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Microwave-enhanced nucleophilic fluorination in the synthesis of fluoropyridyl derivatives of [3,2-*c*]pyrazolo-corticosteroids, potential glucocorticoid receptor-mediated imaging agents

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Abstract—Fluoropyridyl derivatives of [3,2-*c*]pyrazolo-corticosteroids have high affinity for the glucocorticoid receptor (GR) and are highly active glucocorticoids. They are thus considered to be excellent candidates for PET imaging of GR containing tissues when labeled with fluorine-18 ($t_{1/2}$ = 110 min). Previously reported syntheses of these fluorinated glucocorticoids were accomplished by conventional thermal nucleophilic halogen exchange reactions with chloropyridyl precursors. These reactions were found to proceed at rates too slow for feasible application to radiosynthesis using [^{18}F]fluoride. We have applied microwave-heating methods to these reactions and found that significant rate enhancements can be realized. Kinetic experiments showed an average relative rate ratio of 3/1 for microwave versus conventional heating and preparative experiments showed an average relative conversion ratio of 4.5/1 during the initial 120 min, a period approximating one half-life of the isotope. The microwave method described was used to prepare previously unreported 2′-(2-fluoro-4-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxo-pregn-4-eno-[3,2-*c*]-pyrazole, which was evaluated for biological activity.

It has been demonstrated that fluoropyridyl derivatives of [3,2-*c*]pyrazolo-corticosteroids of structural types **I** and **II** (Fig. 1) are active glucocorticoids and have great potential as high affinity positron emission tomography (PET) imaging agents of glucocorticoid receptor (GR) containing tissues when labeled with fluorine-18.[1] We previously reported receptor binding and biological activity data for **Ia** and **Ib**. We now report the synthesis (Scheme 1)and comparative biological evaluation (Table 1) of compound **II**. Effective radiosyntheses of these ligands are best accomplished by nucleophilic displacement of appropriately placed leaving groups using [^{18}F]fluoride. Typical conditions involve heating the substrate with K^{18}F in DMSO in the presence of complexing agent kryptofix-222.[2]

While pyridine rings are generally activated to nucleophilic substitution, our experience with pyridylpyrazolo systems such as these shows that they take place at slow rates even at high temperatures. This is likely due to the electron-donating pyrazolo substituent, which deactivates the pyridine ring. Also, since leaving groups at position 4 relative to the pyridine ring nitrogen are more reactive than those at position 2,[3] slower rates are expected for reactions leading to compounds **Ia**, **Ib**, and **II**. The advent of microwave-heating methods, especially the introduction of focused microwave chemical reactors, has led to several reports of efficient microwave-assisted nucleophilic substitutions in heteroaromatic systems. These have been discussed in a recent review.[4] Microwave heating has also been demonstrated to be advantageous over conventional heating in nucleophilic radiohalogenation.[5–7]

In our reported synthesis of fluoropyridyl derivatives of [3,2-*c*]pyrazolo-corticosteroids we employed nucleophilic displacement of chloride using KF/kryptofix/DMSO under conventional-heating conditions. Not surprisingly these reactions, while successful in producing

