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Novel and efficient syntheses of 3′,5′-diamino derivatives of 2′,3′,5′-trideoxycytidine and 2′,3′,5′-trideoxyadenosine. Protonation behavior of 3′,5′-diaminonucleosides

Iván Lavandera, Susana Fernández, Miguel Ferrero and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33006 Oviedo, Spain

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Abstract—High yielding synthetic routes to 3′,5′-diamino-2′,3′,5′-trideoxycytidine and 3′,5′-diamino-2′,3′,5′-trideoxyadenosine are described. In addition, the protonation behavior of 3′,5′-diamino-2′,3′,5′-trideoxycytidine, 3′,5′-diamino-2′,3′,5′-trideoxyadenosine, 3′,5′-diamino-3′,5′-dideoxythymidine, and 3′,5′-diamino-2′,3′,5′-trideoxyuridine has been studied by means of pH-metric measurements and NMR spectroscopy. The ionization constants and the sequence of protonation sites have been determined.

1. Introduction

In the last few years the ever-growing interest in the field of nucleosides and nucleotides has led to renewed efforts in the synthesis of analogues.[1] Modifications in the sugar moiety are one of the most important kind of nucleoside derivatives that can lead to promising chemotherapeutic agents.[2] These compounds have attracted much attention as potential antiviral and anticancer agents.[3] Due to their use as building-blocks to prepare anti-sense and anti-gene oligonucleotides, they also emerge as potential and selective inhibitors of gene expression.[4] Particularly, amino sugar nucleosides are important bioactive molecules,[5] of which puromycin (3′-amino-2′,3′-dideoxyadenosine) is one of the most important examples. One of the major strategies for the synthesis of sugar-modified nucleosides is the modification of the intact nucleosides.[6] Although there has been a growing demand for the development of nucleoside-base therapeutics, 3′,5′-diamino-2′,3′,5′-trideoxynucleosides remains to some extent unexplored mainly due to the difficult synthetic accessibility. There is very little in the literature concerning the synthesis of such nucleosides.[7]

In our ongoing study to elucidate the effects of amino-derivatized nucleosides on critical biosynthetic pathways as well as their use as monomers for the preparation of antisense oligonucleotides, we first reported the synthesis of 3′,5′-diamino-3′,5′-dideoxythymidine and 3′,5′-diamino-2′,3′,5′-trideoxyuridine.[8] With these considerations in mind, we describe here for the first time the preparation of 3′,5′-diamino-2′,3′,5′-trideoxycytidine and an efficient alternative synthesis to *N*-benzoyl-3′,5′-diamino-2′,3′,5′-trideoxyadenosine. Additionally, we perform protonation studies of 3′,5′-diaminonucleoside derivatives of 2′-deoxycytidine, 2′-deoxyadenosine, thymidine, and 2′-deoxyuridine with the aim of finding the ionization constants and the sequence of protonation sites on these molecules.

2. Results and discussion

Starting from 2′-deoxyuridine (**1**) we previously synthesized 3′,5′-diazido-2′,3′,5′-trideoxyuridine (**2**) through a simple and efficient three step procedure.[8] This diazido compound was treated[9] with phosphorus oxychloride, 1,2,4-triazole, and triethylamine in acetonitrile at room temperature for 1 h to afford the 4-triazolylpyrimidinone derivative **3** (Scheme 1). Subsequent treatment with aqueous ammonia in 1,4-dioxane solution give the cytidine derivative **4**. Hydrogenation of **4** in EtOH in the presence of 10% Pd/C yielded 3′,5′-diamino-2′,3′,5′-trideoxycytidine (**5**) with 29% overall yield.

For the synthesis of *N*-benzoyl-3′,5′-diamino-2′,3′,5′-trideoxyadenosine (**12**), 5′-*O*-protected adenosine derivative **6** (commercially available) was subjected to Mitsunobu conditions (Scheme 2). Thus, esterification of the hydroxyl group at 3′ position takes place with inversion of configuration giving place to ester **7**. Methanolysis of the *p*-nitrobenzoate ester and cleavage of the dimethoxytrityl group with catalytic amounts of formic acid in $CHCl_3$ afforded *N*-benzoyl-2′-deoxyxyloadenosine (**9**). Mesylation

Keywords: 3′,5′-diaminonucleosides; protonation studies; ionization constants.

* Corresponding author. Tel.: +34-985-103-448; fax: +34-985-103-451; e-mail: vgs@sauron.quimica.uniovi.es

