Synthesis of Halogen-Substituted Pyridyl and Pyrimidyl Derivatives of [3,2-*c*]Pyrazolo Corticosteroids: Strategies for the Development of Glucocorticoid Receptor Mediated Imaging Agents

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Ligands for the glucocorticoid receptor labeled with high-energy isotopes are highly desired for their potential applications in nuclear medical studies of the brain where the dysregulation of this receptor system is thought to be involved in various neurodegenerative disorders. Analogues of the glucocorticoid cortivazol have previously been prepared as target compounds for labeling with high-energy isotopes. However, the phenyl rings of arylpyrazoles of this type are not sufficiently activated for nucleophilic substitution reactions that are generally required for the synthesis of radiohalogenated analogues. Since suitably substituted aromatic nitrogen heterocyclic groups are amenable to nucleophilic substitution, the goal of this study was the synthesis of pyridylpyrazolo and pyrimidylpyrazolo analogues similar to cortivazol that could be labeled with radiohalogens in the pyridine or pyrimidine rings. We describe the synthesis of several [3,2-*c*]pyrazolo steroids containing pyridyl, halopyridyl, and pyrimidyl substituents at the 2′ position of the pyrazole ring. These compounds were tested for binding to the glucocorticoid receptor and for biological activity in glucocorticoid responsive HeLa cells grown in tissue culture. Of the pyridyl and pyrimidyl derivatives, 2′-(3-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole showed superior activity in both assays and it was used as the basis for the synthesis of several analogues that were halogenated in the pyridine ring. These halogenated compounds were all tested for their binding to the glucocorticoid receptor and for their biological activity. One, a fluorinated compound 2′-(2-fluoro-5-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole had excellent activity, considerably better than the potent glucocorticoid dexamethasone. Most importantly, fluorination was achieved using a nucleophilic exchange reaction, a method that is adaptable to radiolabeling with the positron-emitting isotope fluorine-18. Thus, considering its superior biological activity and adaptability for facile radiosynthesis, this target compound has the potential for imaging of glucocorticoid receptor containing tissues using positron emission tomography.

Introduction

Cortivazol **1** (Figure 1) and similar arylpyrazolo steroids have been extensively investigated as antiinflammatory agents[1,2] and have been found to be powerful glucocorticoids rivaling the potent corticoid dexamethasone.[3,4] [³H]Cortivazol has been prepared and demonstrated to be a high-affinity ligand for the glucocorticoid receptor.[5] To develop glucocorticoid receptor mediated imaging agents, we have reported the synthesis of several analogues of arylpyrazoles similar to cortivazol **1**.[6] These compounds were synthesized under the supposition that substituents at the phenyl ring would not interfere with binding to the glucocorticoid receptor because this ring structure is distant from the A ring present in the naturally occurring corticoids. This notion proved to be incorrect because most of the compounds were found to be poor ligands for the glucocorticoid receptor. However, one of them, the *p*-fluorophenyl analogue, 2′-(4-fluorophenyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole (4-FPhP), was an excellent ligand for the receptor, and it displayed very high biological activity.

The challenge was to develop a method to incorporate radioactive fluorine (¹⁸F) into this compound. The initially obvious synthetic route to the ¹⁸F-substituted steroid using electrophilic fluorination methods is not attractive as a means to the radiopharmaceutical. While a variety of electrophilic ¹⁸F-labeled radiofluorination reagents are available, their use is inefficient because of lower specific activities and radiochemical yields than can be attained with nucleophilic methods employing [¹⁸F]fluoride.[7] Two studies have reported attempts to use nucleophilic methods to synthesize ¹⁸F-labeled phenylpyrazoles as glucocorticoid receptor ligands. Both used compounds with a simplified side chain containing 17β-hydroxy-17α-alkynyl groups (based on the glucocorticoid WIN 44577[8]). Although Wüst et al. synthesized a nonradioactive *p*-fluoroaryl-*N*-pyrazolo steroid that

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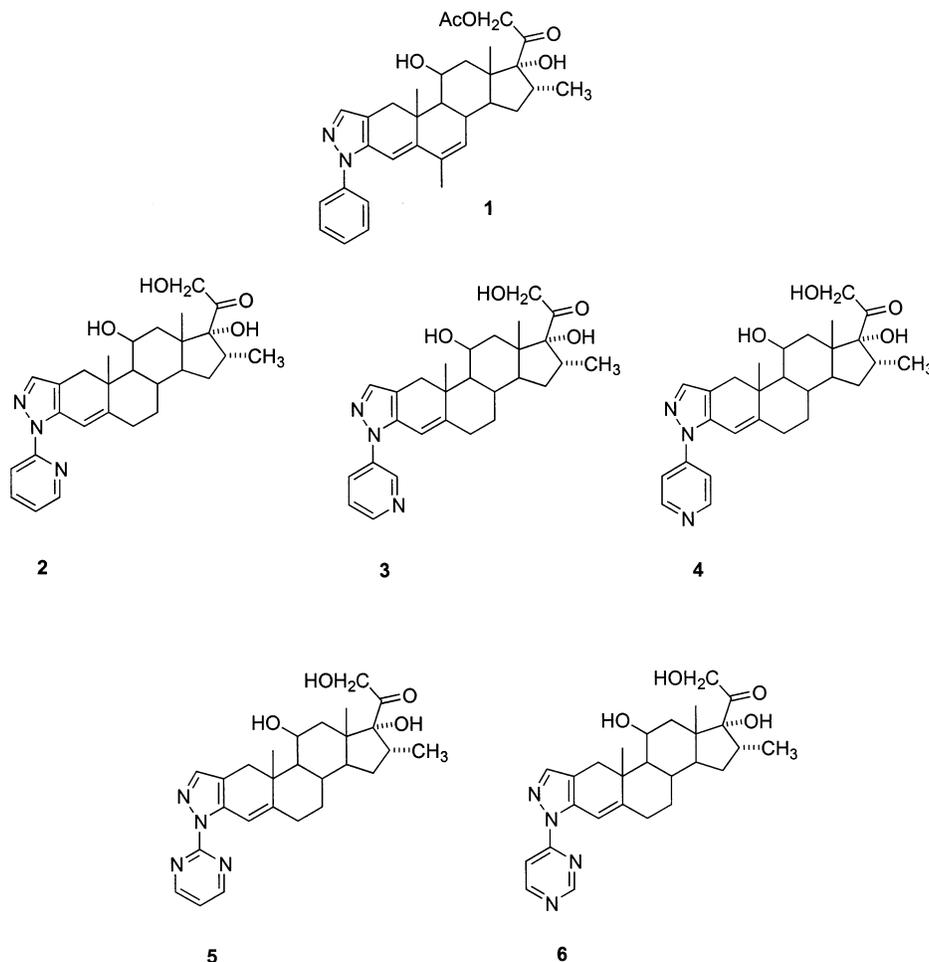† State University of New York.
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Figure 1. Structure of cortivazol **1** and related pyridylpyrazolo steroids **2**−**4** and pyrimidylpyrazolo steroids **5**−**6**.

showed high glucocorticoid activity, their attempt to synthesize the ^{18}F-labeled steroid by [^{18}F]fluoride substitution of the phenyliodonium tosylate produced the desired product in only a 0.2% yield (decay-corrected).[9] The authors noted that the very low yield "indicates the challenging task to incorporate [^{18}F]fluoride into non-activated aromatic systems containing a delicate organic structure". Feliu produced the *p*-[^{18}F]fluorophenyl-*N*-pyrazolo steroid (WIN 44577) by a different route, through the synthesis of *p*-[^{18}F]fluoronitrobenzene, reduction to the corresponding aniline, conversion to the diazonium salt, reduction to the arylhydrazine, and finally condensation with the steroidal keto aldehyde precursor to form the desired [^{18}F]fluoroglucocorticoid.[10] This five-step radiosynthesis was accomplished in 100 min with a 6% yield. The biological properties of this tracer were not reported, and further studies have not been published.

