Reactions of Ethyl and Phenyl Chloroformate with Adenosine Derivatives as an Entry to N^6-Ureido-Linked Spin-Labeled Adenosine and Other Modified Adenosines

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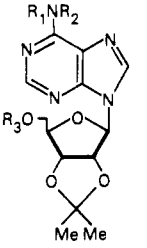
2′,3′,5′-*O*-Triacetyl-N^6,N^6-bis(phenoxycarbonyl)adenosine (**10**) reacted readily with 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy to afford an 87% yield of ureido compound **16**, which was then deacetylated to give spin-labeled adenosine derivative **19** in 56% yield. Adenosine derivatives **24–27** were prepared from **10** in a similar manner. Treatment of 2′,3′-*O*-isopropylideneadenosine (**2**) with phenyl chloroformate gave $O^{5'}$,8-cycyloadenosines **13** and **14**; structure **13** was assigned on the basis of long-range selective proton-decoupled (LSPD) ^{13}C NMR spectra. $O^{5'}$,8-Cycloadenosine **8** was similarly prepared from **2** and ethyl chloroformate. Reaction of 2′,3′,5′-*O*-triacetyladenosine (**9**) with phenyl chloroformate in the presence of dimethylformamide afforded amidine derivative **12** (74%).

One of the most fruitful approaches to the study of biological membranes and specific sites on proteins has been the spin-label method.[2,3] The central role in biochemistry that is played by adenosine and its nucleotides has led to considerable interest in the synthesis of their spin-labeled analogues, and it is now possible to attach stable nitroxide free-radicals to various positions in these key biochemicals, namely, at C-2,[4,5] C-6,[6] C-8,[5,7] N^6,[5,8-10] 3′-O,[11] and the terminal phosphate.[12] Despite this progress, further work is needed; some of these syntheses are relatively difficult, and overall yields of the spin-labeled materials are frequently quite low. Moreover, since the site and nature of substituents in modified adenosine and ATP analogues are known[13,14] to markedly influence the behavior of such compounds, it is desirable to have access to a relatively wide assortment of spin-labeled analogues that differ in both the location of the spin label and the nature of the linkage between the "reporter group" and the molecular component of interest. These circumstances, together with our employment of spin-labeled adenosine nucleotides to study glutamine synthetase[15] and platelet membranes,[4] have prompted an investigation of new synthetic methods for conveniently obtaining variously structured spin-labeled nucleosides and nucleotides. The present report deals with the use of a protected N^6,N^6-bis(phenoxycarbonyl)adenosine derivative for obtaining, relatively easily, a prototype spin-labeled adenosine analogue having an N^6-ureido linkage to the 4-position of the 2,2,6,6-tetramethylpiperidinyl-1-oxy radical. Several other modified forms of adenosine obtained during the course of this synthesis are also reported herein.

Results and Discussion

Previously reported studies have established that N^6-ureido derivatives of adenosine are accessible by reaction of amino compounds with either N^6-(ethoxycarbonyl)-2′,3′,5′-*O*-triacetyladenosine[16a] or N^6,N^6-bis(phenoxycarbonyl)adenosine[16b] and that the latter substance is more reactive toward nucleophilic substitution. To extend this chemistry to the synthesis of an N^6-linked spin-labeled adenosine analogue, we initially investigated the reactions of 5′-*O*-acetyl-2′,3′-*O*-isopropylideneadenosine (**1**) and



1, R_1=R_2=H, R_3=Ac
2, R_1=R_2=R_3=H
3, R_1=R_2=EtOC(O), R_3=Ac
4, R_1=H, R_2=EtOC(O), R_3=Ac
5, R_1=R_2=R_3= EtOC(O)
6, R_1=H, R_2=R_3=EtOC(O)
7, R_1=R_2=H, R_3=EtOC(O)
15, R_1=H, R_2=CHO, R_3=PhOC(O)

2′,3′-*O*-isopropylideneadenosine (**2**) with ethyl chloroformate. Partial reaction (62%) of **1** with EtOC(O)Cl in pyridine led to the isolation of bis- and monoethoxycarbonylated derivatives **3** (17%) and **4** (29%), respectively. The analogous reaction (70%) of **2** afforded derivatives **5** (4%), **6** (9%), and **7** (14%), as well as material having an elemental composition, a mass spectral parent ion, and NMR (^1H/^{13}C) spectral parameters (vide infra) that were consistent with $O^{5'}$,8-cycloadenosine structure **8** (5%), which has heterotopic EtOC(O) substituents. The

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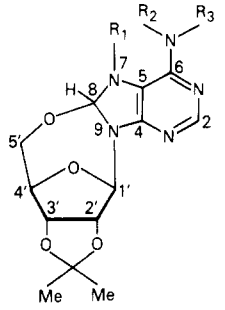
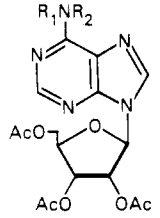


