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Synthesis and [^{11}C]-radiolabelling of dechloro-epibatidine and 2PABH, two potential radioligands for studying the central nAChRs *in vivo*.

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SUMMARY

The two epibatidine (EPB) analogues dechloro-epibatidine (DCl-EPB) and 2PABH were synthesised *via* a two step reaction starting from N-methoxycarbonyl-7-azabicyclo[2.2.1]heptene. The radiochemical syntheses of [^{11}C]N-methylated derivatives was performed using the classical methylating agent [^{11}C]CH_3I in acetonitrile. The radiochemical yield ranged from 5 to 10 % (decay corrected from [^{11}C]CH_3I) and was fully sufficient for small animal experiments. High specific activities in the range of 140 to 360 GBq/μmol at EOS (end of synthesis) were obtained. The radiosynthesis, semipreparative HPLC, formulation and quality control were completed in an average time of 35 min.

Key Words: nAChR, epibatidine, dechloro-epibatidine, 2PABH, carbon-11, positron emission tomography

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels (LGIC), which mediate a variety of physiological functions (1) including neurotransmitter release (2, 3) and control of cerebral blood flow (4). Beside these

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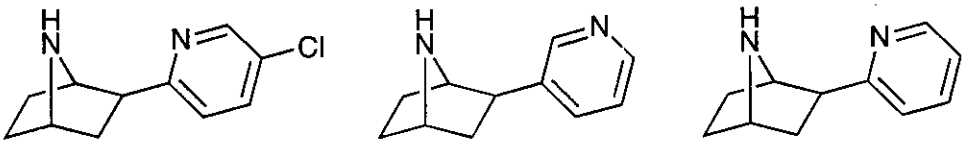
central nervous system (CNS) functions they play a major role in some pathophysiological phenomena like smoking cessation, schizophrenia, anxiety as well as Alzheimer's and Parkinson's disease (3, 5, 6).

A number of these findings were accelerated by the discovery of the alkaloid epibatidine (EPB)(see Fig. 1), which is a highly potent nAChR agonist (7). Displaying a special preference for $\alpha4\beta2$ and $\alpha2\beta3$ nAChR subtypes (8), EPB revealed a consistent deficit in the concentration of central $\alpha4\beta2$ receptors in Alzheimer's disease (9 - 11). These facts suggest EPB may be of diagnostic use. An excellent method for tomographic imaging *in vivo* and studying neuroreceptors in human beings is positron emission tomography (PET). In consequence, efforts were focused on the development of suitable radioligands based on the structure of EPB (12 - 16). Despite the fact that several EPB analogues labelled with carbon-11 and fluorine-18 have been synthesised in the last years, the binding characteristics of nAChRs are not yet well understood. As it is extremely difficult to predict *in vivo* radioligand behaviour from *in vitro* data, it is important to develop radioligands for collecting and comparing *in vivo* PET data.



EPB DCL-EPB 2PABH

Figure 1: Structures of epibatidine and its analogues.

Recently two EPB analogues have been reported, which have attracted special interest from a basic point of view. One is dechloro-epibatidine (DCL-EPB) (Fig. 1), which shows a slightly reduced affinity but higher levels of specific binding *in vivo* (17, 18). Switching the pyridine nitrogen of the dechloro-compound from the *meta* to the *ortho* position results in *exo*-2-(2-pyridyl)-7-azabicyclo[2.2.1]heptane (2PABH) (Fig. 1). By comparison with EPB, 2PABH in electrophysiological studies exhibits totally different characteristics with extreme selectivity towards the $\alpha7$ nAChR subtype (19).

In this study we report the novel syntheses of DCL-EPB, 2PABH and their methylated derivatives as well as carbon-11 labelling, purification and quality control of the [^{11}C]N-methyl-derivatives.

RESULTS AND DISCUSSION

The reaction scheme for the synthesis of DCL-EPB and 2PABH is shown in Figure 2. The N-methoxycarbonyl-7-azabicyclo[2.2.1]heptene (**1**) was synthesised according to Clayton et al (20). The key step in the precursor synthesis is the Heck reaction of the bicycloheptene with the appropriate pyridine (**2a**, **2b**) derivatives. In this palladium catalysed reaction tetrakis(triphenylphosphine)palladium was used because it tolerates a broad spectrum of halides and does not require anhydrous conditions. With this synthetic approach ligands with different pyridine moieties were easily accessible using appropriate halopyridines.

