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Novel epimerization of aromatic *C*-nucleosides with electron-withdrawing substituents with trifluoroacetic acid –benzenesulfonic acid using mild conditions☆

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Abstract—Trifluoroacetic acid and benzenesulfonic acid in dichloromethane at ambient temperature have been found to be efficient co-catalysts for the epimerization of *C*-nucleosides with electron-withdrawing substituents. This approach provides a convenient route to the β anomer of the nucleosides with high yields in the range 54–78%. Synthesis of the 3′-phosphoramidite of 2,4-difluorophenyl *C*-nucleoside suitable for solid-phase DNA synthesis is described.

Chemical approaches have proven to be powerful tools for understanding the basis of specificity and catalysis of DNA polymerases and DNA repair glycosylases.[1,2] One of the first examples was the use of non-polar nucleoside analogues to probe the basis of nucleotide recognition by DNA polymerases.[1,3] These analogues lack the hydrogen bond donor–acceptor groups of normal DNA molecules, yet mimic their shape, or even the shape of the entire base pair.[1,2] Thus, Kool and colleagues have shown that DNA polymerases will specifically incorporate the non-polar dTTP analogue, 2,4-difluorotoluenyl nucleotide triphosphate (dFTP) opposite to adenine on the template strand, showing that high specificity can occur in the absence of hydrogen bonding.[3] They also found that polymerases can specifically incorporate a pyrenyl (Y) nucleotide triphosphates (dYTP) opposite to a DNA site that lacks a base, confirming that steric complimentarity is an important component of high fidelity DNA replication.[1,4] Some interesting results from Wiebe and coworkers[5] suggest that similar difluorophenyl *C*-nucleoside derivatives could also be very useful biopharmaceuticals. The most remarkable properties of *C*-nucleosides are their cell permeability, their ability to bind to proteins with high affinities, and their stability towards metabolic deglycosylation, which make these compounds potential anticancer, antiviral and diagnostic imaging agents.

We are actively using chemical approaches to understand the recognition and catalytic mechanism of the DNA repair enzyme uracil DNA glycosylase (UDG), which removes unwanted deoxyuridine residues from DNA.[2,6–11] To better understand the role of base shape and hydrogen bonding in uracil recognition by UDG, we wished to incorporate a 2,4-difluorophenyl *C*-nucleoside uracil analogue in DNA.[12] Although the efficient synthesis of the difluorotoluenyl *C*-nucleoside phosphoramidite has been described and is commercially available, the synthesis of the difluorophenyl derivative has never been reported.[13,14] We initially attempted synthesis of the difluorophenyl *C*-nucleoside phosphoramidite according to Kool's method, but the yield was very low in the epimerization step (<20%). Although we reported previously that trifluoroacetic acid is an effective catalyst in dichloromethane for the epimerization of a variety of *C*-nucleosides with *electron-donating* substituents,[15] we found that TFA alone is not an efficient catalyst for the epimerization of *C*-nucleosides that contain *electron-withdrawing* substituents, requiring the development of new synthetic methods for these compounds.

In this work, we report that TFA and benzenesulfonic acid are efficient, convenient and economical co-catalysts for epimerization of a variety of *C*-nucleosides at ambient temperature in dichloromethane (Table 1), while benzenesulfonic acid by itself is oxidative and

Keywords: epimerization; *C*-nucleosides; trifluoroacetic acid; benzenesulfonic acid; dichloromethane.

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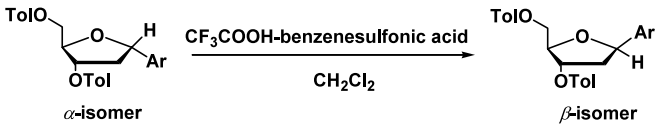
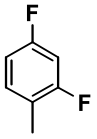
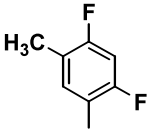
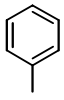
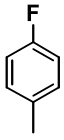
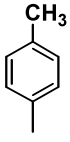
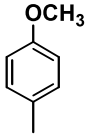
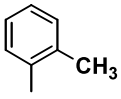
Table 1. Epimerization of *C*-nucleosides with trifluoroacetic acid–benzenesulfonic acid in CH_2Cl_2[a]

