clinical investigation. The preparation of (S)-N-(2,6-[2-methyl-\textsuperscript{14}C]dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride monohydrate (6, [\textsuperscript{14}C]-LEA 103) with a specific activity of 12.44 mCi/mmole is described. The key step in the synthesis is a Pd(II)-mediated reaction of (S)-1-benzoyloxy carbonyl-N-(2-methylphenyl)piperidine-2-carboxamide (3) with [\textsuperscript{14}C]methyl iodide. Also described is the preparation of compound 3 from (S)-2-piperidinecarboxylic acid.

Key-words: ropivacaine, local anaesthetic agent, carbon-14 labelling, Pd(II)-mediated ortho-alkylation.

INTRODUCTION
The (S)-enantiomer of N-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride monohydrate (LEA 103, ropivacaine hydrochloride monohydrate) was chosen as a candidate in the search for a long acting local anaesthetic agent with low heart toxicity. In order to study the metabolism and pharmacokinetics of this drug a radioactively labelled compound was required. The present paper describes the synthesis of \textsuperscript{14}C-labelled ropivacaine.
RESULTS AND DISCUSSION

Tremont and Rahman recently reported (1) that acetanilides can be ortho-alkylated with reactive alkyl halides in the presence of Pd(OAc)$_2$. An example of this reaction is given in Scheme 1. Since, ropivacaine is a N-(2,6-dimethylphenyl)carboxamide derivative, we decided to apply this reaction type when introducing carbon-14 in ropivacaine. This would provide a compound containing the label in what would most probably be a metabolically stable position, which further supports the soundness of this synthetic strategy.

According to a preliminary experiment, when reacting 2-methylacetanilide with 1.2 equiv of CH$_3$I and 1.5 equiv of Pd(OAc)$_2$ in CH$_3$COOH at 60$^\circ$C, the desired 2,6-dimethyl compound was formed in a yield of about 70%. This showed that the ortho-alkylation reaction is not dependent on the large excess of CH$_3$I (15 equiv) reported in reference 1. Since it is desirable to introduce carbon-14 as late as possible in the synthetic pathway, efforts were made to ortho-alkylate compound 1 as described above. However, as this failed, probably due to the presence of the free amino function in 1, we decided to prepare a N-protected starting compound. (S)-Pipelicinic acid was reacted with benzyl chloroformate to give the carbamate 2, using standard methods. Compound 2 was coupled with ortho-toluidine, by the use of a method described by Arrieta et al (2).

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Preliminary results using the racemic analogue of 3 in the Pd(II)-mediated reaction (1.2 equiv of CH$_3$I and 1.5 equiv of Pd(OAc)$_2$ at 80$^\circ$C), indicated that the desired compound was formed in a yield of about 70%. Furthermore, applying the method on chiral material, i.e. 3, followed by depro-
tection (HBr/CH₃COOH) and alkylation with n-propyl iodide (cf. Scheme 2), no racemization in the formed ropivacaine was observed, as determined by HPLC using a chiral α₁,4-acid glycoprotein column (3). Application of the method using [¹⁴C]methyl iodide gave the desired carbon-14 labelled LEA 103 in an overall yield of 37% (Scheme 2). The specific activity was, after addition of cold material, 12.44 mCi/mmol. The enantiomeric purity was 100±0.3%, as determined by HPLC.

Scheme 2

![Scheme 2](image)

Reagents: a = PhCH₂OCOCl, NaOH; b = DMAP, SOCl₂ and 2-CH₃C₆H₄NH₂, DMAP; c = Pd(OAc)₂, CH₃COOH, ¹⁴CH₃I; d = HBr, CH₃COOH; e = n-C₃H₇I, K₂CO₃.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. Optical rotations were obtained on a AA-100 polarimeter from Optical Activity Ltd. ¹H and ¹³C NMR spectra were recorded at 199.5 MHz and 50.1 MHz, respectively, on a JEOL FX 200 spectrometer using Me₄Si as the internal standard. Radiochemical purity was determined by scanning alumina-TLC plates.
using a Berthold LB 283 TLC Linear Analyzer. Enantiomeric purity was determined by HPLC using a LKB EnantioPac column eluted (0.4ml/min) with 8 mM phosphate buffer (pH=7.2) containing 0.1 M NaCl and 6% 2-propanol. The column was connected to a Berthold LB504 radioactivity monitor and to a LDC/ Milton Roy UV-spectromonitor operating at 220 nm. Radioactivity was measured in a Packard Tri-Carb 460 C liquid scintillator spectrometer. [1^4C]Methyl iodide was purchased from Amersham, U.K. (S)-Piperidinecarboxylic acid [\(\alpha\)D]20° =-26.40° c=1, H2O), was purchased from Jansen, Belgium.

