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Synthesis and Properties of New Nucleotide Analogues Possessing Squaramide Moieties as New Phosphate Isosters

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Dedicated to Professor Wojciech J. Stec on the occasion of his 65th birthday

Keywords: Squaric acid / Acidity / Nucleotide analogues / Cyclic nucleotide analogues

New analogues of 2′-deoxynucleotides and ribonucleotides incorporating a unique squaramide structure were synthesized. Because of the strong acidity of this moiety (pK_a = 2.3), these nucleotide analogues exist in a monoanionic form, which can be regarded as an electronic isoster of 5′-nucleotides under physiological conditions. The synthesis of the nucleotide analogues was achieved through the condensation of 5′- or 3′-aminonucleosides with dimethyl squarate, whilst the selective removal of the methyl group was effectively accomplished by treatment with sodium bromide. In addition, we also synthesized 3′,5′-cyclic nucleotide analogues from the 3′,5′-diazidonucleoside derivatives. NMR analysis revealed that their ribose puckering was of an N-type form, identical to that in cAMP and cGMP. Because of the unique structural, electronic, and conformational properties of squaramide-type nucleotide analogues, these analogues should be quite interesting as potential biologically active compounds such as antiviral and anticancer agents.
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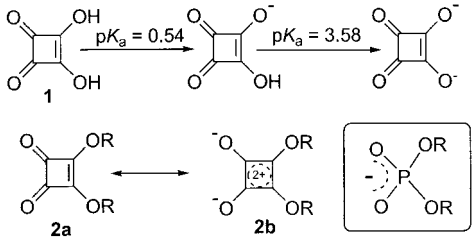
Introduction

Squaric acid (**1**) is a bibasic acid with a cyclobutene-3,4-dione structure (Scheme 1). The unique properties of squaric acid derivatives (**2a**) are characterized by the highly polarized carbonyl group; this is attributable to the stability of the resonance structure (**2b**),[1] which is aromatic according to the Hückel rule (number of π electrons = $4n + 2$: n = 0 for **2b**). Such a highly polarized electronic structure, together with the strong acidity of squaric acid (pK_{a1} = 0.54; pK_{a2} = 2.2),[2] makes such derivatives useful in medicinal chemistry as isoelectronic isosters of carboxylic acids[3] and sulfonic acids.[4] Recently, we have proposed similarities in the electronic structures of squaric acid and phosphoric acid, and have reported the synthesis of artificial DNAs containing 3′–5′ and 2′–5′ squaryl dimide linkages, which displayed an unusual bent structure and G–T mismatch stabilization, respectively.[5,6] The potential of squaric acid as a phosphate mimic has also been proposed by Seto and co-workers[7] in the development of protein tyrosine phosphatase inhibitors.



Scheme 1. Structures of squaric acid derivatives.

In this paper we report the synthesis of new 5′- and 3′-nucleotide analogues utilizing the properties of squaric acid described above for the replacement of the phosphoric acid residues of natural nucleotides with squaric acid skeletons. In view of the chemical instability of squaric acid esters under hydrolytic conditions, we designed squaramide derivatives of nucleosides **3**–**9**, as shown in Scheme 2. Although Glusenkamp and co-workers[8] have reported the synthesis of an 5′-adenosine squaramide derivative by condensation of the corresponding 5′-aminonucleoside and diethyl squarate, the removal of the ethyl group and the synthesis of other nucleotide derivatives have not been reported. Because of the importance of nucleotides – 5′,3′-, and 5′,3′-cyclic nucleotides, for example – in living systems, many nucleotide and cyclic nucleotide analogues[9] of phosphorus-modified compounds have been developed as biochemical tools and drugs.[10] There has, however, been little study

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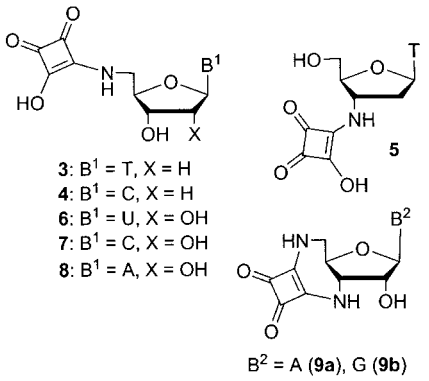
of analogues not containing phosphorus atoms, especially on analogue design involving carbon acids.[11] A new class of nucleotide analogues, such as that reported in this paper, should therefore be useful as biochemical and molecular biological tools and for pharmaceutical applications.



Scheme 2. Structures of squaramide-type nucleotide analogues.

Results and Discussion

pK_a of *N*-Methylsquaramide

Although the acidity of squaric acid has been well characterized,[2] there has been no reported quantitative study of the pK_a values of squaric acid derivatives such as squaryl monoamides. In order to confirm the similarity between squaramide compounds and phosphates, the pK_a value of *N*-methylsquaramide (**10**) was measured by alkaline titration. As shown in Figure 1, compound **10** proved to be a monobasic acid and its pK_a was determined to be 2.3. Comparison of the pK_a of **10** with that of methyl phosphate (pK_a < 1) revealed that the acidity of the former was weaker than that of the latter, but it should be noted that the above pK_a did indicate the existence of **10** in monoanionic form under physiological conditions. Because phosphate monoesters also exist in their monoanionic forms under physiological conditions, the results suggest the potential of the squaramide moiety as a phosphate mimic.

Synthesis of 5′-Squaramides of Deoxynucleosides and of Thymidine 3′-Squaramide

First of all, to establish a general procedure for the synthesis of 5′-nucleotide analogues **3**, the synthesis of the 5′-thymidylate analogue was carried out as shown in Scheme 3. The squaryl group was introduced onto the amino group of 5′-amino-5′-deoxythymidine (**11a**)[12] by condensation with dimethyl squarate[13,14] in the presence of a catalytic amount of *N*,*N*-diisopropylethylamine (DIEA), as shown in Scheme 3. The reaction was complete within 15 min and the target compound **12a** was obtained in 87% yield.

Next, removal of the methyl group was examined. Because the esters of squaric acid are fairly stable to aqueous acids we first tested the deprotection under mild alkaline conditions. The methyl group could be removed at room temperature within 4 h to give **3** in 57% yield. Although the hydrolysis of a small portion of the squaramide moiety was also observed during the reaction, the target material could be separated from the side-products by reversed-phase column chromatography.

The synthesis of 2′-deoxycytidine derivative **4** is also shown in Scheme 3. The 5′-amino derivative[15] **11b** was condensed with dimethyl squarate to give the methyl squarate derivative **12b** in 66% yield. The methyl ester of **12b** was cleaved by treatment with aqueous NaOH to give the desired compound **4** in 77% yield.

