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Synthesis of Two Fluoro Analogues of the Nicotinic Acetylcholine Receptor Agonist UB-165

Andrew Sutherland,[†] Timothy Gallagher,*[,†] Christopher G. V. Sharples,[‡] and Susan Wonnacott[‡]

School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom, and Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom

t.gallagher@bristol.ac.uk

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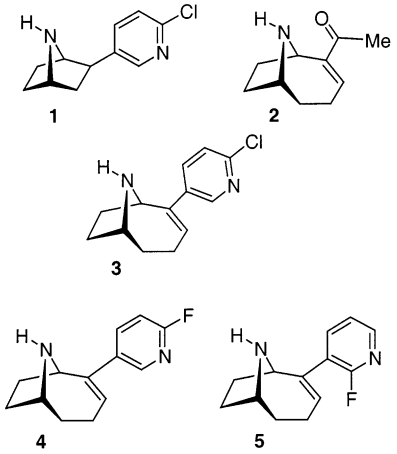
Abstract: Two racemic fluoropyridine analogues **4** and **5** of the potent nicotinic agonist UB-165 have been synthesized. Halogenated pyridines **7** and **12** provided the organometallic reagents needed for the Negishi and Suzuki coupling reactions used for the preparation of **4** and **5**, and the *N*-vinyloxycarbonyl protecting group of **8** and **15** was cleaved using a novel trifluoroacetic acid-mediated deprotection protocol. Analogue **4** retained high binding affinity at rat brain α4β2 and α7 nicotinic receptors.

The azabicyclic alkaloids, (−)-epibatidine (**1**) and (+)-anatoxin-a (**2**), have been identified as ligands with high affinity for the nicotinic acetylcholine receptors (nAChRs) and in particular the α4β2* subtype, which is widely distributed in brain tissue.[1−4] These observations have stimulated interest in targeting nAChRs for the development of drugs for Parkinson's disease, Alzheimer's disease, anxiety, Tourette's syndrome, and ulcerative colitis.[5] While epibatidine (**1**) is 200 times more potent than nicotine as an analgesic agent, this ligand is extremely toxic and therefore has limited therapeutic potential.[6] However, both epibatidine and anatoxin-a represent important probes for the location and function of nAChR subtypes and ultimately as leads for drug candidates.

Using these two molecules as starting points for novel ligand design, we[7] described the synthesis of a novel potent nAChR ligand, UB-165 (**3**), which combines two important structural features of epibatidine (**1**) and anatoxin-a (**2**), i.e., the 3′-chloropyridyl and the 9-azabicyclo[4.2.1]nonene moieties of **1** and **2**, respectively. Although a potent agonist, UB-165 shows little selectivity for different nAChR receptors, a trend that is also observed with **1** and **2**.[8] Therefore, structural analogues of UB-165 are desirable for the development of subtype-selective nicotinic agonists, which would allow the targeting and activation of particular receptor isoforms.



Recent reports[9] describing the preparation of fluoro analogues of epibatidine have prompted our study toward the synthesis of similar derivatives of UB-165. Herein, we report the synthesis of **4** and **5**, the 2-fluoro-5-pyridyl and the 2-fluoro-3-pyridyl analogues, respectively, of UB-165, together with a novel method for cleaving the *N*-vinyloxy (*N*-Voc) protecting group.

The synthetic strategy employed for the synthesis of 2-fluoro-5-pyridyl analogue **4** was based on our previous approach to the synthesis of UB-165 derivatives.[7,10] This involved a Negishi[11] Pd(0)-mediated cross-coupling of the synthetically versatile enol triflate **6**[7,10] with the pyridyl zinc halide derived by Li−Br exchange from 5-bromo-2-fluoropyridine (**7**), which gave the desired adduct **8** in 41% yield (Scheme 1). However, a second product, the 5-bromo-2-fluoro-3-pyridyl adduct **9**, was also isolated in 19% yield. After carrying out this coupling reaction under several different sets of conditions, we noted that slow (≥2 min, conditions a, Scheme 1) dropwise addition of BuLi to 5-bromo-2-fluoropyridine (**7**) at −78 °C led to increasing amounts of **9** (up to 33% yield, but always with **8** also being formed), while rapid addition (<5 s, condi-

* Corresponding author. Phone: (44-117)9288260. Fax: (44-117)-9298611.
[†] University of Bristol.
[‡] University of Bath.

