Available online at www.sciencedirect.com
SCIENCE DIRECT®
Steroids 68 (2003) 177–191
Steroids
www.elsevier.com/locate/steroids

Synthesis of novel arylpyrazolo corticosteroids as potential ligands for imaging brain glucocorticoid receptors

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

[a] *Institut für Bioanorganische und Radiopharmazeutische Chemie, FZ-Rossendorf e.V., Postfach 510119, Dresden 01314, Germany*
[b] *Institut für Interdisziplinäre Isotopenforschung an der Universität Leipzig, Leipzig, Germany*
[c] *Department of Chemistry, University of Illinois, Urbana, IL 61801, USA*

Received 15 July 2002; received in revised form 8 November 2002; accepted 11 November 2002

Abstract

Corticosteroids regulate a variety of essential physiological functions, such as mineral balance and stress. The great interest in these steroids, especially the glucocorticoids, stems from roles they are thought to play in neuropsychiatric disorders, such as severe depression and anxiety.

The development of glucocorticoid receptor (GR) ligands which are appropriately labeled with short-lived positron-emitting radioisotopes would allow the non-invasive in-vivo imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context we have synthesized a series of novel arylpyrazolo steroids exhibiting different substitution patterns at the D-ring of the steroid skeleton, as ligands for brain GRs. Special attention was given to 4-fluorophenyl pyrazolo steroids, which are known to display high binding affinity toward the GR. The compounds were evaluated in a competitive radiometric receptor binding assay to determine their relative binding affinities (RBA) to the GR. Some compounds show good binding affinities of up to 56% in comparison to dexamethasone (100%). In initial experiments, selected candidates were labeled with the positron emitter fluorine-18 and in one case with the γ-emitter iodine-131.

Keywords: Arylpyrazolo corticosteroids; Glucocorticoid receptor binding; Radiolabeling; Positron emission tomography (PET)

1. Introduction

Endogenous corticosteroids exert a wide range of physiologic, biochemical, and behavioral effects in the central nervous system where the hormonal response is organized [1–3]. Corticosteroid hormones are produced in the adrenal gland, and their production is controlled via the hypothalamo-pituitary-adrenocortical (HPA) axis, a classical closed-loop endocrine system. They are released into the circulation particularly in high amounts after periods of stress. Due to their lipophilic nature, corticosteroids not only act on peripheral organs but also readily enter the brain. In neurons, they bind to two different intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). MRs and GRs are co-located in brain regions that are involved in the regulation of fear and anxiety, such as hippocampus, septum, and amygdala. GRs are also distributed in other parts of the brain, and the highest concentrations are found in regions involved in the feedback regulation of the hormonal stress response, such as the paraventricular hypothalamus, hippocampus and pituitary [4,5]. There is accumulating evidence that corticosteroids, in addition to noradrenaline, corticotropin releasing factor and serotonin, play an important role in stress and the pathophysiology of anxiety disorders and depression. It has been suggested that a disturbed HPA axis regulation may be a primary causal factor in depression. Since feedback inhibition of glucocorticoids is decreased in many depressive patients, it has been hypothesized that disturbances in the MR/GR balance may have deleterious consequences for regulation of the stress response, and it may increase vulnerability to depression.

The development of GR ligands which are appropriately labeled with short-lived positron-emitting radioisotopes would allow the non-invasive in vivo imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context, PET studies of brain GRs would provide important information on the neurobiological

* Corresponding author. Tel.: +49-351-260-3694; fax: +49-351-260-2915.
E-mail address: wuest@fz-rossendorf.de (F. Wüst).

0039-128X/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0039-128X(02)00171-X

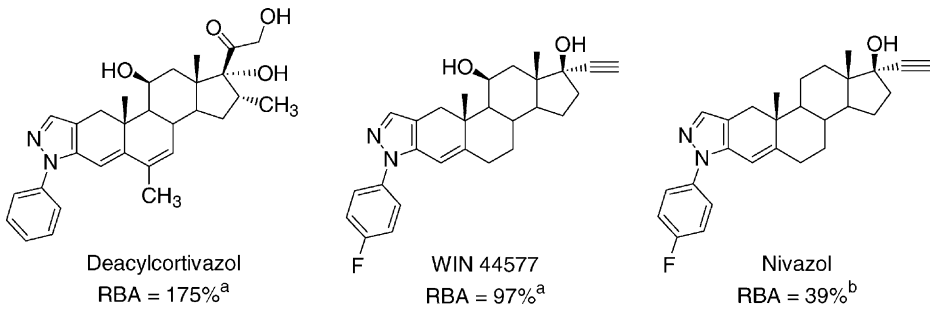

Fig. 1. High-affinity arylpyrazolo glucocorticoids (a) K.E. Carlson and J.A. Katzenellenbogen, unpublished results; (b) Rat liver cytosol, [14].

basis of GR-mediated functional abnormalities of HPA axis function in depression [6,7].

Several attempts have been made to synthesize glucocorticoids labeled with the short-lived positron emitter fluorine-18 ($t_{1/2}$ = 109.6 min) to image brain GRs [8–13]. Some of the compounds exhibited excellent in vitro GR binding, in some cases even significantly higher than the high-affinity glucocorticoid dexamethasone. However, none of the investigated compounds were suitable for in vivo visualization of brain GRs with PET. Rapid in vivo defluorination and the inability to cross the blood–brain-barrier are possible reasons for the lack of any accumulation of these compounds in the brain.

A unique set of synthetic arylpyrazolo steroids, exemplified by deacylcortivazol, nivazol and WIN 44577 (Fig. 1), have been shown to be very high-affinity ligands for the GR [14,15]. The strong GR binding affinity of compounds like WIN 44577, as well as their stable aryl-bound fluorine and their higher lipophilicity (for an anticipated enhanced brain uptake), make this class of compounds attractive for the development of novel fluorine-18 labeled ligands for PET studies of brain GR.

Intrigued by the high GR binding affinity of several arylpyrazolo steroids, we have synthesized a series of novel pyrazolo steroids exhibiting different substitution patterns at the D-ring of the steroid skeleton. The binding affinity of these compounds for the GR was determined in a competitive radiometric receptor binding assay, and in initial experiments potential candidates were labeled with the positron emitter fluorine-18 and in one case with the γ-emitter, iodine-131.

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. [16] using Merck silica-gel (0.040–0.063 mm). ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra were recorded on a Varian Inova-400 at 400, 100 and 376 MHz, respectively, with $CDCl_3$ and $DMSO_{d6}$. Low resolution mass spectra were recorded on a Micromass tandem quadrupole mass spectrometer system (1100 series Hewlett-Packard) and on Mariner mass spectrometer system (Applied Biosystems). Analytical HPLC was conducted using LaChrom systems from Merck–Hitachi. The chemical purity was determined with a PHENOMENEX LUNA C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase was MeOH/water (90/10) (A) and acetonitrile/water (70/30) (B). The system was run with a flow rate of 1.0 ml/min.

Solvents and reagents were purchased from Sigma, Fluka, or Aldrich. THF was distilled from sodium/benzophenone ketyl prior to use. Other reagents were used as received. Iodophenylhydrazine [17] and 4-hydroxy(tosyloxy)iodotoluene [18] were prepared according to literature procedures.

2.2. Organic chemistry

2.2.1. 11β-Hydroxy-17α,20:20,21-bis-(methylenedioxy)-pregn-4-ene-3-one (2)

To a suspension of hydrocortisone **1** (2.54 g, 7 mmol) in CH_2Cl_2 (100 ml) was added formalin (40 ml, 40%) and concentrated HCl (40 ml). The bi-layer system was vigorous stirred at room temperature for 3 h. The organic layer was separated, washed with saturated $NaHCO_3$ solution and flushed through a silica-gel plug. After evaporation of the solvent under reduced pressure, 2.9 g (102%) of **2**, contaminated with the 11β-MOM-ether (20%), was obtained as a wax. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H; 18-CH_3), 1.43 (s, 3H; 19-CH_3), 3.98 (m, 2H; 21-CH_2), 4.47 (m, 1H; 11α-H), 4.98 (m, 4H; O–CH_2–O from bis(methylenedioxy)), 5.66 (s, 1H; 4-H), LRMS (ESI positive) 405 [M + H].

2.2.2. 11β-Hydroxy-2-hydroxymethylene-17,20:20,21-bis-(methylenedioxy)-pregn-4-ene-3-one (3)

Steroid **2** (2.8 g, contaminated with 11β-MOM-ether) was dissolved in dry toluene (15 ml) and methyl formate (2 ml). Then, NaH (600 mg, 15 mmol, 60% dispersion in mineral

oil) was added. After stirring at room temperature for 4 h 1N HCl (100 ml) was added and the mixture was extracted several times with methylene chloride. The methylene chloride was extracted several times with 1N NaOH. After acidification of the NaOH extract with HCl and subsequent re-extraction with methylene chloride the solvent was removed to give 1.8 g (60%) of **3** as yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H; 18-CH_3), 1.33 (s, 3H; 19-CH_3), 4.00 (m, 2H; 21-CH_2), 4.40 (m, 1H; 11α-H), 5.03 (m, 4H; O–CH_2–O from bis(methylenedioxy)), 5.73 (d, J = 1.8 Hz, 1H, 4-H), 7.35 (s, 1H=CHOH). LRMS (ESI positive) 433 [M + H].

2.2.3. 2′-(4-Fluorophenyl)-11β-hydroxy-2-hydroxymethylene-17,20:20,21-bis-(methylenedioxy)-pregn-4-eno[3,2-c]pyrazole (4)

Compound **3** (1.04 g, 2.4 mmol) in HOAc (20 ml) was added to a solution of NaOAc (197 mg, 2.4 mmol) and 4-fluorophenylhydraziniumchloride (390 mg, 2.4 mmol) in HOAc (10 ml) and water (5 ml). The reaction mixture was stirred at room temperature overnight. 1N HCl (100 ml) was added followed by extraction with methylene chloride. The organic layer was washed with $NaHCO_3$-solution followed by water. Drying (Na_2SO_4) and evaporation of the solvent gave 1.06 g (84%) of steroid **4** as a brown foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.14 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu$ = 128.7 Hz, J = 15.46 Hz, 2H; 1α/β-H), 4.00 (m, 2H; 21-CH_2), 4.47 (m, 1H; 11α-H), 5.03 (m, 4H; O–CH_2–O), 6.06 (d, J = 2.2 Hz, 1H, 4-H), 7.12–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 524 [M + H].

