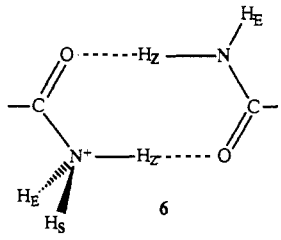
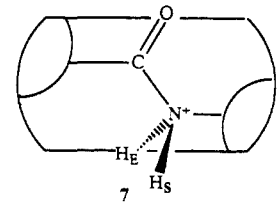


6

of microscopic reversibility, the initial labeling must be as shown (H_S from solvent). Then exchange of H_Z is retarded by the necessity for rotation out of the intramolecular hydrogen bond. That hydrogen bond would appear to be quite strong, imposing a significant barrier to rotation about the C–N^+ bond. Nevertheless there seems to be hardly any barrier to rotation.

Comparison of Acid- and Base-Catalyzed Exchange. Why does internal hydrogen bonding retard base-catalyzed exchange of H_Z ca. 30-fold but acid-catalyzed exchange hardly at all? Indeed, the ratio k_{ES}/k_{ZS} for acid-catalyzed exchange is comparable to that found for ordinary primary amides,[14] so that the hydrogen bonding in **6** does not retard exchange of H_Z. This result is consistent with observations on rotation of ammonium ion within its solvation shell.[15] This rotation is extremely fast, especially in water, despite the necessity for breaking and remaking hydrogen bonds. Nevertheless, it is remarkable that the internal hydrogen bond in **1** is so much more resistant to breaking than that in **6**.

This comparison clarifies an aspect of NH exchange in proteins. Acid-catalyzed exchange had long been thought to occur by the N-protonation mechanism. However, substituent effects in model *N*-methyl amides[16] and considerations of solvent accessibility to nitrogen and oxygen[17] indicate that the imidic acid mechanism is dominant. However, many of these NH are in α-helices, β-sheets, or other environments where the nitrogen is accessible from only one face. Protonation on that face produces an intermediate **7** that can lose only H_S, the proton that came from solvent. Loss



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of the original NH proton is impossible, since H_E is now embedded in the protein and inaccessible to solvent. In a primary amide, even **1**, the corresponding intermediate **6** can undergo rotation about the C–N single bond and render any NH proton accessible to solvent. However, in a protein the backbone resists such twisting, and **7** cannot lead to proton exchange. In contrast, both the base-catalyzed and imidic acid mechanisms permit removal of the proton without this complication. Thus the N-protonation mechanism is quite unlikely for acid-catalyzed exchange of secondary NH in proteins.

Conclusions. The internal hydrogen bond in diamide **1** retards base-catalyzed exchange of H_Z ca. 30-fold. Exchange is viewed as occurring by direct abstraction of the proton from the hydrogen bond, and this may be the first example in which this one-step mechanism predominates. In contrast, the internal hydrogen bond retards the acid-catalyzed exchange of H_Z not at all. This is a consequence of the nearly free rotation about the C–N single bond of the N-protonated intermediate. However, this mechanism cannot be operative in proteins.

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Synthesis of Covalently Linked Double-Helical Cross Sections Representative of Purine–Pyrimidine, Purine–Purine, and Pyrimidine–Pyrimidine Duplexes†

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Abstract: Here described are the syntheses of (1) covalently linked cross sections with molecular architecture similar to Watson–Crick hydrogen-bonded purine–pyrimidine base pairs in RNA, DNA, and RNA/DNA double helices; (2) covalently linked purine–purine cross sections with dimensions such as would be produced in the pairing of A with I or G, generating a bulge in double-helical RNA or DNA; and (3) covalently linked pyrimidine–pyrimidine cross sections with dimensions such as might be produced in the hypothetical pairing of C with U or T, namely, a pinched-in RNA or DNA cross section.

In two preliminary communications,[1,2] we have introduced the concept of covalently linked double-helical cross sections that are representative of purine–pyrimidine, purine–purine, and pyrimidine–pyrimidine duplexes. We described briefly how these complex molecules in the bis(ribonucleoside) and bis(deoxyribonucleoside) series can be synthesized conveniently from the natural ribo- and deoxyribonucleosides in only three steps plus initial O-protection and final O-deprotection. We now provide further rationale and full details of the synthesis and spectroscopic

† Dedicated to the memory of Professor Roger Adams in the centennial year of his birth.
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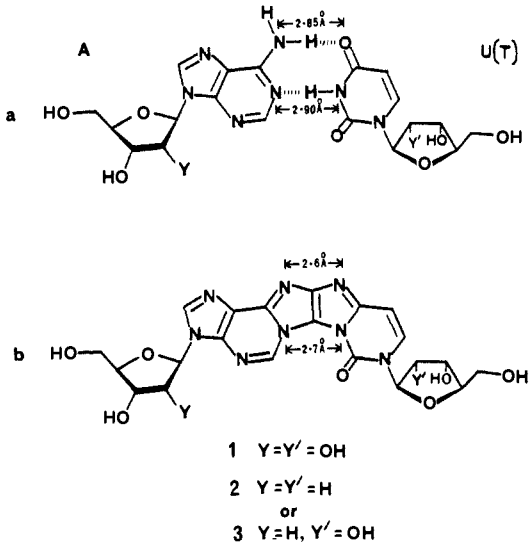

Figure 1. Comparison of the geometry of (a) a Watson–Crick A·U (A·T) base pair with (b) a double-helical cross section containing a 1,3,4,6-tetraazapentalene linking system.

properties of the target molecules.

The classical Watson–Crick double-helical model of DNA/RNA possesses well-defined hydrogen bonds that hold the two strands in complementarity (Figure 1a).[3] Our idea was to substitute for the central eight-membered ring containing the hydrogen bonds a coplanar array of two unsaturated five-membered rings (Figure 1b). The terminal purine and pyrimidine rings would be held thereby in correct register by the central 1,3,4,6-tetraazapentalene linking system, the geometry of which mimics closely that of the natural hydrogen-bonding system. Moreover, the terminal rings (Figure 1b) could not be pulled apart easily in biological systems unless unforeseen enzymes exist that can act upon the five-ring N-heterocyclic system.

The close similarity of the overall molecular geometry is indicated by the respective interatomic distances in Figure 1a and b, which differ only by ~0.2 Å. The same small difference would hold for the interatomic distances between the purine C1′ and the pyrimidine C1′ in the two representations. The dimensions shown in Figure 1b have not yet been determined but have been calculated from a composite structure consisting of two separate entitites in the formula, i.e., 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine, the dimensions of which have been determined by single-crystal X-ray structure analysis.[4] In the simple tetracyclic molecule analogous to that in Figure 1b, namely, dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene,[5] the corresponding N–N distances, top and bottom, have been determined by X-ray analysis to be 2.54 and 2.59 Å, respectively.[6]

It might be argued that although the dimensions for the central eight-membered ring in Figure 1a and the central two five-membered rings in Figure 1b are in close conformity, the N for O substitution in the latter detracts from the excellence of the analogy. To the contrary, both N and O present an electron pair in the base-pair plane in the major groove (when **1**, **2**, or **3** is in a polynucleotide structure). A trivalent nitrogen rather than a divalent oxygen is necessary for the five-membered ring construction. It is a moot point at this time whether the central ring system in Figure 1b is unprotonated or protonated in aqueous solution (cf. Figure 1a). In any event, the close resemblance of the two entities in Figure 1 suggests that it may be possible to incorporate the covalent cross section into polynucleotides or

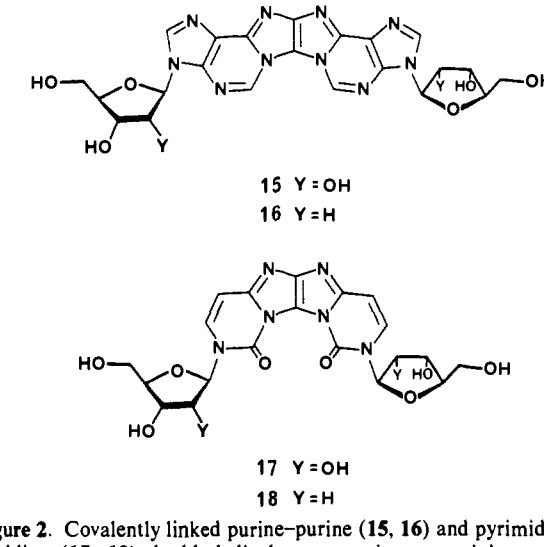

Figure 2. Covalently linked purine–purine (**15**, **16**) and pyrimidine–pyrimidine (**17**, **18**) double-helical cross sections containing a central 1,3,4,6-tetraazapentalene.

polydeoxynucleotides and thus provide constructs that would resist separation, i.e., prevent replication at the DNA level within a cell. Thus, the synthesis of covalently linked base pairs offered not only a challenge but a worthwhile goal because of potential biological applications.

The concept described above can also be applied to covalently linked cross sections that are distorted in their overall dimensions from those of the Watson–Crick hydrogen-bonded base pairs in a double helix. Great interest in the effect of DNA distortion on binding and biological activity has stimulated us to provide, for example, a covalently linked purine–purine cross section (Figure 2, **15** and **16**) with dimensions such as would be produced in the pairing of A with I or G, capable of generating a bulge when incorporated in a double-helical DNA or RNA. We also provide a covalently linked cross section with dimensions such as might be produced in the hypothetical pairing of C with T or U, namely, a pinched-in DNA or RNA cross section (Figure 2, **17** and **18**). There is the potential in these molecules, when phosphorylated and incorporated in a double-helical polynucleotide sequence, of showing just what the biological effects would be of a bulge or a narrowing of the helix under different circumstances. This manuscript describes the details of the methodology that culminated in the synthesis of the target molecules, from which point the biological investigations can now proceed.

The following strategic requirements were set for the synthesis of a covalently linked bis(deoxyribosyl) or bis(ribosyl) cross section. (A) The starting materials should be readily available and related to the normal base-pairing entities of DNA or RNA. (B) The deoxyribosyl and ribosyl groups should already be attached and in the correct stereochemistry in the starting materials. Any attempt at later attachment to the unsubstituted N-heterocyclic ring system would be complicated by isomer separation and requisite structure elucidation. (C) A nucleoside annelating agent should be used that closes onto one of the rings, adenine or cytosine, preferentially so that only one step remains necessary to close the second five-membered ring pictured in Scheme I. (D) An efficient oxidizing agent must be found for the second ring closure. We have chosen the di-*O*-acetyl derivatives of deoxyadenosine and deoxycytidine and the tri-*O*-acetyl derivatives of adenosine and cytidine as convenient starting materials and chloroketene diethyl acetal[7] as the nucleoside annelating agent.

The heating of 2′,3′,5′-tri-*O*-acetyladenosine (**4a**)[8] with chloroketene diethyl acetal (**5**) in ethyl acetate in the presence of *p*-toluenesulfonic acid afforded the chloroimidate **8a** in quantitative yield[9-11] via the adduct **6a** (Scheme I). The subsequent con-

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Scheme I

densation of **8a** with 1 equiv of 2′,3′,5′-tri-*O*-acetylcytidine (**9a**)[12] in benzene/acetonitrile with *p*-toluenesulfonic acid under reflux yielded (27%) the bis(riboside) derivative **11a**[1,11] via the putative intermediate **10a**. The low yield in this step was the result, in part, of the reversion of **8a** to **4a** and the cyclization of **8a** to **6a**.[1,9] The direction of closure of the new five-membered ring was established by ^{1}H NMR guidelines.[9] Whereas annelation of the five-membered ring onto a cytidine unit decreases the chemical shift difference between the pyrimidine ring protons, annelation onto an adenosine unit causes a marked downfield shift of the original purine 2-proton. It was evident from the downfield shift of the original 2-proton of the adenosine moiety (**8a**) after reaction with **9a** that ring closure had occurred on the adenosine side of **10a** and that the structure of the product could be represented as **11a**. The proton NMR spectrum (Figure 3a) displayed five aromatic signals plus those due to two tri-*O*-acetylribofuranosyl moieties. The FAB mass spectrum of **11a** exhibited a pseudo molecular ion $(M + H)^+$ peak at m/z 785 and two fragment ion peaks at m/z 527 and 269 due to the loss of one and two sugar units, respectively. The ^{13}C NMR spectrum and the ^{1}H/^{13}C short-range correlation studies gave further confirmation of the assigned structure.

The corresponding bis(deoxyribonucleoside) derivative **11b** was synthesized in a similar manner from 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**4b**)[13a] and 3′,5′-di-*O*-acetyl-2′-deoxycytidine (**9b**)[13b] and was characterized by similar spectroscopic means. The compound of hybrid type (**11c**) was synthesized from **4b** and **9a**.