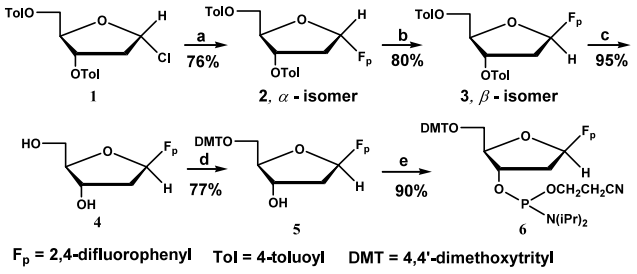


Compound	Ar	Conditions	β-isomer (%)	α-isomer (%)
2		20 % catalyst[b] 40 °C, 40 h	58	37
7		12 % catalyst 40 °C, 20 h	54	35
8		12 % catalyst 40 °C, 20 h	54	38
9		12 % catalyst 40 °C, 20 h	65	27
10		5 % catalyst 40 °C, 20 h	64	30
11		5 % catalyst 23 °C, 2 h	78	20
12		5 % catalyst 40 °C, 20 h	63	32

[a]Both the β and α isomers were isolated by silica-gel column chromatography. The characterization of the new compounds **9**α,[21] **9**β,[22] **12**α,[23] and **12**β[24] is listed in the References.
[b] Percent of the total reaction volume contributed from the combination catalyst.

causes substrate decomposition and dehydration.[13,14] It was found that **2** was converted to **3** in the presence of the combination catalysts TFA–benzenesulfonic acid (5:1 molar ratio)[16] at 40°C for 40 h to afford β isomer **3** (58%) and α isomer **2** (37%). The α isomer **2** was retreated using identical conditions to give more β-isomer **3** (22%). Thus, the combined yield of the β isomer was 80% after two epimerizations. The total synthesis of 2,4-difluorophenyl *C*-nucleoside phosphoramidite is shown in Scheme 1.[17] The results for the epimerization of difluorophenyl and other aromatic *C*-nucleosides are listed in Table 1.

^{19}F NMR measurements suggest the formation of carbocation intermediate during the epimerization reaction (Fig. 1). The chemical shifts of 1-bromo-2,4-difluorobenzene in $CDCl_3$ show only a small up-field shift (0.09 ppm) when the co-catalysts were added, indicating that these catalysts do not alter the electronic structure of the aromatic ring. However, upon addition



Scheme 1. Total synthesis of the difluorophenyl *C*-nucleoside phosphoramidite. *Reagents and conditions*: (a) $Cd(Fp)_2$/THF, rt; (b) TFA–benzenesulfonic acid (5:1), CH_2Cl_2, 40°C, 48 h; (c) NaOMe/MeOH, 23°C; (d) 4,4′-dimethoxytrityl chloride, DMAP, pyridine, CH_2Cl_2, 23°C; (e) *N*,*N*′-diisopropyl-2-*O*-cyanoethyl phosphonamidic chloride, DIPEA, CH_2Cl_2, 5 min, 23°C.

of the co-catalysts, the ^{19}F NMR chemical shifts of **2** showed 0.48 and 1.15 ppm down-field shifts for the fluorine atoms at the 2 and 4 positions, respectively, indicating that the oxygen in the sugar ring is protonated with the possible formation of a benzylic cation (Scheme 2).[18,19] In contrast with previous procedures,[13,14] no dehydration, carbonization or decomposition is observed, and the solvent CH_2Cl_2 is easily removed. The yields of products are about fourfold better than when benzenesulfonic acid alone is utilized with toluene as the solvent at 110°C for 8 h.[13] Although an alternative epimerization method using BF_3 in nitromethane has been reported, this approach requires high temperature.[20]

In conclusion, we provide an efficient new method for the epimerization of difluorophenyl and other *C*-nucleosides with electron-withdrawing substituents using the co-catalysts TFA-benzenesulfonic acid (5:1 molar ratio) in dichloromethane. This method should find wide application in the synthesis of such compounds, which may be incorporated into DNA via phosphoramidite chemistry for structure–activity studies in biological systems.

