Elemental Fluorine to 8-Fluoropurines in One Step

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Abstract: An efficient regiocontrolled approach to the synthesis of 8-fluoropurines by direct fluorination of purines with dilute elemental fluorine is described. The one-step procedure produced regiospecific substitution of the C(8)-hydrogen of purine derivatives in isolated yields close to 30% with protected purines in $CHCl_3$. Fluorination yields with unprotected purines in EtOH were reduced to less than 10%. The electrophilic fluorination procedure has a broad scope of applicability and permits a ready and easy access to 8-fluoropurine derivatives, for the first time, for evaluation of their biochemical and pharmacological properties.

Introduction

The interest in fluorinated purine and pyrimidine derivatives in the last four decades stems from the unique properties displayed by fluorine-substituted bioactive molecules.[1] In terms of size, replacement of a hydrogen for fluorine would produce minimum steric perturbations upon the binding of the analogue molecules to receptors or enzymes. However, the strong electron-withdrawing properties of fluorine may substantially, yet in a predictable manner, alter the chemical stability[2] or enzymatic activity[3] of substrate molecules. Stimulated by this combination, and the increased stability of the carbon-fluorine bond relative to the carbon-hydrogen bond, new procedures for the synthesis of fluoropurines,[1,4,6−10] and fluoropyrimidines (and their nucleosides)[1,5] with anticancer and antiviral activities[1] were developed. The fluorine atom has been successfully introduced at the 2- and 6-positions of the purine ring system[1,4] and the sugar[2] side chain, but access to 8-fluoropurines has remained limited.[11] As a result, knowledge on the enzymatic and/or pharmacological activities of 8-fluoropurines has been hampered by the difficulties encountered in their synthesis. We now report the first reaction of purines with elemental fluorine to generate efficiently 8-fluoropurines.[12]

Results and Discussion

Chemistry. A variety of novel fluorinating reagents has been introduced for site-specific fluorination in the past 15 years.[13] However, regiospecific fluorination reactions involving elemental fluorine are still rare. Surprisingly, the reaction of 9-substituted purines **1** with F_2 (1% in He) in polar solvents proceeds smoothly at room temperature. In general, the C(8)-fluorinated derivative was the major isolated product in moderate yields. The simplicity of this one-step fluorination reaction, however, makes it very attractive to produce otherwise inaccessible 8-fluoropurine derivatives. The fluorination reactions are easily carried out with regular glassware using mixtures of F_2 and He. The flow rate of the F_2/He mixture is controlled (∼5−10 μmol of F_2/min) to assure a fine dispersion of the gas mixture in the solvents used.[14] Progress of the reaction, namely the conversion of **1a**−**f** to **2a**−**f** (Table 1), was monitored by TLC, ^{1}H NMR, and ^{19}F NMR. Disappearance of the C(8)-hydrogen singlet at 7.7−8.0 ppm in ^{1}H NMR and the concomitant appearance of a singlet in the ^{19}F NMR at −102.3 to −108.2 ppm (Table 2) enabled the progress of the reaction and incidentally confirmed a reaction at carbon-8 of the purine ring.

The protected oxopurines **1b**,**d**,**e** and adenosine **1f** all reacted cleanly with F_2, and the corresponding 8-fluoro counterparts (**2b**,**d**,**e**,**f**) were obtained in 25−30% isolated yields. Unreacted starting material (≥20%) was always present at the end of the reaction. Attempts to consume most of the starting material(s) led to a decrease in the isolated product (**2**) yields, presumably due to multiple fluorination and/or formation of oxidation products. For example, in the fluorination of **1f**, a byproduct with a molecular weight of 446 (FAB MS) and a ^{19}F NMR

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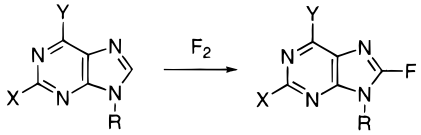
Table 1



1 **2**

compd[a]	R	X	Y	isolated yields (%)
a	β-D-ribofuranosyl	NH_2	OH	7
b	2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl	NHAc	OH	30
c[b]	(2-hydroxyethoxy)methyl	NH_2	OH	10
d	(2-acetoxyethoxy)methyl	NHAc	OH	28
e	2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl	H	OH	27
f	2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl	H	NH_2	25

[a] The cyclic amide tautomers of the indicated hydroxy(lactim) heterocycles predominate in aqueous solution. [b] Reaction also performed with acetyl hypofluorite in HOAc (see the Experimental Section).