Next, as an example of the 3′-nucleotide analogue, thymidine 3′-squaramide (**5**) was synthesized as shown in Scheme 4. Treatment of 5′-*O*-DMTr-3′-amino-3′-deoxythymidine (**13**)[16] with dimethyl squarate gave the squaramide derivative **14** in 76% yield. Subsequently, the DMTr group of **14** was removed by treatment with 80% acetic acid to give the 5′-hydroxy derivative **15** in 60% yield. Finally, com-

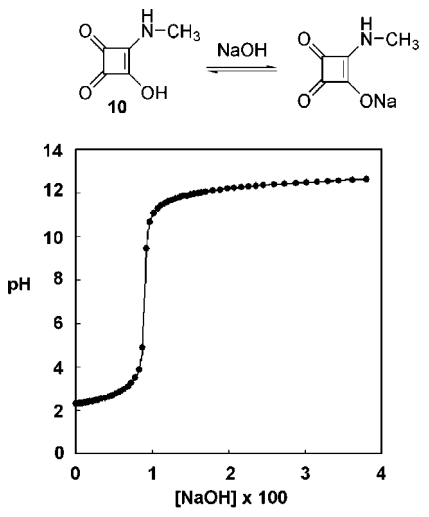
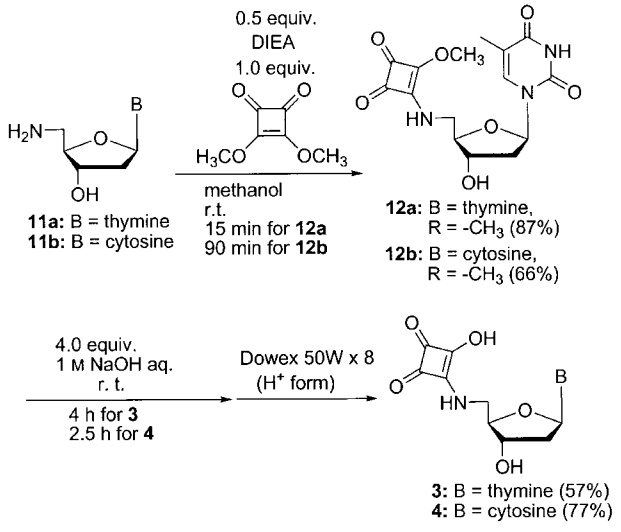


Figure 1. Alkaline titration of squaramide.



Scheme 3. Synthesis of deoxynucleoside 5′-phosphate analogues.

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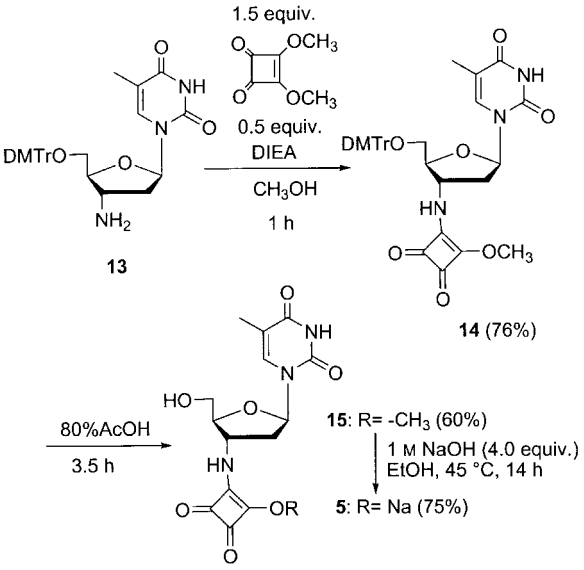
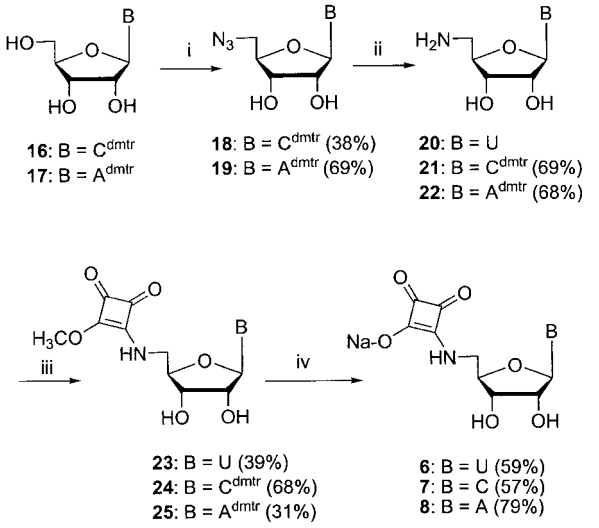
pound **15** was converted to thymidine 3′-squaramide (**5**) in 75% yield by alkaline hydrolysis at 45 °C.



Scheme 4. Synthesis of thymidine 3′-phosphate analogues.



Reagents and conditions: i. PPh_3 (1.2 equiv.)-LiN_3 (5.0 equiv.)-CBr_4 (1.2 equiv.), r.t., 1 h. ii. PPh_3 (2.0 equiv.), pyridine, r.t., 6 h for **21** and 24 h for **22**. iii. dimethyl squarate (1.0 equiv.), DIEA (0.5 equiv.), CH_3OH, r. t. 1 h for **23** and **24**, 3 h for **25**. iv. NaI (1.2 equiv.), 2-hexanone, 120 °C, 13 h for **6**, 19 h for **7**, 15 h for **8**.

Scheme 5. Synthesis of ribonucleoside 5′-phosphate analogues.

Synthesis of Ribonucleoside 5′-Squaramides

In addition to the deoxynucleotide analogues described above, we also tried to synthesize the 5′-ribonucleotide analogues **6**–**8** (Scheme 2). Because ribonucleosides have higher polarities than deoxynucleosides, we employed the 4,4′-dimethoxytrityl (DMTr) group for protection of the exocyclic amino groups of cytosine and adenine, to increase the lipophilicity of these materials. Introduction of the lipophilic DMTr group allowed the purification of synthetic intermediates by silica gel chromatography; the reaction scheme is shown in Scheme 5. As an example, N^4-(dimethoxytrityl)-cytidine (**16**) was converted into the 5′-azide derivative **18** by treatment with PPh_3-LiN_3/CBr_4.[17] The azide group of **18** was converted into the amino derivative **21** by treatment with PPh_3. Substitution of dimethyl squarate with the amino group of **21** gave the squaramide derivative **24** in 68% yield.

For the synthesis of the ribonucleotide analogues, we searched for reaction conditions suitable for the removal of both the DMTr group and the methyl group in order to simplify the reaction scheme. Previously, several researchers had reported removal of methyl groups from methyl phosphate derivatives by use of sodium iodide under anhydrous conditions.[18] Because of the isoelectronic properties of phosphoric acid and squaric acid, we expected that this procedure should also be effective for removal of methyl groups from the squaric acid derivatives. Consequently, we found that the methyl group of **24** was removed by treatment with NaI in hexan-2-one at 120 °C to give the sodium salt of the desired uridine 5′-squaramide **9** in 57% yield. It should be noted that the DMTr group could also be removed during this procedure, probably due to the instability of this protecting group at such high temperature.

Other 5′-ribonucleoside derivatives **6** and **8** were synthesized by essentially the same procedure. In the case of the uridine derivative **6**, the base-unprotected derivative **20**[12] could be used because of the less polar nature of uridine.

Analogues of cAMP and cGMP with Squardiamide Linkages

Finally, we tried to synthesize cAMP and cGMP analogues incorporating squaryl diamide linkages. The key intermediates, the 3′,5′-diazido-3′,5′-dideoxynucleosides **28** and **29**, were synthesized by the procedure reported by Gottikh,[19] with a slight modification. In our modified procedure, we employed bis(trifluoromethylsulfonyl) derivative **26** in place of the di(methylsulfonyl) derivative reported in the original procedure. The use of the triflic acid moiety as a leaving group enabled unfavorable olefin formation to be reduced and the yield of **27** improved. Compound **27** was further converted into the diazidonucleosides **28** and **29** by the original procedure.[19] The base moieties of **28** and **29** were protected with DMTr groups to give the DMTr derivatives **30** and **31**, and the azide compounds were converted into the amino derivatives **32** and **33** by use of triphenylphosphane. In spite of the highly polar natures of the diamino structures of **32** and **33**, they could be purified by silica gel column chromatography, because of the lipophilicity of the DMTr group. Subsequent condensation of **32** and **33** with dimethyl squarate gave the DMTr derivatives of cAMP (**34**) and cGMP (**35**) analogues, which could be converted into the desired products, **9a** and **9b**, by removal of the DMTr groups (Scheme 6).