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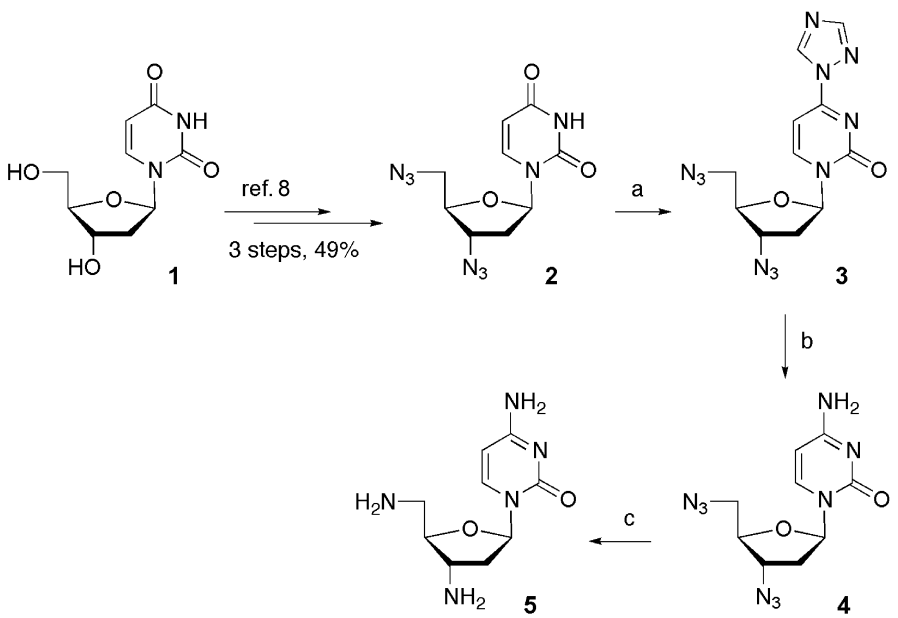
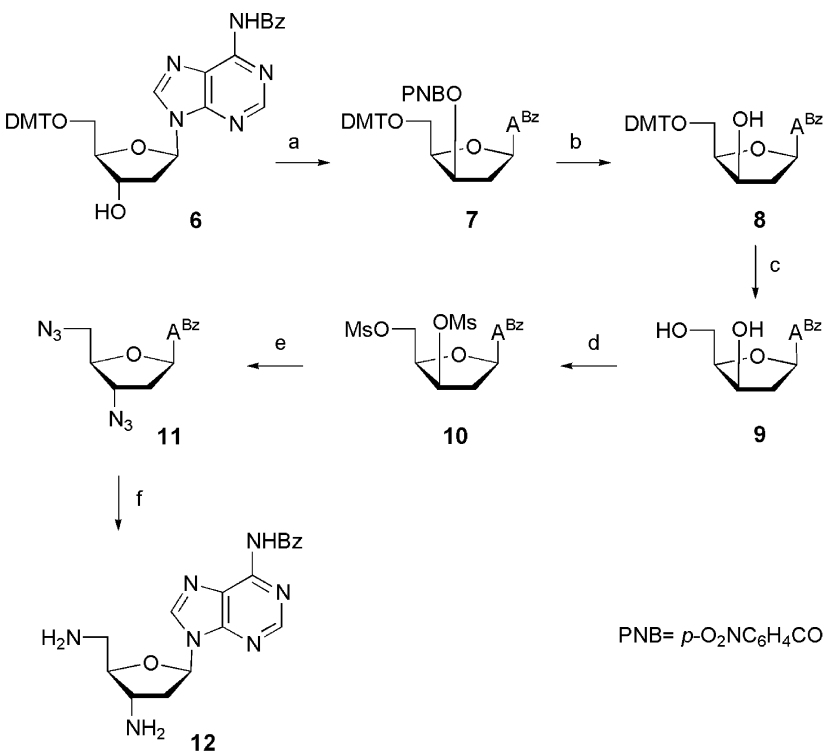


a. 1,2,4-Triazole, $POCl_3$, Et_3N, MeCN, rt (93%); b. NH_3, 1,4-dioxane, rt (89%); c. H_2, Pd/C, EtOH, rt (71%).

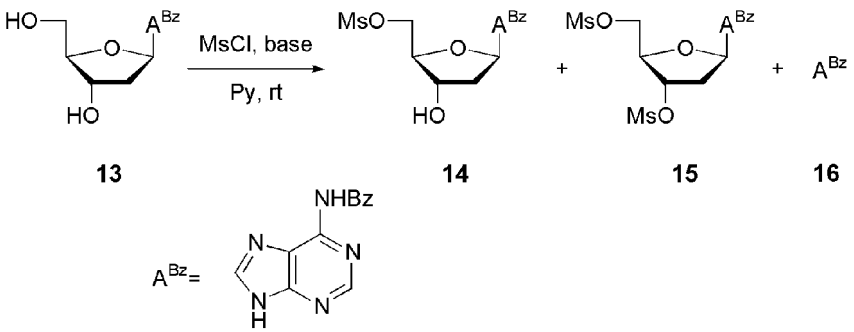
Scheme 1. Synthesis of 3′,5′-diamino-2′-deoxycytidine.



PNB= p-$O_2NC_6H_4CO$

a. PNBOH, Ph_3P, DEAD, THF, rt (70%); b. NaOMe, MeOH, 0 °C (97%); c. HCO_2H, $CHCl_3$, rt (94%); d. MsCl, DMAP, Py, rt (75%); e. NaN_3, DMF, 65 °C (75%); f. H_2, Pd black, EtOH, rt (81%).

Scheme 2. Synthesis of 3′,5′-diamino-2′-deoxyadenosine *N*-benzoylated.



Scheme 3. Mesilation reaction with model nucleoside, 2′-deoxyadenosine *N*-benzoylated.

Table 1. Mesylation reaction of *N*-benzoyl-2′-deoxyadenosine (**13**)

Entry	Base (equiv.)	Equiv. of MsCl	*t* (h)	**13** (%)[a]	**14** (%)[a]	**15** (%)[a]	**16** (%)[a]
1	Et_3N[b]	4	13	50	50		
2	Et_3N (6)	4	23		70	30	
3	Et_3N (1:1 v/v)	6	66		30	30	40
4	Et_3N (1:2 v/v)	6	66		20	40	40
5	Et_3N (1:4 v/v)	6	66		12[c]	65[c]	
6	Imidazol (10)	6	72		Complex	Mixture	
7	DMAP (10)	6	2			99[c]	

[a] Calculated by ^{1}H NMR.
[b] As base and solvent.
[c] Isolated yields after flash chromatography.

of **9** proved difficult, the 5′-*O*-mesyl derivative and fragments corresponding to the hydrolysis of the glycosidic bond being observed when the reaction was carried out with 2.2 equiv. of mesyl chloride in pyridine at 0°C.