Table 2. ^{19}F and ^{1}H NMR Chemical Shifts for Purine and Anomeric (or N−CH_2−O) Protons

	^{19}F NMR[a]	^{1}H NMR			
compd	C(8)-F	C(8)-H	C(2)-H	C(1′)-H	solvent
1a		7.93		5.68	DMSO-d_6
2a	−102.3			5.61	DMSO-d_6
1b		7.77		6.10	$CDCl_3$
2b	−107.5			6.08	$CDCl_3$
1c		7.80[b]		5.33[c,d]	DMSO-d_6
2c	−108.2			5.32[c,d]	DMSO-d_6
1d		7.78		5.47[c]	$CDCl_3$
2d	−107.8			5.39[c]	$CDCl_3$
1e		8.00	8.26	6.15	$CDCl_3$
2e	−103.3		8.20	6.05	$CDCl_3$
1f		7.95	8.36	6.17	$CDCl_3$
2f	−102.9		8.34	6.05	$CDCl_3$

[a] Typical chemical shifts for fluoropurines are as follows: C(2)-F, −50 ppm; C(6)-F, −60 to −79 ppm.[49,11] [b] C(8)-H chemical shift in CD_3OD: 7.76. [c] Chemical shifts for the N−CH_2−O protons (singlet). [d] C(1′)-H chemical shifts in CD_3OD: **1c**, 5.40; **2c**, 5.32.

chemical shift of −51.24 was also observed. Thus, the isolated yields reported herein reflect these conditions.

Solubility of the unprotected purines **1a**,**c** precluded the use of the most favorable solvent conditions (e.g., $CHCl_3$), but we found that these fluorination reactions could be carried out in EtOH with acceptable results [the addition of base improved their solubility in EtOH (see the Experimental Section)].[15] We have also evaluated the effectiveness of acetyl hypofluorite (AcOF) for selective C(8)-hydrogen substitution with **1c**. The milder AcOF (generated in-situ)[16] also reacted but with lower efficiency. However, in our hands, initial attempts to fluorinate these substituted purines with XeF_2 and other recently introduced fluorinating agents failed. Thus, reactions of **1b**,**d**,**e**,**f** with XeF_2,[17] *N*-fluoropyridinium triflate, *N*-fluoro-3,5-dichloropyridinium triflate, *N*-fluoro-2,4,6-trimethylpyridinium triflate,[18] and *N*-fluoro-*N*-(chloromethyl)triethylenediamine bis (tetrafluoroborate) (Select fluor reagent)[19] were all unsuccessful.

In the reaction with F_2, a key element in the success of the fluorination reaction resides in the modulation of the electrophilic character of F_2 reactivity.[20] The use of polar solvents for the fluorination reactions lowers the activation energy of the transition state intermediate below that of homolytic cleavage of the F−F bond (39 kcal/mol).[20d] It also provides a hydrogen acceptor to the counterion of the electrophile (e.g., fluoride ion with F_2 and acetate ion for AcOF).[20b,c]

The effective use of polar solvents for fluorine substitution reactions has been observed earlier. The synthesis of 5-fluorouracil[21] and its nucleosides[21,22] using elemental fluorine in polar solvents has been proposed as initiated by *syn* addition of fluorine across the C(5)−C(6) double bond with solvent-assisted eliminations of fluoride ion. The use of $CHCl_3$ also allowed the controlled fluorination of benzoate esters,[23] but similar aromatic substitutions with diluted elemental fluorine (<1% F_2 in N_2) in solvents favoring homolytic F_2 cleavage (e.g., $CFCl_3$) produced very low yields (<0.1%) of fluorinated products.[24] Nevertheless, regiospecific monofluorinations of aromatic substrates with elemental fluorine (or AcOF) are unfavored and frequently require the directing effects of Group IVA metals (Si, Ge, Sn).[25,26] Even more rare are reports of selective F_2-mediated monofluorination of nitrogen-containing heterocyclic compounds.[27] It is noteworthy in this regard that fluorination of both imidazole and stannylated derivatives thereof produced essentially trace fluorinated products as judged by ^{19}F NMR.[13,28] In any event, it is quite conspicuous that the direct fluorination of purines has not yet been reported while other halogens have been successfully used in their elemental form to produce regiospecific substitution of the C(8)-hydrogen of purines[29] and in many instances their use constitutes the most convenient procedure for regiospecific C(8)-halogen substitution.

