Lithium [^{11}C]methyl(2-thienyl)cuprate•LiCN in 1,4-Additions to α,β-Unsaturated Ketones. ^{11}C and ^{13}C Labelling of the Androgen Mesterolone

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Summary

Conjugate 1,4-addition of the [^{11}C]methyl group to α,β-unsaturated ketones was achieved by the use of lithium [^{11}C]methyl(2-thienyl)cuprate•LiCN in a mixture of tetrahydrofuran and diethylether. The ^{11}C labelling procedure was applied successfully to three model compounds and the androgen mesterolone. Mesterolone labelling was also performed with ^{13}C in order to confirm the position of the label. The labelled products were obtained in radiochemical yields of 31-50% within 35-51 minutes of the end of radionuclide production. In a typical synthesis, 1.5 GBq [1α-*methyl*-^{11}C]mesterolone was synthesised starting from an 18 μA•h cyclotron irradiation.

Key words: mesterolone, androgen receptor, Michael addition, ^{11}C, [^{11}C]methyl(2-thienyl)cuprate.

Introduction

1,4-Addition of alkyl, alkenyl and aryl groups *via* organocuprates to α,β-unsaturated ketones is an established and widely used synthetic procedure (1). The most suitable organocuprates for conjugate additions so far seem to be alkyl-, alkenyl- and aryl(2-thienyl)cuprates with an iodo (2) or cyano group (3) as a ligand, although β-silyl organocuprates show great promise (4). Activating agents like trimethylsilyl chloride (TMSCl) (5), trimethylsilyl iodide (6) and boron trifluoride (7) added to the organocuprate improve reaction rates and chemical yields of 1,4-additions.

Addition of lithium (2-thienyl)cyanocuprate (LTCC) and lithium (2-thienyl)iodocuprate (LTIC), respectively, to a no-carrier-added solution of [^{11}C]methyllithium has been reported to yield a lithium [^{11}C]methyl(2-thienyl)cuprate reagent with a corresponding iodo or cyano ligand. These [^{11}C]methylcuprates were then reacted with carboxylic acid chlorides (8,9) and 1-iodoheptane (8) to form ^{11}C-labelled ketones and octane, respectively, in good yields.

While 1,4-additions in ^{11}C labelling synthesis have been reported for the reaction of hydrogen [^{11}C]cyanide on ethylacrylate (10) and acrylonitrile (11), in this paper we report the first method using lithium [^{11}C]methyl(2-thienyl)

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cuprate·LiCN **1** for 1,4-addition of the ^{11}C-methyl functionality to α,β-unsaturated ketones **2**, **4** and **6** as shown in Scheme 1.

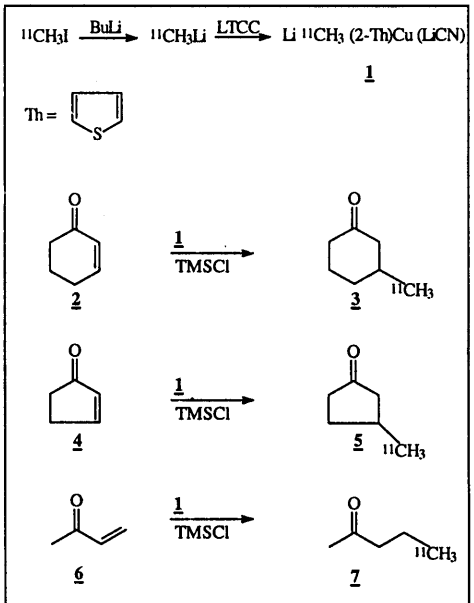
The procedure developed was applied to the ^{11}C labelling of the androgen 17β-hydroxy-1α-methyl-5α-androstan-3-one (mesterolone) (12,13), a potential candidate for *in vivo* PET imaging of the androgen receptor (AR) in prostate cancer (14,15). The same procedure was also utilised for the ^{13}C labelling of mesterolone, which confirmed, through ^{13}C-NMR, the location of the carbon label at the 1α-methyl position.

