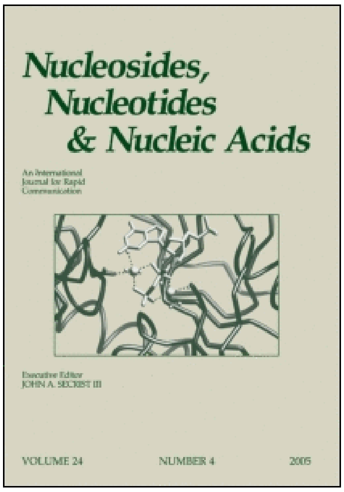


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Convenient Regioselective Formation of 2'-O-Tosyladenosine

B. M. Ratnayake Bandara[a]; Carl Bernofsky[a]
[a] Department of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana

To cite this Article Bandara, B. M. Ratnayake and Bernofsky, Carl(1990) 'Convenient Regioselective Formation of 2'-O-Tosyladenosine', Nucleosides, Nucleotides and Nucleic Acids, 9: 2, 189 — 195
To link to this Article: DOI: 10.1080/07328319008045131
URL: http://dx.doi.org/10.1080/07328319008045131

NUCLEOSIDES & NUCLEOTIDES, 9(2), 189-195 (1990)

CONVENIENT REGIOSELECTIVE FORMATION OF 2'-O-TOSYLADENOSINE

B. M. Ratnayake Bandara and Carl Bernofsky

Department of Biochemistry, Tulane University School of Medicine,
New Orleans, Louisiana 70112

Abstract - 2'-O-Tosyladenosine (1) was obtained in 50% yield from the reaction of adenosine and sodium p-toluenesulfinate in the presence of sodium hypochlorite. The sequential intermediacy of N^6-monochloroadenosine (2) and p-toluenesulfonyl chloride during this process is proposed on the basis of their individual reactions with sodium p-toluenesulfinate and adenosine, respectively.

INTRODUCTION

The usefulness of modified nucleosides and nucleotides in biology and medicine has led to a widespread interest in procedures for their synthesis.[1] The preparation of such derivatives often requires the use of intermediates in which hydroxyl groups in the sugar moiety are either protected or activated, and thus it is important to have methods by which these groups can be selectively modified. We describe here a one pot method for the regioselective formation of 2'-O-tosyladenosine (1) under aqueous conditions starting from the parent nucleoside. Tosylation at the 2'-position has been previously achieved by use of 2'3'-O-(dibutylstannylene)adenosine,[2] 5'-O-(monomethoxytrityl)adenosine,[3] and 5'-O-acetyladenosine[4] under nonaqueous conditions. The present method does not require prior modification of any hydroxyl group and thus is experimentally more convenient.

RESULTS AND DISCUSSION

A white precipitate, homogeneous by high-pressure liquid chromatography (HPLC), formed when sodium hypochlorite was added to an

TABLE 1

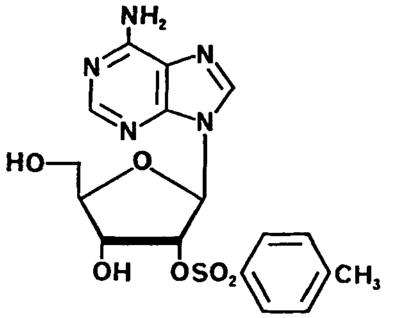
^{13}C- and ^{15}N-NMR Spectral Data for 2'-O-Tosyladenosine (1)

Carbon	Chemical shift*	Nitrogen	Chemical shift*
2	152.76	1	237.31
4	148.67	3	220.87
5	120.32	7	243.46
6	156.85	9	165.17
8	140.70	NH_2	84.43
1'	88.19		
2'	79.75		
3'	70.66		
4'	85.73		
5'	62.42		
1" (tosyl)	145.83		
2", 6"	130.19		
3", 5"	127.52		
4"	132.04		
4"-Me	21.93		