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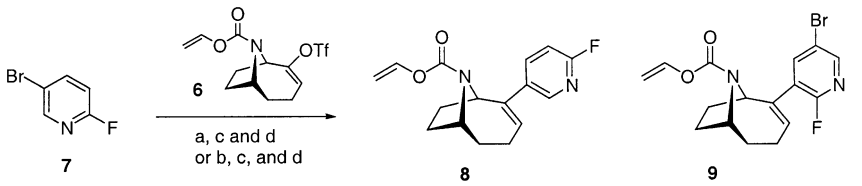
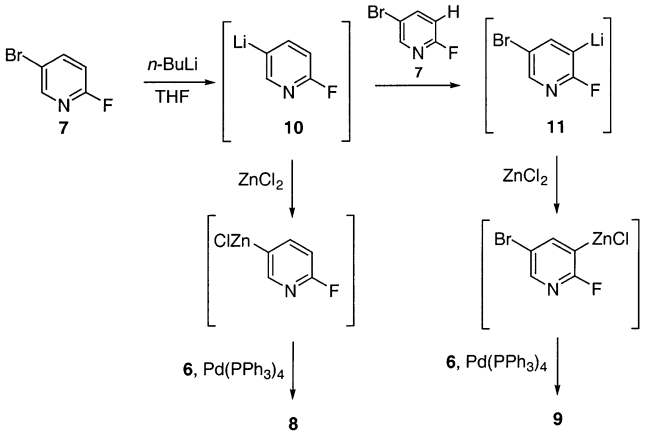
SCHEME 1[a]



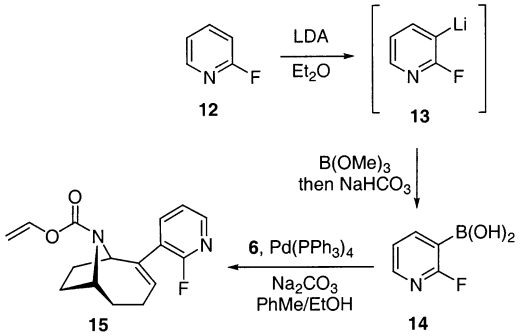
Slow addition of BuLi (a, c and d):	41%	19% (see text)
Fast addition of BuLi (b, c and d):	77%	0%

[a] Reagents and conditions: (a) BuLi (slow addition over 2 min), THF, −78 °C; (b) BuLi (fast addition, <5 sec), THF, −78 °C; (c) $ZnCl_2$, THF, from −78 °C to rt; (d) **6**, $Pd(PPh_3)_4$, THF, Δ.

SCHEME 2



SCHEME 3



tions b, Scheme 1) of BuLi (again at −78 °C) gave only the desired adduct **8** in 77% yield.

These results suggest that slow (2 min) addition of BuLi leads to halogen−metal exchange to give **10**, which is the kinetically favored process (Scheme 2). However, during the time taken for addition, the resulting lithiated species **10** facilitates a competing halo-directed lithiation of the starting 5-bromo-2-fluoropyridine (**7**) to give 3-lithio-5-bromo-2-fluoropyridine (**11**). Both lithiated intermediates **10** and **11** then undergo Li−Zn transmetalation and subsequent cross-coupling with enol triflate **6** to give adducts **8** and **9**, respectively.

Related halogen−lithum exchange processes are known. The synthesis of 2-fluoropyridine-5-boronic acid has been achieved starting from **7**, and clean bromine−lithium exchange was carried out using *inverse* addition of BuLi to the halopyridine **7**.[12] The iodine−lithium exchange of 5-iodo-2-fluoropyridine has also been recently reported[9a] using a slow addition of BuLi to achieve iodine−lithium exchange. However, these authors do not discuss complications associated with competing side reactions involving intermediate **10**.