Results and Discussion

With an interest to develop a tracer for imaging the AR in prostate cancer, the steroid mesterolone was identified as a target compound. This anabolic androgen binds to the rat prostatic AR with a relative binding affinity (RBA) of 0.25 (methyltrienolone RBA = 1, 5α-dihydrotestosterone RBA = 0.46, testosterone RBA = 0.15) and to human serum sex hormone-binding globulin (SHBG) with a RBA of 4.4 (methyltrienolone RBA < 0.01, 5α-dihydrotestosterone RBA = 1, testosterone RBA = 0.19) (13). With moderate AR affinity and high affinity for SHBG, which has been suggested to be desirable in AR- and oestrogen receptor-based imaging agents (15), labelled mesterolone could be a suitable candidate for human AR imaging with PET (14). The 1α-methyl group was selected as a potential ^{11}C labelling position in mesterolone and introduction of a methyl group by a 1,4-addition reaction on the steroidal enone ZK 5777 (17β-acetoxy-5α-androst-1-en-3-one) **8** was chosen as a labelling approach. Three model compounds were selected in order to develop the labelling method, and the method was applied in the synthesis of [1α-*methyl*-^{11}C]mesterolone **9**.

The conjugate addition method was first developed for the synthesis of 3-[^{11}C]methylcyclohexanone **3** from 2-cyclohexenone **2**. Two different lithium [^{11}C]methyl(2-thienyl)cuprates were used in reactions with **2** in various solvents and with or without TMSCl as an activating agent. The conditions which provided the highest radiochemical yields were then applied to substrates 2-cyclopentenone **4** and methyl vinyl ketone **6**.

Lithium [^{11}C]methyl(2-thienyl)cuprate•LiCN **1** and lithium [^{11}C]methyl(2-thienyl)cuprate•LiI were prepared by adding the appropriate cuprate reagent (LTCC or LTIC) to a



Scheme 1. The [^{11}C]cuprate **1** provided the model ^{11}C Michael addition products **3**, **5** and **7** from α,β-unsaturated ketones **2**, **4** and **6**.

no-carrier-added solution of [^{11}C]methyllithium, which was obtained in an exchange reaction on [^{11}C]methyl iodide using excess butyllithium (BuLi) (16). Since the radiochemical yields of the desired addition product **3** were higher using the [^{11}C]methylcuprate obtained from LTCC than those from LTIC, the former was used in subsequent experiments. Furthermore, LTCC is commercially available in tetrahydrofuran (THF) while LTIC is not.

To circumvent the potential problem of 1,2-addition, which could be a dominant side reaction resulting from incomplete transmetallation between [^{11}C]methyllithium and LTCC, 2 equivalents of LTCC were used (based on the amount of added BuLi which reacts with LTCC and LTIC to form the corresponding butyl cuprate), resulting in higher radiochemical yields as compared to the results using one equivalent of LTCC.

The amount of α,β-unsaturated ketone **2** was found to influence the radiochemical yields of the desired product **3**. The conjugate addition required 4 equivalents of the enone **2** relative to the amount of added BuLi. With 1.25 equivalents of **2**, the radiochemical yields dropped to less than 10%. LTCC was poorly soluble in solvents other than THF, so THF was present in all experiments. However the highest radiochemical yields of the addition products were obtained only when diethylether was present. THF was selected for dissolving the enone-TMSCl mixtures on account of its superior solvating properties over diethylether (particularly in the case of the steroidal substrate **8**). In our experiments, the substitution of dimethylsulfide for diethylether when trapping [^{11}C]methyl iodide gave no difference in radiochemical yield. For practical reasons diethylether was selected as the trapping solvent. However, in reported reactions of various monocuprates with 2-cyclohexenone, dimethylsulfide was found to provide higher reaction yields than diethylether (17).