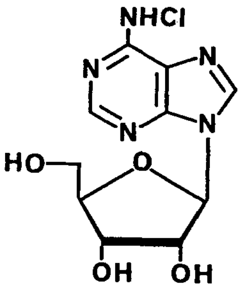
* ^{13}C-NMR chemical shifts are δ ppm relative to an external reference of tetramethylsilane in DMSO-d_6. ^{15}N-NMR chemical shifts are referenced to an external standard of [^{15}N]NH_3 (liq) as determined with a secondary standard of [^{15}N]NO_3^- in D_2O.

aqueous solution of adenosine and sodium p-toluenesulfinate. Fast-atom-bombardment mass spectroscopy (FABMS) and microanalytical data indicated the molecular formula to be $C_{17}H_{19}N_5O_6S$, suggesting that the product was a tosyl derivative of adenosine. Natural abundance ^{15}N-nuclear magnetic resonance (NMR) chemical shifts (Table 1) were in the same range as those of adenosine and adenine,[5] indicating that the tosylation had taken place in the ribose moiety. Three tosyl isomers of adenosine at 2' (m.p. 229-230°C),[2] 3' (m.p. 162-165°C)[3] and 5' (m.p. 155-156°C)[6] are known. The melting point and the ^{1}H-NMR data of the product closely resembled those reported for 2'-O-tosyladenosine (1).[2] The ^{13}C-NMR signals of the adenine and ribose carbons of the product were assigned by comparison with those of adenosine;[7] the remaining signals were readily assignable to the tosyl group (Table 1).

The tosylation presumably involved the initial formation of 9-(β-*D*-ribofuranosyl)purine-6-chloramine (2) as a primary intermediate (Eq 1). We have previously isolated and characterised this relatively unstable



1



2

chloramine from the reaction of adenosine with hypochlorite.[8] It is known that hypochlorite reacts with amines at a much faster rate than with arenesulfinates, and this led Scully and Bowdring[9] to propose the intermediacy of chloramines in the formation of arenesulfonamides from amines and arenesulfinates in the presence of hypochlorite. In the present work, the reaction of 2 with aqueous sodium p-toluenesulfinate gave the 2'-O-tosylate (1) as the only product, which is consistent with the proposal that adenosine chloramine (2) is involved as an intermediate.

Chloramine 2, once formed, apparently reacted with p-toluenesulfinate to form p-toluenesulfonyl chloride (Eq 2) as the second intermediate which in turn selectively tosylated adenosine at 2'-position (Eq 3). This sequence was verified by a separate experiment in which adenosine was reacted with p-toluenesulfonyl chloride under aqueous conditions. The solid that separated from the reaction mixture in 26% yield was found to be homogeneous by HPLC and identified as 2'-O-tosyladenosine (1). For reasons that are not clear, the yield (50%) of 1 from the tosylation through the chloramine intermediate (2) was appreciably higher than that (26%) from the direct incubation of adenosine with p-toluenesulfonyl chloride. The 5'-monophosphate derivative of adenosine is also known to furnish the corresponding 2'-O-tosylate selectively in 61% yield from its reaction with p-toluenesulfonyl chloride in aqueous base.[10]

$$\mathrm{AdNH_2\ (adenosine) + HOCl \rightarrow AdNHCl\ (2) + H_2O} \quad (1)$$

$$\mathrm{2 + p\text{-}MeC_6H_4SO_2H \rightarrow AdNH_2 + p\text{-}MeC_6H_4SO_2Cl} \quad (2)$$