Several variants of the aryl moiety of cortivazol, including 3- and 4-pyridyl groups (**3** and **4**, Figure 1), have been investigated and found to provide compounds with high glucocorticoid potential.[2] These are particularly relevant because appropriately substituted pyridyl groups lend themselves well to nucleophilic substitution by [^{18}F]fluoride, the favored method of introducing this isotope for the preparation of radiolabeled ligands intended for positron emission tomography (PET) imaging. Our strategy therefore was to produce a substituted pyrazolo glucocorticoid containing a heterocyclic ring in which [^{18}F]fluoride could be incorporated directly by

nucleophilic attack with [^{18}F]fluoride in a one-step radiochemical reaction requiring, subsequently, only deprotection. To accomplish this, we synthesized the pyridylpyrazolo steroids **2**−**4** and tested their biological activity as glucocorticoids by measuring their binding to the glucocorticoid receptor and the induction of alkaline phosphatase (AlkP) in HeLa S3 cells. The analogous pyrimidyl compounds **5** and **6**, which have not been previously reported, were also synthesized and evaluated because the heterocyclic pyrimidyl ring, when appropriately substituted, is also a potential site for nucleophilic substitution by [^{18}F]fluoride. One of these compounds, the 3-pyridylpyrazolo steroid **3**, had very high biological activity and served as the model for the synthesis of several chloropyridylpyrazolo analogues **15a**−**c**. The chloropyridylpyrazolo steroids were used for the synthesis of the corresponding fluoropyridylpyrazolo steroids related to **3** through nucleophilic substitution by fluoride. These fluoro compounds and their chlorinated precursors were evaluated for their biological activity.

Chemistry

The synthesis of the pyridylpyrazolo and pyrimidylpyrazolo steroids requires the preparation of the precursor hydrazines **8a**−**c** (Scheme 1) and **13a**−**d** (Scheme 2), which are then condensed with 11β-hydroxy-2-hydroxymethylene-16α-methyl-17, 20:20,21-bis-(methylenedioxy)pregn-4-en-3-one **14**.[1] Removal of the protecting group (Scheme 3) or displacement of the

Scheme 1. Synthesis of Heteroaryl Hydrazines **8a−c** by Direct Displacement of Halogen
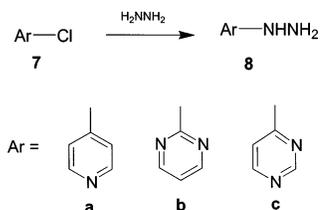
Scheme 2. Synthetic Sequence Leading to Heteroaryl Hydrazines **13a−d**
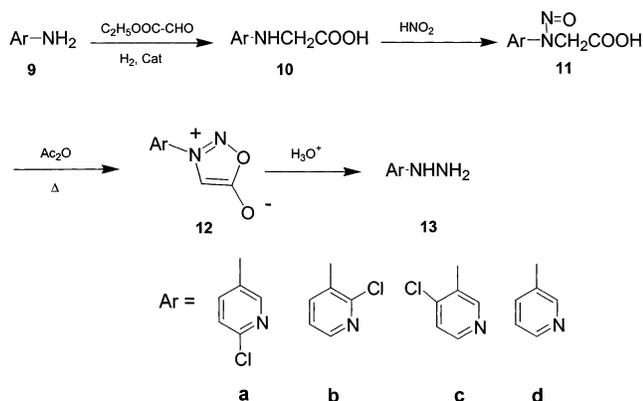
substituent with fluoride followed by removal of protecting groups (Scheme 4) affords the target compounds. The requisite hydrazines were prepared by two general methods. Pyrimidyl hydrazines **8b** and **8c** as well as 4-pyridylhydrazine **8a** were prepared by direct displacement of halogen by hydrazine in appropriately substi-

tuted heteroaryl chlorides **7** (Scheme 1).[11−13] 3-Pyridylhydrazine and hydrazines containing the substituted 3-pyridyl moiety were prepared by hydrolysis of the precursor *N*-(3-pyridyl)sydnones **12** (Scheme 2). The synthesis and hydrolysis of *N*-(3-pyridyl)sydnone itself were originally described by Hunsberger and co-workers.[14] Subsequently, the methods were applied to the synthesis of substituted pyridylsydnones.[15] Generally, the method involves the reductive amination of ethyl glyoxylate with a substituted 3-aminopyridine derivative **9** producing a substituted *N*-(3-pyridyl)glycine **10** that is converted to its *N*-nitroso derivative **11**. Cyclodehydration of the latter by heating in acetic anhydride gives the desired substituted *N*-(3-pyridyl)sydnone. Using these methods, we have prepared sydnones **12a−d** (Scheme 2) and have converted them to pyridylhydrazines **13a−d.** These hydrazines were not isolated, but were used directly in excess as ethereal solutions in the following step.

The precursor hydrazines **8a−c** and **13a−d** were each successfully condensed with steroid **14**, resulting in side chain protected N-substituted pyrazolo steroids **15** (Scheme 3). These were deprotected using aqueous acid as shown to give the chloropyridylpyrazolo products **16a−c** and nonhalogenated compounds **2−6**. The protected chloropyridylpyrazolo compounds **15a−c** were successfully fluorinated to give the protected fluoropyridylpyrazolo compounds **17a−c** as shown in Scheme 4. The fluorination method employs potassium fluoride with Kryptofix in dimethyl sulfoxide as solvent. This methodology has been successfully applied in radiofluorination procedures using [^{18}F]fluoride and thus is

Scheme 3. Synthesis of Chlorinated and Unsubstituted Heteroaryl Pyrazolo Steroids **2−6** and **16a−c**
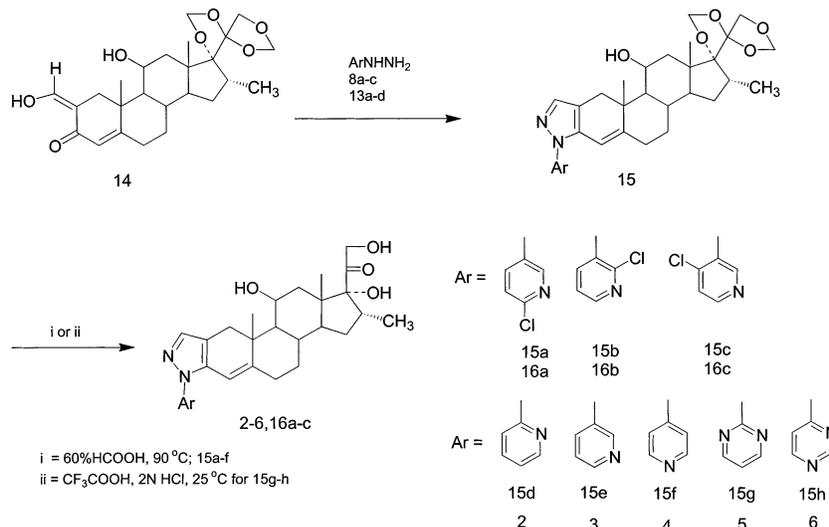
Scheme 4. Synthesis of Fluoropyridyl Pyrazolo Steroids **18a−c**
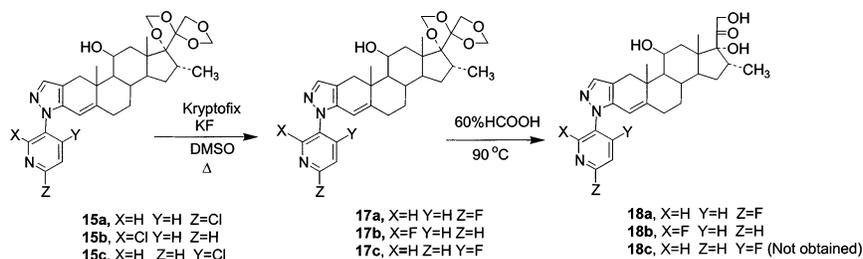
applicable to the preparation of these fluorinated products as radiotracers.[7]

In the course of these studies, it was determined that the hydrolytic conditions (60% aqueous formic acid, 100 °C for 30 min) normally used for the removal of the bis-(methylenedioxy) protecting group (BMD) in **17a**−**c** result in a significant amount of unwanted hydrolysis of the fluoropyridyl products. This leads to hydroxypyridyl (pyridone) side products as detected by HPLC and HRMS. Defluorination could be limited except in the case of **17c** by using lower reaction temperatures and shortening the reaction time (see Results and Discussion). By use of these modifications, the deprotected fluoropyridylpyrazolo products **18a** and **18b** were prepared. All attempts, however, at deprotection of **17c** using a variety of conditions and reagents (see Results and Discussion) resulted exclusively in hydrolytic loss of fluoride. Attempts to directly convert the deprotected chloropyridylpyrazolo steroid **16c** to **18c** using the KF/Kryptofix method were also not successful and gave only chlorinated products resulting from degradation of the corticoid side chain due to the high basicity of fluoride in this medium.

