Simple Models of Nucleic Acid Interactions. 6. Synthesis and Base-Base Interactions of 1-(Adenosin-N^6-yl)-2-(cytidin-N^4-yl)ethane and 1-(Adenosin-N^6-yl)-4-(cytidin-N^4-yl)butane[1a,b]

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Reaction of cytidine (1) with 1,2-diaminoethane in the presence of aqueous $NaHSO_3$ at pH 7 and 37 °C gave N^4-(2-aminoethyl)cytidine (**2a**). Similarly, treatment of 1 with 1,4-diaminobutane or ethylamine afforded N^4-(4-aminobutyl)cytidine (**2b**) or N^4-ethylcytidine (**2c**). Condensation of **2a** or **2b** with 6-chloro-9-(β-D-ribofuranosyl)purine (**3**) in the presence of triethylamine in dimethylformamide at room temperature gave the title compounds **4a** or **4b**. The UV spectra in water at pH 7 show a small hypochromism in Cyd$(CH_2)_2$Ado (**4a**) and virtually none in Cyd$(CH_2)_4$Ado (**4b**). The CD spectra exhibit a slightly increased molecular ellipticity and a narrower Cotton band in **4a** relative to the equimolar mixture of model compounds **2c** and N^6-ethyladenosine. Compound **4b** shows an even smaller increase in ellipticity, and the width of the corresponding Cotton band is almost identical with that of the reference mixture. By contrast, a considerable hypochromism is found in compound **4a** at pH 2, while **4b** shows a slight hyperchromism. The CD spectrum of **4a** shows a large increase in molecular ellipticity at pH 2 relative to that of the corresponding reference mixture. Derivative **4b** exhibits a considerably less intense Cotton band. The extent of base stacking in **4a** and **4b** is compared with that in Ado$(CH_2)_2$Ado and Ado$(CH_2)_4$Ado, and the effect of protonation on the UV and CD spectra of **4a** and **4b** is discussed.

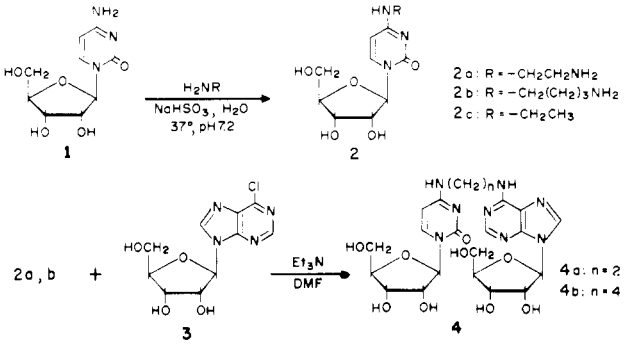
Previous studies have shown that the N^6–N^6-bridged nucleosides Ado$(CH_2)_2$Ado and Ado$(CH_2)_4$Ado[2] are, after proper functionalization,[3] valuable spacer probes for active sites of enzymes such as snake venom phosphodiesterase[4] and ribosomal peptidyltransferase.[5] The latter finding provided evidence that N^6–N^6-bridged adenosines resemble the 3′ terminus of tRNA. It was therefore of interest to study bridged nucleosides which would simulate more closely the 3′ terminal sequence of tRNA (C-A), the title compounds **4a** and **4b**.

Although synthetic avenues to the bridged derivatives of adenosine are available,[6] the corresponding nucleosides with two different bases in the molecule have not yet been reported. The synthesis and spectroscopic investigation of the first of two such derivatives, **4a** and **4b**, are the subject of this paper.

Results and Discussion

Synthesis. The preparation of the title compounds **4a** and **4b** followed a general procedure used previously[6b] for the synthesis of Ado$(CH_2)_2$Ado and Ado$(CH_2)_4$Ado. The starting materials, ω-aminoalkylcytidines **2a** and **2b**, were conveniently obtained by a transamination method described briefly some time ago.[7] The reaction, which consists in the treatment of cytidine (**1**) with excess amine in aqueous $NaHSO_3$ at pH 7, was applied more recently to the synthesis of the corresponding ω-aminoalkylcytidine 2′(3′)-phosphates.[8] However, the products obtained were characterized only by paper electrophoresis and a positive ninhydrin test.