$$\mathrm{AdNH_2 + p\text{-}MeC_6H_4SO_2Cl \rightarrow 1 + HCl} \quad (3)$$

The relative acidities of the OH groups of adenosine may be responsible for the regioselectivity of the tosylation reaction. The weak acidity ($pK_a \approx 12.5$)[11] of adenosine is attributed to the 2',3'-*cis*-diol system that is capable of intramolecular hydrogen bonding.[12] The ratios of O-monomethylated derivatives[13,14] from the reaction of adenosine with diazomethane or iodomethane, and their relative mobilities on ion-exchange columns,[13] suggest that 2'-OH is more acidic than 3'-OH which in turn is more acidic than 5'-OH. The inductive effect of the adenine moiety appears to contribute to the enhanced acidity of the 2'-OH group.[13] Presumably, the relative acidities of the OH groups are sufficiently distinctive to effect the tosylation selectively at the 2'-position under the present conditions. The formation and distribution of isomeric tosyl derivatives in the product mixture also appear to be influenced by reaction conditions and the steric properties of the reactants. For example, the reaction of 8-bromoadenosine with 2,4,6-triisopropylbenzenesulfonyl chloride in an anhydrous alkaline medium was reported to yield both 2'- and 3'-O-monosulfonylated compounds as major products; however, the ratio of 2'- to 3'-O-sulfonyl isomer increased when 5'-OH was substituted with bulky groups.[15]

EXPERIMENTAL

Melting points were recorded on a Fisher-Johns apparatus and are uncorrected. Elemental analysis was performed by Galbraith Laboratories (Knoxville, Tenn.). HPLC analyses were performed on a Phase-Sep ODS2 4.6x250-mm column using a Model 9533 liquid chromatograph (IBM Instruments); the mobile phase was 50% aqueous methanol flowing at 1 mL/min. UV spectra were obtained at ambient temperature with a Hewlett Packard, Model 8452 diode array spectrophotometer and 10-mm light-path quartz cuvettes. NMR spectra were obtained in DMSO-d_6 with a Brucker AM 500 spectrometer. FAB Mass spectra were obtained in an acidified (HCl) glycerol matrix with a Model 70SQ spectrometer (VG Instruments). All chemicals were the best commercial grade available, and solutions were prepared with Pyrex-distilled water that was previously deionized.

<u>Reaction of adenosine with sodium p-toluenesulfinate in the presence of sodium hypochlorite</u>. Adenosine (1.603 g, 6 mmol) was dissolved in water (200 mL) at 55-60°C. Sodium p-toluenesulfinate (2.14 g, 12 mmol) was

added with magnetic stirring. To the warm clear solution was added NaOCl (13 mL, 6 mmol) in small aliquots over a period of 5 min, and stirring was continued at 50°C for an additional 30 min. The warm reaction mixture was filtered through a sintered glass funnel under suction and the white precipitate washed successively with water (2 x 100 mL), 95% EtOH (2 x 50 mL) and $CHCl_3$ (2 x 50 mL). The solid was subjected to HPLC analysis as described above. A portion of the vacuum-dried solid (1.25 g, 50%) was recrystallized from hot MeOH, m.p. 231-233°C (dec.); UV λ_{max}(MeOH) nm (ϵx10^{-3}) 262 (10.3), 230 (9.5), 218 (8.0); ^{1}H-NMR (DMSO-d_6) δ 8.15 (1H, s, H-8), 7.97 (1H, s, H-2), 7.41 (brs, NH_2), 7.35 (2H, d, J=8.2 Hz, H-2",6"), 6.97 (2H, d, J=8.2 Hz, H-3",5"), 6.06 (1H, d, J=7.2 Hz, H-1'), 6.06 (d, J=4.7 Hz, OH-3'), 5.80 (m, OH-5'), 5.45 (1H, dd, J=7.3 and 4.9 Hz, H-2'), 4.34 (1H, m, J=4.8 Hz, H-3'), 4.06 (1H, brs, H-4'), 3.64 (1H, d, J=12.5 Hz, H-5'), 3.54 (1H, m, J=12.4 and 2.8 Hz, H-5'), 2.25 (3H, s, Me-4"); ^{15}N- and ^{13}C-NMR spectral data are given in Table 1; FABMS 422 (MH^+). Calculated for $C_{17}H_{19}N_5O_6S$: C, 48.45; H, 4.54; N, 16.62; S, 7.61. Found: C, 48.32; H, 4.77; N, 16.47; S, 7.83.

Reaction of p-toluenesulfonyl chloride with adenosine. Adenosine (534 mg, 2 mmol) was dissolved in water (100 mL) at 40-50°C and p-toluenesulfonyl chloride (418 mg, 2.2 mmol) was added with magnetic stirring. NaOH (1 mL, 1 mmol) was added and the stirring continued overnight. The reaction mixture was centrifuged, and the residue washed with water (3 x 50 mL) and 95% EtOH (4 x 20 mL). The vacuum-dried residue (220 mg, 26%) was found by HPLC analysis and mixed melting point determination to be identical to the solid described above.