Effects of C(8) Fluorine Substitution. The lack of easy access to 8-fluoropurines has limited the understanding of their biochemical and pharmacological properties. It could be anticipated, for example, that the electronegative effects of the fluorine atom at C(8) in these analogues may be significant on the substrate activity of 8-fluoropurine ribosides with several enzymes (e.g., *N*-ribosylhydrolases and transferases that cleave the C−N ribosidic bond by an S_N1-like mechanism).[2,30,31]

Theoretical and experimental approaches[32] have indicated the existence of a marked flexibility about the glycosidic bond of

(15) With protected purines (**1b,d,e,f),** about 80% of the starting material was consumed under the conditions described in the Experimental Section. For reaction with purines **1a,c** in EtOH, larger amounts of starting material could be recovered from the reaction mixture. Longer reaction times, however, revealed the formation of side products in detriment of the yield of the C(8)-fluorinated products. Also, solvents such as water and HOAc provided much lower yields than that observed in EtOH medium.

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8-substituted purine analogues with substituents having a van der Waals radius of less than 2 Å.[33] The conformation of the base about the glycosidic bond (e.g., *syn or anti*) is frequently related to the chemical shift of C(1′)H, but this chemical shift is also markedly dependent on the anisotropic effect of the C(8) substituent.[31d] In this regard, the solvents effects cannot be excluded either.[31d] Therefore, little information about their potential as enzyme substrates can be extracted at the present time from the observed chemical shifts of the β-D-ribofuranosyl anomeric proton (C(1′)H) (or the CH_2–N(9) proton) in 8-fluoropurines, when compared with the C(8)-H substituted counterparts (Table 2).[34] It is also unlikely that the preferential conformer population based on torsion around the C(1′)−N(9) bond may play a significant role in the ability of 8-fluoropurine analogues to bind to enzymes, transporters, or receptor sites. More likely, modification of biological activity could be attributed to the electron-withdrawing properties of the fluorine atom, rather than to any conformational changes around the glycosidic bond.[31d,32]

To gain initial insight into the significance of 8-fluoro substitution, we evaluated the 8-fluoroacycloguanine (**2c**) in its ability to act as a substrate for Herpes Virus Simplex I thymidine kinase (HSV tk).[35,36] The specificity of **2c** for HSV tk and its unique in-vivo stability and rapid plasma clearance have also permitted the use of fluorine-18-labeled **2c**[37] to image with positron emission tomography the expression of HSV tk transplanted genes in living animals as reported in detail elsewhere.[36]

Conclusion. The use of elemental fluorine provides for the first time a procedure of broad applicability for the synthesis of 8-fluoropurine derivatives. This reliable and direct synthetic approach now makes accessible a variety of 8-fluoro-substituted purines for determination of their biochemical and pharmacological properties. As an indication of the potential of these derivatives, the ability of the 8-fluoroacycloguanine derivative **2c** to act as a substrate for HSV tk has already provided a new approach to monitor gene expression in-vivo.[36]

Experimental Section

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. The ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded with a 360 MHz instrument. The ^{1}H and ^{13}C chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS). The ^{19}F chemical shifts are referenced to an external fluorotrichloromethane standard. The concentrations of all the samples for NMR analysis were maintained at 50 mM. Electron impact and direct chemical ionization (DCI) high-resolution mass spectral (HRMS) data were recorded on a VG Analytical Autospec mass spectrometer. Fast atom bombardment (FAB) high-resolution mass spectral data were obtained on a ZAB SE mass spectrometer. The preparative HPLC purification were carried out on a Beckman 110 system equipped with a UV detector. Ultraviolet spectra were recorded with a Beckman DU-640 spectrophotometer. TLC were run on silica gel plates (Whatman PE SIL G/UV) in $CHCl_3$:CH_3OH:H_2O. Solvent proportions were as follows: **1a**, **2a**, **1c**, **2c** (60:36:4); **1b**, **2b**, **1d**, **2d** (80:18:2); **1f**, **2f** (90:9:1); and **1e**, **2e** (100% EtOAc).