Results and Discussion

The goal of this study was to synthesize a glucocorticoid for PET imaging with [18]F. There were two major hurdles to overcome: the design of a compound that had high biological activity, and one that could be synthesized by common radiochemical techniques, e.g., substitution with [^{18}F]fluoride. To accomplish this, we synthesized several pyridyl- and pyrimidylpyrazolo steroids, **2**−**6**, compounds that when substituted with an appropriate leaving group in the activated pyridyl or pyrimidyl ring would produce the fluorinated analogue by exchange reactions. These pyrazolo steroids were tested in in vitro assays to determine which of those structures would be most likely to produce a highly active glucocorticoid when labeled with fluorine. The compounds were tested in two different assays: competition for the binding to the glucocorticoid receptor and induction of a glucocorticoid responsive gene (induction of AlkP) in HeLa cells. The binding assay provides a good measure of the intrinsic glucocorticoid potential of each compound, while the HeLa cell assay assesses additional relevant biological factors, such as susceptibility or resistance to metabolism, or whether the compound is an antagonist rather than an agonist. As can be seen in Table 1, the three unsubstituted pyridyl compounds **2**, **3**, and **4** are good ligands for the glucocorticoid receptor and they are also potent glucocorticoids, as is evident from their induction of AlkP in HeLa cells. The results further show that compound **3** is more potent than **2** and **4**. This is in agreement with the in vivo biological response in rats previously reported for these pyridyl steroids.[2] In contrast, the 2-pyrimidyl analogue **5** did not bind measurably to the glucocorticoid receptor, and the 4-pyrimidyl analogue **6** was also a relatively poor ligand. As would be expected from these results, neither showed appreciable activity in the HeLa cell assay, and it appeared that further syntheses of pyrimidyl pyrazoles would be unlikely to lead to useful glucocorticoids.

On the basis of the above results, we synthesized several halopyridyl derivatives of compound **3** (2′-(3-

Table 1. Glucocorticoid Action of Steroidal Pyridyl- and Pyrimidylpyrazoles

compound	glucocorticoid receptor (RBA[a])	HeLa Cell AlkP (RSA[b])
dexamethasone	100	100
4-FPhP	236 ± 50	483 ± 274
2	35 ± 9	30 ± 18
3	183 ± 65	255 ± 153
4	86 ± 50	135 ± 87
16b	166 ± 47	97 ± 59
16a	7 ± 2	24 ± 11
16c	78 ± 32	33 ± 33
18b	93 ± 31	121 ± 51
18a	304 ± 167	436 ± 160
5	0.2 ± 0.2	0
6	6 ± 3	0

[a] RBA, relative binding activity. Inhibition of [^{3}H]dexamethasone binding to glucocorticoid receptor in rat liver cytosol compared to dexamethasone. [b] RSA, relative stimulatory activity, i.e., induction of alkaline phosphatase (AlkP) in HeLa cells compared to dexamethasone. RBA and RSA are ±SD from at least three separate experiments performed in duplicate. The RSA and RBA are calculated using the curve-fitting program Prism.

pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-c]pyrazole) including two fluorinated derivatives **18a** and **18b** as potential glucocorticoid receptor imaging agents. As noted earlier, all attempts to prepare **18c** by aqueous acid deprotection of **17c** resulted exclusively in hydrolytic loss of fluoride. Interestingly hydrolysis of halide was observed for all of the fluorinated pyridylpyrazoles **17a**−**c** but not for the chlorinated analogues **15a**−**c**. Dehalogenation could be minimized for **17a** and **17b** by lowering reaction temperatures to 90 °C and shortening reaction time to 15 min. Even lower temperatures failed to allow isolation of **18c**, and the corresponding hydroxylated product was identified. In fact, at 40 °C in 60% formic acid, hydrolysis of fluoride was observed while the BMD ether protecting group remained intact. A lower temperature procedure for cleavage of the BMD ether group using trifluoroacetic acid and 0.1 N hydrochloric acid at 25 °C (see compound **5** in the Experimental Section) also resulted in hydrolysis of fluoride with no evidence of **18c**. In a nonacidic aqueous environment, neither hydrolysis of fluoride nor cleavage of the BMD ether group was observed at 60 °C over a period of 20 h. The observation of hydrolysis of the fluorinated compounds but not the chlorinated analogues under acidic conditions is consistent with earlier studies of halopyridines[16,17] and is due to nucleophilic attack by water on the more electrophilic fluorine-substituted protonated pyridine ring, resulting in a complex from which loss of covalent HF is competitive with loss of water. In the chlorinated analogues, attack by water on the less electrophilic ring may be slower and loss of (likely) ionic chloride may be less competitive with loss of water, resulting in retention of the halogen.[16−18]

In addition to the fluoropyridyl- and unsubstituted pyridyl- and pyrimidylpyrazolo steroids, the biological activities of the chloropyridyl compounds **16a**−**c** were determined under the conjecture that they might serve as models for compounds substituted with radiohalogens other than fluorine (I and Br). One of the chloro compounds, **16a**, showed poor binding to the glucocorticoid receptor, while two of them, **16b** and **16c**, were good ligands with relative binding affinities (RBAs) approaching the potent glucocorticoid dexamethasone

(166% and 78%, respectively). Neither **16b** nor **16c** exhibited the biological activity in the HeLa cell assay (97% and 33%, respectively) that might have been expected from their affinity for the glucocorticoid receptor. In fact, we have previously seen that the *p*-fluorophenyl analogue (see Table 1) has a greater biological activity in the HeLa cell assay in comparison to dexamethasone than would have been expected on the basis of receptor affinity, possibly because of metabolic protection. On this basis, it might logically have been predicted that the chloro analogues **16b** and **16c** would have produced a biological response of a greater magnitude than their receptor binding potency. Although there is usually a direct relationship between receptor binding and biological activity, this disconnect, especially for **16c**, may be caused by several factors including instability in biological media and metabolic inactivation. Moreover, receptor binding does not always mirror the conformational changes of the receptor that are required for the transcriptional stimulation of steroid responsive genes.[19] Regardless of the mechanism, the results of the HeLa cell assay indicate that non-fluorine substitution (at least with chlorine) of the 3-pyridylpyrazole is detrimental to its biological activity.

However, as can be seen in Table 1, the fluorinated analogues **18a** and **18b** were excellent ligands, with **18b** having a similar RBA for the glucocorticoid receptor as dexamethasone and with **18a** having an RBA approximately twice that of dexamethasone. Their biological activity reflected their affinity for the receptor, with **18b** having about the same potency as dexamethasone in the HeLa cell assay and with **18a** being considerably more potent, approximately 4-fold. Compound **18a** thus has great potential for imaging of glucocorticoid receptor containing tissues using positron emission tomography when labeled with the fluorine-18 isotope. Work is currently in progress to synthesize and test the radiolabeled steroid.

Summary and Conclusions

We have prepared several analogues of the well-known glucocorticoid cortivazol **1** in which the phenyl group has been replaced by the heterocyclic pyridyl and pyrimidyl groups. Substitution by the pyrimidyl group was found to seriously detract from glucocorticoid activity, while substitution by the pyridyl groups gave compounds with significant (compounds **2** and **4**) and enhanced (compound **3**) activity. Fluoropyridyl analogue **18a** derived from the structure of compound **3** was found to have enhanced potency as a glucocorticoid. It was prepared by methods that are adaptable to labeling with the positron-emitting isotope fluorine-18. Compound **18a** thus has great potential for PET imaging of glucocorticoid receptor containing tissues, such as the brain, where it may contribute to the understanding of several neurodegenerative disorders.

