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Expeditious synthesis of steroids containing a 2-methylsulfanyl-acetyl side chain as potential glucocorticoid receptor imaging agents

Frank Wuest[a,*], *Kathryn E. Carlson*[b], *John A. Katzenellenbogen*[b]

[a] *Institut für Radiopharmazie, Forschungszentrum Dresden-Rossendorf e.V., Postfach 510119, 01314 Dresden, Germany*

[b] *Department of Chemistry, University of Illinois, Urbana, IL 61801, USA*

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ABSTRACT

In our effort to develop imaging agents for brain glucocorticoid receptors, we have prepared several novel glucocorticoids possessing a 2-methylsulfanyl-acetyl side chain. The synthesis was accomplished via a Mitsunobu reaction with thiobenzoic acid starting from cortisol, prednisolone, dexamethasone and triamcinolone acetonide to give the corresponding *S*-thiobenzoates in 75–82% yield. Subsequent saponification and reaction with methyl iodide afforded C-21 methylthioethers in 68–82% yield. All compounds were tested in an in vitro glucocorticoid receptor-binding assay. Triamcinolone acetonide-based compound **12** showed promising binding affinity of 144% relative to dexamethasone (100%). Compound **12** was selected for radiolabeling with the short-lived positron emitter carbon-11. The radiolabeling was carried out starting from *S*-thiobenzoate **8** and in situ formation of the corresponding sodium thiolate, which was further reacted with [^{11}C]methyl iodide. The obtained radiochemical yield was 20–30%. The specific activity was determined to be 20–40 GBq/μmol at the end-of-synthesis, and the radiochemical purity exceeded 98%.

1. Introduction

Corticosteroids and their corresponding receptors regulate a broad variety of physiological functions in the peripheral and in the central nervous system. Their production is controlled via the hypothalamus-pituitary-adrenocortical (HPA) axis. In response to stress, corticosteroids are released from the adrenals in high amounts, and these circulating glucocorticoids, in stress-range concentrations, mediate the negative feedback inhibition of the HPA axis through binding to the glucocorticoid receptor (GR). There is strong evidence that stress-induced dysfunction of the HPA axis is involved in the pathogenesis of psychiatric disorders such as anxiety and severe depression [1–5].

Glucocorticoid receptor ligands that are labelled with short-lived positron emitters such as carbon-11 (^{11}C, $t_{1/2}$ = 20.4 min) and fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min) would be very useful for in vivo imaging of the levels of brain GRs by means of positron-emission-tomography (PET). Various attempts have been made over the last two decades to prepare ^{11}C- and ^{18}F-labeled PET radiotracers in order to study the pathological and physiological mechanisms behind GR-mediated abnormalities of the HPA axis function and regulation [6–13]. This research has mainly focused on

* *Corresponding author.*
E-mail address: f.wuest@fzd.de (F. Wuest).

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steroidal glucocorticoids, including classical GR ligands like cortisone, prednisolone, dexamethasone, as well as aryl-[3,2-c]pyrazoles, as potential GR imaging agents. Recently, non-steroidal compounds based on a benzopyrano-quinoline structure were envisaged for the synthesis of potential PET radiotracers [14]. However, to date all reported ^{11}C- and ^{18}F-labeled compounds for imaging brain GRs have failed to meet the requirements for suitable PET radiotracers, mainly due to their metabolic instability, insufficient blood-brain-barrier penetration and/or high non-specific binding.

For the synthesis of PET radiotracers for imaging GR and other receptors, the method used for radiolabeling should be simple and robust to give the potential radiotracers in sufficient and reliable radiochemical yields and at high effective specific activities. In this respect, heteroatom methylation reactions with the readily available labeling precursors [^{11}C]methyl iodide or [^{11}C]methyl triflate are especially attractive. Thiol groups in particular are excellent nucleophiles, giving the corresponding ^{11}C-labeled S-methyl ethers in good radiochemical yields under mild reaction conditions [15,16].

Recently, a novel class of glucocorticoids containing a modified C-17 side chain has been identified in a screening effort to discover functionally dissociated GR modulators. These steroids bind to the GR causing a conformational change in the receptor that allows it to transrepress gene transcription but have little or no transactivation activity [17,18]. For the design and synthesis of radiotracers for imaging GR by means of PET, this functional distinction is not relevant. The compounds showed GR binding affinities comparable to that of the potent glucocorticoid dexamethasone. A selection of representative examples for functionally dissociated GR modulators and dexamethasone is given in Fig. 1.