(S)-1-Benzylcarbonyl-piperidine-2-carboxylic acid (2)

To a solution of (S)-piperidine-2-carboxylic acid (500 mg, 3.87 mmol) in 2M NaOH (1.9 ml, 3.8 mmol) at 4°C was added 0.660 ml (4.6 mmol) of benzyl chloroformate together with a simultaneous addition of 2.3 ml (4.6 mmol) of 2M NaOH. The ice-bath was then removed and the mixture was stirred at 20°C overnight, whereafter water (10 ml) was added. The mixture was washed with ether (2x15 ml) and acidified (pH 2) with 2M H2SO4. The acidic solution was extracted with ether (3x15 ml) and the combined extracts were dried (Na2SO4). Evaporation of the solvent gave 796 mg (78.3%) of the title compound. Mp 108-109°C. [\(\alpha\)]D22° =-40.7° (c=1,CH3OH).

HNMR (CDCl3)δ 1.28 - 1.46 (m, 2H), 1.61- 1.80 (m,3H), 2.18- 2.37 (m, 1H), 3.02 (q,1H), 4.09 (t, 1H), 4.94 (dd, 1H), 7.32 (s, 2H), 7.35 (s, 3H), 8.65 (broad s, 1H). 13C NMR (CD3OD) δ 21.58, 25.66, 27.68, 42.89, 55.66, 68.33, 128.61, 128.95, 129.41, 137.92, 158.04, 174.51.

(S)-1-Benzylcarbonyl-N-(2-methylphenyl)-piperidine-2-carboxamide (3)

73 µl (1.0 mmol) of SOCl2 was added to a solution of 4-dimethylaminopyridine (DMAP, 113 mg, 0.924 mmol) in CH2Cl2 (5 ml) at 20°C. The reaction mixture was stirred for 30 min and the acid 2 (222 mg, 0.84 mmol) in CH2Cl2 (2 ml) was added. The temperature was kept at -20°C and the stirring was continued for another 30 min. A solution of o-toluidine (90 µl, 0.84 mmol) and DMAP (113 mg, 0.924 mmol) in 2 ml of CH2Cl2 was slowly added at -20°C. The reaction mixture was stirred for another 18 h while the temperature slowly reached room temperature. After the addition of CH2Cl2 (10 ml), the organic phase was washed with 1M HCl, sat. Na2CO3 solution and finally water, dried over Na2SO4, and concentrated in vacuum. Purification on silica gel (eluent CH3Cl/ EtOAc 9.5:0.5 v/v) followed by freeze drying afforded 130 mg (44%) of a white solid. M.p. 68-69
(S)-1-Benzoyloxycarbonyl-N-(2,6-[2-methyl-14C]dimethylphenyl)-piperidine-2-carboxamide (4)

10 mCi (0.167 mmol) of 14CH3I was transferred, via a vacuum line, to a stirred mixture of compound 3 (58 mg, 0.167 mmol), Pd (OAc)2 (56 mg, 0.25 mmol), and CH3COOH (1 ml). After breaking the vacuum, the mixture was heated in a sealed tube at 90°C for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (3 ml) and 2M NH3 (3 ml). The organic phase was separated and the aqueous phase was extracted with EtOAc (2x3 ml). The combined extracts were dried (Na2SO4) and evaporation of the solvent gave crude 4. TLC (SiO2, CH2Cl2/ EtOAc 9.5: 0.5 v/v) indicated that 61% of the radioactivity was present in the title compound.

(S)-N-(2,6-[2-Methyl-14C]dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride monohydrate (6)

The crude product 4 obtained from the previous reaction was dissolved in CH3COOH (1 ml) and treated with 30% HBr in CH3COOH (0.5 ml) for 15 h at 20°C. The solvent was evaporated and the residue was dissolved in 2M HCl (2.5 ml). The acidic water phase was washed with ether/ EtOAc 1:1 v/v (2x2.5 ml) and basified with 45% NaOH. Extraction with EtOAc (3x3 ml), drying over Na2SO4, and evaporation of the solvent gave 35 mg of crude and uncharacterized 5.

To a solution of crude 5 (35 mg) in 2 ml of CH3CN was added K2CO3 (25 mg, 0.18 mmol), and n-C3H7I (15 µl, 0.15 mmol). The mixture was stirred in a sealed tube for 18 h at 80°C. The solvent was evaporated and 2M NaOH (2.5 ml) was added to the reaction mixture, and this was extracted with EtOAc (3x2.5 ml). After drying (Na2SO4), the solvent was removed and the crude compound was purified on a short alumina column with ether/ hexane (1:1 v/v) as the eluent. The obtained base was converted to a HCl-salt by the addition of 4M HCl i ether (40 µl). Evaporation of the solvent gave 19 mg (37% overall yield from 3) of the hydrochloride analogue of 6. 50 mg of cold 6 was added and the combined salts were recrystallized from
acetone/ water (9:1 v/v) to give 48.2 mg of the title compound. The specific activity was 12.44 mCi/mmol and the overall radiochemical yield was 19%. The radiochemical purity was 98.7% as determined by TLC (Al₂O₃, ether/ hexane 1:1 v/v) and scanning. The radiochemical enantiomeric purity was 100% ee as determined by radio-HPLC. The enantiomeric purity was 100±0.3% ee as determined by HPLC using the UV-detector (the detection limit is 0.3% at a concentration of about 0.7 μmol/ ml). The retention times for 6 and its corresponding R-isomer were 28.9 min and 19.2 min, respectively.

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