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Short Communication

An Alternative Method for the Preparation of 11-Derivatized Progesterone

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Steroids with additional functional groups are often needed for biochemical, microbiological and medicinal applications.[1] For example, in immunoassays and affinity chromatography haptens are coupled covalently to a carrier prior to immunization. In competitive immunoassays, haptens coupled to a label molecule are used as tracers. Most commonly a spacer arm with a reactive ω-substituent (amino, thiol, acid) is introduced into the steroid structure before the coupling reaction. Since the coupling site as well as the length and chemical structure of the spacer arm play a very important role in antibody recognition, a wide range of methods for steroid tethering have been developed.

Derivatization of progesterone at C11 can be performed by using commercially available and cheap (+)-11-α-hydroxyprogesterone (**1**) as the starting material. Most commonly, **1** has been converted into its ω-substitued ester derivatives.[2] However, the ester linkage has the known problem of chemical lability. Although an elegant method for etherification of 3- and 17-hydroxysteroids via alkyl triflates has been reported,[3] applicability of the method for (+)-11-α-hydroxyprogesterone derivatization has not been demonstrated. I present here a method for the preparation of a chemically stabile C11 derivative of progesterone. Its suitability for further derivatization with a luminescent europium(III) chelate is also demonstrated.

Results and discussion

The methylthiomethyl ether, **2**, is the known by-product of oxidation of (+)-11-α-hydroxyprogesterone (**1**) in a mixture of DMSO and acetic anhydride.[4] It is also known that methylthiomethyl ether formation can be markedly increased by addition of acetic acid to the reaction mixture.[5,6] Indeed, treatment of **1** with a mixture of DMSO, acetic acid and acetic anhydride for two days at ambient temperature gave the methylthiomethyl derivative **2** in 89% isolated yield (Scheme 1); only traces of 11-ketoprogesterone, the product of oxidation, were formed. It is worth noting that methylthiomethylation of **1** with dimethyl sulfide in the presence of benzoyl peroxide, as described for testosterone,[7] was not successful.

For the attachment of spacer arms to methylthiomethyl ethers, the latter can be easily converted *in situ* into more reactive halomethyl ethers, from which the halogen may be substituted with a wide range of nucleophiles.[8–13] Two alternative methods for this have been reported. Matteucci[8] and Zavgorodni *et al.*[9] converted the methylthiomethyl ether first into the corresponding bromomethyl derivative, from which the bromine ion was subsequently displaced with a nucleophile using a base as a catalyst. The method of van Boom[10] consists of treatment of methylthiomethyl ether and alcohol with *N*-iodosuccinimide (NIS) and a catalytic amount of trifluoromethanesulfonic acid (TfOH). In the present case, the latter method was chosen. Accordingly, short treatment of **2** with methyl glycolate in the the presence NIS and TfOH yielded the desired progesterone derivative **3** together with some **1**, the product of methythiomethyl ether hydrolysis, which were easily separated on silica gel. Finally, saponification of the ester function gave the progesterone derivative bearing a carboxymethoxymethoxy tether arm at C11 (**4**).

In order to demonstrate the suitability of **4** for further derivatization needed for non-radioactive immunoassays, it was allowed to react with a europium(III) chelate of 2,2′,2″,2‴{[4-[(4-aminophenyl)ethynyl]pyridine-2,6-diyl}bis(methylenenitrilo)]tetrakis(acetic acid) (**5**)[14] under standard conditions.[15] The desired tracer (**6**) was successfully isolated by precipitation from acetone followed by TLC purification.

