Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 85–88

TETRAHEDRON LETTERS

Efficient epimerization of pyrene and other aromatic *C*-nucleosides with trifluoroacetic acid in dichloromethane☆

Yu Lin Jiang and James T. Stivers*

Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205-2185, USA

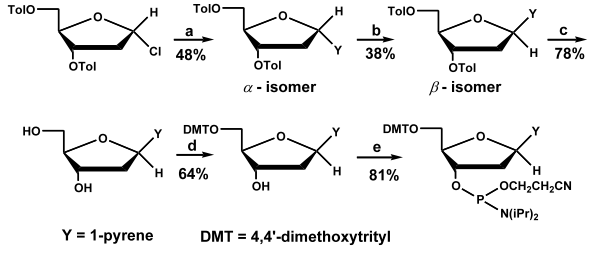
Received 18 September 2002; revised 30 October 2002; accepted 5 November 2002

Abstract—Trifluoroacetic acid has been found to be an efficient and non-oxidative catalyst for the epimerization of pyrene and other *C*-nucleosides in dichloromethane at ambient temperature. Aspects of the epimerization mechanism are also elucidated.

Recently, non-polar nucleoside analogs have attracted much interest as probes of protein-nucleic acid recognition, because they lack the hydrogen bond donor–acceptor groups of the natural DNA bases, yet mimic the shape of natural bases or even base pairs. Importantly, the pioneering work of Kool and colleagues has shown that DNA polymerase will specifically incorporate a pyrene (Y) nucleoside triphosphate (dYTP) opposite to DNA sites that lack bases, confirming that steric complimentarity is an important component of high fidelity DNA replication.[1,2] In our laboratory, pyrene has been found to be a very useful tool in the studies of DNA base flipping by uracil DNA glycosylase (UDG), and the rational engineering of a DNA glycosylase that is specific for an unnatural C:Y base pair.[4] In general, placement of a pyrene nucleotide opposite to a damaged base in duplex DNA forces the base into an extrahelical conformation and enhances enzymatic damage recognition. The flipping of DNA bases and chemical rescues of base flipping mutations of enzymes by pyrene have also been reported.[3–6]

Although pyrene nucleoside phosphoramidite and related *C*-nucleosides can be prepared as described previously (Scheme 1), the yield is very low in the epimerization step. The reaction requires high temperature and the oxidative catalysts benzenesulfonic acid and concentrated sulfuric acid, causing dehydration and carbonization side reactions.[7,8]

We report here that trifluoroacetic acid is a very effective catalyst for the epimerization of a variety of *C*-nucleosides in dichloromethane solvent. Trifluoroacetic acid has been found to be a catalyst for the isomerization of indolo[2,3-*a*]quinolizidine derivatives,[9] 3-indolyl sulfides to 2-indolyl sulfides[10,11] and α-aryloxymethylcinnamic acids.[12] Most importantly, trifluoroacetic acid is non-oxidative but it is also a strong acid. Typically, to a solution of α-isomer (0.180 g, 0.32 mmol) in 6 ml dichloromethane, were added trifluoroacetic acid (60 μl). The mixture was stirred for 18 h at room temperature. The reaction was quenched by adding onefold excess of triethylamine (200 μl). The solvents were removed in vacuo and the residue was purified on silica gel (acetone/CH_2Cl_2/hexanes, 3:9:88) to give β-isomer



Scheme 1. Published synthesis of pyrene nucleoside phosphoramidite and the yield at each step.[7,8] (a) CdY_2/THF/reflux; (b) benzenesulfonic acid–H_2SO_4–H_2O–toluene, reflux, 6 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 23°C.

Keywords: epimerization; pyrene nucleoside; *C*-nucleosides; trifluoroacetic acid and dichloromethane.

☆ This work was supported by NIH grant RO1 GM56834 (J.T.S.).