Scheme I



The interaction of cytidine (**1**) and excess 1,2-diaminoethane in aqueous $NaHSO_3$ at pH 7.2 at 37 °C for 3 days led to N^4-(2-aminoethyl)cytidine (**2a**) in 60% yield (Scheme I). Sodium bisulfite was removed with Dowex 50 (H^+), and the resin was washed with water followed by 5% pyridine and 5% NH_4OH. The latter eluent afforded the product **2a** as a syrup which solidified on trituration with ethanol. Similarly, cytidine (**1**) and 1,4-diaminobutane afforded N^4-(4-aminobutyl)cytidine (**2b**) in 92% yield. The reaction of **1** with ethylamine was less favorable, providing N^4-ethylcytidine (**2c**) in only 20% yield. This can be partly explained by a decreased statistical factor. Product **2c** was needed along with previously reported[6b] N^6-ethyladenosine as a model for spectroscopic studies (vide supra). Compound **2c** has been described previously,[9] but it was characterized only by a qualitative UV spectrum and paper chromatography. The structures of **2a–c** were confirmed by quantitative UV and NMR spectra; nucleosides **2a** and **2b** gave a violet coloration with ninhydrin.

The coupling of **2a** with chloro derivative **3** was effected in 3 days at room temperature on treatment with triethylamine in dimethylformamide (DMF). The bridged nucleoside **4a** was isolated in 60% yield after ion-exchange

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(2) Abbreviations throughout the paper conform with recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochim. Biophys. Acta* **1971**, *247*, 1.

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Table I. Hypochromism (H), Hypochromicity (h), and CD Maxima ($[\Theta]_{max}^{D} - [\Theta]_{max}^{M}$) of Bridged Nucleosides[a]

	pH 7			pH 2		
compd	H	h	$10^{-3}([\Theta]_{max}^{D} - [\Theta]_{max}^{M})$	H	h	$10^{-3}([\Theta]_{max}^{D} - [\Theta]_{max}^{M})$
$Cyd(CH_2)_2Ado$ (**4a**)	0.4	5.6	6.2	3.2	13.2	12.6
$Cyd(CH_2)_4Ado$ (**4b**)	−4.1	−1.1	5.5	−7.5	−7.3	5.1
$Ado(CH_2)_2Ado$[b]	19.2	30.6	12.6[c]	14.8	29.3	4.3[c]
$Ado(CH_2)_4Ado$[b]	8.2	12.3	19.0[c]	none	none	3.4[c]

[a] $[\Theta]_{max}^{D}$ is the molecular ellipticity of the bridged nucleosides and $[\Theta]_{max}^{M}$ is the sum of molecular ellipticities of the corresponding reference compounds. [b] The values were taken from ref 6b, unless stated otherwise. [c] Calculated from the corresponding CD spectra in ref 6b.

chromatography on Dowex 50 (H^+). Similarly, compound **4b** was obtained by the reaction of **2b** with chloro derivative **3** in 55% yield. Nucleosides **4a** and **4b** were characterized by UV, CD, and NMR spectra. The NMR spectra showed the presence of two distinct $H_{1'}$ doublets typical for purine and pyrimidine ribofuranose moieties along with the expected patterns of heterocyclic protons.

UV and CD spectra. As in the previous cases of $Ado(CH_2)_2Ado$ and $Ado(CH_2)_4Ado$,[6b] the UV and CD studies provided a wealth of information about the interaction of cytosine and adenine residues in $Cyd(CH_2)_2Ado$ (**4a**) and $Cyd(CH_2)_4Ado$ (**4b**). The N^4-ethylcytidine (**2c**) and N^6-ethyladenosine[6b] were used as spectroscopic reference models. The measurements were performed at ca. 10^{-4} M concentrations, which excluded intermolecular interactions.[6b] Compound **4a** shows surprisingly little hypochromism in water at pH 7. In addition, the shape of the UV absorption bands is essentially symmetrical, and no shift of the UV maxima was observed in contrast to $Ado(CH_2)_2Ado$ and $Ado(CH_2)_4Ado$.[6b] The UV spectrum of **4b** (Figure 1) is virtually superimposable with that of an equimolar mixture of **2c** and N^6-ethyladenosine. As expected, compound **4a**, in which the time-averaged distance between the bases is shorter, exhibits a slightly greater hypochromism than **4b**. In sharp contrast, $Ado(CH_2)_2Ado$ and $Ado(CH_2)_4Ado$ showed significant hypochromism, and the differences between both derivatives are much more pronounced[6b] (Table I). However, the results are in qualitative agreement with a general trend of stacking observed in aqueous solutions of free nucleosides,[10] dinucleoside phosphates,[11] and bridged nucleic acid bases,[12] i.e., purine–purine > purine–pyrimidine. It is important to note that absence of hypochromism in **4b** does not necessarily imply that there is no interaction between the bases. Nevertheless, a comparison of the corresponding dinucleoside phosphate (C-A), which exhibits a considerable hypochromism,[11] and $Cyd(CH_2)_4Ado$ (**4b**) reveals a striking difference despite the fact that space-filling models show a similar distance between the bases.