Caution: Fluorine and acetyl hypofluorite are highly toxic and reactive gases. However, they can be handled safely by following the procedures developed specifically for such gases.[38]

Direct Fluorination of Unprotected Purine Nucleosides with F_2. Fluorine (1% in He, 0.6 mmol) was bubbled into a solution of the unprotected purine derivative (0.3 mmol) in absolute ethanol (6.0 mL) and tetraethylammonium hydroxide (0.34 mL of 20% aqueous solution) at room temperature over a period of 1 h. The reaction mixture was neutralized with 1 N HOAc (0.46 mL), concentrated, and chromatographed on silica gel ($CHCl_3$:CH_3OH:H_2O = 90:9:1). Earlier fractions contained the required fluoro analogue, and from the later fractions, the unreacted starting material was recovered. Fractions containing product were pooled, and the solvents were evaporated to give the 8-fluoropurine nucleoside analogue, which was further purified by preparative HPLC (column: Alltech Econosil, C-18, 5μ, 50 × 1 cm; mobile phase: 5% CH_3OH in water, flow rate: 5 mL/min; UV: 254 nm).

8-Fluoro-9-[(2-hydroxyethoxy)methyl)]guanine (8-Fluoroacyclovir) (2c). 10% isolated yield (52% yield based on the starting material recovered); mp 212 °C (dec). ^{1}H NMR (CD_3OD): δ 3.53−3.62 (m, 4H), 5.31 (s, 2H) ppm. UV (H_2O) λ_{max} (H_2O): 242 nm (ϵ 9530), 275 (7100). Electron impact HRMS calcd for $C_8H_{10}N_5O_3F$: 243.0768. Found: 243.0772.

8-Fluoroguanosine (2a). 7% yield (46% yield based on the starting material recovered); mp 238 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.42−3.56 (m, 2H), 3.81−3.84, 4.02−4.06 (2m, 2H), 4.54−4.59 (m, 1H), 4.88 (t, J = 5.8 Hz, 1H), 5.15 (d, J = 5.0 Hz, 1H), 5.49 (d, J = 5.7 Hz, 1H), 5.61 (d, J = 6.6 Hz, 1H), 6.58 (br s, 2H), 10.83 (br s, 1H) ppm. UV (methanol) λ_{max}(H_2O): 243 nm (ϵ 9500), 275 (7100). FAB HRMS $(M^+H)^+$ calcd for $C_{10}H_{13}N_5O_5F$: 302.0901. Found: 302.0905.

Fluorination of Acyclovir (1c) with AcOF. To a solution of AcOF[16] [prepared by bubbling of 0.12 mmol of 1% F_2 in He into a solution of aqueous ammonium hydroxide (0.03 mL) in glacial acetic acid (5 mL)] was added 25 mg of acyclovir (**2c**)[39] (0.11 mmol) in 1 mL of acetic acid. The reaction mixture was stirred at room temperature