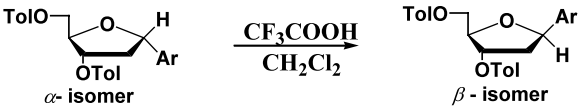
* Corresponding author: Tel.: 410-502-2758; fax: 410-955-3023; e-mail: jstivers@jhmi.edu

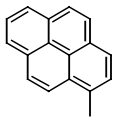
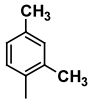
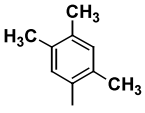
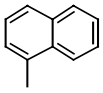
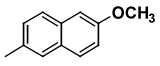
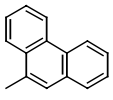
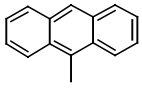
0040-4039/03/$ - see front matter
PII: S0040-4039(02)02481-4

(0.124 g) and α-isomer (0.052 g). The α-isomer was resubjected to identical conditions to give more β-isomer (0.033 g). The combined yield of β-isomer was 87% after two epimerization steps.

The results for epimerization of pyrene and other aromatic *C*-nucleosides are listed in Table 1. Our goal was to make these *C*-nucleosides by the most efficient and economical method for common use. These results show the effectiveness and convenience of using trifluoroacetic acid in CH_2Cl_2 at room temperature for this epimerization. In contrast with previous procedures,[7,8] no dehydration, carbonization or decomposition was observed, and the solvent CH_2Cl_2 was easily removed. The yields of products were increased by more than twofold.[7,8] Trifluoroacetic acid is also an effective catalyst for the reverse epimerization of the β-isomer to the α-isomer (Fig. 1). Therefore, the epimerization is reversible and the equilibrium constant K is 2.3 ($k_\alpha = 2.3\times10^{-4}$ s^{-1} and $k_\beta = 1.0\times10^{-4}$ s^{-1}). We note that the relative rates of epimerization in Table 1, and the optimal concentrations of TFA that are required for efficient epimerization, are likely related to the electron donating ability of the aromatic group, which would serve stabilize the proposed carbocation intermediate (see below). We routinely convert these *C*-nucleosides

Table 1. Epimerization of *C*-nucleosides with trifluoroacetic acid (TFA) in CH_2Cl_2[a]



No	Ar	Conditions	β-isomer (%)	α-isomer (%)
1		1% TFA, 25 °C, 18h	69	29
2		5% TFA, 40 °C, 18h	75	22
3		1% TFA, 40 °C, 18h	60	38
4		5% TFA, 40 °C, 18h	60	38
5		% TFA, 40 °C, 4.5h	50	50
6		5% TFA, 40 °C, 18h	46	50
7		% TFA, 25 °C, 18h	62	35

[a] Both the β- and α-isomers were isolated by silica-gel column chromatography. The characterizations of the new compounds **5**α,[14] **5**β,[15] **7**α[16] and **7**β[17] are reported in the references.

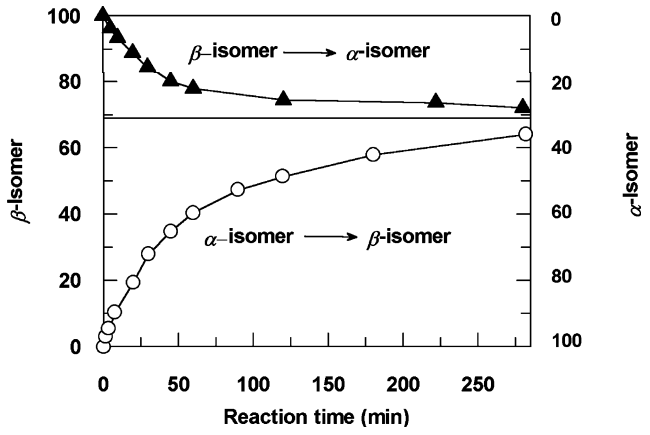


Figure 1. Reaction curves of the epimerization of pyrene nucleosides (52 mM) either from α-isomer to β-isomer (lower panel) or from β-isomer to α-isomer (upper panel) in CH_2Cl_2 in the presence of 1% trifluoroacetic acid and at room temperature. For each point, 20 μL sample was taken from reaction mixture and immediately quenched by 40 μl 5% triethylamine in CH_2Cl_2. After evaporation to dryness using a N_2 stream, the sample was dissolved in 0.6 ml $CDCl_3$ and analyzed ^1H NMR spectroscopy (400 MHz). The relative amounts of α-isomer and β-isomer were quantitated by integration of characteristic NMR peaks over time. The curves are single exponential fits to the data ($k_{obsd}=k_\alpha+k_\beta$). The equations are $K=k_\beta/k_\alpha$ and $k_\beta=k_{obsd}/(1/K+1)$.[13]