Compounds RU 24782 and RU 24858 show a relative binding affinity (RBA) of 85 and 128%, respectively, relative to dexamethasone (RBA = 100%). The promising RBA value and the 21-methyl-thioether substitution pattern make compound RU 24782 an interesting lead structure for the design and synthesis of a series of 21-methylthioether group-containing compounds capable of being labelled with the short-lived positron emitter ^{11}C ($t_{1/2}$ = 20.6 min).

In this report, we describe the synthesis of a set of glucocorticoids possessing a 2-methylsulfanyl-acetyl side chain at position C-17, and we have evaluated their receptor binding properties toward the GR. The introduction of a 21-methylthioether group was easily accomplished through a Mitsunobu reaction followed by a saponification–methylation sequence as an expeditious route for steroids containing a 2-methylsulfanyl-acetyl side. One candidate was selected for labeling with ^{11}C by the reaction of [^{11}C]methyl iodide with the corresponding C-21 thiol group-containing compound as labeling precursor.

2. Experimental

2.1. General

Melting points were determined on a BOËTIUS melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 plastic plates, with visualization under UV (254 nm). Flash chromatography was performed as described by Still et al. [19] using Merck silica gel (0.040–0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-400 at 400 and 100 MHz, respectively. $CDCl_3$ was used as solvent. Elemental analyses were obtained on a LECO CHNS 932 elemental analyser. Solvents and reagents were purchased from Sigma, Fluka, or Aldrich. Cortisol **1**, prednisolone **2**, dexamethasone **3** and triamcinolone acetonid **4** were purchased from Sigma.

2.2. Chemistry

2.2.1. General procedure for the synthesis of S-thiobenzoates 5–8

Diisopropyl-azodicarboxylate (DIAD) (1.3 ml, 6 mmol) was added via syringe to a solution of PPh_3 (1.57 g, 6 mmol) in THF (20 ml) at 0 °C. The mixture was stirred for 30 min. Then, 21-hydroxy steroid **1–4** (3 mmol) and thiobenzoic acid (738 μl, 6 mmol) in THF (10 ml) were slowly added. The reaction mixture was stirred for 1 h at 0 °C and for one additional hour at room temperature. Saturated $NaHCO_3$ solution was added, and the mixture was extracted with ethyl acetate. After evaporation of the solvent the residue was purified by flash-chromatography (50% EtOAc/petroleum ether).

21-(S)-Benzoylthio-11β,17α-dihydroxy-4-pregnene-3,20-dione **5**. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (s, 3H; 18-CH_3), 1.45 (s, 3H; 19-CH_3), 4.11 (AB quartet, Δν = 304 Hz, *J* = 16.6 Hz, 2H; CH_2–SBz), 4.50 (m, 1H; 11α-H), 5.69 (bs, 1H; 4-H), 7.46 (m, 2H; Ar–H), 7.60 (m, 1H; Ar–H), 7.96 (m, 2H; Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.5, 20.9, 23.7, 31.4, 32.0, 32.6, 33.8, 34.3, 34.9, 36.7, 39.2, 40.0, 47.2, 51.8, 55.9, 68.3, 90.7, 122.3, 127.4, 128.7, 133.8, 136.1, 172.4, 191.8, 199.7, 205.7. Melting point: 212–215 °C. Analysis calculated for $C_{28}H_{34}O_5S$: C, 69.68; H, 7.10; S, 6.64; found: C, 69.43; H, 6.88; S, 6.19.

21-(S)-Benzoylthio-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione **6**. Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (s, 3H; 18-CH_3), 1.47 (s, 3H; 19-CH_3), 4.09 (AB quartet, Δν = 293 Hz, *J* = 16.8 Hz, 2H; CH_2–SBz), 4.54 (m, 1H; 11α-H), 6.03 (bs, 1H; 4-H),

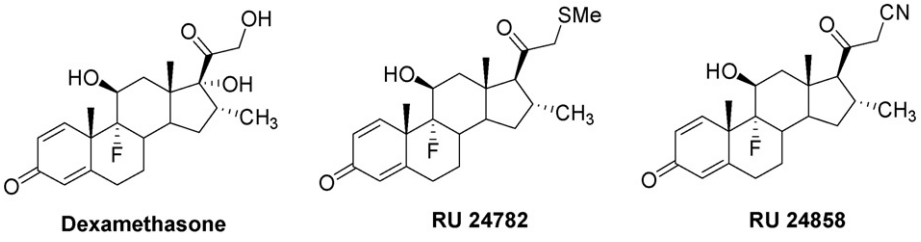


Fig. 1 – Structure of dexamethasone and glucocorticoids containing a modified C-17 side chain.