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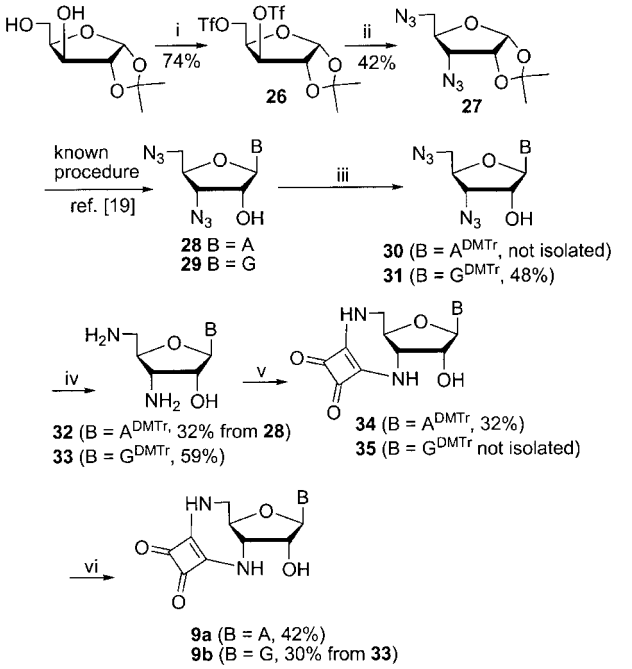

Reagents and conditions: i. *N,N*-diisopropylethylamine (2.7 equiv.), Tf_2O (2.8 equiv.), CH_2Cl_2, -78 °C, 1 h. ii. LiN_3 (5.0 equiv.), DMF, 40 °C, 4 h. iii. TMSCl (2.5 equiv.), pyridine, r.t. 1 h, then DMTrCl (1.2 equiv.), r.t., 24 h for **30** and 8.5 h for **31**. iv. PPh_3 (4.0 equiv.), pyridine, r.t., 14 h for **32** and 24 h for **33**, then 28% NH_3, r. t. v. dimethyl squarate (1.0 equiv.), DIEA (0.5 equiv.), CH_3OH, r.t., 1 h for **34**, 2 h for **35**. vi. 1%TFA/CH_2Cl_2 7 h for **9a**,18 h for **9b**

Scheme 6. Synthesis of cyclic nucleotide analogues.

Conformation Properties of the Cyclic Nucleotide Analogue 9b

As described above, the 2′-deoxyribo- and ribonucleotide analogues **3**–**8** and the cyclic nucleotide analogues **9a** and **9b** were successfully synthesized. The cyclic nucleotide analogues differ from naturally occurring cyclic AMP and GMP in that the oxygen atoms at the 5′- and the 3′-positions are replaced by nitrogen atoms and the phosphorus atoms are replaced by a cyclobutene ring. It should therefore be interesting to elucidate the effects of these two modifications on the sugar conformations of the cyclic nucleotide analogues. The proton–proton coupling constants $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ of the ribose moieties in cAMP and cGMP have been reported to be 0.7–0.8 Hz, 5.2–5.7 Hz, and 9–10 Hz, respectively.[20] The splitting pattern of these cyclic nucleotides cAMP and cGMP is characteristic of N-type sugar puckering. Significantly, the coupling constants $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ in **9b** were quite similar to those of the cyclic nucleotides and proved to be 0 Hz, 5.4 Hz, and 9.0 Hz, respectively. This splitting pattern is characteristic of the N-type conformation. These results suggested that the change of the two hydroxy groups on the ribose ring and the phosphorus atom for the squaramide-type structure produced a ribose conformation identical to that in the 3′,5′-cyclic nucleotide.

Conclusions

We have synthesized new analogues of 2′-deoxynucleotides and ribonucleotides incorporating a unique squaramide structure. Because of the strong acidity (pK_a = 2.3) of squaramide, these nucleotide analogues exist in monoanionic forms, which should act under physiological conditions as electronic isosters of 5′-nucleotides. The synthesis of the nucleotide analogues **3**–**8** was achieved by the condensation of 5′- or 3′-aminonucleosides with dimethyl squarate. The selective removal of the methyl group was effectively carried out by treatment with sodium iodide. We also synthesized 3′,5′-cyclic nucleotide analogues **9a** and **9b**, starting from the 3′,5′-diazidonucleoside derivatives. NMR analysis of **9b** showed its ribose puckering to have the N-type conformation, which is identical to that in cAMP and cGMP. Because of these unique structural, electronic, and conformational properties of squaramide-type nucleotide analogues, these analogues are quite interesting as potential biologically active compounds such as antiviral and anticancer agents. Biological studies of the compounds synthesized in this study are in progress and will be reported elsewhere.

Experimental Section

General Remarks: ^1H and ^{13}C spectra were obtained on a Varian Unity INOVA instrument at 500 and 126 MHz, respectively. The chemical shifts were measured from tetramethylsilane in $CDCl_3$ (δ = 0 ppm), DMF in [D_7]DMF (δ = 2.76 ppm), DMSO in [D_6]-DMSO (δ = 2.49 ppm), and DDS in D_2O (δ = 0 ppm) for ^1H NMR, and $CDCl_3$ (δ = 77.0 ppm), [D_7]DMF (δ = 162.5 ppm), [D_6]-DMSO (δ = 39.7 ppm) and dioxane in D_2O (δ = 67.4 ppm) for ^{13}C NMR spectroscopy. Column chromatography was performed with Wako silica gel C-200. Amino-modified silica gel was purchased from Fuji-silycia Co., Ltd. Recycle HPLC was performed on a JALGEL GS-310 column with use of CH_3CN as a solvent. TLC was performed with Merck silica gel 60 (F_{254}) plates. ESI mass spectra were measured on a Mariner™ instrument.

***N*-Methylsquaramide (10):** Diethyl squarate (1 g, 5.9 mmol) was dissolved in ethanol (30 mL). Methylamine (40% in methanol, 0.6 mL, 5.9 mmol) was added, and the resulting solution was stirred for 15 min at ambient temperature. The solvents were removed under reduced pressure, and the residue was dissolved in NaOH (1 M, 5.9 mL, 5.9 mmol). After 12 h, the solution was concentrated under reduced pressure and was then diluted with methanol (10 mL). Dowex 50 W × 8 (H^+ form, 5.9 mmol) was added and removed by filtration. The solvent was removed under reduced pressure to give **10** (5.7 mmol, 97%). ^1H NMR (270 MHz, [D_6]-DMSO): δ = 3.30 (s, 3 H), 4.59 (br., 1 H) ppm. ^{13}C NMR (67.8 MHz, [D_6]DMSO): δ = 29.7, 182.0, 188.4 ppm. ESI-MS calcd. for $C_5H_5NO_3$ [M + H]$^+$: 127.0269; found 127.0270.

General Procedure 1. Reactions between 5′- or 3′-Aminonucleoside and Dimethyl Squarate

5′-Amino-5′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-5′-deoxythymidine (12a): 5′-Amino-5′-deoxythymidine (**11a**, 300 mg, 1.2 mmol) was dissolved in methanol (4 mL), and *N*,*N*-diisopropylethylamine (110 μL, 0.62 mmol) and 3,4-dimethoxy-3-cyclobutene-1,2-dione (dimethyl squarate, 180 mg, 1.2 mmol) were added. The resulting solution was stirred at ambient temperature for 15 min.