2.2.4. 2′-(4-Fluorophenyl)-11β,17α,21-trihydroxy-20-oxo-pregn-4-eno[3,2-c]pyrazole (5)

Steroid **4** (1.06 g, 2.02 mmol) was refluxed in 50% formic acid (100 ml) for 45 min. The formic acid was evaporated and the residue was re-dissolved in MeOH (75 ml) and 1N NaOH (15 ml). After stirring for 1 h at room temperature the solution was acidified with 1N HCl (150 ml). Extraction with methylene chloride, evaporation of the solvent and subsequent purification by flash chromatography (80% EtOAc–hexane) gave 780 mg (80%) of the desired product **5**. HPLC-analysis mobile phase B: t_R = 5.61 min, 98% chemical purity, m.p. 187–189 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H; 18-CH_3), 1.30 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu$ = 123.8 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.48 (AB quartet, $\Delta\nu$ = 137.1, J = 19.9 Hz, 2H; CH_2–OH). 4.51 (m, 1H; 11α-H), 6.05 (d, J = 2.2 Hz, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 481 [M + H].

2.2.5. 2′-(4-Fluorophenyl)-11β-hydroxy-androst-4-ene-17-one[3,2-c]pyrazole (6)

To a solution of steroid **5** (220 mg, 0.457 μmol) in EtOH–CH_2Cl_2 (10 ml, 1:1) was added $NaBH_4$ (9 mg, 0.23 mmol). After 1 h, acetone (1 ml) and water (3 ml) was added followed by $NaIO_4$ (244 mg, 1.14 mmol) and the mixture was stirred at room temperature overnight. Water (50 ml) was added and the mixture was extracted with EtOAc. The EtOAc layer was flushed through a silica-gel plug and the solvent was evaporated to give 177 mg (92%) of **6** as a solid. HPLC-analysis mobile phase B: t_R = 10.15 min, 97% chemical purity, m.p. 223–226 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.17 (s, 3H; 18-CH_3), 1.33 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu$ = 123.8 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.50 (m, 1H; 11α-H), 6.07 (d, J = 2.2 Hz, ^{1}H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, ^{1}H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 421.

2.2.6. 2′-(4-Fluorophenyl)-17α(1-propyn-1-yl)-11β, 17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (7)

To a solution of ketone **6** (193 mg, 0.46 mmol) in dry THF (10 ml) was added 1-propynylmagnesium bromide (9.14 ml, 4.57 mmol, 0.5 M in THF). The solution was stirred at room temperature under an nitrogen atmosphere overnight. Then, saturated NH_4Cl-solution (100 ml) was added and the mixture was thoroughly extracted with EtOAc. The solvent was removed under reduced pressure and subsequent purification by flash chromatography (50% EtOAc/hexane) afforded 212 mg (57%) of steroid **7** as a solid. HPLC-analysis mobile phase A: t_R = 5.56 min, 95% chemical purity, m.p. 95–98 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 1H; C≡CCH_3), 2.85 (AB quartet, $\Delta\nu$ = 115.5 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.05 (d, J = 2.2 Hz, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 461.

2.2.7. 2′-(4-Fluorophenyl)-17α[(3-hydroxy)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (8)

Tetrahydro-2-(2-propynyloxy)-2H-pyran (1.05 g, 7.5 mmol) in THF (15 ml) was treated with *n*-BuLi (4.68 ml, 7.5 mmol, 1.6 M in hexane) at 0 °C. After 1 h at 0 °C, ketone **6** (315 mg, 0.75 mmol) in THF (8 ml) was added and the mixture was stirred at room temperature overnight. 1N HCl (1 ml) was added and stirring was continued for 10 min at 60 °C. After the addition of water (50 ml) the solution was extracted with EtOAc. The EtOAc was washed with $NaHCO_3$-solution, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (10% MeOH–CH_2Cl_2) to afford steroid **8** (222 mg, 62%) as a solid. HPLC-analysis mobile phase B: t_R = 5.21 min, 98.5% chemical purity, m.p. 147–150 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.14 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.85 (AB quartet, $\Delta\nu$ = 115.3 Hz, J = 15.4 Hz, 2H; 1α/β-H), 4.32 (s, 2H; CH_2–OH), 4.49 (m, 1H; 11α-H), 6.05 (d, J = 2.1 Hz, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.42 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 477.

*2.2.8. 2′-(4-Fluorophenyl)-17α[(3-methansulfonyl)oxy-1-propyn-1-yl]-11β, 17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (**9**)*

To a solution of alcohol **8** (47.8 mg, 0.1 mmol) in THF (5 ml) and Et_3N (30.4 μl, 0.22 mmol) was added MsCl (15.7 μl, 0.2 mmol) at 0 °C. After stirring overnight the solvent was partially removed and the residue was purified by flash chromatography (80% EtOAc–hexane) to give 48 mg (86.5%) of mesylate **9**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.86 (AB quartet, $\Delta\nu = 112.4$ Hz, $J = 15.3$ Hz, 2H; 1α/β-H), 3.08 (s, 3H; OSO_2CH_3), 4.51 (m, 1H; 11α-H), 4.89 (s, 2H; CH_2-OMs), 6.06 (d, $J = 1.8$ Hz, 1H, 4-H), 7.13–7.18 (m, 2H: Ar–H), 7.42 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 555.

*2.2.9. 2′-(4-Fluorophenyl)-17α[(3-fluoro)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole (**10**)*

To a solution of mesylate **9** in THF (1.5 ml) was added TBAF (150 μl, 150 μmol, 1 M in THF) and the resulting mixture was heated to 60 °C for 30 min. The solvent was evaporated and the residue was purified by flash chromatography (60% EtOAc–hexane) to give 7.5 mg (38%) of fluoride **10**. HPLC-analysis mobile phase A: $t_R = 4.85$ min, 95.5% chemical purity, m.p. 125–127 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.86 (AB quartet, $\Delta\nu = 113$, $J = 15.4$ Hz, 2H; 1α/β-H), 4.50 (bs, 1H; 11α-H), 5.00 (d, $J_{HF} = 45.9$ Hz, 2H; CH_2-F), 6.06 (bs, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). ^{19}F-NMR (376 MHz, $CDCl_3$): δ −214.24 (*t*, $J_{HF} = 47$ Hz), −115.33 (m). LRMS (ESI positive) 479 [M + H].

*2.2.10. General procedure for the preparation of amine-containing steroids **11**, **12** and **13***

To a solution of Na_2CO_3 (50 mg, 0.47 mmol) in water (250 μl) was added the amine (1.0 mmol) followed by mesylate **9** (45 mg, 81 μmol) in acetone (1.5 ml). The solution was stirred for 2 h at room temperature. Saturated $NaHCO_3$-solution (20 ml) was added and EtOAc was used for extraction. After drying (Na_2SO_4) and evaporation of the solvent the residue was purified by flash chromatography (10% MeOH–CH_2Cl_2) to yield the corresponding amine.

*2.2.11. 2′-(4-Fluorophenyl)-17α[(3-N-pyrrolidino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole (**11**)*

Yield: 47%. HPLC-analysis mobile phase A: $t_R = 5.85$ min, 98.5% chemical purity, m.p. 196–198 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.82 (m, 4H; pyrrolidine), 2.67 (m, 4H; pyrrolidine), 2.84 (AB quartet, $\Delta\nu = 127.9$, $J = 15.2$ Hz, 2H; 1α/β-H), 3.48 (s, 2H; C≡CCH_2-pyrrolidine), 4.50 (bs, 1H; 11α-H), 6.05 (d, $J = 1.8$ Hz, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 530 [M + H].

*2.2.12. 2′-(4-Fluorophenyl)-17α[(3-N-piperidino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole (**12**)*

Yield: 77%. HPLC-analysis mobile phase A: $t_R = 6.24$ min, 96.5% chemical purity, m.p. 200–203 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.61 (quint., $J = 5.4$ Hz, 2H; piperidine), 1.94 (m, 4H; piperidine), 2.49 (m, 4H; piperidine), 2.84 (AB quartet, $\Delta\nu = 129.8$, $J = 15.2$ Hz, 2H; 1α/β-H), 3.31 (s, 2H; C≡CCH_2-piperidine), 4.50 (bs, 1H; 11α-H), 6.06 (bs, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.48 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 544 [M + H].

*2.2.13. 2′-(4-Fluorophenyl)-17α[(3-N-morpholino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole (**13**)*

Yield: 70%. HPLC-analysis mobile phase B: $t_R = 6.29$ min, 96.5% chemical purity, m.p. 96–99 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.55 (m, 4H; morpholine), 2.82 (AB quartet, $\Delta\nu = 126.4$, $J = 15.2$ Hz, 2H; 1α/β-H), 3.34 (s, 2H; C≡CCH_2-morpholine), 3.74 (m, 4H; morpholine), 4.50 (bs, 1H; 11α-H), 6.05 (d, $J = 1.9$ Hz, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 546 [M + H].

*2.2.14. 2′-(4-Fluorophenyl)-21-(methansulfonyl)oxy-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (**14**)*

A solution of steroid **5** (200 mg, 0.416 mmol) in dry pyridine (4 ml) was cooled to 0 °C and MsCl (65.4 μl, 0.832 mmol) was slowly added. After stirring at 0 °C for 3 h, the solution was poured into 1N HCl (100 ml) followed by extraction with EtOAc. After drying (Na_2SO_4) the solvent was evaporated and the residue was purified by flash chromatography (80% EtOAc/hexane) to yield 182 mg (78%) of mesylate **14** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.97 (s, 3H; 18-CH_3), 1.30 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu = 128$, $J = 15.3$ Hz, 2H; 1α/β-H), 3.24 (s, 3H; OSO_2CH_3), 4.52 (m, 1H; 11α-H), 5.16 (AB quartet, $\Delta\nu = 108.6$, $J = 17.8$ Hz, 2H; CH_2-OMs), 6.05 (d, $J = 1.7$ Hz, 1H, 4-H), 7.13–7.18 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 559 [M + H].

*2.2.15. Oxetan-3′-one (**15**)*

Mesylate **14** (31 mg, 55.5 μmol) was dissolved in dry THF (750 μl) and potassium *tert*-butoxide (9.34 mg, 83.2 μmol) in THF (400 μl) was added at room temperature. A clear yellow solution occurs. After 5 min, 1N HCl (100 μl) was added to quench the reaction followed by water (10 ml). Extraction with $CHCl_3$, drying (Na_2SO_4), evaporation of the solvent and subsequent flash chromatography (30%

EtOAc/hexane) gave 4 mg (16%) of oxetanone **15** as a white foam. HPLC-analysis mobile phase B: $t_R = 15.13$ min, 96% chemical purity, m.p. 217–220 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu = 129.3$, $J = 15.1$ Hz, 2H; 1α/β-H), 4.55 (m, 1H; 11α-H), 4.98 (AB quartet, $\Delta\nu = 51.4$, $J = 14.5$ Hz, 2H; oxetanone), 6.05 (d, $J = 2.1$ Hz, 1H, 4-H), 7.13–7.18 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 463 [M + H].