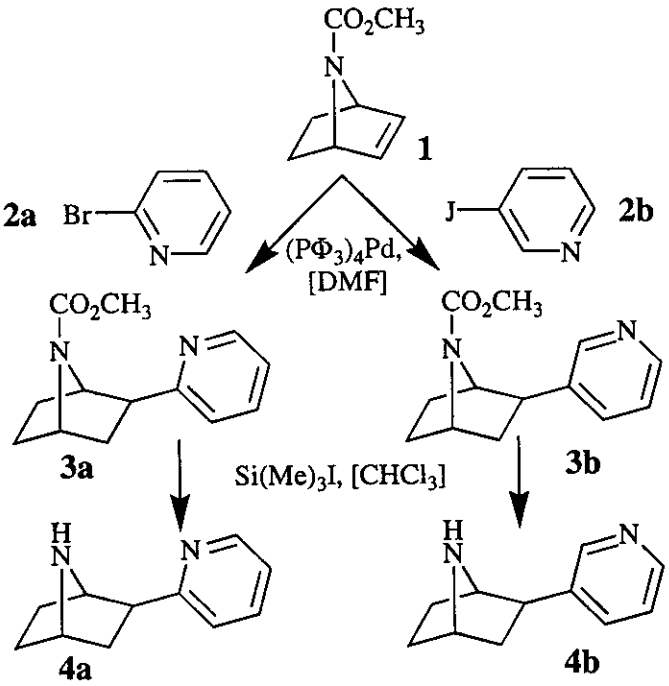
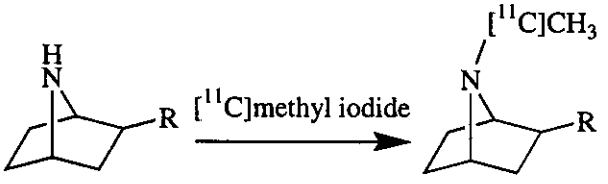


Figure 2: Synthesis of DCL-EPB and 2PABH.

Methylation was performed with CH_3I in acetonitrile. The yields ranged between 35 and 45 % and non reacted precursor could be fully recovered.

The radiosynthesis of [^{11}C]N-methyl-DCl-EPB and [^{11}C]N-methyl-2PABH (see Fig. 3) was accomplished by N-methylation of DCl-EPB or 2PABH, respectively. As methylation agent [^{11}C]methyl iodide was used, which was obtained from the gas phase reaction of [^{11}C]methane with iodine (21). With acetonitrile as the solvent, at 100 °C for 10 minutes under anhydrous conditions the radiochemical yields of the [^{11}C]N-methyl ligands were in the range of 5 to 10 % corrected for decay referred to [^{11}C]iodomethane. This yield was acceptable for *in vivo* rodent experiments and no further optimisation was performed.



R = 2-pyridyl-, 3-pyridyl-

Figure 3: Scheme of radiosynthesis.

For the semipreparative HPLC separation an Aluspher AL column (250 mm x 10 mm, 5µm, Merck) was chosen. Although Al_2O_3 based material does not possess the separation performance and the reproducibility of silica based material, it offers a broader eluent range and more separation methods. The products [^{11}C]N-methyl-DCL-EPB and [^{11}C]N-methyl-2PABH eluted with high radiochemical purity (> 98 %) at 5.2 min. and 5.0 min., respectively (Fig. 4). The precursors were washed from the column after 20 min. With this experimental set-up high specific activities from 140 to 360 GBq/µmol at EOS were obtained. The average time of synthesis including semipreparative HPLC and formulation was 35 min. *In vivo* investigations with both radioligands are in progress.

