Educación y Ciencia to R.U. and M.I. are gratefully acknowledged.

Registry No. **3**, 65750-57-4; **8**, 18707-60-3; **9**, 103-26-4; **10a**, 138234-97-6; **10b**, 138234-98-7; **10c**, 138234-99-8; **10d**, 138235-00-4; **11a**, 138235-01-5; **11b**, 138235-02-6; **11c**, 138235-03-7; **14**, 72079-55-1; *anti*-**15a**, 128474-84-0; *syn*-**15a**, 128571-95-9; *anti*-**15b**, 138235-04-8; *syn*-**15b**, 138331-54-1; *anti*-**15c**, 138235-05-9; *syn*-**15c**, 138331-55-2; *anti*-**15d**, 138235-06-0; *syn*-**15d**, 138331-56-3; *anti*-**17**, 135683-14-6; *syn*-**17**, 135758-02-0; **18**, 138235-07-1; **19**, 138331-57-4; (*E*)-**20**, 131533-35-2; (*Z*)-**20**, 131533-34-1; **21**, 138235-08-2; *anti*-**22**, 138235-09-3; *syn*-**22**, 138331-58-5; *anti*-**23**, 138235-10-6; **29a**, 128571-96-0; **29b**, 131533-36-3; **29c**, 131533-38-5; **29d**, 131533-37-4; **30b**, 131614-09-0; **30d**, 138331-59-6; **33**, 131533-32-9; **34**, 138235-11-7; **35**, 131614-08-9; **37**, 131533-33-0; **38**, 138235-12-8; **39a**, 138235-13-9; **39b**, 138235-14-0; **41a**, 138235-15-1; **41b**, 138235-16-2; **42a**, 138235-17-3; **42b**, 138235-18-4; **43a**, 138331-60-9; **43b**, 138331-61-0; **43c**, 138235-19-5; **45a**, 138331-62-1; **45b**, 138331-63-2; **45c**, 138235-20-8; **46a**, 138331-64-3; **46b**, 138331-65-4; **46c**, 138235-21-9.

Supplementary Material Available: Characterization data for **10a**, **10b**, **10d**, **11a**, **11b**, **11c**, *anti*-**15a**, *syn*-**15a**, *anti*-**15b**, *syn*-**15b**, *anti*-**15c**, *syn*-**15c**, *anti*-**15d**, *syn*-**15d**, *anti*-**22**, *syn*-**22**, **23**, **29a**, **29b**, **29c**, **29d**, **37**, **38**, **41a**, **41b**, **45a**, **45b**, and **45c** (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Reaction of Nucleic Acid Bases with α-Acetylenic Esters. 5.[1] Synthesis and Properties of Adenosine and Cytidine Derivatives

Pierre Roques,[2a,c] Jean Yves Le Gall,[2a] Liliane Lacombe,[2b] and Martin Olomucki*[,2a]

Laboratoire de Biochimie cellulaire and Laboratoire de Chimie des interactions moléculaires, Collège de France, 11, pl. Marcelin Berthelot, 75231 Paris Cedex 05, France

Received September 16, 1991

α-Acetylenic esters are able to react under mild experimental conditions with the base moiety of adenosine and cytidine, while guanosine is unreactive. A double reaction of the triple bond and the ester group of the reagent with the NH_2 group and the adjacent ring nitrogen of the base yields derivatives in which an additional pyrimidone ring is fused to the original base. These derivatives can exist in two isomeric forms. In alkaline solution, or by prolonged heating in water, the medium pyrimidine ring of adenosine derivatives opens by loss of carbon 5. If the derivatization is performed with chlorotetrolic (4-chloro-2-butynoic) acid esters, the modified nucleobases contain a chloromethyl side chain. Tests of the alkylating abilities of the latter in the two isomeric adenosine derivatives show that the chlorine can be easily substituted by a thiol in the presence of alkali; a partial Dimroth rearrangement of one of the reaction products is observed. The reaction with amines is accompanied by ring opening. Nucleic acids containing these alkylating base derivatives can be cross-linked to other macromolecules such as solid supports or contact proteins.

Introduction

Nucleic acid bases having an exocyclic NH_2 and an adjacent ring nitrogen are able to react with certain types of electrophilic bifunctional reagents, yielding derivatives in which an additional five- or six-membered heterocycle is fused to the original purine or pyrimidine. Many of these reagents contain a halogen atom and/or an unsaturated group including C=O,[3-8] C=C,[9-12] C≡N,[13-16] and N=C=O.[17-19] The long known chlorotetrolic (4-chloro-2-butynoic) acid esters, $ClCH_2C{\equiv}CCOOR$,[20-22] have such functions and therefore, like other bifunctional nucleobase reagents, should be able to react with the amidine -N=C(NH_2)- system of adenine or cytosine or the guanidine -N=C(NH_2)NH- system of guanine. This expectation was strengthened by our earlier works which have shown that methyl chlorotetrolate can be used as a bifunctional protein modifier, reacting through its chloromethyl group and the triple bond with protein nucleophiles such as amine, thiol, or imidazole.[23] A similar behavior toward

(1) For part 4, see ref 41a.
(2) (a) Biochimie cellulaire. (b) Chimie des interactions moléculaires. (c) Present address: Laboratoire de neuropathologie expérimentale et virologie, CEA Fontenay-aux-Roses, 92265 Fontenay-aux-Roses, France.
(3) Shapiro, R.; Cohen, B. I.; Shivey, S. J.; Maurer, H. *Biochemistry* **1969**, *8*, 238.
(4) Moschel, R. C.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 294.
(5) Kochetkov, N. K.; Shibaev, V. N.; Kost, A. A. *Tetrahedron Lett.* **1971**, 1993.
(6) (a) Barrio, J. R.; Secrist, J. A.; Leonard, N. J. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 597. (b) Kayasuga-Mikado, K.; Hashimoto, T.; Negishi, T.; Negishi, K.; Hayatsu, H. *Chem. Pharm. Bull.* **1980**, *28*, 932.
(7) Sattsangi, P. D.; Leonard, N. J.; Frihart, C. R. *J. Org. Chem.* **1977**, *42*, 3292.
(8) Leonard, N. J.; Cruickshank, K. A. *J. Org. Chem.* **1985**, *50*, 2480.
(9) (a) Chung, F. L.; Young, R.; Hecht, S. S. *Cancer Res.* **1984**, *44*, 990. (b) Chung, F. L.; Roy, K. R.; Hecht, S. S. *J. Org. Chem.* **1988**, *53*, 14.
(10) Meerman, J. H. N.; Pearson, P. G.; Meier, G. P.; Nelson, S. D. *J. Org. Chem.* **1988**, *53*, 30.
(11) Neudecker, T.; Lutz, D.; Eder, E.; Henschler, D. *Mutat. Res.* **1981**, *91*, 27.
(12) Laib, R. J.; Bolt, H. M. *Arch. Toxicol.* **1978**, *39*, 235.
(13) Furukawa, Y.; Miyashita, O.; Honjo, M. *Chem. Pharm. Bull.* **1974**, *22*, 2552.
(14) Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1457.
(15) Agasimundin, Y. S.; Oakes, F. T.; Kostuba, L. J.; Leonard, N. J. *J. Org. Chem.* **1985**, *50*, 2468; *J. Am. Chem. Soc.* **1984**, *106*, 6847.
(16) Agasimundin, Y. S.; Oakes, F. T.; Leonard, N. J. *J. Org. Chem.* **1985**, *50*, 2474.
(17) Kumar, S.; Leonard, N. J. *J. Org. Chem.* **1988**, *53*, 3959.
(18) (a) Camus, P. Doctorate Thesis, University of Grenoble, 1988. (b) Camus, P.; Lhomme, M. F.; Lhomme, J. *Tetrahedron Lett.* **1989**, *30*, 467.
(19) Nair, V.; Turner, G. A. *Tetrahedron Lett.* **1984**, *25*, 247.
(20) (a) Olomucki, M. *C. R. Acad. Sci.* **1958**, *246*, 1877. (b) Olomucki, M.; Marszak, I. *Bull. Soc. Chim. Fr.* **1959**, 315. (c) *Angew. Chem.* **1959**, *71*, 314. (d) Olomucki, M. *Ann. Chim. (Paris)* **1960**, *5*, 845.
(21) Olomucki, M.; Le Gall, J. Y.; Barrand, I. *J. Chem. Soc., Chem. Commun.* **1982**, 1290.
(22) Olomucki, M.; Le Gall, J. Y. *Org. Synth.* **1987**, *65*, 47.
(23) (a) Olomucki, M.; Diopoh, J. *C. R. Acad. Sci., Ser. D.* **1977**, *284*, 2293. (b) Diopoh, J.; Olomucki, M. *Bioorg. Chem.* **1982**, *11*, 463.

