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Nucleosides and oligonucleotides containing 1,2,3-triazole residues with nucleobase tethers: Synthesis via the azide-alkyne 'click' reaction

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ABSTRACT

A series of novel 1,2,3-triazole nucleosides linked to DNA nucleobases were prepared via copper(I)-catalyzed 1,3-dipolar cycloaddition of *N*-9 propargylpurines or *N*-1 propargylpyrimidines with the tolouyl protected 1-azido-2-deoxyribofuranose **2** followed by treatment with NaOMe/MeOH or aq NH_3. The antiviral activity of such compounds against selected RNA viruses is reported. The strongly fluorescent 1,2,3-triazole compounds **16** and **17** were synthesized from propargylated uracil **1a** and propargylated adenine **1c** with coumarin azide **15,** and the fluorescence properties were studied. The nucleosides **4** and **6** were incorporated into DNA using the phosphoramidite building blocks and employed in solid-phase synthesis. Melting experiments demonstrated that such 1,2,3-triazole nucleosides have a negative impact on the duplex stability when they are placed opposite to the canonical bases as well as abasic sites. The nucleobases attached to the triazole ring cannot involve in the base pair formation with the opposite bases because of the structural variations induced by the triazole ring. The stacking of such nucleosides when positioned at the end of oligonucleotides retains the stability of DNA duplexes. The duplex structures were studied by molecular modelling which support the results of melting experiments.

1. Introduction

The copper(I)-catalyzed Huisgen–Sharpless–Meldal 1,3-dipolar cycloaddition ('click' chemistry) between alkynes and azides resulting in the formation of 1,4-disubstituted 1,2,3-triazoles has gained significant importance because of its wide range of applications in various fields of drug discovery,[1] bioconjugation,[2,3] and material or surface science.[4–7] Five-membered triazole nucleosides are of special interest because of their pronounced biological activities. Among them, a 1,2,4-triazole ring derivative ribavarin (virazole) and 1,2,3-triazole TSAO analogues are used for the treatment of hepatitis C and HIV-1 virus.[8–10]

Various 1,2,3-triazole acyclonucleosides were synthesized from the propargylated nucleobases[11] using the copper free Huisgen 1,3-dipolar cycloaddition to evaluate their anti-HIV activity.[12] Recently, 2-ethynylfuro[2,3-*b*]pyrazines were coupled with various sugars using microwave-assisted 'click' reaction to form 1,2,3-triazole derivatives.[13] Moreover, a related class of modified nucleosides called 'fleximers' were developed by Seley and co-workers. In this class of molecules the nucleobases were 'split' but still retained the key recognition sites of DNA bases.[14] In this manuscript, we wish to report on the synthesis of novel 1,2,3-triazole nucleosides that are linked to DNA nucleobases as recognition elements. They were prepared by the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction between a terminal C≡C bond of propargylated purine or pyrmidine bases with the azide moiety of the sugar component **2** (Scheme 1). The antiviral activity of propargylated bases and 1,2,3-triazole nucleosides is evaluated. The 1,2,3-triazole nucleoside conjugates **4** and **6** are incorporated into DNA by solid-phase synthesis and the duplex stability was studied.

2. Results and discussion

2.1. Synthesis of the monomers

The Huisgen 1,3-dipolar cycloaddition[15] of alkynes and azides is used for the synthesis of the nucleobase linked triazole nucleosides. Previously 1,2,3-triazole acyclonucleosides were prepared via 1,3-dipolar cycloaddition reaction using toluene as solvent at elevated temperatures (110 °C) yielding a mixture of regioisomers.[12] Cu(I) dramatically enhances the rate of reaction and controls the product formation at room temperature. Thus, the copper assisted cycloaddition proceeds in a chemoselective way with the formation of 1,4-disubstituted 1,2,3-triazoles as the sole products.[16,17]

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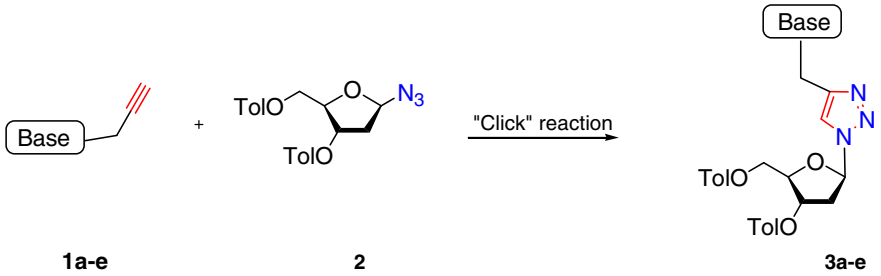
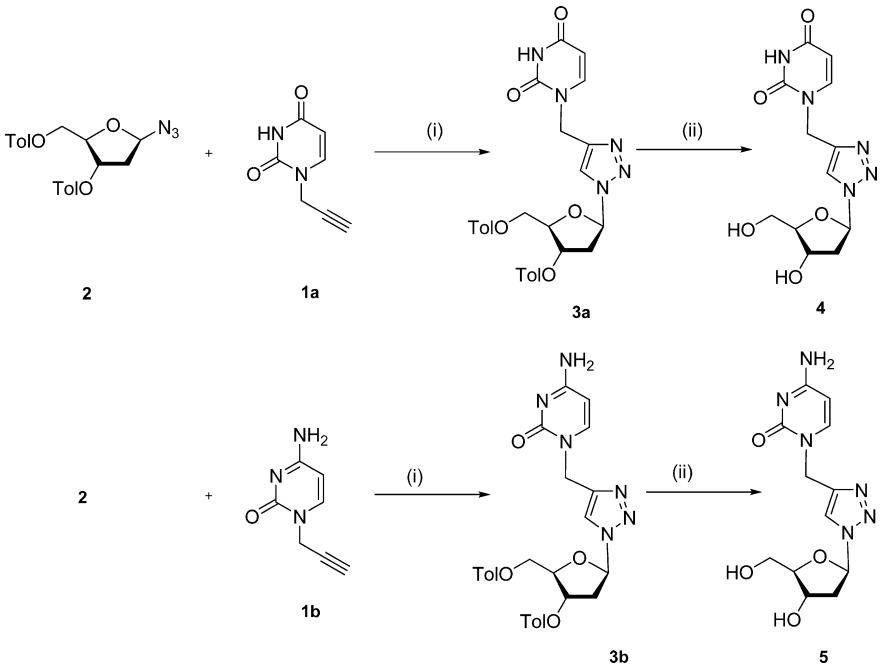


Scheme 1. Copper(I)-catalyzed 'click' reaction between propargylated bases and the azido sugar **2**.