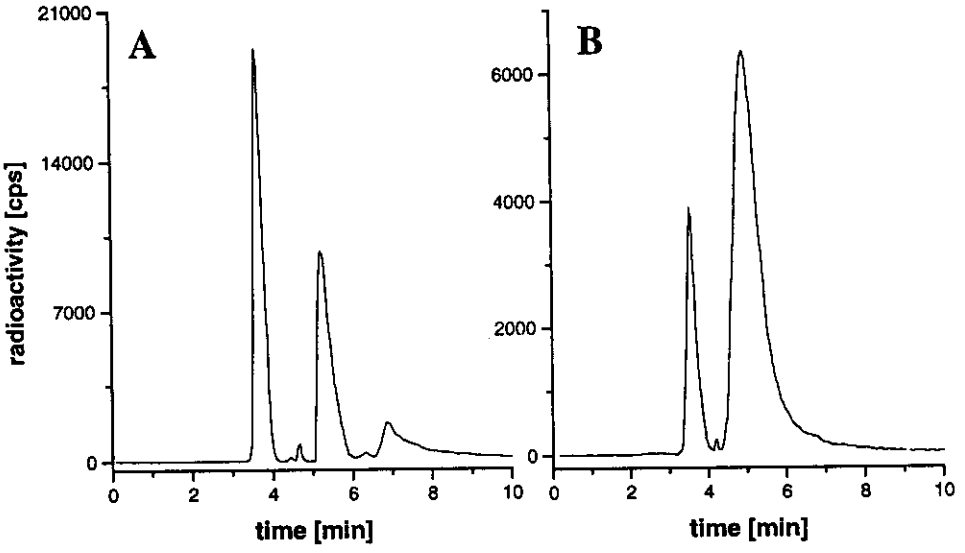


Figure 4: Radiochromatograms from the semipreparative HPLC of [^{11}C]N-methyl-DCl-EPB (panel **A**) and [^{11}C]N-methyl-2PABH (panel **B**) using a Aluspher AL HPLC column (250 mm x 10 mm, 5µm, Merck) at a flow rate of 5 ml/min.

EXPERIMENTAL

Unless otherwise stated reagents were obtained in analytical grade from commercial sources (Fluka, Aldrich, Sigma, Merck).

Analytical Methods

Quality controls of DCL-EPB and 2PABH as well as controls of the methylated and the [^{11}C]methylated derivatives were performed on a Merck-Hitachi LaChrom HPLC-system (L7100 pump, L7200 autosampler, L7400 UV-detector, D7000 interface). For both compounds a Waters µ-Bondapak C18 column (300 mm x 3.9 mm) was used at a flow rate of 2.0 ml/min. As mobile phases acetonitrile/triethylamine/phosphate buffer pH 7 (0.0052 M KH_2PO_4, 0.0082 M Na_2HPO_4) were used with ratios 70/0.014/30 (v/v/v) and 55/0.011/45 (v/v/v) for 2PABH and DCL-EPB, respectively. Standardisation of the methods was performed with the nonmethylated compounds. The standard substances were compared with the

corresponding methylated derivatives *via* quantification of the UV absorption at 262 nm, the maximum absorption of the pyridine group. Radioactivity was measured with a BIOSCAN Flow-Count and a NaI detector (1 inch) connected to a D7000 interface. Data analyses of the chromatograms were carried out with Merck HPLC Manager software.

^{1}H and ^{13}C NMR-spectra were obtained on a VARIAN Gemini 300 spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on a Trio 2000 spectrometer (VG ORGANIC, UK) using electrospray in positive ion mode (ES+). Elementary microanalysis was performed on a LECO CHN-900.

Chemistry

Methoxycarbonyl-DCL-EPB: N-methoxycarbonyl-7-azabicyclo[2.2.1]-heptene (**1**) was prepared according to Clayton et al (20). The heptene (200 mg, 1.31 mmol), 3-iodo-pyridine (**2b**, 669 mg, 3.26 mmol, 2.5eq.) and tetrakis(triphenyl-phosphine)palladium (76 mg, 0.07 mmol, 5 mol%) were dissolved in dry N,N-dimethylformamide (2.0 ml). Piperidine (334 mg, 3.93 mmol, 3 eq.) and formic acid (181 mg, 3.93 mmol, 3eq.) were added and the reaction mixture was stirred under argon at 80°C for 6.5 h. After cooling down 15 ml water was added and the aqueous mixture was extracted with ether (4 x 20 ml). The organic extracts were washed with water, dried (sodium sulfate) and evaporated to give an oil (0.53 g), which was purified on silica by flash chromatography (45 x 1000 mm, ethylacetate/ethanol 90/10, v/v) to give N-methoxycarbonyl-DCL-EPB (**3a**) R_f:0.39 (168 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (dd, *J*=2.1, 2.1 Hz, 1H), 8.44 (dd, *J*=1.7, 4.8 Hz, 1H), 7.62 (ddd, *J*=1.8, 2.0, 7.8 Hz, 1H), 7.55 (ddd, *J*=0.6, 4.8, 7.9 Hz, 1H), 4.47 (dd, *J*=3.8, 3.8 Hz, 1H), 4.42 (d, 2.1 Hz, 1H), 3.67 (s, 3 H), 2.93 (dd, *J*= 5.0, 9.0 Hz, 1H), 2.10-1.40 (m, 6H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 156.0, 149.2, 147.8, 140.8, 134.1, 123.6, 62.1, 56.2, 52.4, 46.2, 44.7, 10.9, 30.4, 28.9 ppm. Mass spectrum (electro spray, cone voltage +30 V) m/z 233 $[M+1]^+$.