6.28 (dd, J = 10.1 Hz, J = 1.9 Hz, 1H; 2-H), 7.27 (d, J = 10.1 Hz, 1H; 1-H), 7.46 (m, 2H; Ar–H), 7.60 (m, 1H; Ar–H), 7.96 (m, 2H; Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.4, 21.1, 23.9, 31.2, 32.0, 33.9, 34.4, 36.6, 40.0, 44.1, 47.4, 51.3, 55.2, 70.3, 90.7, 122.4, 127.4, 127.8, 128.7, 133.9, 136.1, 156.23, 170.1, 186.6, 191.8, 205.7. Melting point: 214–217 °C. Analysis calculated for $C_{28}H_{32}O_5S$: C, 69.97; H, 6.71; S, 6.67; found: C, 69.39; H, 6.43; S, 6.41.

21-(S)-Benzoylthio-9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione **7**. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 7.3 Hz, 3H; 16α-CH_3), 1.07 (s, 3H; 18-CH_3), 1.56 (s, 3H; 19-CH_3), 4.03 (AB quartet, $\Delta\nu$ = 351 Hz, J = 16.8 Hz, 2H; CH_2–SBz), 4.42 (m, 1H; 11α-H), 6.13 (bs, 1H; 4-H), 6.35 (dd, J = 9.9 Hz, J = 1.8 Hz, 1H; 2-H), 7.21 (d, J = 9.9 Hz, 1H; 1-H), 7.46 (m, 2H; Ar–H), 7.60 (m, 1H; Ar–H), 7.95 (m, 2H; Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.7, 16.2, 17.0, 19.5, 22.9 (d, J = 5.7 Hz), 27.3, 31.0, 32.4 (d, J = 19.5 Hz), 34.2, 35.6, 36.5, 37.3, 43.8, 48.0 (d, J = 22.8 Hz), 72.3 (d, J = 38.5 Hz), 90.5, 91.8, 100.2 (d, J = 176.5 Hz), 111.1, 125.2, 127.4, 128.7, 129.9, 133.9, 136.0, 151.8, 166.4, 187.7, 205.3. Melting point: 184–188 °C. Analysis calculated for $C_{29}H_{33}FO_5S$: C, 67.95; H, 6.49; S, 6.26; found: C, 67.61; H, 6.06; S, 5.98.

21-(S)-Benzoylthio-9α-fluoro-16α-17-O-isopropylidene-11β,16α,17α-trihydroxy-1,4-pregna-diene-3,20-dione **8**. Yield: 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H; 18-CH_3), 1.22 (s, 3H; CH_3), 1.46 (s, 3H; CH_3), 1.56 (s, 3H; 19-CH_3), 4.23 (AB quartet, $\Delta\nu$ = 57 Hz, J = 18.3 Hz, 2H; $-CH_2$–SBz), 4.47 (m, 1H; 11α-H), 5.04 (d, J = 5.1 Hz, 1H), 6.14 (bs, 1H; 4-H), 6.36 (dd, J = 10.2 Hz, J = 1.8 Hz, 1H; 2-H), 7.21 (d, J = 10.2 Hz, 1H; 1-H), 7.48 (m, 2H; Ar–H), 7.61 (m, 1H; Ar–H), 7.99 (m, 2H; Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.5, 16.9, 22.9 (d, J = 5.7 Hz), 25.7, 26.5, 27.5, 30.9, 33.2 (d, J = 19.6 Hz), 33.5, 38.1, 43.1, 45.3, 48.1 (d, J = 22.9 Hz), 72.0 (d, J = 38.4 Hz), 82.0, 100.0 (d, J = 176.6 Hz), 111.4, 125.2, 127.4, 128.7, 129.9, 133.9, 136.2, 151.8, 165.7, 186.4, 190.8, 203.8. Melting point: 230–234 °C. Analysis calculated for $C_{31}H_{35}FO_6S$: C, 67.13; H, 6.36; S, 5.78; found: C, 66.74; H, 6.02; S, 5.43.

2.2.2. *General procedure for the synthesis of C-21 methylthioethers* **9**–**12**

S-Thiobenzoate **5**–**8** (0.2 mmol) was stirred in MeOH (5 ml) and 1 N NaOH (0.5 ml) at room temperature for 30 min. Then, MeI (30 μl) was added and the mixture was stirred for 3 h at room temperature. Water (50 ml) was added and the mixture was extracted with ethyl acetate. After evaporation of the solvent the residue was purified by flash-chromatography (50% EtOAc/petroleum ether).