Experimental Section

General. Purification by flash chromatography was performed according to the procedure of Still[20] using 230−400 mesh silica gel (EM Science, Darmstadt, Germany). Melting points were obtained in a Mel-Temp apparatus or in a Koffler hot stage. Infrared spectra were recorded in potassium bromide disks on a MIDAC Prospect FT-IR instrument. NMR spectra were obtained at 100 MHz with a Bruker WP100SY FT instrument, at 400 MHz with a Bruker Aspect 3000 spectrometer, or at 500 MHz with a Bruker AM500 (500 MHz), and chemical shifts are reported relative to residual solvent signals. GC−MS spectra were obtained with a Hewlett-Packard system consisting of a model 5890 GC interfaced with a model 5972 mass-selective detector at 70 eV. The injector temperature was 250 °C, and the detector temperature was 280 °C. The GC column was operating at a flow rate of 1 mL/min (He) at an initial temperature of 200 °C for 5 min and then a temperature gradient of 10 °C/min for 5 min to 250 °C and held. High-resolution mass spectra were obtained by electrospray ionization on a Micromass Q-Tof spectrometer by Dr. Walter J. McMurray at the Yale University Comprehensive Cancer Center using either PEG with NH_4OAc or NaI as an internal standard. Elemental analyses were performed by Schwarzkopf Micro Analytical Laboratory, Woodside, NY. Thin-layer chromatography was performed on silica gel coated glass plates (Merck F254 type). UV-absorbing spots were detected using a short wavelength (254 nm) lamp. UV-nonabsorbing spots were detected by iodine visualization or by heating the plate after spraying with a cesium sulfate−sodium molybdate spray. The computer program Prism was purchased from GraphPad Software Inc. (San Diego, CA). Cell culture reagents were obtained from Gibco-BRL (Grand Island, NH). 2-Hydrazinopyridine dihydrochloride, 3-aminopyridine, 5-amino-2-chloropyridine, and 3-amino-2-chloropyridine were obtained commercially from Aldrich Chemical Co. Unless otherwise indicated, solvents (analytical or HPLC grade) and reagents were used as supplied and all reactions were carried out under nitrogen.

High-performance liquid chromatography (HPLC) was performed on a Beckman model 334 gradient system equipped with a model 421 controller, an Altex CR-1A integrator−recorder, and a Hitachi model 100-10 variable-wavelength detector or on a Beckman System Gold with a model 168 detector. The following systems were used: (H1) Ultrasphere-ODS (5 μm, 1 cm × 25 cm), 70% $CH_3OH−H_2O$, 3 mL/min, 260 nm; (H2) Ultrasphere-ODS (5 μm, 1 cm × 25 cm), 80% CH_3-$OH−H_2O$, 3 mL/min, 260 nm; (H3) Ultrasphere-ODS column (5 μm, 4.6 mm × 25 cm), 85% $CH_3OH−H_2O$, 1 mL/min, 254 and 280 nm; (H4) Ultrasphere-ODS column (5 μm, 4.6 mm × 25 cm), 80% $CH_3OH−H_2O$, 1 mL/min, 254 and 280 nm; (H5) Ultrasphere-ODS column (5 μm, 4.6 mm × 25 cm), 65% CH_3-$OH−H_2O$, 1 mL/min, 254 and 280 nm; (H6) Ultrasphere-ODS column (5 μm, 4.6 mm × 25 cm), 65% $CH_3OH−H_2O$ for 30 min, followed by a linear gradient to 80% $CH_3OH−H_2O$ over 2 min, 1 mL/min, 254 and 280 nm; (H7) Lichrosorb diol column (10 μm, 4.6 mm × 25 cm), CH_2Cl_2/isooctane/2-propanol (70:25:5), 1 mL/min, 260 nm; (H8) Lichrosorb diol column (10 μm, 4.6 mm × 25 cm), CH_2Cl_2/2-propanol (95:5), 1 mL/min, 254 nm; (H9) Lichrosorb diol column (10 μm, 4.6 mm × 25 cm), CH_2-Cl_2/2-propanol (97:3), 1 mL/min, 254 nm; (H10) Lichrosorb diol column (10 μm, 4.6 mm × 25 cm), CH_2Cl_2/2-propanol (99:1), 1 mL/min, 254 nm; (H12) RP C18 (5 μm, 4.6 mm × 25 cm), CH_3-CN/H_2O (1:1), 1 mL/min, 254 nm; (H13) Lichrosorb CN column (10 μm, 4.6 mm × 25 cm), hexanes/2-propanol (85:15), 1 mL/min, 260 nm; (H14) RP C18 (5 μm, 4.6 mm × 25 cm), CH_3-CN/H_2O (57:43), 1 mL/min, 280 nm.

***N*-(2-Chloro-5-pyridyl)glycine Hydrochloride (10a).** A solution of 5-amino-2-chloropyridine (4.54 g, 35 mmol), ethyl glyoxylate (Fluka, 50% in toluene, (7.24 g, 35 mmol), 18 mL of 95% ethanol, and 22 mL of concentrated HCl was allowed to stand at 25 °C for 1 h in a hydrogenation bottle. Catalyst (550 mg of 5% Pt on carbon) was added, and the mixture was hydrogenated on a Parr low-pressure reaction apparatus charged initially at 50 psi until no further absorption of hydrogen was evident (2 h). Distilled water (25 mL) was added, and the mixture was heated on a water bath to dissolve the white precipitate that formed during the reaction. Removal of the catalyst by filtration of the warm mixture and evaporation of the filtrate gave the glycine hydrochloride as a light-tan solid. Concentrated HCl was added, and the mixture was heated at 100 °C for 20 min and then cooled to 25 °C (during this treatment the solid did not dissolve) and allowed to stand for 5 days. Collection of the solid by vacuum filtration gave

5.87 g of tan powder. Concentration of the filtrate gave an additional 0.33 g of product with the same melting point (total yield 80%). Data for **10a**: mp 214−215 °C (dec); IR (KBr) 3358, 2600−3000 br, 1733 cm^{-1}; ^{1}H NMR (100 MHz, D_2O): δ 7.62 (s, 1H, H-6), 7.26 (m, 2H, H-3 and H-4), 3.84 (s, 2H, CH$_2$). Anal. (C$_7$H$_8$N$_2$O$_2$Cl$_2$) C, H, N, Cl.

N-Nitroso-N-(2-chloro-5-pyridyl)glycine (11a). Sodium nitrite (1.48 g, 21.5 mmol) was added in small portions over 5 min to a stirred suspension of the hydrochloride salt of **10a** (4.4 g, 19.7 mmol) in 50 mL of water was at 0 °C in an ice−saltwater bath. The reaction mixture was stirred at 0 °C for 1 h, and the tan precipitate that formed was collected by filtration. Crystallization after treatment with charcoal from water with a small amount of acetone gave 1.5 g (35%) of nitroso product **11a**. Data for **11a**: mp 146−148 °C (dec); IR (KBr) 3448, 1724, 1484, 1387 cm^{-1}; ^{1}H NMR (500 MHz, THF-d_8) δ 10.8 (s, 1H, COOH), 8.63 (d, 1H, J = 2.8 Hz, H-6), 8.05 (dd, 1H, J = 2.8, 8.7 Hz, H-3), 7.54 (d, 1H, J = 8.7 Hz, H-4). Anal. (C$_7$H$_6$N$_3$O$_3$Cl). C: calcd, 38.99; found, 39.88. H: calcd, 2.81; found, 2.59. N: calcd, 19.49; found, 19.64.

2-Chloro-5-pyridylsydnone (12a). A solution of N-nitroso-N-(2-chloro-5-pyridyl)glycine **11a** (1.31 g, 6.09 mmol) in 13 mL of acetic anhydride was heated with stirring at 100 °C for 30 min in a flask equipped with a condenser and a drying tube. Evaporation of the solvent gave a brown tarry residue that was stirred in 2 mL of 1:3 ethanol/water at 0 °C for 1 h. Filtration gave a 0.7 g of tan solid, mp 126−130 °C. A further 0.16 g was obtained from the filtrate upon treatment with charcoal and concentration, giving a total yield of 71%. Crystallization in water gave **12a**. Data for **12a**: mp 128−131 °C; IR (KBr) 1750 cm^{-1}; GC−MS: parent peak (*m/z*) 197, (*m/z* + 2) 199, 167, 139; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.83 (s, 1H, H-6), 8.07 (d, 1H, J = 8.8 Hz, H-3), 7.65 (d, 1H, J = 8.8 Hz, H-4), 6.79 (s, 1H, sydnone ring H). Anal. (C$_7$H$_4$N$_3$O$_2$Cl) C, H, N.