8, $R_1=R_2=EtOC(O)$, $R_3=H$
13, $R_1=R_2=R_3=PhOC(O)$
14, $R_1=R_2=PhOC(O)$, $R_3=H$

formation of **8** can be rationalized by extension of the findings reported by Leonard and co-workers.[17]

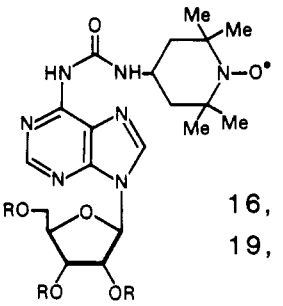
Reactions of thermally labile[4] 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxyl with bis(ethoxycarbonyl) derivatives **3** and **8** required forcing conditions (refluxing PrOH) and led to intractable product mixtures. Consequently, a more reactive precursor, **10**,[16b] was prepared in good yield



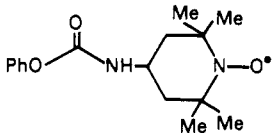
9, $R_1=R_2=H$
10, $R_1=R_2=PhOC(O)$
11, $R_1=H$, $R_2=CHO$
12, $R_1=:$, $R_2=Me_2NCH$

(74%) from 2′,3′,5′-*O*-triacetyladenosine (**9**) and PhOC(O)Cl in 2:1 pyridine/dimethylformamide (DMF). The DMF, which was used to solubilize **9**, also led to the formation of two minor byproducts that were identified as N^6-formylated material **11** (8%) and amidine **12** (5%) on the basis of their respective elemental analyses, mass spectral parent ions, and ^1H NMR spectra. Formation of **12**, which has precedence in chemistry reported by Zemlička and Owens,[18] was the primary event in DMF as solvent without pyridine: 74% **12** and 2% **11**. In view of these results and on consideration of the above findings for the reaction of **2** with EtOC(O)Cl, it was not surprising that treatment of **2** with PhOC(O)Cl in pyridine–DMF gave products having elemental compositions and NMR spectra that were consistent with structures **13** (6%), **14** (29%), and **15** (17%). The ^{13}C–^1H scalar couplings in **13** that operate through ≥2 bonds were determined from 400-MHz ^1H long-range selective proton-decoupled (LSPD[19]) ^{13}C NMR spectra. The magnitudes of these long-range coupling constants (Table I), especially those for C-2 and C-8, were in accord with an $O^{5'}$,8-cycloadenosine structure and were inconsistent with an alternative $O^{5'}$,2-cycloadenosine framework. Product **13** was thus used as the structural "anchor" compound for the connectivities assigned to products **8** and **14**, since **8**, **13**, and **14** gave rise to virtually identical D-ribofuranosyl, C-2, and C-8 $^{13}C\{^1H\}$ NMR signals (Table II). In addition, compounds **8**, **13**, and **14** gave rise to ^1H NMR signals for H-2 and H-8 in the range of δ 8.40–8.43 and 6.43–6.68, respectively.

As expected, the reaction of 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxyl with diphenoxy derivative **10** proceeded smoothly at room temperature to afford spin-labeled ureido compound **16** (87%), which was separated



16, R=Ac
19, R=H



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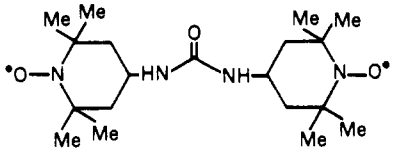

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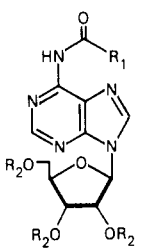
from byproducts **17** and **18** by column chromatography. Deacetylation of **16** with aqueous $NaHCO_3$ gave the desired, spin-labeled, adenosine analogue **19** (56%). The overall yield of **19** from starting material **9** was 36%. The scope of the substitution chemistry for **10** was briefly examined with several other amino compounds. 4-Amino-2,2,6,6-tetramethylpiperidine and propylamine likewise reacted with **10** at room temperature to give **20** and **21**, respectively, whereas 1,1-dimethylhydrazine and L-alanine



20, R_1=2,2,6,6-tetramethylpiperidine-4-yl, R_2=Ac
21, R_1=PrNH, R_2=Ac
22, $R_1=Me_2NNH$, R_2=Ac
23, R_1=EtOC(O)C(Me)HNH, R_2=Ac
24, R_1=2,2,6,6-tetramethylpiperidine-4-yl, R_2=H
25, R_1=PrNH, R_2=H
26, $R_1=Me_2NNH$, R_2=H
27, R_1=EtOC(O)C(Me)HNH, R_2=H

ethyl ester required more vigorous reaction conditions (refluxing $CHCl_3$, 7–20 h) to produce, respectively, **22** and **23**. The acetyl protecting groups in **20–23** were easily removed with aqueous $NaHCO_3$ to give the corresponding ureido nucleosides **24–27**.