The problem of cyclization of compounds **11a–c** to **14a–c** is essentially one of forming a bond between two electron-rich centers, namely, the pyrimidine ring nitrogen and the carbon on the etheno bridge, which is actually the β-carbon of an enamine system. This necessitates a reversal of polarization at one center. Reaction conditions were first developed with a model compound. It was discovered that the oxidative cyclization of 2-(2-pyridylamino)imidazo[1,2-*a*]pyridine to dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene could be effected[5,6] by means of iodobenzene diacetate [(diacetoxyiodo)benzene, iodosobenzene diacetate][14] in 2,2,2-trifluoroethanol. However, these conditions failed to bring about the cyclization of **11a** to **14a**, due partially to the poor nucleophilicity of the endocyclic nitrogen of the cytidine moiety. The only product isolated was an adduct of trifluoroethanol with the reactive intermediate (e.g., **13a**).[15] Thus, we considered it necessary to use a stronger, complexing oxidant with a bulkier fluorinated alcohol as one component of a high-dielectric, nonnucleophilic solvent. Oxidative cyclization of **11a** to **14a** was effected by means of 2-nitroiodobenzene diacetate[16] in a solvent consisting of 1,1,1,3,3,3-hexafluoro-2-propanol or 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol and nitromethane (1:2 v/v) at

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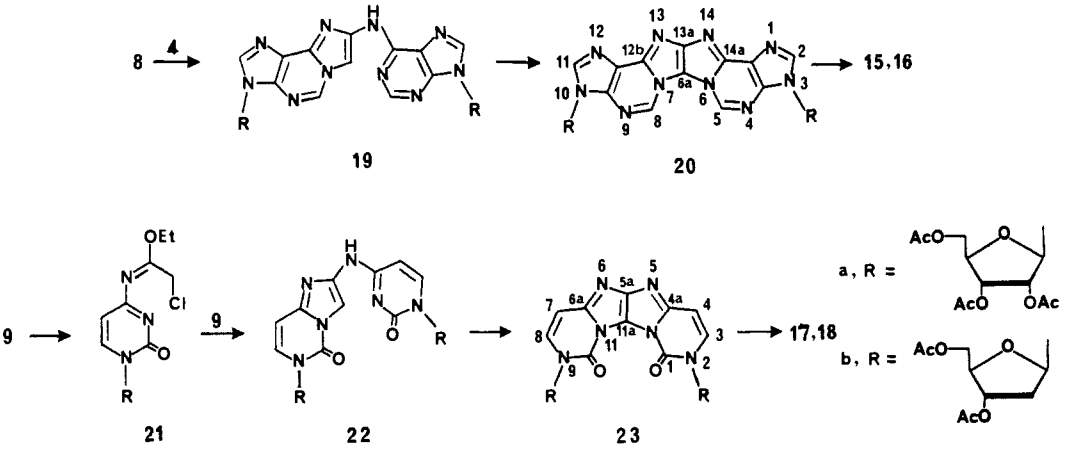
Scheme II



−10 °C. The oxidative cyclization did not proceed in either solvent alone. Under the improved reaction conditions, the highly fluorescent product **14a** was obtained in 36% yield. Compounds **14b** and **14c** were obtained by this methodology in yields of 40 and 26%, respectively. It is pertinent to note that the oxidative ring closure of **11a** did not proceed in the presence of tris(*p*-bromophenyl)aminium hexachloro stibnate, which is a potent one-electron acceptor.[17,18] This observation, together with the experience gathered in the iodobenzene diacetate oxidation of model compounds,[5,19] favors an ionic pathway (i.e., via **13**) for the ring closure.

The structure elucidation of **14a–c** was achieved by ^1H and ^{13}C NMR spectroscopy, $^1H/^{13}C$ short-range[20] and long-range[21,22] correlation studies, and FAB mass spectrometry. The proton NMR spectrum of **14a** revealed the presence of two tri-*O*-acetylribosyl moieties and four protons attached to unsaturated carbon atoms (Figure 3b) of the aglycon portion. The 5-proton in the bay region displayed a marked downfield chemical shift of ~1 ppm when it was compared with the corresponding proton in the precursor **11a**. This feature, which is due to the anisotropy of the proximal carbonyl group, is at the same time a confirmation of the direction of ring closure. The chemical shift difference between the 10- and 11-protons was diminished appreciably (~0.5 ppm) upon oxidative cyclization. This change is indicative of the occurrence of the second etheno annelation on the cytidine moiety.[9] Compounds **14b** and **c** exhibited similar proton NMR patterns. The ^{13}C NMR spectra of **14a–c** displayed seven quaternary carbon and four unsaturated C(H) resonances for the aglycon portion. The unambiguous assignments of the ^{13}C NMR signals were facilitated by the analysis of the proton-coupled ^{13}C NMR spectra and long-range $^1H/^{13}C$ heteronuclear correlations, as shown for **14c** (Experimental Section, Figures 4 and 5). The complete assignment of carbon and proton resonances for **14c**, as given in Figure 6a is representative for compounds **14a–c**.

The FAB mass spectra of **14a–c** displayed characteristic fragmentation patterns similar to those exhibited by their respective precursors **11a–c**, that is, intense pseudo molecular ion peaks, $(M + H)^+$, at m/z 783, 643, and 725, respectively. The elemental compositions of **14a–c** were confirmed by high-resolution FAB mass spectrometry.

The final phase in the synthesis of compounds **1**, **2**, and **3** required treatment with methanolic ammonia at 0 °C for a period of 3–4 h followed by careful reaction workup. It was observed that complete deacetylation could also be effected by treatment with *tert*-butylamine[23] (0.15 M) in methanol, which furnished cleaner products than the NH_3/MeOH conditions. The products were purified by recrystallization from either water or aqueous methanol, and their structures, indicated by their respective precursors, were confirmed by their proton NMR spectra and by low- and high-resolution FAB mass spectrometry. The overall accomplishment is the short synthesis of three representative compounds having a high degree of complexity: five N-heteroaromatic rings containing a total of eight nitrogens; ribofuranosyl or deoxyribofuranosyl groups on the appropriate nitrogens for cross-sectional analogy (RNA, DNA, and DNA/RNA); and, pro forma, eight, six, or seven asymmetric carbons.

If, indeed, the actual N–N distances in the dual five-membered ring system common to **1–3** are close to those shown in Figure 1 and found in the model dipyrido-1,3,5,6-tetraazapentalene[6] and the pentacyclic ring system is flat, these compounds represent an accurate dimensional mimic (ca. ±0.2 ppm) of a natural base-paired cross section. Distortion to a wider (**15**, **16**) or narrower (**17**, **18**) covalent cross section has been achieved by synthesis of the compounds shown in Figure 2.[2] The methodology used for **1–3** was found to be applicable to the preparation of the covalently linked purine–purine bis(nucleosides) **15** and **16** (Scheme II). The synthesis of **15** began with the formation of the chloroimidate **8a**, followed by reaction with 1 equiv of 2′,3′,5′-tri-*O*-acetyladenosine (**4a**) in a solvent consisting of benzene/dichloromethane/acetonitrile (3:2:1, v/v) in the presence of 0.5 equiv of *p*-toluenesulfonic acid at 60 °C. By repeated flash chromatographic separations, the fluorescent bis(ribonucleoside) derivative **19a** was isolated and most of the unreacted **4a** was recovered. The structure of **19a**, as in cases of the compounds described above and to follow, was established by ^1H NMR spectroscopy, $^1H/^{13}C$ short-range and long-range heteronuclear correlation studies, and FAB mass spectrometry. The oxidative cyclization of **19a** to the fluorescent product **20a** was effected in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-propanol or 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol and nitromethane at −10 °C. The presence of a plane of symmetry within the hexacyclic nitrogen ring system in the oxidation product was evident from the dramatic simplification of its ^1H NMR spectrum in comparison with that of its immediate precursor **19a**. Particularly diagnostic is the significant downfield shift of the NMR signal for the proton on the pyrimidine portion of the purine ring system observed in the first cyclization, **8a** to **19a** (0.46 ppm), and again in the second cyclization, **19a** to **20a** (0.61 ppm). The ^{13}C NMR spectrum of **20a** was also indicative of the symmetry achieved. The chemical shifts of the different junctional carbons 6a and 13a appeared at 111.41 and 152.51 ppm, respectively, and that of the identical junctional carbons 12b and 14a appeared at 141.45 ppm. The bis(deoxyribonucleoside) derivative **20b** was synthesized by a similar experimental protocol

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that started with 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**4b**).

An improvement in the complete deacetylation of **20a** and **b** over that mentioned in the earlier communication,[2] namely, treatment with methanolic ammonia, has been found in the use of *tert*-butylamine (0.15 M) in methanol at 0 °C for 3 h followed by 1.5 h at room temperature. The products, 3,10-di-β-D-ribofuranosylpurino[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purine (**15**) and 3,10-bis(2′-deoxy-β-D-ribofuranosyl)purino-[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purine (**16**), exhibit blue fluorescence. They are covalently linked analogues of an A–I base-pair cross section (RNA, DNA) that is hydrogen bonded in an *extended* Watson–Crick manner. While there is some ambiguity about the interbase hydrogen bonding in helical poly(A)·poly(I),[24-27] in the three-stranded helical complex poly-(A)·poly(I)·poly(I),[24,26-31] one set of base pairs is believed to be of an extended Watson–Crick type, N1 to N1 and N6 to O6, and the other, of the Hoogsteen variety, N7 to N1 and N6 to O6.[32] The extended or "long"[27] base pair I·A, by modeling, would have a longer C1′–C1′ distance (13.0 Å) than a standard Watson–Crick base pair (10.67 Å).[27] The base pair I·A within ordered duplexes has been shown to be less stable than I·C[33] and to be strongly affected by the neighboring bases in the sequence.[33] Compounds **15** and **16** also serve as models for the corresponding G·A mismatch base pairs. X-ray structure determination shows that the two purine–purine mispairings at the center of the decamer duplex GCAA*GA*TTGC are in the anti, anti conformation,[34] in agreement with NMR evidence for the decamer in solution.[35] The same geometry of the "long" base pair, which produces a bulge, was observed by NMR for mismatched G·A pairs in the dodecamer duplex CG*A*GAATTC*G*CG[36] and by X-ray in the anticodon stem of tRNA.[37,38]

The versatility of the methodology was demonstrated also in the synthesis (Scheme II) of a covalently linked double-helical cross section (**17**, **18**) representative of a pyrimidine–pyrimidine duplex, which is unlike any structural feature presently observed in Nature. The synthesis of 2,9-di-β-D-ribofuranosylpyrimido-[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[1,2-*c*]pyrimidine-1,10-dione (**17**) started with 2′,3′,5′-tri-*O*-acetylcytidine (**9a**) and that of 2,9-bis(2′-deoxy-β-D-ribofuranosyl)pyrimido[1″,6″:1′,2′]-imidazo[4′,5′:4,5]imidazo[1,2-*c*]pyrimidine-1,10-dione (**18**) started with 3′,5′-di-*O*-acetyl-2′-deoxycytidine (**9b**) and proceeded (through **21a,b** → **22a,b** → **23a,b** → **17**, **18**) as in the earlier examples. The course of the crucial oxidation step, **22a,b** → **23a,b**, was obvious from the resulting simplification of the proton NMR spectra due to the presence of a plane of symmetry in the N-heterocyclic portion of **23**. The structural change was substantiated by the ^{13}C NMR spectrum of **23a**, for example, in which the junctional carbons 5a and 11a appeared at 153.52 and 116.51 ppm, respectively, and the now identical carbons 4a and 6a appeared at 147.31 ppm (Experimental Section, Figure 6b). Here, as in the other cases involving final deacetylation, methanolic *tert*-butylamine (0.15 M) at 0 °C for 3 h was found to be effective and is the method of choice. Full details of the reaction conditions and the yields are given in the Experimental Section. Both **17** and **18** exhibit blue fluorescence.

Table I. Fluorescence Emission Maxima of Precursors and Final Products

compd	λ_{max}^{em} [a] nm	Φ[b]	compd	λ_{max}^{em} [a] nm	Φ[b]	compd	λ_{max}^{em} [a] nm	Φ[b]
1	422	0.14[c]	**14b**	420	0.18[c]	**19a**	418	0.06[c]
2	420	0.12[c]	**14c**	418	0.14[c]	**19b**	418	0.05[c]
3	423	0.14[c]	**15**	424	0.08[c]	**20a**	423	0.15[c]
11a	421	0.001[d]	**16**	427	0.11[c]	**20b**	424	0.15[c]
11b	419	0.002[d]	**17**	386	0.16[c]	**23a**	390	0.17[c]
14a	418	0.14[c]	**18**	390	0.15[c]	**23b**	392	0.15[c]

[a] Excitation at 325 nm in absolute ethanol. [b] Quantum yield calculated relative to coumarin in absolute ethanol, Φ = 0.56 at 325 nm. [c] ±0.02. [d] ±0.001 (standard deviations).