21-Methylthio-11β,17α-dihydroxy-4-pregnene-3,20-dione **9**. Yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (s, 3H; 18-CH_3), 1.44 (s, 3H; 19-CH_3), 2.11 (s, 3H; SCH_3), 3.38 (AB quartet, $\Delta\nu$ = 84 Hz, J = 13.5 Hz, 2H; $-CH_2$–SMe), 4.46 (m, 1H; 11α-H), 5.68 (bs, 1H; 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.8, 17.9, 21.0, 23.9, 29.7, 31.4, 32.0, 32.7, 33.8, 34.7, 39.2, 39.7, 39.9, 48.0, 51.5, 55.9, 68.4, 89.5, 122.3, 172.0, 199.5, 206.2. Melting point: 217–219 °C. Analysis calculated for $C_{22}H_{32}O_4S$: C, 67.31; H, 8.22; S, 8.17; found: C, 66.93; H, 7.89; S, 7.82.

21-Methylthio-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione **10**. Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (s, 3H; 18-CH_3), 1.45 (s, 3H; 19-CH_3), 2.11 (s, 3H; SCH_3), 3.37 (AB quartet, $\Delta\nu$ = 82 Hz, J = 13.5 Hz, 2H; $-CH_2$–SMe), 4.49 (m, 1H; 11α-H), 6.02 (bs, 1H; 4-H), 6.27 (dd, J = 9.9 Hz, J = 1.8 Hz, 1H; 2-H), 7.24 (d, J = 9.9 Hz, 1H; 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.8, 17.8, 21.1, 24.2, 29.7, 29.8, 31.2, 33.9, 34.7, 39.7, 39.9, 48.2, 50.9, 55.2, 70.3, 90.7, 122.5, 127.9, 136.3, 155.9, 187.0, 203.2. Melting point: 237–239 °C. Analysis calculated for $C_{22}H_{30}O_4S$: C, 67.66; H, 7.74; S, 8.21; found: C, 67.32; H, 7.49; S, 7.89.

21-Methylthio-9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione **11**. Yield: 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 7.3 Hz, 3H; 16α-CH_3), 1.06 (s, 3H; 18-CH_3), 1.54 (s, 3H; 19-CH_3), 2.08 (s, 3H; SCH_3), 3.28 (AB quartet, $\Delta\nu$ = 212 Hz, J = 12.8 Hz, 2H; $-CH_2$–SMe), 4.35 (m, 1H; 11α-H), 6.11 (bs, 1H; 4-H), 6.33 (dd, J = 9.9 Hz, J = 1.8 Hz, 1H; 2-H), 7.19 (d, J = 9.9 Hz, 1H; 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.6, 15.5, 17.3, 22.9 (d, J = 5.7 Hz), 27.3, 31.0, 32.3 (d, J = 19.4 Hz), 34.2, 35.9, 37.1, 39.1, 43.8, 48.3 (d, J = 22.8 Hz), 72.1 (d, J = 38.4 Hz), 90.7, 91.4, 100.2 (d, J = 176.5 Hz), 125.0, 129.7, 152.3, 166.4, 186.7, 205.5. Melting point: 176–180 °C. Analysis calculated for $C_{23}H_{31}FO_4S$: C, 65.38; H, 7.39; 15.15; S, 7.59; found: C, 65.11; H, 7.21; S, 7.24.

21-Methylthio-9α-fluoro-16α-17-O-isopropylidene-11β,16α,17α-trihydroxy-1,4-pregna-diene-3,20-dione **12**. Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H; 18-CH_3), 1.15 (s, 3H; CH_3), 1.42 (s, 3H; CH_3), 1.54 (s, 3H; 19-CH_3), 2.19 (s, 3H; SCH_3), 3.51 (AB quartet, $\Delta\nu$ = 23 Hz, J = 16.1 Hz, 2H; $-CH_2$–SMe), 4.41 (m, 1H; 11α-H), 5.02 (d, J = 5.8 Hz, 1H), 6.13 (bs, 1H; 4-H), 6.35 (dd, J = 10.2 Hz, J = 1.8 Hz, 1H; 2-H), 7.17 (d, J = 10.2 Hz, 1H; 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.1, 16.9, 22.9 (d, J = 5.7 Hz), 25.7, 26.5, 27.5, 30.9, 33.2 (d, J = 19.5 Hz), 37.6, 39.8, 43.1, 45.4, 48.1 (d, J = 22.7 Hz), 72.5 (d, J = 38.6 Hz), 82.0, 98.4, 100.8 (d, J = 179.5 Hz), 111.1, 125.2, 129.9, 151.7, 166.0, 186.4, 204.7. Analysis calculated for $C_{25}H_{33}FO_5S$: C, 64.63; H, 7.16; S, 6.90; found: C, 64.31; H, 6.90; S, 6.57.