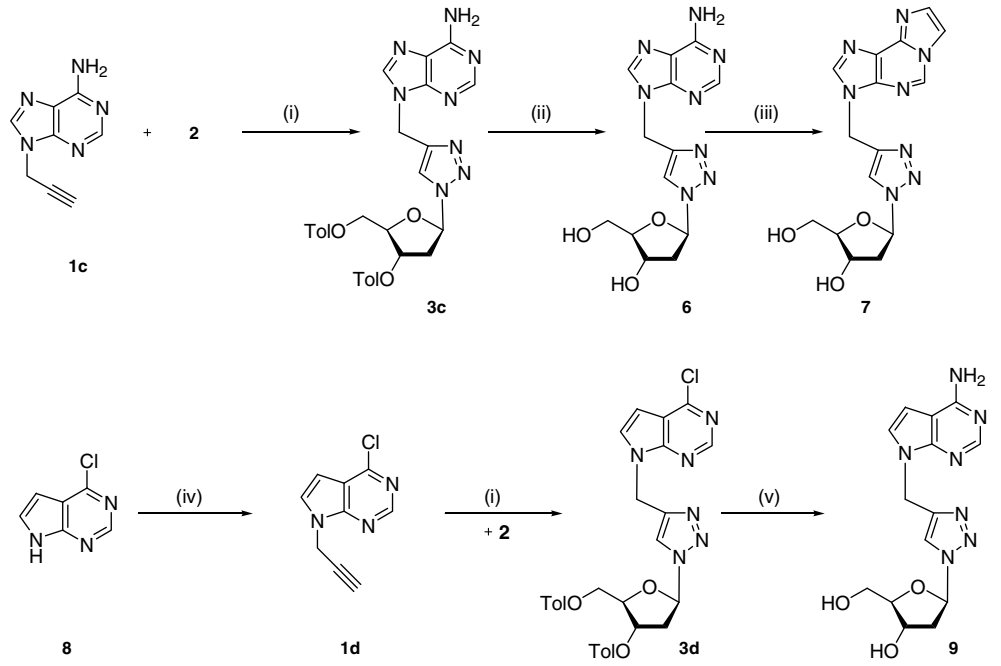
We decided to explore the feasibility of the 'click' chemistry for the construction of novel 1,2,3-triazole nucleosides with pyrimidine and purine tethers connected via a flexible methylene linker. At first, the pyrimidine 1,2,3-triazole nucleosides **4** and **5** were synthesized. For this the pyrimidine nucleobases uracil and cytosine were used as starting materials which were treated with propargyl bromide in the presence of K_2CO_3 and DMF[11,12] to give *N*-1 isomers **1a** and **b** exclusively (71% and 75% yield). Next, the terminal C≡C bonds of propargylated nucleobases **1a** and **b** were ligated to the azide residue of the toluoyl protected 2-deoxyribose **2**[18,19] using the copper(I)-catalyzed 1,3-dipolar cycloaddition to afford the click products **3a** and **b** (Scheme 2). The reaction was performed in THF/H_2O/*t*-BuOH (3:1:1) in the presence of sodium ascorbate and copper sulfate with a little excess of azide at room temperature leading to the product yields 80–85%. Finally, the sugar protecting groups of the triazoyl nucleosides **3a** and **b** were removed using 0.2 M NaOMe/MeOH to generate the nucleosides **4** and **5**.

In a similar fashion 1,2,3-triazole nucleosides containing the adenine and 7-deazapurine bases **1c** and **d** at the 4-position of the triazole moiety were prepared using the above reaction conditions. For that purpose, adenine or 6-chloro-7-deazapurine[20] (**8**) was treated with propargyl bromide to form **1c**[11,12] and **d** followed by cycloaddition with the azido sugar **2** thereby forming the protected nucleosides **3c** and **d**. Then, the protected nucleoside **3c** or **d** was converted (sodium methoxide or aqueous ammonia in an autoclave at 90 °C) to produce nucleosides **6** or **9** (Scheme 3). The etheno nucleoside **7** was prepared from the 1,2,3-triazole nucleoside **6**, which was reacted with chloroacetaldehyde (pH 4.5–5) to give the strongly fluorescent N^6-etheno derivative **7**. The reaction was performed in a similar way as reported for the synthesis of the etheno derivatives of adenine and cytosine.[21]

In addition to the above nucleosides we also demonstrated the application of 'click' reaction in the 7-deazaguanine series using the 2-amino-6-chloro-7-deazapurine (**10**)[22] as starting material. This was converted into the *N*9-propargylated base **1e** by using the same reaction conditions discussed above. Compound **1e** was ligated to the azido sugar **2** to yield the protected nucleoside **3e**. The displacement of the 6-chloro group as well as the deblocking of the toluoyl protecting groups of **3e** was performed with 0.2 M NaOMe/MeOH affording the nucleoside **11**, while deblocking of the protected nucleoside **3e** with methanolic ammonia (saturated at 0 °C at rt) gave compound **13**. The 2,6-dia-



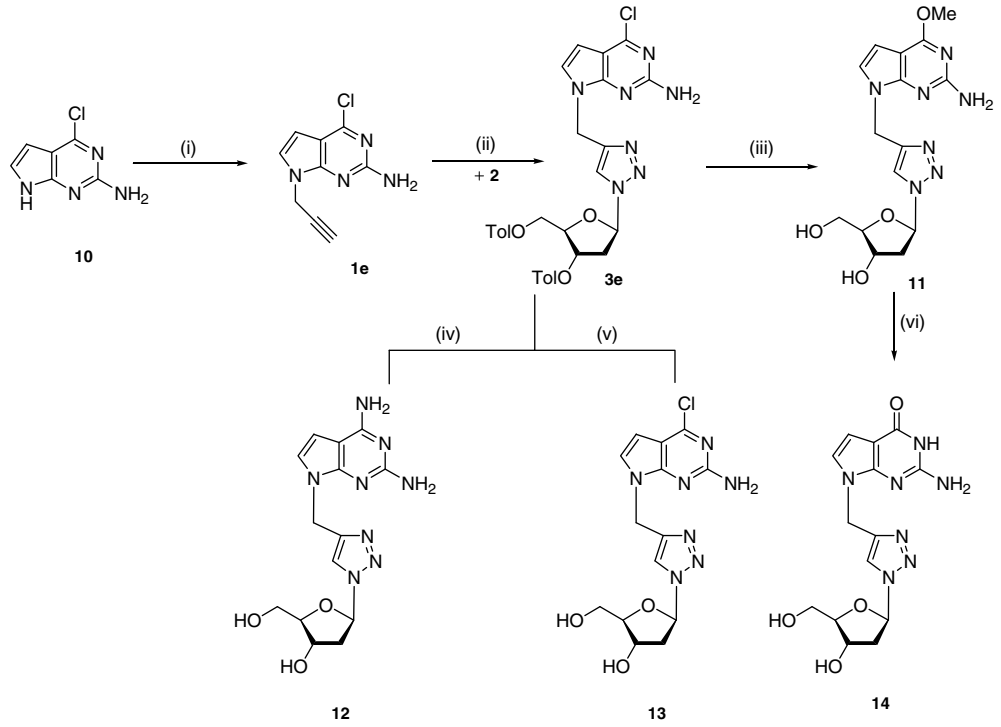
Scheme 2. Reagents and conditions: (i) $CuSO_4$, Na-ascorbate, THF/H_2O/*t*-BuOH (3:1:1), rt; (ii) 0.2 M NaOMe/MeOH, rt.