The difference in the pK_a's of cytidine and adenosine is relatively small,[13] and their magnitudes (4.1 and 3.6) safely exclude protonation of **4a** and **4b** at pH 7. It is also of interest that, whereas the base stacking in C-A was established beyond reasonable doubt,[11,14] the C-A terminus of phenylalanine-specific tRNA from yeast is unstacked,

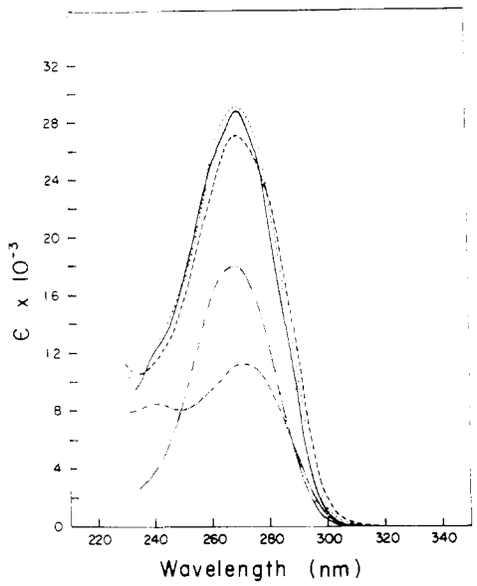

Figure 1. UV spectra at pH 7: $Cyd(CH_2)_2Ado$, – – –; $Cyd(CH_2)_4Ado$, ···; sum of N^4-EtCyd (**2c**) and N^6-ethyladenosine (N^6-EtAdo), —; N^4-EtCyd (**2c**), – · –; N^6-EtAdo, —··— (taken from ref 6b).

according to an X-ray diffraction study.[15] A similar arrangement was found in *E. coli* serine tRNA.[16] Although no explanation for this destacking phenomenon has been given to date, the possibility that crystal packing forces may be responsible for the difference cannot be excluded. Our models, **4a** and **4b**, seem to resemble C-A more as a part of the polynucleotide chain in tRNA rather than the "free" molecule. It may be argued that the joining of bases in **4a** and **4b** by an alkyl chain is different from C-A, affecting the extent of the base stacking. However, biochemical results indicate surprising similarity between $Ado(CH_2)_2Ado$[5a,b] and particularly $Ado(CH_2)_4Ado$[5c] on one hand and the 3′ terminus of tRNA on the other.

A considerably weaker base–base interaction in nucleosides **4a** and **4b** is also reflected in the CD spectra at pH 7 (Figure 2). Unlike in the hypochromism studies, some base stacking can be detected in both compounds. This may be caused by the difference in sensitivity of UV and CD methods. However, as pointed out previously,[6b] the CD spectra can also include other factors such as conformational changes, differences in modes of stacking, etc., which may not necessarily contribute to hypochromism. In addition, the fact that $Ado(CH_2)_nAdo$ has a plane of symmetry, whereas compounds **4a** and **4b** do not, must be considered. Compounds **4a** and **4b** have an increased molecular ellipticity relative to an equimolar mixture of **2c** and N^6-ethyladenosine. In accord with hypochromism measurements, this increase is slightly larger in **4a** with

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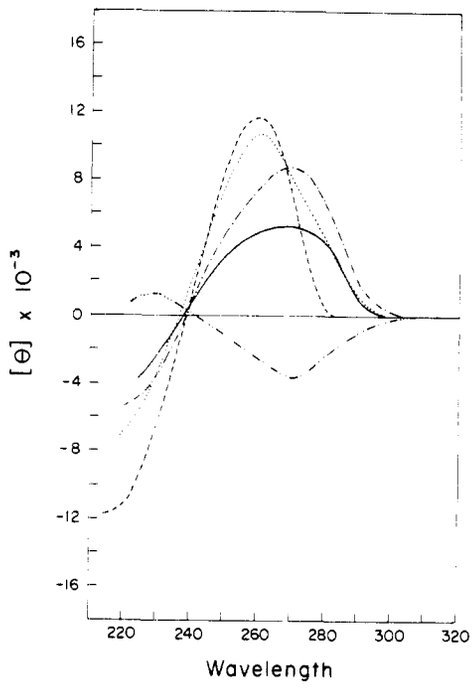

Figure 2. CD spectra at pH 7 (line identification as in Figure 1).