This hydrolysis could be explained by initial protonation of the base moiety followed by dissociation of the protonated nucleoside to a glycosyl oxocarbenium ion and the free purine.[10]

Although the pK_a of pyridine (5.29) is greater than the pK_a of adenine (4.25), it seems that the difference is not enough to shift the equilibrium toward the protonation of pyridine. For this reason, we carried out the reaction in a medium of triethylamine (pK_a=10.75). As the model nucleoside we choose *N*-benzoyl-2′-deoxyadenosine (**13**, Scheme 3).

However, the process was slower than before, and a mixture of starting material and 5′-*O*-mesyl derivative **14** was obtained (entry 1, Table 1). Next, the reaction was carried out with Et_3N as base and pyridine as solvent (entries 2–5, Table 1). The best result was observed with a ratio Et_3N/Py 1:4 (v/v), the desired compound **15** being isolated in 65% yield after flash chromatography. In addition, monomesyl derivative **14** was obtained in 12% yield. The use of imidazol (pK_a=7.0) as base gave rise to a complex mixture (entry 6, Table 1). However, when the process was performed with 10 equiv. of DMAP (pK_a=9.7) in pyridine solution and 6 equiv. of MsCl, 3′,5′-di-*O*-mesyl nucleoside **15** was achieved exclusively after 2 h in quantitative yield (entry 7, Table 1). DMAP makes the reaction faster and this avoids the hydrolysis of the glycosidic bond in solution.

Extension of this reaction to the xylonucleoside **9** led to the formation of di-*O*-mesyl derivative **10** in 75% yield (Scheme 2). Subsequent treatment of **10** with sodium azide in DMF solution afforded diazido **11**. Hydrogenation of the latter in EtOH in the presence of Pd black gave *N*-benzoyl-3′,5′-diamino-2′,3′,5′-trideoxyadenosine (**12**). This efficient six-step procedure from readily available starting material gave **12** in 29% overall yield.

Additionally, we report here the protonation behavior of both 3′,5′-diamino nucleosides **5** and **12**, and the previously reported[8] 3′,5′-diamino-3′,5′-dideoxythymidine (**17**) and 3′,5′-diamino-2′,3′,5′-trideoxyuridine (**18**) (Fig. 1) using pH-metric measurements and NMR spectroscopy. To determine the stepwise protonation sites we recorded their ^{1}H and ^{13}C NMR spectra at different pD values (pD=pH+0.4). As a general rule, when pD is decreased, proton atoms in the α-position to the protonated nitrogen move downfield while β-C signals move upfield.[11]

For all diaminonucleosides, in the range pD 12–10, no appreciable variations in the chemical shifts of ^{1}H and ^{13}C NMR spectra occurred, but between pD 10 and 8 a significant downfield of H-5′ and an upfield of C-4′ were observed (Figs. 2 and 3). The shift of C-4′ can be ascribed to protonation at N-5′ or N-3′, but the fact that C-2′ did not

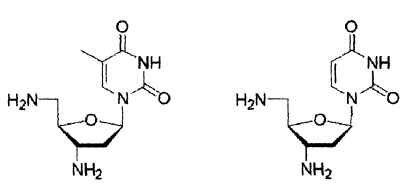


Figure 1. 3′,5′-Diamino-3′,5′-dideoxythymidine and 3′,5′-diamino-2′,3′,5′-trideoxyuridine.

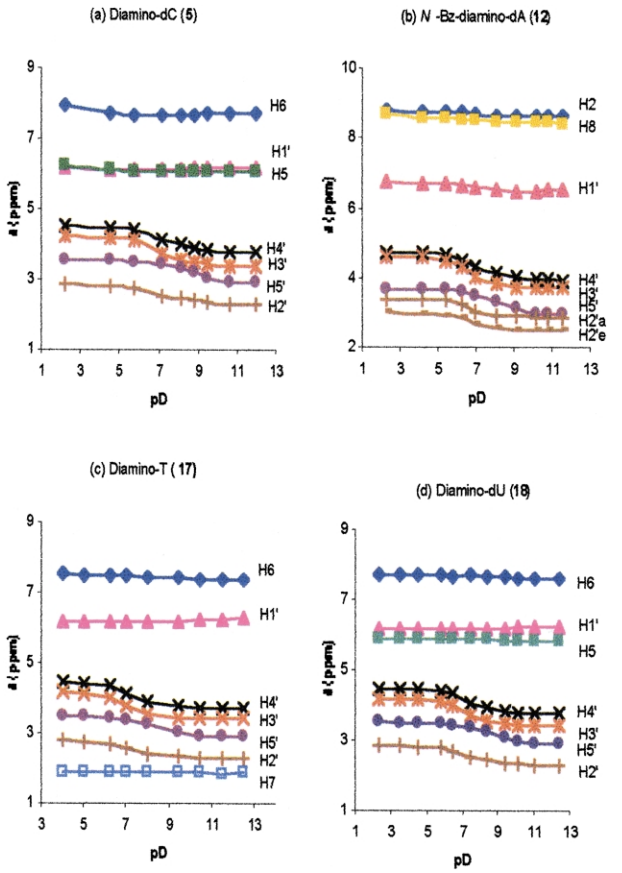


Figure 2. ^{1}H NMR chemical shift of: (a) diamino-dC, **5**; (b) *N*-Bz-diamino-dA, **12**; (c) diamino-T, **17**; (d) diamino-dU, **18** as a function of pD.

