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Syntheses of [21-^{11}C] and (21-^{13}C)Progesterone

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Summary

[21-^{11}C]Progesterone was synthesised *via* the cross-coupling 17β-carboxylic acid chloride-4-androsten-3-one with lithium [^{11}C]methyl(2-thienyl)cuprate (LiCN). The title compound was obtained in 30-35% decay corrected radiochemical yield, based on [^{11}C]methyl iodide, within 35 minutes after end of radionuclide production. The specific radioactivity was 14 GBq/μmole. (21-^{13}C)Progesterone was synthesised using the same procedure for determination of the position of the label.

key words: [21-^{11}C]Progesterone, [^{11}C]methyl(2-thienyl)cuprate, cross-coupling, ^{11}C, (21-^{13}C)Progesterone

Introduction

Many breast tumours express the progesterone receptor, PgR, and the oestrogen receptor, ER. The presence of such receptors has been shown to be a good indicator of the likelihood for response to hormonal therapy (1). *In vivo* imaging of these receptor systems using positron emission tomography (PET) offers a non-invasive tool of high prognostic value (2). The appropriate imaging agent might enable determination of the receptor status of metastatic tumours and lymphnodes which are inaccessible to needle or surgical biopsy.

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Oestrogen receptor based imaging agents have been developed and used successfully in clinical trials (5). However, a PgR imaging agent would be preferable for two reasons: a) PgR positive tumours show better correlation to hormone-responsiveness than do ER positive tumours (1), b) PgR status can be determined even during hormonal therapy by oestrogens or antioestrogens, since PgR, in contrast to ER, will remain unoccupied (3). Imaging of the PgR also has an application beyond breast cancer. Both myomas and meningiomas frequently express PgR (4). However, no attempts to study these tumours using PET have been performed.

A few ^{18}F labelled progestins with relatively high target to tissue selectivity in rats have been developed (6), but to our knowledge no successful imaging of PgR in humans has been reported. 21-[^{18}F]fluoro-19nor-16α-ethyl-progesterone, the most promising candidate, has been evaluated in humans. Unfortunately, the compound suffered from rapid defluorination and high non-specific binding (7).

The short half-life of ^{11}C opens up the possibility for multitracer protocols where a system can be studied with different perturbations or different tracers within a reasonable time-frame. Furthermore, the use of ^{11}C can give access to labelled endogenous substances. Only two examples can be found where ^{11}C have been used for the labelling of oestrogens or progestins (8), and no reports on the use of ^{11}C labelled steroids *in vivo* or *in vitro* have been made. Due to the inherent lipophilicity of many steroids, ^{18}F has been the commonly used nuclide. A high lipophilicity can be accompanied by high non-specific binding and a slow build-up of target to non-target ratios.

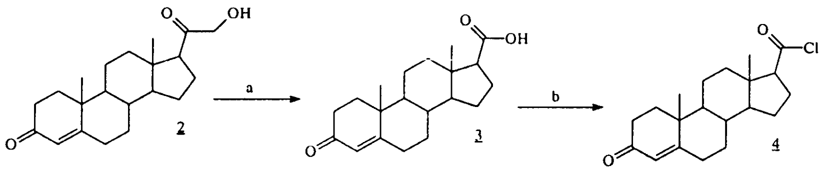
In an ongoing study to image the PgR using the ^{11}C-labelled antiprogestins [N-methyl-^{11}C]RU 38 486 and [N-methyl-^{11}C]ZK 98 299, it was of interest to compare these compounds with the ^{11}C-labelled endogenous substrate for the PgR, ^{11}C -progesterone. A suitable labelling position in progesterone is the 21-methyl group located in the side chain of the steroid D-ring. [21-^{11}C]Progesterone has previously been synthesised by the methylation of a tosylated isocyanide using [^{11}C]methyl iodide (8a). This synthesis suffered from low radiochemical yield, long synthesis time and low specific radioactivity, thus we decided to use a novel route for the synthesis. Recently, we described the synthesis of the ^{11}C-precursors lithium [^{11}C]methyl(2-thienyl)cuprates with either iodo or cyano as ligand, and their applications to cross-coupling, nucleophilic substitution and addition reactions (9). Using these reagents, ^{11}C labelled methyl ketones, fatty acids and compounds resulting from the conjugate addition to α,β-unsaturated ketones have been synthesised. Lithium [^{11}C]methyl(2-thienyl)cyanocuprate 1 was synthesised in two steps from [^{11}C]methyl iodide. [^{11}C]Methyl lithium was prepared by an exchange reaction with excess BuLi and was subsequently converted to 1 using lithium (2-thienyl)cyanocuprate, LTCC, Scheme 1.