ACKNOWLEDGMENTS

The authors are indebted to A. R. Garber, H. J. Cohen, and M. Walla, University of South Carolina, for NMR and mass spectroscopy. This work was supported by grants to C. B. from the National Institutes of Health (AI-24003) and Air Force Office of Scientific Research, Air Force Systems Command, USAF (AFOSR 89-0468). B. M. R. Bandara is a visiting scientist from the Department of Chemistry, University of Peradeniya, Sri Lanka. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either express or implied, of the Air Force Office of Scientific Research or the U. S. Government.

REFERENCES

1. **Nucleotide Analogues as Antiviral Agents**, Martin, J.C., Ed., American Chemical Society, Washington, DC, 1989, 186 pp.; **Adenosine and Adenine Nucleotides: Physiology and Pharmacology**, Paton, D.M., Ed., Taylor and Francis, London, Philadelphia, 1988, 305 pp.; **Physiology and Pharmacology of Adenosine Derivatives**, Daly, J.W., Kuroda, Y., Phillis, J.W., Shimizu, H. and Ui, M., Eds., Raven Press, New York, 1983, 299 pp.; **Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques**, Townsend, L.B. and Tipson, R.S., Eds., John Wiley & Sons, New York, Chichester, Brisbane, Toronto, 1978, 1067 pp.; **Chemistry, Biology, and Clinical Uses of Nucleoside Analogs**, Bloch, A., Ed., Ann. NY Acad. Sci., Vol 255, The NY Academy of Sciences, New York, 1975, 610 pp.
2. Wagner, D., Verheyden, J.P.H. and Moffatt, J.G., **J. Org. Chem.**, 1974, *39*, 24-30.
3. Herdewijn, P., Pauwels, R., Baba, M., Balzarini J. and De Clercq, E., **J. Med. Chem.**, 1987, *30*, 2131-2137.
4. Todd, A. and Ulbricht, T.L.V., **J. Chem. Soc.**, 1960, 3275-3277.
5. Gonnella, N.C., Nakanishi, H., Holtwick, J.B., Horowitz, D.S., Kanamori, K., Leonard, N.J. and Roberts, J.D., **J. Am. Chem. Soc.**, 1983, *105*, 2050-2055.
6. Kuhn, R. and Jahn, W., **Chem. Ber.**, 1965, *98*, 1699-1704.
7. Jones, A.J., Grant, D.M., Winkley, M.W. and Robins, R.K., **J. Am. Chem. Soc.**, 1970, *92*, 4079-4087.
8. Bernofsky, C., Bandara, B.M.R., Hinojosa, O. and Strauss, S., **Free Rad. Res. Comm.**, 1989, in press.
9. Scully, F.E.,Jr. and Bowdring, K., **J. Org. Chem.**, 1981, *46*, 5077-5081.
10. Ikehara, M. and Uesugi, S., **Tetrahedron**, 1972, *28*, 3687-3694.
11. Levene, P.A., Simms, H.S. and Bass, L.W., **J. Biol. Chem.**, 1926, *70*, 243-251.
12. Izatt, R.M., Hansen, L.D., Rytting, J.H. and Christensen, J.J., **J. Am. Chem. Soc.**, 1965, *87*, 2760-2761.
13. Gin, J.B. and Dekker, C.A., **Biochemistry**, 1968, *7*, 1413-1420.
14. Yano, J., Kan, L.S. and Ts'O, P.O.P., **Biochim. Biophys. Acta**, 1980, *629*, 178-183; Martin, D.M.G., Reese, C.B. and Stephenson, G.F.,

Biochemistry, 1968, *7*, 1406-1412; Broom, A.D. and Robins, R.K., **J. Am. Chem. Soc.**, 1965, *87*, 1145-1146.

15. Ikehara, M. and Kaneko, M., **Tetrahedron**, 1970, *26*, 4251-4259.

Received August 28, 1989.