To increase the reactivity of the [^{11}C]methylcuprate **1** and to minimise the formation of side products in the conjugate addition, the activating agent TMSCl was required. Without TMSCl the radiochemical yield of the 1,4-addition product **3** dropped to only a few percent and purification of the reaction mixture by semi-preparative HPLC was difficult due to the large number of ^{11}C-labelled products present.

In one series of experiments benzoyl chloride was added following the addition of the enone-TMSCl mixture. Since benzoyl chloride has been shown to form [*methyl*-^{11}C]acetophenone quantitatively from **1** (8), this study served to estimate the amount of **1** which remained in the reaction mixture unreacted to the enone. No [*methyl*-^{11}C]acetophenone was observed under the conditions used which gave the best radiochemical yields, indicating that all the reactive [^{11}C]methylcuprate was consumed rapidly during the conjugate addition reaction. However [^{11}C]methane was always a major side product in these reactions, regardless of enone substrate, suggesting that the [^{11}C]methyl cuprate was quenched by a proton source in the substrate mixture. 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 sec prior to HPLC purification in order to improve the radiochemical purity of the labelled products.

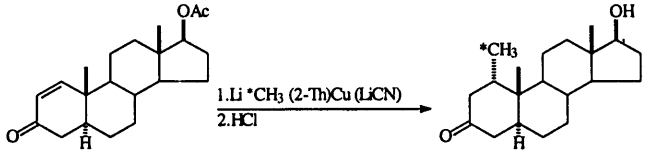
The highest radiochemical yields for the [^{11}C]methyl 1,4-additions were obtained using the [^{11}C]methylcuprate 1 derived from LTCC in reactions with α,β-unsaturated ketones 2, 4 and 6 in a mixture of THF and ether with TMSCl as activating agent. The addition products 3, 5 and 7 were obtained in 35-50% radiochemical yield, counted from [^{11}C]methyl iodide, within 35 min of the end of radionuclide production (Table 1).

Table 1. Decay-corrected radiochemical yields of ^{11}C-labelled addition products.

Substrate	Product	Radiochemical Yield (%)[a]
2-cyclohexenone (2)	3-[^{11}C]methylcyclohexanone (3)	35 ± 6
2-cyclopentenone (4)	3-[^{11}C]methylcyclopentanone (5)	42 ± 7
methyl vinyl ketone (6)	[5-^{11}C]pentan-2-one (7)	50 ± 11
ZK 5777 (8)	[1α-*methyl*-^{11}C]mesterolone (9)	31 ± 1

a. Decay-corrected radiochemical yield ± standard deviation based on HPLC-purified fractions. LC analysis indicated a radiochemical purity >98%. Based on at least 3 experiments.

The strategy used for labelling the model compounds was used in the synthesis of [1α-*methyl*-^{11}C]mesterolone 9 as shown in Scheme 2. After the Michael addition to the steroid, the acetyl protecting group in the 17 position was removed efficiently by acid hydrolysis. The radiochemical yield of 9 (31%) was lower than the yields of the model compounds 3, 5 and 7. The lower yield for 9 can be attributed to the formation of a major side-product which was non-volatile and extremely lipophilic (18).



8

* = ^{11}C 9
* = ^{13}C 10

Scheme 2. Synthesis of the androgen mesterolone ^{11}C- (9) or ^{13}C- (10) labelled in the 1α position.

In the ^{11}C labelling of mesterolone, adequate radiochemical yields were obtained with 1.25 equivalents of the starting material 8. The use of more than 4 equivalents of 8 did not increase the radiochemical yield, unlike in the case of the model compounds. The specific radioactivity of 9, measured at the end of HPLC purification, was determined to be about 40 GBq/μmol, which is sufficient for receptor studies both *in vitro* and *in vivo* with PET (19).