0022-3263/92/1957-1579$03.00/0

nucleobases would incorporate onto the latter an additional five-membered ring. Actually it was found that the reaction involves the ester group instead of the chlorine and results in compounds having a six-membered heterocycle fused to the original base. The synthesis and properties of such derivatives were investigated.

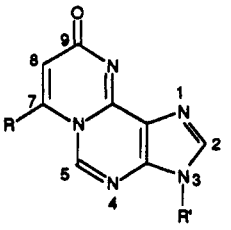
Results and Discussion

When *p*-nitrophenyl chlorotetrolate was reacted with adenosine in DMF for 48 h at 5 °C, a new product, mp 230 °C, precipitated in 82% yield. In TLC (silica gel, 1-butanol/acetic acid/water, 4:2:1), this derivative had a lower (0.39) R_f than adenosine (0.45), gave a pink spot when irradiated at 254 nm, and was not revealed in 366-nm light. The product dissolved in DMSO and in acetic acid but was only sparingly soluble in DMF and in water and practically insoluble in other solvents. Its IR spectrum showed neither the acetylenic 2260-cm^{-1} band nor the CO band at 1740 cm^{-1} of chlorotetrolic acid ester, but instead a new CO absorption appeared at 1670 cm^{-1}, while the C–Cl band at 750 cm^{-1} was still present. The elemental analysis also showed the presence of chlorine and indicated an empirical formula $C_{14}H_{14}N_5O_5Cl$ resulting from addition of 1 molar equiv of chlorotetrolic acid ester to adenosine with loss of *p*-nitrophenol.

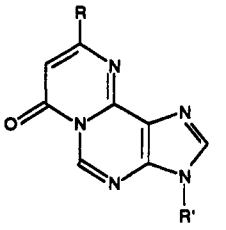
The same product precipitated when adenosine was treated with methyl or ethyl chlorotetrolate in a 50% hydroalcoholic solution of apparent pH 4–5,[24] 7 h under reflux or 8 days at room temperature. However, under these conditions the reaction was more complex, the product was formed in about 25% yield, and different byproducts appeared, mainly a compound visualized in TLC at R_f 0.51 as a pink spot on irradiation with 254-nm light and as a bright-blue fluorescent spot under a 366-nm lamp. When the reaction medium was maintained at pH 7 under a pH-stat, no precipitate formed and the new fluorescent compound could be isolated in 31% yield by elution with water from an Amberlite IRC 50 column. It had the same empirical formula as the former product but a lower (170 °C) mp, a higher (1710 cm^{-1}) IR carbonyl absorption, and a better solubility: it was more soluble in water and also slightly soluble in organic solvents such as methanol or acetone. Its fluorescence properties proved low: quantum yield 5%, lifetime 2.5 ns. The mass spectra of both compounds showed the peak of the ribose fragment.

It was therefore obvious that (i) as expected, chlorotetrolic acid esters are able to react under mild conditions with the adenine ring of adenosine, (ii) the bifunctional reaction does not involve the triple bond and the chlorine but rather the triple bond and the ester group, and (iii) the reaction can yield two isomeric derivatives.

Inspection of the literature has shown that such a reaction was not unprecedented. Reactions of α-acetylenic esters with 2-aminopyridine[25] and some other heterocycles having a primary amino group in the vicinity of one[26–29] or two[30,31] ring nitrogens have been described. They consist in an initial[25–28,30,31,33] addition of nitrogen across the triple bond giving an ethylenic intermediate mainly[34] of the cis form[26,28,30–33] in which the ester group is favorably disposed for ring closure by formation of an amide with the other nitrogen; a new pyrimidone ring is thus fused to the original heterocycle. In most cases the primary attack is performed by the ring nitrogen, and therefore the carbonyl group of the additional pyrimidone ring is connected to the nitrogen atom which was exocyclic in the original heterocycle.[25–30,33] However, formation of isomeric pyrimidone derivatives having the CO group connected to the nitrogen atom common to both cycles was also described;[26,31,33] no isomerization between these two types of pyrimidones was so far observed.[30,33] Fused pyrimidones of the first type are generally characterized by a higher mp, a lower solubility,[31b,35] and a lower value of the IR CO band[27,30,36] than their isomers. Accordingly, in our case the two adenosine derivatives would be 3-β-D-ribofuranosyl-9*H*-9-oxo-7-(chloromethyl)pyrimido[2,1-*i*]purine (**1a**) and 3-β-D-ribofuranosyl-7*H*-7-oxo-9-(chloromethyl)pyrimido-[2,1-*i*]purine (**2a**).



1 a - d, g



2 a, e - i

a, R = $ClCH_2$; R' = ribose

b, R = H; R' = ribose

c, R = $COOCH_3$; R' = ribose

d, R = $ClCH_2$; R' = triacetylribose

e, R = $ClCH_2$; R' = 5'-mono-, di- or triphosphoribose

f, R = $ClCH_2$; R' = 2'-deoxy-5'-triphosphoribose

g, R = $C_6H_5CH_2$-S-CH_2; R' = ribose

h, R = ICH_2; R' = ribose

i, R = n-$C_5H_{11}NH$-CH_2; R' = ribose

This assignment was further corroborated by other spectral data. In ^1H NMR, the base moiety of compound **1a** showed a H_5 signal at δ 8.95 with a 42% NOE on irradiation of $ClCH_2$ (26% for H_8) while in isomer **2a** this signal was strongly deshielded (δ 9.54) and showed practically no NOE on irradiation of $ClCH_2$. The chloromethyl group appeared at 5.25 in **1a** and at 4.73 in **2a**. The other protons were similar for the two derivatives. On the other hand, in earlier works, UV spectra were most commonly used to distinguish between isomeric fused pyrimidones. The absorption maxima of these compounds fall in three regions, the long wavelength band (appearing in the range

(24) DMF cannot be used in this case due to strong darkening of the solutions of alkyl chlorotetrolates in this solvent.

(25) Lappin, G. R. *J. Org. Chem.* **1961**, *26*, 2350.
(26) Reimlinger, H.; Peiren, M. A.; Merenyi, R. *Chem. Ber.* **1970**, *103*, 3252.
(27) Dunwell, D. W.; Evans, S. *J. Chem. Soc. C* **1971**, 2094.
(28) Reimlinger, H. *Chem. Ber.* **1971**, *104*, 2232.
(29) Reimlinger, H.; Billiau, F.; Peiren, M. A.; Merenyi, R. *Chem. Ber.* **1972**, *105*, 108.
(30) Reimlinger, H.; Peiren, M. A. *Chem. Ber.* **1970**, *103*, 3266.
(31) Reimlinger, H.; Peiren, M. A.; Merenyi, R. *Chem. Ber.* **1972**, (a) *105*, 103; (b) *105*, 794.
(32) Herbig, K.; Huisgen, R.; Huber, H. *Chem. Ber.* **1966**, *99*, 2546.
(33) Reimlinger, H.; Jacquier, R.; Daunis, J. *Chem. Ber.* **1971**, *104*, 2702.