Scheme 3. Reagents and conditions: (i) $CuSO_4$, Na-ascorbate, THF/H_2O/*t*-BuOH (3:1:1), rt; (ii) 0.2 M NaOMe/MeOH, rt; (iii) $ClCH_2CHO$, 1 M aq NaOAC buffer (iv) Propargyl bromide, K_2CO_3, DMF, rt; (v) 28% NH_4OH-dioxane (4:1), 90 °C.

mino nucleoside **12** was obtained from **3e** when treated with aq NH_3 (90 °C); the guanosine analogue **14** became accessible from compound **11** (Scheme 4).

The structure of all compounds was confirmed on the basis of ^1H, ^{13}C NMR spectra as well as by elemental analysis. The regioselective formation of the N1/N9 of pyrimidines/purines toward propargylation was determined on the basis of gated-decoupled spectra (Table 1). The terminal alkynyl C-atom of the *N*1/*N*9 propargylated bases **1a** and **c** shows C, H couplings of $^1J_{C,H}$ = 252 Hz, ($^2J_{C,H}$) = 51 Hz and the CH_2 group of the propargyl side chain shows a coupling of ($^1J_{C,H}$) = 147 Hz (Table 2). The propargyl side chain coupling constants are similar in both cases. The site of alkylation was determined to be *N*1/*N*9 based on the ^{13}C NMR chemical shifts of related *N*1/*N*9 substituted uracil/adenine bases



Scheme 4. Reagents and conditions: (i) propargyl bromide, K_2CO_3, DMF, rt; (ii) $CuSO_4$, Na-ascorbate, THF/H_2O/*t*-BuOH (3:1:1), rt; (iii) 0.2 M NaOMe/MeOH, rt; (iv) 28% NH_4OH-dioxane (4:1), 90 °C; (v) NH_3/MeOH, rt; (vi) 2 N NaOH, reflux.

Table 1
^{13}C NMR chemical shifts (δ) of nucleobases measured in DMSO-d_6 at 298 K

Compound	C(2)[a] C(2)[b]	C(4)[a] C(7a)[b]	C(5)[a] C(4a)[b]	C(6)[a] C(4)[b]	C(7)[a] C(5)[b]	C(8)[a] C(6)[b]	CH_2	Triple bonds	
								C≡CH	C≡CH
1[a]	150.4	–	–	163.6	101.7	144.5	36.6	78.1	75.8
1b	155.2	–	–	166.0	94.1	144.8	37.4	79.5	75.3
Ade[d]	152.4	151.3	117.6	155.3	–	139.3	–	–	–
1c	152.8	149.1	118.5	156.0	–	140.2	32.3	78.4	76.0
8	150.3[c]	151.9	116.6	150.3[c]	98.8	128.3	–	–	–
1d	150.2[c]	150.8[c]	116.8	150.6[c]	99.1	130.8	33.9	78.3	76.0
10	159.4	154.7	108.7	151.0	98.8	123.2	–	–	–
1e	159.5	153.2	108.6	151.4	99.3	125.8	33.2	79.0	75.5

[a] Purine numbering.
[b] Systematic numbering.
[c] Tentative.
[d] Adenine.

Table 2
Coupling constants $J_{C,H}$ (Hz) of nucleobases measured in DMSO-d_6 at 298 K

	C(5)[a]/C(7)[b]		C(6)[a]/C(8)[b]		CH_2		CH_2—**C**≡CH		CH_2—C≡**C**H	
	($^1J_{C,H}$)	($^3J_{C,H}$)	($^1J_{C,H}$)	($^3J_{C,H}$)	($^1J_{C,H}$)	($^3J_{C,H}$)	($^2J_{C,H}$)	($^2J_{C,H}$)	($^1J_{C,H}$)	($^3J_{C,H}$)
1a	175.9	2.3	182.1	4.1	146.7	3.6	50.6	8.8	252.7	4.0
1b	172.4	3.7	179.7	3.9	145.5	3.7	50.5	8.9	252.0	4.0
1c	–	–	212.6	3.5	146.2	3.6	50.9	9.1	252.9	4.0
1d	181.9	7.3	190.9	3.9	146.3	3.4	50.8	9.1	252.9	4.1
1e	179.5	7.3	189.7	3.9	146.9	3.5	50.6	8.9	252.4	4.0

[a] Pyrimidine.
[b] Purine.

or nucleosides.[22–25] According to ^{1}H NMR spectra of the 'click' products the terminal triple bonded proton signal (δH = 3.3 ppm) of the alkynyl base disappeared and the newly formed triazole was now observed at 8–9 ppm. The triazole ring formation can also be identified from the ^{13}C-spectra with the new signals of the olefinic C-atoms of the 1,2,3-triazole moiety at δ(C5 = 122–124 ppm) and δ(C4 = 142–144 ppm). The large difference in the chemical shifts (δC4–δC5 = 20 ppm) indicates the formation of 1,4-regioisomers (Table 3). An unambiguous assignment was performed on nucleoside **4** on the basis of the ^{1}H/^{13}C heteronuclear long-range correlations spectra (HMBC). Figure 1 displays the essential part of the spectrum (complete spectrum not shown). The triazole C5 shows $^3J_{C,H}$ couplings with the anomeric proton C1′-H as well as with the methylene group of the linker. In contrary C4 does not show any long-range coupling with C1′-H confirming the formation of the 4-substituted triazole moiety.