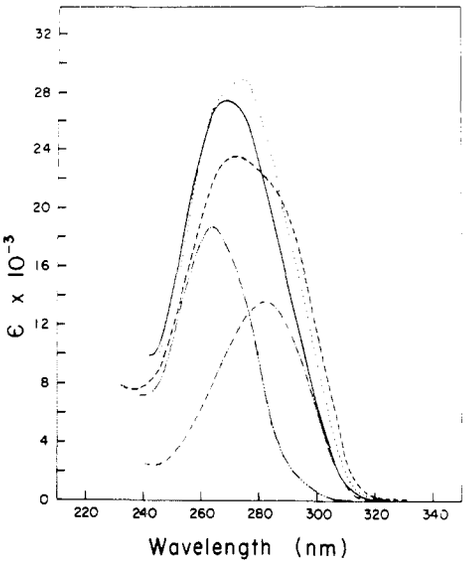

Figure 3. UV spectra at pH 2 (line identification as in Figure 1).

a shorter time-averaged distance between the bases than in **4b**. The same trend is reflected in the widths of the corresponding Cotton bands (Figure 2). As can be seen, the overall shape of the Cotton band of **4b** resembles that of the reference mixture to a greater extent than the band of **4a**. Small differences between **4a** and **4b** reflected in both UV and CD spectra are particularly striking. In summary, both UV and CD spectra show an extensive destacking in **4a** and **4b** at pH 7 relative to C-A, Ado-$(CH_2)_2$Ado, or Ado$(CH_2)_4$Ado (Table I). At the present time, it is not possible to offer an unambiguous interpretation of this phenomenon, though the results are in accord with the general rule of stacking, i.e., purine–purine > purine–pyrimidine.

An entirely different situation is encountered in the case of UV and CD spectra of models **4a** and **4b** at pH 2. Thus, the UV spectrum of **4a** (Figure 3) indicates a significant hypochromism and a shoulder at ca. 290 nm which is not seen in the corresponding equimolar mixture of **2c** and

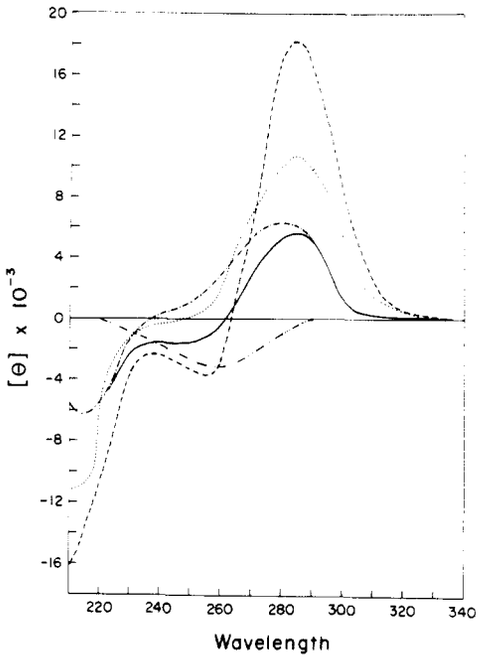

Figure 4. CD spectra at pH 2 (line identification as in Figure 1).

N^6-ethyladenosine. It seems safe to assume that monoprotonated species are responsible for the increased base stacking in **4a** at pH 2 relative to that at pH 7. It can also be expected, on the basis of ionization constants of the parent nucleosides cytidine and adenosine,[13] that protonation of the cytosine residue would take precedence over the adenine moiety. The diprotonated species, if present, should, in any case, be unstacked.[6b] The UV spectrum of **4b** at pH 2 resembles that of Ado$(CH_2)_4$Ado.[6b] A slight hyperchromism was observed, accompanied by a bathochromic shift of the UV absorption maximum relative to that of the reference mixture (Figure 3). It is noteworthy that hyperchromism was also observed in the UV spectrum[11] of C-A at pH 1.

Essentially the same pattern is seen in the CD spectra at pH 2. Thus, the spectra of **4a** and the corresponding reference mixture (Figure 4) are very different. The molecular ellipticity of **4a** is almost 3 times higher, and the main Cotton band is bathochromically shifted (Table I). As expected, a significant decrease in ellipticity was observed in **4b**, and also the shape of the Cotton band is much more similar to that of the reference mixture.

In summary, the differences in UV and CD spectra of cytosine–adenine- and adenine–adenine-bridged nucleosides which reflect the extent of base stacking are striking. It is also interesting that such large differences are not observed in the corresponding dinucleoside phosphates[11] C-A and A-A. Variations in the biochemical properties of suitably functionalized derivatives of Cyd$(CH_2)_2$Ado (**4a**) and Cyd$(CH_2)_4$Ado (**4b**) will be the subject of future work.