2.2.16. 2′-(4-Fluorophenyl)-21-iodo-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (16)

To a solution of mesylate **14** (129 mg, 86 μmol) in acetone (1 ml) was added NaI (129 mg, 860 μmol) in acetone (5 ml). After being stirred overnight while protected from light the solution was poured into water (20 ml) and 0.1N $Na_2S_2O_3$-solution (20 ml). Extraction with EtOAc, drying (Na_2SO_4), evaporation of the solvent and subsequent flash chromatography (40% EtOAc/hexane) afforded 43 mg (85%) of iodide **16** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.00 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu = 121.8$, $J = 15.3$ Hz, 2H; 1α/β-H), 4.10 (AB quartet, $\Delta\nu = 75.4$, $J = 11.3$ Hz, 2H; CH_2-I). 4.52 (m, 1H; 11α-H), 6.05 (d, $J = 2.1$ Hz, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 591 [M + H].

2.2.17. 2′-(4-Fluorophenyl)-21-fluoro-20-oxo-11β, 17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (17)

To a solution of mesylate **14** (182 mg, 0.325 mmol) in acetonitrile (10 ml) was added TBAF (450 μl, 450 mmol, 1 M in THF). After stirring for 15 min, at room temperature the solution was heated to 60 °C and an additional 450 μl of TBAF was added after 40 min. Addition of saturated $NaHCO_3$-solution, extraction (EtOAc), drying (Na_2SO_4), evaporation of the solvent and subsequent preparative TLC (60% EtOAc–hexane) gave 9 mg (5.8%) of fluoride **17** as a white solid. HPLC-analysis mobile phase B: $t_R = 9.04$ min, 93% chemical purity, m.p. 165–168 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.99 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu = 125$, $J = 13.9$ Hz, 2H; 1α/β-H), 4.53 (m, 1H; 11α-H), 5.20 (AB quartet, $\Delta\nu = 77$, $J_{HH} = 16.5$ Hz, with additional doublet splitting due to $J_{HF} = 47.5$ Hz, 2H; CH_2-F), 6.06 (s, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). ^{19}F-NMR (376 MHz, $CDCl_3$): δ −231.13 (t, $J_{HF} = 48$ Hz), −115.28 (m). LRMS (ESI positive) 483 [M + H].

2.2.18. 11β-Hydroxy-androst-4-ene-3,17-dione (18)

A solution of cortisol **1** (1.06 g, 2.92 mmol) in EtOH–CH_2Cl_2 (20 ml, 1:1) was treated with $NaBH_4$ (44.2 mg, 1.17 mmol). After 2 h acetone (5 ml) was added followed by water (5 ml) and $NaIO_4$ (1.56 g, 7.3 mmol). The solution was stirred at room temperature overnight followed by the addition of water (100 ml), extraction with $CHCl_3$ and flushing through a silica-gel plug. After evaporation of the solvent 877 mg (100%) of ketone **18** was isolated a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H; 18-CH_3), 1.42 (s, 3H; 19-CH_3), 4.49 (bs, 1H; 11α-H), 5.64 (s, 1H, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 219.71, 199.82, 172.29, 122.26, 67.36, 56.39, 52.10, 46.57, 40.46, 39.08, 35.05, 34.57, 33.49, 31.58, 31.21, 30.67, 21.35, 20.68, 15.49. LRMS (ESI positive) 303 [M + H].

2.2.19. 3-(N-Pyrrolidinyl)-3,5-androstadien-11β-ol-17-one (19)

Ketone **18** (877 mg, 2.9 mmol) in toluene (70 ml) was refluxed with pyrrolidine (2.5 ml) and $TsOH{\cdot}H_2O$ (45 mg) while removing the formed water. After 4 h the solvent was evaporated and ether was added. Further evaporation of the solvent gave 1 g (97%) of **19** as a yellowish-brown foam which was efficiently pure. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.25 (s, 3H; 19-CH_3), 3.14 (m, 4H; CH_2), 4.47 (m, 1H; 11α-H), 4.74 (s, 1H; 4-H), 4.96 (m, 1H; 6-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 220.15, 144.03, 142.82, 111.01, 95.32, 68.15, 53.63, 52.37, 46.98, 46.86, 40.52, 35.25, 34.92, 33.47, 30.47, 27.75, 24.79, 24.25, 21.94, 21.52, 15.60. LRMS (ESI positive) 356 [M + H].

2.2.20. 17α(1-Propyn-1-yl)-androst-4-ene-11β, 17β-diol-3-one (20)

To a solution of enamine **19** (194 mg, 0.54 mmol) in dry THF (2 ml) was added 1-propynylmagnesium bromide (5.7 ml, 2.86 mmol, 0.5 M in THF). The solution was stirred at room temperature under an nitrogen atmosphere overnight. Saturated NH_4Cl-solution (150 ml) was added and the mixture was thoroughly extracted with EtOAc. The EtOAc was removed under reduced pressure and the residue was redissolved in a solution of NaOAc (3 g) in water (3 ml), HOAc (2 ml) and MeOH (20 ml). The resulting mixture was refluxed for 1 h. Afterwards, water (200 ml) was added followed by extraction with EtOAc. Evaporation of the solvent and subsequent purification by flash chromatography (75% EtOAc/hexane) gave 96 mg (52%) of steroid **20** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H; 18-CH_3), 1.45 (s, 3H; 19-CH_3), 1.84 (s, 3H; CH_3), 4.44 (m, 1H; 11α-H), 5.68 (s, 1H, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 200.05, 172.84, 122.24, 82.46, 82.24, 80.05, 68.09, 55.88, 51.20, 45.83, 42.31, 39.15, 38.70, 34.72, 33.67, 32.03, 31.92, 31.82, 22.99, 20.77, 15.22, 3.53. LRMS (ESI positive) 343 [M + H].

2.2.21. General procedure for the synthesis of steroids 21 and 22

To a solution of steroid **20** (103 mg, 0.3 mmol) in toluene (5 ml), pyridine (1.5 ml) and methyl formate (300 μl) was

added 60% NaH (72 mg, 1.8 mmol) followed by methanol (150 μl). The solution was stirred overnight while the color changed to dark red. Addition of 1N HCl (50 ml) and extraction with methylene chloride gave a yellow solution. The methylene chloride was extracted several times with 1N NaOH. After acidification of the NaOH extract with HCl and subsequent re-extraction with methylene chloride the solvent was removed to give the corresponding 2-hydroxymethylene compound of **20** as a yellow foam. The foam was dissolved in HOAc (10 ml) and a solution of 4-bromophenylhydraziniumchloride or 4-iodophenylhydrazine (0.3 mmol) [17] and NaOAc (0.3 mmol) in HOAc (5 ml) and water (2 ml) was added. The dark red colored solution was stirred at room temperature overnight. Addition of water, extraction with $CHCl_3$ and subsequent purification by flash chromatography (60% EtOAc/hexane) gave compounds **21** or **22**, respectively.

2.2.22. 2′-(4-Bromophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (21)

Yield: 48%. HPLC-analysis mobile phase A: t_R = 8.12 min, 97% chemical purity, m.p. 130–132 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.85 (AB quartet, Δν = 114.9, J = 15.3 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.08 (d, J = 2.2 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.38 and 7.58 (2d of AA′BB′ system, J = 8.8 Hz, 4H; Ar–H). LRMS (ESI positive) 522 [M + H].

2.2.23. 2′-(4-Iodophenyl)-17α(1-propyn-1-yl)-11β, 17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (22)

Yield: 50%. HPLC-analysis mobile phase A: t_R = 8.79 min, 96.5% chemical purity, m.p. 104–106 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.85 (AB quartet, Δν = 115, J = 15.2 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.08 (d, J = 1.8 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.25 and 7.78 (2d of AA′BB′ system, J = 8.7 Hz, 4H; Ar–H). LRMS (ESI positive) 569 [M + H].

2.2.24. 2′-(4-Trimethylstannylphenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (23)

A solution of steroid **22** (160 mg, 0.28 mmol) in toluene (3 ml) was refluxed with Sn_2Me_6 (250 mg, 0.76 mmol) and $Pd[PPh_3]_4$ (4.8 mg, 4.15 μmol) for 3 h. After removal of the solvent the residue was purified by flash chromatography (60% EtOAc/hexane) to afford 100 mg (59%) of steroid **23**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.32 (s, 9H; $Sn(CH_3)_3$), 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.86 (AB quartet, Δν = 108.8, J = 15.2 Hz, 2H; 1α/β-H), 4.50 (bs, 1H; 11α-H), 6.14 (d, J = 1.9 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.46 (AA′BB′, J_{AB} = 8.2 Hz, 2H, Ar–H), 7.57 (AA′BB′, J_{AB} = 8.2 Hz, 2H, Ar–H). LRMS (ESI positive) 607 [M + H].

2.2.25. General procedure for the synthesis of iodonium salts 24 and 25

A solution of steroid **23** (115 mg, 0.19 mmol) and [hydroxy(tosyloxy)iodo]benzene or [hydroxy(tosyloxy)iodo] toluene (0.19 mmol) [18] in methylene chloride (1 ml) was stirred at room temperature for 2 h. The solvent was evaporated by means of a nitrogen stream. The residue was treated with ether resulting in the formation of a white solid. The solid was filtered off, washed with ether and dried under vacuo to yield the iodonium salt **24** and **25**.

2.2.26. Diaryliodonium tosylate (24)

Yield: 28%. ^{1}H-NMR (400 MHz, $DMSO_{d6}$): δ 0.29 (s, 3H; CH_3), 0.50 (s, 3H; CH_3), 1.04 (s, 3H; CH_3), 1.53 (s, 3H; CH_3), 2.05 (AB quartet, Δν = 150, J = 15.5 Hz, 2H; 1α/β-H), 3.60 (bs, 1H; 11α-H), 5.35 (d, J = 1.8 Hz, 1H, 4-H), 6.42 and 6.89 (2d of AA′BB′ system, J_{AB} = 8.3 Hz, 4H; Ar–H), 6.75 (m, 3H; Ar–H), 6.68 (s, 1H; H-pyrazole), 7.64 and 7.13 (2d of AA′BB′ system, J_{AB} = 9 Hz, 4H, Ar–H), 7.4–7.5 (m, 2H; Ar–H). LRMS (ESI positive) 645 [M-OTs].