Conclusions

The conversion of readily available 2′,3′,5′-*O*-triacetyladenosine (**9**) into bis(phenoxycarbonyl) derivative **10** represented a convenient entry to the synthesis of various N^6-ureido analogues of adenosine, which included the novel spin-labeled structure **19**. The reaction conditions needed for introduction of the ureido linkage were markedly dependent on the nature of the amine reagent and may therefore pose problems for unusually labile amines. Biochemical studies with **19** and related spin-labeled nucleoside/nucleotide analogues will be reported elsewhere.

Experimental Section

Elemental analyses were performed by Galbraith Laboratories, Inc. Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Unless specified otherwise, ^1H NMR refers to 60 MHz spectra obtained with a Varian EM-360A spectrometer by using $CDCl_3$ solutions and

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Table I. Long-Range ^{13}C-^1H Scalar Coupling Constants (J) for $O^{5'}$,8-Cycloadenosine 13[a]

^{13}C position	^1H position	J, Hz
4	1′	<1
	2	11.0
	8	4.9
5	2	≤2
	8	2.4
6	2	13.4
8[b]	1′	4.9
	$5_a'$	6.1
	$5_b'$	8.5
5′	4′	≤2

[a] Determined from 400-MHz ^1H long-range selective proton-decoupled (LSPD) ^{13}C NMR spectra recorded in $CDCl_3/C_6D_6$ at ca. 20 °C. See structure 13 for the numbering of the ^{13}C and ^1H positions. [b] Selective low-power irradiation of H-1′ gave a doubled "triplet" for C-8 due to the residual coupling with H-8 and the approximately equal ^3J values with H-$5_a'$ and H-$5_b'$, which was confirmed by simultaneous low-power irradiation of H-1′, H-$5_a'$, and H-$5_b'$ (doublet for C-8).

Table II. ^{13}C NMR Chemical Shifts for the D-Ribofuranosyl, C-2, and C-8 Positions in $O^{5'}$,8-Cycloadenosines 8, 13, and 14[a]

	shift,[b] δ		
position	8	13	14
C-1′	91.84	92.32	91.88
C-2′	88.82	89.77	88.95
C-3′	85.06	85.75	85.15
C-4′	82.24	82.83	82.26
C-5′	69.84	70.47	70.12
C-2	154.24	154.72	154.47
C-8	97.76	98.88	97.97

[a] See structures 8, 13, and 14 for the numbering of the ^{13}C positions. [b] Measured in $CDCl_3$ at ca. 20 °C with Me_4Si as an internal reference; assignments were based on long-range selective proton-decoupled (LSPD) ^{13}C spectra recorded for 13 (cf. Table I). The $C(CH_3)_2$ moiety in each compound gave signals at δ ~114, 26, and 25.

internal Me_4Si for chemical shift (δ) measurement. *^1H NMR data are reported only for compounds having relatively unusual structures, although all materials without a spin label exhibited NMR spectra in accord with their assigned structure.* ^{13}C NMR spectra at 25 and 75 MHz were recorded as previously described;[20] the LSPD method reported by Uzawa and co-workers[19] was employed without modifications by using a JEOL 400-MHz spectrometer. UV spectra were recorded with a Cary 15 instrument. TLC plates were coated with a 250-μm layer of silica gel GF, and Baker 60–200-mesh silica gel was used for column chromatography. Chromatographic solvent compositions are reported as volume/volume ratios; "4:1 to 1:1" indicates a gradient elution. The word "evaporation" refers to rotary evaporation under reduced pressure (water aspirator), and "chilled" refers to use of an ice–water bath.

5′-*O*-Acetyl-N^6,N^6-bis(ethoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (3) and 5′-*O*-Acetyl-N^6-(ethoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (4). To a chilled solution of 1 (450 mg, 1.29 mmol) in pyridine (10 mL) was added EtOC(O)Cl (1 g, 9.2 mmol), and the mixture was stirred overnight at room temperature. Unreacted EtOC(O)Cl was quenched with an aqueous solution of $NaHCO_3$. The pyridine was removed by evaporation, and the products were extracted with EtOAc. TLC showed the presence of three UV-absorbing compounds that were then separated by column chromatography. The least polar compound was eluted with 1:1 C_6H_6–EtOAc and was identified as bisethoxycarbonylated product 3: yield 110 mg (17%); syrup from C_6H_6–pentane; mass spectrum, m/z 493 (M^+).

Anal. Calcd for $C_{21}H_{27}N_5O_9$: C, 51.11; H, 5.50; N, 14.20. Found: C, 51.40; H, 5.63; N, 14.10.

Monoethoxycarbonylated product 4 was then eluted with EtOAc: yield, 155 mg (29%); syrup from C_6H_6–pentane.