Experimental Section

General Procedures and Starting Materials. See ref 6b. Samples for analysis were dried over P_2O_5 at 80 °C and 0.05 mm. Thin-layer chromatography (TLC) was performed on 6 × 2 cm glass plates coated with microcrystalline cellulose (Avicel, average particle size 19 μm) containing 1% of a fluorescent indicator (Brinkmann Instruments) in solvents S_1 (2-propanol–concentrated NH_4OH–water, 7:1:2) and S_2 (1-butanol–acetic acid–water, 5:2:3). TLC in solvent S_3 (chloroform–methanol, 1:1) was conducted on 8 × 2 cm, precoated, silica gel 60 F-254 aluminum foils (Merck, Darmstadt, Germany). Preparative TLC was performed on 20 × 20 cm glass plates covered with a 2-mm-thick layer of Avicel; for the preparation of layers see ref 17. Paper electrophoresis

Table II. R_f Values and Electrophoretic Mobilities of Products[a]

compd	$R_f(S_1)$	$R_f(S_2)$	$R_f(S_3)$	mobility[b] in phosphate	mobility[b] in citrate	mobility[b] in borate
2a	0.38	0.54	0	3.57	1.13	0
2b	0.51	0.59	0	4.20	1.38	−0.71
2c	0.74	0.65		1.13	0.96	0.86
4a	0.43	0.63	0.40	0.77	0.38	1.07
4b	0.60	0.69	0.49			0.98

[a] For details see General Procedures and Starting Materials. [b] Relative to mobility of cytidine = 1.00.

was conducted on an electrophoresis flat plate cooled to 15 °C (Savant Instruments) in 0.02 M Na_2HPO_4 (pH 7), 0.05 M sodium citrate (pH 3.5), and 0.05 M sodium borate (pH 9) at 40 V/cm for 1 h. For R_F values and electrophoretic mobilities see Table II. NMR spectra were obtained with an FT spectrometer (JEOL FX-100) in CD_3SOCD_3; DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) was used as internal reference.

Ultraviolet (UV) and Circular Dichroism (CD) Measurements. For conditions of the measurements see ref 6b. A minimum of two sets of data were obtained in each case. In addition, the CD measurements were performed at two concentrations. The UV and CD data were plotted as molecular extinction coefficients, ϵ, or molecular ellipticity, $[\theta]$, against the wavelength. The UV and CD spectra of the reference samples **2c** and N^6-ethyladenosine[6b] were added graphically. The results are summarized in Figures 1–4. To obtain the hypochromism, H, the recorded UV spectra were integrated by a computer[6b] from the vicinity of the absorption minimum (ca. 230 nm) to the zero absorption at long wavelengths in 2.5-nm intervals. The values of H were then calculated from the corresponding oscillator strengths $f = (4.32 \times 10^{-9}) \int \epsilon\lambda/\lambda^2 d\lambda$ and $H = 100(1 - f^D/f^M))$, where f^D is the oscillator strength of bridged nucleoside **4a** or **4b** and f^M the sum of the oscillator strengths of compound **2c** and N^6-ethyladenosine. Hypochromicity values, h, were obtained from the expression $h = 100(1 - (\epsilon_{max}{}^D/\epsilon_{max}{}^M))$, where $\epsilon_{max}{}^D$ is the extinction coefficient of **4a** or **4b** at λ_{max} and $\epsilon_{max}{}^M$ the sum of extinction coefficients of **2c** and N^6-ethyladenosine.