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The solvent was removed under reduced pressure, and the residue was chromatographed on a column of silica gel with chloroform/methanol (100:6, v/v) to give **12a** (380 mg, 87%). ^{1}H NMR (270 MHz, D_2O): δ = 2.27–2.43 (m, 2 H), 3.46–3.87 (m, 3 H), 4.15, 4.18 (2×s, 3 H), 4.29, 4.42 (m, 1 H), 6.01–6.06 (t, 1 H), 7.20, 7.22 (2×s, 1 H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 12.4, 12.5, 40.1, 47.1, 61.1, 72.2, 72.4, 86.1, 86.3, 86.5, 111.8, 137.8, 138.0, 152.1, 166.1, 174.9, 178.2, 185.0, 185.3, 189.4 ppm. ESI-MS calcd. for $C_{15}H_{17}N_3O_7$ [M + H]$^+$: 351.1067; found 351.1216.

General Procedure 2. Synthesis of Nucleoside 5′-Squarate by Alkaline Hydrolysis. 5′-Amino-5′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-5′-deoxythymidine (3) from 12a: Compound **12a** (100 mg, 0.28 mmol) was dissolved in methanol (2.0 mL), aqueous NaOH (1 M, 1.1 mL, 1.1 mmol) was added, and the resulting solution was stirred at ambient temperature for 4 h. The reaction mixture was neutralized by addition of Dowex 50W×8 (2 mL, free form). The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a C18 reversed-phase column with CH_3CN/H_2O (100:5, v/v) to give **3** (54 mg, 57%). ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 1.76 (s, 3 H), 2.03–2.05 (m, 2 H), 3.59–3.79 (m, 3 H), 4.19 (m, 1 H), 6.09–6.14 (t, ^3J(H,H) = 6.8 Hz, 1 H), 7.19 (br., 1 H), 7.45 (s, 1 H), 11.26 (br., 1 H) ppm. ^{13}C NMR (67.8 MHz, CD_3OD): δ = 12.1, 44.5, 70.4, 83.7, 85.7, 109.6, 135.64, 150.3, 163.6, 181.2, 188.3 ppm. ESI-MS calcd. for $C_{14}H_{16}N_3O_7$ [M + H]$^+$: 338.0988; found 338.0998.

5′-Amino-5′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-2′,5′-dideoxycytidine (12b): Compound **11b** (100 mg, 0.44 mmol) was converted into the title compound **12b** (120 mg, 66%) as described in General Procedure 1. ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 1.89–2.12 (m, 2 H), 3.44–3.68 (m, 2 H), 3.80 (m, 1 H), 4.14 (m, 1 H), 4.26 (2×s, 3 H), 5.36 (br., 1 H), 5.72–5.74 (m, 1 H), 6.11–6.16 (t, $^3J_{H,H}$ = 6.8 Hz, 1 H), 7.16, 7.23 (br., 2 H), 7.51 (d, $^3J_{H,H}$ = 7.3 Hz, 1 H), 8.76, 8.96 (br., 1 H) ppm. ^{13}C NMR (67.8 MHz, [D$_6$]DMSO): δ = 45.8, 46.2, 60.0, 60.2, 70.8, 79.2, 84.6, 84.8, 94.2, 140.8, 154.8, 165.3, 172.0, 172.5, 176.9, 177.5, 182.1, 182.3, 188.9, 189.3 ppm. ESI-MS calcd. for $C_{14}H_{17}N_4O_6$ [M + H]$^+$: 337.1148; found 337.1078.

5′-Amino-5′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-2′,5′-dideoxycytidine (4): Compound **12b** (100 mg, 0.3 mmol) was converted into the title compound **4** (74 mg, 77%) as described in General Procedure 2. ^{1}H NMR (270 MHz, D_2O): δ = 2.13–2.35 (m, 2 H), 3.56–3.81 (m, 2 H), 3.90–3.97 (m, 1 H), 4.14–4.33 (m, 1 H), 5.90 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 6.03, 6.07 (2×t, $^3J_{H,H}$ = 6.3 Hz, 6.7 Hz, 1 H), 7.50 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H) ppm. ^{13}C NMR (67.8 MHz, [D$_6$]-DMSO): δ = 41.0, 41.2, 47.3, 51.5, 51.7, 61.9, 73.2, 87.3, 87.5, 88.3, 88.6, 98.6, 98.9, 133.2, 143.5, 143.9, 153.3, 158.9, 159.1, 167.8, 175.7,184.0, 188.8, 190.4, 197.4, 205.4 ppm. ESI-MS calcd. for $C_{13}H_{14}N_4O_6Na$ [M + Na]$^+$: 345.0811; found 345.0835.

3′-Amino-5′-*O*-(4,4′-dimethoxytrityl)-3′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-3′-deoxythymidine (14): 3′-Amino-5′-*O*-(4,4′-dimethoxytrityl)-3′-deoxythymidine (**13**, 200 mg, 0.37 mmol) was converted into the title compound (180 mg, 76%) as described in General Procedure 1. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.70 (s, 3 H), 2.22–2.27 (m, 2 H), 3.79 (s, 6 H), 4.22 (s, 2 H), 4.84 (s, 3 H), 6.09–6.14 (m, 1 H), 6.83–6.86 (m, 4 H), 7.62–7.65 (m, 10 H), 7.69 (2×s, 1 H), 8.45–8.48, 8.88, 9.07 (m, 1 H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 11.6, 55.3, 56.6, 63.6, 79.4,84.5, 86.2, 87.2, 113.0, 127.3, 127.9, 128.1, 130.0,134.6, 143.7, 151.8, 158.7,163.0, 170.4, 178.1, 181.1, 190.6 ppm. ESI-MS calcd. for $C_{36}H_{36}N_3O_9$ [M + H]$^+$: 654.2452; found 654.2488.

3′-Amino-3′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-3′-deoxythymidine (15): Compound **14** (180 mg, 0.28 mmol) was dissolved in acetic acid (80%, 2.0 mL). The reaction mixture was stirred at ambient temperature for 3.5 h. The solvent was removed under reduced pressure, and the residue was coevaporated twice with water (2×5 mL). The residue was chromatographed on a column of silica gel with chloroform/methanol (100:7, v/v) to give **15** (59 mg, 60%). ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 1.70 (s, 3 H), 2.22–2.27 (m, 2 H), 3.55 (m, 2 H, m), 4.22 (s, 2 H), 4.84 (s, 3 H), 6.09–6.14 (m, 1 H), 7.65, 7.69 (2×s, 1 H), 8.45–8.48, 8.88–9.07 (br., 1 H), 11.21 (br., 1 H) ppm. ^{13}C NMR (67.8 MHz, [D$_6$]DMSO): δ = 12.5, 37.6, 44.6, 53.5, 60.7, 83.4, 84.4, 84.5, 109.3, 136.2, 150.3, 163.6, 171.6, 174.2, 177.3, 177.8, 182.3, 182.7, 187.0, 188.9 ppm. ESI-MS calcd. for $C_{15}H_{18}N_3O_7$ [M + H]$^+$: 352.1145; found 352.1158.