2.2.27. Diaryliodonium tosylate (25)

Yield: 57%. ^{1}H-NMR (400 MHz, $DMSO_{d6}$): δ 0.29 (s, 3H; CH_3), 0.50 (s, 3H; CH_3), 1.00 (s, 3H; CH_3), 1.55 (s, 3H; CH_3), 1.60 (s, 3H; CH_3), 2.04 (AB quartet, Δν = 150.4, J = 15.5 Hz, 2H; 1α/β-H), 3.59 (bs, 1H; 11α-H), 5.34 (d, J = 1.8 Hz, 1H, 4-H), 6.41 and 6.56 (2d of AA′BB′ system, J_{AB} = 7.9 Hz, 4H, Ar–H), 6.67 (s, 1H; H-pyrazole), 6.82 and 7.45 (2d of AA′BB′ system, J_{AB} = 9.0 Hz, 4H, Ar–H), 6.88 and 7.4 (2d of AA′BB′ system, J_{AB} = 8.3 Hz, 4H, Ar–H). LRMS (ESI positive) 659 [M-OTs].

2.3. Radiochemistry

2.3.1. General

^{18}F was produced by the $^{18}O(p, n)^{18}F$ reaction on an enriched water target using an IBA CYCLONE 18/9 cyclotron. The [^{18}O] water containing [^{18}F]fluoride (ca. 5 mCi, 185 MBq) was transferred to a 5 ml Vacutainer® containing a solution of Kryptofix [2.2.2] (6.0 mg, 16.2 μmol) and K_2CO_3 (1.5 mg, 10.9 μmol). Water was azeotropically evaporated using HPLC grade acetonitrile (3× 0.5 ml) in a 100 °C oil bath under a stream of nitrogen. Potassium [^{18}F]fluoride was resolubilized into 500–1000 μl of anhydrous solvent (acetonitrile or DMF) and transferred to a glass vial pre-charged with the labeling precursor (2–10 mg). Progress of the reaction was monitored by means of radio-TLC. A pre-purification was performed by passing the diluted reaction mixture through a silica-gel plug (a Pasteur pipet with ca. 150 mg silica-gel). Radioiodination with iodine-131 was performed using a solution of [^{131}I]NaI (Nycomed-Amersham) in NaOH.

*2.3.2. 2′-([^{18}F]-4-Fluorophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole ([^{18}F]-**7**)*

The reaction was employed using 10 mg of iodonium salts **24** or **25**. After resolubilization the radioactivity (ca. 3–4 mCi, 110–150 MBq) was transferred to the labeling precursor **24** or **25** in DMF (500 μl). The vial was sealed and heated for 40 min at 120 °C. Radio-TLC (R_f = 0.38; 80% EtOAc/hexane) showed the formation of 0.2% (with iodonium salt **24**) and 2% (with iodonium salt **25**) of the desired compound [^{18}F]-7 together with large amounts of non-reacted [^{18}F]fluoride and [^{18}F]fluorobenzene and [^{18}F]fluorotoluene, respectively.

*2.3.3. 2′-(4-Fluorophenyl)-17α-(3-[^{18}F]fluoro)-1-propyn-1-yl)]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole ([^{18}F]-**10**)*

The reaction was employed using 2–3 mg of mesylate **9**. After resolubilization the radioactivity (4.0 mCi, 150 MBq) was transferred to mesylate **9** in acetonitrile (500 μl). The vial was sealed and heated for 20 min at 65 °C. After cooling and addition of ethyl acetate (2 ml) the solution was passed through a silica-gel plug. The eluate (11 MBq, 9%, decay-corrected) was analyzed by radio-TLC (R_f = 0.43; 80% EtOAc/hexane) indicating a radiochemical purity of >93%.

*2.3.4. 2′-(4-Fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-dihydroxy-pregn-4-eno [3,2-c]pyrazole ([^{18}F]-**17**)*

The reaction was employed using 2–4 mg of mesylate **14** or iodo precursor **16**. After resolubilization the radioactivity (3–5.4 mCi, 110–200 MBq) was transferred to the labeling precursor **14** or **16** in acetonitril (500 μl). The vial was sealed and heated for 20 min at 65 °C. After cooling and addition of ethyl acetate (2 ml) the solution was passed through a silica-gel plug. The eluate (3.1 MBq, 4%, decay-corrected for precursor **14** and 21.8 MBq, 14%, decay-corrected for precursor 16) was analyzed by radio-TLC (R_f = 0.55; 80% EtOAc/hexane) indicating a radiochemical purity of >95%.

*2.3.5. 2′-([^{131}I]-4-Iodophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole ([^{131}I]-**22**)*

The reaction was employed using 1.5 mg of organostannane **23**. [^{131}I]NaI (2 μCi, 74 kBq) was added to a solution consisting of 50 μl buffer solution (5% NaOAc in HOAc) and 50 μl oxidizing solution (30% H_2O_2/HOAc 2:1). Tin precursor **23** in 200 μl MeOH was added and the solution was stirred at room temperature for 30 min. Then, 70 μl of a 0.1 M $Na_2S_2O_3$-solution was added to stop the reaction. Radio-TLC (R_f = 0.47; 80% EtOAc/hexane) revealed the formation of 78% of the desired product [^{131}I]-**22** besides non-reacted [^{131}I]iodide. After the addition of ethyl acetate (2 ml) and silica-gel purification the radiochemical purity of the eluate was >95%.

2.4. Biological methods

2.4.1. Determination of the receptor binding affinity

Compounds tested in the GR-binding assay were analyzed by HPLC indicating a chemical purity of 93% for compound **17** and a chemical purity ≥ 95% for compounds **5**–**8**, **10**–**13**, **15** and **21**–**22**. The binding affinity of the arylpyrazolo steroids for the GR was determined by a modification of the method reported for the estrogen receptor [8,19]. 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 unlabeled competitor together with [^{3}H]dexamethasone at 0 °C for 18–24 h. The unlabeled arylpyrazolo steroid competitor was prepared in 1:1 dimethylformamide-buffer to ensure solubility. The free steroid was adsorbed on dextran-coated charcoal as described by Katzenellenbogen et al. [20]. [^{3}H]Dexamethasone was [1,2,4,-^{3}H]dexamethasone (Amersham Biosciences, Piscataway, NJ).

3. Results

3.1. Synthesis of 4-fluorophenylpyrazolo steroids containing 17α-alkynyl side chains

Natural corticosteroids possess the characteristic 17α,21-dihydroxy-20-keto substitution pattern of the pregnane side chain, as exemplified by cortisol **1**. However, several reports on glucocorticoids have shown that the D-ring is tolerant toward different substitution patterns at position C17 of the steroid skeleton while retaining high binding affinity to the GR [21]. Moreover, D-ring modified derivatives often possess greatly reduced binding affinities for the mineralocorticoid receptor, thus constituting steroids called “pure glucocorticoids” [21,22]. Since 17α-alkynyl substituted steroids are easily accessible by 1,2 addition of lithium or magnesium based organometallic acetylides to 17-keto steroids [23,24], we undertook the synthesis of a series of novel 4-fluorophenylpyrazole steroids in which the characteristic corticosteroid 1,3-dihydroxyacetone side chain was replaced by a 17α-alkynyl-17β-hydroxy moiety. The resulting steroids (**7**, **8**, **10**–**13**) combine the beneficial effects of the 17α-alkynyl-17β-hydroxy and 4-fluorophenyl pyrazole moieties for high and selective binding to the GR. Furthermore, the modification of the 17α-alkynyl side chain by either neutral (Me, OH, F) or basic (cyclic aliphatic amines) substituents results in interesting effects on biologically important parameters like receptor binding and lipophilicity (vide infra).

The synthesis of 17-keto steroid **6** as prerequisite starting material for 17α-alkynyl substituted steroids via organometallic 1,2 addition is shown in Fig. 2.

Starting from commercially available cortisol **1**, the synthesis commenced by protecting the 1,3-dihydroxyacetone side chain of **1** by treatment with formaldehyde and

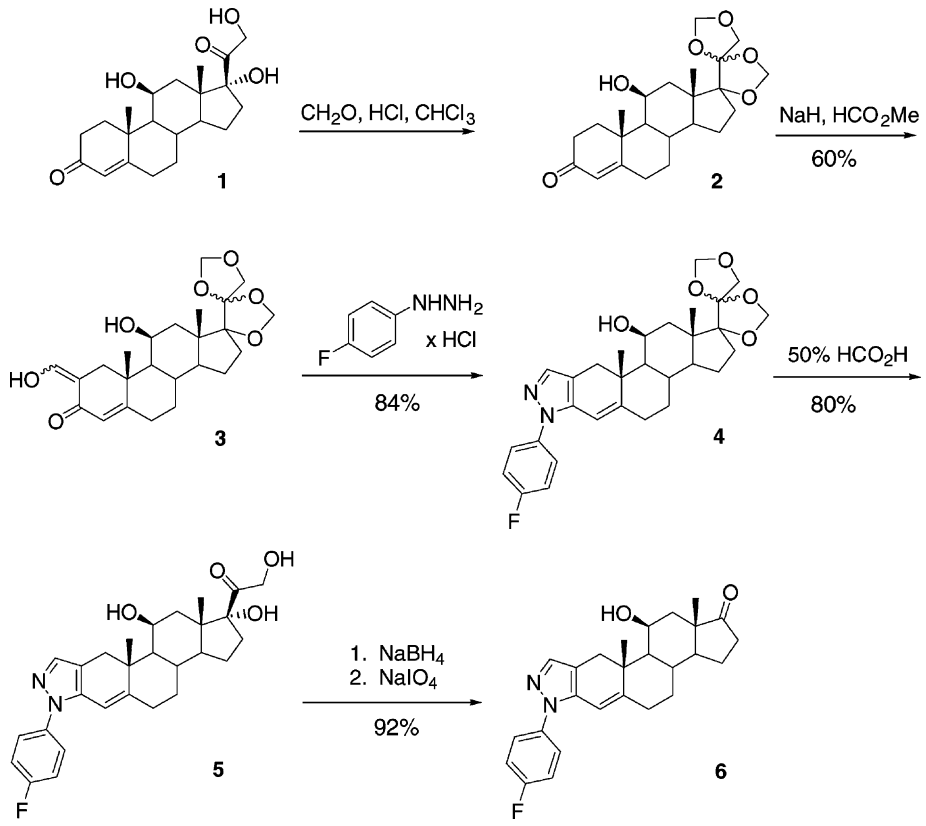

Fig. 2. Synthesis of 2′ (4-fluorophenyl)-11β-hydroxy-androst-4-ene-17-one[3,2-c]pyrazole **6**.

concentrated HCl in chloroform to afford cortisol bis(methylenedioxy)ether **2**. It was observed that a portion of the product was also converted to the 11β-MOM ether of **2** under these reaction conditions.