$$^{11}CH_3I \xrightarrow{\text{BuLi}} {}^{11}CH_3Li \xrightarrow{\text{LTCC}} Li\,{}^{11}CH_3(2\text{-thienyl})Cu\ (LiCN)\quad \underline{1}$$

Scheme 1. Preparation of lithium [^{11}C]methyl(2-thienyl)cuprate(LiCN) 1.

Results and Discussion

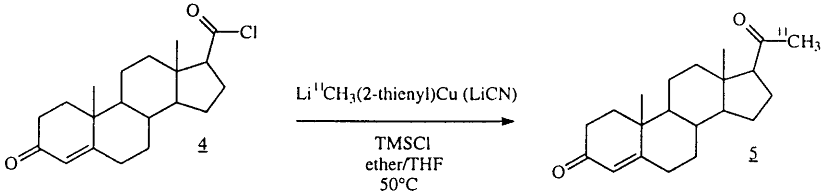
[21-^{11}C]Progesterone was prepared from 17β-carboxylic acid chloride-4-androsten-3-one 4, which was synthesised in two steps from desoxycorticosterone (21-hydroxy-4-pregnen-3,20-dione) 2, Scheme 2.



Scheme 2. Preparation of 17β-carboxylic acid chloride 4-androsten-3-one 3. a) $NaIO_4$, MeOH, H_2O. b) $(CH_2COCl)_2$, DMF, Toluene.

The side chain of 2 was shortened by one carbon through periodate cleavage in aqueous methanol (10). The crude 17β-carboxylic-acid-4-androsten-3-one 3 was shown to be 99% pure as determined by HPLC and was used as such in the next step. The acid 3 was then treated with oxalylic chloride in toluene catalysed by dimethylformamide (DMF) forming the acid chloride 4. After completion of the reaction, toluene and excess reagent was removed under vacuum and the residue dissolved in tetrahydrofuran (THF). The purity of 3 was determined by derivatisation with ethanolamine, and was shown to be >98% pure by HPLC.

The ^{11}C-labelling was performed *via* the cross-coupling of 4 with lithium [^{11}C]methyl(2-thienyl)cuprate (LiCN) 1, Scheme 3.



Scheme 3. Synthesis of [21-^{11}C]progesterone, 5.

[21-^{11}C]Progesterone 5 was obtained in 30-35% decay-corrected radiochemical yield, based on [^{11}C]methyl iodide trapped, within 35 minutes after end of radionuclide production. The specific radioactivity of 5 was 14 GBq/μmole as determined by HPLC. The method described herein, provides 5 with a higher radiochemical yield and a higher specific radioactivity in a shorter reaction time as compared to the previously described method (8a).

In the synthesis of 5, the addition of trimethylsilylchloride, TMSCl, was found to be crucial to the radiochemical yield. Without TMSCl the synthesis of 5 was accompanied by the formation of several by-products and the radiochemical yield never exceeded 10%. The same observation was made when the [^{11}C]methylcuprate 1 was used in conjugate addition to α,β-unsaturated ketones (9c). TMSCl has been proposed to increase the reactivity of the cuprate (11).