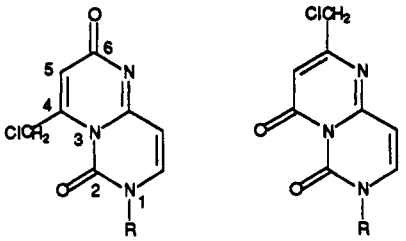
(34) In some cases the simultaneous formation of a certain (generally small) proportion of the noncyclizable *trans* derivative has been mentioned.[25–28,30–33]

(35) Adams, R.; Pachter, I. J. *J. Am. Chem. Soc.* **1952**, *74*, 5491.
(36) Allen, C. F. H.; Beilfuss, H. R.; Burness, D. M.; Reynolds, G. A.; Tinker, J. F.; VanAllan, J. A. *J. Org. Chem.* **1959**, *24*, 779.

of 280–360 nm) being more intense than the medium λ band in isomers having the carbonyl group connected to the nitrogen atom common to two cycles, while in the other isomer the ε value of this band never exceeds one-third of that of the medium band, or sometimes this peak is completely absent.[26-31,33,35,36] Actually product **1a** had a two-band UV spectrum (240 and 297 nm) and product **2a** had a three-band spectrum (240, 307, and 346 nm) with ε values consistent with these rules (see the Experimental Section). The value of the earlier spectral (UV, IR) methods used to distinguish between such isomers was finally confirmed (to our knowledge for the first time) by establishing their crystal structure by X-ray diffraction.[37] The mass spectra of compounds **1a** and **2a** did not show the molecular peak but, in addition to the peak of ribose, gave those due to the base moiety and to the fragmentation of the latter.

The reaction of adenosine with other α-acetylenic esters was also tested. Treatment of the nucleoside with an excess of ethyl propiolate or dimethyl acetylenedicarboxylate in a hydroalcoholic solution for several days at room temperature gave derivatives of type **1**, respectively **1b** and **1c**. Both products precipitate spontaneously in the reaction medium: **1b** in the form of well-developed prisms of pure product in 61% yield and **1c** as a crude powder in 35% yield. On the other hand, the reaction with chlorotetrolic acid ester was also applied to 2′,3′,5′-triacetyladenosine, obtained by treatment of adenosine with acetic anhydride in the presence of a small amount of 4-(dimethylamino)pyridine. When a THF solution of triacetyladenosine and *p*-nitrophenyl chlorotetrolate was kept 4 days at room temperature, pure product **1d** deposited in 74% yield. This compound is much more soluble than derivatives of adenosine with unsubstituted ribose. Also in contrast to the latter its mass spectrum shows fragmentation peaks of triacetylribose rather than those of the base moiety.

Tests with other nucleosides have shown that cytidine reacts at room temperature with chlorotetrolic acid esters to give a 50% yield of derivative **3** (R = ribose). However, guanosine proved unreactive, at least under the usual experimental conditions. This statement was in agreement with some earlier, so far unexplained, differences in reactivity between these nucleobases; thus, while bifunctional reagents able to incorporate an additional heterocycle on the base, such as chloro- or bromoacetaldehyde, react easily with adenosine or cytidine, they do not react[5,6a] or react only with difficulty[6b,7] with guanosine. The low reactivity of deoxyguanosine with methyl vinyl ketone or 2-cyclohexen-1-one was also reported.[9b]



3 **4**

This new derivatization of nucleobases was further extended to mono- and polynucleotides. The reaction of methyl chlorotetrolate with AMP, ADP, and ATP in hydroalcoholic solution, which had to be performed at pH 7 because of the solubility characteristics of these nucleotides, led to derivatives **2e** in about 40% yield.[38] The synthesis of a similar derivative of dATP, **2f**, is described in the Experimental Section. These nucleotide analogues were tested for their ability to substitute for the natural substrates or cofactors of different enzymes[38] and for their possible anti-HIV activities.[39]

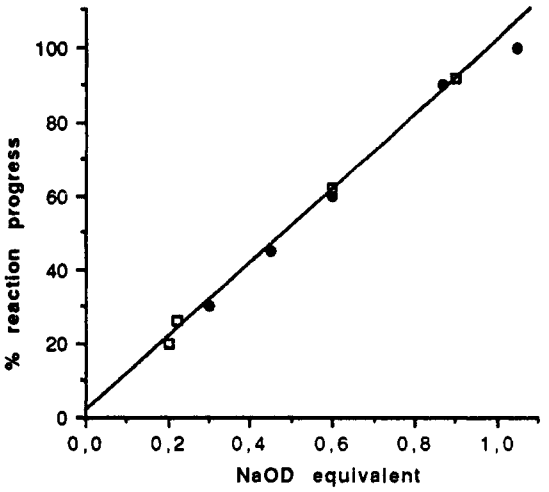


Figure 1. Reaction of compounds **1a** and **2a** with benzyl mercaptan in $(CD_3)_2SO$. Relationship between the amounts of NaOD introduced into the solution and the reaction progress, as determined by integration of NMR spectra: **1a** (□), **2a** (●).

An interesting feature of the reaction with methyl chlorotetrolate was observed when it was applied to single-stranded polynucleotides instead of small molecules. In this case the isomeric nature of the resulting base derivative depends mainly on the conformation of the nucleic acid, presumably because of the steric requirements of the linear and rigid reagent. At pH 7, adenine residues in poly(A)[40] and in single-stranded fragments of tRNA[41] are converted to derivatives of type **1** when the bases are normally stacked, while isomers **2** form when the conformation is more disordered. In the case of poly(C), the formerly unknown isomer **4** of modified cytidine was formed.[40]

The presence of a chloromethyl group in some of the above base derivatives could provide them with alkylating properties useful for cross-linking of biological macromolecules. Earlier observations have shown that the CH_2Cl group reacts easily with thiols. Thus, in poly(A) or poly(C) treated with chlorotetrolic acid ester, the number of modified residues could be determined by back-titration with a thiol (cysteamine).[40] On the other hand, modified poly(A) could be immobilized on thiolated polysaccharides.[40] In the present work, the reaction of thiols with chlorotetrolate-modified adenosine was tested more in detail, using benzyl mercaptan as a model. No reaction occurred in equimolar solutions of the latter and compound **1a** or **2a** in DMSO even after 18 h, as shown by the absence of any evolution of ^1H NMR spectra of the mixtures. In contrast, a very rapid reaction occurred when 0.15 N NaOD was added to the solutions. The conversion was proportional to the amount of the introduced OD^- ions and, hence, of the RS^- ions generated in the medium (Figure

(37) Olomucki, M.; Le Gall, J. Y.; Colinart, S.; Durant, F.; Norberg, B.; Evrard, G. *Tetrahedron Lett.* **1984**, *25*, 3471.

(38) Thomé, F.; Blois, F.; Olomucki, A.; Olomucki, M. *Eur. J. Biochem.* **1987**, *162*, 433.

(39) (a) Roques, P. Doctorate Thesis, University of Paris, 1989. (b) Clayette, P.; Merrouche, Y.; Gharbaoui, T.; Roques, P.; Beugelmans, R.; Olomucki, M.; Dormont, D. *Antiviral Chem. Chemotherapy*, in press.

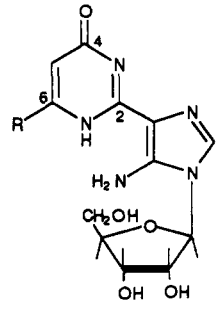
(40) (a) Roques, P.; Olomucki, M. *Eur. J. Biochem.* **1987**, *167*, 103. (b) Roques, P.; Olomucki, M. *Nucleosides Nucleotides* **1990**, *9*, 455.

(41) (a) Roques, P.; Thomé, F.; Olomucki, M. *Biochem. Biophys. Acta* **1988**, *951*, 71. (b) Roques, P.; Olomucki, M. *Biochem. Pharmacol.* **1988**, *37*, 1823.