The position of the ^{11}C label was confirmed by analysis of the ^{13}C-NMR spectrum of (1α-*methyl*-^{13}C)mesterolone **10**. Compound **10** was synthesised by the same method used for **9** through addition of (^{13}C)methyl iodide immediately after the completion of [^{11}C]methyl iodide trapping. The ^{13}C signal at δ 10.4 ppm corresponded to the ^{13}C signal of the 1α-methyl group of authentic mesterolone. The (^{13}C)androgen **10** was also analysed by LC-MS, indicating a molecular ion peak at m/z 306 (M+1), which corresponded with the molecular ion peak at m/z 305 (M+1) observed with authentic mesterolone.

In summary, a method for [^{11}C]methyl 1,4-additions to α,β-unsaturated ketones was developed. The radiochemical yields of the addition products were moderate and reproducible. The labelling method was applied to the ^{11}C labelling of mesterolone which is a potential candidate for the *in vivo* PET imaging of the AR in prostate cancer. [1α-*methyl*-^{11}C]Mesterolone **9** was obtained in 31% radiochemical yield with a specific radioactivity of 40 GBq/μmol, 51 min from the end of radionuclide production.

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 using 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 (20), was used for [^{11}C]methyl iodide production, HPLC injection and fraction collection.

HPLC was performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV absorbance detector (280 nm) in series with a β^+-flow detector (21). Analytical HPLC was performed by co-injection of authentic reference standards. ^{13}C-NMR spectra were recorded on a Varian XL 300 spectrometer at 75.4 MHz with chloroform-d_1 as internal standard. LC-MS was performed using a Micromass VG Quattro equipped with atmospheric pressure chemical ionisation (probe T = 200°C), a Beckman 126 solvent delivery module and a CMA 240 autosampler. The column used was a Beckman Ultrasphere ODS C_{18}, 5 μm, 250 x 4.6 mm ID. A post-column split was used with 10% of the flow to the MS and 90% to a β^+-flow detector. Mobile phases were 0.025 M ammonium formate, pH 3.5/methanol 20:80, 1 ml/min.

Lithium (2-thienyl)cyanocuprate (0.25 M in THF), 2-cyclohexenone, 2-cyclopentenone, 3-methylcyclohexanone, 3-methylcyclopentanone, and 2-pentanone were purchased from Sigma-Aldrich. BuLi and methyl vinyl ketone were purchased from Lancaster. (^{13}C)Methyl iodide was purchased from Larodan AB, Sweden. Mesterolone and its labelling precursor, ZK 5777, were supplied by Schering AG, Berlin, Germany. 2-Cyclohexenone, 2-cyclopentenone, methyl vinyl ketone and TMSCl were distilled before use. THF and ether were distilled under nitrogen from sodium/benzophenone.

[^{11}C]Methyl iodide (22)

[^{11}C]Carbon dioxide was delivered in a stream of nitrogen gas (100 ml/min) to a solution of lithium aluminium hydride (12.5-25 µmol, 0.25 ml THF). After evaporation of THF, hydroiodic acid (57%, 0.4 ml) was added and the reaction mixture was heated to 130°C. During this process the [^{11}C]methyl iodide was transferred by a stream of nitrogen gas (25 ml/min) to the reaction vessel.

3-[^{11}C]Methylcyclohexanone (3)

[^{11}C]Methyl iodide was trapped in a reaction vial (3 ml) containing ether or THF (100 µl) cooled to -72°C. Immediately after trapping, a solution of BuLi in hexane (15 µl, 1.6 M, 24 µmol) was added. After 1 min LTCC (200 µl, 0.25 M in THF, 50 µmol) was added. The reaction vial was then cooled to 0°C over 1-2 min and the substrate mixture containing 2-cyclohexenone 2 (10 µl, 103 µmol), TMSCl (20 µl, 158 µmol), and ether or THF (80 µl) was added. The reaction mixture was, in sequence, heated to 70°C over 5 min, cooled to 0°C, quenched with hydrochloric acid (0.3 M, 0.5 ml) and diluted with methanol (0.5 ml). The resulting solution was sparged with helium (20 ml/min, 15 s). Crude 3 was then purified by semi-preparative HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 × 10 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/methanol 60:40, linear gradient to 90% methanol from 5-10 min, 5 ml/min). Purified 3 was analysed by HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 x 4.6 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water (50/7, v/v)) 70:30, 2 ml/min), t_R = 7.5 min.