Anal. Calcd for $C_{18}H_{23}N_5O_7$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.96; H, 5.68; N, 16.39.

The most polar compound was then eluted with 9:1 EtOAc–MeOH and was identical with starting material 1 (173 mg, 38%).

Reaction of 2′,3′-*O*-Isopropylideneadenosine (2) with Ethyl Chloroformate. EtOC(O)Cl (4 g, 37 mmol) was added to a chilled solution of 2 (1.5 g, 4.9 mmol) in pyridine (60 mL), and the reaction mixture was stirred at room temperature overnight. The pyridine was removed by evaporation, and the residue was distributed between $CHCl_3$ and an aqueous solution of $NaHCO_3$. TLC of the organic layer indicated five UV-absorbing compounds that were then separated by column chromatography with 2:1 to 1:1 C_6H_6–EtOAc followed by EtOAc and then 9:1 to 4:1 EtOAc–MeOH. The products (in their order of elution) were as follows.

***N^6,N^6,5′-O*-Tris(ethoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (5)**: yield 101 mg (4%); mp 170–172 °C (C_6H_6–pentane).

Anal. Calcd for $C_{22}H_{29}N_5O_{10}$: C, 50.47; H, 5.58; N, 13.38. Found: C, 50.41; H, 5.74; N, 13.38.

Diethyl 2′,3′-*O*-isopropylidene-*O*$^{5'}$,8-cycloadenosine-*N*,7-(8*H*)-dicarboxylate (8):[21] yield 105 mg (5%); mp 185–189 °C (EtOAc–pentane); faint UV absorption (TLC); mass spectrum, m/z 451 (M^+); ^1H NMR δ 1.32 (t, J = 7 Hz, 2 CH_3CH_2), 1.30 and 1.47 (2 s, isopropylidene methyls), 3.85 (m, 2 H, H-5′), 4.22 (q, J = 7 Hz, CH_3CH_2), 4.35 (q, J = 7 Hz, CH_3CH_2), 4.5–4.7 (m, 2 H, H-2′ and H-3′), 5.80 (s, 1 H, H-1′), 6.43 (s, 1 H, H-8), 8.40 (s, 1 H, H-2). ^{13}C NMR (25 MHz) δ 14.24 and 14.46 (2 CH_3CH_2O), 61.53 and 64.04 (2 CH_3CH_2O); for other resonances, see Table II.

Anal. Calcd for $C_{19}H_{25}N_5O_8 \cdot H_2O$: C, 48.61; H, 5.80; N, 14.92. Found: C, 48.99; H, 5.91; N, 15.24.

***N^6,O*$^{5'}$-Bis(ethoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (6)**: yield 209 mg (9%); glassy solid from C_6H_6–pentane; mass spectrum, m/z 451 (M^+); ^1H NMR δ 6.20 (d, 1 H, J = 3 Hz, H-1′), 8.15 and 8.75 (2 s, H-2 and H-8).

Anal. Calcd for $C_{19}H_{25}N_5O_8$: C, 50.55; H, 5.58; N, 15.52. Found: C, 50.41; H, 5.17; N, 15.31.

***O*$^{5'}$-(Ethoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (7)**: yield 253 mg (14%); mp 111–113 °C (toluene); UV spectrum similar to that of adenosine, λ_{max} 260 nm (MeOH).

Anal. Calcd for $C_{16}H_{21}N_5O_6$: C, 50.65; H, 5.58; N, 18.46. Found: C, 50.45; H, 5.64; N, 18.59.

The last compound that eluted was identical with starting material 2 (450 mg, 30%).

Reaction of 2′,3′,5′-*O*-Triacetyladenosine (9) with Phenyl Chloroformate in Pyridine–DMF. A solution of 9 (9.43 g, 24 mmol) and PhOC(O)Cl (12 g, 77 mmol) in a mixture of pyridine (120 mL) and DMF (60 mL) was stirred at room temperature overnight. The solvent was removed by evaporation, and the resultant material was distributed between $CHCl_3$ and 0.5 N $NaHCO_3$. The organic layer was washed with water and concentrated, and the resultant material was subjected to column chromatography. The main product, **N^6,N^6-bis(phenoxycarbonyl)-2′,3′,5′-*O*-triacetyladenosine (10)**,[16b] was eluted with 4:1 C_6H_6–EtOAc: yield 11.3 g (74%); mass spectrum, m/z 540 ($M^+ - C_6H_5O$); ^{13}C NMR (25 MHz) δ 20.47 and 20.74 (overlapping acetyl methyls, 2:1 intensity ratio), 62.97 (C-5′), 70.49, 73.13, and 80.56 (C-2′, C-3′ and C-4′, specific resonances were not assigned), 86.79 (C-1′), 121.30 (2 ortho C), 126.44 (1 para C), 129.43 (2 meta C), 144.00 (C-8), 152.58 (C-2); the remaining carbons were not