DCL-EPB: A solution of the methyl carbamate (**3a**) (134 mg, 0.58 mmol) and iodotrimethylsilane (144 mg, 0.72 mmol) in chloroform (4 ml) was heated under reflux for 5 h under argon. Methanol (2 ml) was added and the volatile components

were removed under reduced pressure. The residue was dissolved in 1N NaOH (4 ml) and the base generated was extracted with chloroform. The organic layer was dried ($NaSO_4$) and evaporated. The residue was chromatographed on silica, eluting with ethylacetate/ethanol/triethylamine 82/4/4 (v/v/v) to give the racemic DCL-EPB (**4a**) R_f:0.26 (72 mg, 71 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, *J*=2.3 Hz, 1H), 8.43 (dd, *J*=1.7, 4.7 Hz, 1H), 7.72 (ddd, *J*=1.8, 2.2, 7.9 Hz, 1H), 7.29 (bs, 1H), 7.20 (ddd, *J*=0.7, 4.8, 7.9 Hz, 1H), 3.80 (dd, *J*=3.9, 3.9 Hz, 1H), 3.60 (d, 2.2 Hz, 1H), 2.82 (dd, *J*= 5.1, 8.8 Hz, 1H), 1.93 (dd, *J*=1.9, 12.1 Hz, 1H), 1.75-1.20 (m, 5H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ 149.3, 147.5, 142.0, 134.4, 123.4, 62.7, 56.4, 45.3, 40.1, 31.2, 29.9 ppm. Mass spectrum (electro spray, cone voltage +40 V) m/z 175 $[M+1]^+$. Elementary microanalysis, Calcd for $C_{11}H_{14}N_2$: C 75.82 %, H 8.10 %, N 16.08 %; found C 73.92 %, H 7.74 %, N 15.52 %.

N-Methyl-DCL-EPB: DCL-EPI (**4a**) (30 mg, 0.17 mmol) was dissolved in acetonitrile (5 ml) and iodomethane (34 mg, 0.24 mmol) was added. The reaction mixture was heated at 80 °C for 30 minutes. The solvent was evaporated off and the product was purified by PTLC on silica gel 60 F_{254} plates (Merck) using ethylacetate/ethanol/triethylamine 82/4/4 (v/v/v) as mobile phase R_f:0.61 (14 mg, 43 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (d, *J*=2.2 Hz, 1H), 8.40 (dd, *J*=1.8, 4.8 Hz, 1H), 7.86 (ddd, *J*=1.7, 2.2, 8.0 Hz, 1H), 7.19 (ddd, *J*=0.8, 4.8, 7.9 Hz, 1H), 3.41 (dd, *J*=3.9, 3.9 Hz, 1H), 3.29 (d, 2.0 Hz, 1H), 2.70 (dd, *J*= 4.9, 8.7 Hz, 1H), 2.28 (s, 3 H), 2.05-1.13 (m, 6 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ 149.2, 147.4, 142.4, 134.7, 124.1, 67.5, 61.2, 46.2, 41.5, 34.6, 26.6, 25.4 ppm.

2PABH (exo-2-(2-pyridyl)-7-azabicyclo[2.2.1]heptane): In a similar manner, which is described in detail elsewhere (20) 2PABH (**4b**) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (ddd, *J*=0.9, 1.8, 5.5 Hz, 1H), 7.56 (ddd, *J*=1.9, 7.4, 7.4 Hz, 1H), 7.15 (ddd, *J*=0.8, 1.0, 7.7 Hz, 1H), 7.09 (ddd, *J*=1.1, 4.8, 7.4 Hz, 1H), 3.77 (dd, *J*=3.3 3.3 Hz, 1H), 3.57 (d, *J*=4.1 Hz, 1H), 2.99 (dd, *J*=5.1, 8.1 Hz, 1H), 2.76 (s, 1H), 2.00-1.84 (m, 2H), 1.76-1.71 (m, 2H), 1.52-1.35 (m, 2H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.4, 149.4, 136.2, 122.3, 121.1, 63.2, 56.2, 49.9, 39.3, 29.6, 29.4 ppm; mass spectrum (electro spray, cone voltage +40 V) m/z 175 $[M+1]^+$.