3′-Amino-3′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-3′-deoxythymidine (5): Aqueous NaOH (1.0 M, 1.1 mL) was added to a solution of compound **15** (100 mg, 0.28 mmol) in ethanol (2 mL), and the solution was stirred at 45 °C for 14 h. The reaction mixture was neutralized by addition of Dowex 50W×8 (2 mL), filtered, and concentrated under reduced pressure. The residue was chromatographed on a C18 reversed-phase column with CH_3CN/H_2O (100:1, v/v) to give **5** (71 mg, 75%). ^{1}H NMR (270 MHz, [D$_6$]-DMSO): δ = 1.70 (s, 3 H), 2.22–2.27 (m, 2 H), 3.55 (m, 2 H), 4.22 (s, 2 H), 6.09–6.14 (m, 1 H), 7.69 (s, 1 H), 8.46, 8.88 (br., 1 H), 11.21 (br., 1 H) ppm. ^{13}C NMR (67.8 MHz, [D$_6$]DMSO): δ = 12.5, 37.6, 44.6, 53.5, 83.7, 84.7, 109.5, 136.5, 150.3, 163.6, 182.3, 188.0, 198.3 ppm. ESI-MS calcd. for $C_{14}H_{16}N_3O_7$ [M + H]$^+$: 338.0988; found 338.0972.

***N*^{4}-(4,4′-Dimethoxytrityl)cytidine (16):** Cytidine (973 mg, 4 mmol) was dissolved in pyridine (20 mL), and chlorotrimethylsilane (3.8 mL, 30 mmol) was added. After 1 h, DMTrCl (1.6 g, 4.8 mmol) was added, and the resulting solution was stirred for 8 h. The reaction was quenched by addition of aq. $NaHCO_3$, and the mixture was diluted with ethyl acetate (60 mL). The organic layer was washed twice with saturated aqueous NaCl (30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform/methanol (100:3, v/v) to give **16** (1.7 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.70–3.80 (m, 2 H), 3.77 (s, 6 H), 4.08 (m, 1 H), 4.27 (t, $^3J_{H,H}$ = 4.9 Hz, 1 H), 4.37 (t, $^3J_{H,H}$ = 4.6 Hz, 1 H), 5.11 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 5.57 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H), 6.80 (d, $^3J_{H,H}$ = 8.1 Hz, 4 H), 7.09–7.29 (m, 9 H), 7.47 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 50.5, 55.1, 55.1, 63.0, 70.8, 72.3, 73.7, 77.2, 87.7, 91.2, 113.1, 121.6, 123.9, 126.8, 127.8, 128.6, 129.9, 130.0, 136.8, 139.9, 145.0, 147.0, 149.2, 151.6, 154.3, 158.2, 158.2 ppm. ESI-MS calcd. for $C_{30}H_{32}N_3O_7$ [M + H]$^+$: 546.2240; found 546.9822.

***N*^{6}-(4,4′-Dimethoxytrityl)adenosine (17):** Adenosine (1.1 g, 4 mmol) was converted into **17** (1.4 g, 61%) as described for **16**. ^{1}H NMR (500 MHz,$CDCl_3$): δ = 3.58–3.67 (m, 1 H), 3.71 (s, 6 H), 3.80 (d, $^3J_{H,H}$ = 12.4 Hz, 1 H), 4.15 (brs, 2 H), 4.83 (brs, 1 H), 5.19 (br., 1 H), 5.57 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H), 6.71–6.74 (m, 4 H), 7.14–7.26 (m, 9 H), 7.57 (s, 1 H), 7.94 (s, 1 H) ppm. ^{13}C NMR (125.7 MHz,$CDCl_3$): δ = 55.1, 63.0, 70.8, 72.3, 73.7, 87.7, 91.3, 113.1, 121.7, 125.2, 126.8, 127.8, 128.2, 128.7, 129.0, 130.0, 130.0, 136.95, 139.9, 145.0, 147.1, 151.7, 154.3, 158.3 ppm. ESI-MS calcd. for $C_{31}H_{32}N_5O_6$ [M + H]$^+$: 570.2353; found 570.2294.

5′-Azido-5′-deoxy-*N*^{4}-(4,4′-dimethoxytrityl)cytidine (18): Compound **16** (3.0 g, 5.5 mmol) was dissolved in DMF (6 mL), triphenylphosphane (2.9 g, 11 mmol), lithium azide (1.4 g, 27.5 mmol), and tetrabromomethane (3.6 g, 11 mmol) were added, and the resulting solution was stirred for 1 h. Water (3 mL) and then ethyl acetate (10 mL) were added. The aqueous layer was removed, and the organic layer was washed five times with water

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(10 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform/methanol (100:4, v/v) to give **18** (1.2 g, 38%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.50–3.78 (m, 2 H), 3.79 (s, 6 H), 4.06–4.08 (m, 1 H), 4.14–4.16 (m, 1 H), 4.19–4.22 (m, 1 H), 5.15 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H), 5.75–5.76 (d, 1 H, $^3J_{H,H}$ = 4.2 Hz), 6.82–6.84 (m, 4 H), 7.12–7.50 (m, 13 H) ppm. ^{13}C NMR (67.8 MHz, [D$_6$]DMSO): δ = 12.5, 37.6, 44.6, 53.5, 60.7, 83.4, 84.4, 84.5, 109.3, 136.2, 150.3, 163.6, 171.6, 174.2, 177.3, 177.8, 182.3, 182.7, 187.0, 188.9 ppm. ESI-MS calcd. for $C_{15}H_{18}N_3O_7$ [M + H]$^+$: 352.1145; found 352.1158.

5′-Azido-5′-deoxy-*N*^{6}-(4,4′-dimethoxytrityl)adenosine (19): Compound **17** (8.5 g, 15 mmol) was converted into **19** (4.4 g, 50%) as described for **18**. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.48–3.66 (m, 2 H), 3.76 (s, 6 H), 4.28–4.29 (m, 2 H), 4.47–4.49 (t, $^3J_{H,H}$ = 4.88 Hz, 1 H), 5.89–5.90 (d, $^3J_{H,H}$ = 5.13, 1 H), 6.77–6.79 (m, 5 H), 7.01–7.97 (m, 8 H), 8.00 (s, 1 H), 8.03 (s, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 52.4, 55.4, 71.0, 71.7, 72.8, 75.2, 75.4, 77.5, 84.3, 90.2, 113.4, 121.2, 127.1, 128.1, 129.0, 130.3, 137.4, 138.2, 145.3, 147.9, 152.0, 154.5, 158.5 ppm. ESI-MS calcd. for $C_{31}H_{31}N_8O_5$ [M + H]$^+$: 595.2417; found 595.2466.

5′-Amino-5′-deoxy-*N*^{4}-(4,4′-dimethoxytrityl)cytidine (21): Compound **18** (1.1 g, 2.0 mmol) was dissolved in pyridine (20 mL), triphenylphosphane (1.0 g, 3.9 mmol) was added, and the resulting solution was stirred for 6 h. Concentrated ammonia (10 mL) was added, and the solution was stirred for 12 h. The solvents were removed under reduced pressure, and the residue was chromatographed on an amino-modified silica gel column with chloroform/methanol (100:3, v/v) to give **21** (627 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.86–2.90 (dd, $^3J_{H,H}$ = 4.9 Hz, 5.4 Hz, 1 H), 2.98–3.01 (dd, J = 3.9 Hz, 3.2 Hz, 1 H), 3.78 (s, 6 H), 4.00–4.03 (m, 2 H), 4.15 (t, $^3J_{H,H}$ = 4.0 Hz, 1 H), 5.09 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 5.67 (s, $^3J_{H,H}$ = 2.9 Hz, 1 H), 6.81–6.83, (m, 5 H), 7.11–7.69 (m, 14 H) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 43.4, 55.1, 69.5, 70.5, 73.4, 79.3, 84.7, 88.9, 96.7, 112.9, 113.6, 126.2, 126.5, 127.6, 128.6, 129.6, 129.7, 129.7, 130.1, 132.1, 132.1, 132.2, 133.8, 137.1, 140.6, 145.3, 154.4, 157.6, 163.4 ppm. ESI-MS calcd. for $C_{30}H_{33}N_4O_6$ [M + H]$^+$: 545.2400; found 545.2419.