Compounds **17** and **18** mimic a hypothetical C–U or dC–dU "short" base pair in which the carbonyls are constrained to proximity. This is unlike the structural feature observed in natural RNA (U–U in the R17 virus[39,40]) or in a synthetic oligomer [C's between runs of poly(A) and poly(U)[41]], where the pyrimidine–pyrimidine bases are turned outward.[42] In a thorough study of mismatches by the thermodynamics of double-helix formation, pyrimidine–pyrimidine oppositions such as T·C[43] were found to be strongly destabilizing.[44] Compounds **17** and **18** do offer the advantage over intercalating models[45] of providing a fixed cross section with an established (derived)[46,47] short distance, 8.2 Å, between C1′ and C1′ of the sugar moieties. They are spatially similar to *inter*molecular T_{keto}·T_{enol} pairs (C1′–C1′ distance, 8.6 Å) observed in crystals of the hairpin hexadecamer CGCGCGTTT*T*CGCGCG.[48]

The fluorescence properties of compounds **1–3**, **15**, **16**, and **17**, **18** (Table I) render them suitable covalent cross-sectional probes with C1′–C1′ interatomic distances of approximately 10.4+, 13.0, and 8.2 Å, respectively.

We have phosphorylated these compounds, and we are attempting to incorporate them in double-helical polynucleotide sequences by a combination of enzymatic and chemical methodology. Thus incorporated, they will present three dimensionally specific types of cross section: normal, that is, within 0.2–0.3 Å of normal in width; wider, corresponding to a bulge; and narrower, corresponding to a pinching in of the double helix. Such distortions are of fundamental interest for determination of the influence of local structure on representative enzyme binding and biochemical and biophysical behavior.

Biological Studies. Compounds **1–3** and **15–18** when tested in vitro[49] for antiviral (HSV-1), antiyeast (*Saccharomyces cer-*

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evisiae), and antibacterial[50] (*Escherichia coli*, *Micrococcus luteus*, and *Bacillus subtilis*) activity were found to be inactive. This was also the case when dimethyl sulfoxide was present in the medium and/or was used as a solvent for the application of the compound to the medium. Accordingly, it is somewhat uncertain as to whether these inseparable cross-sectional compounds can be transported into cells in a normal process. All seven compounds were also found to be noncytotoxic at 10 μg/6.35 mm filter disk against the CV-1 monkey kidney cell line.[51] Furthermore, the biochemical induction assays[52] of these compounds at 10 μg/mL were found to be negative,[53] which inferred that there was no damage of the permeabilized *E. coli* DNA.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Josef Nemeth and his staff at the University of Illinois. ^1H, ^{13}C NMR, and two-dimensional HETCOR spectra were recorded on a GE-300 MHz FT NMR spectrometer in deuteriochloroform (unless otherwise mentioned) using tetramethylsilane as internal standard. All chemical shifts (δ) are reported in ppm downfield from Me_4Si and the J values are reported in hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Protons exchangeable with D_2O are abbreviated as "ex".

Mass spectra were obtained by fast atom bombardment technique with a VG ZAB-1HF mass spectrometer. Ultraviolet (UV) absorption spectra were recorded on a Beckman Acta MVI spectrophotometer. FT-IR spectra were recorded on a Nicolet 289B instrument. Fluorescence spectra were measured on a Spex Fluorolog 111C spectrofluorometer coupled with a Datamate microprocessor.

Thin-layer chromatography (TLC) was performed on plastic sheets precoated with silica gel (Merck Kieselgel 60, F254) using chloroform/methanol (9:1, v/v), A, or EtOAc/CH_2Cl_2 (1:1, v/v), B, as the solvent systems. After development, the compounds were visualized by UV light. Column chromatography separations were carried out on silica gel (Alfa silica gel, 58 μm) under pressure (flash chromatography) (6 psi). All reactions were carried out under anhydrous conditions.

Crystals of **1–3** and **15–18** obtained thus far were unsatisfactory for X-ray analysis.

Materials. Ethyl acetate was distilled from phosphorous pentoxide and stored over molecular sieves (4 Å). Acetonitrile was distilled from P_2O_5 and freshly distilled from CaH_2. Benzene was purified by distillation from sodium and stored over molecular sieves (4 Å). DMF was distilled under reduced pressure from CaH_2 and stored over molecular sieves (3 Å). *p*-Toluenesulfonic acid monohydrate was purchased from Aldrich Chemical Co. Hexafluoro-2-propanol (purchased from Aldrich) and hexafluoro-2-methyl-2-propanol (purchased from PCR Chemical) were distilled from P_2O_5. Nitromethane was distilled from P_2O_5. Adenosine, 2′-deoxyadenosine, cytidine, and 2′-deoxycytidine were purchased from Sigma Chemical Co. *tert*-Butylamine (purchased from Aldrich) was purified by distillation over NaOH pellets. Methanolic ammonia was prepared by passing anhydrous ammonia for 20 min into cold (0 °C) anhydrous methanol.

N^6-(1,1-Diethoxy-2-chloroethyl)-2′,3′,5′-tri-*O*-acetyladenosine (7a). A mixture of tri-*O*-acetyladenosine (**4a**;[8] 0.39 g, 1 mmol), chloroketene diethyl acetal (**5**)[7,54] (0.6 g, 4 mmol), and *p*-toluenesulfonic acid monohydrate (0.03 g, 0.16 mmol) in ethyl acetate (5 mL) was stirred for 16 h at room temperature under an atmosphere of nitrogen. During this period the conversion to the adduct **7a** was found to be complete as revealed by TLC (system A) of the reaction mixture. The ethyl acetate was distilled (bath temperature 40–45 °C) under reduced pressure, and excess of **5** was removed by repeated distillation with DMF (6 × 5 mL) under reduced pressure to give a pale yellow syrup. This was purified by silica gel (15 g) chromatography using CH_2Cl_2/EtOAc (3:1, v/v) to afford **7a** as a colorless thick syrup (0.5 g, 100%): R_f 0.52 (system B); ^1H NMR δ 1.23 (t, 6, J = 7.05 Hz, CH_3), 2.1, 2.13, and 2.15 (3 s, 9, $COCH_3$), 3.64 (2 q, 4, OCH_2), 4.39 (s, 2, CH_2Cl), 4.36–4.5 (m, 3, 4′-H, 5′-H), 5.69 (t, 1, 3′-H, $J_{2'3'}$ = 5.2 Hz, $J_{3'4'}$ 4.75 Hz), 5.96 (t, 1, $J_{1'2'}$ = $J_{2'3'}$ = 5.2 Hz), 6.18 (d, 1, 1′-H, J = 5.2 Hz) 6.23 (s, 1, NH, ex), 7.95 (s, 1, 8-H), 8.47 (s, 1, 2-H); low-resolution FAB MS, m/z (relative intensity) 544 (MH^+, 29), 498 (82), 394 amu (100); high-resolution FAB MS, m/z 544.1815 ($C_{22}H_{31}ClN_5O_9$ requires 544.1812 amu).

N^5-(1-Ethoxy-2-chloroethylidene)-2′,3′,5′-tri-*O*-acetyladenosine (8a).[9-11] A stirred mixture of tri-*O*-acetyladenosine (**4a**; 0.2 g, 0.5 mmol), chloroketene diethyl acetal (**5**; 0.3 g, 2 mmol), and *p*-toluenesulfonic acid (0.075g, 0.4 mmol) in ethyl acetate (8 mL) was heated at reflux (80 °C) for 15 h under an atmosphere of nitrogen. The TLC of the reaction mixture (system A) indicated the formation of a major product that was less polar than the starting material. The solution was concentrated under reduced pressure, and excess of **5** was removed by codistillation with DMF (3 × 5 mL) to give a viscous residue. The material was purified by silica gel (10 g) column chromatography using CH_2Cl_2/EtOAc (25–40%, v/v) gradient. Fractions containing **8a** were combined, concentrated under reduced pressure (rotary evaporator), and dried under high vacuum to give pure **8a** (0.24 g, 96%) as a colorless viscous oil; high-resolution FAB MS, m/z 498.1403 ($C_{20}H_{25}ClN_5O_8$ requires 498.1394 amu).

N^6-(1,1-Diethoxy-2-chloroethyl)-3′,5′-di-*O*-acetyl-2′-deoxyadenosine (7b). A mixture of 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**4b**;[13a] 0.300 g, 0.9 mmol), chloroketene diethyl acetal (**5**; 0.6 g, 4 mmol), and *p*-toluenesulfonic acid (0.03 g, 0.16 mmol) in dry ethyl acetate (10 mL) was stirred at room temperature under an atmosphere of nitrogen. TLC of the reaction mixture (system B) after 16 h revealed quantitative conversion to a less polar product. Ethyl acetate was distilled under reduced pressure, and excess of **5** was removed by codistillation in the DMF (3 × 5 mL) under reduced pressure to give a viscous residue. This was subjected to purification by silica gel (15 g) column chromatography using CH_2Cl_2/EtOAc (3:1, v/v) as the eluent to give **7b** (0.39 g, 90%) as colorless amorphous solid: R_f 0.4 (system B); ^1H NMR δ 1.23 (2 t, 6, CH_3CH_2O), 2.1 and 2.14 (2 s, 6, $COCH_3$), 2.63 (m, 1, 2′a-H), 3.0 (m, 1, 2′b-H), 3.64 (m, 4, OCH_2), 4.4 (m, 5, 4′-H, 5′-H, CH_2Cl), 5.45 (m, 2, 3′-H), 6.28 (br s, 2, NH, ex), 6.43 (2d, 1, 1′-H, J = 5.95 Hz), 7.98 (s, 1, 8-H), 8.47 (s, 1, 2-H); low-resolution FAB MS, m/z (relative intensity) 486 (MH^+, 45), 440 (90), 336 (100); high-resolution FAB MS, m/z 486.1749 ($C_{20}H_{29}ClN_5O_7$ requires 486.1758 amu).

N^6-(1-Ethoxy-2-chloroethylidene)-3′,5′-di-*O*-acetyl-2′-deoxyadenosine (8b). A stirred mixture of 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**4b**; 0.165, 0.49 mmol), chloroketene diethyl acetal (**5**; 0.3 g, 2 mmol), and *p*-toluenesulfonic acid (0.06 g, 0.3 mmol) in ethyl acetate (7 mL) was heated at 60 °C for 16 h under an atmosphere of nitrogen. The completion of the reaction was confirmed by TLC analysis (system B). The reaction mixture was concentrated under reduced pressure, and excess of **5** was removed by repeated distillation with DMF (3 × 5 mL) under reduced pressure to give a pale yellow viscous oil. This was purified by silica gel (10 g) column chromatography. Elution with CH_2Cl_2/EtOAc (3:2, v/v), gave 0.15 g (68%) of **8b** as colorless viscous material: R_f 0.26 (system B); ^1H NMR δ 1.44 (t, 3, J = 7.09 Hz), 2.1 and 2.17 (2 s, 6, $COCH_3$), 2.66 (m, 1, 2′a-H), 3.03 (m, 1, 2′b-H), 4.18 (s, 2, CH_2Cl), 4.3–4.6 (m, 5, 4′-H, 5′-H, and CH_2O), 5.45 (m, 1, H3′), 6.51 (2 d, 1′-H, $J_{1'2'a}$ = 6.04 Hz, $J_{1'2'b}$ = 6.12 Hz), 8.17 (s, 1, 8-H), 8.73 (s, 1, 2-H); low-resolution FAB MS, m/z 440.1339 ($C_{18}H_{23}ClN_5O_6$ requires 440.1335 amu).