2.3. *Radiochemistry*

2.3.1. *General*

$[^{11}C]CO_2$ was produced by the $^{14}N(p,a)^{11}C$ reaction on a IBA CYCLONE 18/9 cyclotron. The synthesis was carried out in a remotely controlled synthesis apparatus (Nuclear Interface, Münster, Germany). [^{11}C]Methyl iodide was prepared according to Crouzel et al. [20]. Semi-preparative HPLC-separation and analytical HPLC were performed using a JASCO HPLC system (JASCO, HPLC-pump PU-1580) equipped with a UV–vis detector (JASCO UV-1575) and gamma detector (Raytest Gabi).

21-Methylthio-9α-fluoro-16α-17-O-isopropylidene-11β,16α,17α-trihydroxy-1,4-pregna-diene-3,20-dione ***[^{11}C]12***. The thiolate intermediate required for the methylation reaction was generated 15 min prior to the distillation of [^{11}C]methyl iodide. [^{11}C]Methyl iodide was transferred in a stream of nitrogen into the reaction vessel containing thiobenzoate **8** (0.5 mg) in MeOH (400 μl) and 5 N NaOH (30 μl) at room temperature. The formation of the corresponding desmethyl precursor occurred in situ immediately after the addition of NaOH to thiobenzoate **8** in MeOH at room temperature. After completion of the [^{11}C]methyl iodide transfer, the reaction vessel was sealed and heated at 50 °C for 5 min. The reaction mixture was diluted with eluent (1 ml) and the mixture was transferred from the reaction vessel onto a semi-preparative C-18 column (Phenomenex Luna C18(2), 250 mm × 10 mm, 10 μm) using CH_3CN:H_2O (55:45) as the mobile phase at a flow rate of 9 ml/min. The

product eluted between 6 and 7 min. The fraction containing the ^{11}C-labeled steroid **[^{11}C]12** was diluted with 30 ml of water and passed through a SPE-cartridge RP-18E (40–63 μm). The product was eluted from the cartridge with EtOH (1 ml). An aliquot was taken for quality control using radio-HPLC (SUPELCO Discovery C18, 150 mm × 4.6 mm, 5 μm, acetonitrile/water (55:45, v/v), flow rate: 1 ml/min, t_R = 6.1 min). Compound **[^{11}C]12** was obtained in 20–30% decay-corrected radiochemical yield (related to [^{11}C]CO_2). The radiochemical purity exceeded 98%, and the specific activity was determined to be 20–40 GBq/μmol at the end-of-synthesis.

2.4. *Biological methods*

2.4.1. *Determination of the receptor binding affinity*

The binding affinity of steroids **5–12** towards the (GR) was determined by a modification of the method reported for the estrogen receptor [6,21]. The radiotracer was [^{3}H]dexamethasone and adrenalectomized male rat liver cytosol was used as the protein. The cytosol was incubated with buffer or several concentrations of steroids **5–12** as competitors together with [^{3}H]dexamethasone at 0 °C for 18–24 h. Compounds **5–12** were used in dimethylformamide/buffer (1:1) to ensure complete solubility. Free steroid was adsorbed on dextran-coated charcoal as described by Katzenellenbogen et al. [22]. [^{3}H]Dexamethasone was [1,2,4,-^{3}H]Dexamethasone (Amersham Biosciences, Piscataway, NJ).

3. Results and discussion

3.1. *Synthesis of glucocorticoids containing a C-17 2-methylsulfanyl-acetyl side chain*

The endogenous corticosteroid cortisol **1** possesses the characteristic 17α,21-dihydroxy-20-keto substitution pattern at the D-ring of the steroid backbone. The C-21 hydroxy group can easily be transformed into a wide variety of different functionalities. Prominent examples include C-21 halides and C-21 esters [23–25]. Moreover, the development of GR imaging probes for positron-emission-tomography (PET) has led to the synthesis of several C-21 ^{18}F-substituted steroids as potential radiotracers, such as 21-[^{18}F]fluoro-21-deoxy-dexamethasone [6], 21-[^{18}F]fluoro-21-deoxyprednisone [9] and 21-[^{18}F]fluoro-21-deoxy-triamcinolone acetonide [6].