5′-Amino-5′-deoxy-*N*^{6}-(4,4′-dimethoxytrityl)adenosine (22): Compound **19** (3.4 g, 5.7 mmol) was dissolved in pyridine (10 mL), triphenylphosphane (3.0 g, 11.5 mmol) was added, and the resulting solution was stirred for 4 h. Concentrated ammonia (10 mL) was added, and the solution was stirred for 14 h. The mixture was diluted with ethyl acetate (100 mL) and was then washed with water (100 mL) and twice with saturated NaCl (100 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was chromatographed on an amino-modified silica gel column with chloroform/methanol (100:5) to give **22** (2.7 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.85–2.99 (m, 2 H), 3.74 (s, 6 H), 4.09–4.12 (m, 1 H), 4.29–4.27 (m, 1 H), 4.58–4.61 (t, $^3J_{H,H}$ = 5.25 Hz, 1 H), 5.80–5.81 (d, $^3J_{H,H}$ = 5.37 Hz, 1 H), 6.75–6.78 (m, 4 H), 7.17–7.31 (m, 9 H), 7.91(s, 1 H), 7.95 (s, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 45.4, 55.1, 70.6, 71.5, 74.4, 85.9, 89.8, 113.0, 121.2, 126.8, 127.8, 128.7, 130.0, 137.2, 138.7, 145.1, 147.9, 151.9, 154.2, 158.2 ppm. ESI-MS calcd. for $C_{31}H_{33}N_6O_5$ [M + H]$^+$: 569.2512; found 569.2557.

5′-Amino-5′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-5′-deoxyuridine (23): Compound **20** (1.2 g, 5.0 mmol) was condensed with dimethyl squarate for 1 h as described in General Procedure 1 to give **23** (695 mg, 39%). ^{1}H NMR (500 MHz, [D$_7$]DMF): δ = 3.70–3.77 (m, 2 H, 5′-H, 5′′-H), 3.93–3.95 (m, 1 H, 4′-H), 4.03–4.04 (m, 1 H, 3′-H), 4.04–4.06 (m, 1 H, 3′-H), 4.33–4.35 (m, 3 H), 5.63–5.68 (m, 1 H), 5.90–5.91 (d, $^3J_{H,H}$ = 4.6 Hz, 1 H), 7.73–7.75 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 8.27, 8.93 (br., 1 H, NH), 11.37 (br., 1 H, NH) ppm. ^{13}C NMR(125.7 MHz, [D$_7$]DMF):δ = 46.5, 47.0, 60.5, 60.6, 71.6, 73.7, 73.8, 83.5, 83.8, 89.8, 90.1, 102.6, 102.6, 141.8, 151.6, 163.8, 173.4, 173.9, 178.2, 178.8, 183.5, 183.8, 189.9, 190.4 ppm. ESI-MS calcd. for $C_{14}H_{16}N_3O_8$ [M + H]$^+$: 354.0937; found 354.0939

5′-Amino-5′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-5′-deoxy-4-*N*-(4,4′-dimethoxytrityl)cytidine (24): Compound **21** (544 mg, 1.0 mmol) was condensed with dimethyl squarate for 1 h as described in General Procedure 1 to give **24** (448 mg, 68%). ^{1}H NMR (500 MHz, [D$_6$]DMSO,75 °C): δ = 3.59–3.74 (m, 2 H, 5′-, 5′′-H), 3.74 (s, 6 H, OMe of DMTr), 3.82–3.88 (m, 2 H, 4′-H, 3′-H), 3.96 (m, 1 H, 2′-H), 4.23 (s, 3 H, OMe of squaric acid), 4.86 (br., 1 H, OH), 5.06 (br., 1 H, OH), 5.63, 5.64 (d, $^3J_{H,H}$ = 4.9 Hz, 1 H, 1′-H), 6.83–7.28 (14 H, m, 6-H, DMTr), 7.55 (1 H, br., NH) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 75 °C): δ = 45.7, 54.9, 59.4, 59.7, 69.4, 70.5, 72.7, 81.8, 90.1, 112.8, 126.1, 127.3, 128.3, 129.2, 129.6, 136.7, 144.9, 153.9, 157.6, 169.9, 172.5, 182.5, 189.0 ppm. ESI-MS calcd. for $C_{35}H_{35}N_4O_9$ [M + H]$^+$: 655.24; found 655.3326.

5′-Amino-5′-*N*-(2-methoxy-3,4-dioxocyclobuten-1-yl)-5′-deoxy-6-*N*-(4,4′-dimethoxytrityl)adenosine (25): Compound **22** (853 mg, 1.5 mmol) was condensed with dimethyl squarate for 3 h as described in General Procedure 1 to give **25** (319 mg, 31%). ^{1}H NMR ([D$_6$]DMSO, 75 °C): δ = 3.70–3.77 (m, 8 H, 5′H, 5′′H, Me of DMTr), 4.05–4.08 (m, 1 H, 4′-H), 4.19 (m, 4 H, 3′-H, OMe), 5.08–5.09 (m, 1 H, 2′-H), 5.29 (br., 1 H, OH), 5.30 (br., 1 H, OH), 5.80–5.88 (d, $^3J_{H,H}$ = 5.86 Hz, 1 H, 1′-H), 6.83–6.89 (m, 4 H, DMTr), 7.12–7.31 (m, 11 H, DMTr), 7.96 (br., 1 H, NH) 8.24 (s, 1 H, 2-H), 8.29 (s, 1 H, 8-H), 8.92 (br., 1 H, NH) ppm. ^{13}C NMR (125.7 MHz, [D$_7$]DMF): δ = 55.5, 60.3, 70.9, 72.1, 73.9, 84.7, 113.4, 113.7, 127.1, 127.9, 128.2, 128.4, 129.3, 129.7, 130.6, 138.3, 141.1, 141.2, 141.5, 146.3, 149.0, 152.1, 154.9, 159.1, 173.7, 178.4, 183.8, 190.0 ppm. ESI-MS calcd. for $C_{36}H_{35}N_6O_8$ [M + H]$^+$: 679.2516; found 679.2541.

5′-Amino-5′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-5′-deoxycytidine Sodium Salt (7): Compound **24** (178 mg, 0.27 mmol) and NaI (49 mg, 0.32 mmol) were dissolved in hexan-2-one. The reaction mixture was stirred at 120 °C for 19 h. The solvent was removed under reduced pressure, and the resulting residue was chromatographed on a C-18 column with water/CH_3CN (5:95, v/v) to give **7** (52 mg, 57%). ^{1}H NMR (500 MHz, D_2O): δ = 4.27–4.28, 4.30, 4.31–4.40, 4.43, (m, 2 H), 4.61–4.63 (dd, $^3J_{H,H}$ = 4.9 Hz, 11.2 Hz, 1 H), 4.72 (dd, $^3J_{H,H}$ = 3.9 Hz, 4.6 Hz, 1 H), 5.13–5.14 (m, 1 H), 6.3 (d, $^3J_{H,H}$ = 3.7 Hz, 1 H), 6.4 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 7.99–8.00 (d, 1 H, $^3J_{H,H}$ = 7.6 Hz) ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 44.2, 69.7, 73.3, 81.9, 90.1, 96.1, 141.2, 157.2, 165.8, 181.5, 188.1, 195.0 ppm. ESI-MS calcd. for $C_{13}H_{15}N_4O_7$ [M + H]$^+$: 339.09407; found 340.2517.