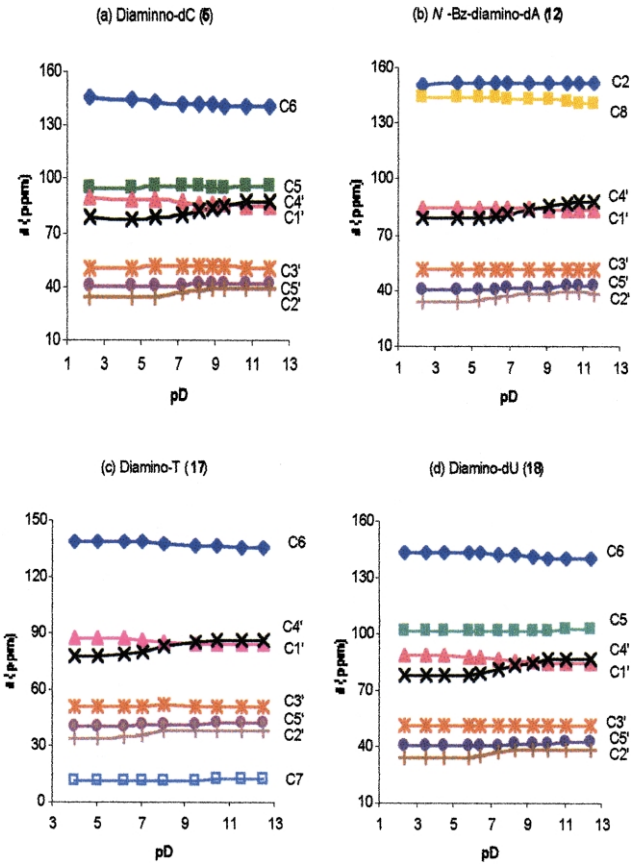


Figure 3. ^{13}C NMR chemical shift of: (a) diamino-dC, **5**; (b) *N*-Bz-diamino-dA, **12**; (c) diamino-T, **17**; (d) diamino-dU, **18** as a function of pD.

experience a clear shift suggests that the first proton binding to the molecule occurs mainly at the amino group of the 5′-position. In the pD range 8–5, the most significant shifts are shown by H-3′, C-4′, and C-2′, which confirms the second protonation to be predominantly at the N-3′ position.

An additional protonation is expected for 3′,5′-diamino-2′-deoxycytidine (**5**) due to the presence of an extra amino group on the base. Nucleoside **5** lacks a hydrogen in the α-position to this amino group. However, a downfield of H-5 (β-position) was observed at pD<5. Since in **18** no appreciable shift of that hydrogen was evident, the shift of H-5 in **5** can be imputed to the base protonation. This fact is confirmed by a slight upfield of C-5 (Fig. 3(a)).

The potentiometric titrations were carried out in 0.1 M Me_4NCl aqueous solution at 298 K. In Table 2, the basicity constants of **5**, **12**, **17**, and **18** are presented. The comparison of the pK_a values indicated that the basicity is similar for purine and pyrimidine nucleosides. In the case of 3′,5′-diamino-2′,3′,5′-trideoxycytidine a third pK_a of 3.67 appears, corresponding to protonation at the exocyclic amino base, in addition to the pK_as for protonation at N-3′ and N-5′.

From the ionization constants the distributions of the protonated species of the diaminonucleoside derivatives formed as a function of pH were calculated using the computer program SUPERQUAD[12] (Fig. 4). The free amine predominates at pH>10 whereas at a pH of ca. 7.5–8.5 the main species in solution is the monoprotonated form. Below pH 5, the diprotonated form exists for dA, T, and dU derivatives (Fig. 4(b), (c) and (d)) almost exclusively. This is also the main species at pH=4.5–6 in 3′,5′-diamino-dC (**5**), which predominates in its fully protonated form (LH_3^+) at pH<3 (Fig. 4(a)).

The distribution of protonated species at different pHs can be used as a guide to chemical reactivity. Thus, when we carried out the enzymatic acylation of 3′,5′-diamino-thymidine (**17**), a poor yield of acylated derivative was obtained. However, if the process was performed in the presence of molecular sieves, the isolated yield of 5′-acetylnucleoside was increased up to 80%.[8] To explain this fact, a measurement of pH to a water solution of **17** before and after the addition of molecular sieves (1:1 w/w) was carried out. We observed a slight increase of pH from 8.8 to 9.2, in which at least 50% of free amine was present. The addition of molecular sieves to the enzymatic acylation

Table 2. Logarithms of the protonation constants of **5**, **12**, **17**, and **18**

	log K^b			
Reaction[a]	**5**	**12**	**17**	**18**
L+H=HL	8.74 (7)	9.21 (4)	8.99 (3)	9.06 (5)
HL+H=H_2L	6.49 (7)	6.57 (4)	6.59 (3)	6.78 (5)
H_2L+H=H_3L	3.67 (7)			

[a] Charges have been omitted for clarity.
[b] Values in parentheses are standard deviation in the last significant figure.