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(21) Although not officially sanctioned by either IUPAC or *Chemical Abstracts*, the naming of 8 (and 13 and 14) as an $O^{5'}$,8-cycloadenosine derivative is regarded by *Chemical Abstracts* as an acceptable alternative to the current *Chemical Abstracts* name: ethyl 8-[(ethoxycarbonyl)-amino]-3a,4,13,13a-tetrahydro-2,2-dimethyl-4,13-epoxy-5*H*-1,3-dioxolo-[5,6][1,3]oxazocino[3,2-*e*]purine-7(6a*H*)-carboxylate. The preferred name for 8 in line with the IUPAC rules of organic nomenclature and the *Chemical Abstracts* 1967–71 index period is as follows: (3a*R*,4*R*,13*R*,13a*R*)-7-carboxy-3a,4,6a,7,13,13a-hexahydro-2,2-dimethyl-4,13-epoxy-5*H*-1,3-dioxolo[5,6][1,3]oxazocino[3,2-*e*]purine-8-carbamic acid, diethyl ester.

observed due to their long T_1 relative to the pulse-repetition time.

N^6-Formyl-2′,3′,5′-O-triacetyladenosine (11) was then eluted with 2:1 C_6H_6–EtOAc: yield 720 mg (8%); mp 156 °C (BuOAc); mass spectrum, m/z 422 ($M^+ + 1$); ^1H NMR δ 6.25 (d, 1 H, H-1′), 8.55 and 8.65 (2 s, H-2 and H-8), 9.90 (d, 1 H, J = 10 Hz, NH-CHO, collapsed to a singlet after NH exchange with D_2O).

Anal. Calcd for $C_{17}H_{19}N_5O_8$: C, 48.45; H, 4.76; N, 16.62. Found: C, 48.17; H, 4.97; N, 16.51.

Elution with 19:1 EtOAc–MeOH then afforded starting material **9** (340 mg, 3%). The last component was then eluted with 9:1 EtOAc–MeOH and was identified as **6-(N,N-dimethylamidino)-9-(2′,3′,5′-O-triacetyl-β-D-ribofuranosyl)purine (12)**: yield 340 mg (3%); syrup from EtOAc–pentane; λ_{max} 310 nm (MeOH); ^1H NMR δ 2.06, 2.10, and 2.13 (3 s, 3 acetyls), 3.20 and 3.26 (2 s, dimethylamino), 4.43 (m, 3 H, H-5′ and H-4′), 5.68 (dd, 1 H, H-3′, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 2 Hz), 5.93 (t, 1 H, H-2, $J_{1',2'}$ = 5 Hz, $J_{2',3'}$ = 5 Hz), 6.25 (d, 1 H, H-1′, $J_{1',2'}$ = 5 Hz), 8.00 and 8.53 (2 s, H-2 and H-8), 8.90 (s, 1 H, amidino proton); mass spectrum m/z 448 (M^+), 433 ($M^+ - CH_3$), 405 ($M^+ - CH_3CO$).

Anal. Calcd for $C_{19}H_{24}N_6O_7$: C, 50.89; H, 5.39; H, 18.74. Found: C, 50.36; H, 5.21; N, 18.36.

Reaction of 2′,3′,5′-O-Triacetyladenosine (9) with Phenyl Chloroformate in DMF. A solution of **9** (786 mg, 2 mmol) and PhOC(O)Cl (1 g, 6.4 mmol) in DMF (10 mL) was allowed to stand at room temperature overnight. Pyridine (5 mL) was added, and the solution was then evaporated to dryness. The residue was distributed between an aqueous solution of $NaHCO_3$ and $CHCl_3$, and the separated organic layer was then washed with water. TLC indicated one major product and one minor product, which were then separated by column chromatography (EtOAc). The less polar compound was identical with **11**; yield 20 mg (2%). The more polar compound was identical with amidine **12**; yield 662 mg (74%).

Reaction of 2 with Phenyl Chloroformate in Pyridine–DMF. To a solution of **2** (1.535 g, 5 mmol) in a mixture of pyridine (50 mL) and DMF (25 mL) was added PhOC(O)Cl (3.13 g, 20 mmol), and the mixture was stirred overnight at room temperature. The residue that was obtained by concentration of the reaction mixture was distributed between an aqueous solution of $NaHCO_3$ and $CHCl_3$. The organic layer was washed with water and concentrated, and the product mixture was separated by silica gel chromatography. Elution of diphenyl carbonate (973 mg) with 9:1 C_6H_6–EtOAc was followed by isolation of **triphenyl 2′,3′-O-isopropylidene-O^5′,8-cycloadenosine-N,N,7(8H)-tricarboxylate (13)**: mp 126–130 °C (C_6H_6–pentane); yield 212 mg (6%); ^1H NMR δ 1.32 and 1.52 (2 s, isopropylidene methyls), 3.95 (m, 2 H, H-5′), 4.2–4.7 (m, 3 H, H-2′, H-3′, and H-4′), 5.88 (s, 1 H, H-1′), 6.67 (s, 1 H, H-8), 7.1–7.3 (m, 15 H, aromatic), 8.43 (s, 1 H, H-2). For ^{13}C NMR data, see Tables I and II.