N-[3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purin-8-yl]cytidine 2′,3′,5′-Tri-*O*-acetate (11a). Compound **8a** obtained from tri-*O*-acetyladenosine (**4a**; 6 g, 15.3 mmol), chloroketene diethyl acetal (10.5 g, 7.0 mmol), and *p*-toluenesulfonic acid (0.5 g, 2.6 mmol) was dried under high vacuum for 4 h. Then 2′,3′,5′-tri-*O*-acetylcytidine (**9a**;[12] 5.6 g, 15.3 mmol) dissolved in benzene (40 mL) and acetonitrile (40 mL) was added, and the mixture was heated at 80 °C for 48 h under nitrogen. The TLC (system A) of the reaction mixture indicated the formation of a fluorescent product along with other UV-active compounds, including unreacted **9a** and tri-*O*-acetyladenosine **4a**. The reaction mixture was concentrated to dryness on a rotary evaporator to give a dark brown residue. This was purified by flash chromatography using $CHCl_3$/MeOH (8%, v/v). The progress of chromatography was monitored by TLC of the fractions (25 mL). Fractions containing **11a** were combined and concentrated to dryness under reduced pressure and purified by recrystallization from ethyl acetate to give **11a** (0.41 g, 17%) as a pale yellow powder: mp 182–184 °C; R_f 0.43 (system A); FTIR (KBr) 3120, 1738, 1650, 1569, 1492, 1358, 1224, 1040 cm^{-1}; ^1H NMR (see Figure 3) δ 8.84 (s, 1, 5-H), 8.68 (br s, 1, 7-H), 8.21 (s, 1, 2-H), 7.45 (d, 1, J = 6.2 Hz, 6″-H), 6.46 (d, 1, J = 6.21 Hz, 5″-H), 6.27 (d, 1, J = 5.08 Hz, 1′-H), 6.14 (d, 1, J = 4.55 Hz, 1‴-H), 6.03 (dd, 1, J = 5.08, 5.32 Hz, 2″-H), 5.70 (dd, 1, J = 4.55, 5.14 Hz, 2‴-H), 5.34–5.42 (m, 2, 3′-H and 3‴-H), 4.31–4.52 (m, 6, 4′-H and 5‴-H), 2.26, 2.17, 2.12, 2.11, 2.11, and 2.05 (s, 18, $COCH_3$); ^{13}C NMR (75.2 Hz) δ 170.28, 170.18, 169.50, 169.27, 161.05 (C-4″), 139.94 (C-6″), 139.66 (C-2), 138.93 (C-3a), 137.60 (C-8), 122.92 (C-9b), 100.10 (C-7), 97.44 (C-5″), 88.87, (C-1″), 86.85 (C-1′), 79.48, 73.46, 73.14, 70.48, 70.14, 63.13, 62.98,

(49) We are grateful to Dr. Tom G. Holt for performing the antiviral, antibacterial, and cytotoxicity assays.

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(54) *Caution! Chloroketene diethyl acetal is a mutagen and should be handled with caution in a well-ventilated hood, with suitable trapping.*

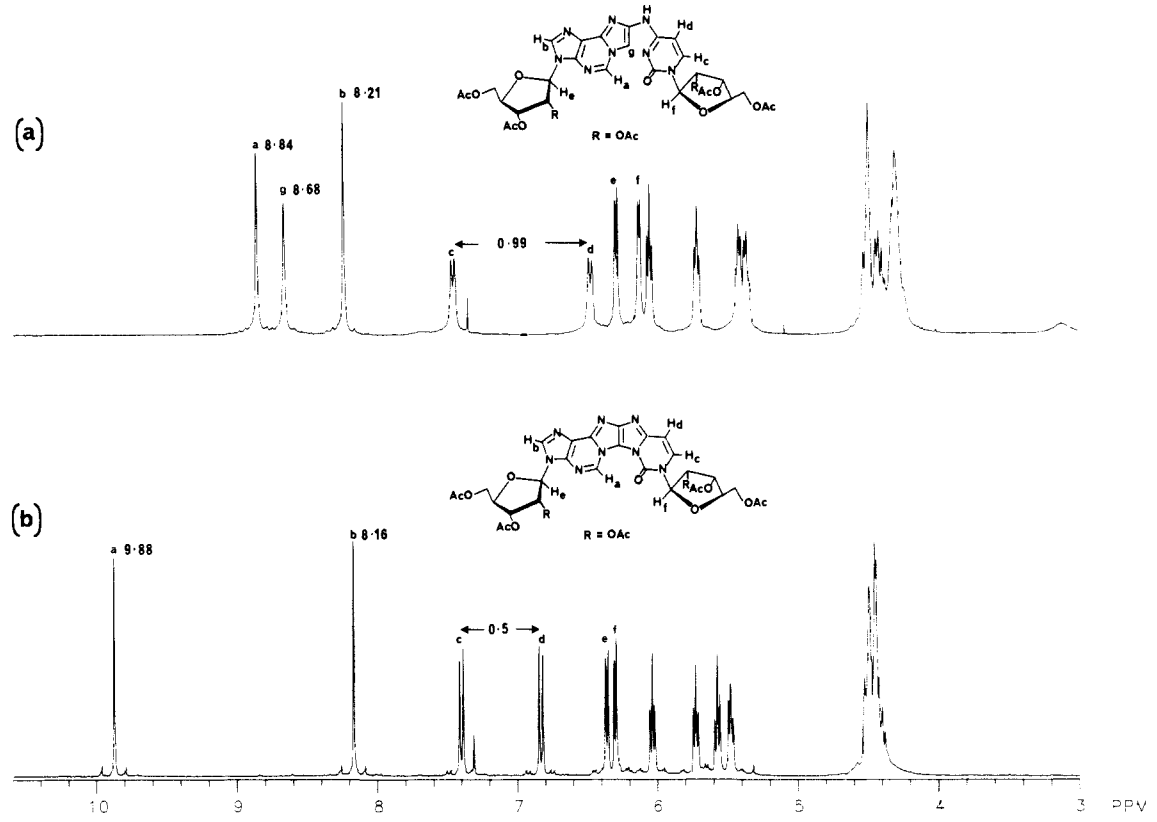

Figure 3. Comparisons of the ^{1}H NMR spectra of compounds (a) **1a** and (b) **14a**, indicating the simplification of the spectrum, the increase in the chemical shift value (δ) of the purine 2-proton (H_a), and the compression of the chemical shift difference between the pyrimidine protons (H_c and H_d) upon cyclization.

20.68, 20.64, 20.61, 20.43, 20.38, 20.36; UV λ_{max} (MeOH) 322 nm (ϵ 13 300), 291 (27 800) 254 (29 100), 247 (29 500); low-resolution FAB MS, *m/z* (relative intensity) 785 (MH^+, 85), 525 (58), 269 (100); high-resolution FAB MS, *m/z* 785.2385 ($C_{33}H_{37}N_8O_{15}$ requires 785.2378), 269.0905 ($C_{11}H_9N_8O$ requires 269.0899 amu). Anal. Calcd for $C_{33}H_{36}N_8O_{15}\cdot H_2O$: C, 49.37; H, 4.74; N, 13.99. Found: C, 49.09; H, 4.40; N, 13.96.

3,9-Bis(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (14a). To a cold (−10 °C) solution of **11a** (0.3 g, 0.38 mmol) in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol (11 mL) and nitromethane (24 mL) was added dropwise a solution of 2-nitroiodobenzene diacetate (**12**; 0.25 g, 0.68 mmol) in the same solvent mixture (8 mL) over a period of 25 min. The reaction was stirred at −10 °C for 1 h and at 0 °C for 30 min under an atmosphere of nitrogen. During this period, all the starting material had reacted to give a highly blue fluorescent product as indicated by TLC analysis. The solvents were removed by distillation at 35 °C under reduced pressure, and the residue was purified by column chromatography on silica gel (10 g) using a methanol/CH_2Cl_2 (0–2%) gradient. The fluorescent product **14a** eluted in CH_2Cl_2/MeOH (1%); the fractions were combined and concentrated under reduced pressure to give 0.16 g of amorphous material. This was purified further by column chromatography to afford 0.11 g (36%) of **14a**: R_f 0.33 (system A); IR (KBr) 1745, 1630, 1372, 1223, 1100, 1069 cm^{-1}; ^{1}H NMR (Figure 3) δ 9.88 (s, 1, 5-H), 8.17 (s, 1, 2-H), 7.37 (d, 1, J = 8.07 Hz, 10-H), 6.86 (d, 1, J = 8.03 Hz, 11-H), 6.35 (d, 1, J = 5.37 Hz, 1′-H), 6.28 (d, 1, J = 5.09 Hz, 1″-H), 6.04 (dd, 1, J = 5.24, 5.34 Hz, 2′-H), 5.72 (dd, 1, J = 4.96, 5.13 Hz, 2″-H), 5.55 (dd, 1, J = 5.61, 5.72 Hz, 3′-H), 5.47 (dd, 1, J = 4.43, 5.63 Hz, 3″-H), 4.38–4.53 (m, 6, 4′-H, 4″-H, 5′-H, 5″-H), 2.20, 2.18, 2.18, 2.15, 2.14, and 2.1 (s, 18, $COCH_3$); ^{13}C NMR (75.2 MHz) δ 170.23, 169.44, 169.18, 153.48 (C-12a), 145.74 (C-11a), 145.13 (C-8), 142.68 (C-13a), 139.45 (C-2), 138.08 (C-3a), 135.84 (C-5), 127.55 (C-10), 124.13 (C-13b), 114.55 (C-6a), 101.08 (C-11), 88.66 (C-1″), 86.90 (C-1′), 80.22, 73.22, 73.11, 70.50, 70.18, 63.01, 62.96, 20.65, 20.39, 20.31, 20.27; UV λ_{max} (MeOH) 325 nm (ϵ 11 800), 289 (23 000), 280 (23 600), 273 (23 300), 250 (26 000), 230 (25 300); low-resolution FAB MS, *m/z* (relative intensity) 783 (MH^+, 100), 525 (22), 267 (28); high-resolution FAB MS, *m/z* 783.2217 ($C_{33}H_{35}N_8O_{15}$ requires 783.2222), 525.1471 ($C_{22}H_{21}N_8O_8$, requires 525.1484), 267.0732 ($C_{11}H_7N_8O$ requires 267.0743 amu).

3,9-Di(β-D-ribofuranosyl)-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]-imidazo[2,1-*i*]purin-8(9*H*)-one (1). Method A. To a cold solution of **14a** (35 mg, 0.045 mmol) in methanol (2 mL) was added methanolic ammonia (5 mL). The reaction flask was stoppered and stirred at 0 °C for 4 h. The solution was concentrated to dryness at room temperature under reduced pressure, the residue was triturated with methanol (5 mL), and the mixture was concentrated to dryness in vacuo. The yellow substance thus obtained was dissolved in hot methanol and a few drops of water, and the solution was allowed to cool. The solid that separated was filtered, washed with methanol and dried to give 15 mg (65%) of **1**. Crystallization from water or water/ethanol afforded analytically pure product: mp 240–242 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 9.77 (s, 1, 5-H), 8.67 (s, 1, 2-H), 7.98 (d, 1, J = 7.85 Hz, 10-H), 6.93 (d, 1, J = 7.85 Hz, 11-H), 6.16 (d, 1, $J_{1',2'}$ = 4.47 Hz, 1′-H), 6.1 (d, 1, $J_{1',2'}$ = 5.38 Hz, 1″-H), 5.6 (d, 1, J = 5.88 Hz, ex, OH), 5.56 (d, 1, J = 5.4 Hz, ex, OH), 5.28 (t, 2, J = 4.86 Hz, ex, OH), 5.22 (d, 1, J = 4.89 Hz, ex, OH), 5.12 (m, 1, ex, OH), 4.62 (m, 1, 2′-H), 4.22 (m, 2, 2″-H and 3′-H), 4.1 (m, 1, 3″-H), 4.00 (m, 2, 4′-H, 4″-H), 3.80–3.60 (m, 4, 5″-H, 5″-H); FTIR (KBr) 3400, 1704, 1615, 1500, 1400, 1387, 1337, 1224, 1041 cm^{-1}; UV λ_{max} (H_2O) 350 nm (ϵ 6200), 327 (9300), 290 (12 600), 280 (12 700), 250 (15 700), 232 (17 000); low-resolution FAB MS, *m/z* (relative intensity) 531 (MH^+, 12), 399 (4), 267 (4), 157 (100); high-resolution FAB MS, *m/z* 531.1585 ($C_{21}H_{23}N_8O_9$ requires 531.1588 amu).

Method B. A mixture of **14a** (0.095 g, 0.12 mmol) in methanolic *tert*-butylamine (0.15 M, 15 mL) was stirred at 0 °C for 4 h followed by stirring at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with dry CH_2Cl_2, filtered, and washed thoroughly with ethanol and dried to give 50 mg (78%) of **1**, recrystallized from aqueous ethanol.