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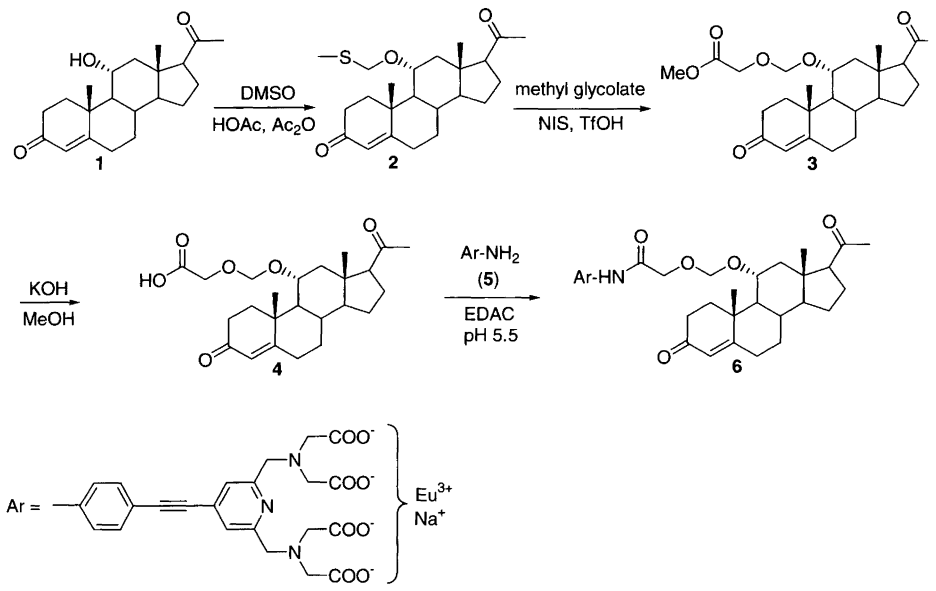

Scheme 1.

In summary, a simple method for the attachment of a linker arm to the sterically hindered 11 position of progesterone is described. The tether arm of **4** is not labile under basic conditions or in the presence of esterases, which are the known problems associated with the most commonly used steroid hemisuccinates. If desired, different tethers can be introduced simply by changing the nucleophile used. Although the present approach for steroid tethering is demonstrated only for **1**, the method should, in all likelihood, be applicable to other hydroxysteroids as well.

Experimental

Adsorption column chromatography was performed on columns packed with silica gel 60 (Merck). Analytical and preparative TLC was carried out on silica gel 60 F_{254} plates (Merck) with the following eluents A, CH_2Cl_2–MeOH 97:3 (v/v); B, CH_2Cl_2–MeOH 7:3 (v/v); C, MeCN–H_2O 4:1 (v/v). NMR spectra were recorded on a Jeol LA-400 spectrometer operating at 399.8 and 100.5 MHz for ^1H and ^{13}C, respectively. Me_4Si was used as an internal reference. Coupling constants are given in Hz. For ^1H NMR only the most characteristic signals are reported. IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrophotometer. Fast atom bombardment mass spectra were recorded on a VG ZabSpec-ao TOF instrument in the positive detection mode.

11-α-Methylthiomethoxyprogesterone **2**. 11-α-Hydroxyprogesterone (1.0 g, 3.03 mmol) was dissolved in the mixture of DMSO (6 ml), acetic acid (2 ml) and acetic anhydride (6 ml), stirred for 2 days at ambient temperature and then concentrated (oil pump). The residue was suspended in sat. $NaHCO_3$ and the crude product was separated as an oil. Purification on silica gel (eluent $CHCl_3$) yielded 1.06 g (89%) of **2**. R_f (A) 0.39. ^1H NMR ($CDCl_3$): δ 5.73 (1 H, s, H-4), 4.68 (2 H, s, OCH_2S), 3.92 (1 H, m, H11), 2.22 (3 H, s, COMe), 2.14 (3 H, s, SCH_3), 1.33 (3 H, s, 19-CH_3), 0.74 (3 H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$): δ 208.60, 199.77, 170.54, 124.50, 74.78, 72.55, 62.92, 56.82, 55.17, 43.62, 39.86, 36.89, 35.03, 34.06, 33.40, 31.60, 31.22, 24.18, 23.05, 18.39, 15.61, 14.30. IR (film): 1709, 1667, 1613, 1042. MS: *m/z* 391 ($M^+ + H$).