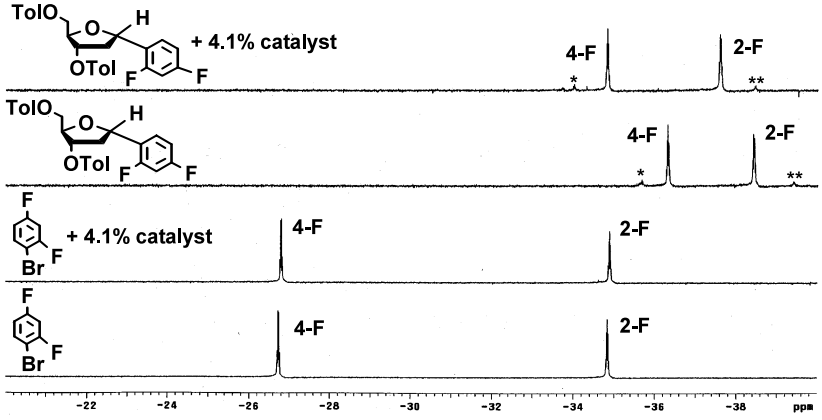
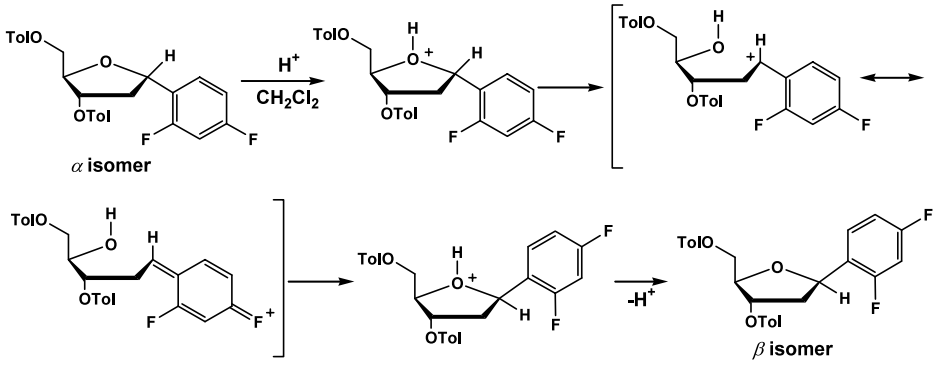


Figure 1. ^{19}F NMR spectra of 15 mM 1-bromo-2,4-difluorobenzene and α isomer **2** in the absence and presence of 4.1% (v/v) combination catalyst in $CDCl_3$ at rt (370 MHz ^{19}F frequency). TFA was used as an internal reference for the fluorine chemical shifts. The asterisk symbols * and ** are the 4-F and 2-F atoms of the β isomers **3**, which were assigned from an authentic sample of this isomer.



Scheme 2. Proposed mechanism for the epimerization of aromatic *C*-nucleosides containing fluorine in presence of the combination catalyst TFA–benzenesulfonic acid in CH_2Cl_2.