Anal. Calcd for $C_{34}H_{29}N_5O_{10}$: C, 61.16; H, 4.38; N, 10.49. Found: C, 61.42; H, 4.46; N, 10.34.

Diphenyl 2′,3′-O-isopropylidene-O^5′,8-cycloadenosine-N,7(8H)-dicarboxylate (14) was then eluted with 4:1 C_6H_6–EtOAc: yield 790 mg (29%); glassy solid from C_6H_6–pentane; ^1H NMR δ 1.32 and 1.52 (2 s, iospropylidene methyls), 3.95 (m, 2 H, H-5′), 4.5–4.7 (m, 3 H, H-2′, H-3′, and H-4′), 5.58 (s, 1 H, H-1′), 6.68 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 10.0 (br, 1 H, NH, exchangeable with D_2O). For ^{13}C NMR data, see Table II.

Anal. Calcd for $C_{27}H_{25}N_5O_8$: C, 59.23; H, 4.60; N, 12.79. Found: C, 59.28; H, 4.82; N, 12.52.

N^6-Formyl-2′,3′-O-isopropylidene-5′-O-(phenoxycarbonyl)adenosine (15) was then eluted by using 1:1 C_6H_6–EtOAc: yield 378 mg (17%); glassy solid from C_6H_6–pentane; ^1H NMR δ 9.90 (d, 1 H, NH–CHO J = 9 Hz, collapsed to a singlet after NH exchange with D_2O).

Anal. Calcd for $C_{21}H_{21}N_5O_7$: C, 55.76; H, 4.45; N, 15.40. Found: C, 55.38; H, 4.65; N, 15.38.

Reaction of 10 with 4-Amino-2,2,6,6-tetramethylpiperidinyl-1-oxy. A solution of **10** (223 mg, 0.35 mmol) and the free radical (172 mg, 1 mmol) in $CHCl_3$ (10 mL) was allowed to stand at room temperature overnight. The concentrated reaction mixture was then chromatographed on silica gel. **4-[(Phenoxycarbonyl)amino]-2,2,6,6-tetramethylpiperidinyl-1-oxy (17)** was eluted with 2:1 C_6H_6–EtOAc: yield 70 mg; mp 166–167 °C dec (EtOAc–pentane); mass spectrum, m/z 292 ($M^+ + 1$).

Anal. Calcd for $C_{16}H_{23}N_2O_3$: C, 65.95; H, 7.96; N, 9.62. Found: C, 65.79; H, 8.15; N, 9.31.

4,4′-(Carbonyldiimino)bis(2,2,6,6-tetramethylpiperidinyl-1-oxy) (18) was then eluted with 1:1 C_6H_6–EtOAc: yield, 20 mg; mp 116–118 °C (EtOAc–pentane); mass spectrum, m/z 370 ($M^+ + 2$).

Anal. Calcd for $C_{19}H_{36}N_4O_3$: C, 61.92; H, 9.85; N, 15.21. Found: C, 61.45; H, 9.95; N, 14.92.

2,2,6,6-Tetramethyl-4-[[[[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]amino]carbonyl]amino]-piperidinyl-1-oxy (16) was then eluted with 19:1 EtOAc–MeOH: amorphous powder from EtOAc–pentane; yield 180 mg (87%); mass spectrum, m/z 590 (M^+).

Anal. Calcd for $C_{26}H_{36}N_7O_9$: C, 52.87; H, 6.14; N, 16.60. Found: C, 52.84; H, 6.36; N, 16.24.

2,2,6,6-Tetramethyl-4-[[[[9-(β-D-ribofuranosyl)-9H-purin-6-yl]amino]carbonyl]amino]piperidinyl-1-oxy (19). A solution of **16** (500 mg, 0.85 mmol) in a mixture of MeOH (20 mL) and 0.25 N $NaHCO_3$ (20 mL) was allowed to stand at room temperature for 1 h. Methanol was removed by evaporation, and the aqueous solution was then extracted with $CHCl_3$. Silica gel chromatography (19:1 EtOAc–MeOH) of the extract afforded red fractions that were combined to give analytically pure **19** after crystallization from EtOAc: yield 265 mg (56%); mp 190–191 °C.

Anal. Calcd for $C_{20}H_{30}N_7O_6 \cdot CH_3CO_2C_2H_5$: C, 52.16; H, 6.93; N, 17.75. Found: C, 52.25; H, 6.99; N, 17.99.