We intended to prepare the isomeric 2-fluoro-3-pyridyl analogue **5**[13] by direct *ortho*-lithiation of 2-fluoropyridine **12**, a process that is well-precedented.[14] Lithiation would then be followed by transmetalation with zinc(II) chloride and the usual Pd(0)-mediated Negishi cross-coupling reaction with enol triflate **6**. However, using our standard conditions [LDA, THF, −78 °C, then $ZnCl_2$ (from −78 °C to rt), and then **6**, Pd(0), reflux], no cross-coupling to enol triflate **6** was observed. Examination of the various steps involved in this sequence revealed that while *ortho*-lithiation of **12** with LDA proceeded smoothly, either the resulting lithiated species **13** was not able to undergo transmetalation (with $ZnCl_2$) or the resulting organozinc species was unstable to the reaction conditions.[15] The 3-lithiated intermediate **13** did, however, undergo reaction with trimethylborate to give, after hydrolysis, boronic acid **14**[16] in 62% yield. This species was subsequently coupled under Suzuki conditions[17] with enol triflate **6** to give adduct **15** in 74% yield (Scheme 3). It should be noted that the 2-fluoro-3-pyridyl adduct **15** can also be made from the bromo analogue **9**. Accordingly, treatment of **9** with Reike activated zinc, followed by an aqueous quench, resulted in clean C−Br reduction to give **15** in 65% yield.[18]

(12) Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 2885−2890.

(13) 2-Chloro-3-substituted isomer of epibatidine (cf. **5**) has been described. Senokuchi, K.; Nakai, H.; Kawamura, M.; Katsube, N.; Nonaka, S.; Sawaragi, H.; Hamanaka, N. *Synlett* **1994**, 343−344.

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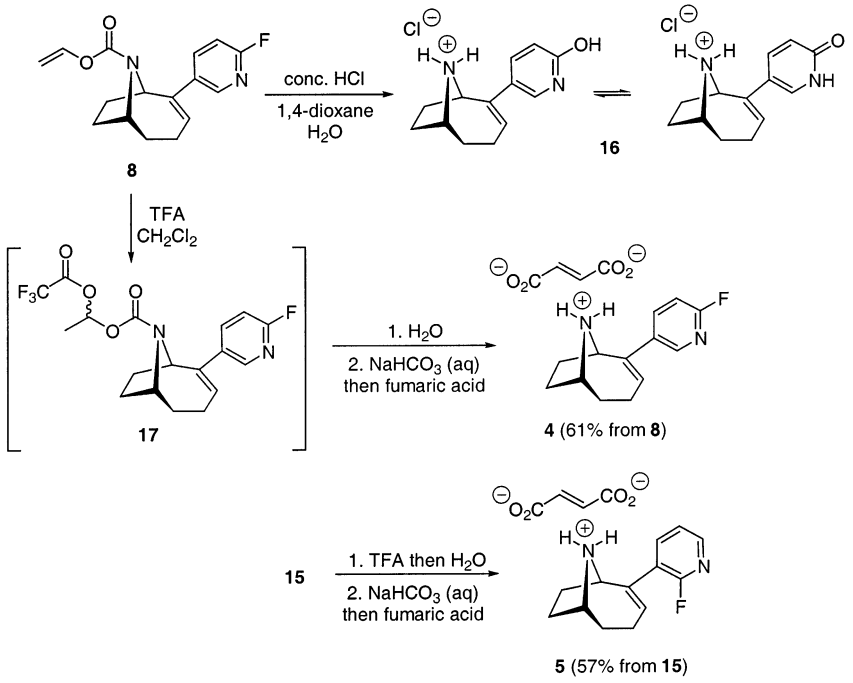
(15) Lithiation at C(3) of 2-fluoropyridine was successful, on the basis of our use of this chemistry to prepare boronic acid **14**, but attempts to carry out the Li−Zn exchange, followed by Pd(0)-mediated cross-coupling to enol triflate **6**, failed. Unlike 2-fluoropyridine, 2-bromopyridine does undergo directed deprotonation (with LDA), Li−Zn exchange, and Pd(0)-mediated cross coupling successfully.[18] Formation of **9** from **7** implicates the intermediacy of a 2-fluoro-3-pyridylzinc species (Scheme 2), yet we were unable to generate this species using LDA (to achieve direct deprotonation of **7**) followed by transmetalation with $ZnCl_2$.