2.2. Antiviral activity

Triazole nucleosides show pronounced biological activities. The five-membered 1,2,4-triazole nucleoside ribavarin (virazole) was the first synthetic nucleoside showing a broad spectrum of antivi-

Table 3
^{13}C NMR chemical shifts (δ) of modified nucleosides and their derivatives measured in DMSO-d_6 at 298 K

	C(2)[a] C(2)[b]	C(4)[a] C(7a)[b]	C(5)[a] C(4a)[b]	C(6)[a] C(4)[b]	C(7)[a] C(5)[b]	C(8)[a] C(6)[b]	Triazole[c]		C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
3a	150.8	–	–	163.8	101.3	145.5	142.9	123.3	87.8	[d]	74.6	82.3	63.9
4	150.8	–	–	163.8	101.3	145.6	142.7	122.2	88.1[e]	[d]	70.5	88.3[e]	61.6
3b	155.7	–	–	166.1	93.8	145.9	143.6	123.5	87.8	[d]	74.7	82.3	64.0
5	155.8	–	–	166.2	93.9	146.1	143.6	122.5	88.1[e]	[d]	70.7	88.4[e]	61.8
6	152.8	149.4	118.6	156.0	–	140.9	142.9	122.5	88.2[e]	[d]	70.6	88.4[e]	61.7
7	138.5	140.7	122.6	141.1	–	137.0	142.8	122.3	88.1[e]	[d]	70.4	88.3[e]	61.5
3d	150.1[e]	150.7[e]	116.8	150.4[e]	98.9	131.2	143.0	123.2	87.9	[d]	74.6	82.3	63.9
9	150.2	148.9	102.2	156.4	99.6	124.5	143.6	122.2	88.0	[d]	70.5	88.3	61.6
3e	159.4	153.3	108.6	151.3	99.0	123.2	143.6	123.2	87.8	[d]	74.6	82.3	64.0
13	159.5	153.4	122.4	151.4	99.2	126.3	143.5	122.4	88.2[e]	[d]	70.6	88.4[e]	61.8
11	159.7	153.9	98.5	163.2	97.2	122.6	144.0	122.4	88.2[e]	[d]	70.7	88.4[e]	61.8
12	157.6	151.5	100.0	156.1	95.5	121.0	143.9	122.2	88.0[e]	[d]	70.5	88.3[e]	61.7
14	152.8	150.5	102.1	159.6	100.1	120.7	144.2	122.6	88.4[e]	[d]	70.8	88.5[e]	62.0
20	150.8	–	–	163.8	101.3	145.3	142.4	123.0	86.1[e]	[d]	70.4	87.5[e]	64.0
22	151.9	150.3	125.2	152.4	–	144.9	142.6	122.7	88.4[e]	[d]	70.6	88.5[e]	61.8
23	151.6	150.2	125.1	152.2	–	144.7	142.2	123.0	87.6[e]	[d]	70.4	86.1[e]	64.1
16	150.8	–	–	162.5	101.3	145.5	142.5	123.2	–	–	–	–	–
17	152.6	149.3	118.6	155.9	–	140.7	142.7	124.5	–	–	–	–	–

[a] Purine numbering.
[b] Systematic numbering.
[c] Triazole carbons.
[d] Superimposed by the signal of DMSO-d_6.
[e] Tentative.

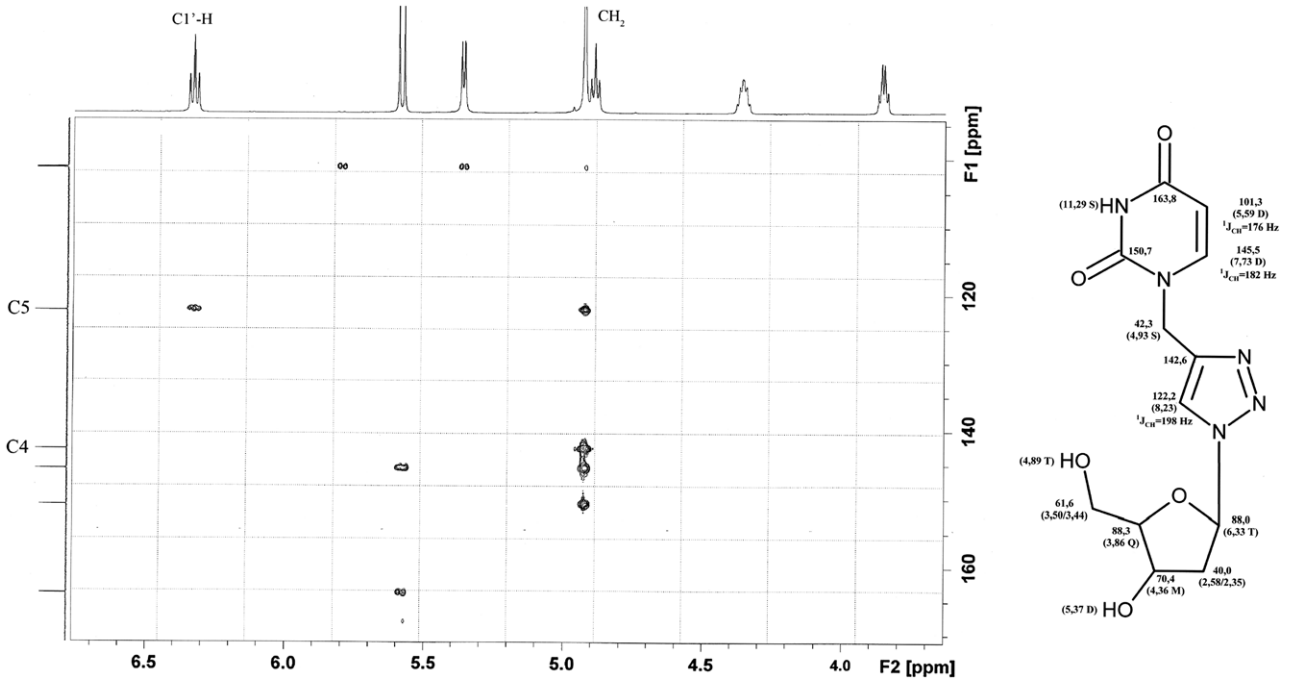


Figure 1. HMBC spectrum of **4** measured in DMSO-d_6.

ral activities against many RNA and DNA viruses.[26] This prompted us to evaluate the antiviral activity of the related 1,2,3-triazole nucleosides as well as propargylated bases. Among the tested viruses, representatives of flavivirus which belong to the class of positive-sense single-stranded RNA (ss RNA$^+$) viruses were selected. These are *Hepacivirus* (hepatitis C; HCV), yellow fever virus (YFV), dengue virus type 2 (DENV-2), and West Nile virus (WNV). Unfortunately, none of the compounds show selective antiviral activity against the tested ssRNA$^+$ viruses. First, regarding the activity in the HCV replicon system a series of compounds were selected for testing (Table 4). Their ability to inhibit the HCV replicon replications at the test concentration of 15 μM was evaluated by an HCV replicon assay (ELISA) as previously described.[27] The cellular toxicity was determined at the test concentration of 15 μM of drug in GS4.1 cells. For all compounds inhibition of the HCV replicon replication is accompanied by cytotoxicity. Only the toluoyl protected 7-deazaadenine and 7-deazaguanine nucleosides (**3d** and **e**) as well as its propargylated bases **1d** and **e** develop less cytotoxicity than other triazole compounds in the HCV replicon system. Next, these compounds were tested against YFY, DENV, WNV measured by the inhibition of virus-induced cytopathogenicity in acutely infected baby hamster kidney (BHK-21) cells. The data are summarized in Table 5. However, in all the compounds the antiviral activity is accompanied by cytotoxicity for the host cell lines (BHK-21) within the same concentration range.