N^4-(2-Aminoethyl)cytidine (2a). A solution of cytidine (**1**; 0.24 g, 1 mmol), 1,2-diaminoethane (1.23 g, 12.5 mmol), and $NaHSO_3$ (1.68 g, 16.2 mmol) in water (10 mL) whose pH was 7.1 was kept at 37 °C for 75 h. Electrophoresis (phosphate) showed that the reaction was almost complete. The mixture was added with stirring to 25 mL of Dowex 50 (H^+) in the hood (evolution of SO_2), and the suspension was allowed to stand for 1 h at room temperature. Then it was added to 25 mL of Dowex 50 (H^+) in a chromatographic column, and the resin was eluted successively with water, 5% aqueous pyridine, and 5% NH_4OH (500 mL each). The latter eluent afforded after evaporation syrupy **2a** containing 1,2-diaminoethane as shown by electrophoresis (phosphate). This product was chromatographed on a Dowex 50 (NH_4^+) column (60 mL) by using a linear gradient of water (1 L) and 3% NH_4OH (1 L). The elution was monitored with a Uvicord II fraction collector coupled with a UV detector (LKB, Uppsala, Sweden). Evaporation of the appropriate fractions gave **2a** as a colorless gum (0.2 g, 67%). Trituration with ethanol afforded a white amorphous solid [0.18 g (60%), mp above 150 °C (foaming, transition point)] which was uniform on TLC (S_1) and electrophoresis (phosphate and borate): UV max (water) 270 nm (ϵ_{max} 9250); NMR (CD_3SOCD_3) δ 7.77 (d, 1, H_6, $J_{6,5}$ = 7.8 Hz), 5.79 (d, 1, $H_{1'}$, $J_{1',2'}$ = 3.9 Hz), 5.77 (d, 1, H_5, $J_{5,6}$ = 7.8 Hz), the remaining ribose protons are overlapped with hydroxy and methylene signals. Anal. Calcd for $C_{11}H_{18}N_4O_5 \cdot 0.3H_2CO_3$: C, 44.51; H, 6.15; N, 18.38. Found: C, 44.82, H, 6.42; N, 18.24.

N^4-(4-Aminobutyl)cytidine (2b). The experiment was performed as above with cytidine (**1**; 0.48 g, 2 mmol) and 1,4-diaminobutane instead of 1,2-diaminoethane. The workup of the reaction mixture also followed the procedure given for compound **2a**. The linear gradient used was 50% methanol (2 L) and 5% NH_4OH in 50% methanol (2 L). The resultant syrupy **2b** was dissolved in water (50 mL), and the solution was lyophilized to give 0.48 g (92%) of white amorphous hygroscopic solid homogeneous on TLC (S_1) and electrophoresis (phosphate and borate): UV max (water) 272 nm (ϵ_{max} 9500); NMR (CD_3SOCD_3) δ 7.78 (d, 1, H_6, $J_{6,5}$ = 7.6 Hz), 5.74 (d, 1, H_5, $J_{5,6}$ = 7.6 Hz), $H_{1'}$ is overlapped with H_5. Anal. Calcd for $C_{13}N_{22}N_4O_5 \cdot 0.2H_2CO_3$: C, 48.52; H, 6.91; N, 17.15. Found: C, 48.67; H, 6.63; N, 16.70.

N^4-Ethylcytidine (2c). The pH of a solution of 70% aqueous ethylamine (2.06 g, 32 mmol) and $NaHSO_3$ (6.2 g, 60 mmol) in water (40 mL) was adjusted to 7.2 by addition of more ethylamine (0.5 mL). Cytidine (1, 0.96 g, 4 mmol) was added, and the resultant mixture was kept at 37 °C for 18 days. TLC (S_1) showed about half of the original amount of 1 remaining. The workup followed the procedure outlined for **2a** above. The Dowex resin was washed with water and 5% aqueous pyridine (2 L each). Evaporation of the pyridine eluate gave a syrup which showed two spots on TLC (S_1): cytidine (1) and product **2c**. The mixture was applied to a cellulose column (Avicel PH-101, FMC Corp., average particle size 50 μm, 3 × 84 cm). Elution with solvent S_1 monitored with Uvicord II gave, after evaporation of the appropriate fractions, oily product **2c**. Trituration with ether containing ethanol gave an off-white amorphous hygroscopic solid: 0.21 g (20%); homogeneous on TLC (S_1 and 1-butanol–ethanol–water, 16:2:5); UV and CD spectra, cf. Figures 1–4; NMR (CD_3SOCD_3) δ 7.78 (d, 1, H_6, $J_{6,5}$ = 7.3 Hz), 3.29 (q, 2, CH_2 of ethyl), 1.12 (t, 3, CH_3 of ethyl), all other signals were overlapped. Anal. Calcd for $C_{11}H_7N_3O_5 \cdot 0.65H_2O$: C, 46.68; H, 6.52; N, 14.85. Found: C, 46.39; H, 6.67; N, 15.25.