to the corresponding nucleoside phosphoramidites for use in solid phase DNA synthesis and ultimately biological studies in our laboratory.[3–6]

Other aliphatic acids and solvents were also tested for the epimerization of pyrene *C*-nucleoside. No reaction was observed using formic acid or acetic acid, and the reaction was very slow using the popular DNA synthesis reagent trichloroacetic acid or dichloroacetic acid. No epimerization was observed using chloroform, toluene, THF, ethyl acetate, acetone, methanol or ethanol as the solvent. The poor ability of TCA to catalyze epimerization is fortuitous given the wide use of this reagent in solid-phase DNA synthesis.

The addition of trifluoroacetic acid to CH_2Cl_2 was found to dramatically quench the pyrene fluorescence of the α and β isomers. Surprisingly, trifluoroacetic acid did not quench the fluorescence of pyrene itself. The selective fluorescence quenching of the pyrene nucleoside isomers may directly or indirectly reflect the protonation of the oxygen in the sugar ring. This is a key step in the epimerization process, allowing the benzylic cation of pyrene to be formed (Scheme 2).[8]

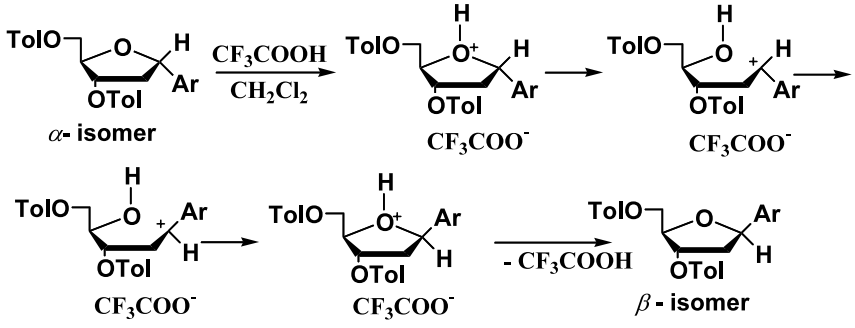
In conclusion, we have shown an efficient new method for the epimerization of pyrene and other air-sensitive *C*-nucleosides using dichloromethane solvent and trifluoroacetic acid catalyst. This procedure makes these very useful compounds readily accessible in high yields, and should find wide application in the studies which incorporate these analogs into DNA via phosphoramidite chemistry.

Acknowledgements

This work was supported by National Institutes of Health Research Grant RO1GM56834 (J.T.S.).

References

1. Matray, T. J.; Kool, E. T. *Nature* **1999**, *399*, 704.
2. Morales, J. C.; Kool, E. T. *Nature Struct. Biol.* **1998**, *5*, 950.
3. Jiang, Y. L.; Kwon, K.; Stivers, J. T. *J. Biol. Chem.* **2001**, *276*, 42347.
4. Stivers, J. T.; Kwon, K.; Jiang, Y. L. *Biophys. J.* **2002**, *82*, 595.
5. Jiang, Y. L.; Drohat, A. C.; Ichikawa, Y.; Stivers, J. T. *J. Biol. Chem.* **2002**, *277*, 15385.
6. Jiang, Y. L.; Stivers, J. T.; Song, F. *Biochemistry* **2002**, *41*, 11248.
7. Ren, R. X.-F.; Chaudhuri, N. C.; Paris, P. L.; Rumney, S., IV; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 7671.
8. Chaudhuri, N. C.; Ren, R. X. F.; Kool, E. T. *Synlett* **1997**, 341.
9. Lounasmaa, M.; Berner, M.; Brunner, M.; Suomalainen, H.; Tolvanen, A. *Tetrahedron* **1998**, *54*, 10205.
10. Hamel, P.; Girard, Y.; Atkinson, J. G. *J. Org. Chem.* **1992**, *57*, 2694.
11. Hamel, P.; Girard, Y.; Atkinson, J. G. *J. Chem. Soc., Chem. Commun.* **1989**, 63.