2′-Deoxy-*N*-[3-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purin-8-yl]cytidine 3′,5′-Di-*O*-acetate (11b). The synthesis was similar to that for **11a**. Compound **8b** made from 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**4b**; 5 g, 14.9 mmol), chloroketene diethyl acetal (**5**; 9 g, 60 mmol), and *p*-toluenesulfonic acid (0.15 g, 0.8 mmol) in ethyl acetate (85 mL) was dissolved in benzene (60 mL), and *p*-toluenesulfonic acid (0.15 g, 0.8 mmol), 3′,5′-di-*O*-acetyl-2-deoxycytidine (**9b**;[13b] 4.6 g, 14.8 mmol), and dichloromethane (30 mL) were added. The resulting mixture was heated to 80 °C under nitrogen for 48 h. The TLC analysis (system A) revealed the presence of several UV-active products. One of the products was fluorescent with slightly higher R_f value than that of **4b**. The solution was filtered, and the residue was washed thoroughly with benzene/CH_2Cl_2 (1:1) and dried to give 2.3 g of **9b**. The filtrate was concentrated under reduced pressure to a thick syrup and purified by flash chromatography using a methanol/chloro-

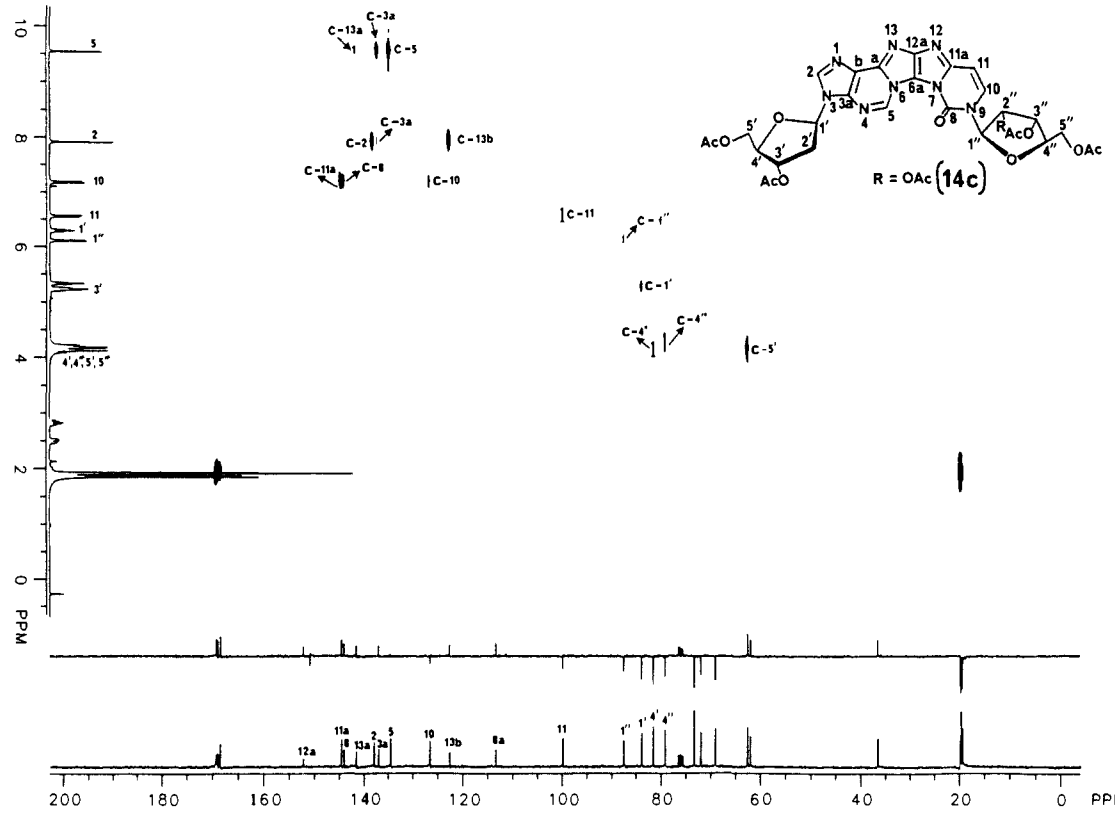

Figure 4. Assignments of the ^{13}C NMR signals for **14c** by proton coupling.

form (0–8%, v/v) gradient. The desired product **11b** eluted after the ethoxyetheno derivative **6b**. The fractions containing **11b** were combined and concentrated under reduced pressure to give a brown amorphous substance. This was purified twice by flash chromatography using $CHCl_3$/MeOH (9%, v/v) to give 0.465 of **11b** as amorphous but homogeneous material. Further purification by recrystallization from aqueous ethanol gave analytically pure **11b**: mp 130–132 °C; R_f 0.48 (system A); FTIR (KBr) 3120, 1750, 1650, 1570, 1500, 1360, 1230, 1110, 1055, 940, 780 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 10.91 (s, 1, ex, NH), 8.84 (s, 1, 4-H), 8.71 (s, 1, 7-H), 8.26 (s, 1, 2-H), 7.58 (d, 1, J = 6.25 Hz, 6″-H), 6.48–6.55 (dd, 2, 1′-H, 1″-H, J = 6.41, 7.79 Hz), 6.34 (d, 1, J = 6.25 Hz, 5″-H), 5.51 (m, 1, 3′-H), 5.22 (m, 1, 3‴-H), 4.30–4.50 (m, 6, 4′-H and 4″-H and 5′-H and 5‴-H), 3.11 and 2.73 (m, 4, 2′-H and 2‴-H), 2.17, 2.12, 2.08, and 2.05 (s, 12, $COCH_3$); UV λ_{max} (MeOH) 340 nm (ϵ 12 700), 298 (29 700), 290 (30 200), 253 (31 900), 247 (31 800); low-resolution FAB MS, m/z (relative intensity) 669 (MH^+, 55), 469 (58), 269 (100); high-resolution FAB MS, m/z 669.2289 ($C_{29}H_{33}N_8O_{11}$ requires 669.2271), 469.1580 ($C_{20}H_{21}N_8O_6$ requires 469.1586 amu).

3,9-Bis(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (14b). The oxidation procedure was similar to that used for **14a**. The reaction mixture was stirred at −10 °C under a nitrogen atmosphere for 1.5 h when the TLC (system A) of the reaction mixture indicated complete conversion to a highly fluorescent product. The solvents were distilled under reduced pressure (bath temperature <35 °C), and the residue was charged on a silica gel column. Elution with CH_2Cl_2/MeOH (0–2%, v/v) gradient gave **14b** (56%) as a pale yellow amorphous material: R_f 0.36 (system A); FTIR (KBr) 3100, 1740, 1690, 1640, 1620, 1500, 1370, 1340, 1230, 1100 cm^{-1}; ^1H NMR ($(CD_2)_2SO$) δ 9.80 (s, 1, 5-H), 8.17 (s, 1, 2′-H), 7.45 (d, 1, J = 8.08 Hz, 10-H), 6.76 (d, 1, J = 8.08 Hz, 11-H), 6.60 (m, 2, 1′-H, 1″-H), 5.50 (m, 1, 3′-H), 5.37 (m, 1, 3″-H), 4.3–4.5 (m, 6, 4′-H, 4″-H, 5′-H, 5″-H), 3.05–2.2 (m, 4, 2′-H and 2″-H), 2.18, 2.16, 2.12 (s, 12, $COCH_3$); ^{13}C NMR (75.2 MHz) δ 170.29, 170.17, 153.12 (C-12a), 145.76 (C-11a), 144.90 (C-8), 142.71 (C-13a), 138.75 (C-2), 138.07 (C-3a), 135.58 (C-5), 126.82 (C-10), 123.82 (C-13b), 114.36 (C-6a), 100.55 (C-11), 86.41 (C-1″), 84.85 (C-1′), 82.68, 82.62, 74.40, 74.09, 63.67, 63.63, 37.74, 37.93, 20.89, 20.72, 20.67; UV λ_{max} (MeOH) 324 nm (ϵ 8800), 289 (17 500), 280 (17 800), 272 (17 200), 249 (24 700), 230 (18 400); low-resolution FAB MS, m/z (relative intensity) 667 (MH^+, 20), 467 (15), 119 (100); high-resolution FAB MS, m/z 667.2108 ($C_{29}H_{31}N_8O_{11}$ requires 667.2105), 267.0735 ($C_{11}H_7N_8O$ requires 267.0743 amu).

3,9-Bis(2′-deoxy-β-D-ribofuranosyl)-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (2). Methods A and B were used for the deacetylation as in the preparation of **1**. A better yield (72%) was obtained with methanolic *tert*-butylamine than with methanolic ammonia: mp >300 °C; ^1H NMR ($(CD_3)_2SO$) δ 9.74 (s, 1, 5-H), 8.62 (s, 1, 2-H), 7.92 (d, 1, J = 7.88 Hz, 10-H), 6.91 (d, 1, J = 7.88 Hz, 11-H), 6.53 (d, 2, $J_{1',2'}$ = 6.01 Hz, 1′-H and 1″-H), 5.40 (m, 2, ex, OH), 5.17 (br s, 1, ex, OH), 5.02 (br s, 1, ex, OH), 4.46 (m, 1, 3′-H), 4.35 (m, 1, 3″-H), 3.91 (m, 2, 4′-H and 4″-H), 3.8–3.43 (m, 4, 5′-H and 5″-H), 2.8–2.2 (m, 4, 2′-H and 2″-H); UV λ_{max} (H_2O) 350 nm (ϵ 15 000), 332 (21 600), 280 (12 000), 272 (13 700), 232 (28 600); low-resolution FAB MS, m/z (relative intensity) 499 (MH^+, 22), 383 (6), 267 (10), 119 (100); high-resolution FAB MS, m/z 499.1695 ($C_{21}H_{23}N_8O_7$ requires 499.1690 amu).

***N*-[3-(3,5-Di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purin-8-yl]cytidine 2′,3′,5′-Tri-*O*-acetate (11c).** This was prepared from **4b** and **9a** in a condensation similar to that used for **11b**. The solvents were removed by distillation under reduced pressure, and the residue was purified by flash chromatography using a $CHCl_3$/MeOH (7–10%, v/v) gradient. The progress of separation was monitored by TLC (system A). The desired product **11c** eluted soon after the ethoxyetheno derivative **6b**. Fractions containing **11c** were combined, concentrated under reduced pressure, and subjected twice to flash chromatography using $CHCl_3$/MeOH (7%, v/v). Recrystallization from ethanol furnished an analytically pure sample: mp 142–143 °C; R_f 0.48 (system A); FTIR (KBr) 3120, 1739, 1647, 1563, 1499, 1365, 1224, 1041 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 10.48 (s, 1, ex, NH), 8.81 (s, 1, 5-H), 8.68 (s, 1, 7-H), 8.18 (s, 1, 2-H), 7.49 (d, 1, J = 7.26 Hz, 6″-H), 6.52 (dd, 1, J = 7.02, 6.66 Hz, 1′-H), 6.39 (d, 1, J = 7.26 Hz, 5″-H), 6.17 (d, 1, $J_{1',2'}$ = 4.18 Hz, 1‴-H), 5.5–5.37 (m, 3, 2‴-H, 3′-H), 4.49–4.3 (m, 6, 4′-H, 4‴-H, 5′-H, 5‴-H), 3.1–3.05 (m, 1, 2′a-H), 2.72–2.66 (m, 1, 2′b-H), 2.17, 2.11, 2.1, and 2.07 (s, 15, $COCH_3$); UV λ_{max} (MeOH) 330 nm (ϵ 12 500), 291 (28 700), 252 (29 000), 247 (29 300); fluorescence λ^{em}_{max} 418 nm, λ^{ex}_{max} 325 nm (absolute ethanol); low-resolution FAB MS, m/z (relative intensity) 727 (MH^+, 100), 527 (12), 469 (32), 269 (80); high-resolution FAB MS, m/z 727.2310 ($C_{31}H_{35}N_8O_{13}$ requires 727.2324), 469.1598 ($C_{20}H_{21}N_8O_6$ requires 469.1586), 269.0904 ($C_{11}H_9N_8O$ requires 269.0902 amu).