N^6-(2,2,6,6-Tetramethylpiperidin-4-yl)-2′,3′,5′-O-triacetyladenosine (20). A solution of **10** (317 mg, 0.5 mmol) and 4-amino-2,2,6,6-tetramethylpiperidine (200 mg, 1.28 mmol) in $CHCl_3$ (10 mL) was allowed to stand at room temperature for 1 h, and the product was then isolated by silica gel chromatography with 9:1 to 4:1 EtOAc–MeOH: yield 210 mg (73%); amorphous powder from EtOAc–pentane; mass spectrum, m/z 575 (M^+). The acetic acid salt, which was obtained as a glassy solid from AcOH–EtOAc–pentane, was used for elemental analysis.

Anal. Calcd for $C_{26}H_{37}N_7O_8 \cdot CH_3CO_2H$: C, 52.90; H, 6.50; N, 15.42. Found: C, 52.69; H, 6.74; N, 15.35.

N^6-(Propylcarbamoyl)-2′,3′,5′-O-triacetyladenosine (21). A solution of **10** (483 mg, 0.76 mmol) and propylamine (100 mg, 1.7 mmol) in $CHCl_3$ (10 mL) was allowed to stand at room temperature for 1 h, and the product was then isolated by silica gel chromatography with 19:1 EtOAc–MeOH: yield 330 mg (91%); glassy solid from EtOAc–pentane.

Anal. Calcd for $C_{20}H_{26}N_6O_8$: C, 50.20; H, 5.48; N, 17.57. Found: C, 49.96; H, 5.31; N, 17.30.

N^6-[(Dimethylamino)carbamoyl]-2′,3′,5′-O-triacetyladenosine (22). A solution of **10** (317 mg, 0.5 mmol) and 1,1-dimethylhydrazine (140 mg, 2.3 mmol) in $CHCl_3$ (10 mL) was refluxed for 20 h. The product was isolated by silica gel chromatography with 9:1 to 4:1 EtOAc–MeOH: mp 76–80 °C (EtOAc–pentane); yield 210 mg (88%).

Anal. Calcd for $C_{19}H_{25}N_7O_8$: C, 47.59; H, 5.26; N, 20.45. Found: C, 47.40; H, 5.30; N, 20.21.

N^6-[[1-(Ethoxycarbonyl)ethyl]carbamoyl]-2′,3′,5′-O-triacetyladenosine (23). A solution of L-alanine ethyl ester hydrochloride (175 mg, 1.14 mmol) in a mixture of Et_3N (0.5 mL) and $CHCl_3$ (20 mL) was refluxed for 7 h. The product was isolated by silica gel chromatography with EtOAc and then 19:1 EtOAc–MeOH: yield 135 mg (50%); glassy solid from EtOAc–pentane.

Anal. Calcd for $C_{22}H_{28}N_6O_{10}$: C, 49.25; H, 5.26; N, 15.67. Found: C, 49.11; H, 5.24; N, 15.67.

N^6-[(2,2,6,6-Tetramethylpiperidin-4-yl)carbamoyl]-adenosine (24). A solution of **20** (100 mg, 0.16 mmol) in a mixture of MeOH (10 mL) and 0.25 N $NaHCO_3$ (10 mL) was allowed to stand at room temperature for 10 min. The MeOH was removed by evaporation, and the product was extracted with $CHCl_3$. The monohydrate of compound **24** was obtained as a glassy solid from EtOAc–pentane; yield 25 mg (35%). The acetic acid salt of **24** was prepared in C_6H_6.

Anal. Calcd for $C_{20}H_{31}N_7O_5 \cdot H_2O$: C, 51.38; H, 7.11; N, 20.97. Found: C, 51.85; H, 6.90; N, 21.00.

Anal. Calcd for $C_{20}H_{31}N_7O_5 \cdot CH_3CO_2H \cdot H_2O$: C, 50.08; H, 7.07; N, 18.58. Found: C, 49.89; H, 6.97; N, 18.46.

N^6-(Propylcarbamoyl)adenosine (25). A solution of **21** (150 mg, 0.31 mmol) in a mixture of MeOH (5 mL) and 0.25 N $NaHCO_3$

(5 mL) was allowed to stand at room temperature for 10 min, and the solution was then concentrated to a final volume of 1 mL. Acetone (30 mL) was added, and the precipitate was removed by suction filtration. The filtrate was taken to dryness, and the product was obtained as a powder from EtOH–C_6H_6: mp 109–111 °C; yield 63 mg (58%).

Anal. Calcd for $C_{14}H_{22}N_6O_6 \cdot H_2O$: C, 45.82; H, 5.60; N, 23.01. Found: C, 45.40; H, 5.99; N, 22.70.