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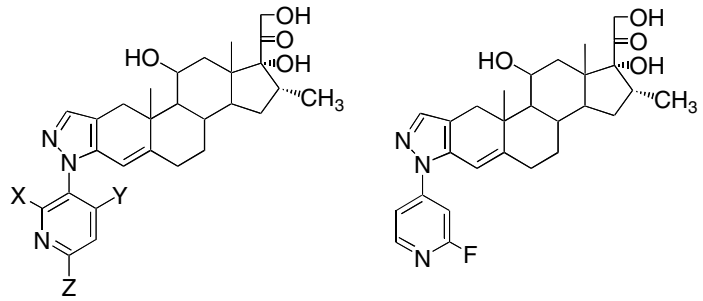
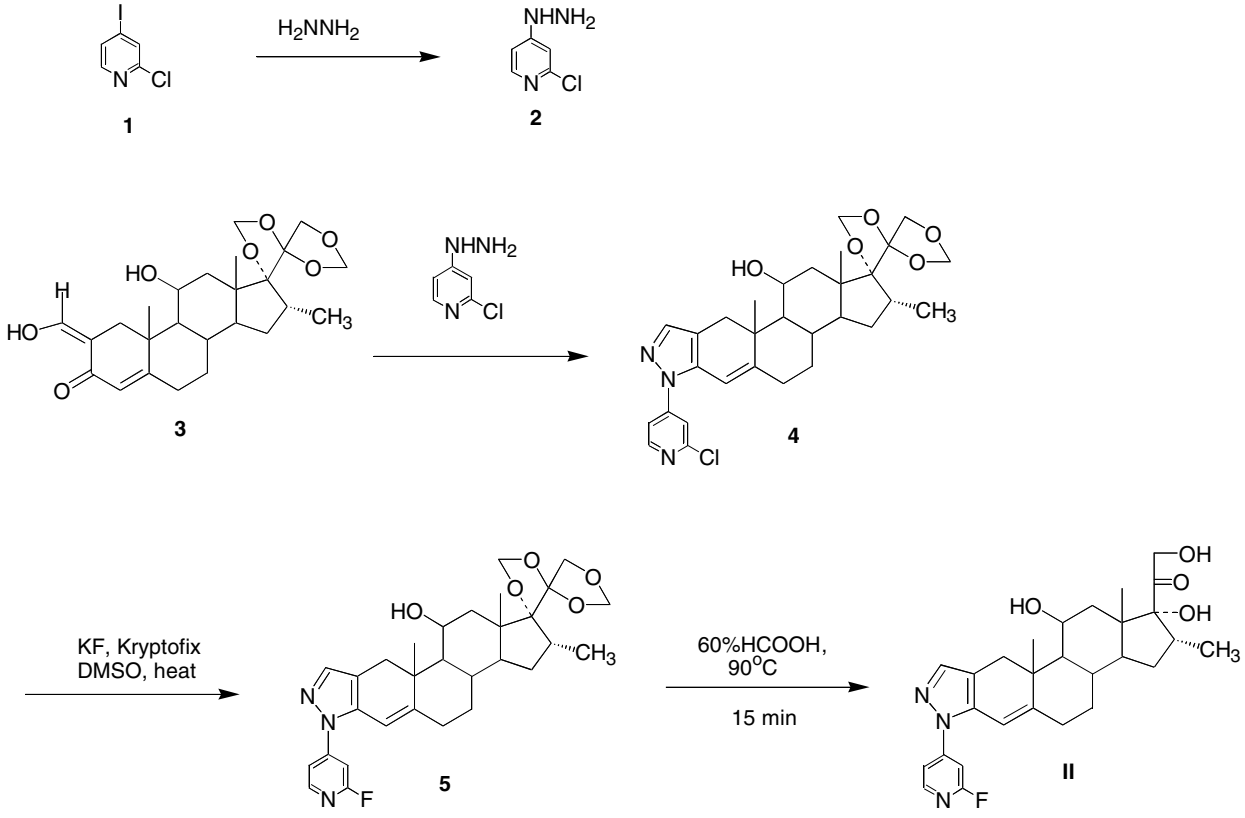


Ia, X=Y=H, Z=F
Ib, X=F, Y=Z=H
Ic, X=Z=H,Y=F

II

Figure 1.