2-Chloro-5-hydrazinopyridine (13a). To 197 mg (1.00 mmol) of N-(2-chloro-5-pyridyl)sydnone **12a** in an N$_2$ atmosphere at 25 °C was slowly added 1 mL of concentrated HCl. After evolution of CO$_2$ ceased, a fine yellow precipitate appeared on continued stirring. The slurry was cooled to −11 °C (ice−saltwater) and 2.2 mL of 20% NaOH was added whereupon the color became red. Solid anhydrous K$_2$CO$_3$ was added to saturate the solution, and it was extracted with ether (6 × 10 mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered, and stored under N$_2$ over solid anhydrous K$_2$CO$_3$. Compound **13a** was characterized as its oxalate salt by treatment of 12 mL of the above ethereal solution with 1.5 mL of 10% oxalic acid solution in absolute ethanol. This produced 15 mg of pale-yellow solid. Data for **13a**-oxalate: mp 156.5−158.5 °C; IR (KBr) 3443, 3274, 3162, 1630, 1592, 1487, 1310 cm^{-1}. The mass of the oxalate salt allows an estimate of the yield (unoptimized) of **13a** to be at least 40%. Elemental analysis was consistent with an oxalate salt containing a 2:1 ratio of the hydrazine to oxalic acid (C$_5$H$_6$ClN$_3$)$_2$·H$_2$C$_2$O$_4$. Anal. (C$_{12}$H$_{14}$N$_6$O$_4$Cl$_2$) C, H, N.

2′-(2-Chloro-5-pyridyl)-11β-hydroxy-16α-methyl-17,20:20,21-bis(methylenedioxy)pregn-4-eno[3,2-c]pyrazole (15a). A solution of compound **14**[1] (93 mg, 0.21 mmol) and **13a** (evaporated from 35 mL of the ether solution described above) in absolute ethanol (3 mL) was stirred and heated at reflux for 3 h under N$_2$. Evaporation of the solvent and purification by flash chromatography on a 2 cm × 18 cm column of silica gel with elution with 5% 2-propanol in chloroform gave 39 mg (33%) of **15a** as an oil. Data for **15a**: UV λ_{max} = 265 nm; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.58 (d, 1H, J = 2.5 Hz, pyridine H-6), 7.87 (dd, 1H, J = 2.5 and 8.5 Hz, pyridine H-3), 7.49 (s,1H, pyrazole-H), 7.45 (d, 1H, J = 8.5 Hz, pyridine H-4), 6.09 (s, 1H, H-4), 5.1 (m, 4H, −OCH$_2$O-), 4.46 (m, 1H, H-11α), 4.01 (s, 2H, H-21), 1.33 (s, 3H, H-19), 1.19 (s, 3H, H-18), 0.96 (d, 3H, J = 6.9 Hz, 16α-CH$_3$); HRMS calcd for C$_{30}$H$_{36}$ClN$_3$O$_5$ 554.2422, found 554.2427.

2′-(2-Fluoro-5-pyridyl)-11β-hydroxy-16α-methyl-17,20:20,21-bis(methylenedioxy)pregn-4-eno[3,2-c]pyrazole (17a). A solution of **15a** (40 mg, 0.0724 mmol), KF (21.7 mg, 0.375 mmol), and Kryptofix (37.1 mg, 0.0989 mmol) in DMSO (7.6 mL) was heated at 130 °C in a screw-cap test tube for 2 h. Additional portions of KF and Kryptofix were added, and heating continued for a total of 16 h. The entire reaction mixture was submitted to flash chromatography on a 5.5 cm × 16 cm column of silica gel, and eluting with 2:3 toluene/EtOAc gave 22.6 mg (58%) of **17a** as an oil. An analytical sample of the product was obtained by crystallization in methylene chloride−petroleum ether. Data for **17a**: mp 117−121 °C; UV λ_{max} = 260 nm; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.37 (m,1H, pyridine H-6), 8.01 (ddd, 1H, J = 9 Hz, $J_{F,H}$ = 8 Hz and J = 3 Hz, pyridine H-3), 7.48 (s, 1H, pyrazole H), 7.07 (dd, 1H, J = 9, 3 Hz, pyridine H-4), 6.06 (s, 1H, H-4), 5.10 (m, 4H, −OCH$_2$O−), 4.47 (m, 1H, H-11α), 4.01 (s, 2H, H-21), 1.33 (s, 3H, H-19), 1.19 (s, 3H, H-18), 0.96 (d, 3H, J = 7 Hz, 16α-CH$_3$); HRMS calcd for C$_{30}$H$_{36}$FN$_3$O$_5$ 538.2717, found 538.2725.

2′-(2-Fluoro-5-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-c]pyrazole (18a). A solution of **17a** (6.2 mg, 0.012 mmol) in 60% formic acid (6.2 mL) was heated at 90 °C in a screw-cap test tube for 15 min. The mixture was cooled and concentrated using portions of ethanol (3 × 10 mL) to assist in removing formic acid. Purification by HPLC system H1 (t_R = 14.2 min) followed by crystallization in methylene chloride−petroleum ether gave 1.6 mg (28%) of **18a**. Data for **18a**: mp 234−238 °C; UV λ_{max} = 260 nm; IR (KBr) 3430, 1711, 1099 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.36 (m, 1H, pyridine H-6), 8.00 (ddd, 1H, J = 9 Hz, $J_{(F,H)}$ = 8 Hz, and J = 3 Hz, pyridine H-3), 7.48 (s, 1H, pyrazole H), 7.08 (dd, 1H, J = 9, 3 Hz, pyridine H-4), 6.06 (s, 1H, H-4), 4.51 (m, 1H, H-11α), 4.63 and 4.30 (AB quartet, J = 20 Hz, 2H, H-21), 1.31 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, J = 7.2 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{34}$FN$_3$O$_4$ 495.2612, found 495.2613; analytical HPLC in systems H5 (t_R = 25 min) and H7 (t_R = 7.2 min), >97% pure.

2′-(2-Chloro-5-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-c]pyrazole (16a). A solution of **15a** (10 mg, 0.018 mmol) in 60% formic acid (10 mL) was hydrolyzed as described above for compound **17a** to give 4.5 mg (49%) of **16a** after HPLC in system H1 (t_R = 23.5 min). Crystallization in methylene chloride−petroleum ether gave 2.4 mg (26%) of **16a**. Data for **16a**: mp 142−143 °C; UV λ_{max} = 266 nm; IR (KBr) 3435, 1713, 1098 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.53 (d, 1H, J = 2.4 Hz, pyridine H-6), 7.84 (dd, 1H, J = 8.8, 2.4 Hz, pyridine H-3), 7.46 (s, 1H, pyrazole-H), 7.42 (d, 1H, J = 8.8 Hz, pyridine H-4), 6.07 (s, 1H, H-4), 4.49 (m, 1H, H-11α), 4.59 and 4.28 (AB quartet, J = 20 Hz, 2H, H-21), 1.29 (s, 3H, H-19), 1.04 (s, 3H, H-18), 0.92 (d, 3H, J = 7.2 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{34}$ClN$_3$O$_4$ 512.2316 (M + H), found 512.2312 (M + H); analytical HPLC in systems H5 (t_R = 29.7 min) and H10 (t_R = 9.4 min), >98% pure.

N-(2-Chloro-3-pyridyl)glycine Hydrochloride (10b). A mixture of 3-amino-2-chloropyridine (3.0 g, 23 mmol), ethyl glyoxylate (50% in toluene, 8.0 g, 39 mmol), 12 mL of water, 14 mL of concentrated HCl, and 400 mg of 5% Pt on carbon was prepared and hydrogenated as described for **10a** above. After removal of the catalyst and evaporation the glycine hydrochloride, **10b** was obtained as an oil, 5.4 g. Data for **10b**: IR (KBr) 3387, 2600−3200 br, 1731 cm^{-1}; ^{1}H NMR (100 MHz, D$_2$O) δ 7.8 (m, 1H, pyridine), 7.5 (m, 1H, pyridine), 7.2 (m, 1H, pyridine), 3.92 (m, 2, CH$_2$).

N-Nitroso-N-(2-chloro-3-pyridyl)glycine (11b). A solution of sodium nitrite (0.85 g, 0.012 mmol) in H$_2$O (9 mL) was gradually added to a solution of glycine hydrochloride **10b** (2.5 g, 0.011 mmol) in 8 mL of water at −7 °C (ice−saltwater bath) and stirred at −7 °C for 2 h. The precipitate that formed was filtered and dried in a vacuum desiccator, giving **11b**, 1.4 g (58%) (the material gave a positive Liebermann test).[21] Data for **11b**: mp 139−143 °C; IR (KBR) 3200−3600, 1731, 1461 cm^{-1}. Anal. (C$_7$H$_6$N$_3$O$_3$Cl). C: calcd, 38.99; found, 38.31. H: calcd, 2.81; found, 3.15. N: calcd, 19.49; found, 17.24.