5′-Amino-5′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-5′-deoxyuridine Sodium Salt (6): Compound **23** (106 mg, 0.3 mmol) was treated with NaI in hexan-2-one for 13 h as described for **7** to give **6** (67 mg, 59%). ^{1}H NMR (500 MHz, D_2O): δ = 3.92–3.96 (dd, $^3J_{H,H}$ = 5.6, 14.6 Hz, 1 H), 4.07–4.10 (dd, $^3J_{H,H}$ = 3.7 Hz, 14.6 Hz, 1 H), 4.26–4.29 (dd, $^3J_{H,H}$ = 5.7 Hz, 9.6 Hz, 1 H), 4.36–4.38 (t, $^3J_{H,H}$ = 5.7 Hz, 1 H), 4.51–4.53 (t, $^3J_{H,H}$ = 4.4 Hz, 5.1 Hz, 1 H), 5.92 (d, $^3J_{H,H}$ = 3.7 Hz, 1 H), 5.92–5.94 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H), 7.69–7.71 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H) ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 47.2, 72.6, 75.8, 85.2, 92.8, 105.2, 144.8, 154.2, 168.7, 184.4, 190.8, 197.9, 205.7 ppm. ESI-MS calcd. for $C_{13}H_{14}N_3O_8$ [M + H]$^+$: 340.0781; found 340.0802.

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5′-Amino-5′-*N*-(2-hydroxy-3,4-dioxocyclobuten-1-yl)-5′-deoxyadenosine Sodium Salt (8): Compound **25** (102 mg, 0.15 mmol) was treated with NaI in hexan-2-one for 15 h as described for **7** to give **8** (45 mg, 79%). ^{1}H NMR (500 MHz, D_2O): δ = 3.70–3.84 (m, 2 H), 4.17–4.20 (m, 1 H), 4.42–4.44 (t, $^3J_{H,H}$ = 5.1 Hz, 5.4 Hz, 1 H), 4.77–4.79 (m, 1 H), 5.92 (d, $^3J_{H,H}$ = 5.1 Hz, 1 H), 8.07 (s, 1 H), 8.13 (s, 1 H) ppm. ^{13}C NMR (500 MHz, D_2O): δ = 43.9, 70.2, 72.9, 83.1, 88.0, 118.7, 140.1, 148.5, 152.5, 155.2, 181.4, 187.7, 194.7 ppm. ESI-MS calcd. for $C_{14}H_{14}N_6O_6$ [M + H]$^+$: 362.0975; found 362.0668.

1,2-*O*-Isopropylidene-3,5-*O*-bis(trifluoromethylsulfonyl)-α-D-xylose (26): 1,2-*O*-Isopropylidene-α-D-xylose (11.4 g, 60 mmol) was rendered anhydrous by repeated coevaporation with toluene, and CH_2Cl_2 (360 mL) and *N*,*N*-diisopropylethylamine (28 mL, 162 mmol) were added. The resulting mixture was cooled to –78 °C, and trifluoromethanesulfonic anhydride (29 mL, 171 mmol) was then added dropwise over 1 h. After the reaction was complete, the organic solution was washed twice with water (100 mL), and then with saturated $NaHCO_3$. The organic layer was dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexane/ethyl acetate (100:1, v/v) to give **26** (20 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 3 H, CH_3), 1.52 (s, 3 H,CH_3), 4.59–4.79 (m, 3 H, 3-H, 4-H, 5-H), 5.24–5.25 (dd, $^3J_{H,H}$ = 2.2 Hz, 1 H, 2-H), 6.04 (d, $^3J_{H,H}$ = 3.7 Hz, 1-H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 26.1, 26.5, 70.4, 75.6, 83.0, 86.7, 104.7, 113.7, 117,0, 119.6, 119.7 ppm. $C_{10}F_6O_9S_2{\cdot}H_2O$: C 25.43, H 2.99, S 13.58, F 24.13; found C 25.45, H 2.85, S 13.84, F 24.86.

1,2-*O*-Isopropylidene-3,5-diazido-α-D-xylose (27): Compound **26** (454 mg, 1 mmol) was dissolved in DMF (1 mL), lithium azide (245 mg, 5 mmol) was added, and the resulting mixture was stirred at 40 °C for 4 h. The solution was diluted with diethyl ether (10 mL) and was then washed with water (10 mL) and three times with saturated aqueous NaCl (10 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with hexane/diethyl ether (100:3, v/v) to give **27** (84 mg, 42%). The structure was confirmed by comparison of the spectroscopic data with those reported in the literature.[18]

***N*^{2}-(4,4-Dimethoxytrityl)-3,5-diazidoguanosine (31):** Compound **29** (457 mg, 1.4 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was dissolved in dry pyridine (13 mL), and chlorotrimethylsilane (433 μL, 3.4 mmol) was added. After 1 h, 4,4′-dimethoxytrityl chloride (555 mg, 1.6 mmol) was added, and the resulting mixture was stirred for 24 h. The reaction was quenched by addition of saturated $NaHCO_3$ (13 mL). The material was extracted twice with ethyl acetate (50 mL), and the organic layers were combined, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on an amino-modified silica gel column with chloroform/methanol (100:5, v/v) to give **31** (510 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.22–3.39 (m, 2 H), 3.43 (s, 6 H), 3.91 (m, 2 H), 4.54 (m, 1 H), 5.53 (s, 1 H), 6.45–6.47 (m, 4 H), 6.79–7.01 (m, 9 H), 7.63 (s, 1 H), 7.68 (s, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 52.0, 55.3, 62.0, 70.9, 75.8, 77.5, 81.2, 90.4, 90.4, 113.3, 121.2, 127.0, 128.0, 128.9, 130.2, 137.4, 137.4, 138.5, 145.3, 147.8, 152.2, 152.2, 154.4, 158.4 ppm. ESI-MS calcd. for $C_{31}H_{30}N_{11}O_5$ [M + H]$^+$: 636.2431; found 636.2416.

***N*^{6}-(4,4-Dimethoxytrityl)-3,5-diaminoadenosine (32):** Compound **28** (738 mg, 2.3 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. Additional pyridine (24 mL) and chlorotrimethylsilane (737 μL, 5.8 mmol) were added to the residue, and the resulting solution was stirred at ambient temperature. After 1 h, 4,4′-dimethoxytrityl chloride (948 mg, 2.8 mmol) was added, and the mixture was stirred at ambient temperature for 8.5 h. The reaction was quenched by portionwise addition of saturated $NaHCO_3$ (100 mL). After 1 h, the products were extracted twice with ethyl acetate (20 mL), and the organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform/hexane (5:1, v/v) to give crude **30**. The crude material was dissolved in pyridine (120 mL), triphenylphosphane (950 mg, 3.6 mmol) was added, and the mixture was stirred at ambient temperature for 14 h. Aqueous ammonia (28%, 12 mL) was added, and the solution was stirred for 23 h. The solvents were removed under reduced pressure, and the residue was dissolved in ethyl acetate (30 mL), washed three times with saturated aqueous NaCl (50 mL), and concentrated under reduced pressure. The residues was chromatographed on a silica gel column with chloroform/methanol/triethylamine (100:10:1, v/v/v) to give **32** (32% from **28**). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.97–3.11 (m, 2 H), 3.76–3.80 (m, 7 H), 3.88–3.91 (m, 1 H), 4.45–4.47 (m, 1 H), 5.89 (d, $^3J_{H,H}$ = 3.17 Hz, 1 H), 6.78–6.80 (m, 5 H), 7.21–7.33 (m, 8 H), 7.97 (s, 1 H), 8.01 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 43.3, 53.6, 55.0, 70.4, 75.1, 85.6, 90.8, 112.9, 121.2, 126.6, 127.7, 128.6, 129.9, 137.2, 138.4, 145.1, 147.7, 151.9, 154.0, 158.1 ppm. ESI-MS calcd. for $C_{31}H_{34}N_7O_4$ [M + H]$^+$: 568.2672; found 568.2672.