The amount of the 11β-MOM ether contamination was determined to be 20% by means of ^{1}H-NMR using the 11α-H signals at 4.47 ppm (OH) and 4.25 ppm (MOM). However, the following steps are not affected by the 11β-MOM ether group, and **2** could be used as a mixture of the 11β-OH and 11β-MOM compound. The introduction of the 2-hydroxymethylene functionality in **3** was achieved by reacting the sodium enolate of **2** with methyl formate in toluene. Subsequent condensation of **3** with 4-fluorophenylhydrazine hydrochloride in the presence of NaOAc in acetic acid gave 4-fluorophenyl pyrazole **4** in 84% yield. By using these reaction conditions, the desired 2′-fluorophenylpyrazole steroid **4** was formed exclusively, and the corresponding 1′-fluorophenylpyrazole isomer was not observed. The bis(methylenedioxy) ether protecting group and the partially present 11β-MOM ether were removed in boiling 50% formic acid. The resulting 1,3-dihydroxyacetone side chain in **5** was transformed into the 17-keto steroid **6** by reduction of the 20-keto group in **5** with $NaBH_4$ and subsequent oxidative cleavage of the intermediate 17,20,21-trihydroxy side chain with $NaIO_4$. The total yield of **6** was 37%, based on cortisol **1**. The reaction pathway for the introduction of 17α-alkynyl side chains and their further functionalization is depicted in Fig. 3.

Treatment of ketone **6** with 1-propynylmagnesium bromide in THF at room temperature gave steroid **7** in 57% yield. The 1,2 addition of the lithium derivative of tetrahydro-2-(2-propynyloxy)-2*H*-pyran to ketone **6** afforded alcohol **8** in 62% yield after removal of the THP-ether protecting group. Alcohol **8** was used as a suitable starting material for further modifications of the 17α-alkynyl side chain. For this purpose the primary hydroxyl group in **8** was first converted into the corresponding mesylate to obtain a good leaving group. This approach allows the convenient and flexible terminal functionalization of the alkynyl side chain with a variety of substituents by displacement of the mesylate with several nucleophiles. Thus, treatment of mesylate **9** with TBAF in THF afforded the fluoride-substituted steroid **10** in 38% yield. In another set of reactions, mesylate **9** was treated with several aliphatic cyclic amines in the system acetone-Na_2CO_3 to provide steroids **11**–**13** in 47, 77 and 70% yield, respectively.

The steroids obtained according to the reaction scheme in Fig. 3 can be subdivided into two classes: one set of compounds (**7**, **8**, and **10**) possess neutral substituents (Me in **7**, OH in **8**, F in **10**) in the 17α-alkynyl side chain, whereas compounds **11**–**13** contain pyrrolidine, piperidine or morpholine as basic amine substituents.

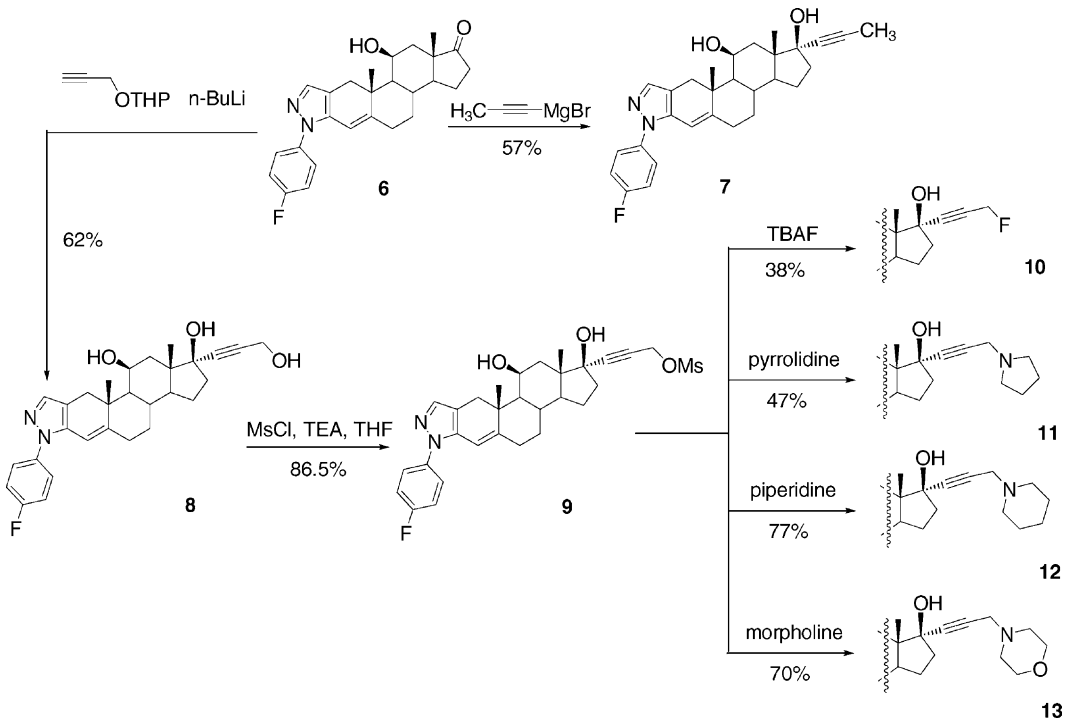

Fig. 3. Synthesis of 17α-alkynyl-17β-hydroxy steroids.

3.2. Synthesis of 4-fluorophenylpyrazolo steroids containing an oxetan-3′-one moiety and modified 1,3-dihydroxyacetone side chains

As another set of compounds, we have synthesized several 4-fluorophenyl pyrazolo steroids containing an intact and modified 1,3-dihydroxyacetone side chain, as well as a spiro-oxetanone. Steroid **5** possessing the 1,3-dihydroxyacetone side chain was obtained as an intermediate in the reaction sequence for the synthesis of ketone **6** (vide supra, Fig. 2). Steroid **5** was used to convert the 1,3-dihydroxyacetone side chain into the oxetan-3′-one **15** and to form iodide **16** and fluoride **17** (Fig. 4).

Although several methods are known for preparing oxetan-3-ones (e.g. by oxidation [25], by acid-catalyzed decomposition of diazo ketones [26], by [2 + 2] cycloaddition [27]), only intramolecular displacement reactions [28] have been used to prepare steroidal oxetane-3′-ones. In the case of C17 spiro-oxetan-3′-ones, the reaction seems especially challenging, since forcing reaction conditions are required, and the reaction only proceeds in poor yields. However, Pons and Simons [29] have described a general method to overcome these problems and to provide the desired C17 spiro-oxetan-3′-ones in good yields. Following this procedure, first mesylate **14** was prepared from triol **5** using MsCl in pyridine in 78% yield. An intramolecular oxetan-3′-one ring closure reaction of mesylate **14** using potassium *tert*-butoxide in THF at room temperature provided the desired spiro-oxetan-3′-one **15** in 16% yield. It is noteworthy that this intramolecular displacement reaction was also observed as a competitive reaction in the synthesis of several fluorine-18 containing corticosteroids starting from the corresponding α-keto mesylates [8,11].

The formation of 21-fluoro steroid **17** proved to be challenging, and α-keto mesylate **14** could be converted into 21-fluoro steroid **17** using TBAF in acetonitrile only in very a low yield (5.8%). Oxetanone **15** was always inevitably formed as a side product, because of the strong basic character of the fluoride anion under these conditions. Adaptation of this procedure for the formation of the corresponding F-18 labeled compound also gave disappointing results. Better results in the F-18 fluorination could be achieved using the 21-iodo compound **16** rather than the α-keto mesylate **14**. The required 21-iodo steroid 16 was easily prepared by the reaction of mesylate 14 with NaI in acetone in 85% yield.

3.3. Synthesis of 4-bromophenylpyrazolo steroid *21* and 4-iodophenylpyrazolo steroid *22*

Within the series of 17α-alkynyl-17β-hydroxy steroids, we also wanted to study the influence of 4-bromophenyl and 4-iodophenyl derivatives in terms of their GR binding, since bromine and iodine also have radioisotopes (Br-76, I-123 or I-124, respectively) suitable for in vivo imaging. Therefore, we set up the synthesis of two additional 17α-(1-propyn-1-yl)-17β-hydroxy steroids **21** and **22**, containing bromine or iodine in the arylpyrazolo moiety (Fig. 5).

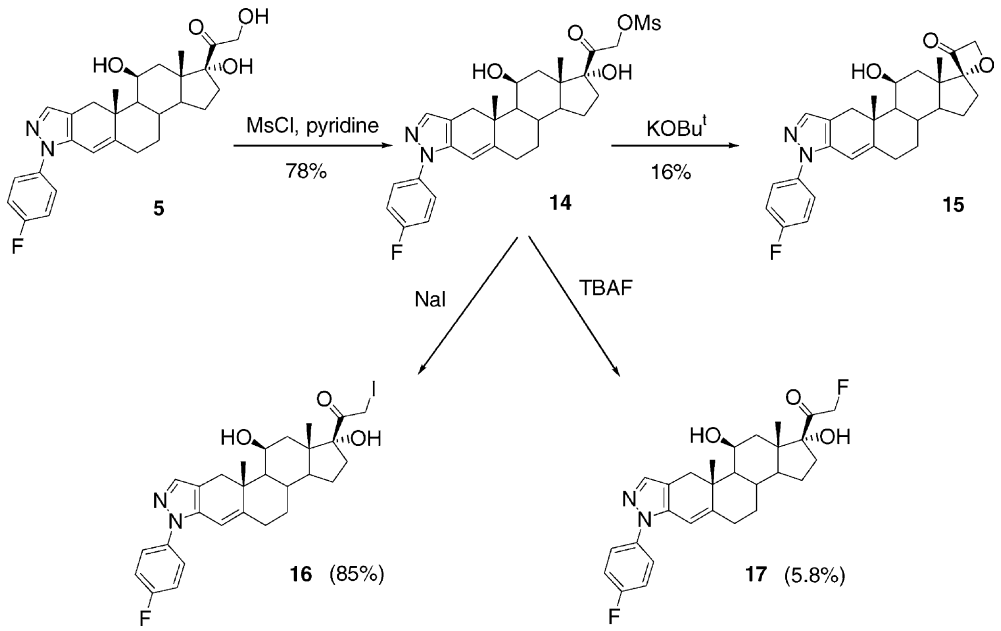

Fig. 4. Synthesis of steroids containing an oxetan-3′-one moiety and modified 1,3-dihydroxyacetone side chains.