(16) Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 3323−3328.

(17) Suzuki, A. *Pure Appl. Chem.* **1994**, *66*, 213−222.

SCHEME 4



The final stage for the preparation of analogues **4** and **5** required deprotection of the *N*-vinyloxycarbonyl (Voc) protecting group (Scheme 4). In our original synthesis of UB-165, the *N*-Voc group was removed using aqueous acid (at reflux) to give the hydrochloride salt of the final secondary amine.[7,10] However, attempts to apply these aqueous conditions to the corresponding fluoro analogue **8**, gave pyridone **16** in 97% yield. To prevent hydrolysis of the 2-fluoropyridyl moiety, a mild nonaqueous deprotection procedure for the *N*-Voc group was required. We reasoned that treatment of **8** with TFA would lead to protonation of the *N*-Voc group and trapping with trifluoroacetate to give intermediate **17**. This O,O-diacylated acetal intermediate would be expected to undergo mild hydrolytic cleavage and subsequent fragmentation (to give ethanal and CO_2) to provide the target amine. In the event, reaction of **8** with TFA (1:1 TFA/CH_2Cl_2, reflux, 24 h) followed by an aqueous quench gave amine **4** (TFA salt), which validates this novel process for the deprotection of an *N*-Voc carbamate. The crude TFA salt was transformed to the corresponding fumaric acid salt, which was recrystallized from propan-2-ol to give the target compound **4** in 61% overall yield from **8**. Similarly, treatment of **15** with TFA and subsequent formation of the fumarate salt gave our second target compound **5** in 57% yield.

Initial assessment of the biological activity of UB-165 analogues **4** and **5** in radioligand binding assays[10] shows that **4** and **5** compete for [^{3}H]nicotine binding to $\alpha4\beta2$ nicotinic receptors in rat brain membranes with K_i values of 0.82 ± 0.18 and 306 ± 219 nM, respectively. At rat brain $\alpha7$ nicotinic receptors, labeled with [^{3}H]methyllycaconitine, **4** and **5** interacted with K_i values of 0.30 ± 0.04 and 21 ± 14 μM, respectively. Thus, **4** retains the binding potency observed with the parent molecule **3**,[8] whereas the ability of fluoro isomer **5** to interact with these nicotinic receptor subtypes is substantially diminished. Pyridone **16** showed a 100-fold loss of binding potency at $\alpha4\beta2$ nicotinic receptors (compared to UB-165) and was essentially without binding activity at $\alpha7$ nicotinic receptors. The biological profiles of **4** and **5**, as well as the pyridone analogue **16**, will be reported in full in due course.

Experimental Section

2-(2-Fluoro-5-pyridyl)-9-vinyloxycarbonyl-9-azabicyclo-[4.2.1]non-2-ene (8). A solution of 5-bromo-2-fluoropyridine (**7**) (0.7 g, 3.97 mmol) in THF (10 mL) was cooled to −78 °C under a nitrogen atmosphere, and BuLi (2.5 M in hexanes, 1.58 mL, 3.97 mmol) was added dropwise over 2 min. After an additional 20 min at −78 °C, a solution of anhydrous zinc(II) chloride (0.43 M in THF, 9.13 mL, 3.97 mmol) was added and the reaction mixture was allowed to warm to room temperature. Enol triflate **6**[7,10] (0.5 g, 1.47 mmol) in THF (5 mL) was then added followed by a solution of $Pd(PPh_3)_4$ (0.02 g, 0.02 mmol) in THF (5 mL). The resulting solution was heated under reflux for 4 h and then allowed to cool, and the reaction was quenched with saturated aqueous ammonium chloride solution (20 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed in vacuo. Purification by flash column chromatography, eluting with 20% ethyl acetate in light petroleum, gave 2-(5-bromo-2-fluoro-3-pyridyl)-9-vinyloxycarbonyl-9-azabicyclo[4.2.1]non-2-ene (**9**) (0.11 g, 19%) as a colorless oil. For spectroscopic details of **9**, see Supporting Information.