1). In the case of compound **2a** the final fluorescent product **2g** was homogeneous, remained unchanged even in the presence of excess of thiol, and could be isolated and purified on a preparative scale. On the other hand, the equally reactive compound **1a** yielded an unstable nonfluorescent derivative **1g** which within several hours partly isomerized to product **2g**. Pure compound **1g** could therefore not be isolated, although spectral data could be recorded on a freshly prepared sample. ^{1}H NMR spectra of isomers **2g** and **1g** showed the same characteristics as the starting products, in particular different chemical shifts of protons 5 and 1′. In addition, the position of phenyl protons was different in products **2g** and **1g** (δ 7.23 and 7.10 ppm, respectively), presumably due to a difference in stacking of the phenyl ring over the heterocyclic base. Isomer **1g** is probably partly stabilized by stacking, since the Dimroth rearrangement thus observed was not complete. An equilibrium of about 65% **1g** and 35% **2g** was reached after 24 h and remained unchanged for over 10 days even in the presence of an excess of sodium hydroxide. Such an equilibrium of isomers was observed for *N*-methyladenosine in neutral pH.[42] Although no Dimroth rearrangement was observed in the case of derivatives **1a** or **2a** in neutral aqueous buffer,[40a] these products, devoid of an aromatic substituent, underwent a complete **1a** to **2a** rearrangement in DMSO at room temperature (half-life 24 h) in the presence of 1.5 equiv of sodium hydroxide.

The chlorine atom in compound **2a** could be easily exchanged for iodine by treatment with sodium iodide in boiling acetone; the iodomethyl derivative **2h** was thus obtained in 90% yield. This reaction could not be applied to **1a** because of the very low solubility of this product in acetone.

Further studies of the chemical properties of our adenosine derivatives were related to their interaction with amines. Product **2a** rapidly disappeared in 50% aqueous DMF at 40 °C in the presence of 2 equiv of 1-amylamine, taken as a model; under these conditions its half-life was 20 min, as determined by HPLC. The major reaction product **5a** precipitated in the medium. Such a ring-opened compound also formed in the presence of ammonia in 15% aqueous methanol. This ring opening by loss of carbon 5 is relatively easy, even in the absence of amine or ammonia, since it also occurred when methyl chlorotetrolate was refluxed with adenosine in 50% aqueous methanol for time periods longer than those necessary for the synthesis of **1a** or **2a**. Product **5a** thus obtained was fully characterized and proved identical with those resulting from treatment of **2a** with amine or ammonia.



5

a, R = $ClCH_2$

b, R = H

c, R = n-$C_5H_{11}NH$-CH_2

Similarly, prolonged heating of adenosine with ethyl propiolate in hydroalcoholic solution yielded 90% of an analogous ring-opened product **5b** (together with some 1,3,5-tris(ethoxycarbonyl)benzene, resulting from trimerization of ethyl propiolate under these conditions). Compound **5b** also formed when a pure sample of the tricyclic base derivative **1b** was heated in water to 80 °C.

Such ring-opening reactions with loss of a carbon atom in tricyclic bases[43] and in substituted purines[44] have been described, and it has been observed that the reaction is slower in apolar solvents such as dichloromethane.[43] The solvent used in the above reaction of **2a** with 1-amylamine apparently favored ring fission over substitution of chlorine, and the ring-opened product **5a**, due to its low solubility, immediately precipitated, thus preventing a possible reaction of the amine with the chloromethyl group. A nonaqueous, less polar solvent was therefore sought. Actually, when the reaction of **2a** with amylamine was tried in a 9:1 mixture of methanol and DMF (apparent pH 7.5, 70 h at 40 °C until the starting material disappeared), substitution of chlorine took place; however, different products formed which could not be separated. ^{1}H NMR analysis of fractions isolated by HPLC showed that the mixture probably contained the tricyclic aminomethyl derivative **2i** [in $(CD_3)_2SO$ δ 9.55 (H_5), 8.78 (imidazol CH), 6.5 (H_8), 6.00 ($H_{1'}$)], and the ring-opened compound **5c** [no signal in the 9 ppm region, 7.50 (imidazol CH), 6.70 (NH_2), 6.02 (H_5), 5.55 ($H_{1'}$)]. Furthermore, while in solvents such as methanol or acetone no ring opening was observed, substitution of chlorine did not occur either. Thus, in contrast to hydroalcoholic ammonia, compound **2a** remained unchanged in dry methanolic ammonia. It therefore seemed advisable to perform the reaction in such solvents in order to avoid ring opening and to use the more reactive iodo derivative **2h** to improve the substitution of the side chain. Product **2h**, either prepared as mentioned above or obtained in situ from **2a**, was heated for 2 h in acetone in the presence of equivalent amounts of 1-amylamine and potassium carbonate. As expected, the integrity of the tricyclic base was preserved in this reaction. However, in addition to the (1-amylamino)methyl derivative **2i**, a higher amount of a tertiary amine, resulting from a double N-alkylation of amylamine by two nucleoside radicals, was also obtained as shown by spectral data; the latter product also formed when an excess of amine was used.

Thus the nucleoside derivatives described in the present paper exhibit a broad spectrum of chemical properties. Their reactivity involves modifications of the base fragment and, for those having a halomethyl side chain, the alkylating ability of this group. These reactions therefore occurred in competition in the above model tests. However, it should be pointed out that the formation of products of different kinds is not objectable when the use of such base derivatives for cross-linking of biomolecules is considered. Actually in this case the exact chemical nature of binding is less important than the basic fact that a covalent bond can be created. Thus, for example, a nucleic acid modified by chlorotetrolic acid ester can be bound to a contact protein, as shown in our earlier work in which modified $tRNA^{Phe}$ could be bound to phenylalanyl-tRNA synthetase, and the binding areas of these macromolecules have been determined.[45]

(42) Wilson, M. H.; Mc Closkey, A. *J. Org. Chem.* **1973**, *38*, 2247.

(43) Odijk, W. M.; Koomen, G. J. *J. Chem. Soc., Perkin Trans. 2* **1987**, 733.

(44) Fujii, T.; Saito, T.; Inoue, I.; Kumazawa, Y.; Tamura, K. *Chem. Pharm. Bull.* **1988**, *36*, 107.

Experimental Section

Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Reaction progress and product purity were monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} plastic sheets with fluorescent indicator, using the following solvents (v/v): A, 1-butanol/acetic acid/water, 4:2:1; B, 1-butanol/water/acetone/5% ammonia/acetic acid, 9:4:3:2:2; C, methanol/formic acid/ethyl acetate, 5/3/2; D, benzene/methanol/acetic acid, 12:2:1; E, chloroform/ethanol, 8:2. The spots were visualized in UV light (254 and 366 nm). Infrared spectra (KBr disks) were obtained with a Perkin-Elmer 720 spectrometer. Ultraviolet spectra were measured on Zeiss PMQ II or Shimadzu UV 160 apparatus. Unless otherwise stated, nuclear magnetic resonance spectra were recorded on a Bruker AM 200 SY at 200 MHz for ^1H and 50 MHz for ^{13}C or a Varian FT 80A spectrometer at 80 MHz for ^1H and 20 MHz for ^{13}C. Chemical shifts (δ) in $(CD_3)_2SO$ with a trace of D_2O are reported in parts per million (ppm) downfield from TMS as an internal standard. Routine low-resolution mass spectra and exact mass data were measured on a AEI MS-50 instrument at an ionizing potential of 70 eV. HPLC was carried out on a LKB instrument using a reversed phase 4.6- × 250-mm ODS-120T (5 μm) column from Toyo Soda Manufacturing Co.; isocratic elutions in 0.05 M acetate buffer, pH 5, containing 20% methanol were performed at a flow rate of 1 mL/min with nucleoside detection at 275 nm. Solvent evaporations were performed at reduced pressure on a Büchi Rotavapor-R rotary evaporator. Ethyl propiolate and dimethyl acetylenedicarboxylate were obtained from Aldrich and were used without further purification. Methyl chlorotetrolate (chloro-4-butyn-2-oate) was prepared according to Olomucki et al.[21,22]

***p*-Nitrophenyl 4-Chloro-2-butynoate.** A solution of dicyclohexylcarbodiimide (3.5 g, 17 mmol) in 10 mL of THF was added to an ice-cold solution of 4-chloro-2-butynoic acid[20] (2 g, 17 mmol) and *p*-nitrophenol (2.36 g, 17 mmol) in 20 mL of THF. The mixture was stirred at rt overnight, and the precipitated solid was filtered off and washed with THF. The filtrate was evaporated, the residual oil was dissolved in 30 mL of ether, and 10 mL of petroleum ether (bp 40–70 °C) was added. The product, 2.7 g (66%), mp 80 °C, crystallized on cooling: IR 2260 (C≡C), 1740 (C=O), 1530 and 1355 cm^{-1} (aryl NO_2). Anal. Calcd for $C_{10}H_6NO_4Cl$: C, 50.12; H, 2.52; N, 5.85; O, 26.71; Cl, 14.80. Found, C, 50.1; H, 2.6; N, 5.8; O, 26.8; Cl, 14.8.