Employing the same oxidative cleavage reaction as exemplified by the transformation of steroid **5** to 17-keto steroid **6**, cortisol **1** was converted into steroid **18** in quantitative yield. Further reaction of compound **18** requires the selective protection of the C3 keto group in the presence of the C17 keto group. Such a selective protection of the 3-keto group in **18** could conveniently be achieved through formation of the corresponding 3-enamine by refluxing 3,17-dienone **18** in benzene with pyrrolidine and a trace of TsOH, while removing the water generated by this reaction. Subsequent 1,2-addition of 1-propynylmagnesium bromide to the 17-keto group in **19**, followed by restoration of the 3-enone structure by deprotection with NaOAc/HOAc, gave compound **20** in a total yield of 50% based on cortisol **1**. Pyrazole formation was performed according to the described procedure (vide supra, Fig. 2), using the condensation of the corresponding 1,3-dicarbonyl compound (derived from **20** by treatment with NaH and methyl formate in toluene) with 4-bromo- and 4-iodophenylhydrazine [17] in acetic acid. This procedure afforded the desired arylpyrazolo steroids **21** and **22** in 48% and 50% yield, respectively.

3.4. Synthesis of diaryliodonium salts *24* and *25*

The use of [^{18}F]fluoride in nucleophilic aromatic substitution reactions represents a very important and widely applied reaction in fluorine-18 chemistry [30,31]. Nucleophilic aromatic substitutions with [^{18}F]fluoride proceed efficiently when a suitable good leaving group (nitro, trialkylammonium, etc.) is attached ortho- or para to an

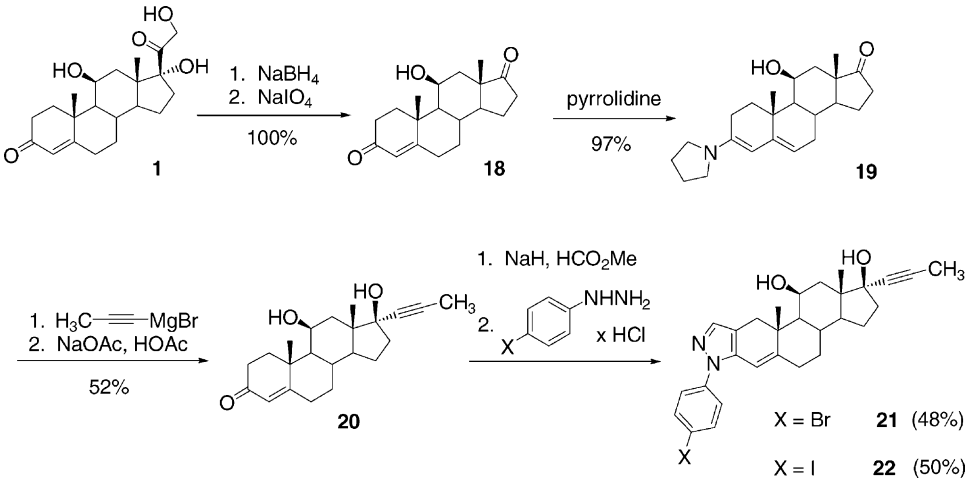

Fig. 5. Synthesis of 4-bromo- and 4-iodophenylpyrazolo steroids **21** and **22**.

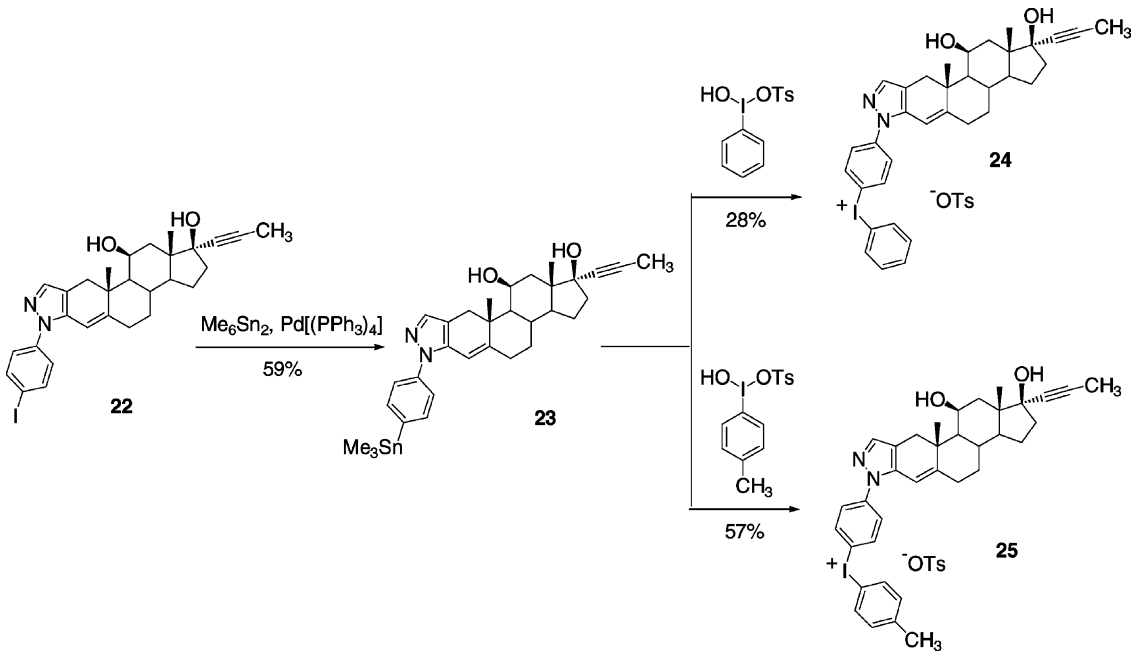

Fig. 6. Synthesis of diaryliodonium salts **24** and **25**.

electron-withdrawing group (nitro, CHO, CN, etc.) essential to activate the aromatic ring. In the case of arylpyrazolo steroids, the pyrazole moiety is not well suited to activate the aromatic ring efficiently for a nucleophilic aromatic substitution with [^{18}F]fluoride. However, several diaryliodonium salts have been shown to react with [^{18}F]fluoride to generate the corresponding aryl fluorides in reasonable yield, even from non-activated aromatic rings [32]. Therefore, we have synthesized diaryliodionium tosylates **24** and **25** as precursors for the introduction of [^{18}F]fluoride into the aromatic ring. The synthesis of aryliodonium salts **24** and **25** is given in Fig. 6.

Several methods have been reported for the preparation of diaryliodonium salts that comprise the reaction of either aryltrialkylsilanes [33], aryltrialkylstannanes [34] or arylboronic acids [35] with hydroxy(tosyloxy)iodobenzenes, as well as electrochemical methods [36]. We choose the reaction employing aryltrialkylstannanes. For this purpose, the required trimethylstannyl-containing steroid **23** was prepared via a Pd-catalyzed cross-coupling reaction between iodoaryl steroid **22** and hexamethyldistannane. Treatment of steroid **23** with hydroxy(tosyloxy)iodobenzene (Koser's reagent) or hydroxy(tosyloxy)-iodotoluene [18] resulted in an electrophilic aromatic substitution (ipso-destannylation) reaction that provided the desired diaryliodonium tosylates **24** and **25** in 28 and 57% yield, respectively.

3.5. Radiochemistry I—^{18}F-labeling on aliphatic side chains

The synthesis of ^{18}F-labeled steroids [^{18}F]-**17** and [^{18}F]-**10** is depicted in Fig. 7. The incorporation of fluorine-18 proceeded by [^{18}F]fluoride ion displacement of mesylate precursors **14** and **9** or iodo precursor **16**. For the synthesis of [^{18}F]-**17**, resolubilized [^{18}F]fluoride (Kryptofix®/potassium carbonate, [37]) in acetonitrile was transferred to a glass vial containing the appropriate precursor **14** or **16**, and the reaction mixture was heated at 65 °C for 20 min. To remove non-reacted [^{18}F]fluoride, the reaction was diluted with ethyl acetate (2000 μl) and applied to a silica "plug" (a Pasteur pipet with ca. 150 mg silica-gel) and eluted into a flask. Analysis of the product by radio-TLC (R_f = 0.55; 80% EtOAc/hexane) indicated a radiochemical purity >95%. The average radiochemical yield (decay-corrected) was 4% starting from mesylate precursor **14**. However, the radiochemical yield could be increased up to 14% (decay-corrected) when iodo precursor **16** was used.

Employing the same protocol, steroid [^{18}F]-**10** was obtained in 9% decay-corrected radiochemical yield after the reaction of mesylate **9** with resolubilized [^{18}F]fluoride. Radio-TLC (R_f = 0.43; 80% EtOAc/hexane) indicated a radiochemical purity of >93% after purification by silica-gel filtration.

*3.6. Radiochemistry II—^{18}F-labeling using diaryliodonium salts **24** and **25***

In order to incorporate the F-18 label into the metabolically stable position of the aromatic ring, we made use of diaryliodonium salts **24** and **25** as suitable labeling precursors (Fig. 8). Diaryliodonium salts **24** and **25** differ in the functionalization of the aryl ring, being phenyl and tolyl, respectively. Radiochemistry was performed using the powerful nucleophilic radiofluorinating agent K[^{18}F]F

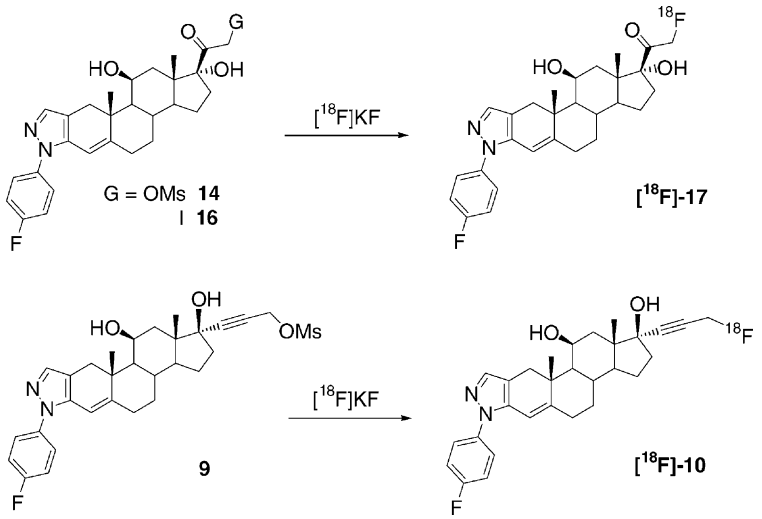

Fig. 7. Radiosynthesis of ^{18}F-labeled steroids [^{18}F]-**17** and [^{18}F]-**10**.