3-[^{11}C]Methylcyclopentanone (5)

3-[^{11}C]Methylcyclopentanone 5 was prepared from 2-cyclopentenone 4 (8.6 µl, 103 µmol) in the same manner described above for 3. Crude 5 was purified by semi-preparative HPLC and purified 5 was analysed by HPLC (t_R = 4.5 min) using the same conditions used for 3.

[5-^{11}C]Pentan-2-one (7)

[5-^{11}C]Pentan-2-one 7 was prepared as described for 3 starting from methyl vinyl ketone 6 (7.0 µl, 100 µmol). Crude 7 was purified by semi-preparative HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 × 10 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water (50/7, v/v)) 80:20, 5 ml/min). Purified 7 was analysed by HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 x 4.6 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water (50/7, v/v)) 80:20, 2 ml/min), t_R = 6.2 min.

17β-Hydroxy-1α-[^{11}C]methyl-5α-androstan-3-one ([1α-methyl-^{11}C]mesterolone) (9)

[^{11}C]Methyl iodide was trapped in a reaction vial containing ether (100 µl) cooled to -72°C. A solution of BuLi in hexane (15 µl, 1.6 M, 24 µmol) was added. After 1 min LTCC (200 µl, 0.25 M in THF, 50 µmol) was added. The reaction vial was then cooled to 0°C over 1-2 min and the substrate mixture containing ZK 5777 **8** (10.4 mg, 30 µmol), TMSCl (20 µl, 158 µmol) and THF (80 µl) was added. The resulting mixture was then heated to 70°C over 5 min. After cooling to 0°C, hydrochloric acid was added (0.4 ml, 6 M), followed by ethanol (0.3 ml) and the vial was sparged with He (20 ml/min, 15 sec) and then heated to 90°C over 5 min. After the reaction vial was cooled to 0°C NaOH (0.5 ml, 5 M) was added. Crude **9** was purified by semi-preparative HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 × 10 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/ethanol 50:50 and a linear gradient to 90% ethanol from 5-15 min, 5 ml/min). Purified **9** was analysed by HPLC (Beckman Ultrasphere ODS C_{18}, 5 µm, 250 x 4.6 mm ID column, eluted with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water (50/7, v/v)) 30:70, 2 ml/min), t_R = 4.0 min. In order to determine the specific radioactivity of **9**, the amount of unlabelled mesterolone co-produced in the radiosynthesis was quantified by LC-MS.

17β-Hydroxy-1α-([^{13}C)methyl-5α-androstan-3-one ((1α-methyl-^{13}C)mesterolone) (10)

[^{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 µmol) was added, followed by BuLi (30 µl, 1.6 M in hexane, 48 µmol). After 1 min, a solution of LTCC in THF (400 µl, 0.25 M, 100 µmol) was added. The reaction vial was then cooled to 0°C over 2 min. The substrate mixture containing ZK 5777 **8** (20.8 mg, 60 µmol) and TMSCl (40 µl, 316 µmol) in THF (160 µl) was added. The reaction mixture was heated to 70°C over 5 min. After cooling the vial to 0°C, hydrochloric acid was added (0.8 ml, 6 M), followed by ethanol (0.6 ml) and the vial was sparged with He (20 ml/min, 15 sec) and then heated to 90° C over 5 min. After cooling to 0°C NaOH (1.0 ml, 5 M) was added. Crude [1α-*methyl*-^{11}C/^{13}C]mesterolone was purified by semi-preparative HPLC employing the same conditions used for **9** and the purified ^{11}C/^{13}C product was analysed by LC-MS. After decay of the ^{11}C radioactivity, the collected fraction was concentrated to dryness *in vacuo*. The residue was dissolved in chloroform-d_1 (0.7 ml) and analysed by ^{13}C-NMR.

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