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16. **Combination catalyst**. Trifluoroacetic acid (0.50 ml, 0.74g, 6.4 mmol) was added to benzenesulfonic acid (0.22g, 1.4 mmol). The mixture was vortexed and centrifuged to give a clear solution (0.58 ml). The residue was found to be about 0.06 g.
17. Total synthesis of 2,4-difluorophenyl *C*-nucleoside phosphoramidite:
    2 and **3**. To dry THF (10 ml) in a two-necked round-bottomed flask equipped with a condenser, magnesium turnings (0.288 g, 12 mmol), 1-bromo-2,4-difluorobenzene (1.35 ml, 12 mmol) and two crystals of iodine were added consecutively. The mixture was stirred and heated slightly to achieve slow reflux for 0.5 h using magnetic stirrer. Dry cadmium chloride was then added to the above warm solution in one portion. The mixture was heated for a few minutes until most of the $CdCl_2$ was dissolved. To the reaction mixture, a suspension of 1′-α-chloro-3′,5′-di-*O*-toluyl-2′-deoxyribose (3.0 g, 7.8 mmol) in 10 ml THF suspension were added dropwise at rt over 10 min. The mixture was stirred at 40°C for 2 h, and then rt overnight. The solvent was removed in vacuo and the residue was purified on silica gel (EtAc/hexanes, 4:96) to give **2** (1.82 g, 76%) and **3** (0.24 g, 11%). **2** ^{1}H NMR ($CDCl_3$, ppm) δ 7.97 (d, 2H, $J=8.0$ Hz); 7.66 (d, 2H, $J=8.0$ Hz); δ 7.56 (m, 1H); 7.26 (d, 2H, $J=7.2$ Hz); 7.18 (d, 2H, $J=7.6$ Hz); 6.89 (m, 1H); 6.80 (m, 1H); 5.60 (dd, 1H, $J=3.2$ Hz); 5.56 (m, 1H); 4.72 (m, 1H); 4.56 (m, 2H); 2.44 (m, 1H); 2.42 (s, 3H); 2.39 (s, 3H); 2.30 (m, 0.5H); 2.27 (m, 0.5H). ^{19}F NMR ($CDCl_3$, ppm) δ −38.43 (2-F); −36.33 (4-F). HRMS (MALDI-FTMS) calcd for $C_{27}H_{24}F_2O_5Na$ (M+Na) 489.148, found 489.146.
    3: In addition, **3** can be prepared by epimerization of **2**. To a solution of **2** (0.180 g, 0.37 mmol) in 2 ml CH_2Cl_2, was added 0.5 ml of the combination catalyst. The homogenous mixture was sealed in a sample vial and shaken for 40 h at 40°C, and then washed using 15 ml 5% $NaHCO_3$. The solvents were removed in vacuo and the residue was purified on silica gel (ethyl acetate/hexanes, 4:96) to give **3** (0.105 g) and **2** (0.068 g), the latter was treated again to give more **3** (0.040 g). The overall yield was 80%. ^{1}H NMR ($CDCl_3$, ppm) δ 7.99 (d, 2H, $J=8.4$ Hz); 7.91 (d, 2H, $J=8.4$ Hz); 7.50 (m, 1H); 7.29 (d, 2H, $J=7.6$ Hz); 7.22 (d, 2H, $J=8.4$ Hz); 6.81 (m, 2H); 5.61 (m, 1H); 5.47 (dd, 1H, $J=5.2$ Hz); 4.67 (m, 2H); 4.54 (m, 1H); 2.62 (m, 1H); 2.42 (s, 3H); 2.40 (s, 3H); 2.16 (m, 1H). ^{19}F NMR ($CDCl_3$, ppm) δ −39.41 (2-F); −35.70 (4-F). HRMS (MALDI-FTMS) calcd for $C_{27}H_{24}F_2O_5Na$ (M+Na) 489.148, found 489.146.
    4: To a solution of **3** (0.90 g, 1.8 mmol) in 5 ml CH_2Cl_2, were added 20 ml MeOH and 0.25 ml NaOMe (in MeOH, 25%). The mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo and the residue was purified on silica gel using EtAc then acetone to give **4** (0.40 g, 95%). **4** ^{1}H NMR ($CDCl_3$, ppm) δ 7.43 (m, 1H); 6.83 (m, 1H); 6.79 (m, 1H); 5.34 (dd, 1H, $J=5.6$ Hz); 4.48 (m, 1H); 4.03 (m, 1H); 3.85 (m, 1H); 3.78 (m, 1H); 2.32 (m, 0.5H); 2.30 (m, 0.5H); 2.05 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{11}H_{12}F_2O_3Na$ (M+Na) 253.065, found 253.065.