(generated by treatment of cyclotron-produced [^{18}F]fluoride with Kryptofix/potassium carbonate) in DMF as the solvent at 120 °C for 40 min. The use of phenyl-functionalized iodonium salt **24** gave the desired product [^{18}F]-**7** in only 0.2% yield. Radio-TLC analysis after silica-gel purification (vide supra) showed the desired product [^{18}F]-**7** ($R_f = 0.38$; 80% EtOAc/hexane) together with [^{18}F]fluorobenzene. Because of its volatility, the amount of [^{18}F]fluorobenzene could not be quantitated.

The observation of [^{18}F]fluorobenzene formation is consistent with the findings in the literature when non-symmetric phenyl-functionalized iodonium salts were used for fluorinations with [^{18}F]fluoride [32]. Since the attack of the fluoride ion preferentially occurs on the more electron-deficient aromatic ring, the use of the more electron-donating tolyl-fuctionalized iodonium salt **25** should favor the formation of F-18 labeled steroid [^{18}F]-**7**. This assumption was confirmed, because using this precursor, the radiochemical yield of [^{18}F]-**7** could be increased up to 2%. However, radio-TLC analysis also indicated the formation of large amounts of 4-[^{18}F]fluoro-toluene as a by-product.

*3.7. Radiochemistry III—^{131}I-labeling with trimethylstannyl steroid **23***

Several iodine isotopes such as iodine-120, -123, -124 possess decay properties suitable for in vivo imaging by positron or single-photon emission tomography (PET or

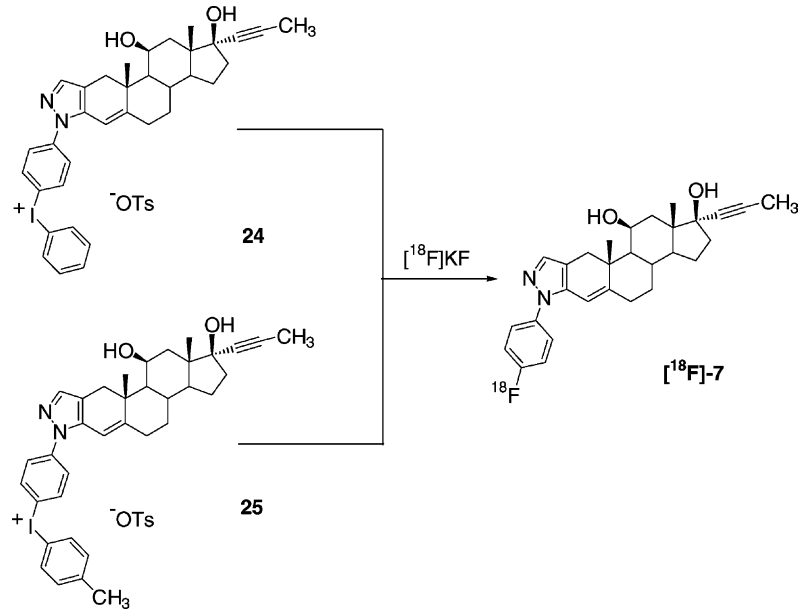

Fig. 8. Radiosynthesis of ^{18}F-labeled steroid [^{18}F]-**7** via diaryliodonium salts **24** and **25**.

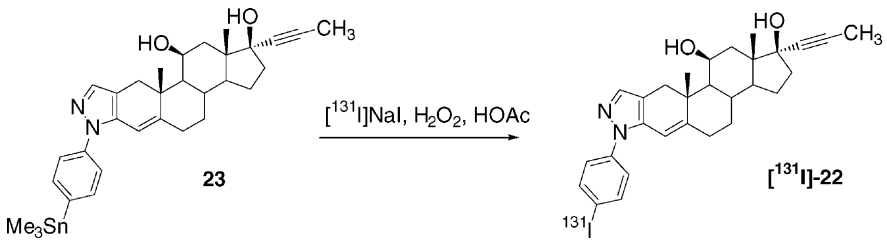

Fig. 9. Radiosynthesis of ^{131}I-labeled steroid [^{18}F]-**22**.

SPECT). The convenient half-life of iodine-131 ($t_{1/2}$ = 8.04 days) makes this isotope ideal for the elaboration of labeling protocols using iodine isotopes. Several radioiodination reactions using organometallic intermediates have been shown to be very effective, rapid and site-specific methods for the incorporation of iodine isotopes into small molecules like steroids [38]. In this context, the use of organostannanes has provided especially good results for the radioiodination of aromatic rings via iodo-destannylation reactions. Thus, we used organostannane **23** as a precursor for radiolabeling with iodine-131 (Fig. 9). For this purpose, [^{131}I]NaI (ca. 2 μCi, 75 kBq) in a buffer solution (5% NaOAc in HOAc) was oxidized with H_2O_2 in HOAc. The progress of the reaction with tin precursor 23 was monitored by radio-TLC. The reaction was completed after 30 min, at which point a solution of $Na_2S_2O_3$ was added to stop the reaction. After silica-gel purification (vide supra), the eluate was analyzed by radio-TLC (R_f = 0.47; 80% EtOAc/hexane), which indicated a radiochemical purity of >95% for [^{131}I]-**22**. Radio-TLC analysis revealed the formation of 78% of [^{131}I]-**22** besides non-reacted [^{131}I]iodide.

3.8. Glucocorticoid receptor binding affinities and log $P_{o/w}$ values of arylpyrazolo corticosteroids

The GR binding affinities of all arylpyrazolo 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 liver cytosol from adrenalectomized rats, with [^{3}H]dexamethasone as radiotracer and unlabeled dexamethasone as standard (RBA = 100%). All steroids tested in this work show lower GR binding in comparison to the synthetic and high-affinity ligand dexamethasone. However, some compounds still exhibit appreciable binding, up to 56% that of dexamethasone, which is considerably better than the binding affinity of the natural hormone cortisol **1** (RBA = 8.4%). Moreover, all synthesized arylpyrazolo steroids show an increased lipophilicity (expressed as calculated log $P_{o/w}$ values) by at least one order of magnitude compared to dexamethasone or cortisol **1**. The enhanced lipophilicity might be a benefit for improved blood–brain-barrier penetration when appropriately radiolabeled compounds are used for brain imaging studies.

Within the series of 17α-alkynyl substituted steroids, the character of the terminal substitutent shows an interesting effect on the RBA value. In the case of the neutral terminal substituents F (10), OH (8) and Me (7), the RBA values vary between 37, 31 and 56%, respectively. These values represent relatively good binding to the GR, and the binding is significantly higher compared to steroids **11**–**13** containing basic groups. Thus, the morpholine-containing steroid **13** reaches an RBA of about 17.5%, whereas the RBA drops markedly when more basic cyclic amines are used, being 7.5% for pyrrolidine **11** and 8.5% for piperidine **12**. These findings indicate that neutral side chains support GR binding better than basic side chains. Nevertheless, all measured RBA values within the series of 17α-alkynyl substituted steroids display at least the same (compounds **11** and **12**) or better GR binding (compounds **7**, **8**, **10** and **13**) compared to the natural occurring steroid cortisol **1**.

In the second set of compounds, the 1,3-dihydroxyacetone side chain containing steroid **5** shows the highest RBA value, 50% relative to dexamethasone. In contrast, the corresponding 21-fluoro compound **17** displays a reduced binding affinity of 11%, which is comparable to that of cortisol **1**. On the other hand, structurally very different ketone **6** and oxetanone **15** exhibit moderate to good binding of 18 and 22%, respectively.

Table 1
Relative binding affinities (RBAs) of arylpyrazolo steroids for the GR

Compound	RBA (0 °C) (%)	Log $P_{o/w}$[a]
Dexamethasone	100	2.06
Cortisol **1**	8.4[b]	1.43, (1.61[c])
5	50	3.46
6	18	3.30
7	56	4.50
8	31	3.34
10	37	4.31
11	7.5	4.82
12	8.5	5.38
13	17.5	3.91
15	22	3.08
17	11	3.87
21	6.5	5.22
22	1.5	5.48

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 [8].
[c] Literature value [39].

Comparison of the RBA values within a third series of compounds reveal a strong influence of the halogen attached to the phenyl ring on the GR binding affinity. By far the best binding affinity is observed with 4-fluorophenyl pyrazole **7**, being 56%. With bromine in compound **21** or iodine in compound **22**, the RBA drops to 6.5 and 1.5%, respectively. The low RBA values for bromine- and iodine-containing steroids **21** and **22** in comparison to fluorine-containing steroid **7** confirm the beneficial effect of the 4-fluorophenyl moiety in terms of high binding affinity to the GR.

4. Discussion

In our effort to develop imaging agents for brain GRs, we have prepared several novel arylpyrazolo steroids. The compounds that we have synthesized can be divided into three classes: 4-fluorophenyl pyrazoles possessing a 17α-alkynyl side chain, 4-fluorophenyl pyrazoles containing a selection of different substitution patterns in the D-ring, and compounds which differ with respect to the halogen attached to the arylpyrazole moiety. All steroids were tested for binding to the GR, and their lipophilicity ($\log P_{o/w}$ values) was calculated.

We found that all steroids containing a 4-fluorophenyl pyrazole moiety possess at least the same binding capability to the GR as the natural stress-induced hormone cortisol **1**. Relatively high binding of 50 and 56%, relative to dexamethasone, was observed for compounds **5** and **7**, which contain the intact 1,3-dihydroxyacetone side chain or the relatively small and neutral 17α-propynyl side chain, respectively. These binding affinities are 6–7 times better than the GR binding of cortisol **1**. This finding also shows that the 1,3-dihydroxyacetone side chain is not necessary for high GR binding and can be replaced by neutral 17α-alkynyl side chains. By comparing the measured RBA values, it also became evident that basic substituents like morpholine and to a greater extent pyrrolidine and piperidine diminish GR binding significantly. The GR seems to tolerate neutral 17α-alkynyl side chains rather than basic side chains. Apparently, the basic character of the cyclic amines seems to have more influence on the GR binding than the steric bulk when the RBA values of the strong basic pyrrolidine **11** (RBA = 7.5%) and piperidine **12** (RBA = 8.5%) are compared with the less basic morpholine **13** (RBA = 17.5%).