2-Chloro-3-pyridylsydnone (12b). Sydnone **12b** was prepared using **11b** (0.3 g, 1.4 mmol) and acetic anhydride as described for **12a**. Purification by flash chromatography and eluting with 3:1 chloroform/acetonitrile followed by crystal-

lization from methylene chloride−petroleum ether gave 198 mg (72%) of **12b**. Data for **12b**: mp 100−101 °C; IR (KBr) 1746, 1420 cm^{-1}; GC−MS parent peak (*m/z*) 197, (*m/z* + 2) 199, 167, 139; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.75 (dd, 1H, *J* = 1.8, 4.8 Hz, H-6), 8.02 (dd, 1H, *J* = 1.8, 8.0 Hz, H-4), 7.59 (dd, 1H, *J* = 4.8, 8.0 Hz, H-5), 6.76 (s, 1H, sydnone). Anal. (C$_7$H$_4$N$_3$O$_2$Cl) C, H, N.

2′-(2-Chloro-3-pyridyl)-11β-hydroxy-16α-methyl-17,20: 20,21-bis(methylenedioxy)pregn-4-eno[3,2-*c*]pyrazole (15b). Compound **15b** was prepared as described above for **15a** (**12a** →**13a** →**15a**) using sydnone **12b** (65 mg, 0.33 mmol) and concentrated HCl (0.5 mL) to produce **13b**. The resulting solution of **13b** in ether was reacted with **14** (84 mg, 0.19 mmol) in the same manner as above (for **15a**), giving a clear yellow oil after evaporation. The residue was dissolved in methylene chloride and washed with 2% aqueous NaOH and then H$_2$O. The organic layer was dried over Na$_2$SO$_4$, filtered, and evaporated. Purification of the residue by flash chromatography eluting with 1:1 toluene/EtOAc gave 47.5 mg (44%) of **15b**. The product was crystallized by methylene chloride and petroleum ether. Data for **15b**: mp 267−274 °C; UV λ$_{max}$= 264 nm; IR (KBr) 3433, 2931, 2874, 1621, 1099, 1015, 941 cm^{-1}; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.5 (m, 1H, pyridine H-6), 7.8 (m, 1H, pyridine H-4), 7.5 (s, 1, pyrazole), 7.3 (m, 1H, pyridine H-5), 6.09 (s, 1H, H-4), 5.10 (m, 4H, −OCH$_2$O−), 4.00 (s, 2H, H-21), 1.32 (s, 3H, H-19), 1.19 (s, 3H, H-18), 0.93 (d, *J* = 6.7 Hz, 3H, 16α-CH$_3$); MS 554 (M$^+$), 556 (M$^+$+ 2).

2′-(2-Fluoro-3-pyridyl)-11β-hydroxy-16α-methyl-17,20: 20,21-bis(methylenedioxy)pregn-4-eno[3,2-*c*]pyrazole (17b). A solution of compound **15b** (15 mg, 0.027 mmol), KF (7.84 mg, 0.135 mmol), and Kryptofix (13.9 mg, 0.037 mmol) in 2.8 mL of dimethyl sulfoxide was reacted as described above for **17a**. At 45 and 90 min, additional molar equivalents of KF and Kryptofix were added. After 135 min, the entire reaction mixture was submitted to flash chromatography. Elution with 1:2 hexanes/EtOAc gave 12.7 mg (87.4%) of **17b**. Data for **17b**: UV λ$_{max}$ = 272 nm; MS 538 (M$^+$); ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.24 (m, 1H, pyridine H-6), 7.97 (m, 1H, pyridine H-5), 7.50 (s, 1H, pyrazole C−H), 7.45 (m, 1H, pyridine, H-4), 5.86 (s, 1H, H-4), 5.10 (m, 4H, −OCH$_2$O−), 3.99 (s, 2H, H-21), 1.31 (s, 3H, H-19), 1.26 (s, 3H, H-18), 0.95 (d, 3H, *J* = 7 Hz, 16α-CH$_3$).

2′-(2-Fluoro-3-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole (18b). Compound **18b** was prepared from **17b** (12.7 mg, 0.023 mmol) and 60% formic acid (10 mL) by the procedure used to prepare **18a**. Purification by flash chromatography and eluting with 10% 2-propanol−chloroform gave 9.8 mg (84%) of **18b**. An analytical sample of **18b** was obtained by HPLC purification in system H1 (*t*$_R$ = 14.2 min) followed by crystallization in methylene chloride−petroleum. Data for **18b**: mp 225−230 °C; UV λ$_{max}$ = 272 nm; IR (KBr) 3420, 1711, 1056 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.27 (m, 1, pyridine H-6), 7.99 (m, 1H, pyridine H-4), 7.52 (s, 1H, pyrazole C−H), 7.36 (m, 1H, pyridine H-5), 5.90 (s, 1H, H-4), 4.52 (m,1H, H-11α), 4.64 and 4.31 (AB quartet, *J* = 20 Hz, 2H, H-21), 1.32 (s, 3H, H-19), 1.08 (s, 3H, H-18), 0.95 (d, 3H, *J* = 7.5 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{34}$FN$_3$O$_4$ 496.2611 (M + H), found 496.2631; HPLC systems H10 (*t*$_R$ = 21.6 min) and H12 (*t*$_R$ = 9.4 min), >99% pure.

2′-(2-Chloro-3-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole (16b). Compound **16b** was prepared from **15b** (15 mg, 0.027 mmol) and 60% formic acid (10 mL) by the procedure used to prepare **18a**. Purification of the residue by flash chromatography and eluting with 10% 2-propanol in chloroform gave 12.4 mg (89.4%) of **16b**. Data for **16b**: mp 200−205 °C (CH$_2$Cl$_2$−petroleum ether); UV λ$_{max}$ = 271 nm; IR (KBr) 3423, 1709, 1053 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.50 (d, 1H, *J* = 4.6 Hz, pyridine H-6), 7.81 (d, 1H, *J* = 7.9 Hz, pyridine H-4), 7.51 (s, 1H, pyrazole H), 7.41 (m, 1H, *J* = 7.9, 4.6 Hz, pyridine H-5), 5.72 (s, 1H, H-4), 4.53 (m, 1H, H-11α), 4.63 and 4.30 (AB quartet, *J* = 20 Hz, 2H, H-21), 1.32 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, *J* = 7.2 Hz, 16α-CH$_3$). HRMS calcd for C$_{28}$H$_{34}$ClN$_3$O$_4$ 512.2316 (M + H),

found 512.2310 (M + H); HPLC systems H5 (*t*$_R$ = 17.4 min) and H7 (*t*$_R$ = 7.8 min), >98% pure.

N-(4-Chloro-3-pyridyl)glycine Hydrochloride (10c). Compound **10c** was prepared using 3-amino-4-chloropyridine (1.59 g, 12.3 mmol), ethyl glyoxylate (50% in toluene, 4.2 g, 20 mmol), 6.2 mL of H$_2$O, 7.4 mL of concentrated HCl, and 184 mg of 5% Pt on carbon as described for **10a** above. After removal of the catalyst and evaporation, the glycine hydrochloride **10c** was obtained as a clear yellow oil (2.79 g, 100%). Data for **10c**: IR (KBr) 2700−3400 br, 1736 cm^{-1}; ^{1}H NMR (100 MHz, D$_2$O) 7.96 (m, 1H, pyridine), 7.75 (m, 1H, pyridine), 7.69 (m, 1H, pyridine), 3.95 (m, 2H, CH$_2$).

N-Nitroso-N-(4-chloro-3-pyridyl)glycine (11c). Compound **11c** was prepared using sodium nitrite (3.82 g, 55.4 mmol) and glycine·HCl **10c** (11.2 g, 50.4 mmol) using the procedure described for **11b**, giving 4.4 (41%) of **11c**, which gave a positive Liebermann test.[21] Data for **11c**: mp 109.5−112 °C; IR (KBr) 1732, 1471, 1219, 950, 834, 732 cm^{-1}. Anal. (C$_7$H$_6$N$_3$O$_3$Cl). C: calcd, 38.99; found, 38.47. H: calcd, 2.81; found, 3.06. N: calcd 19.49; found, 19.03.