Scheme 1.

the desired product, were too slow to be applicable to the synthesis of ^{18}F-labeled compounds. Fluorine-18 has a half-life of 110 min. Ideally, following the production of the isotope, synthetic incorporation into the compound of interest should not extend much beyond one half-life. Thus, conversions need to approach about 50% within about 120 min (2 h) for fluorine-18. Reaction times to this level of conversion using conventional-heating methods were typically 24–48 h. Herein we report the results of our application of controlled microwave-heating methods to these halogen exchange reactions and demonstrate that sufficient rate enhancements are realized. Since compound **II**, 2′(2-fluoro-4-pyridyl)-11β,17,21-trihydroxy-16α-methyl-20-oxo-pregn-4-eno[3,2-*c*]pyrazole, has not been previously reported, its synthesis, chemical characterization, and biological activity are reported here. Compound **II** was prepared as shown in Scheme 1 by methods analogous to those we have used for compounds **Ia**, **Ib** and similar pyrazolo steroids.[1] Briefly, 2-chloro-4-hydrazinopyridine **2** was prepared in 85% yield by selective displacement of iodine by anhydrous hydrazine in 2-chloro-4-iodopyridine (Aldrich Chemical Co.). It was then condensed with 11β-hydroxy-2-hydroxymethylene-16α-methyl-17, 20:20, 21-bis-(methylenedioxy)-pregn-4-en-3-one, **3**, to give compound **4** in 57% yield. Reaction of **4** with KF, kryptofix-222, in DMSO under microwave heating gave a mixture of 65% **5** and 35% unreacted **4**(Table 3), from which **5** was separated using flash

Table 1. Glucocorticoid action of steroidal pyridylpyrazoles

Compound	GR[a] (RBA)	HeLa AlkP[b] (RSA)	log $P_{o/w}$[c]
Dexamethasone	100	100	1.87 ± 0.63
Cortisol	6 ± 1	19 ± 5	1.43 ± 0.47
II	129 ± 6	35 ± 2	3.62 ± 1.08
Ia	250 ± 30	237 ± 68	3.62 ± 1.09
Ib	93 ± 31	121 ± 51	3.97 ± 1.09

[a] GR, glucocorticoid receptor; RBA, relative binding affinity where dexamethasone K_a 4.6 ± 0.1 nM = 100%.

[b] HeLa AlKP, HeLa cell alkaline phosphatase bioassay; RSA, relative stimulatory activity where dexamethasone EC_{50} 5.4 ± 1.2 nM = 100%. Values are averages of three experiments performed in duplicate ± SD. The results for **Ib** are taken from our previous publication[1] and are presented for illustrative purposes.

[c] log $P_{o/w}$, octanol–water partition coefficients based on ACDLabs computed predictions.

chromatography (2:3 toluene–ethyl acetate on flash grade silica gel). Deprotection of **5** (60% formic acid, 90 °C, 15 min) gave 2′-(2-fluoro-4-pyridyl)-11β,17,21-trihydroxy-16α-methyl -20-oxo-pregn-4-eno-[3,2-*c*]-pyrazole, **II**, in 44% yield. An analytical sample was purified by semi-preparative HPLC (25 × 1 cm Ultrasphere-ODS column eluted at 3 mL/min with 70% methanol–water, t_R = 21 min). Compound **II** was characterized by its spectral properties.[8]

As shown in Table 1 compound **II** was found to bind to the GR (RBA 129%) slightly better than dexamethasone and less than **Ia** (RBA = 250%). While the results of both assays are somewhat lower than we previously reported for **1a**, they are not statistically different. We had previously reported that compound **1b** had about the same glucocorticoid receptor binding affinity and biological potency as dexamethasone. On the basis of binding affinity for GR, it is not surprising that **II** would have weaker biological potency in the HeLa cell assay when compared to **Ia** (35% vs 237%). Contrariwise, while **II**, as would be expected, is more potent than cortisol, it is less potent than dexamethasone even though **II** has a slightly greater affinity than dexamethasone for GR. One likely explanation for this difference in GR binding and glucocorticoid action may be that **II** is metabolized and inactivated in the HeLa cell faster than either dexamethasone or **Ia**. Nevertheless, compound **II** is a potent glucocorticoid more powerful than cortisol and thus it has potential as an in vivo-imaging agent when labeled with fluorine-18.