3-(3′,5′-Di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (14c). Compound **14c** was synthesized by the same oxidation method that was used for **14a** and **14b** in 25% yield: R_f 0.36 (system A); IR (KBr) 1746, 1670, 1630, 1570, 1492, 1365, 1224, 1048 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 9.75 (s, 1, 5-H), 8.1 (s, 1, 2-H), 7.33 (d, 1, J = 8.03 Hz, 10-H), 6.75 (d, 1, J = 8.03 Hz, 11-H), 6.48 (dd, 1, $J_{1',2'}$ = 6.9 Hz, 1′-H), 6.28 (1, d, J = 5.46 Hz, 1″-H), 5.51 (dd, 1, $J_{1',2'}$ = 5.46 Hz, $J_{2',3'}$ = 5.63 Hz, 2″-H), 5.44–5.38 (m, 2, 3′-H,

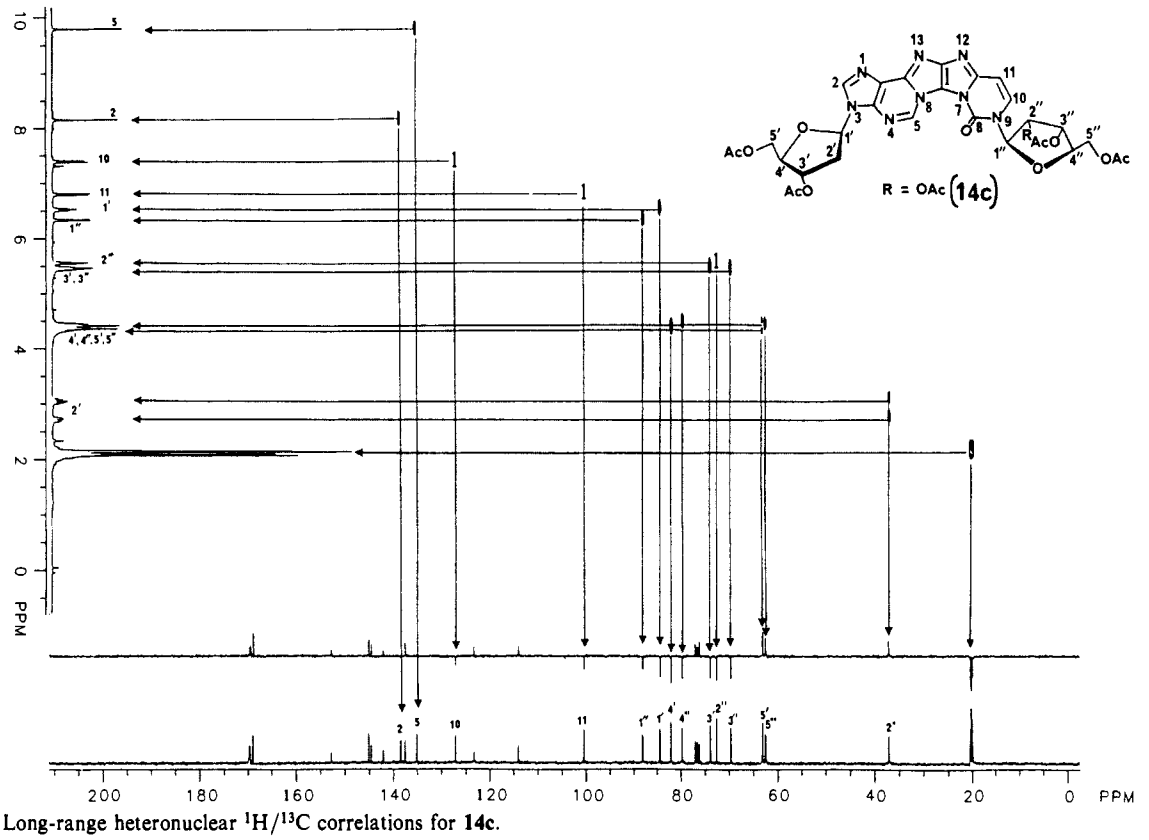

Figure 5. Long-range heteronuclear $^1H/^{13}C$ correlations for **14c**.

3″-H), 4.41–4.26 (m, 6, 4′-H, 4″-H), 5′-H, 5″-H), 3.02–2.95 (m, 1, 2′a-H), 2.7–2.63 (m, 1, 2′b-H), 2.11, 2.06, and 2.03 (s, 15, $COCH_3$); ^{13}C NMR δ 170.31, 170.15, 170.01, 169.46, 153.26 (C-12a), 145.59 (C-11a), 145.06 (C-8), 142.67 (C-13a), 138.93 (C-2), 138.00 (C-3a), 135.54 (C-5), 127.50 (C-10), 123.72 (C-13b), 114.44 (C-6a), 100.98 (C-11), 88.56 (C-1″), 84.85 (C-1′), 82.51 (C-4′), 80.15 (C-4″), 74.34 (C-3′), 73.02 (C-2″), 70.11 (C-3″), 63.60 (C-5′), 62.98 (C-5″), 37.54 (C-2′), 20.81, 20.67, 20.65, 20.38, 20.33 (see Figures 4–6); UV λ_{max} (MeOH) 325 nm (ϵ 12 500), 290 (23 200), 273 (23 200), 250 (27 300), 230 (26 200); low-resolution FAB MS, m/z (relative intensity) 725 (MH^+, 80), 525 (30), 467 (12), 267 (88), 119 (100); high-resolution FAB MS, m/z 725.2154 ($C_{31}H_{33}N_8O_{13}$ requires 725.2167), 525.1487 ($C_{21}H_{21}N_8O_8$ requires 525.1482), 267.0741 ($C_{10}H_7N_8O$ requires 267.0743 amu).

3-(2′-Deoxy-β-D-ribofuranosyl)-9-(β-D-ribofuranosyl)-3*H*-pyrimido-[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (3). Methods A and B were used for the deacetylation of the pentaacetate as with the hexaacetate **14a** and tetraacetate **14b**. A better yield (78%) was obtained with methanolic *tert*-butylamine than with methanolic ammonia: mp 252 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 9.76 (s, 1, 5-H), 8.62 (s, 1, 2-H), 7.96 (d, 1, J = 8.04 Hz, 10-H), 6.92 (d, 1, J = 8.04 Hz, 11-H), 6.54 (dd, 1, $J_{1',2'}$ = 6.7 Hz, 1′-H), 6.16 (d, 1, $J_{1',2'}$ = 5.05 Hz, 1″-H), 5.55 (1, d, J = 5.4 Hz, ex, OH), 5.39 (d, 1, J = 4.18 Hz, ex, OH), 5.26 (t, 1, J = 4.93 Hz, ex, OH), 5.21 (d, 1, J = 5.14 Hz, ex, OH), 5.00 (t, 1, J = 5.39 Hz, ex, OH), 4.46 (m, 1, 2″-H), 4.22 (m, 1, 3′-H), 4.08 (m, 1, 3″-H), 3.98 (m, 1, 4′-H), 3.73 (m, 1, 4″-H), 3.8–3.5 (m, 4, 5′-H and 5″-H), 2.76 (m, 1, 2′a-H), 2.43 (m, 1, 2′b-H); FTIR (KBr) 3400, 1697, 1619, 1500, 1485, 1407, 1337, 1302, 1224, 1076, 740 cm^{-1}; UV λ_{max} (H_2O) 326 nm (ϵ 10 700), 288 (13 200), 280 (13 800), 250 (17 100), 230 (19 100); low-resolution FAB MS, m/z (relative intensity) 515 (MH^+, 10), 399 (5), 267 (10), 119 (100); high-resolution FAB MS, m/z 515.1633 ($C_{21}H_{23}N_8O_8$ requires 515.1638 amu).

***N*-[3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purin-8-yl]adenosine 2′,3′,5′-Tri-*O*-acetate (19a).** A mixture of 2′,3′,5-tri-*O*-acetyladenosine (**4a**; 6 g, 15.3 mmol), chloroketene diethyl acetal (**5**; 9 g, 60 mmol), and *p*-toluenesulfonic acid (0.4 g, 2 mmol) in ethyl acetate (125 mL) was stirred at room temperature for 16 h under nitrogen atmosphere. The TLC analysis (system A) revealed quantitative conversion to **7a**. The ethyl acetate was distilled under reduced pressure, and excess of **5** was removed by repeated distillation with DMF (6 × 15 mL) to give a pale yellow syrup. This was dried in vacuo for 3 h and dissolved in benzene (60 mL), and **4a** (6 g, 15.3 mmol) and *p*-toluenesulfonic acid (0.4 g, 2 mmol) were added. This mixture was dissolved by the addition of CH_2Cl_2 (40 mL) and acetonitrile (20 mL) and heated at 80 °C under nitrogen. After 48 h an additional 0.8 g (4 mmol) of *p*-toluenesulfonic acid was added. The heating was continued for another 24 h, when TLC of the reaction mixture revealed that most of the chloroimidate had reacted. The solution was concentrated under reduced pressure, when some **4a** separated. The mixture was cooled and filtered, and the residue was washed thoroughly with benzene/CH_2Cl_2 (3:1) and dried to give 5.5 g of unreacted **4a**. The filtrate and the washings were combined and concentrated, when more of **4a** crystallized. Concentration, filtration, washing with benzene/CH_2Cl_2 (3:1), and drying yielded another 3.0 g. The filtrate and washings were combined and concentrated under reduced pressure, and the residue was purified by flash chromatography using $CHCl_3$/MeOH (6%, v/v). The desired product **19a** had slightly higher R_f value than that of **4a** and was fluorescent. Various fractions containing **19a** were combined and concentrated under reduced pressure on a rotary evaporator to give 1 g of amorphous material. Further purification by flash chromatography using $CHCl_3$/MeOH (8%, v/v) and recrystallization from ethanol afforded **19a** as a pale yellow powder: mp 123–125 °C; R_f 0.38 (system A); ^1H NMR ($CDCl_3$) δ 9.67 (s, 1, ex, NH), 8.87 (s, 1, 8″-H), 8.53 (s, 1, 7-H), 8.15 (s, 1, 2-H), 6.37 (d, 1, $J_{1',2'a}$ = 5.76 Hz, 1′-H), 6.27 (d, 1, $J_{1',2'b}$ = 5.17 Hz, 1‴-H), 6.17 (dd, 1, J = 5.73 Hz, 2′-H), 6.07 (dd, 1, J = 5.35 Hz, 2″-H), 5.82 (dd, 1, J = 5.12, 4.35 Hz, 3′-H), 5.73 (dd, 1, J = 4.99, 4.88 Hz, 3‴-H), 4.48 (m, 6, 4′-H, 4‴-H, 5′-H, 5‴-H), 2.05, 2.09, 2.11, 2.16, and 2.17 (s, 18, $COCH_3$); ^{13}C NMR ($CDCl_3$) 170.29, 169.46, 169.34, 152.62 (C-2″), 150.49 (C-6″), 149.69 (C-4″), 142.06 (C-8), 141.07 (C-8′), 139.51 (C-2), 138.62 (C-3a), 137.87 (C-9a), 134.90 (C-5), 123.43 (C-9b), 120.78 (C-5′), 97.63 (C-7), 86.72 (C-1″), 85.76 (C-1′), 80.35 and 80.12 (C-4′ and C-4″), 73.08 and 73.03 (C-2′ and C-2″), 70.69 and 70.55 (C-3′ and C-3″), 63.15 and 63.05 (C-5′ and C-5″), 20.62, 20.54, 20.43, 20.28; UV λ_{max} (MeOH) 320 nm (ϵ 11 300), 286 (33 900), 252 (30 900); low-resolution FAB MS, m/z (relative intensity) 809 (MH^+, 60), 551 (21), 293 (42), 119 (100); high-resolution FAB MS, m/z 809.2487 ($C_{34}H_{37}N_{10}O_{14}$ requires 809.2490 amu).

3,10-Bis(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*,10*H*-purino-[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purine (20a). To a cold (−10 °C) solution of **19a** (0.27 g, 0.334 mmol) in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-propanol and nitromethane (25 mL, 1:5 M) was added dropwise a solution of 2-nitroiodobenzene diacetate (0.19 g, 0.52 mmol) in 5 mL of the same solvent mixture. The solution was stirred at −10 °C under nitrogen for 1 h and at room temperature for 30 min. Solvents were removed by distillation under reduced pressure (bath temperature, 35 °C), and the dark residue was purified by flash chromatography using $CHCl_3$/MeOH (9%, v/v) as solvent. Fractions containing the fluorescent product **20a** were combined, concentrated under reduced pressure on a rotary evaporator, and dried under high vacuum to give

0.115 g (45%) as amorphous material that was homogeneous on TLC: R_f 0.20 (system A); ^{1}H NMR ($CDCl_3$) δ 9.46 (s, 1, 5-H), 7.86 (s, 1, 2-H), 6.2 (d, $J_{1',2'}$ = 4.21 Hz, 1'-H), 5.9 (dd, 1, $J_{2',3'}$ = 4.67 Hz, 2'-H), 5.57 (dd, 1, $J_{3',4'}$ = 4.96 Hz, 3'-H), 4.39–4.22 (m, 3, 4'-H and 5'-H), 2.05, 2.05, 2.04 (s, 9, $COCH_3$); ^{13}C NMR (75.2 MHz) ($CDCl_3$) δ 170.41, 169.56, 152.51 (C-13a), 141.45 (C-12b and C-14a), 138.81 (C-11), 137.51 (C-3a), 133.52 (C-5), 122.48 (C-12a), 111.41 (C-6a), 86.58 (C-1'), 79.89 (C-4'), 73.74 (C-2'), 70.36 (C-3'), 62.96 (C-5'), 20.59, 20.41; UV λ_{max} (MeOH) 320 nm (ϵ 10400), 276 (80900), 236 (19700); low-resolution FAB MS, m/z (relative intensity) 807 (MH^+, 32), 549 (22), 291 (30), 19 (100); high-resolution FAB MS, m/z 807.2355 ($C_{34}H_{35}N_{10}O_{14}$ requires 807.2337 amu).