1-(Adenosin-N^6-yl)-2-(cytidin-N^4-yl)ethane (4a). Compound **2a** (50 mg, 0.16 mmol) was dissolved in warm DMF (1 mL). The solution was brought to room temperature, and chloro nucleoside **3** (50 mg, 0.17 mmol) was added followed by triethylamine (0.05 mL, 0.4 mmol). The mixture was stirred for 72 h whereupon TLC (S_3) showed the reaction was almost completed. The solution was evaporated, and the residue was chromatographed on a column of Dowex 50 (H^+, 25 mL). The elution was performed successively with water, 5% aqueous pyridine, and 5% NH_4OH. Evaporation of the ammonia eluate gave an amorphous solid [**4a**, 56 mg (60%)] which was further purified by preparative TLC on one 20 × 20 cm, 2-mm-thick layer of Avicel in solvent S_1. The major UV absorbing band was eluted with water, and the filtered solution was lyophilized to afford 36 mg (39%) of **4a** as an amorphous solid homogeneous on TLC (S_1–S_3) and electrophoresis (phosphate and borate): UV and CD spectra, cf. Figures 1–4; NMR (CD_3SOCD_3) δ 8.36 and 8.26 (2 s, 2, purine H_8 and H_2), 7.90 (d, 1, pyrimidine H_6, $J_{6,5}$ = 7.8 Hz), 5.92 (d, 1, "adenosine" $H_{1'}$, $J_{1',2'}$ = 6.4 Hz), 5.86 (d, 1, "cytidine" $H_{1'}$, $J_{1',2'}$ = 3.4 Hz), 5.78 (d, 1, pyrimidine H_5, $J_{5,6}$ = 7.8 Hz), the remaining signals overlapped. Anal. Calcd for $C_{21}H_{28}N_8O_9 \cdot 1.25H_2O$: C, 45.12; H, 5.50; N, 20.05. Found: C, 44.80; H, 5.24; N, 20.35.

Evaporation of the ammonia eluate gave 8 mg (16%) of the starting material **2a** identified by TLC (S_1 and S_2).

1-(Adenosin-N^6-yl)-4-(cytidin-N^4-yl)butane (4b). The reaction was performed on the same scale as in the case of **4a** by using compound **2b** instead of **2a** as the starting material. The workup of the reaction mixture and chromatography also followed the above procedure. Evaporation of the pyridine eluate gave a gummy residue which after lyophilization afforded a white amorphous solid [**4b**, 63 mg (70%)]. This product was further purified by TLC as given for **4a** to give 49 mg (55%) of **4b**, homogeneous on TLC (S_1 and S_2) and electrophoresis (phosphate and borate): UV and CD spectra, cf. Figures 1–4; NMR (CD_3SOCD_3) δ 8.35 and 8.21 (2 s, 2, purine H_8 and H_2), 7.86 (d, 1, pyrimidine H_6, $J_{6,5}$ = 8 Hz), 5.89 (d, 1, "adenosine" $H_{1'}$, $J_{1',2'}$ = 6.6 Hz), 5.77 (d, 1, "cytidine" $H_{1'}$, $J_{1',2'}$ = 2.9 Hz), 5.71 (d, 1, pyrimidine H_5, $J_{5,6}$ = 8 Hz), the rest of the signals overlapped.

(17) Chládek, S.; Žemlička, J. *J. Org. Chem.* **1974**, *39*, 2187.

Anal. Calcd for $C_{23}H_{32}N_8O_9 \cdot 1.25H_2O$: C, 46.69; H, 5.96; N, 18.94. Found: C, 46.32; H, 5.93; N, 18.70.

Evaporation of the ammonia eluate gave **2b** (9 mg, 18%) as identified by TLC (S_1 and S_2). A similar workup of the aqueous portion afforded **3** (16 mg, 32%).

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Registry No. 1, 65-46-3; **2a**, 73611-47-9; **2b**, 73611-48-0; **2c**, 22342-50-3; **3**, 5399-87-1; **4a**, 73611-49-1; **4b**, 73611-50-4; 1,2-diaminoethane, 107-15-3; 1,4-diaminobutane, 110-60-1; ethylamine, 75-04-7.

Notes

Synthesis of Spiro-Activated Cyclopropanes from Alkenes via the Irradiation of Isopropylidene Diazomalonate. A Reinvestigation

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"Spiro-activated" cyclopropanes 1 have been demonstrated to be valuable intermediates in organic synthesis.[1] Recently, we reported[2] a simple method for the preparation of these intermediates by the direct irradiation (253.7 nm) of isopropylidene diazomalonate (**2**) in the presence of an olefin. A number of olefins were investigated, including 1,3,3-trimethylcyclohexene which was reported to afford adduct **3**. However, recent attempts to employ **3** as an intermediate in a projected synthesis led us to doubt the structure (**3**) assigned originally. Accordingly, we undertook an X-ray crystallographic analysis of this substance. Crystallographic data were obtained at 115 K with a Syntex P1 diffractometer equipped with a locally constructed low-temperature device.[3] Experimental and data-handling techniques were analogous to those described previously.[4] Experimental parameters are summarized in footnote 5. Direct-methods (MULTAN), difference-Fourier, and least-squares refinement techniques were used in the solution of the structure. In the final refinement, positions and anisotropic thermal parameters of all nonhydrogen atoms were refined along with the positions of the hydrogen atoms. Isotropic temperature factors of the hydrogen atoms were fixed at 2.0 Å^2. As Figure 1 reveals, this compound was not cyclopropane **3** as reported previously, but rather the cyclobutanone **4a**.