(31) (a) In the only example available to date on the ability of an 8-fluoropurine to act as a substrate for an enzymatic reaction (Ikehara, M.; Fukui, T. *Biochim. Biophys. Acta* **1974**, *338*, 512), it was reported that adenosine deaminase (EC 3.5.4.4), an enzyme known to bind adenosine in the *anti* conformation,[31b,c] hydrolyzed 8-fluoroadenosine only at 1% of the rate of adenosine. Caution should exist in the interpretation of these data, however. Kobayashi *et al.*[9] reported that the product claimed by Ikehara and Yamada[8] was not 8-fluoroadenosine. Further, Stolarski *et al.*[31d] reported their unsuccessful attempts to synthesize 8-fluoroadenosine by following the procedure of Ikehara and Yamada.[8] Our spectroscopic data for 8-fluoroadenosine (**2f**) agree with those reported by Kobayashi *et al.*[9] (see the Experimental Section). (b) Hampton, A.; Harper, P. J.; Sasaki, T. *Biochemistry* **1972**, 11, 4736. (c) Zemlicka, J. *J. Am. Chem. Soc*. **1975**, *97*, 5896. (d) Stolarski, R.; Pohorille, A.; Dudycz, L.; Shugar, D. *Biochim. Biophys. Acta* **1980**, *610*, 1.

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(36) With K1735M2 murine melanoma cells stably transfected with the HSV tk gene, **2c** was 6 times more effective than **1c** in competing for HSV tk dependent incorporation of [^{3}H]**1c** into cells. Moreover, **2c** effectively killed cells expressing HSV tk (50% survival at 0.2 μM), with no substantial killing of cells transfected with the empty vector. *In-vivo* in mice, **2c** showed unusual metabolic stability. ^{19}F NMR analysis of urine accumulated for 60 min after intravenous administration showed the presence of a single molecular entity [^{19}F NMR (CD_3OD/$CFCl_3$): δ −108.2 ppm] consistent with unaltered **2c**. Srinivasan, A.; Gambhir, S.; Barrio, J. R.; Wu, L.; Namavari, M.; Satyamurthy, N.; Sharfstein, S.; Cherry, S.; Green, A.; Berk, A.; Phelps, M. E.; Herschman, H.R. Submitted for publication in *Science*.

(37) Radiofluorinated **2b,c,d** were synthesized by bubbling cyclotron-produced [^{18}F]F_2 (Bishop, A. J.; Satyamurthy, N.; Bida, G. T.; Hendry, G.; Phelps, M. E.; Barrio, J. R. *Nucl. Med. Biol*. **1996**, *23*, 189) (1% in Ar) with **1b,c,d,** respectively (specific activity: 2.5 Ci/mmol; $t_{1/2}$ of ^{18}F is 109.7 min).

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for 15 min and evaporated to dryness. 8-Fluoroacyclovir (**1c**) (5% isolated yield; 43% yield based on the starting material recovered) was purified by column chromatography followed by preparative HPLC (see above).

Direct Fluorination of Protected Purine Nucleosides with F_2. Fluorine (1% in He, 0.6 mmol) was bubbled into a solution of the protected purine derivative (**1b**,**d**,**e**,**f**) (0.4 mmol) in $CHCl_3$ (6.0 mL) at room temperature over a period of 1 h. The reaction mixture was concentrated and chromatographed (silica gel). The initial fraction afforded the fluoronucleoside, and from the later fractions, the unreacted starting material was recovered.

***N*$_2$-Acetyl-8-fluoro-9-[(2-acetoxyethoxy)methyl]guanine (2d).** Acetonitrile was used as the solvent for the fluorination. Chromatography eluent: $CHCl_3$:CH_3OH:H_2O (95:4.5:0.5); 28% yield; mp 196−198 °C. ^{1}H NMR (CD_3OD): δ 1.97 (s, 3H), 2.23 (s, 3H), 3.82 (t, J = 4.5 Hz, 2H), 4.15 (t, J = 4.5 Hz, 2H), 5.47 (s, 2H) ppm. ^{13}C NMR (DMSO-d_6): δ 20.38, 23.70, 62.53, 67.08, 70.97, 113.50 (d, J = 12.2 Hz), 147.29, 148.68, 149.31 (d, J = 244.1 Hz), 153.92, 170.09, 173.53 ppm. DCI HRMS $(M^+H)^+$ calcd for $C_{12}H_{15}N_5O_5F$: 328.1057. Found: 328.1056.