3,10-Di(β-D-ribofuranosyl)-3*H*,10*H*-purino[1'',6'':1',2']imidazo-[4',5':4,5]imidazo[2,1-*i*]purine (15). Either methanolic ammonia or methanolic *tert*-butylamine could be used for deacetylation of **20a** to **15** (yield ≤83%): mp 275 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 9.98 (s, 1, 5-H), 8.71 (s, 1, 2-H), 6.14 (d, 1, $J_{1',2'}$ = 5.43 Hz, 1'-H), 5.63 (d, 1, J = 5.8 Hz, ex, OH), 5.33 (d, 1, J = 4.90 Hz, ex, OH), 5.16 (t, 1, J = 5.18 Hz, OH), 4.66 (m, 1, 2'-H), 4.23 (m, 1, 3'-H), 4.02 (m, 1, 4'-H), 3.72 (m, 1, 5'-H), 3.64 (m, 1, 5'-H); UV λ_{max} (H_2O) 322 nm (ϵ 12400), 275 (58500), 270 (57900), 238 (20500); low-resolution FAB MS, m/z (relative intensity) 555 (MH^+, 12), 423 (10), 291 (5), 119 (100); high-resolution FAB MS, m/z 555.1703 ($C_{22}H_{23}N_{10}O_8$ requires 555.17029 amu).

2'-Deoxy-*N*-[3-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purin-8-yl]adenosine 3',5'-Di-*O*-acetate (19b). A mixture of 3',5'-di-*O*-acetyl-2'-deoxyadenosine (**4b**; 5 g, 14.9 mmol), chloroketene diethyl acetal (**5**; 9 g, 60 mmol), and *p*-toluenesulfonic acid (2.6 mmol) in ethyl acetate (125 mL) was stirred at room temperature for 16 h under nitrogen. After the removal of ethyl acetate under reduced pressure, the excess of **5** was removed by repeated codistillation with DMF (6 × 15 mL), and the residue was dried in vacuo for 4 h. This material was dissolved in dry benzene (50 mL), and *p*-toluenesulfonic acid (0.9 g, 4.7 mmol) and **4b** (5 g, 14.9 mmol) were added. The resulting mixture was dissolved by the addition of CH_2Cl_2 (15 mL) and CH_3CN (15 mL) and was heated at 80 °C for 48 h under nitrogen. The TLC (system A) of the reaction mixture revealed the presence of several UV-active products of which the major component was **4b**. There were two fluorescent spots, and one had a slightly higher R_f value than that of **4b**. The solvents were removed under reduced pressure, and the residue was triturated with ethyl acetate, which caused some of the unreacted **4b** to separate. The mixture was cooled and filtered, and the solid was washed with ethyl acetate and dried to give 3.0 g of **4b**. The filtrate and the washings were combined and concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel using chloroform/methanol (7.5%, v/v). The desired product **19b** eluted after the ethoxyetheno derivative **6b**. The fractions containing **19b** were combined and concentrated in vacuo to give brown amorphous material. Further elution of the column gave 4.5 g of **4b**. Compound **19b** was again purified by flash chromatography on silica gel using chloroform/methanol (8%, v/v) to afford 0.41 g of amorphous material. Crystallization from aqueous alcohol gave **19b** as a pale yellow powder: mp 143–145 °C; R_f 0.33 (system A); ^{1}H NMR ($CDCl_3$) δ 9.34 (s, 1, ex, NH), 8.82 (s, 1, 8''-H), 8.76 (s, 1, 5-H), 8.68 (s, 1, 2''-H), 8.57 (s, 1, 7-H), 8.16 (s, 1, 2-H), 6.58 (dd, 1, $J_{1',2'a}$ = 5.89 Hz, $J_{1',2'b}$ = 8.61 Hz, 1'-H), 6.52 (t, 1, $J_{1',2'a}$ = 5.80, $J_{1',2'b}$ = 8.68 Hz, 1'''-H), 5.58 (m, 1, 3'-H), 5.50 (m, 1, 3'''-H), 4.40 (m, 6, 4'-H, 4'''-H, 5'-H, 5'''-H), 3.23, 3.02, and 2.68 (m, 4, 2'-H, 2'''-H), 2.17, 2.16, 2.12, and 2.05 (s, 12 $COCH_3$); UV λ_{max} (MeOH) 320 nm (ϵ 12300), 286 (32900), 250 (29900); low-resolution FAB MS, m/z (relative intensity) 693 (MH^+, 30), 493 (22), 293 (61), 119 (100); high-resolution FAB MS, m/z 693.2361 ($C_{30}H_{33}N_{10}O_{10}$ requires 693.2381 amu). Anal. Calcd for $C_{30}H_{32}N_{10}O_{10}\cdot H_2O$: C, 50.70; H, 4.64; N, 19.74. Found: C, 50.60; H, 4.48; N, 19.93.

3,10-Bis(3',5'-di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)-3*H*,10*H*-purino[1'',6'':1',2']imidazo[4',5':4,5]imidazo[2,1-*i*]purine (20b). To a stirred cold (−10 °C) solution of **19b** (0.28 g, 0.4 mmol) in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol and nitromethane (1:2, v/v; 25 mL) was added dropwise a solution of 2-nitroiodobenzene diacetate (0.22 g, 0.6 mmol) in 7 mL of the same solvent mixture. The reactants were stirred at −10 °C for 1 h and at room temperature for 30 min under nitrogen, when the TLC (system A) of the reaction mixture indicated complete conversion of **19b**. The solvents were removed by distillation under reduced pressure (bath temperature 35 °C) and the dark residue was purified by flash chromatography using chloroform/methanol (8%, v/v). The progress of separation was followed by TLC of the fractions (10 mL). Fractions containing **20b** were combined, concentrated under reduced pressure, and finally dried to give 0.10 g (35%) as homogeneous material, which was recrystallized from methanol: mp 133–135 °C; R_f 0.14 (system A); ^{1}H NMR ($CDCl_3$) δ 9.28 (s, 1, 5-H), 8.03 (s, 1, 2-H), 6.44 (dd, 1, $J_{1',2'a}$ = 6.61 Hz, $J_{1',2'b}$ = 6.81 Hz 1'-H), 5.45 (m, 1, 3'-H), 4.38 (m, 3, 4'-H and 5'-H), 3.07 and 2.79 (m, 2, 2'-H), 2.15 and 2.07 (s, 6, $COCH_3$); ^{13}C NMR (75.2 MHz) ($CDCl_3$) δ 170.47, 170.34, 153.20 (C-13a), 133.21 (C-5), 122.82 (C-12a), 111.48 (C-6a), 84.81 (C-1), 82.45 (C-4'), 74.32 (C-3'), 63.71 (C-5'), 37.47 (C-2'), 20.88, 20.71; UV λ_{max} (MeOH) 320 nm (ϵ 10400), 276 (80900), 237 (19400); low-resolution FAB MS, m/z (relative intensity) 691 (MH^+, 90), 491 (73), 291 (100); high-resolution FAB MS, m/z 691.2221 ($C_{30}H_{31}N_{10}O_{10}$ requires 691.2227 amu).

3,10-Bis(2'-deoxy-β-D-ribofuranosyl)-3*H*,10*H*-purino[1'',6'':1',2']imidazo[4',5':4,5]imidazo[2,1-*i*]purine (16). Deacetylation of **20b** was accomplished with methanolic ammonia at 0 °C for 1 h, 20 °C for 3 h (68%), or with methanolic *tert*-butylamine at 0 °C, 20 °C for 2 h (66%). Purification was effected by crystallization from aqueous methanol to give colorless **16**: mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 9.93 (s, 1, 5-H), 8.65 (s, 1, 2-H), 6.58 (dd, 1, $J_{1',2'}$ = 6.53 Hz, 1'-H), 5.42 (d, 1, J = 2.55 Hz, ex, 3'-OH), 5.02 (b, 1, J = 4.66 Hz, ex, 5'-OH), 4.49 (m, 1, 3'-H), 3.94 (m, 1, 4'-H), 3.66 (m, 1, 5'-H), 3.59 (m, 1, 5'-H), 2.82 (m, 1, 2'-H), 2.45 (m, 1, 2'-H); FTIR (KBr) 3400, 1633, 1499, 1471, 1393, 1351, 1323, 1217, 1182, 1147, 1083, 1048, 921, 631 cm^{-1}; UV λ_{max} (H_2O) 322 nm (ϵ 13000), 274 (65100), 270 (64000), 238 (22600); low-resolution FAB MS, m/z (relative intensity) 523 (MH^+, 18), 407 (10), 291 (18), 155 (100); high-resolution FAB MS, m/z 523.1811 ($C_{22}H_{23}N_{10}O_6$ requires 523.1802 amu).

***N*⁴-(1-Ethoxy-2-chloroethylidene)-3',5'-di-*O*-acetyl-2'-deoxycytidine (21b).** A mixture of 3',5'-di-*O*-acetyl-2'-deoxycytidine (**9b**;[13b] 0.3 g, 0.96 mmol) and chloroketene diethyl acetal (**5**; 0.6 g, 4 mmol) in acetonitrile (8 mL) was stirred under an atmosphere of nitrogen at room temperature for 16 h. During the period, conversion to the chloroimidate **21b** was complete as revealed by TLC. The solution was concentrated under reduced pressure, and excess of **5** was removed by codistillation with DMF (5 × 5 mL) of the reaction mixture under reduced pressure to give a thick syrupy material. This was purified by silica gel (15 g) column chromatography. Elution with CH_2Cl_2/EtOAc (30%, v/v) gave **21b** (0.35 g, 84%) as a pale yellow viscous oil: R_f 0.25 (system B); ^{1}H NMR δ 1.37 (t, 3, J = 7.1 Hz), 2.09 and 2.12 (2 s, 6, $COCH_3$) 2.8–2.9 (m, 2, 2'-H), 4.2–4.33 (m, 5, 4'-H', 5'-H, and CH_2O), 4.37 (s, 2, CH_2Cl), 5.23 (m, 1, 3'-H), 6.09 (d, 1, J = 7.16 Hz, 5-H), 6.27 (dd, 1, $J_{1',2'}$ = 5.71 Hz, 1'-H), 7.9 (d, 1, J = 7.16 Hz, 4-H); high-resolution FAB MS, m/z 416.1223 ($C_{17}H_{23}ClN_3O_7$ requires 416.1225 amu).

***N*-[5,6-Dihydro-5-oxo-6-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[1,2-*c*]pyrimidin-2-yl]cytidine 2',3',5'-Tri-*O*-acetate (22a).** A mixture of tri-*O*-acetylcytidine (**9a**; 4.0 g, 10.8 mmol) and chloroketene diethyl acetal (**5**; 6.6 g, 44 mmol) in acetonitrile (100 mL) was stirred at room temperature for 16 h under a nitrogen atmosphere. Acetonitrile was removed by distillation under reduced pressure, and excess of **5** was removed by codistillation under reduced pressure with DMF (8 × 10 mL) to give a thick syrup. This was dried under high vacuum for 3 h, more **9a** (4 g, 10.8 mmol) was added, together with *p*-toluenesulfonic acid (0.25 g, 0.68 mm). The mixture was dissolved by the addition of benzene (40 mL) and acetonitrile (10 mL) and heated at 80 °C for 20 h under nitrogen. TLC (system A) of the reaction mixture indicated the formation of a fluorescent product that had a higher R_f value than that of **9a**. The reaction mixture was concentrated to dryness on a rotary evaporator to give a dark brown residue that was purified by flash chromatography using $CHCl_3$/MeOH (5%, v/v) as the eluent. The progress of chromatography was followed by TLC analysis (system A) of the fractions (25 mL). Fractions containing **22a** were pooled and concentrated under reduced pressure to give 1.06 g of amorphous substance, which was contaminated with other minor impurities. Further elution of the silica gel column gave 3.7 g of unreacted **9a**. Compound **22a** was again purified by flash chromatography using $CHCl_3$/MeOH (5%, v/v) and recrystallized from ethanol/water to give 0.75 g (17%) as a pale yellow, fluffy substance: mp 138 °C; R_f 0.50 (system A); FTIR (KBr) 1732, 1682, 1619, 1492, 1379, 1218, 1041 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 360 MHz) δ 10.77 (s, 1, NH, ex), 8.09 (s, 1), 7.84 (d, 1, J = 7.30 Hz), 7.61 (d, 1, J = 7.9 Hz), 6.77 (d, 1, J = 7.94 Hz), 6.24 (d, 2, $J_{1',2'}$ = 5.4 Hz, 1'-H), 6.19 (d, 1, J = 7.62 Hz), 5.96 (d, 1, $J_{1',2'}$ = 4.9 Hz, 1'-H), 5.60–5.34 (m, 4, 2'-H and 3'-H), 4.40–4.26 (m, 6, 4'-H and 5'-H), 2.11 and 2.06 (s, 18, $COCH_3$); UV λ_{max} (MeOH) 310 nm (ϵ 22500), 256 (17800), 237 (23600); fluorescence λ^{em}_{max} 417 nm, λ^{ex}_{max} 325 nm (absolute ethanol); low-resolution FAB MS, m/z (relative intensity) 761 (MH^+, 28), 503 (24), 245 (100); high-resolution FAB MS, m/z 761.2257 ($C_{32}H_{37}N_6O_{16}$ requires 761.2266 amu). Anal. Calcd for $C_{32}H_{36}N_6O_{16}\cdot 0.5H_2O$: C, 50.00; H, 4.81; N, 10.94. Found: C, 50.08; H, 4.76; N, 10.72.