Further elution with 30% ethyl acetate gave the title compound **8** (0.17 g, 41%) as a colorless oil. IR (film) ν (cm^{-1}): 2932, 1709, 1646, 1482, 1417, 1145, 826. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26−8.17 (1.5 H, m, ArH), 7.84 (0.5 H, ddd, J = 8.9, 8.1, 2.4 Hz, ArH), 7.25 (0.5 H, dd, J = 7.6, 6.3 Hz, Voc), 7.22 (0.5 H, dd, J = 7.6, 6.3 Hz, Voc), 6.90 (1 H, m, ArH), 5.85 (1 H, m, 3-H), 4.89−4.78 (1.5 H, m, Voc and 1-H), 4.66−4.57 (1.5 H, m, Voc and 6-H), 4.49 (0.5 H, dd, J = 6.3, 1.7 Hz, Voc), 4.41 (0.5 H, dd, J = 6.3, 1.7 Hz, Voc), 2.53−1.64 (8 H, m, 4-H_2, 5-H_2, 7-H_2 and

(18) Karig, G.; Spencer, J. A.; Gallagher, T. *Org. Lett.* **2001**, *3*, 835−838. Reike zinc provides a convenient method for reduction of bromopyridines.

8-H_2). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 162.2 (d, J = 241.3 Hz, C), 151.1/151.0 (C), 145.5/145.3 (CH), 144.3 (C), 142.4/142.3 (CH), 140.2/140.1 (CH), 136.1 (C), 130.1/129.6 (CH), 109.1/108.8 (CH), 95.5/95.3 (CH_2), 59.4/59.3 (CH), 56.9/56.8 (CH), 31.9/31.8 (CH_2), 31.2/31.1 (CH_2), 29.1/28.7 (CH_2), 24.2/24.1 (CH_2). ^{19}F NMR (283 MHz, $CDCl_3$): δ −70.6 and −70.7 (2 × br d, $J \approx 7$ Hz). HRMS: calcd for $C_{16}H_{18}FN_2O_2$ (MH^+), 289.1352; found, 289.1351. Proton and carbon assignments are based on two-dimensional and DEPT experiments.

Optimized Procedure for the Halogen−Metal Exchange of 7 (to Give 8). Using exactly the same scale as described above, rapid addition (<5 s) of BuLi, followed by transmetalation and cross-coupling, led to the isolation of **8** as a colorless oil in 77% yield. None of byproduct **9** was isolated using these "fast addition" conditions.

2-(2-Fluoro-3-pyridyl)-9-vinyloxycarbonyl-9-azabicyclo-[4.2.1]non-2-ene (15). To a solution of the enol triflate **6**[7,10] (0.1 g, 0.32 mmol) and $Pd(PPh_3)_4$ (0.02 g, 0.02 mmol) in 9:1 toluene/ethanol (10 mL) were added 2-fluoro-3-pyridylboronic acid (**14**)[16] (0.08 g, 0.64 mmol) and an aqueous solution of sodium carbonate (0.1 g, 0.96 mmol in 4 mL of water). The reaction mixture was heated under reflux for 3 h. The reaction mixture was cooled to room temperature, diluted with water (20 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification by flash column chromatography, eluting with 30% ethyl acetate in light petroleum, gave the title compound **15** (0.062 g, 74%) as a colorless oil. IR (film) ν (cm^{-1}): 2932, 1713, 1646, 1423, 1333, 1146. ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.11 (0.5 H, ddd, J = 4.9, 3.2, 1.9 Hz, ArH), 8.07 (0.5 H, ddd, J = 4.9, 3.2, 1.9 Hz, ArH), 7.97 (0.5 H, ddd, J = 9.9, 7.6, 1.9 Hz, ArH), 7.62 (0.5 H, ddd, J = 9.9, 7.6, 1.9 Hz, ArH), 7.27−7.11 (2 H, m, ArH and Voc), 5.84 (1 H, m, 3-H), 4.78 (0.5 H, dd, J = 14.2, 1.9 Hz, Voc), 4.72 (1 H, m, 1-H), 4.57 (1 H, m, 6-H), 4.45 (0.5 H, dd, J = 6.3, 1.3 Hz, Voc), 4.31 (0.5 H, dd, J = 2.6, 1.3 Hz, Voc), 4.27 (0.5 H, dd, J = 6.3, 1.3 Hz, Voc), 2.61−1.63 (8 H, m, 4-H_2, 5-H_2, 7-H_2 and 8-H_2). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 160.2 (d, J = 243.2 Hz, C), 150.6 (C), 146.1/145.9 (CH), 144.1 (C), 142.6 (C), 142.5/142.0 (CH), 140.2/140.1 (CH), 132.7/132.6 (CH), 121.5/121.4 (CH), 95.3/95.0 (CH_2), 59.1/59.0 (CH), 57.1/56.9 (CH), 31.8/31.7 (CH_2), 30.2 (CH_2), 28.4/28.3 (CH_2), 24.2/24.1 (CH_2). ^{19}F NMR (283 MHz, $CDCl_3$): δ −67.6 and −69.5 (2 × br d, $J \approx 10$ Hz). HRMS: calcd for $C_{16}H_{18}FN_2O_2$ (MH^+), 289.1352; found, 289.1346.