Further evidence for the GR tolerance toward modifications of the steroidal D-ring was revealed by the relatively good binding affinities of ketone **6** (RBA = 18%) and oxetanone **15** (RBA = 22%). However, only moderate binding affinity of 11% was found for 21-fluoro steroid **17**, which represents a compound with a modified 1,3-dihydroxyacetone side chain. The beneficial effect of 4-fluorophenyl pyrazoles on the GR was confirmed by comparison of the RBA values of fluorine **7** (RBA = 56%), bromine **21** (RBA = 6.5%), and iodine **22** (RBA = 1.5%). By introduction of the halogens bromine and iodine into the aromatic ring the electronic and steric characteristics apparently prevent any sufficient binding to the GR. This effect is especially distinct when iodine is used.

Selected candidates were chosen to investigate the labeling of arylpyrazolo steroids with the short-lived positron emitter fluorine-18. The fluorine-18 label could be easily introduced into aliphatic side chains to afford radiolabeled steroids [^{18}F]-**17** and [^{18}F]-**10** in radiochemical yields of 14 and 9%, respectively. It is noteworthy that the incorporation of fluorine-18 proceeds well despite the free OH-groups present in precursors **9**, **14** or **16**.

In an effort to label arylpyrazolo steroids in a metabolically stable position, we attempted the synthesis of fluorine-18 labeled steroid [^{18}F]-**7**. Since the aromatic ring is not sufficiently activated by a strong electron-withdrawing group, we used diaryliodonium salts **24** and **25** as precursors for the incorporation of fluorine-18. The experiments show the influence of the structure of the iodonium salt used on the yield and product distribution. Iodonium salt **25** contains a more electron releasing tolyl substituent compared to the phenyl ring in **24**. This change causes an improved yield in the attack of the [^{18}F]fluoride to the steroidal part of the iodonium salt. Despite the low decay-corrected radiochemical yields (0.2 and 2.0%, respectively), the metabolically stable fluorine-18 labeled glucocorticoid [^{18}F]-**7** represents an interesting agent to investigate further for the study of brain GR by means of PET.

Acknowledgments

The authors thank Prof. Dr. G. Stöcklin for helpful discussions. Financial support to FW by the Deutsche Forschungsgemeinschaft and to JAK and KEC by a grant from the Department of Energy (DE FG02 86ER60401) is gratefully acknowledged.

References

[1] De Kloet ER, Sutanto W, editors. Neurobiology of steroids, vol. 22. New York: Academic Press; 1994.
[2] Joëls M, Vreugdenhil E. Corticosteroids in the brain. Mol Neurobiol 1998;17:87–108.
[3] De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. Endocr Rev 1998;19:269–301.
[4] Korte SM. Corticosteroids in relation to fear, anxiety and psychopathololgy. Neurosci Biobehav Rev 2001;25:117–42.
[5] Reul JMHM, De Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 1985;117:2505–11.
[6] Holsboer F. The rational for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. Psychiatr Res 1999;33:181–214.
[7] De Kloet ER. Brain corticosteroids receptor balance and homeostatic control. Front Neuroendocrinol 1991;12:95–164.
[8] Pomper MG, Kochanny MJ, Thieme AM, Carlson KE, VanBrocklin HF, Mathias CJ, et al. Fluorine-substituted corticosteroids: synthesis and evaluation as potential receptor-based imaging agents for positron emission tomography of the brain. Nucl Med Biol 1992;19:461–80.

[9] Hoyte RM, Labaree DC, Fede J-M, Harris C, Hochberg RB. Iodinated and fluotinated steroid 2′-aryl-[3,2-c] pyrazoles as potential glucocorticoid receptor imaging agents. Steroids 1998;63:595–602.
[10] DaSilva JN, Crouzel C, Stulzaft O, Khalili-Varasteh M, Hantraye P. Synthesis, tissue distribution in rats and PET studies in baboon brain of no-carrier-added [^{18}F]RU 52461: in vivo evalution as a brain glucocorticoid receptor radioligand. Nucl Med Biol 1992;19:167–73.
[11] Feliu AL, Rottenberg DA. Synthesis and evaluation of fluorine-18 21-fluoroprednisone as a potential ligand for neuro-PET studies. J Nucl Med 1986;28:998–1005.
[12] Visser GM, Krugers HJ, Luurtsema G, van Waarde A, Elsinga PH, de Kloet ER, et al. Synthesis and organ distribution of [^{18}F]fluoro-Org6141 in the rat: a potential glucocorticoid receptor ligand for positron emission tomography. Nucl Med Biol 1995;22:915–20.
[13] Feliu AL. Synthetic studies with [^{18}F]*p*-fluorobenzenediazonium chloride: application to the synthesis of radiolabelled glucocorticoid [^{18}F] Win 4457. J Labelled Cpd Radiopharm 1988;25:1245–54.
[14] Schlechte JA, Simons Jr SS, Lewis DA, Thompson EB. [^{3}H]Cortivazol: a unique high affinity ligand for the glucocorticoid receptor. Endocrinology 1885;117:1355–62.
[15] Winneker RC, Russell MM, Might CK, Schane HP. The interaction of nivazol with the glucocorticoid receptor from rat and rhesus monkey target tissues. Steroids 1985;42:447–57.
[16] Still WC, Kahn M, Mitra A. Rapid chromatographic technique for preparative separations with moderate resolution. J Org Chem 1978;43:2923–5.
[17] Baloniak S, Thiel U, Pacholczyk M. Synthesis of 1-(4-iodophenyl)-3-hydroxypyridazin-6-one derivatives. Acta Pol Pharm 1976;33:73–9.
[18] Koser GF, Wettach RH. [Hydroxy(tosyloxy)iodo]benzene, a versatile reagent for the mild oxidation of aryl iodides at the iodine atom by ligand transfer. J Org Chem 1980;45:1542–3.
[19] Katzenellenbogen JA, Johnsonm Jr HJ, Myers HN. Photoaffinity labels for estrogen binding proteins of rat uterus. Biochemistry 1973;12:4085–92.
[20] Katzenellenbogen JA, Johnson Jr HJ, Carlson KE, Myers HN. Photoreactivity of some light-sensitive estrogen derivatives. Use of an exchange assay to determine their photointeraction with the rat uterine estrogen binding protein. Biochemistry 1974;13:2986–94.
[21] Teutsch G, Costerousse G, Deraedt R, Benzoni J, Fortin M, Philibert D. 17α-Alkynyl-11β,17-dihydroxyandrostane derivatives: a new class of potent gluco-corticoids. Steroids 1981;38:651–65.
[22] Ojasoo T, Doré J-C, Gilbert J, Raynaud J-P. Binding of steroids to the progestin and glucocorticoid receptors analyzed by correspondence analysis. Steroids 1988;31:1160–9.
[23] Wüst F, Spies H, Johannsen B. Synthesis of 17α-substituted mercaptoalkynyl derivatives of 3,17β-estradiol. Tetrahedron Lett 1998; 38:2931–2.
[24] El Amouri H, Vessiéres A, Vichard D, Top S, Gruselle M, Jaouen G. Syntheses and affinities of novel organometallic-labeled estradiol derivatives: a structure–affinity relationship. J Med Chem 1992;35:3130–5.
[25] Wojtowitcz JA, Polak R. 3-Substituted oxetanes. J Org Chem 1973;38:2061–6.
[26] Ducruix A, Pascard C, David S, Fischer J-C. Crystal and molecular structure of a carbohydrate-derived oxetan-3-one. Definitive evidence for a novel participation of a pyranose ring oxygen atom. J Chem Soc Perkin Trans 1976;2:1678–82.
[27] Schroeter S, Orlando CM. Photocycloaddition of various ketones and aldehydes to vinyl ethers and ketene diethyl acetals. J Org Chem 1969;34:1181–7.
[28] Rowland AT, Drawbough RS, Dalton JR. Steroid oxetanones. Part 3. Synthesis of 5,7-alpha-epoxy-5-alpha-cholestan-6-ones. J Org Chem 1977;42:487–90.
[29] Pons M, Simons Jr SS. Facile, high-yield synthesis of spiro C-17 steroidal oxetan-3′-ones. J Org Chem 1981;46:3262–4.
[30] Berridge MS, Tewson TJ. Chemistry of fluorine-18 radiopharmaceuticals. Appl Radiat Isot 1986;37:685–93.
[31] Kilbourn RM. Fluorine-18 labeling of radiopharmaceuticals. Nuclear Science Series NAS-NS-32203. Washington DC: National Acadamy Press; 1990.
[32] Shah A, Pike VW, Widdowson DA. The synthesis of [^{18}F]fluoroarenes from the reaction of cyclotron-produced [^{18}F]fluoride ion with diaryliodonium salts. J Chem Soc Perkin Trans 1998;1: 2043–6.
[33] Koser GF, Wettach RH, Smith CS. New methodology in iodonium salt synthesis. Reactions of [hydroxy(tosyloxy)iodo]arenes with aryltrimethylsilanes. J Org Chem 1980;45:1543–4.
[34] Pike VW, Butt F, Shah A, Wissowson DA. Facile synthesis of substituted diaryliodonium tosylates by treatment of aryltributylstannanes with Koser's reagent. J Chem Soc Perkin Trans 1999;1: 245–8.
[35] Carroll MA, Pike VW, Widdowson DA. New synthesis of diaryliodonium sulfonates from arylboronic acids. Tetrahedron Lett 2000;41:5393–6.
[36] Peacock MJ, Pletcher D. The electrosynthesis of diaryliodonium salts. Tetrahedron Lett 2000;41:8995–8.
[37] Coenen HH, Klatte B, Knöchel A, Schüller M, Stöcklin G. Preparation of n.c.a. 17-[^{18}F]fluoroheptodecanoic acid in high yields via aminopolyether supported nucleophilic fluorination. J Labelled Cpd Radiopharm 1986;23:455–67.
[38] Wilbur DS. Radiohalogenations of proteins: an overview of radionuclides. Bioconjugate Chem 1992;3:433–70.
[39] Alvarez Nuñez FA, Yalkowsky SH. Correlation between log P and Clog P for some steroids. J Pharm Sci 1997;86:1187–9.