11-α-Methoxycarbonylmethoxymethoxyprogesterone **3**. Compound **2** (0.2 g, 0.51 mmol) and methyl glycolate (90 µl, 1.0 mmol) were dissolved in dry 1,2-dichloroethane (5 ml) containing powdered 3 Å molecular sieves, and the mixture was cooled on an ice–water bath. A freshly prepared solution of *N*-iodosuccinimide (0.11 g, 0.51 mmol) and TfOH (10 µl) in dry diethyl ether–1,2-dichloroethane (10 ml; 1:1, v/v) was added and the mixure was stirred for 10 min after which it was diluted with methylene chloride (20 ml) and poured into aqueous sodium thiosulfate. The organic layer was separated, dried (Na_2SO_4) and concentrated. Purification on silica gel (eluent A) yielded the title compound as an oil (0.14 g, 64%). R_f (A): 0.35. ^1H NMR ($CDCl_3$): δ 5.74 (1 H, s, H-4), 4.89 (1 H, d, *J* 6.8, OCH_aO), 4.85 (1 H, d, *J* 6.8, OCH_bO), 4.22 (2 H, AB, *J* 7.84, $OCOCH_2O$), 3.94 (1 H, m, H-11), 3.77 (3 H, s, $COOCH_3$), 2.13 (3 H, s, $COCH_3$), 1.29 (3 H, s, 19-CH_3), 0.69 (3 H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$): δ 208.55, 199.77, 170.45, 170.19, 124.58, 94.75, 77.23, 64.80, 63.07, 56.81, 55.08, 51.95, 46.01, 43.64, 39.88, 37.05, 35.02, 34.10, 33.49, 31.61, 31.28, 24.22, 22.81, 18.42, 14.34. IR (film): 1757, 1702, 1670, 1055. MS: *m/z* 433 ($M^+ + H$).

11-α-Carboxymethoxymethoxyprogesterone **4**. Compound **3** (0.10 g, 0.23 mmol) was dissolved in 0.5 M methanolic

KOH containing 2% (v/v) water (5 ml) and stirred for 1 h at ambient temperature. The reaction mixture was diluted with water (10 ml) and washed with ether (10 ml). The aqueous layer was acidified with 10% citric acid and extracted with chloroform. The organic layer was dried over molecular sieves and concentrated to give quantitatively **4**. R_f (B): 0.34. ^{1}H NMR ($CDCl_3$): δ 7.17 (1 H, br, COOH), 5.77 (1 H, s, H-4), 4.90 (1 H, d, *J* 6.8, OCH_aO), 4.79 (1 H, d, *J* 6.8, OCH_bO), 4.24 (2 H, AB, *J* 16.6, $HOOCCH_2O$), 3.95 (1 H, m, H-11), 2.13 ($COCH_3$), 1.29 (3 H, s, 19-CH_3), 0.69 (3 H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$): δ 209.19, 200.57, 173.24, 171.32, 124.49, 94.77, 77.23, 64.59, 63.06, 56.77, 55.02, 45.99, 43.68, 39.94, 36.97, 35.01, 33.97, 33.56, 31.59, 31.29, 24.21, 22.88, 18.44, 14.33. IR (film): 1740, 1701, 1667, 1054. MS: *m/z* 419 ($M^+ + H$).

Labelling of **4** *with an aminochelate* **5**. Compound **4** (20 mg, 25 μmol) was dissolved in dioxane (300 μl). Morpholin-4-ylethanesulfonic acid buffer (0.5 M, pH 5.5; 600 μl), compound **5** (50 mg, 75 μmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (10 mg, 50 μmol) were added, and the mixture was stirred for 1 h at ambient temperature. Precipitation from acetone yielded crude **6**, which was purified on a preparative TLC plate (eluent C). R_f (C): 0.35. MS: *m/z* 1079 ($M^+ + Eu^{3+} 2Na^+$).

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