N-(4-chloro-3-pyridyl)sydnone (12c). Sydnone **12c** was prepared using **11c** (2.15 g, 10 mmol) and acetic anhydride as described for **12a**. Purification by flash chromatography and eluting with CHCl$_3$/CH$_3$CN (3:1) gave 521 mg (26%) of **12c**. Data for **12c**: mp 129−132 °C; IR (KBr) 1737, 1490, 1408, 835, 731 cm^{-1}; GC−MS parent peak (*m/z*) 197, (*m/z* + 2) 199; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.83 (d, 1H, *J* = 5.4 Hz, H-6), 8.80 (s, 1H, H-2), 7.67 (d, 1H, *J* = 5.4 Hz, H-5), 6.71 (s, 1H, sydnone).

4-Chloro-3-hydrazinopyridine (13c). Compound **13c** was prepared using **12c** (120.5 mg, 0.61 mmol) and 0.6 mL of concentrated HCl by the procedure used for **13a**. Extraction of the product required 10 × 15 mL portions of ether. Compound **13c** was characterized as its oxalate salt as in **13a**. Data for **13a**-oxalate: mp 140−143 °C; IR (KBr) 3460, 1723, 1625 cm^{-1}.

2′-(4-Chloro-3-pyridyl)-11β-hydroxy-16α-methyl-17,20: 20,21-bis(methylenedioxy)pregn-4-eno[3,2-*c*]pyrazole (15c). Pyrazole **15c** was prepared using **14** (162 mg, 0.364 mmol) and **13c** (70.7 mg, 0.492 mmol) as described for **15b**. Purification by flash chromatography and eluting with toluene/EtOAc (1:2) gave 108 mg (54%) of **15c**. Data for **15c**: mp 191−201 °C; UV λ$_{max}$ = 268 nm; IR (KBr) 3433, 2932, 1497, 1429, 1098, 942 cm^{-1}; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.64 (s, 1H, pyridine H-2), 8.57 (d, *J* = 4.1 Hz, 1H, pyridine H-6), 7.52 (s, 1H, pyrazole C−H) overlapping with 7.50 (d, *J* = 4.1 Hz, 1H, pyridine H-5), 5.68 (s, 1H, H-4), 5.09 (m, 4, −OCH$_2$O−), 3.99 (s, 2H, H-21), 1.32 (s, 3H, H-19), 1.18 (s, 3H, H-18), 0.94 (d, 3H, *J* = 7 Hz, 16α-CH$_3$); MS 554.2 (M$^+$), 556 (M$^+$ + 2).

2′-(4-Chloro-3-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-*c*]pyrazole (16c). Pyrazole **16c** was prepared using **15c** (20 mg, 0.036 mmol) and 60% formic acid (20 mL) as described for **18a**. Purification by flash chromatography and eluting with 10% 2-propanol−chloroform gave 6.6 mg (36%) of **16c**. An analytical sample of **16c** was obtained by HPLC in system H1 (*t*$_R$ = 16.4 min) followed by crystallization in CH$_2$Cl$_2$−petroleum ether. Data for **16c**: mp 200−205 °C; UV λ$_{max}$ = 266 nm; IR (KBr) 3423, 2929, 1714, 1643, 1135, 986 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.64 (s, 1H, pyridine H-2), 8.57 (d, 1H, *J* = 5.3 Hz, pyridine H-6), 7.52 (s, 1H, pyrazole H), 7.50 (d, 1H, *J* = 5.3 Hz, pyridine H-5), 5.69 (s, 1H, H-4), 4.53 (m, 1H, H-11α), 4.63 and 4.30 (AB quartet, *J* = 20 Hz, 2H, H-21), 1.32 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, *J* = 7.4 Hz, 16α-CH$_3$); MS 512 (M$^+$), 514 (M$^+$ + 2); HRMS calcd for C$_{28}$H$_{34}$ClN$_3$O$_4$ 512.2316 (M + H), found 512.2311 (M + H); HPLC systems H8 (*t*$_R$ = 11.6 min) and H12 (*t*$_R$ = 10.4 min), >99% pure.

2′-(4-Fluoro-3-pyridyl)-11β-hydroxy-16α-methyl-17,20: 20,21-bis(methylenedioxy)pregn-4-eno[3,2-*c*]pyrazole (17c). Pyrazole **17c** was prepared using **15c** (25 mg, 0.045 mmol), KF (13.5 mg, 0.23 mmol), Kryptofix (23.2 g, 0.062 mmol), and 4.42 mL of dimethyl sulfoxide as described for **17a**. Purification by flash chromatography using 1:5 hexanes/EtOAc yielded 21.1 mg of a mixture of starting material and product

in a 2:1 ratio. An analytical sample of **17c** was obtained by semipreparative HPLC in system H2 (t_R = 20.4 min). Data for **17c**: UV λ_{max} = 260 nm; IR (KBr) 3445, 2932, 1622, 1511, 1098, 942, 758 cm^{-1}; ^{1}H NMR (500 MHz, CD$_2$Cl$_2$) δ 8.76 (d, 1H, J = 10 Hz, pyridine H-6), 8.58 (m, 1H, pyridine H-2), 7.53 (s, 1H, pyrazole-H), 7.24 (dd, 1H, J = 5, J = 10 Hz, H-5), 5.82 (s, 1H, H-4), 5.06 (m, 4H, $-$O$_2$CH$_2$O), 3.97 (s, 2H, H-21), 1.29 (s, 3H, H-19), 1.16 (s, 3H, H-18), 0.91 (d, 3H, J = 7 Hz, 16α-CH$_3$); HRMS calcd for C$_{30}$H$_{36}$FN$_3$O$_5$ 538.2717, found 538.2703.

2′-(2-Pyridyl)-11β-hydroxy-16α-methyl-17,20:20,21-bis-(methylenedioxy)pregn-4-eno[3,2-c]pyrazole (15d). A solution of **14** (50 mg, 0.12 mmol), 2-hydrazinopyridine dihydrochloride (Aldrich) (27 mg, 0.15 mmol), and anhydrous NaOAc (24 mg, 0.3 mmol) in 3.2 mL of absolute ethanol was stirred and heated at reflux under N$_2$ for 3 h. Distillation of part of the solvent (1 mL) followed by slow addition of water (1.5 mL) induced crystallization to give 30 mg (48%) of **15d**. Data for **15d**: IR (KBr) 2931, 2876, 1592, 1474, 1098, 942, 778 cm^{-1}.

2′-(2-Pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxo-pregn-4-eno[3,2-c]pyrazole (2). Hydrolysis of **15d** (30 mg, 0.054 mmol) was carried out as described for **18a**. Purification by flash chromatography in 5% 2-propanol$-$chloroform gave 14.3 mg (55%) of **2**. An analytical sample was crystallized in CH$_3$OH and water. Data for **2**: mp 140$-$146 °C; UV λ_{max} = 284 nm; IR (KBr) 3445, 2927, 1709, 1592, 1472, 144, 1384, 779 cm^{-1}; ^{1}H NMR (100 MHz, CDCl$_3$) δ 8.43 (d, 1H, J = 4.3 Hz, pyridine H-6), 7.86 (m, 2H, pyridine H-3, H-5), 7.44 (s, 1H, pyrazole C$-$H), 7.13 (m, 1H, pyridine H-4), 6.22 (s, 1H, H-4), 4.52 (s, 1H, H-11α), 4.62 and 4.30 (AB quartet, J = 20 Hz, 2H, H-21), 1.30 (s, 3H, H-19), 1.04 (s, 3H, H-18), 0.93 (d, 3H, J = 7.2 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{35}$N$_3$O$_4$ 478.2706 (M + H), found 477.2725; HPLC systems H6 (t_R = 37 min) and H13 (t_R = 27 min), >95% pure.