Table 1 also includes data (log $P_{o/w}$) for compounds **Ia**, **Ib**, and **II**, which show they are between one and two orders of magnitude more lipophilic than cortisol and dexamethasone. This effect of introducing the arylpyrazolo moiety into cortisol-related structures, which has been previously observed,[9] enhances the likelihood that these compounds will cross the blood–brain barrier in imaging studies utilizing the appropriate fluorine-18 labeled radiotracers.

Nucleophilic fluorination reactivity studies were carried out on the isomeric side-chain protected chloropyridyl precursors of compounds **Ia**–**c** and **II**. These are shown in Figure 2. The reactions were typically conducted as described.[10] Under the conditions used these reactions were found to follow pseudo-first order kinetics based on the concentration of the starting chloropyridyl compounds. The concentration of the nucleophile (fluoride) was essentially constant due to the excess of insoluble KF present throughout the reactions. Kinetic data are summarized in Table 2.

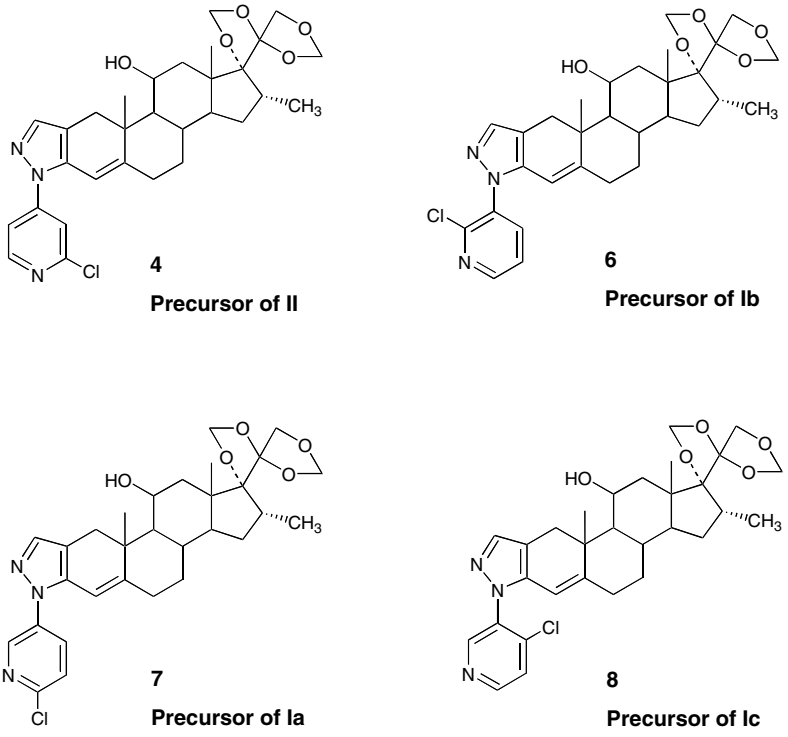


Figure 2.

Table 2. Kinetic data

Isomer	Temp (°C)	$k_c \times 10^3$ min^{-1}	$k_m \times 10^3$ min^{-1}	$k_{m/c}$ (rel rate)
6	150	13	48	3.7
4	150	2.7	5.4	2
7	130	0.6	3.7	6.2
8	110	3.5	5.7	1.6

c, conventional heating.
m, microwave heating.

Table 3. Percent conversion to fluorinated product

Isomer	Temp (°C)	Method	Time (min)	% conversion
6	150	Conventional	60	12
	150	Microwave	60	33
4	150	Conventional	120	11
	150	Microwave	120	65
7	130	Conventional	120	7
	130	Microwave	120	47
8	110	Conventional	120	19
	110	Microwave	120	51

In addition to the kinetic experiments we collected data from preparative runs over extended times to demonstrate the extent of conversion to product under both conventional and microwave-heating conditions. In consideration of the goal of achieving significant conversions at approximately one half-life of the isotope ($t_{1/2}$ ^{18}F = 110 min), we present in Table 3 the degree of conversion to fluorinated products under conventional and microwave heating. The kinetic data and the conversions observed in preparative experiments demonstrate that microwave heating enhanced these halogen exchange reactions. The best enhancement was a 6.2-fold increase observed for isomer **7**, the precursor to fluoropyridyl compound **Ia**. Significantly, **Ia** has been shown to be a high affinity and highly active glucocorticoid (Table 1). Rate enhancements were also demonstrated (Tables 2 and 3) for isomers **6** and **4** leading to the other active glucocorticoids **Ib** and **II**.