This result can be rationalized in terms of a Wolff rearrangement of the initially formed carbene (**5**) to afford ketene **6**[7] which undergoes a remarkably regio- and stereospecific cycloaddition[8] to the olefin to afford cyclo-

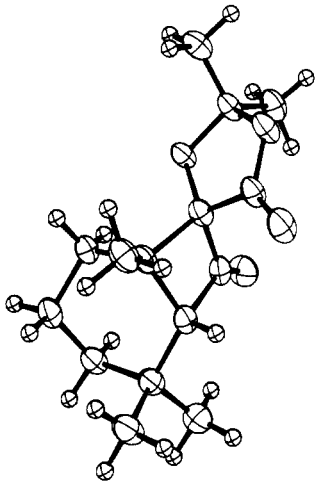
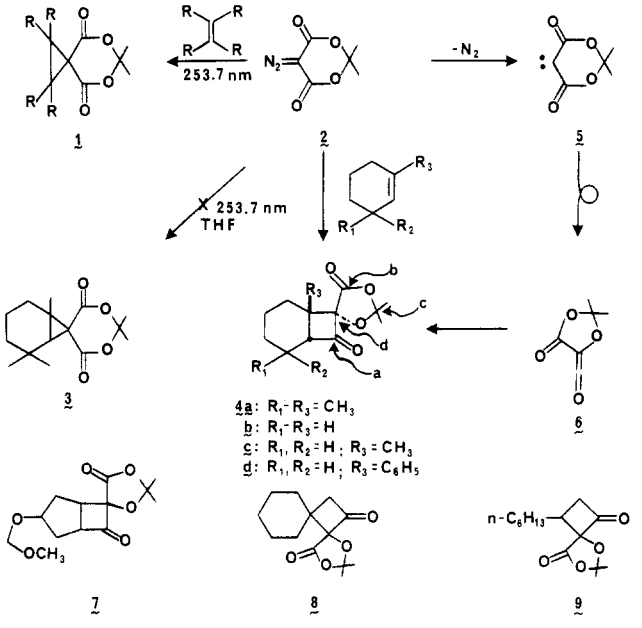


Figure 1.

Scheme I



(1) S. Danishefsky, *Acc. Chem. Res.*, **12**, 66 (1979), and references cited therein.

(2) (a) T. Livinghouse and R. V. Stevens, *J. Am. Chem. Soc.*, **100**, 6479 (1978); (b) R. V. Stevens, *Pure Appl. Chem.*, **51**, 1317 (1979).

(3) C. E. Strouse, *Rev. Sci. Instrum.*, **47**, 891 (1976).

(4) J. Strouse, S. W. Layten, and C. E. Strouse, *J. Am. Chem. Soc.*, **99**, 562 (1977).

(5) Crystal data: space group $P2_1/C$; $Z = 4$; lattice parameters (115 K), $a = 10.966$ (3) Å, $b = 8.128$ (2) Å, $c = 17.805$ (3) Å, $\beta = 113.02$ (2)°; radiation, Cu K crystal monochromatized (1.5418 Å); crystal dimensions, 0.17 × 0.20 × 0.20 mm; absorption coefficient, 7.16 cm^{-1}; $T_{min} = 0.80$, $T_{max} = 0.85$; scan range, 1.0 below Kα_1 to 1.0 above Kα_2; scan rate, 6.0°/min; scan mode, θ-2θ; $2\theta_{max} = 110°$; background time = scan time; observed reflections [**I** greater than 3.0(σ**I**)], 1728, $R = 0.042$, $R_w = 0.059$, error in observation of unit weight of 2.24.

(6) Such a rearrangement in methanol–benzene but at unspecified wavelength has been reported previously: S. L. Kammula, H. L. Tracer, P. B. Shevlin, and M. Jones, Jr., *J. Org. Chem.*, **42**, 2931 (1977).

(7) Ketene **6** has been identified spectroscopically by photolysis of **2** in an argon matrix: private communication from Professor O. L. Chapman and R. Hayes, Department of Chemistry, University of California, Los Angeles.

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