    5: To **4** (0.200 g, 0.87 mmol) in 4 ml pyridine, were added 4-(dimethylamino)pyridine (20 mg, 0.16 mmol) and 4,4′-dimethoxytrityl chloride (0.52 g, 1.53 mmol). If the reaction did not go to completion, more 4,4′-dimethoxytrityl chloride was needed. The reaction mixture was stirred at rt for 1 h, and quenched by adding 20 ml 5% $NaHCO_3$. The solvent was removed in vacuo and the residue was purified on silica gel (Et_3NH_3/EtAc/hexanes, 0.5:24.5:75) to give **5** (0.335 g, 77%). **5** ^{1}H NMR ($CDCl_3$, ppm) δ 7.43 (m, 1H); 7.28 (m, 5H); 7.18 (d, 4H, $J=10.4$ Hz); 6.84 (d, 4H, $J=8.8$ Hz); 6.84 (m, 1H); 6.74 (m, 1H); 5.36 (m, 1H); 4.42 (m, 1H); 3.84 (m, 1H); 3.79 (s, 6H); 3.76 (m, 1H); 3.35 (m, 0.5H); 3.28 (m, 0.5H); 2.34 (m, 1H); 2.01 (m, 1H). Mass (ESI) calcd for $C_{32}H_{30}F_2O_5Na$ (M+Na) 555, found 555.
    6: To **5** (0.320 g, 0.58 mmol) in 4 ml CH_2Cl_2 and 0.44 ml *N*,*N*-diisopropylethylamine, was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.22 ml, 0.96 mmol) in a dropwise fashion. The reaction mixture was stirred at rt for 5 min, and quenched by adding 5 ml 5% $NaHCO_3$. The solvent was removed in vacuo and the residue was purified on silica gel (Et_3NH_3/EtAc/hexanes, 2:25:73) to give **6** (0.39 g, 90%). **6** ^{1}H NMR ($CDCl_3$, ppm) δ 7.21–7.54 (m, 12H); 6.81 (m, 4H); 5.35 (m, 1H); 4.52 (m, 1H); 4.21 (m, 1H); 3.83 (m, 1H); 3.79 (6H, s); 3.69 (m, 1H); 3.60 (m, 2H); 3.29 (m, 2H); 2.60 (m, 1H); 2.46 (m, 2H); 1.95 (m, 1H); 1.16 (m, 12H). ^{31}P NMR ($CDCl_3$, ppm) δ 149.36 (s) and 148.90 (s). Mass (ESI) calcd for $C_{41}H_{47}F_2N_2O_6P$ (M+H, M+Na and M+Cl,) 733, 755 and 767, found 733, 755 and 767, respectively.
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21. **9α**: ^{1}H NMR ($CDCl_3$, ppm) δ 7.97 (d, 2H, $J=8.0$ Hz); 7.81 (d, 2H, $J=8.4$ Hz); 7.41 (m, 2H); 7.25 (d, 2H, $J=8.0$ Hz); 7.20 (d, 2H, $J=8.4$ Hz); 7.04 (m, 2H); 5.59 (m, 1H); 5.34 (m, 1H); 4.69 (m, 1H); 4.57 (m, 2H); 2.93 (m, 1H); 2.41 (s, 3H); 2.40 (s, 3H); 2.26 (m, 1H). ^{19}F NMR ($CDCl_3$, ppm) δ −38.34. HRMS (MALDI-FTMS) calcd for $C_{27}H_{25}FO_5Na$ (M+Na) 471.158, found 471.156.
22. **9β**: ^{1}H NMR ($CDCl_3$, ppm) δ 7.99 (d, 2H, $J=8.4$ Hz); 7.94 (d, 2H, $J=8.0$ Hz); 7.38 (m, 2H); 7.29 (d, 2H, $J=7.6$ Hz); 7.24 (d, 2H, $J=8.0$ Hz); 7.00 (m, 2H); 5.61 (d, 1H, $J=6.0$ Hz); 5.23 (m, 1H); 4.65 (m, 2H); 4.53 (m, 1H); 2.54 (m, 1H); 2.44 (s, 3H); 2.41 (s, 3H); 2.19 (m, 1H). ^{19}F NMR ($CDCl_3$, ppm) δ −37.38. HRMS (MALDI-FTMS) calcd for $C_{27}H_{25}FO_5Na$ (M+Na) 471.158, found 471.156.
23. **12α**: ^{1}H NMR ($CDCl_3$, ppm) δ 7.98 (d, 2H, $J=8.0$ Hz); 7.79 (d, 2H, $J=8.4$ Hz); 7.62 (d, 1H, $J=7.2$ Hz); 7.12–7.25 (m, 7H); 5.62 (m, 1H); 5.51 (t, 1H, $J=6.8$ Hz); 4.77 (m, 1H); 4.59 (m, 2H); 3.00 (m, 1H); 2.41 (s, 3H); 2.39 (s, 3H); 2.39 (s, 3H); 2.30 (s, 3H), 2.15 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{28}H_{28}O_5Na$ (M+Na) 467.183, found 467.182.
24. **12β**: ^{1}H NMR ($CDCl_3$, ppm) δ 7.99 (d, 2H, $J=8.4$ Hz); 7.95 (d, 2H, $J=8.0$ Hz); 7.58 (m, 1H); 7.12–7.28 (m, 7H); 5.61 (d, 1H, $J=4.4$ Hz); 5.44 (m, 1H); 4.68 (m, 2H); 4.53 (m, 1H); 2.59 (m, 1H); 2.44 (s, 3H); 2.39 (s, 3H); 2.39 (s, 3H); 2.35 (s, 3H), 2.15 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{28}H_{28}O_5Na$ (M+Na) 467.183, found 467.182.