3-β-D-Ribofuranosyl-9*H*-9-oxo-7-(chloromethyl)pyrimido[1,2-*i*]purine (1a). A solution of adenosine (2 g, 7.5 mmol) and *p*-nitrophenyl 4-chloro-2-butynoate (1.6 g, 6.7 mmol) in 20 mL of DMF was kept at 5 °C for 1 day. Another portion of 1.6 g of ester was added, and the solution was stored under the same conditions for 2 more days to allow a more complete crystallization of the product. The precipitated crystals were filtered, washed with 20 mL of ethanol and 20 mL of acetone, and dried: yield 2.25 g (82%) of pure **1a**; mp 230 °C dec; R_f 0.39 in solvent A; the spot was pink under a 254-nm lamp and was not revealed in 366-nm light; HPLC, retention volume 10.4 mL; pK_a = 0.66; IR 1670 (C=O), 750 cm^{-1} (C-Cl); UV (water) λ_{max} nm (ϵ) 240 (39 000), 297 (14 000); ^1H NMR δ 3.65 (m, 2 H, 5′-CH_2), 3.99 (q, 1 H, $H_{4'}$), 4.15 (t, 1 H, $H_{3'}$), 4.50 (t, 1 H, $H_{2'}$), 5.25 (s, 2 H, CH_2Cl), 6.00 (d, J = 5.5 Hz, 1 H, $H_{1'}$), 6.67 (s, 1 H, H_8), 8.66 (s, 1 H, H_2), 8.95 (s, 1 H, H_5); irradiation of CH_2Cl gave a 42% NOE for H_5 and 26% for H_8, the H_2 proton remaining unchanged; ^{13}C NMR δ 41.0 interfering with $(CD_3)_2SO$ signal (CH_2Cl), 115.8 (C_8), 144.0 (C_7), 166.5 (C=O); MS m/e (relative intensity) 235 (100), M – ribose, 207 (29), 172 (61), 132 (13), 119 (62). Anal. Calcd for $C_{14}H_{14}N_5O_5Cl$: C, 45.72; H, 3.84; N, 19.04; Cl, 9.64. Found: C, 45.7; H, 4.0; N, 19.0; Cl, 9.6.

3-β-D-Ribofuranosyl-7*H*-7-oxo-9-(chloromethyl)pyrimido[1,2-*i*]purine (2a). A solution of adenosine (5 g, 18.7 mmol) and methyl 4-chloro-2-butynoate (5.3 g, 40 mmol) in 110 mL of water and 50 mL of methanol was kept for 7 h at 50 °C and then for 20 h at rt, the pH being maintained at 7 by means of a pH-stat. The reaction mixture was extracted several times with ether and evaporated in vacuo, and the residue was dissolved in water and chromatographed with water on a 4.8- × 48-cm column of Amberlite IRC 50 to yield 2.13 g of pure product **2a**, mp 170 °C; unreacted adenosine (1.2 g) was subsequently recovered by elution with 2 M acetic acid: yield 31%, or 41% based on the amount of unreacted adenosine; R_f 0.51 in solvent A, 0.82 in solvent C (blue fluorescent spot on irradiation with a 366-nm lamp); HPLC, retention volume 35 mL; pK_a = 1; IR 1710 (C=O), 750 cm^{-1} (C-Cl); UV (water) λ_{max} nm (ϵ) 240 (10 950), 307 (6700), 346 (12 050); ^1H NMR δ 3.67 (m, 2 H, 5′-CH_2), 4.01 (q, 1 H, $H_{4'}$), 4.21 (t, 1 H, $H_{3'}$), 4.55 (t, 1 H, $H_{2'}$), 4.73 (s, 2 H, CH_2Cl), 6.07 (d, J = 5.5 Hz, 1 H, $H_{1'}$), 6.61 (s, 1 H, H_8), 8.78 (s, 1 H, H_2), 9.54 (s, 1 H, H_5); practically no NOE on irradiation of CH_2Cl; ^{13}C NMR δ 45.8 (CH_2Cl), 104.0 (C_8), 157.8 (C_9), 163.7 (C=O); MS m/e (relative intensity) 235 (69), 207 (64), 172 (100), 119 (86). Anal. Calcd for $C_{14}H_{14}N_5O_5Cl$: C, 45.72; H, 3.84; N, 19.04; Cl, 9.64. Found: C, 45.5; H, 3.9; N, 18.9; Cl, 9.3.

3-β-D-Ribofuranosyl-9*H*-9-oxopyrimido[1,2-*i*]purine (1b). A solution of adenosine (267 mg, 1 mmol) and ethyl propiolate (196 mg, 2 mmol) in 15 mL of 33% aqueous ethanol was kept at rt, the apparent pH being periodically adjusted to 6 with 1 N sodium hydroxide. Two additional portions of 1 mmol of ethyl propiolate were introduced after 2 and 4 days. After 6 days, 90 mg of pure product, mp 243–244 °C dec, were collected. Additional amounts of compound **1b** crystallized on standing, giving an overall yield of 195 mg (61%): R_f 0.25 in solvent A; pK_a = 1; IR 1660 cm^{-1} (C=O); UV (water), λ_{max} nm (ϵ) 236 (42 600), 291 (16 300); ^1H NMR δ 3.63 (m, 2 H, 5′-CH_2), 3.98 (q, 1 H, $H_{4'}$), 4.18 (t, 1 H, $H_{3'}$), 4.52 (t, 1 H, $H_{2'}$), 5.97 (d, J = 5.5 Hz, 1 H, $H_{1'}$), 6.42 (d, J = 8 Hz, 1 H, H_8), 8.41 (d, J = 8 Hz, 1 H, H_7), 8.63 (s, 1 H, H_2), 8.86 (s, 1 H, H_5); MS m/e (relative intensity) 187 (29), M – ribose, 132 (18), ribose. Anal. Calcd for $C_{13}H_{13}N_5O_5$: C, 48.90, H, 4.10; N, 21.94. Found: C, 48.7; H, 4.0; N, 21.6.

3-β-D-Ribofuranosyl-9*H*-9-oxo-7-(methoxycarbonyl)pyrimido[1,2-*i*]purine (1c). Adenosine (1 g, 3.7 mmol) was dissolved by warming in 10 mL of water and treated with a solution of dimethyl acetylenedicarboxylate (0.65 g, 7.4 mmol) in 10 mL of ethanol. The mixture was kept at rt; after 36 h a second portion of 0.65 g of diester was added. After 4 days the precipitate that formed was filtered and washed successively with water, ethanol, and acetone (5 mL of each solvent); the mother liquor yielded an additional amount of solid after 48 h at 4 °C. The overall yield of crude product was 0.5 g (35%). A 0.1-g sample was dissolved in 10 mL of boiling water, some insoluble tar was discarded, the filtrate was chilled, and the pure product was precipitated by addition of 1 mL of ethanol, filtered, washed with cold ethanol, and dried. The product had the following properties: mp 239 °C dec; R_f in solvent A 0.19; IR 1750 (CO ester), 1650 cm^{-1} (CO lactam); UV (water) λ_{max} nm (ϵ) 236 (25 400), 300 (9900); ^1H NMR δ 3.62 (m, 2 H, 5′-CH_2), 3.96 (m + s, 4 H, $H_{4'}$ and OCH_3), 4.15 (t, J = 5.5 Hz, 1 H, $H_{3'}$), 4.52 (t, J = 5.5 Hz, 1 H, $H_{2'}$), 5.96 (d, J = 5.5 Hz, $H_{1'}$), 6.86 (s, 1 H, H_8), 8.65 (s, 1 H, H_2), 9.24 (s, 1 H, H_5). Anal. Calcd for $C_{15}H_{15}N_5O_7 \cdot H_2O$: C, 45.57; H, 4.33; N, 17.72. Found: C, 45.8; H, 3.9; N, 17.6.