2′-(3-Pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxo-pregn-4-eno[3,2-c]pyrazole (3). Pyrazole **15e** was prepared using **14** (93 mg, 0.21 mmol) and 3-hydrazinopyridine[14] (0.42 mmol, 24.5 mL of 0.017 M solution in ether), followed by hydrolysis in 60% formic acid as described for **18a** above, giving 55 mg (55%) of **3**. A portion of this material (45 mg) was purified by flash chromatography in 15% 2-propanol$-$chloroform and then by crystallization in aqueous MeOH to give an analytical sample of **3**. Data for **3**: mp 240$-$250 °C; UV λ_{max} = 263; IR (KBr) 3400, 1717, 1131 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.81 (d, 1H, J = 2.3 Hz, pyridine H-2), 8.60 (d, 1H, J = 5.0 Hz, pyridine H-6), 7.9 (d, 1H, J = 7.7 Hz, pyridine H-4), 7.49 (s, 1H, pyrazole-H), 7.43 (m, 1H, pyridine H-5), 6.14 (s, 1H, H-4), 4.51 (m, 1H, H-11α), 4.63 and 4.30 (AB quartet, J = 20 Hz, 2H, H-21), 1.32 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, J = 7.3 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{35}$N$_3$O$_4$ 478.2706 (M + H), found 478.2720; HPLC in systems H5 (t_R =15.4 min) and H7 (t_R = 12.4 min), >98% pure.

2′-(4-Pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxo-pregn-4-eno[3,2-c]pyrazole (4). Pyrazole **15f** was prepared using **14** (100 mg, 0.22 mmol), 4-hydrazinopyridine hydrochloride[11] (65 mg, 0.45 mmol), and sodium acetate (61 mg, 0.45 mmol) as described for **15d**. Purification by flash chromatography in EtOAc gave 37 mg of **15f**, which was hydrolyzed as described for **18a**. Purification by flash chromatography and eluting with 15% 2-propanol$-$chloroform gave 11.5 mg (11%) of **4**. Data for **4**: mp 159$-$162 °C (MeOH$-$H$_2$O); UV λ_{max} = 269; IR (KBr) 3400, 1710, 1596, 1068 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.70 (m, 2H, pyridine H-2, H-6), 7.58 (m, 2H, pyridine H-3, H-5), 7.53 (s, 1H, pyrazole-H), 6.29 (s, 1H, H-4), 4.52 (m, 1H, H-11α), 4.64 & 4.31 (AB quartet, J = 20 Hz, 2H, H-21), 1.32 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, J = 7.7 Hz, 16α-CH$_3$); HRMS calcd for C$_{28}$H$_{35}$N$_3$O$_4$ 478.2706 (M + H), found 477.2711; HPLC systems H5 (t_R =15.4 min) and H9 (t_R = 10.6 min), >97% pure.

2′-(2-Pyrimidyl)-11β-hydroxy-16α-methyl-17,20:20,21-bis(methylenedioxy)pregn-4-eno[3,2-c]pyrazole (15g). A solution of compound **14** (50 mg, 0.12 mmol) and 2-pyrimidyl-hydrazine **8b**[12] (12 mg, 0.11 mmol) in ethanol (6 mL) was refluxed for 1 h. Evaporation of the solvent and purification of the residue by flash chromatography on a 2 cm × 17 cm

column of silica gel using EtOAc as eluent gave 20 mg (34%) of **15g**. Data for **15g**: ^{1}H NMR (400 MHz, CHCl$_3$) δ 8.77 (d, 2H, J = 4.7 Hz, pyrimidine H-4,6), 7.56 (s, 1H, H-pyrazole), 7.17 (t, 1H, J = 4.7 Hz, pyrimidine H-5), 6.95 (d, 1H, J = 2 Hz, H-4), 5.10 (m, 4H, $-$OCH$_2$O$-$), 4.46 (m, 1H, H-11α), 4.00 (s, 2H, H-21), 1.32 (s, 3H, H-19), 1.20 (s, 3H, H-18), 0.97 (d, 3H, J = 7.4 Hz, 16α-CH$_3$).

2′-(2-Pyrimidyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-c]pyrazole (5). A solution of **15g** (20 mg, 0.04 mmol), CF$_3$COOH (1.5 mL), and 2 N HCl (1.5 mL) was stirred for 2 h at 25 °C. After the reaction was quenched with EtOAc (1 mL), the organic layer was separated, washed with saturated aqueous NaHCO$_3$, and H$_2$O and dried (Na$_2$SO$_4$). Purification by flash chromatography on a 2 cm × 17 cm column of silica gel and eluting with CH$_3$OH/EtOAc (1:50) followed by crystallization from acetone gave 7 mg (33%) of **5**. Data for **5**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (d, 2H, J = 4.8 Hz, pyrimidine H-4,6), 7.57 (s, 1H, H-pyrazole), 7.43 (t, 1H, J = 4.8 Hz, pyrimidine H-5), 6.95 (s, 1H, H-4), 4.48 and 4.07 (AB quartet, J = 19 Hz, 2H, H-21), 4.29 (m, 1H, H-11α), 1.19 (s, 3H, H-19), 0.85 (s, 3H, H-18), 0.77 (d, 3H, J = 7.4 Hz, 16α-CH$_3$); HRMS (M + Na$^+$) calcd for C$_{27}$H$_{34}$N$_4$O$_4$Na 501.2478, found 501.2481. Anal. (C$_{27}$H$_{34}$N$_4$O$_4$) C, H, N.

2′-(4-Pyrimidyl)-11β,17,21-trihydroxy-16α-methyl-20-oxopregn-4-eno[3,2-c]pyrazole (6). Pyrazole **15h** was prepared using **14** (57 mg, 0.13 mmol) and 4-pyrimidylhydrazine **8c**[13] (37 mg, 0.33 mmol) as described for **15g**. Purification by flash chromatography on a 2 cm × 17 cm column of silica gel and eluting with n-hexane/EtOAc (3:1) gave 15 mg (22%) of **20**. Hydrolysis of **15h** (13 mg, 0.026 mmol) as described above for **5**, followed by flash chromatography on a 2 cm × 17 cm column of silica gel and eluting with n-hexanes/EtOAc (1:2) and further purification by HPLC system H2 (t_R = 9.5 min) gave 6 mg (43%) of **6**. Data for **6**: ^{1}HNMR (400 MHz, CDCl$_3$) δ 9.02 (d, 1H, J = 0.7 Hz, pyrimidine H-2), 8.71 (d, 1H, J = 6 Hz, pyrimidine H-5), 7.92 (dd, 1H, J = 0.7, 6 Hz, pyrimidine H-6), 7.51 (s, 1H, pyrazole H), 7.31 (d, 1H, J = 1.6 Hz, H-4), 4.62 and 4.30 (AB quartet, J = 20 Hz, 2H, H-21), 4.50 (m, 1H, H-11α), 1.31 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.95 (d, 3H, J = 7 Hz, 16α-CH$_3$); HRMS (M + Na$^+$) calcd for C$_{27}$H$_{34}$N$_4$O$_4$Na 501.2478, found 501.2475; HPLC systems H10 (280 nm, t_R = 10 min) and H14 (t_R = 10 min), >99% pure.

Competitive Binding to the Glucocorticoid Receptor. Binding affinity for the glucocorticoid receptor relative to dexamethasone was determined as previously described using cytosol obtained from adrenalectomized rats.[22] A complete dose response was obtained by incubating cytosol overnight in an ice bath with 2 nM [^{3}H]dexamethasone ± steroidal competitors over a range of concentrations from 10^{-11} to 10^{-6} M. Afterward, a slurry of dextran-coated charcoal was added, mixed thoroughly, and centrifuged. The receptor-bound steroid was quantified in an aliquot of the supernatant by counting in a liquid scintillation spectrophotometer. Relative binding affinity in comparison to dexamethasone was determined by analysis of the displacement curve using the curve-fitting program Prism. Each steroid was tested in at least three experiments performed in duplicate.

Glucocorticoid Potency: HeLa Cell Assay. Biological potency of the steroids was assessed by the induction of alkaline phosphatase in HeLa cells [23] as described[24] with some minor modifications.[6] The cells are grown in the presence of the steroids (over a range of 10^{-12}$-$10^{-6} M) in a 96-well plate for 3 days. The induction of alkaline phosphatase (AlkP) in the cells was determined kinetically at 405 nm through the use of the chromogenic substrate p-nitrophenyl phosphate, as we have described.[25] The relative stimulatory activity (RSA) represents the ratio of 1/EC$_{50}$ of the steroid analogue to that of dexamethasone × 100. The EC$_{50}$ of each compound was determined from the dose response using the curve-fitting program Prism. Each compound was analyzed in at least three separate experiments performed in duplicate.

Note Added after ASAP Posting. This manuscript was released ASAP on 10/26/2002 with errors in Schemes 1 and 2. The correct version was posted on 11/14/2002.

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