Elementary microanalysis, Calcd for $C_{11}H_{14}N_2$: C 75.82 %, H 8.10 %, N 16.08 %; found C 72.39 %, H 8.21 %, N 15.32 %.

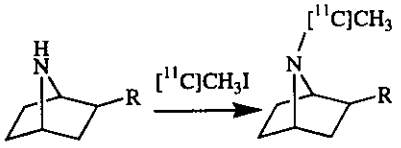
N-methyl-2PABH: According to the procedure which is described for the preparation of N-methyl-DCL-EPB, 2PABH (25 mg, 0.14 mmol) was treated with iodomethane (21 mg, 0.15 mmol) to afford N-methyl-2PABH (10 mg, 36 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (ddd, *J*=0.8, 2.0, 5.2 Hz, 1H), 7.62 (ddd, *J*=1.8, 7.8, 7.8 Hz, 1H), 7.44 (ddd, *J*=0.9, 1.0, 7.9 Hz, 1H), 7.08 (ddd, *J*=1.1, 5.0, 7.4 Hz, 1H), 3.60 (dd, *J*=3.1 3.1 Hz, 1H), 3.44 (d, *J*=4.3 Hz, 1H), 3.01 (dd, *J*=4.9, 8.2 Hz, 1H), 2.31 (s, 3H), 2.14-1.30 (m, 6H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): δ 167.9, 148.6, 136.5, 121.4, 121.1, 66.6, 61.9, 50.4, 38.3, 34.8, 26.9, 25.9 ppm; mass spectrum (electro spray, cone voltage +40 V) m/z 189 $[M+1]^+$.

Radiolabelling:

Radiosynthesis of [^{11}C]CH_3I: [^{11}C]CO_2 was produced *via* the $^{14}N(p,\alpha)^{11}C$ reaction using a 16.5 MeV negative ion cyclotron (General Electric PETtrace, Medical System). After catalytic reduction (Ni) of [^{11}C]CO_2 to [^{11}C]CH_4, [^{11}C]CH_3I was obtained by subsequent gas phase iodination with I_2 at 720 °C according to the procedure described recently (21). Yields of up to 50 % (decay corrected from [^{11}C]CH_4) were obtained with a preparation time of approx. 13 minutes.

Radiosynthesis of [^{11}C]N-methyl-DCL-EPB: The precursors were transferred into a vial which had been oven dried (150 °C). The vial was flashed with argon (45 °C, 30 min.) and 250 µl acetonitrile (Merck, for DNA synthesis) was added under argon. At -30 °C [^{11}C]CH_3I was trapped in the solution. Following the complete addition of [^{11}C]CH_3I the flow was stopped and the reaction vessel was heated to 100 °C for 10 min (see Fig. 3). After cooling the reaction mixture was injected on a Aluspher AL HPLC column (250 mm x 10 mm, 5µm, Merck). With dichloromethane/methanol (96/4,v/v) as mobile phase at a flow rate of 5 ml/minute [^{11}C]N-methyl-DCL-EPB eluted between 5 and 6 minutes (Fig. 4a), while the precursor eluted after 20 minutes. The product fraction was collected and after evaporation of the eluent, dissolved in physiological saline solution. Quality control was performed on the analytical HPLC system described above.

Radiosynthesis of [^{11}C]N-methyl-2PABH: In a similar way as described for the preparation of [^{11}C]N-methyl-DCL-EPB, 2PABH was methylated with [^{11}C]CH_3I. Before the reaction mixture was injected on the HPLC column, unreacted [^{11}C]CH_3I was removed by a stream of nitrogen (30 °C). Using dichloromethane/methanol/diethylamine (95/5/0.004,v/v/v) as solvent the desired product eluted between 4.5 - 7 minutes (Fig. 4b) and precursor after 20 minutes.



R = 2-pyridyl-, 3-pyridyl-

CONCLUSION

With the preparation of the [^{11}C]N-methylated-derivatives of DCL-EPB and 2PABH two novel radioligands for *in vivo* nAChR research have been made accessible. Using a straight phase HPLC-column for the semipreparative separation, the desired carbon-11 products eluted before the precursors, thus yielding high specific activities and high radiochemical purities. These prerequisites allow *in vivo* investigations with both radioligands. All [^{11}C]N-methyl-derivatives of EPB, DCl-EPB and 2PABH are now available and promise highly interesting *in vivo* comparisons.

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