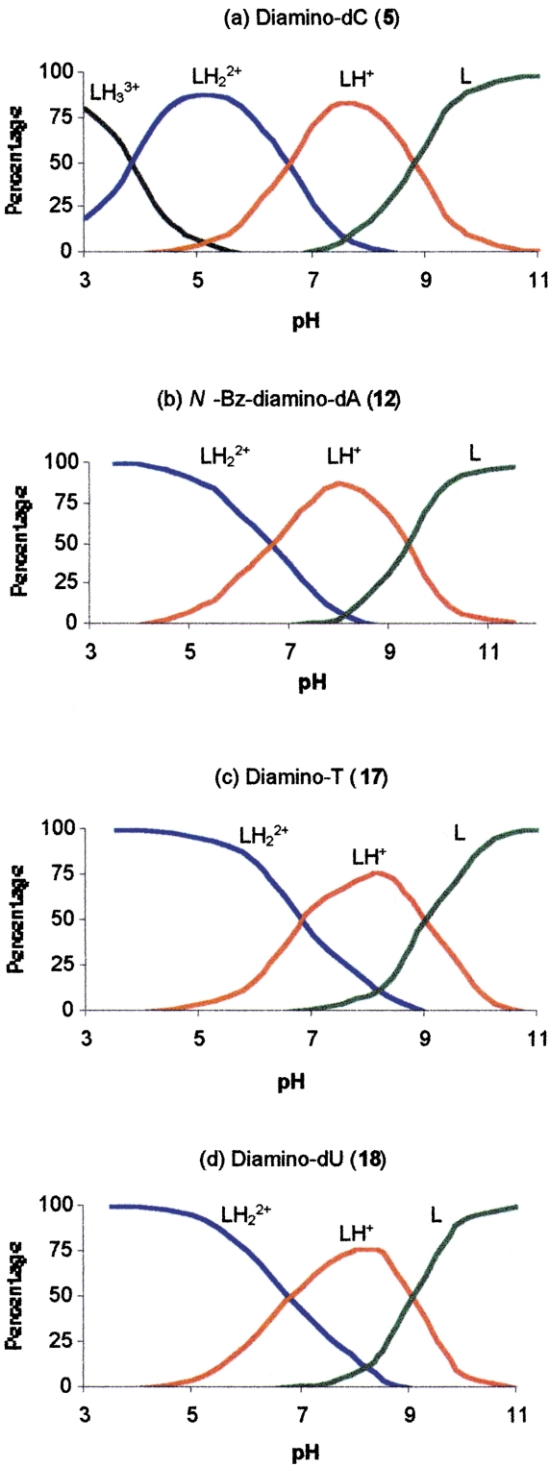


Figure 4. Distribution diagram of protonated species of: (a) diamino-dC, **5** (=L); (b) *N*-Bz-diamino-dA, **12** (=L); (c) diamino-T, **17** (=L); (d) diamino-dU, **18** (=L) as a function of pH.

reaction shifts the equilibrium toward the free amine, and this favors the above process. The acid–base effects of molecular sieves are previously reported in the literature.[13]

In summary, efficient syntheses of 3′,5′-diamino derivatives of 2′-deoxycytidine and 2′-deoxyadenosine have been described. The sequence of the protonation sites and the ionization constants of a series of four diaminonucleoside derivatives has been determined by potentiometric study and analysis of ^{1}H and ^{13}C NMR at different pD values. The first protonation takes place mainly at the 5′-amino group, giving the monoprotonated form as the predominant species at ca. pH 8. The diprotonated species is present mainly at pH 5. In the case of 3′,5′-diaminocytidine, the base protonation occurs at pH values below 3. The distribution diagrams of protonated species allows us to determine the predominant form at different pHs to plan the best way to perform a process. Aminonucleosides **5**, **12**, **16**, and **17** are subjected to biological assays and under investigation as monomers for antisense oligonucleotides.

3. Experimental

3.1. General

Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on an Infrared Fourier Transform spectrophotometer using KBr pellets. Flash chromatography was performed using silica gel 60 (230–400 mesh). ^{1}H, ^{13}C NMR, and DEPT were obtained using AC-200 (^{1}H, 200.13 MHz and ^{13}C, 50.3 MHz), and AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers for routine experiments. An AMX-400 spectrometer operating at 400.13 and 100.61 MHz for ^{1}H and ^{13}C, respectively, was used for the acquisition of $^{1}H-^{1}H$ and $^{1}H-^{13}C$ correlation experiments. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). ESI^{+} was used to record mass spectra (MS). Microanalyses were performed on a Perkin–Elmer model 2400 instrument. For the NMR titrations, the samples were prepared with known amounts of the diaminonucleoside, the pD was adjusted by addition of DCl or NaOD solutions in D_2O and the correction $pD=pH^{*}+0.4$ was used, where pH^{*} is the direct measurement of a pH-meter calibrated with non-deuterated buffer solutions. For the pH-metric titrations, a Metrohm TITRO-PROCESSOR-636 titrimeter was used, the reference electrode was an Ag/AgCl electrode in sat. aq. KCl, the cell was thermostated at 298±0.1 K, and the measurements were performed under nitrogen atmosphere. The protonation constants were determined by titration with 0.1N NaOH of a solution containing 10^{-3} M of the HCl salt of the diamine in the presence of Me_4NCl (0.1 M). The measurements were carried out twice, and the data analysis was performed with the computer program SUPERQUAD.[12]

3.1.1. 3′,5′-Diazido-4-(1,2,4-triazol-1-yl)-2′,3′,5′-trideoxycytidine (3). $POCl_3$ (293 μL, 3.15 mmol) was added to a suspension of 1,2,4-triazole (1.7 g, 25.16 mmol) in dry acetonitrile (21 mL). Triethylamine (3.86 mL, 27.68 mmol) and a solution of **2**[8] (350 mg, 1.26 mmol) in 4 mL of dry acetonitrile were added at 0°C. Then, the reaction was stirred for 1 h at room temperature. After that, 2.4 mL of Et_3N and 0.7 mL of water were added and stirred for 10 min. Later, the solvent was evaporated in vacuo and the crude residue was redissolved in a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$ (3×15 mL). The organic layer was dried with Na_2SO_4, filtered off and the solvent was evaporated under reduced pressure, affording 384 mg (93%) of an hygroscopic solid corresponding to compound **3**. R_f