Various steroid derivatives containing a sulfur moiety at the C-21 position are also known from the literature. The introduction of a sulfur moiety at position C-21 typically exploits the conversion of the C-21 hydroxy group into a good leaving group (e.g., OMs or OTs) followed by the reaction with a sulfur nucleophile such as potassium thiolacetate or thiourea [26–29]. An alternative approach to this conversion, reported by our group, effected the conversion of the C-21 hydroxy group of progesterone into the corresponding *S*-thiobenzoate by means of a Mitsunobu reaction [30]. Subsequent saponification gave the free C-21 thiol group, which was further reacted with oxorhenium(V) complexes to form several rhenium-progesterone mixed-ligand complexes. The straightforward and high yielding synthesis of 21-[(*S*)-benzoylthio]-progesterone via the Mitsunobu reaction of progesterone with PPh_3/DIAD/thiobenzoic acid prompted us to apply this approach for the synthesis of a series of C-21 sulfur-containing glucocorticoids. The general outline of the synthesis of C-21 sulfur-containing glucocorticoids is illustrated in Fig. 2.

Starting from commercially available C-21 hydroxy-containing steroids **1–4**, the synthesis commenced by a Mitsunobu reaction using two equivalents of DIAD, PPh_3 and thiobenzoic acid to afford the corresponding *S*-thiobenzoates **5–8** in good chemical yields of 75–82%. Formation of the *S*-thiobenzoates was confirmed by characteristic AB coupling pattern at 4.03–4.23 ppm in the ^{1}H NMR, which is indicative of the C-21 methylene protons in compounds **5–8**, and the typical chemical shift of the thiocarbonyl group at about 191 ppm as observed in the ^{13}C NMR spectra.

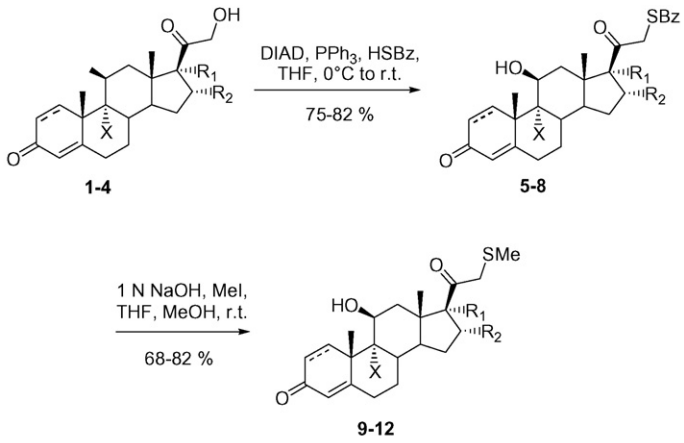
The C-21 *S*-thiobenzoates **5–8** were dissolved in MeOH and 1 N NaOH was added to release the C-21 thiol groups as sodium thiolate intermediates. The in situ formed thiolates were treated with a 2.4-fold excess of methyl iodide to afford the desired C-21 methylthioethers **9–12** in good yields ranging from 68 to 82%. The introduction of the methylthioether group could be monitored by NMR of compounds **9–12** that showed characteristic singlets at 2.08–2.19 ppm in the ^{1}H NMR.

The total yield of C-21 methylthioethers **9–12** was in the range of 50–60% for the two-step reaction sequence. With respect to a potential radiolabeling with the short-lived positron emitter ^{11}C at the C-21 position via *S*-methylation with [^{11}C]methyl iodide, the use of *S*-thiobenzoates **5–8** as labeling precursors offers several advantages. Compared to the corresponding free thiols, *S*-thiobenzoates are fairly stable compounds that can be handled without special precautions. When appropriately stored, the *S*-thiobenzoates can be used without the loss of reactivity and decomposition over months. More important, *S*-thiobenzoates **5–8** can be converted in situ very easily into the corresponding C-21 thiolates by simple treatment with a base such as NaOH. This approach circumvents the isolation and handling of the oxidation sensitive free thiols as labeling precursors.

3.2. *Glucocorticoid receptor binding affinities and log $P_{o/w}$ values of glucocorticoids*

The GR binding affinities of all synthesized steroids and reference compounds dexamethasone and cortisol **1** are listed, along with calculated log $P_{o/w}$, values, in Table 1.