3-(2,3,5-Triacetyl-β-D-ribofuranosyl)-9*H*-9-oxo-7-(chloromethyl)pyrimido[1,2-*i*]purine (1d). A suspension of 2′,3′,5′-triacetyladenosine (0.5 g, 1.26 mmol) in 18 mL of THF containing 0.5 g (2 mmol) of *p*-nitrophenyl 4-chloro-2-butynoate was gently warmed until the solid dissolved. The clear solution was kept at rt for 4 days, and the precipitate that formed was filtered off and washed with THF and ether: yield 0.464 g (74%); mp 142 °C; R_f 0.43 in solvent A, revealed in 254 but not in 366-nm light; IR 1755 (broad, acetyl CO), 1665 cm^{-1} (lactam CO); UV (water) λ_{max} nm (ϵ) 240 (30 750), 297 (11 950); ^1H NMR [250 MHz, $(CD_3)_2SO$] δ 2.04, 2.07, 2.13 (each s, 3 H, CH_3), 4.27 (m, 1 H) and 4.43 (m, 2 H) ($H_{4'}$ and 5′-CH_2), 5.26 (s, 2 H, CH_2Cl), 5.61 (t, J = 5.5 Hz, 1 H, $H_{3'}$), 5.95 (t, J = 5.5 Hz, 1 H, $H_{2'}$), 6.33 (d, J = 5 Hz, 1 H, $H_{1'}$), 6.69 (s, 1 H, H_8), 8.61 (s, 1 H, H_2), 9.01 (s, 1 H, H_5); MS m/e (relative intensity) 259 (0.7) and 258 (0.8), triacetylribose, 198 (16) (exact mass 198.0526), ribose – AcOH (base moiety – HCl would be 198.0415), 156 (53), 139 (10), 114 (51), 97 (40), 60 (21), 43 (100), 38 (11), 36 (31). Anal. Calcd for $C_{20}H_{20}N_5O_8Cl$: C, 48.68; H, 4.08; N, 14.18; Cl, 7.18. Found: C, 48.7; H, 4.3; N, 13.8; Cl, 7.2.

(45) Roques, P.; Thomé, F.; Dubord, C.; Olomucki, M. *Biochem. Biophys. Acta* **1989**, *1009*, 99.

1-β-D-Ribofuranosyl-2,6-dihydro-4-(chloromethyl)-2,6-dioxopyrimido[1,2-*c*]pyrimidine (3, R = Ribose). *p*-Nitrophenyl 4-chloro-2-butynoate (156 mg, 0.65 mmol) was added to 243 mg (1 mmol) of cytidine in 3.5 mL of DMF, and the yellow solution was kept at rt for 2 days, during which two other portions of 84 mg (0.35 mmol) of *p*-nitrophenyl 4-chloro-2-butynoate were added. The solution was evaporated in vacuo, the residue was dissolved in methanol, and the pure product (172 mg, 50%) was precipitated in several crops by careful successive additions of ethanol, acetone, and ether: mp about 150 °C dec; R_f 0.48 in solvent A; HPLC, retention volume 15 mL; pK_a = 1; IR 1730 (CO in the additional cycle), 1660 cm^{-1} (cytidine CO); UV (water) λ_{max} nm (ϵ) 249 (20 200), 307 (9600); ^{1}H NMR (JEOL FX 90Q) δ 3.42 (m, 2 H, 5′-CH_2), 3.71 (m, 1 H, $H_{4'}$), 3.99 (m, 1 H, $H_{3'}$), 4.10 (m, 1 H, $H_{2'}$), 5.18 (s, 2 H, CH_2Cl), 5.78 (d, J = 2.4 Hz, 1 H, $H_{1'}$), 6.26 (d, J = 8 Hz, 1 H, H_9), 6.52 (s, 1 H, H_5), 8.19 (d, J = 8 Hz, 1 H, H_{10}); MS *m/e* 175.038, 147.043, 121.028, 120.032, 119.048, 93.033, 38, 36. Anal. Calcd for $C_{13}H_{14}N_3O_6Cl$: C, 45.43; H, 4.11; N, 12.22; Cl, 10.31. Found: C, 45.2; H, 4.1; N, 11.9; Cl, 10.1.

3-(5-Triphospho-2-deoxy-β-D-ribofuranosyl)-7*H*-7-oxo-9-(chloromethyl)pyrimido[1,2-*i*]purine (2f). A solution of 132 mg (1 mmol) of methyl 4-chloro-2-butynoate in 1.5 mL of methanol was added to 98 mg (0.2 mmol) of dATP in 1.5 mL of water. The solution was brought to pH 7 with 1 N sodium hydroxide and kept at this pH at rt, another 1-mmol portion of methyl 4-chloro-2-butynoate in 0.3 mL of methanol being added after 3 days. TLC in solvent B showed that dATP practically disappeared after 8 days. Methanol was evaporated in vacuo, and the remaining solution was extracted four times with chloroform and chromatographied with solvent B on a 3- × 45-cm Whatmann CF 11 cellulose column to afford 60 mg (45%) of product: mp 160 °C dec; R_f 0.22 in solvent B; IR 1705 cm^{-1}; UV (water) λ_{max} nm (ϵ) 236 (11 100), 307 (6700), 346 (12 000); ^{1}H NMR (D_2O) δ 2.5 and 2.7 (each m, 1 H, $H_{2'}$), 3.95 (m, 2 H, 5′-CH_2), 4.20 (m, 1 H, $H_{4'}$), 4.70 (s, 2 H, CH_2Cl), 6.48 (t, 1 H, $H_{1'}$), 6.54 (s, 1 H, H_8), 8.76 (s, 1 H, H_2), 9.48 (s, 1 H, H_5). Anal. Calcd for $C_{14}H_{14}N_5O_{13}PCl{\cdot}3NH_4{\cdot}H_2O$: C, 25.44; H, 4.26; N, 16.95. Found: C, 25.1; H, 4.0; N, 16.4.

Reaction of Compounds 1a and 2a with Benzyl Mercaptan. The reaction was followed by ^{1}H NMR using the DMSO peak at δ 2.49 as reference. Samples of nucleosides **1a** or **2a** (10 mg, 0.027 mmol) were dissolved in 0.5 mL of $(CD_3)_2SO$ (dissolution of product **1a** was made by 0.5 h shaking at 60 °C). One equivalent of benzyl mercaptan was introduced in the solution by means of a Hamilton microsyringe. The molar ratio of reagents was controlled by integration of the spectra of the mixture.