A potential side-reaction in the synthesis of 5 is 1,4-conjugate addition in the steroid A-ring. However, in the synthesis of (21-^{13}C)progesterone 6 no by-products from this reaction pathway could be observed using LC-MS or ^{13}C-NMR. [^{11}C]Methane was always a major side product in the synthesis of 5, indicating that the [^{11}C]methylcuprate was quenched by a proton source in the substrate mixture. A possible proton source could be residual acid 3 from incomplete conversion to 4. The amount of [^{11}C]methane formed was 32-46% based on [^{11}C]methyl iodide. The [^{11}C]methane, which eluted from the HPLC column before the desired products with considerable tailing, was removed from the reaction mixture by sparging with helium gas for 15 seconds prior to HPLC purification in order to improve the radiochemical purity of the labelled product.

In one experiment, the [^{11}C]methylcuprate 1 was generated with *tert*-BuLi to examine whether any of the by-products were formed from unreacted n-BuLi and hence could be diminished by the use of the less nucleophilic base *tert*-BuLi. The product distribution was found to be almost identical when comparing the two bases, the only difference being large amounts of unreacted [^{11}C]methyl iodide with the use *tert*-BuLi indicating that the exchange reaction had not reached completion.

In order to determine the position of the ^{11}C-label in 5, (21-^{13}C)progesterone was synthesised using the same method as used for [21-^{11}C]progesterone by simultaneous addition of (^{13}C)methyl iodide with [^{11}C]methyl iodide. ^{13}C-NMR analysis of 6 showed a signal at δ 31.5 ppm corresponding to the methyl group of authentic progesterone. The (21-^{13}C) progesterone was also characterised by LC-MS, where the molecular mass was determined to be m/z 316 (M+1), while in authentic progesterone the molecular mass was determined to be m/z 315 (M+1).

In conclusion, [21-^{11}C]progesterone 5 have been synthesised *via* the cross-coupling of lithium [^{11}C]methyl(2-thienyl)cuprate(LiCN) 1 with 17β-carboxylic-chloride-4-androsten-3-one 3. This further establishes 1 as a useful precursor in the synthesis of ^{11}C labelled compounds.

Experimental Section

General

[^{11}C]Carbon dioxide was prepared by the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre by the ^{14}N(p,α)^{11}C reaction in a gas target containing nitrogen (AGA, Nitrogen 6.0) and 0.05% oxygen (AGA, Oxygen 6.0) bombarded with 17 MeV protons. An automated synthesis system, Synthia (12), was used for [^{11}C]methyl iodide production, HPLC injection and fraction collection.

HPLC was performed using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-absorbance detector in series with a β^+-flow detector. In the analysis of the ^{11}C-labelled compound, unlabelled reference substance was added in the HPLC runs using UV-absorbance detection at 242 nm. NMR spectra were recorded on a Varian XL 300 (300 MHz) or Varian Gemini (200 MHz) spectrometer with chloroform-d$_1$ as internal standard. LC-MS was performed using a Micromass VG Quattro or Micromass Platform equipped with atmospheric pressure chemical ionisation (APcI+ or APcI-), a Beckman 126 solvent delivery module, a Beckman 166 variable wavelength UV absorbance detector in series with a β^+-flow detector and a CMA 240 autosampler. The cI probe was held at 200°C. The column was a Beckman Ultrasphere ODS C_{18}, 5 µm, 250 x 4.6 mm ID. A post column split was used with 50% of the flow to the MS and 50% to the UV absorbance and β^+-flow detector respectively. Mobile phases were 0.025 M ammonium formate/methanol 20:80, 1 ml/min.

Lithium (2-thienyl)cyanocuprate (0.25 M in THF), *tert*-BuLi (1.7 M in pentane) and desoxycorticosterone were purchased from Sigma-Aldrich. n-BuLi (1.6 M in pentane) was purchased from Lancaster. TMSCl, toluene, THF and ether were freshly distilled before use. [^{11}C]Methyl iodide was synthesised according to a published procedure (13).