2-(2-Fluoro-5-pyridyl)-9-azabicyclo[4.2.1]non-2-ene Fumaric Acid Salt (4). 2-(2-Fluoro-5-pyridyl)-9-vinyloxycarbonyl-9-azabicyclo[4.2.1]non-2-ene (**8**) (59 mg, 0.2 mmol) was dissolved in dichloromethane (5 mL), and TFA (5 mL) was added. The reaction mixture was heated under reflux for 24 h. The reaction mixture was cooled to room temperature, and the reaction was quenched by the addition of water (10 mL). This solution was concentrated under vacuum, and the resulting residue was redissolved in ethyl acetate (20 mL), washed with water (2 × 10 mL) and then an aqueous saturated solution of sodium hydrogen carbonate (2 × 10 mL), dried (Na_2SO_4), and finally concentrated in vacuo. The resulting oil was dissolved in propan-2-ol (5 mL), and a solution of fumaric acid (26 mg, 0.22 mmol) in propan-2-ol (5 mL) was added. This solution was concentrated in vacuo, and the resulting solid was recrystallized from propan-2-ol to give the title compound **4** (44 mg, 61%) as a colorless solid. IR (film) ν (cm^{-1}): 3332, 2508, 1637, 1465, 1378, 1257, 673. ^{1}H NMR (270 MHz, CD_3OD): δ 8.18 (1 H, d, J = 2.5 Hz, ArH), 7.95 (1 H, ddd, J = 8.4, 7.0, 1.6 Hz, ArH), 7.07 (1 H, dd, J = 8.4, 2.5 Hz, ArH), 6.67 (2 H, s, fumarate), 6.28 (1 H, dd, J = 8.2, 4.1 Hz, 3-H), 4.63 (1 H, d, J = 9.4 Hz, 1-H), 4.33 (1 H, m, 6-H), 2.73−2.48, 2.38−2.15 and 2.01−1.88 (8 H, m, 4-H_2, 5-H_2, 7-H_2, and 8-H_2). ^{13}C NMR (100.5 MHz, CD_3OD): δ 170.1 (C), 160.0 (d, J = 240.1 Hz, C), 144.7 (CH), 139.7 (CH), 139.6 (CH), 139.4 (C), 136.5 (C), 134.9 (CH), 108.8 (CH), 59.4 (CH), 59.4 (CH), 30.6 (CH_2), 28.2 (CH_2), 27.1 (CH_2), 23.0 (CH_2). ^{19}F NMR (283 MHz, CD_3OD): δ −72.8 (br d, J ca. 7 Hz). HRMS: calcd for $C_{13}H_{16}FN_2$ (MH^+), 219.1298; found, 219.1297.

Acknowledgment. The authors thank the EPSRC and BBSRC (Biomolecular Sciences Project Grant 86/B11785) for financial support.

Supporting Information Available: General experimental details, characterization of compound **9**, Reike zinc reduction of **9** (to give **15**), and synthesis and characterization of pyridone **16** and fluoro derivative **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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