***N*$_2$,2′,3′,5′-Tetraacetyl-8-fluoroguanosine (2b).** Chromatography eluent: $CHCl_3$:CH_3OH:H_2O = 98:1.8:0.2; 30% yield (yield based on the recovered starting material: 37%); mp 89−92 °C. ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 2.32 (s, 3H), 4.41 (dd, J = 11.0 and 7.1 Hz, 1H), 4.48 (q, J = 5.4 Hz, 1H), 4.69 (dd, J = 11.0 and 4.8 Hz, 1H), 5.85 (t, J = 4.6 Hz, 1H), 6.06 (t, J = 5.2 Hz, 1H), 6.08 (d, J = 5.4 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 20.40, 20.60, 20.89, 24.23, 63.09, 70.93, 72.33, 80.02, 85.52, 115.81 (d, J = 14.2 Hz), 145.67, 147.68, 149.06 (d, J = 250.0 Hz), 154.46, 169.48, 169.87, 171.74, 171.89 ppm. These data are in agreement with literature values.[10]

2′,3′,5′-Tri-*O*-acetyl-8-fluoroadenosine (2f). Chromatography eluent: EtOAc:hexane (3:1); 25% yield; mp 98−101 °C. ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 6H), 2.15 (s, 3H), 4.29 (dd, J = 12.1 and 5.0 Hz, 1H), 4.39 (q, J = 4.5 Hz, 1H), 4.48 (dd, J = 12.1 and 3.5 Hz, 1H), 5.73 (t, J = 5.4 Hz, 1H), 5.79 (br s, 2H), 6.05 (d, J = 5.6 Hz, 1H), 6.07 (t, J = 5.3 Hz, 1H), 8.34 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 20.42, 20.56, 20.67, 62.95, 70.52, 71.96, 80.39, 85.04, 114.24 (d, J = 12.2 Hz), 148.68 (d, J = 4.0 Hz), 150.83 (d, J = 254.5 Hz), 152.86 (d, J = 3.4 Hz), 154.34, 169.47, 169.58, 170.55 ppm. FAB HRMS $(M + H)^+$ calcd for $C_{16}H_{19}N_5O_7F$: 412.1268. Found: 412.1264. These data are in agreement with literature values.[9]

2′,3′,5′-Tri-*O*-acetyl-8-fluoroinosine (2e). Chromatography eluent: EtOAc:hexane (9:1); 27% yield; mp 80−83 °C. ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 4.29 (dd, J = 11.9 and 4.9 Hz, 1H), 4.39 (q, J = 4.5 Hz, 1H), 4.47 (dd, J = 11.9 and 3.5 Hz, 1H), 5.65 (t, J = 5.5 Hz, 1H), 6.02−6.05 (m, 2H), 8.20 (s, 1H), 13.02 (br s, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 20.31, 20.46, 20.61, 62.82, 70.30, 71.89, 80.34, 85.20, 119.55 (d, J = 12.3 Hz), 145.28, 147.24 (d, J = 3.7 Hz), 150.07 (d, J = 252.9 Hz), 157.74, 169.32, 169.47, 170.39 ppm. FAB HRMS $(M + H)^+$ calcd for $C_{16}H_{18}N_4O_8F$: 413.1109. Found: 413.1104.

General Procedure[35b,40,41] for Deprotection of 2b,d. To the protected 8-fluoropurine derivative (**2b,d**) (0.1 mmol) was added a solution of methanolic ammonia (5 mL of 2 M solution), and the reaction mixture was stirred at room temperature. The solution was evaporated to dryness, and the residue was purified by column chromatography as described above to provide the deblocked 8-fluoronucleosides (**2a**: reaction time 5 h, 50% yield; **2c**: reaction time 2−4 h, 30% yield).[42]

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(42) Literature precedence indicates for a nucleophilic displacement of C(8)-F in methanolic ammonia.[11] Under the experimental conditions for deprotection, **2b,d** defluorinated with a $t_{1/2}$ of decomposition of about 5 h. Defluorination was easily demonstrated with radiofluorinated **2b,d**.[37] Appearance of [^{18}F]fluoride ion, disappearance of starting materials (**2b,d**), and product formation (**2a,c**) was verified by radio thin layer chromatography (silica gel, $CHCl_3$:CH_3OH:H_2O, 60:36:4)). Optimum reaction times for product (**2a,c**) isolation varied from 2 to 5 h.