3-β-D-Ribofuranosyl-7*H*-7-oxo-9-[(benzylthio)methyl]pyrimido[1,2-*i*]purine (2g). A solution of benzyl mercaptan (70 mg, 0.6 mmol) in 1.4 mL of DMSO was added to a solution of compound **2a** (200 mg, 0.54 mmol) in 10 mL of DMSO. One equivalent of 0.1 N sodium hydroxide was then introduced dropwise in 2 h under nitrogen. The reaction was followed by TLC in solvent C. The new product, R_f 0.62, showed a fluorescent blue-green spot in 366-nm light. At the end of the reaction the solvent was evaporated under 0.1 mmHg, and the residual oil was extracted with 30 mL of hot ethanol. The extract was chilled to 4 °C; after 2 h a first precipitate was collected by centrifugation, and one-third of the supernatant was evaporated and chilled, giving a second crop of solid. An additional amount of product was obtained on standing at 4 °C after addition of 60 mL of petroleum ether (bp 40–70 °C). The overall yield of pure compound was 88 mg (35%): mp 115 °C after two recrystallizations in water; UV (H_2O, 5% DMSO) λ_{max} nm (ϵ) 255 (10 000), 300 (7100), 350 (9800), 364 (9500); ^{1}H NMR δ 3.65 (m, 4 H, 5′-CH_2 and CH_2S), 3.80 (s, 2 H, CH_2S), 4.15 (t, 1 H, $H_{3'}$), 4.52 (t, J = 5.5 Hz, 1 H, $H_{2'}$), 6.10 (d, J = 6 Hz, 1 H, $H_{1'}$), 6.35 (s, 1 H, H_8), 7.23 (m, 5 H, phenyl), 8.66 (s, 1 H, H_2), 9.45 (s, 1 H, H_5); MS *m/e* 323, 246, 201, 173, 124, 119, 91, 78, 77, 44. Anal. Calcd for $C_{21}H_{21}N_5O_5S{\cdot}1.5H_2O$: C, 52.27; H, 5.01; N, 14.51. Found: C, 52.7; H, 4.7; N, 14.2.

Reaction of Compound 1a with Benzyl Mercaptan. The same procedure applied to the isomer **1a** gave 117 mg (87%) of a homogeneous nonfluorescent product, R_f 0.4 in solvent C, mp 113–120 °C. When this product was left in solution, it converted to a compound chromatographically identical with **2g**. A small sample of the nonfluorescent product **1g** could be isolated. It showed the following spectral characteristics: ^{1}H NMR δ 3.65 (m, 4 H, 5′-CH_2 and CH_2S), 3.70 (s, 2 H, CH_2S), 4.15 (t, 1 H, $H_{3'}$), 4.52 (t, 1 H, $H_{2'}$), 5.95 (d, J = 6 Hz, 1 H, $H_{1'}$), 6.39 (s, 1 H, H_8), 7.10 (m, 5 H, phenyl), 8.55 (s, 1 H, H_2), 8.97 (s, 1 H, H_5); MS *m/e* 323, 246, 233, 201, 173, 119, 91, 78.

3-β-D-Ribofuranosyl-7*H*-7-oxo-9-(iodomethyl)pyrimido[1,2-*i*]purine (2h). Compound **2a** (1.24 g, 3.36 mmol) was dissolved in 1 h in 110 mL of boiling acetone. Sodium iodide (0.76 g, 5.07 mmol) was added, and the solution was refluxed for 45 min. After cooling to 4 °C, the sodium chloride was filtered off, and the filtrate was concentrated to 40 mL and kept at 4 °C overnight. The precipitated solid was filtered and washed with acetone; the filtrate gave an additional amount of product on standing. The overall yield of analytically pure product, mp about 160 °C dec, was 1.54 g (90%). In TLC it gave a blue fluorescent spot when visualized under 366 nm and showed the same R_f in solvents A–D as the chloromethyl analogue: IR 1705 cm^{-1} (CO); UV (water) λ_{max} nm (ϵ) 236 (25 400), 300 (9900); ^{1}H NMR δ 3.62 (m, 2 H, 5′-CH_2), 3.96 (q, 1 H, $H_{4'}$), 4.14 (t, 1 H, $H_{3'}$), 4.47 (s, 2 H, CH_2I), 4.52 (t, 1 H, $H_{2'}$), 6.06 (d, J = 5.5 Hz, 1 H, $H_{1'}$), 6.59 (s, 1 H, H_8), 8.76 (s, 1 H, H_2), 9.50 (s, 1 H, H_5). Anal. Calcd for $C_{14}H_{14}N_5O_5I$: C, 36.62; H, 3.07; N, 15.25. Found: C, 36.7; H, 3.1; N, 15.1.

6-(Chloromethyl)-2-(1-β-D-ribofuranosyl-5-amino-4-imidazolyl)-4*H*-4-oxopyrimidine (5a). (a) By Reaction of Adenosine with Methyl Chlorotetrolate. A solution of methyl chlorotetrolate (2 g, 15 mmol) in 40 mL of methanol was added to a solution of adenosine (2 g, 7.5 mmol) in 40 mL of water, and the mixture was refluxed until TLC in solvent A showed no further evolution (10 h). The solution was cooled, and the precipitate (0.9 g, mp 152 °C) was filtered, washed, and recrystallized from water, yielding 0.825 g (30%) of product: mp 208 °C dec; R_f 0.51 in solvent A, 0.57 in solvent B; IR 1650 cm^{-1} (CO); UV (water) λ_{max} nm (ϵ) 220 (13 100), 240 (6000), 331 (15 500); ^{1}H NMR [$(CD_3)_2SO$] δ 3.60 (br s, 2 H, 5′-CH_2), 4.0 (br s, 1 H, $H_{3'}$), 4.32 (br s, 1 H, $H_{2'}$), 4.51 (s, 1 H, CH_2Cl), 5.22 (m, 1 H, exchangeable, $OH_{5'}$), 5.43 (q, 2 H, exchangeable, $OH_{2'}$), 5.54 (d, J = 6.6 Hz, 1 H, $H_{1'}$), 6.00 (s, 1 H, H_5), 6.85 (s, 2 H, exchangeable, NH_2), 7.52 (s, 1 H, imidazole CH), 11.1 (s, 1 H, exchangeable, NH); MS *m/e* 225, 133, 114, 38, 36. Anal. Calcd for $C_{13}H_{16}N_5O_5Cl{\cdot}0.5H_2O$: C, 42.57; H, 4.67; N, 19.09. Found: C, 42.4; H, 4.4; N, 18.7.

(b) By Hydrolysis of 2a with Methanolic Ammonia. Ammonium hydroxide (25%, 1.8 mL) was added to a solution of 0.1 g of compound **2a** in 10 mL of methanol. After one night at rt the precipitated solid was collected, washed with water and methanol, and dried. Yield 0.07 g (71%) of product identical (TLC, IR, UV, RMN) with the one described above under (a).

2-(1-β-D-Ribofuranosyl-5-amino-4-imidazolyl)-4*H*-4-oxopyrimidine (5b). A solution of adenosine (1.068 g, 4 mmol) and ethyl propiolate (0.784 g, 8 mmol) in 60 mL of 33% aqueous ethanol was heated under reflux for 75 h. The pH was periodically adjusted to 7 with 1 N sodium hydroxide. The fine yellow needles which precipitated at rt were filtered and washed twice with ethanol. The product, 0.071 g, had mp 128 °C. Recrystallization from ethanol raised the mp to 132–133 °C (for 1,3,5-tris(ethoxycarbonyl)benzene, lit.[46] mp 133–134 °C): IR 3025, 1730, 1610, 1480, 1250, 865, 725 cm^{-1}; ^{1}H NMR δ 1.4 (t, 9 H, 3 CH_3), 4.4 (q, 6 H, 3 CH_2), 8.6 (s, 3 H, aromatic). Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.22; H, 6.16. Found: C, 60.9; H, 6.2.

The filtrate was concentrated in vacuo to half of its volume and extracted with chloroform. Further concentration to a small volume, followed by precipitation with acetone, yielded 1.010 g (90%) of essentially pure **5b**. Recrystallization from DMF–methanol afforded pure product: mp 197–198 °C; R_f 0.21 in solvent A; IR 1677 cm^{-1}; UV (water) λ_{max} nm (ϵ) 214 (17 100), 240 (10 400), 318 (16 700); ^{1}H NMR [$(CD_3)_2SO$] δ 3.60 (d, J = 2.9 Hz, 2 H, 5′-CH_2), 4.00 (m, 2 H, $H_{3'}$ and $H_{4'}$), 4.31 (m, 1 H, $H_{2'}$), 5.34 (br s, 3 H, exchangeable, OH), 5.55 (d, J = 6.5 Hz, 1 H, $H_{1'}$), 5.89 (d, J = 6.6 Hz, 1 H, H_5), 6.70 (m, 2 H, exchangeable, NH_2), 7.54 (s, 1 H, imidazole CH), 7.84 (d, J = 6.6 Hz, 1 H, H_6), 12.0 (m, 1 H, exchangeable, NH); ^{13}C NMR 61.3 ($C_{5'}$), 70.5, 73.0 ($C_{2'}$ and $C_{3'}$), 85.7 ($C_{4'}$), 87.8 ($C_{1'}$), 108.3 (pyrimidone C_5), 110.0 (imidazole

(46) Karabinos, J. V.; Wright, J. I.; Hipsher, H. F. *J. Am. Chem. Soc.* **1946**, *68*, 906.