***N*^{2}-(4,4-Dimethoxytrityl)-3,5-diaminoguanosine (33):** Compound **31** (418 mg, 0.66 mmol) was dissolved in dry pyridine (6 mL), and triphenylphosphane (690 mg, 2.6 mmol) was added. After 24 h, aqueous ammonia (28%, 12 mL) was added, and the mixture was stirred for 15 h. Water (20 mL) was added, and the material was extracted three times with ethyl acetate (10 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform/methanol (100:7, v/v) to give **33** (226 mg, 59%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 2.62–2.74 (m, 2 H), 3.33–3.34 (m, 1 H), 3.72–3.75 (m, 8 H), 5.17–5.18 (m, 2 H), 6.86–6.87 (m, 5 H), 7.19–7.32 (m, 12 H), 7.56 (s, 1 H), 7.81 (s, 1 H), 8.32 (br., 1 H) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 43.7, 54.1, 55.0, 69.4, 73.6, 79.2, 84.9, 89.3, 112.9, 113.0, 117.7, 126.4, 127.7, 128.2, 129.7, 129.7, 136.9, 137.2, 145.2, 149.0, 150.8, 156.5, 157.6 ppm. ESI-MS calcd. for $C_{31}H_{34}N_7O_5$ [M + H]$^+$: 584.2621; found 584.2673.

***N*^{6}-(4,4′-Dimethoxytrityl)-3′,5′-diamino-3′,5′-*N*-(3,4-dioxocyclobutene-1,2-diyl)adenosine (34):** *N*,*N*-Diisopropylethylamine (63 μL, 0.37 mmol) and dimethyl squarate (104 mg, 0.73 mmol) were added to a solution of compound **32** (414 mg, 0.73 mmol) in methanol (1 mL). The mixture was stirred at ambient temperature for 1 h, the solvent was removed under reduced pressure, and the residue was chromatographed on a column of silica gel with chloroform/methanol (100:5, v/v) to give **34** (208 mg, 32%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.77–3.80 (m, 7 H), 3.99–4.01 (m, 1 H), 4.26–4.30 (m, 1 H), 4.37–4.39 (m, 1 H), 5.13–5.14 (m, 1 H), 5.82 (d, $^3J_{H,H}$ = 0.7 Hz, 1 H), 6.77–7.44 (m, 13 H), 7.78 (s, 1 H), 8.02 (s, 1 H) ppm. ^{13}C NMR (125.7 MHz, [D$_7$]DMF): δ = 51.8, 55.1, 62.5, 70.6, 75.9, 81.7, 86.4, 92.7, 113.1, 121.7, 126.6, 126.8, 127.7, 127.8, 128.2, 128.5, 128.7, 128.9, 130.0, 136.1, 137.3, 138.3, 144.9, 145.2, 147.9, 152.6, 154.2, 158.3, 158.3, 167.6, 168.5, 181.7, 182.7 ppm. ESI-MS calcd. for $C_{35}H_{32}N_7O_6$ [M + H]$^+$: 646.2412; found 646.2502.

3′,5′-Diamino-3′,5′-*N*-(3,4-dioxocyclobutene-1,2-diyl)adenosine (9a): Compound **35** (147 mg, 0.23 mmol) was dissolved in trifluoroacetic acid/dichloromethane (1% v/v, 3 mL), and the solution was

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stirred at ambient temperature for 7 h. The product was extracted three times with water (20 mL), and the aqueous solution was concentrated under reduced pressure. The solution was neutralized with dimethylaminomethyl-polystyrene, filtered, and concentrated under reduced pressure. The residue was purified by gel-filtration chromatography. The fraction containing the desired product was concentrated, and the residue was triturated with diethyl ether to give **9a** (33 mg, 42%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.70–3.72 (m, 2 H), 4.07–4.09 (m, 1 H), 4.11–4.22 (m, 1 H), 4.75–4.77 (t, $^3J_{H,H}$ = 5.2 Hz, 1 H), 6.00 (s, 1 H), 6.02–6.03 (d, $^3J_{H,H}$ = 5.4 Hz, 1 H), 7.34 (br., 2 H), 8.15 (s, 1 H), 8.29 (s, 1 H), 8.64–8.70 (br., 2 H) ppm. ^{13}C NMR (125.7 MHz, [D$_7$]DMF): δ = 48.7, 63.8, 76.1, 82.0, 92.0, 120.3, 140.1, 149.9, 153.6, 157.2, 169.0, 169.5, 183.0, 183.7 ppm. ESI-MS calcd. for $H_{14}N_7O_4$ [M + H]$^+$: 344.1107; found 346.1106.

3′,5′-Diamino-3′,5′-*N*-(3,4-dioxocyclobutene-1,2-diyl)guanosine (9b): *N*,*N*-Diisopropylethylamine (27.4 μL, 0.16 mmol) and dimethyl squarate (46 mg, 0.32 mmol) were added to a solution of compound **33** (188 mg, 0.32 mmol) in methanol (3 mL). The mixture was stirred at ambient temperature for 2 h, the solvent was removed under reduced pressure, and the residue was chromatographed on a column of silica gel with chloroform/methanol (100:5, v/v) to give crude **35**. The crude **35** was dissolved in trifluoroacetic acid/dichloromethane (1% v/v, 2 mL), and the solution was stirred at ambient temperature for 18 h. The product was extracted three times with water (10 mL), and the aqueous solution was concentrated under reduced pressure. The solution was neutralized with dimethylaminomethyl-polystyrene, filtered, and concentrated under reduced pressure. The residue was purified by gel-filtration chromatography. The fraction containing the desired product was concentrated and the residue was triturated with diethyl ether to give **9b** (34 mg, 30% from **33**). ^{1}H NMR (500 MHz, [D$_7$]DMF/10% D$_2$O): δ = 3.87 (dd, $^3J_{H,H}$ = 2.4 Hz, 12 Hz, 1 H, 5′-H), 4.35–4.39 (m, 1 H, 4′-H), 4.57 (dd, $^3J_{H,H}$ = 5.4 Hz, 9.0 Hz, 1 H, 3′-H), 4.86 (d, $^3J_{H,H}$ = 5.4 Hz, 1 H, 2′-H), 5.97 (s, 1 H, 1′-H), 7.93 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.7 MHz, [D$_7$]DMF): δ = 48.4, 63.2, 76.1, 81.8, 91.7, 118.0, 136.4, 151.2, 154.6, 157.3, 168.6, 169.2, 182.6, 183.3 ppm. ESI-MS calcd. for $C_{14}H_{14}N_7O_5$ [M + H]$^+$: 360.1056; found 360.1017.

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