These results demonstrate that significant rate enhancements in nucleophilic fluorination of pyridyl substituents by halogen exchange can be realized using microwave heating compared to conventional-heating methods. The applicability of these results to no-carrier-added radiochemical synthesis has not yet been evaluated. However, the very low concentrations of [^{18}F]fluoride under such conditions would be counterbalanced by an excess of the substrate, which can be expected to favor successful radiolabeling. Indeed, experience with similar nucleophilic fluorinations shows that even faster rates are often observed under no-carrier-added conditions compared to those of routine chemical synthesis.[11] Finally, as indicated in the above discussion and in Scheme 1, a deprotection step is required in each case to obtain the biologically active product. As we have shown[1] this can be accomplished efficiently in 15 min and will not add significant time to the synthesis of the radiolabeled material. Therefore, the application of microwave heating to the synthesis of fluoropyridyl derivatives of [3,2-*c*]pyrazolo-corticosteroids will increase the feasibility of producing fluorine-18 labeled versions of these important glucocorticoids for imaging of GR containing tissues using positron emission tomography.

Acknowledgments

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8. Data for **II**: mp 139–144 °C; UV λ_{max} = 270 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, 1H, J = 5.6 Hz, pyridine H-6), 7.50 (s, 1H, pyrazole-H), 7.44 (d, 1H, J = 5.6 Hz, pyridine H-5), 7.15 (s, 1H, pyridine H-3), 6.27 (s, 1H, H-4), 4.51 (m, 1H, H-11α), 4.62 & 4.30 (AB quartet, J = 19.9 Hz, 2H, H-21), 1.31 (s, 3H, H-19), 1.07 (s, 3H, H-18), 0.94 (d, 3H, J = 7.4 Hz, 16α-CH_3); HRMS (M+H) calcd for $C_{28}H_{34}FN_3O_4$: 495.2606, found: 495.2602.
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10. Fluorination reactions were carried out using 5 mg (0.009 mmol) of chloropyridyl compound, 10 mg (0.027 mmol) kryptofix-222, and 5.4 mg (0.093 mmol) KF in 0.5 mL DMSO. In reactions studied using conventional heating the reactants were mixed in a screw-capped test tube that was closed and then heated with stirring in an oil bath at the indicated temperature (Table 2). Reaction progress was monitored by withdrawing small aliquots, which were diluted in methanol and analyzed for fluorinated product by high-performance liquid chromatography (HPLC): Beckman System Gold (Model 126 modular pumps and Model 168 diode-array detector) with 32Karat Software using a 25 cm × 4.4 mm Ultrasphere-ODS column eluted at 1 mL/min with 70% or 85% methanol–water. UV detection was at 270 nm. In reactions studied using microwave heating the reactants were mixed in a 10 mL conical flask fitted with a condenser and mounted in the cavity of a CEM Discover microwave reactor. The reactor was operated with stirring in open-vessel mode under constant temperature conditions. The temperature was maintained by a combination of compressed air cooling and applied microwave power. For example, to maintain a temperature of 130 °C, typically required about 80 W with compressed air at 40 psi.

Reaction progress was monitored by withdrawing small aliquots by syringe through narrow bore Teflon tubing extending through the top of the condenser into the reaction vessel. The aliquots were diluted with methanol and analyzed by HPLC as described above. Kinetic data for these reactions were obtained from concentrations of fluorinated products computed at various time points using integration data from the HPLC analyses above.

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