(EtOAc): 0.54; IR (KBr): ν 3235, 3065, 2932, 2105, 1637 and 1616 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 2.46 (m, 1H, $H_{2'}$), 2.77 (m, 1H, $H_{2'}$), 3.67–3.96 (m, 2H, $H_{5'}$), 4.07 (m, 1H, $H_{4'}$), 4.18 (m, 1H, $H_{3'}$), 6.15 (dd, 1H, $H_{1'}$, $^3J_{HH}$=6.7, 4.9 Hz), 7.12 (d, 1H, H_5, $^3J_{HH}$=7.2 Hz), 8.12 (s, 1H), 8.38 (d, 1H, H_6, $^3J_{HH}$=7.2 Hz), and 9.25 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 38.5 ($C_{2'}$), 51.1 ($C_{5'}$), 59.2 ($C_{3'}$), 82.6 ($C_{4'}$), 87.3 ($C_{1'}$), 94.7 (C_5), 143.1 (CH), 145.9 (C_6), 153.9 (2C, C_2+CH), and 159.3 (C_4); MS (ESI^+, *m/z*): 368 [$(M+K)^+$, 4%] and 352 [$(M+Na)^+$, 100]. Anal. calcd (%) for $C_{11}H_{11}N_{11}O_2$: C, 40.11; H, 3.37; N, 46.80. Found: C, 39.9; H, 3.5; N, 46.6.

3.1.2. 3′,5′-Diazido-2′,3′,5′-trideoxycytidine (4). A solution of **3** (860 mg, 2.61 mmol) in dry 1,4-dioxane (50 mL) was cooled at 0°C under nitrogen, and then an aqueous solution of NH_3 (33%, 15 mL) was added. After reaction for 2 h at room temperature, the solvent was evaporated in vacuo and the crude residue was purified by flash chromatography column (gradient eluent 10% to 15% MeOH/EtOAc) affording 640 mg (89%) of a white solid corresponding to compound **4**. R_f (20% MeOH/EtOAc): 0.28; mp: 138–141°C; IR (KBr): ν 3479, 3414, 3236, 2105, 1637, 1617, 1483, and 1277 cm^{-1}; ^1H NMR (MeOH-d_4, 300 MHz): δ 2.53–2.72 (m, 1H, $H_{2'}$), 3.79–3.93 (m, 2H, $H_{5'}$), 4.18 (m, 1H, $H_{4'}$), 4.50 (m, 1H, $H_{3'}$), 6.12 (d, 1H, H_5, $^3J_{HH}$=7.4 Hz), 6.35 (dd, 1H, $H_{1'}$, $^3J_{HH}$=6.3 Hz), and 7.94 (d, 1H, H_6, $^3J_{HH}$=7.4 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 38.3 ($C_{2'}$), 53.1 ($C_{5'}$), 62.3 ($C_{3'}$), 83.8 ($C_{4'}$), 87.4 ($C_{1'}$), 96.4 (C_5), 142.4 (C_6), 158.0 (C_2), and 167.7 (C_4); MS (ESI^+, *m/z*): 316 [$(M+K)^+$, 11%], 300 [$(M+Na)^+$, 100], and 278 [$(M+H)^+$, 32]. Anal. calcd (%) for $C_9H_{11}N_9O_2$: C, 38.97; H, 4.00; N, 46.48. Found: C, 39.2; H, 4.1; N, 46.3.

3.1.3. 3′,5′-Diamino-2′,3′,5′-trideoxycitydine (5). A solution of compound **4** (100 mg, 0.36 mmol) in EtOH (6 mL) was exposed to a positive pressure of hydrogen gas (balloon) at room temperature for 4 h in the presence of 10% palladium on charcoal (20 mg). The catalyst was removed by filtration on Celite and the filtrate was evaporated to dryness. The crude was purified by flash chromatography column [4% NH_3(aq.)/MeOH] to afford after vacuum drying 58 mg (71%) of a white solid corresponding to compound **5**. R_f [10% NH_3(aq.)/MeOH]: 0.39; mp: 186–187°C (decomposed); IR (KBr): ν 3550, 3480, 3413, 3239, 1638, 1617 and 1490 cm^{-1}; ^1H NMR (D_2O, 200 MHz): δ 2.04–2.29 (m, 1H, $H_{2'}$), 2.72–2.98 (m, 2H, $H_{5'}$), 3.24 (m, 1H, $H_{4'}$), 3.66 (m, 1H, $H_{3'}$), 5.87 (d, 1H, H_5, $^3J_{HH}$=7.6 Hz), 6.01 (dd, 1H, $H_{1'}$, $^3J_{HH}$=7.0, 5.0 Hz), and 7.53 (d, 1H, H_6, $^3J_{HH}$=7.6 Hz); ^{13}C NMR (D_2O, 75.5 MHz): δ 39.5 ($C_{2'}$), 42.3 ($C_{5'}$), 51.7 ($C_{3'}$), 85.7 ($C_{4'}$), 86.2 ($C_{1'}$), 96.1 (C_5), 141.8 (C_6), 157.6 (C_2), and 166.2 (C_4); MS (ESI^+, *m/z*): 264 [$(M+K)^+$, 25%], 248 [$(M+Na)^+$, 33], and 226 [$(M+H)^+$, 100]. Anal. calcd (%) for $C_9H_{15}N_5O_2$: C, 47.97; H, 6.72; N, 31.10. Found: C, 47.8; H, 6.7; N, 31.1.