Scheme 2. Proposed pathway for the epimerization of aromatic *C*-nucleoside by CF_3COOH in CH_2Cl_2.

12. Krishnamoorthy, T. V.; Rajagopalan, K.; Balasubramanian, K. K. *Tetrahedron Lett*. **1985**, *26*, 1747.
13. Sato, Y.; Tateno, G.; Seio, K.; Sekine *Eur. J. Org. Chem*. **2002**, 87.
14. **5α**: ^{1}H NMR ($CDCl_3$, ppm) δ 7.99 (d, 2H, $J=8.0$ Hz); 7.82 (s, 1H); 7.68–7.72 (m, 3H); 7.50 (d, 1H, $J=8.0$ Hz); 7.26 (d, 2H, $J=8.4$ Hz); 7.14 (m, 2H); 7.09 (d, 2H, $J=8.0$ Hz); 5.65 (m, 1H); 5.50 (m, 1H); 4.76 (m, 1H); 4.60 (m, 2H); 3.93 (s, 3H); 3.00 (m, 1H); 2.41 (s, 3H); 2.40 (m, 1H); 2.35 (s, 3H). HRMS (MALDI-FTMS) calcd for $C_{32}H_{30}O_6Na$ (M+Na) 533.193, found 533.193.
15. **5β**: ^{1}H NMR ($CDCl_3$, ppm) δ 8.01 (d, 2H, $J=8.0$ Hz); 7.98 (d, 2H, $J=7.6$ Hz); 7.79 (s, 1H); 7.71 (d, 1H, $J=8.4$ Hz); 7.64 (d, 1H, $J=8.8$ Hz); 7.48 (d, 1H, $J=8.4$ Hz); 7.30 (d, 2H, $J=7.6$ Hz); 7.22 (d, 2H, $J=8.4$ Hz); 7.11 (m, 2H); 5.66 (m, 1H); 5.40 (dd, 1H, $J=10.8$ Hz, $J=5.2$ Hz); 4.70 (m, 2H); 4.59 (m, 1H); 2.59 (m, 1H); 2.44 (s, 3H); 2.39 (s, 3H), 2.37 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{32}H_{30}O_6Na$ (M+Na) 533.193, found 533.193.
16. **7α**: ^{1}H NMR ($CDCl_3$, ppm) δ 8.63 (d, 2H, $J=9.2$ Hz); 8.45 (s, 1H); 8.03, 7.47, 7.25 (m, 12H); 7.90 (d, 2H, $J=8.0$ Hz); 6.80 (m, 1H); 5.88 (m, 1H); 4.89 (m, 1H); 4.69 (m, 1H); 4.50 (m, 1H); 2.81 (m, 1H); 2.45 (s, 3H); 2.40 (s, 3H); 2.35 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{35}H_{30}O_5Na$ (M+Na) 553.198, found 553.197.
17. **7β**: ^{1}H NMR ($CDCl_3$, ppm) δ 8.68 (d, 2H, $J=9.2$ Hz); 8.44 (s, 1H); 8.10, 7.42, 7.25 (m, 14H); 6.66 (dd, 1H, $J=12$ Hz, $J=6.0$ Hz); 5.91 (m, 1H); 4.92 (m, 2H); 4.64 (m, 1H); 2.58 (m, 1H); 2.44 (s, 3H); 2.40 (s, 3H); 2.35 (m, 1H). HRMS (MALDI-FTMS) calcd for $C_{35}H_{30}O_5Na$ (M+Na) 553.198, found 553.197.