2,9-Bis(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrimido[1'',6'':1',2']-imidazo[4',5':4,5]imidazo[1,2-*c*]pyrimidine-1,10(2*H*,9*H*)-dione (23a). To a cold (−10 °C) solution of **22a** (0.3 g, 0.4 mmol) in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol (11 mL) and nitromethane (24 mL) was added dropwise a solution of 2-nitroiodobenzene diacetate

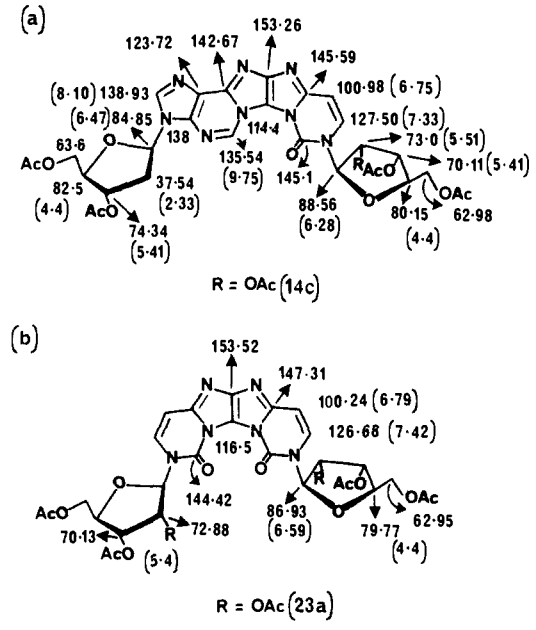

Figure 6. Graphic ^{13}C NMR assignments for (a) **14c** and (b) **23a**. ^{1}H NMR assignments are in parentheses.

(0.25 g, 0.68 mmol) in the same solvent mixture (7 mL). The system was stirred at −10 °C under nitrogen for 1 h, followed by stirring at 0 °C for 30 min. TLC analysis (system A) of the reaction mixture indicated almost complete conversion to a fluorescent product with a higher R_f (0.52) value than the starting material. The solvents were removed by distillation under reduced pressure, and the residue was purified by column chromatography on silica gel (10 g, Brinkmann) using a $CHCl_3$/MeOH (0–2%, v/v) gradient to give 0.16 g of **23a** as amorphous material. This was further purified by silica gel (5 g) chromatography using a $CHCl_3$/MeOH (0–2%, v/v) gradient to afford 0.102 g (34%) of **23a** as a pale yellow solid. Crystallization from CH_2Cl_2/MeOH provided analytically pure **23a** as colorless needles: mp 208–209 °C; R_f 0.52 (system A); FTIR (KBr) 1739, 1626, 1358, 1218, 1063 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.42 (d, 1, J = 8.02 Hz, 3-H), 6.79 (d, 1, J = 8.02 Hz, 4-H), 6.59 (d, 1, $J_{1',2'}$ = 5.43 Hz, 1'-H), 5.4 (m, 2, 2'-H and 3'-H), 4.43 (m, 3, 4'-H and 5'-H's), 2.20, 2.13, 2.08 (s, 9, $COCH_3$); ^{13}C NMR (75.2 MHz) δ 169.88, 169.32, 153.52 (C-5a), 147.31 (C-6a), 144.42 (C-1), 126.68 (C-3), 116.51 (C-11a), 100.24 (C-4), 86.93 (C-1'), 79.77 (C-4'), 72.87 (C-2'), 70.13 (C-3'), 62.95 (C-5'), 20.59, 20.26; UV λ_{max} (MeOH) 350 nm (ϵ 14 650), 333 (19 700), 322 (16 000), 253 (24 000), 216 (24 300); low-resolution FAB MS, m/z (relative intensity) 759 (MH^+, 32), 501 (18), 267 (33), 155 (100); high-resolution FAB MS, m/z 759.2103 $C_{32}H_{35}N_6O_{16}$ requires 759.2100 amu). Anal. Calcd for $C_{32}H_{34}N_6O_{16}\cdot H_2O$: C, 49.48; H, 4.62; N, 10.82. Found: C, 49.48; H, 4.19; N, 10.66.

2,9-Di(β-D-ribofuranosyl)pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]-imidazo[1,2-*c*]pyrimidine-1,10(2*H*,9*H*)-dione (17). A suspension of **23a** (30 mg, 0.04 mmol) in methanolic *tert*-butylamine (0.15 M, 5 mL) was stirred at 0 °C for 3 h and then at room temperature for 2 h. The reaction mixture was cooled and the gelatinous precipitate was filtered, washed with cold methanol, and dried in vacuo to give 16 mg of colorless product, which was recrystallized from aqueous ethanol to give **17** (12 mg, 60%) as a powder: mp 219–222 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 7.86 (d, 1, J = 7.92 Hz), 6.86 (d, 1, J = 7.92 Hz), 6.12 (d, 1, $J_{1',2'}$ = 4.87 Hz, 1'-H), 5.51 (1, d, J = 4.8 Hz, OH, ex), 5.22 (m, 2, OH, ex), 4.18 (m, 1, 2'-H), 4.06 (m, 1, 3'-H), 3.95 (m, 1, 4'-H), 3.65 (m, 2, 5'-H); FTIR (KBr) 3400, 3200, 1696, 1604, 1393, 1091 cm^{-1}; UV λ_{max} (H_2O) 350 nm (ϵ 12 900) (sh), 333 (16 600), 256 (20 900), 220 nm (19 100); low-resolution FAB MS, m/z (relative intensity) 507 (MH^+, 24), 375 (11), 243 (21); high-resolution FAB MS, m/z 507.1490 ($C_{20}H_{23}N_6O_{10}$ requires 507.1476 amu).

2′-Deoxy-*N*-[6-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)-5,6-dihydro-5-oximidazo[1,2-*c*]pyrimidin-2-yl]cytidine 3′,5′-Di-*O*-acetate (22b). A solution of 3′,5′-di-*O*-acetyl-2′-deoxycytidine (**9b**;[13b] 5 g, 16 mmol) and chloroketene diethyl acetal (**5**; 10 g, 67 mmol) in acetonitrile (125 mL) was stirred at room temperature for 21 h under an atmosphere of nitrogen. Acetonitrile was removed by distillation under reduced pressure, and excess of **5** was removed by repeated codistillation with DMF (6 × 15 mL) under reduced pressure. The residue was dried under high vacuum for 5 h, dissolved in a mixture of benzene (40 mL) and acetonitrile (20 mL). *p*-Toluenesulfonic acid (0.3 g, 0.16 mmol) was added, and the mixture was heated at 60 °C for 40 h under nitrogen. TLC (system A) of the reaction mixture indicated the formation of a fluorescent product (R_f 0.43, system A) along with other products. Most of the starting material remained unreacted. The reaction mixture was concentrated under reduced pressure to a thick syrup, charged onto a silica gel column (300 g), and eluted with CH_2Cl_2/MeOH (6%). The progress of separation was followed by TLC (system A) of the various fractions (25 mL). Appropriate fractions (as revealed by fluorescence) were combined and concentrated to dryness under reduced pressure to afford 1 g of pale yellow **22b**. Crystallization from CH_2Cl_2/pentane afforded 0.85 g (17%) of **22b** as analytically pure material: mp 220 °C; R_f 0.43 (system A); IR (KBr) 1750, 1700, 1625, 1565, 1407, 1370, 1240, 1225, 1120 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 10.65 (s, 1, NH, ex), 8.10 (s, 1), 7.80 (d, 1, J = 7.46 Hz), 7.55 (d, 1, J = 7.95 Hz), 6.74 (d, 1, J = 7.90 Hz), 6.45 (dd, 1, $J_{1',2'a}$ = 7.32 Hz, $J_{1',2'b}$ = 6.72 Hz, 1'-H), 6.22 (dd, 1, $J_{1',2'a}$ = 7.7 Hz, $J_{1',2'b}$ = 6.15 Hz, 1'-H), 6.19 (d, 1, J = 7.01 Hz), 5.23 (m, 2, 3'-H), 4.28–4.22 (m, 6, 4'-H and 5'-H), 2.47–2.42 (m, 4, 2'a-H and 2'b-H), 2.4, 2.37, 2.35, 2.32 (s, 12, $COCH_3$); UV λ_{max} (MeOH) 310 nm (ϵ 22 600), 257 (18 900), 238 (24 400); fluorescence λ^{em}_{max} 424 nm, λ^{ex}_{max} 325 nm (EtOH); low-resolution FAB MS, m/z (relative intensity) 645 (MH^+, 70), 445 (40), 245 (100); high-resolution FAB MS, m/z 645.2139 ($C_{28}H_{33}N_6O_{12}$ requires 645.2156 amu). Anal. Calcd for $C_{28}H_{32}N_6O_{12}\cdot 0.5H_2O$: C, 51.53; H, 5.06; N, 12.88. Found: C, 51.39; H, 5.09; N, 12.65.

2,9-Bis(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)pyrimido-[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[1,2-*c*]pyrimidine-1,10(2*H*,9*H*)-dione (23b). To a cold (−10 °C) solution of **22a** (0.13 g, 0.2 mmol) in a solvent mixture of 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol (5 mL) and nitromethane (11 mL) was added dropwise a solution of 2-nitroiodobenzene diacetate (0.11 g, 0.3 mmol) in 4 mL of the same solvent mixture. The mixture was stirred under nitrogen at −10 °C. After 1.5 h the TLC (system A) of the reaction mixture indicated almost complete conversion to a slightly less polar, distinctly blue fluorescent compound. The solution was allowed to warm to ambient temperature for 15 min, and the solvents were removed (bath temperature 40 °C) under reduced pressure. The residue was dried in vacuo and purified by column chromatography on silica gel (5 g, Brinkmann 0.05–0.2 mm) using a CH_2Cl_2/MeOH (0–2%, v/v) gradient. The progress of separation was monitored by TLC (system A) of 5-mL fractions. Appropriate fractions containing the blue fluorescent product were combined, concentrated under reduced pressure, and dried under high vacuum for 3 h to yield 72 mg (56%) of pale yellow **23b**: R_f 0.47; FTIR (KBr) 1740, 1630, 1365, 1220, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, 1, J = 8.05 Hz, 3-H), 6.76 (d, 1, J = 8.05 Hz, 4-H), 6.60 (dd, 1, $J_{1',2'}$ = 5.34 Hz), 5.26 (m, 1, 3'-H), 4.37 (m, 3, 4'-H), 2.73 and 2.21 (m, 2, 2'a-H and 2'b-H), 2.14 and 2.13 (s, 6, $COCH_3$); ^{13}C NMR (75.2 MHz) δ 170.05, 153.57, 147.56, 144.15, 126.36, 116.02, 99.54, 86.51, 82.43, 74.15, 63.61, 38.17, 20.67; UV λ_{max} (MeOH) 350 nm (ϵ 10 000), 332 (11 400), 256 (22 600), 218 (19 800); fluorescence λ^{em}_{max} 392 nm, λ^{ex}_{max} 325 nm, Φ = 0.15 (ethanol) (relative to coumarin in ethanol, Φ = 0.56 at λ^{ex}_{max} = 325 nm); low-resolution FAB MS, m/z (relative intensity) 643 (65), 443 (25), 243 (88), 119 (100); high-resolution FAB MS, m/z 643.2008 ($C_{28}H_{31}N_6O_{12}$ requires 643.2001 amu).

2,9-Bis(2′-deoxy-β-D-ribofuranosyl)pyrimido[1″,6″:1′,2′]imidazo-[4′,5′:4,5]imidazo[1,2-*c*]pyrimidine-1,10(2*H*,9*H*)-dione (18). The deacetylation of **23b** was best accomplished with the preservation of syn geometry[55] by the use of 0.2 M *tert*-butylamine in methanol at −5 to −10 °C for 2 h: mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.81 (d, 1 J = 7.96 Hz), 6.74 (d, 1, J = 7.96 Hz), 6.42 (dd, 1, $J_{1',2'a}$ = 6.57 Hz, $J_{1',2'b}$ = 6.4 Hz, 1'-H), 5.32 (d, 1, J = 4.05 Hz, ex, 3'-OH), 5.12 (t, 1, J = 4.98 Hz, ex, 5'-OH), 4.30 (m, 1, 3'-H), 3.89 (m, 1, 4'-H), 3.64 (m, 2, 5'-H), 2.24 (m, 2, 2'-H); UV λ_{max} (H_2O) 350 nm (ϵ 12 700), 332 (16 900), 320 (13 500), 255 (21 500), 220 nm (21 900); low-resolution FAB MS, m/z 475, 359, 243; high-resolution FAB MS, m/z 475.1577 ($C_{20}H_{23}N_6O_8$ requires 475.1577 amu).

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