C_5), 131.0 (imidazole CH), 144.1, 155.1, 161.2 (pyrimidone C_2 and C_4 and imidazole C_4), 154.8 (pyrimidone C_6); MS *m/e* (relative intensity) 309 (6), M^+, 177 (90), M – ribose. Anal. Calcd for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.65. Found: C, 46.5; H, 5.0; N, 22.65.

The same product was neatly obtained when compound **1b** was heated to 80 °C in water for 48 h.

Determination of Ionization Constants of Modified Nucleosides. The spectrophotometric method described by Albert and Sergeant[47] was used. Standard 10^{-3} M solutions of nucleosides were made in 10^{-2} M borate, phosphate, or acetate buffers which have very low UV absorption.

Acknowledgment. We are indebted to Drs. J. C. Brochon and F. Merola for the determination of the fluorescence properties of compound **2a**.

(47) Albert, A.; Sergeant, E. P. *The Determination of Ionization Constants*; Chapman and Hall Ltd.: New York, 1971.

Notes

One-Step Preparation of Hydrazinium Nitrates from Tertiary Amines and Azaarenes with $H_2NOSO_3H/Ba(NO_3)_2/BaO$ and Conversion to Energetic Amine–Nitroimides[1]

George A. Olah,* Mark B. Sassaman, Miljenko Zuanic, Chandra B. Rao, and G. K. Surya Prakash*

Donald P. and Katherine B. Loker Hydrocarbon Research Institute and Department of Chemistry, University of Southern California, Los Angeles, California 90089-1661

Richard Gilardi,* Judith Flippen-Anderson, and Clifford George

Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375-5000

Received December 20, 1990 (Revised Manuscript Received June 18, 1991)

Hydrazine derivatives, in which one of the nitrogens is quaternized, have been known since 1876, when Emil Fischer first described the isolation of a water-soluble, crystalline material from a complex mixture of products obtained by the reaction of phenylhydrazine with ethyl bromide (eq 1).[2] Since then analogous compounds were prepared with the exclusion of unwanted side products by treating 1,1-disubstituted hydrazines with alkyl halides (eq 2).[3-5]

$$PhNHNH_2 \xrightarrow{EtBr} PhN^+(Et)_2NH_2\ Br^- + \text{other products} \quad (1)$$

$$R_2NNH_2 \xrightarrow{R'X} R_2N^+(R')NH_2\ X^- \quad (2)$$

Hydrazinium salts have also been obtained by the reactions of pyridine with sulfonyl azides;[6] chloramine with tertiary amines;[7] hydroxylamine-*O*-sulfonic acid with tertiary and aromatic heterocyclic amines;[8] by rearrangement of diazepinones;[9] and by the reaction of hydrazine with pyridinones.[10] More recently Tartakovskii et al. have prepared nitrohydrazine and their imido salts by a desilylative nitration procedure.[11a]

The chemistry of these compounds has not been well-studied, although an excellent review has been published.[11b] Relevant to hydrazinium salts is the formation of highly stable, covalently bonded "ylide" or "betaine" compounds, in which the negative charge is stabilized by both Coulombic attraction and by delocalization into an adjacent electron-withdrawing group, referred to as **amine–imides** (zwitterionic structures A) and are pre-

$$\underset{A}{R_3\overset{+}{N}-\overset{-}{N}-Z} \qquad Z = \text{aryl, acyl, sulfonyl, nitro}$$

pared directly from the corresponding hydrazinium salts. Such compounds derived from azaarenes function as 1,3-dipoles in cycloadditions[12] and, in addition, undergo the types of reactions described for the aliphatic series.[13a]

The amine–nitroimides, where $Z = NO_2$, were introduced as a new class of compounds by Katritzky in 1969[13b] and studied in an impressive body of work.[13a-f] The amine–nitroimides were prepared from hydrazinium nitrates in acetic acid–acetic anhydride–nitric acid, trifluoroacetic acid–trifluoroacetic anhydride, or by treatment with nitronium tetrafluoroborate in acetonitrile.[13c] The hydrazinium nitrates, in turn, were obtained from the halides by metathesis with silver nitrate.[7] In all, a three-step process was required to prepare the nitrates and a fourth step used to obtain the amine–nitroimides.

In continuation of our studies on the preparation of nitro compounds we undertook a study of the highly energetic but stable amine–nitroimides, with the goal to find simplified, less costly route (without silver salts) to starting hydrazinium nitrates. These compounds are subsequently

(1) Considered Synthetic Methods and Reactions Part 165. For Part 164, see: Olah, G. A.; Ramos, M. T.; Wang, Q.; Prakash, G. K. S. *Synlett* **1991**, 41

(2) Fischer, E. *Ber.* **1876**, *9*, 880.

(3) Fischer, E. *Ann.* **1879**, *199*, 281.

(4) Knorr, L. *Ann.* **1883**, *221*, 297.

(5) Schneider, W.; Seebach, F. *Ber.* **1921**, *54*, 2285.

(6) (a) Ashley, J. N.; Buchanan, G. L.; Easson, A. P. T. *J. Chem. Soc.* **1947**, 60. (b) Buchanan, G. L.; Levine, R. M. *J. Chem. Soc.* **1950**, 2248.

(7) Omietanski, G. L.; Sisler, H. H. *J. Am. Chem. Soc.* **1956**, *78*, 1211.

(8) (a) Meusen, A.; Gösl, R. *Angew. Chem.* **1957**, *69*, 754. (b) Gösl, R.; Meusen, A. *Ber.* **1959**, *92*, 2521. (c) Gösl, R.; Meusen, A. *Org. Synth.* **1963**, *43*, 1.

(9) Moore, J. A.; Binkert, J. *J. Am. Chem. Soc.* **1959**, *81*, 6045.

(10) El-Kholy, I. E.; Rafla, F. K. *J. Chem. Soc. C* **1969**, 974.

(11) (a) Sisler, H. H.; Omietanski, G. M.; Rudner, B. *Chem. Rev.* **1957**, *57*, 1021. (b) Tartakovskii, V. A.; Ioffe, S. L.; Kalnin, A. V.; Apazov, E. T.; Strelenso, Y. N. *Mendeleev Commun.* **1991**, 95.

(12) (a) For a review of 1,3-dipolar cycloadditions, see: Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565. (b) Beyer, H.; Thieme, E. *J. Prakt. Chem.* **1966**, *31*, 293.

(13) (a) Epsztajn, J.; Lunt, E.; Katritzky, A. R. *Tetrahedron* **1970**, *26*, 1665. (b) Epsztajn, J.; Katritzky, A. R. *Tetrahedron Lett.* **1969**, 4739. (c) Epsztajn, J.; Katritzky, A. R.; Lunt, E.; Mitchell, J. W.; Roch, G. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2622. (d) Katritzky, A. R.; Mitchell, J. W. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2624. (e) Arriau, J.; Deschamps, J.; Duke, J. R.; Epsztajn, J.; Katritzky, A. R.; Lunt, E.; Mitchell, J. W.; Rizvi, S. Q. A.; Roch, G. *Tetrahedron Lett.* **1974**, *44*, 3865. (f) Arriau, J.; Dargelos, A.; Epsztajn, J.; Katritzky, A. R.; Lunt, E.; Mitchell, J. W.; Rizvi, S. Q. A.; Roch, G. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1245.

0022-3263/92/1957-1585$03.00/0