3.1.4. N^6-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*p*-nitrobenzoyl)-2′-deoxyxyloadenosine (7).[14] *p*-Nitrobenzoic acid (254 mg, 1.52 mmol) was added to a solution of **6** (500 mg, 0.76 mmol) in dry THF (50 mL) under nitrogen. Then, Ph_3P (400 mg, 1.52 mmol) and DEAD (237 μL, 1.52 mmol) were added. After reaction for 2 h at room temperature, the solvent was evaporated in vacuo and the crude residue was purified by flash chromatography column (EtOAc) affording 432 mg (70%) of a yellow solid corresponding to compound **7**. R_f (EtOAc): 0.68; mp: 109–111°C; IR (KBr): ν 3413, 2929, 1731, 1638, 1617, 1507, and 1249 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.07 (m, 2H, $H_{2'}$), 3.46 (dd, 1H, $H_{5'}$, $^2J_{HH}$=9.0, 7.4 Hz), 3.69 (dd, 1H, $H_{5'}$, $^2J_{HH}$=9.0, 7.4 Hz), 3.74 (s, 3H, *Me*O), 3.75 (s, 3H, *Me*O), 4.67 (m, 1H, $H_{4'}$), 5.96 (m, 1H, $H_{3'}$), 6.55 (dd, 1H, $H_{1'}$, $^3J_{HH}$=5.4 Hz), 6.71 (m, 4H, $H_{m'}$), 7.18–7.28 (m, 7H, $H_{o'}$+$H_{m''}$+$H_{p''}$), 7.35 (m, 2H, $H_{o''}$), 7.48–7.67 (m, 5H, H_m+H_p+$H_{o'''}$), 8.01 (m, 2H, H_o), 8.20 (m, 3H, H_8+$H_{m'''}$), 8.61 (s, 1H, H_2), and 9.01 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 38.5 ($C_{2'}$), 54.6 (2C, 2*Me*O), 60.2 ($C_{5'}$), 72.9 ($C_{3'}$), 82.0 ($C_{4'}$), 84.0 ($C_{1'}$), 86.0 (C_t), 112.5 ($C_{m'}$), 123.1 (2C, C_5+$C_{m'''}$), 126.4 (CH), 127.3 (CH), 128.3 (CH), 129.4 (CH), 129.5 (CH), 129.9 (CH), 132.4 (C_p), 132.8 (C), 133.5 (C), 134.4 (C), 134.7 (C), 140.2 (C_8), 143.6 ($C_{i''}$), 148.9 (C), 150.0 (C), 150.8 (C), 151.9 (C_2), 157.9 ($C_{p'}$), 162.7 (C_e), and 164.1 (C_a); MS (ESI^+, *m/z*): 845 [$(M+K)^+$, 37%], 829 [$(M+Na)^+$, 33], and 807 [$(M+H)^+$, 100]. Anal. calcd (%) for $C_{45}H_{38}N_6O_9$: C, 66.98; H, 4.75; N, 10.42. Found: C, 67.0; H, 4.6; N, 10.2.

3.1.5. N^6-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyxyloadenosine (8).[15] Compound **7** (360 mg, 0.45 mmol) was dissolved in dry MeOH (50 mL) under nitrogen, and was cooled at 0°C. Then, a solution 0.2 M of NaOMe in MeOH (1.8 mL, 0.36 mmol) was added. After reaction for 1.5 h at 0°C, the solvent was evaporated in vacuo and the crude residue was purified by flash chromatography column (gradient eluent EtOAc-50% MeOH/EtOAc) affording 284 mg (97%) of a white solid corresponding to compound **8**.

3.1.6. N^6-Benzoyl-2′-deoxyxyloadenosine (9).[7c] A catalytic amount of formic acid (3–4 drops) was added to a solution of compound **8** (133 mg, 0.20 mmol) in $CHCl_3$ (14 mL). The reaction was stirred for 24 h at room temperature. Then, a few drops of 1N KOH were added, the solvent was evaporated in vacuo, and the crude residue was purified by flash chromatography (gradient eluent 10–20% MeOH/EtOAc) affording 70 mg (94%) of a white solid corresponding to compound **9**.

3.1.7. N^6-Benzoyl-3′,5′-di-*O*-methanesulfonyl-2′-deoxyxyloadenosine (10). DMAP (688 mg, 5.63 mmol) was added to a solution of compound **9** (200 mg, 0.56 mmol) in dry pyridine (8 mL) under N_2. Then, dry methanesulphonyl chloride (261 μL, 3.38 mmol) was added. After reaction for 2 h at room temperature, the solvent was evaporated in vacuo, and the crude residue was purified by flash chromatography (8% MeOH/EtOAc) to afford 216 mg (75%) of a white solid corresponding to compound **10**. R_f (20% MeOH/EtOAc): 0.65; mp: 75–77°C; IR (KBr): ν 3494, 1602, 1566, and 1251 cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz): δ 3.18–3.35 (m, 8H, $H_{2'}$+*Me*S), 4.82 (s, 3H, $H_{5'}$+$H_{4'}$), 5.74 (m, 1H, $H_{3'}$), 6.83 (dd, 1H, $H_{1'}$, $^3J_{HH}$=7.1, 3.0 Hz), 7.71–7.85 (m, 3H, H_m+H_p), 8.28 (m, 2H, H_o), 8.78 (s, 1H, H_8), and 8.91 (s, 1H, H_2); ^{13}C NMR (MeOH-d_4, 50.3 MHz): δ 37.4 (*Me*S), 38.3 (*Me*S), 40.2 ($C_{2'}$), 68.3 ($C_{5'}$), 80.0 (CH), 81.8 (CH), 85.4 ($C_{1'}$), 125.1 (C_5), 129.4 (CH), 129.8 (CH), 133.9 (C_p), 134.9 (C_i), 143.4 (C_8), 151.1 (C_6),

153.1 (C_4), 153.3 (C_2), and 168.1 (C=O); MS (ESI^+, *m/z*): 512 [$(M+H)^+$, 100%]. Anal. calcd (%) for $C_{19}H_{21}N_5O_8S_2$: C, 44.61; H, 4.14; N, 13.70. Found: C, 44.6; H, 4.2; N, 13.4.