The GR binding affinities were assayed using a liver cytosol preparation from adrenalectomized rats, with [^{3}H]dexamethasone as radiotracer and unlabeled dexamethasone **3** as standard (RBA = 100%). Most compounds synthesized in this work show lower GR binding in comparison to the synthetic and high-affinity GR ligand dexamethasone **3**. However, some of the tested compounds showed relative binding affinities higher (compounds **7**, **8** and **12**) or comparable (compounds **6** and **11**) to that of the endogenous hormone cortisol **1**. Moreover, we were very pleased to find a binding affinity of 144% for compound **12**, which is significantly higher than the binding affinity of dexamethasone **3**. In fact, the GR binding affinity of steroid **12** is in the same range as determined for a related C-21 fluorine-substituted



Compound	X	R^1	R^2	C^1- C^2
1 (Cortisol)	H	OH	H	-
2 (Prednisolone)	H	OH	H	Δ
3 (Dexamethasone)	F	OH	Me	Δ
4 (Triamcinolone acetonide)	F	acetonide	acetonide	Δ
5	H	OH	H	-
6	H	OH	H	Δ
7	F	OH	Me	Δ
8	F	acetonide	acetonide	Δ
9	H	OH	H	-
10	H	OH	H	Δ
11	F	OH	Me	Δ
12	F	acetonide	acetonide	Δ

Fig. 2 – Synthesis of 2-methylsulfanyl-acetyl side chain-containing glucocorticoids.

triamcinolone acetonide (RBA = 174%), which was prepared and tested as ^{18}F-labeled PET radiotracer for imaging brain GR [12].

All steroids, in this work, showed increased lipophilicity (expressed as calculated $\log P_{o/w}$ values) compared with cortisol **1** and dexamethasone **3**. As expected, the *S*-thiobenzoates **5–8** show higher $\log P_{o/w}$ values than the corresponding thiomethylethers **9–12**, being in the range of 4.03–5.38 and 2.43–3.80, respectively. The enhanced lipophilicity might be a benefit for improved blood-brain-barrier penetration, although a high lipophilicity is also often accompanied by high non-specific binding when appropriately radiolabeled compounds are used for brain imaging studies.

Within the series of compounds tested, *S*-thiobenzoates **5–7** show a higher binding affinity compared with the corresponding methylthioether **9–11**. However, this trend is not valid for glucocorticoids **8** and **12** based upon the triamcinolone acetonide structure. Methylthioether **12** shows a significant higher binding affinity (RBA = 144%) than *S*-thiobenzoates **8** (RBA = 27.6%). In this special case, the lack of a free 17α-OH group in compounds **8** and **12** is a structural difference that may serve as a possible explanation for this observation. The methylthioether moiety in steroids **9–12**, which can act as a weak H-bond acceptor, coupled with the presence of a 17α-OH group as a potential H-bond donor in compounds **9–11** may lead to intramolecular H-bond formation, which seems to have a detrimental effect on the receptor binding. This explanation is also consistent with the structure of C-21 methylthioether- and nitrile-containing GR ligands RU 24782 and RU 24858. Both compounds, which lack

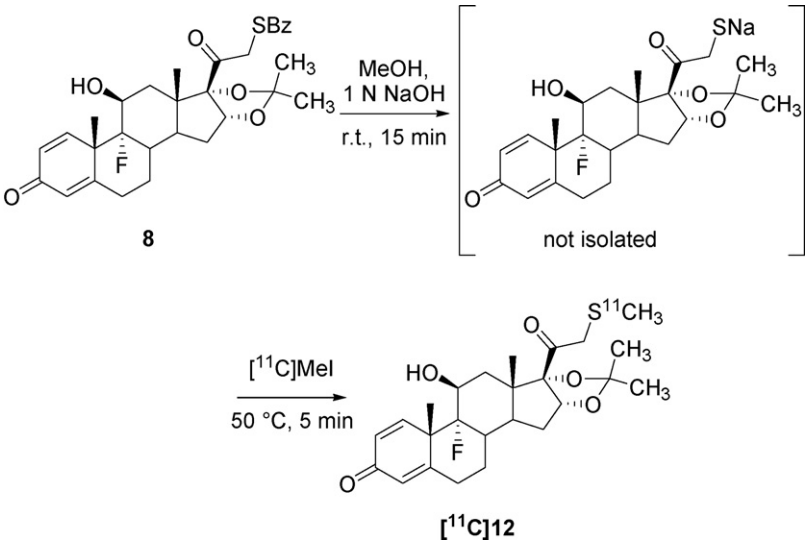


Fig. 3 – Radiosynthesis of [^{11}C]12.