Table 4
Activity of 1,2,3-triazole nucleosides and propargyl bases in the HCV[a] replicon system

Compound	HCV Replicon		Compound	HCV Replicon	
	% Inhib.[b]	% Tox.[c]		% Inhib.[b]	% Tox.[c]
3a	11.32	−4.12	**7**	6.07	−2.24
4	0.04	−5.59	**22**	9.02	−3.55
3b	15.64	−8.12	**3d**	49.25	20.77
5	10.08	−5.59	**9**	−3.8	−3.79
3e	32.06	6.49	**13**	2.93	−3.3
1a	12.09	−8.85	**1c**	2.99	−10.4
1d	69.22	1.84	**1e**	44.82	1.35
6	7.54	−0.61	—	—	—

[a] *Hepacivirus*.
[b] The observed inhibition of HCV replicon replication at the test concentration of 15 μM of drug as determined by the HCV replicon (ELISA) assay.
[c] The observed cellular toxicity at the test concentration of 15 μM of drug.

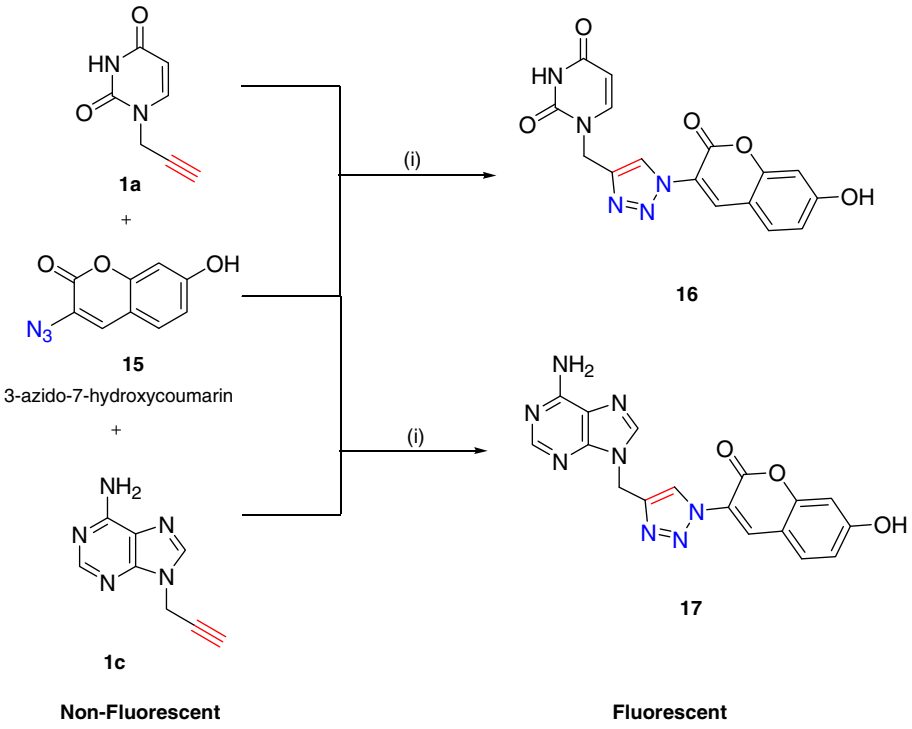
Table 5
Activity of 1,2,3-triazole nucleosides and propargyl bases against selected ssRNA viruses in BHK cell lines

Compound	YFV[a]		DENV[a]		WNV[a]	
	% Inhib.[b]	% Tox.[c]	% Inhib.[b]	% Tox.[c]	% Inhib.[b]	% Tox.[c]
3a	8.56	−1.14	0.82	3.37	−2.84	3.37
4	4.59	−4.57	0.75	9.5	−2.67	9.5
3b	2.03	−1.98	−2.26	4.08	−3.67	4.08
5	7.89	−4.62	0.95	9.41	0.24	9.41
3e	−6.17	2.38	−2.39	0.17	−1.44	0.17
1a	−11.35	0.08	−4.6	2.76	−4.57	2.76
1d	−0.25	−19.45	−0.85	−9.83	3.81	−9.83
6	8.36	−2.12	1.02	7.85	−0.43	7.85
7	4.39	−11.67	2.23	−2.95	−2.44	−2.95
22	8.76	−2.91	1.02	5.54	−1.16	5.54
3d	0.82	−1.39	−1.05	0.31	−2.95	0.31
9	10.98	4.48	3.43	3.14	2.42	3.14
13	13.33	8.55	2.49	1.3	5.71	1.3
1c	−2.81	−7.46	−3.4	3.8	−1.77	3.8
1e	−3.21	0.72	1.56	−2.0	0.07	−2.0

[a] *Flavivirus*.
[b] The observed inhibition against *Flavivirus* at the test concentration 15 μM of drug in acutely infected BHK cell lines as determined by CPE (cytopathic effect) assay.
[c] The observed cellular toxicity at the test concentration of 15 μM of drug in BHK cell lines.