17β-carboxylic-acid-4-androsten-3-one 2

To a solution of $NaIO_4$ (500mg, 2.3 mmole) in water (7.5 ml), desoxycorticosterone, (21-hydroxy-4-pregnen-3,20-dione, 629 mg, 1.9 mmole) in methanol (25 ml) was added dropwise. After 24 hours the methanol was evaporated, the residue diluted with EtOAc and extracted with 0.5M NaOH. The combined aqueous phase was acidified with concentrated HCl. The white precipitate was filtered and thoroughly washed with water (5x10 ml) yielding 502mg (1.6 mmole, 83%) 2 as an off white powder after drying. 2 was characterised by NMR and LC-MS and the purity was 99% by HPLC. ^{1}H(DMSO): δ 1.15(s,3H), 1.30-3.05(m), 3.82(s,3H), 6.10(s,1H), 12.4(s,1H) ^{13}C(DMSO): δ 13.3, 17.1, 20.6, 23.4, 24.2, 31.8, 32.2, 33.8, 35.2, 35.4, 37.8, 38.4, 43.2, 53.3, 54.6, 54.9, 123.4, 171.1, 174.9, 198.2 LC-MS (APcI-): m/z 315 (M-1)

17β-carboxylic-chloride-4-androsten-3-one 3

To a slurry of 2 (200 mg, 0.63 mmole) in toluene (5 ml), oxalylic chloride (250 μl, 2.9 mmole) and DMF (1 μl, 13 μmole) was added. After 30 minutes at room temperature the reaction mixture became clear and after 60 minutes excess oxalylic chloride and toluene was evaporated and the procedure repeated. After the second evaporation, 3 (208 mg, 0.59 mmol, 93%) was isolated as yellow crystals. The crystals were immediately dissolved in THF (1 ml) to give a brightly yellow 0.6 M solution.

[21-^{11}C]Progesterone 5

[^{11}C]Methyl iodide was trapped in a reaction vial containing diethylether (100 µl) at -72 °C. Butyllithium (10 µl, 1.6 M in hexane, 16 µmole) was added and 1 min later LTCC (100 µl, 0.25 M in THF, 25 µmole). The reaction vial was kept at 0 °C for 1-2 min and the substrate mixture containing acid chloride 4 (0.6 M in THF, 100 µl, 60 µ mole), TMSCl (15 µl, 120 µmole), and THF (80 µl) added. The reaction mixture was heated at 50 °C for 5 min. After heating 1 M HCl/EtOH 1:3 (1.0 ml) was added and the vial sparged with He (20 ml/min, 15 s). The reaction mixture was purified by semipreparative HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 × 10 mm ID column, eluting with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water 50:7, v:v) 30:70, 5 ml/min, a linear gradient to 98% acetonitrile/water (50:7, v:v) 10-13.8 min). [21-^{11}C]Progesterone was analysed by HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 x 4.6 mm ID column, 0.05 M ammonium formate, pH 3.5/(acetonitrile/water 50:7, v:v) 40:60, 2 ml/min, $t_R = 5.6$ min).

(21-^{13}C)Progesterone 6

[^{11}C]Methyl iodide was trapped in a reaction vial containing diethyl ether (200 µl) cooled to -72 °C. (^{13}C)Methyl iodide (5 µL, 20% in heptane (v/v), 15 µmole) was added, followed by butyl lithium (30 µl, 1.6 M in hexane, 48 µmole). After 1 min, a solution of LTCC in THF (300 µl, 0.25 M, 150 µmole) was added. The solution was then kept at 0 °C for 2 min. The acid chloride 4 (260 µl, 0.3 M in THF, 78 µmole) was added and the reaction mixture was heated at 50 °C for 3 min. After heating, 1 M HCl/EtOH 1:3 (1 ml) was added and the vial sparged with N_2 (20 ml/min, 15 s). The [21-^{11}C/^{13}C]progesterone was purified by semipreparative HPLC using the same conditions as for 5. The collected fraction was concentrated to dryness under vacuum, the residue dissolved in chloroform-d_1 (0.7 ml) and analysed by ^{13}C-NMR and LC-MS. ^{13}C: δ 31.5, LC-MS: m/z 316(M+1)

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