Table 1 – Relative binding affinities (RBAs) of glucocorticoids 5–12 for the GR related to dexamethasone (RBA = 100%)

Compound	RBA (0 °C)	Log $P_{o/w}$ [a]
1 (Cortisol)	9.6 [6][b]	1.43 (1.61 [31])[c]
3 (Dexamethasone)	100	2.06
5	3.2	4.03
6	6.6	4.30
7	19.1	4.67
8	27.6	5.38
9	1.4	2.48
10	3.5	2.74
11	5.8	3.12
12	144	3.80

Each value represents the mean of two determinations, with a coefficient of variance of 0.3.

[a] Log $P_{o/w}$ values have been calculated based on ACDLabs predictions.

[b] Literature value [6].

[c] Literature value [31].

a 17α-OH group, and therefore, cannot form such a H-bond, show promising RBA values of 85 and 128%, respectively. The promising high RBA value of 144% for steroid **12** makes this compound an interesting candidate for radiolabeling with the short-lived positron emitter ^{11}C.

3.3. *Radiochemical synthesis of 21-[^{11}C]methylthio-9α-fluoro-16α-17-O-isopropylidene-11β,16α,17α-trihydroxy-1,4-pregna-diene-3,20-dione [^{11}C]12*

Compound **[^{11}C]12** was prepared through a ^{11}C-methylation reaction using in situ generated C-21 thiolate and [^{11}C]methyl iodide, as shown in Fig. 3.

Fifteen minutes prior to distillation of [^{11}C]methyl iodide into the reaction vessel, the thiolate intermediate was generated in situ by treatment of 0.5 mg of S-thiobenzoate **8** in MeOH with an excess of 5 N NaOH at room temperature. The formation of the corresponding C-21 sodium thiolate could easily be monitored by a change in the reaction mixture from colorless to yellow. It is noteworthy that the use of MeOH as a protic and polar solvent seems to be essential for a successful S-methylation reaction with [^{11}C]methyl iodide. When DMF or DMSO were used as solvents, only traces of the desired ^{11}C-labeled compound **[^{11}C]12** could be detected. The reaction was completed within 5 min at 50 °C. The product mixture contained more than 70% of **[^{11}C]12** along with unreacted [^{11}C]methyl iodide and some minor unidentified impurities. The complete synthesis time including [^{11}C]methyl iodide preparation, methylation reaction and purification was about 35 min. Compound **[^{11}C]12** was obtained in a radiochemical yield of 20–30% (n = 3, decay-corrected to [^{11}C]carbon dioxide). In a typical experiment, starting from 40 GBq of [^{11}C]carbon dioxide, 2–3 GBq of **[^{11}C]12** could be prepared. The specific activity of **[^{11}C]12** was determined to be 20–40 GBq/μmol at the end-of-synthesis. The radiochemical purity of **[^{11}C]12** exceeded 98% as shown by radio-HPLC analysis. The radiopharmacological evaluation of ^{11}C-labeled compound **[^{11}C]12** is currently in progress and will be reported elsewhere.

4. Summary and conclusion

In our effort to develop imaging agents for brain glucocorticoid receptors, we have prepared several novel glucocorticoids possessing a C-21 sulfur moiety. The synthesis was easily accomplished via a Mitsunobu reaction starting from the corresponding C-21 hydroxy compounds **1–4** to give S-thio-benzoates **5–8** in good chemical yields. This reaction is not limited to thiobenzoic acids, and various different S-thioesters should be accessible with this method. The desired C-21 methylthioethers **9–12** were obtained by subsequent saponification with NaOH and methylation of the sodium thiolate intermediate with methyl iodide. Hence, the applied

Mitsunobu reaction represents a reliable and convenient synthesis approach for the straightforward incorporation of a sulphur moiety into the C-21 position starting from readily available C-21 hydroxy steroids in a single step through C-21 S-thioester formation. Subsequent release of the thiolate offers the opportunity for a broad array of reactions with different electrophiles such as alkyl halides. Moreover, the ease of access, the stability and the facile conversion into the corresponding C-21 thiolates in situ make the C-21 S-thiobenzoates excellent starting material for the radiolabeling with [^{11}C]methyl iodide.

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