2.3. Fluorescence properties of 1,2,3-triazole coumarin conjugates bearing nucleobase tethers

Earlier, the copper(I)-catalyzed 'click' reaction has been used to introduce various reporter groups into DNA.[28,29] Our laboratory has reported on the fluorescence properties of four DNA octadiynyl nucleosides attached with a coumarin dye.[30,31] Coumarin dyes are



Scheme 5. Reagents and conditions: (i) $CuSO_4$, Na-ascorbate, THF/H_2O/*t*-BuOH (3:1:1), rt.

widely used because of their favourable photophysical properties. They are utilized in the blue spectra region with strong emission intensities and applied as nucleobase specific quenchers to determine structural dynamics of DNA.[32] From the previous reports it can be concluded that a dye attached to a nucleobase via a rigid linker (*N*3-propargylated 2′-dU) enhances the fluorescence of nucleosides compared to more flexible linker units (5-octadiynyl-2′dU).[33] In order to test this phenomenon at the nucleobase level we report on the fluorescence properties of nucleobase-coumarin conjugates. The 1,2,3-triazole ring formed from the terminal alkyne and azido coumarin was found to generate fluorescence.[34] In order to study the fluorescence behavior of dye molecule when attached to adenine and uracil bases via a short linker a series of compounds were synthesized. First, the uracil base **1a** containing a N1-terminal alkynyl group was selectively conjugated to the azide residue of coumarin azide **15** to form fluorescent 1,2,3-triazolyl nucleobase-dye conjugate **16**. Next, the terminal triple bond of adenine **1c** was conjugated with the non-fluorescent coumarin azide **15**, leading to the formation of adduct **17** in 78% yield (Scheme 5). The 'click' functionalized compounds were identified by ^1H, ^{13}C NMR, gated-decoupled spectra as well as by mass spectra. The ^1H NMR spectra of click products revealed the disappearance of two terminal C≡C hydrogens (singlet at 3.23 ppm); two new singlets appeared at a range of δ 8–9 ppm attributed to the hydrogens from the newly formed triazole rings. The characteristic signals of the olefinic carbon atoms of the newly formed 1,2,3-triazole moiety were identified in the range of δ(C) 123.2 and 142.5 ppm (Table 3). Moreover, the chemical shifts of the triazole ring carbons are clearly evidenced by ^1H, ^{13}C, DEPT-135 NMR spectra. The DEPT-135 NMR spectra show no signals for the quaternary carbons (δC4) and an inverted signal for the triazole carbon (δC5) at around 125.4 ppm.

Previous reports have shown that the fluorescence of coumarin derivatives is pH dependent, different absorption bands are observed at different pH values due to the presence of neutral and phenolate anionic species. Alkaline pH leads to the predominant existence of phenolate anion.[35] The fluorescence spectrum of uracil-dye compound **16** shows an emission at 476 nm when excited at 346 nm at pH 7.0, Tris–HCl buffer (Fig. 2). Similar emission and excitation wavelengths were found for the adenine dye conju-

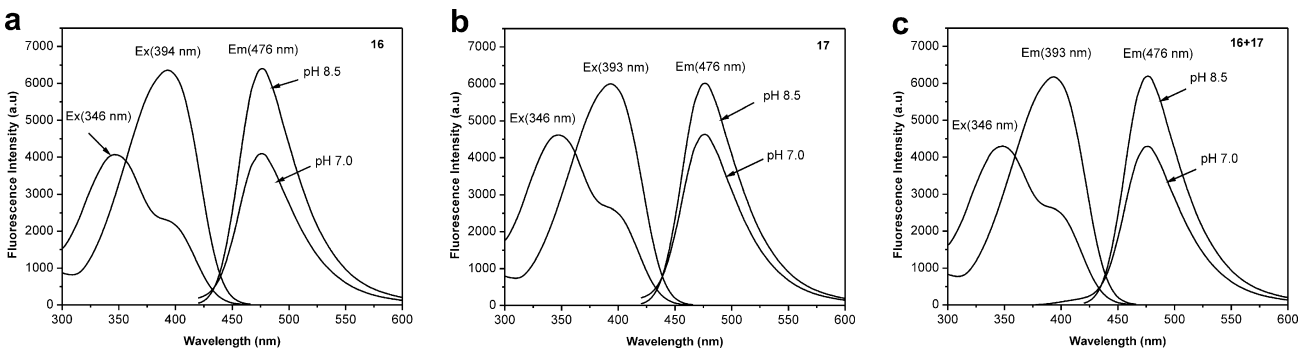


Figure 2. Fluorescence emission and excitation spectra of compounds **16** (a) and **17** (b) and 1:1 mixture of **16** + **17** (c) at an equimolar concentration (2×10^{-5} M) measured in pH 7.0 and pH 8.5 Tris–HCl buffer.

gate **17** (Fig. 2b). Mixing an equal amount of A–U dye conjugates (**16** and **17**) does not lead to any fluorescence quenching, thus base pair formation seems not to occur. Similar results were observed when the above experiment was performed in pH 8.5 Tris–HCl buffer, but the excitation was shifted bathochromically to 394 nm with an identical emission (476 nm), which revealed the existence of phenolate anionic species of coumarin. According to previous reports the fluorescence properties of nucleobase-coumarin pairs are strongly influenced by the length of the side chain between nucleobase and dye resulting in strong interactions between the residues.[36,33] From the above experiments we conclude that 1,2,3-triazolyl dye conjugates **16** and **17** employing a rigid short chain do not lead to significant fluorescence quenching.

In order to compare the fluorescence properties of the dye attached to the nucleobases as well as nucleosides, compounds **16**, **18**, and **19**[33] were chosen. The signal intensity observed in the fluorescence spectra of the dye conjugates is in the order of (**16** > **18** > **19**) with the lowest fluorescence observed for the flexible linker side chain conjugate **19** (Fig. 3). The emission maximum for **18** and **19** is found to be almost identical with a 476 nm emission when excited at 393 nm at pH 8.5. Compared to the nucleoside dye conjugates **18** and **19,** the base conjugate **16** shows the highest fluorescence due to the placement of dye at *N*1 position of the base moiety. Moreover, an increase of the π-system by an electronic coupling between the dye and the nucleobase via the linker moiety can make a favorable contribution.

Next, similar experiments were performed with the etheno nucleosides. The fluorescence properties of etheno nucleosides[37] enable them to be used as structural and functional probes in DNA and RNA.[38] Our laboratory has reported on different 7-deazapurine, 8-azapurine, and 2,8-dideazapurine etheno nucleosides, which possess strong fluorescence properties.[39] So, the photophysical properties of the click product **7** were studied and compared with N^6-ethenoadenosine. Compound **7** shows an emission at 409 nm and an excitation observed at 292 nm with a stoke shift of 118 nm (Fig. 4). A similar emission is observed for the related N^6-ethenoadenosine derivative.[39] In order to understand the influence of the triazole ring on the fluorescence, the quantum yield of the nucleoside **7** was determined under neutral conditions in water. The quantum yield (Φ) of the 1,2,3-triazole etheno nucleoside **7** was found to be 0.51, which is almost similar to that of N^6-ethenoadenosine (0.52).[39] From Figure 3A and B both etheno derivatives show similar/comparable fluorescence.