N^6-[(Dimethylamino)carbamoyl]adenosine (26). A solution of **22** (317 mg, 0.5 mmol) in a mixture of MeOH (20 mL) and 0.25 N $NaHCO_3$ (20 mL) was allowed to stand at room temperature for 1 h. The solution was concentrated to a final volume of 5 mL, acetone (50 mL) was added, and the precipitate was then removed by suction filtration. The filtrate was taken to dryness, and the residue was then washed with water that contained a relatively small amount of acetone: yield 89 mg (50%); mp 143 °C (H_2O).

Anal. Calcd for $C_{13}H_{19}N_7O_5 \cdot H_2O$: C, 42.04; H, 5.70; N, 26.40. Found: C, 42.23; H, 5.58; N, 26.51.

N^6-[[1-(Ethoxycarbonyl)ethyl]carbamoyl]adenosine (27). A solution of **23** (410 mg, 0.76 mmol) in a mixture of MeOH (20 mL) and 0.25 N $NaHCO_3$ (20 mL) was allowed to stand at room temperature for 1 h. The solution was concentrated to a final volume of 3 mL, acetone was added, and the precipitate was then removed by suction filtration. The filtrate was taken to dryness, and the residual material was then precipitated from EtOH–EtOAc. A satisfactory elemental analysis could not be obtained: ^{13}C NMR (25 MHz) δ 16.15 (α-CH_3), 19.67 (CH_3CH_2O), 64.20 (CH_3CH_2), 65.41 (C-5′), 73.24, 76.57, and 88.39 (C-2′, C-3′, and C-4′, specific resonances were not assigned), 82.31 (α-C), 91.14 (C-1′), 144.99 (C-8), 152.07 (C-4), 153.48 (C-2), 157.42 [NC(O)N]; the remaining carbons were not observed due to their long T_1 relative to the pulse-repetition rate.

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Registry No. 1, 15888-38-7; 2, 362-75-4; 3, 82838-77-5; 4, 82838-78-6; 5, 82838-79-7; 6, 82838-80-0; 7, 82838-81-1; 8, 82838-82-2; 9, 7387-57-7; 10, 66386-42-3; 11, 82838-83-3; 12, 82838-84-4; 13, 82848-98-4; 14, 82848-99-5; 15, 82838-85-5; 16, 82838-86-6; 17, 82838-87-7; 18, 15180-53-7; 19, 82838-88-8; 20, 82838-89-9; 21, 82838-90-2; 22, 82838-91-3; 23, 82838-92-4; 24, 82838-93-5; 24 AcOH, 82838-94-6; 25, 66781-63-3; 26, 82849-00-1; 27, 82838-95-7; EtOC(O)Cl, 541-41-3; PhOCC(O)Cl, 1885-14-9; 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy, 14691-88-4; 4-amino-2,2,6,6-tetramethylpiperidine, 36768-62-4; propyl amine, 107-10-8; 1,1-dimethylhydrazine, 57-14-7; L-alanine ethyl ester hydrochloride, 1115-59-9.

Photochemistry of (*o*-Methylphenyl)alkenes and the Stereospecific Trapping of the Resulting *o*-Xylylenes[1a]

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The photochemical behavior of a series of *o*-methylstyrenes with simple alkyl groups in the α or β positions was investigated in order to determine the synthetic potential of the resulting *o*-xylylenes. The major photochemical product of all the styrenes employed (**1**, **9**, **10**, and **11**) was the corresponding *o*-xylylene. The *o*-xylylenes were trapped in acceptable yields by maleic anhydride to give the Diels–Alder adducts. In the case of **9** or **10** and **11** the *o*-xylylenes were produced stereoselectively and trapped stereospecifically to give **15** or **16**, respectively. In the absence of a dienophile or in the presence of a weak dienophile, such as cyclohexene, a slower isomerization of the *o*-methylstyrenes to the meta isomers was observed, presumably via a benzvalene intermediate. In addition, the *o*-xylylene produced from **9** or **10** and **11** underwent geometrical isomerization in the absence of maleic anhydride, resulting in the formation of **10** and **11** upon irradiation of **9** and vice versa.

There has been considerable recent interest in the application of *o*-xylylenes (*o*-quinodimethanes) in organic synthesis.[2] These reactive intermediates are excellent dienes for Diels–Alder cycloadditions and allow the construction of six-membered rings fused to benzene rings. The transient *o*-xylylenes have been generated by a number of methods, including the thermolysis of benzocyclobutenes,[2] Vollhardt's method based on the cobalt-catalyzed preparation of the benzocyclobutenes,[2b,3] various 1,4-eliminations,[2b,d–f,h,4] and the photoenolization of *o*-alkylphenyl ketones.[5] Our interest in this area is the synthetic use of *o*-xylylenes generated photochemically from *o*-alkylstyrene derivatives.[6] We have recently demonstrated that several phenyl-substituted *o*-xylylenes generated by this method can be trapped as Diels–Alder adducts in good yields.[7] Our ultimate goal is to use this method to generate *o*-xylylenes from *o*-alkylstyrenes substituted in the α position

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