3.1.8. N^6-Benzoyl-3′,5′-diazido-2′,3′,5′-trideoxyadenosine (11).[7c] Sodium azide (382 mg, 5.87 mmol) was added to a solution of compound **10** (500 mg, 0.98 mmol) in dry DMF (15 mL) under nitrogen. The reaction was stirred for 5 h at 65°C, then 2 mL of water were added, and the solvent was evaporated in vacuo. The crude residue was purified by flash chromatography (EtOAc) affording 297 mg (75%) of a white solid corresponding to compound **11**.

3.1.9. N^6-Benzoyl-3′,5′-diamino-2′,3′,5′-trideoxyadenosine (12).[16] A solution of compound **11** (50 mg, 0.12 mmol) in EtOH (4 mL) was exposed to a positive pressure of hydrogen gas (balloon) at room temperature for 4 h in the presence of Pd black (20 mg). The catalyst was removed by filtration on Celite and the filtrate was evaporated to dryness. The crude was purified by flash chromatography [1% NH_3(aq.)/MeOH] to afford after vacuum drying 35 mg (81%) of a white solid corresponding to compound **12**. R_f [5% NH_3(aq.)/MeOH]: 0.33; mp: 74–76°C (decomposed); IR (KBr): ν 3454, 3364, 2936, 1636, 1603, 1567, and 1488 cm^{-1}; ^1H NMR (D_2O, 300 MHz): δ 2.32 (m, 1H, $H_{2'}$), 2.64 (m, 1H, $H_{2'}$), 2.90–3.09 (m, 2H, $H_{5'}$), 3.54 (m, 1H, $H_{3'}$), 3.86 (m, 1H, $H_{4'}$), 6.23 (dd, 1H, $H_{1'}$, $^3J_{HH}$=5.7 Hz), 7.22 (m, 2H, H_m), 7.36 (m, 1H, H_p), 7.61 (d, 2H, H_o, $^3J_{HH}$=7.7 Hz), 8.22 (s, 1H, H_8), and 8.35 (s, 1H, H_2); ^{13}C NMR (D_2O, 75.5 MHz): δ 39.4 ($C_{2'}$), 42.6 ($C_{5'}$), 52.4 ($C_{3'}$), 84.6 ($C_{4'}$), 85.8 ($C_{1'}$), 123.7 (C_5), 128.2 (CH), 128.9 (CH), 132.7 (C_i), 133.4 (C_p), 143.3 (C_8), 149.6 (C), 151.2 (C), 152.0 (C_2), and 168.2 (C=O); MS (ESI^+, *m/z*): 376 [$(M+Na)^+$, 5%] and 354 [$(M+H)^+$, 100]. Anal. calcd (%) for $C_{17}H_{19}N_7O_2$: C, 57.76; H, 5.42; N, 27.76. Found: C, 57.6; H, 5.6; N, 27.9.

3.1.10. N^6-Benzoyl-3′,5′-di-*O*-methanesulfonyl-2′-deoxyadenosine (15). The same procedure as the one described for **10** yielded **15** (white solid, 97%). R_f (20% MeOH/EtOAc): 0.78; mp: 57–59°C; IR (KBr): ν 3470, 2942, 1699, 1603, 1566, 1356, 1250, and 1175 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.85 (m, 1H, $H_{2'}$), 3.17 (s, 3H, *Me*S), 3.24–3.38 (m, 4H, $H_{2'}$+*Me*S), 4.52 (s, 3H, $H_{5'}$+$H_{4'}$), 5.58 (m, 1H, $H_{3'}$), 6.59 (dd, 1H, $H_{1'}$, $^3J_{HH}$=7.0 Hz), 7.54 (m, 2H, H_m), 7.64 (m, 1H, H_p), 8.04 (d, 2H, H_o, $^3J_{HH}$=7.3 Hz), 8.69 (s, 1H, H_8), 8.78 (s, 1H, H_2), and 11.26 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz): δ 36.0 ($C_{2'}$), 36.8 (*Me*S), 37.8 (*Me*S), 68.4 ($C_{5'}$), 79.9 (CH), 81.5 (CH), 83.7 (CH), 125.9 (C_5), 128.5 (2C, C_o+C_m), 132.5 (C_p), 133.3 (C_i), 143.2 (C_8), 150.6 (C_6), 151.8 (2C, C_4+C_2), and 165.7 (C=O); MS (ESI^+, *m/z*): 512 [$(M+H)^+$, 100%], and 534 [$(M+Na)^+$, 5%]. Anal. calcd (%) for $C_{19}H_{21}N_5O_8S_2$: C, 44.61; H, 4.14; N, 13.70. Found: C, 44.8; H, 3.9; N, 13.5.

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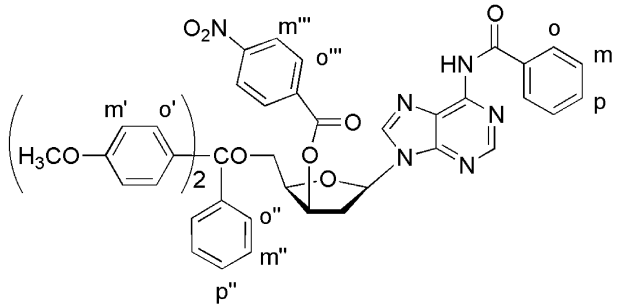
14. Structure of compound **7** is labelled as follows:



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