2.4. Synthesis of phosphoramidites and properties of oligonucleotides

Next, the influence of 1,2,3-triazolyl modified nucleosides **4** and **6** containing uracil and adenine nucleobases on the stability of DNA was studied. For this various oligonucleotides were prepared. The amino group of **6** was protected with the benzoyl residue using the protocol of transient protection.[40] Then, the pyrimidine nucleoside **4** and protected purine compound **22** were converted into the DMTr-derivatives **20** and **23**, and further transformed into the phosphoramidite building blocks **21** and **24** under standard conditions (Scheme 6). The structure of all compounds were characterized by ^{1}H, ^{13}C (see Table 3), and ^{31}P NMR spectroscopy as well as by elemental analysis.

Oligonucleotide synthesis was carried out on solid phase with an ABI 392-08 synthesizer at a 1 μmol scale employing the synthesized phosphoramidites **21** and **24** as well as standard building blocks. The coupling yields were always higher than 95%. The

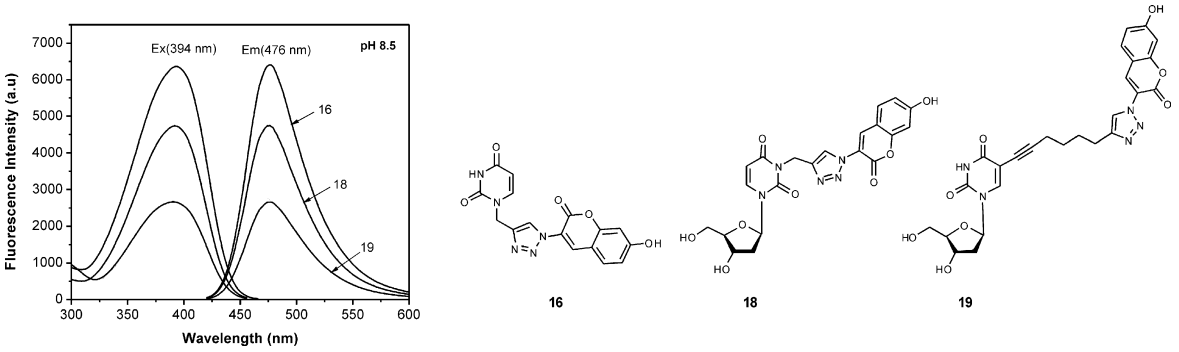


Figure 3. Fluorescence excitation and emission spectra of compounds **16**, **18**, and **19** at an equimolar concentration (2×10^{-5} M) measured in 0.1 M Tris–HCl buffer (pH 8.5).

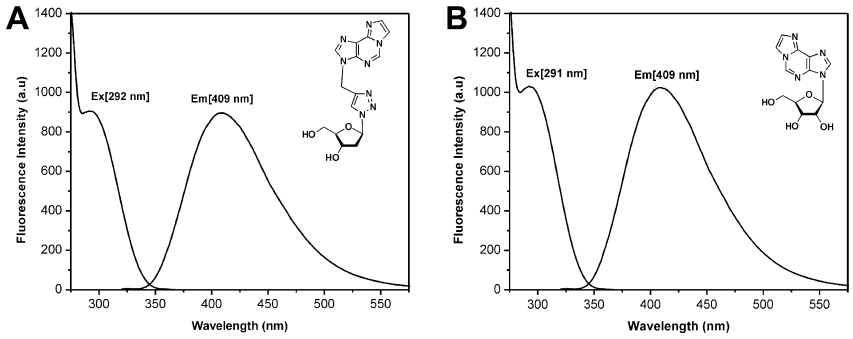
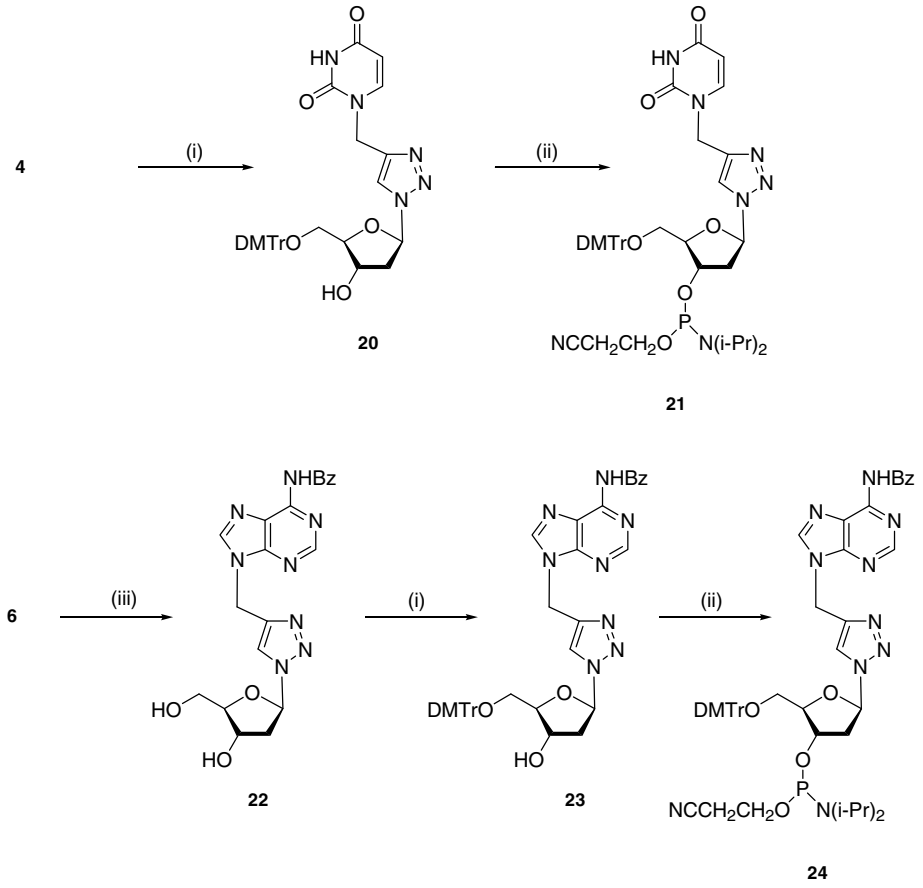


Figure 4. Steady-state excitation and emission spectra of nucleoside **7** (A), and N^6-ethenoadenosine (B) at an equimolar concentration (2.8×10^{-5} M) measured in water at room temperature.



Scheme 6. Reagents and conditions: (i) DMTr-Cl, pyridine, rt; (ii) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$, *i*-Pr_2EtN, CH_2Cl_2, 30 min, rt; (iii) Me_3SiCl, benzoyl chloride, pyridine, 2 h, rt.

synthesis of oligonucleotides was performed by employing the DMT-on mode. After cleavage from the solid support, the oligomers were deprotected in 25% aqueous ammonia solution for 14–16 h at 60 °C. The purification of the 5′-dimethoxytritylated oligomers was carried out by reversed-phase HPLC (see Section 4). The molecular masses of the oligonucleotides were determined by Applied Biosystems Voyager DE PRO with 3-hydroxypicolinic acid (3-HPA) as a matrix. The detected masses were close to the calculated values (see Section 4, Table 7).

Hybridization experiments were performed using the oligonucleotide duplex 5′-d(TTT TTT TTT TTT)-3′ (**25**) and 3′-d(AAA AAA AAA AAA) (**26**) as a reference (see Table 6). One incorporation of 1,2,3-triazole compound **4** replacing dT in the standard oligomer **25** results in a T_m decrease of 12 °C when compared to the standard duplex. A similar result was observed when dA was replaced by **6** in the standard sequence, but this decrease is less when compared to that of **4**. When both nucleosides **4** and **6** are placed opposite to each other the T_m decreases by 27 °C. These results clearly indicate that such 1,2,3-triazole nucleosides with nucleobase tethers when placed in DNA cannot form base pairs because the methylene linked nucleobases are flexible and not stacked. They do not show any base pairing. These results prompted us to evaluate the effect of single and multiple incorporations of 1,2,3-triazole nucleosides in different sequence motifs. For that purpose a self-complementary sequence **29** was used which shows a T_m value of 36 °C. Replacing three dA's with **6** (**30**) results in a T_m of <10 °C. However, when compounds **4** and **6** are located at the 5′-end (**33**·**32**), 3′-end (**34**·**31**), or placed opposite to each other (**33**·**34**) their stability is retained. These results imply that the terminal 1,2,3-triazole nucleosides **4** and **6** develop stacking interaction when they are positioned at the ends without disturbing the duplex structure.

Table 6
T_m values of oligonucleotide duplexes containing 1,2,3-triazole nucleosides[a]

Duplex	T_m [°C]	Duplex	T_m [°C]
5′-d(TTT TTT TTT TTT) **25**	44	5′-d(TTT TT**4** TTT TTT) **27**	32
3′-d(AAA AAA AAA AAA) **26**		3′-d(AAA AAA AAA AAA) **26**	
5′-d(TTT TTT TTT TTT) **25**	35	5′-d(TTT TT**4** TTT TTT) **27**	17
3′-d(AAA AA**6** AAA AAA) **28**		3′-d(AAA AA**6** AAA AAA) **28**	
5′-[d(ATATATATATAT)]$_2$ **29**	36	5′-[d(ATAT**6**T**6**T**6**TAT)]$_2$ **30**	<10
5′-d(T_{24}) **31**	67	5′-d(**4**T_{23}) **33**	67
3′-d(A_{24}) **32**		3′-d(A_{24}) **32**	
5′-d(A_{23}**6**) **34**	67	5′-d(**4**T_{23}) **33**	67
3′-d(T_{24}) **31**		3′-d(**6**A_{23}) **34**	
5′-d(TTT TT**X** TTT TTT) **35**	33	5′-d(TTT TT**X** TTT TTT) **35**	19
3′-d(AAA AAA AAA AAA) **26**		3′-d(AAA AA**6** AAA AAA) **28**	
5′-d(TTT TTT TTT TTT) **25**	36	5′-d(TTT TT**4** TTT TTT) **27**	14
3′-d(AAA AA**X** AAA AAA) **36**		3′-d(AAA AA**X** AAA AAA) **36**	

[a] Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. **X =** abasic site.

Table 7
Molecular masses of oligonucleotides measured by MALDI-TOF mass spectrometry

Oligonucleotide	Mass (calcd)	Mass (found)
5′-d(TTT TT**4** TTT TTT) **27**	3655.4	3655.0
5′-d(AAA AAA **6**AA AAA) **28**	3777.6	3777.1
5′-d(ATAT**6**T**6**T**6**TAT) **30**	3885.7	3886.4
5′-d(**4**T_{23}) **33**	7305.8	7306.7
5′-d(A_{23}**6**) **34**	7536.2	7536.3
5′-d(TTT TT**X** TTT TTT) **35**	3464.3	3464.7
5′-d(AAA AAA **X**AA AAA) **36**	3563.4	3563.5

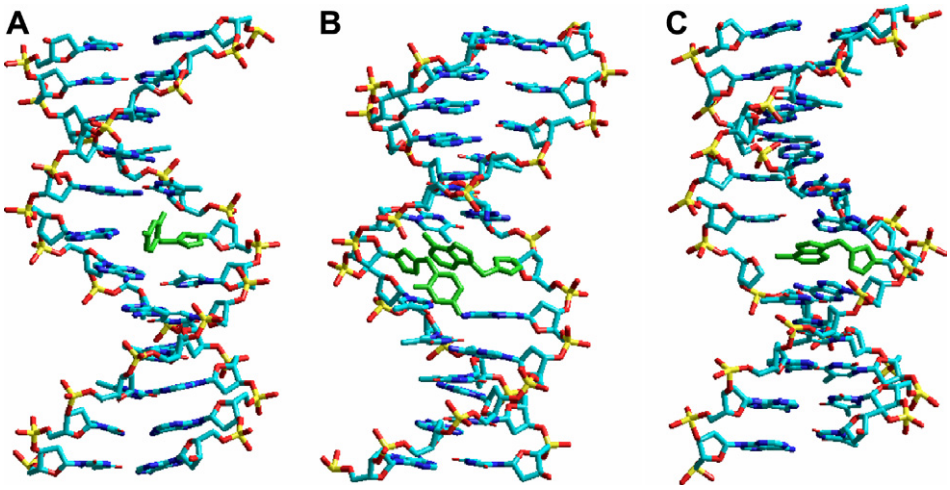


Figure 5. Molecular models (A) of non-self complementary duplex 5′-d(TTT TT**4** TTT TTT)·3′-d(AAA AAA AAA AAA) (**27**·**26**) (B) duplex 5′-d(TTT TT**4** TTT TTT)·3′-d(AAA AA**6** AAA AAA) (**27**·**28**) (C) duplex 5′-d(TTT TT**X** TTT TTT)·3′-d(AAA AA**6** AAA AAA) (**35**·**28**). The models were constructed using Hyperchem 7.0/8.0 and energy minimized using the MM^+ calculations.

To give more space for the nucleoside conjugates **4** and **6** in the DNA duplex, an abasic site was introduced into the standard sequence **25**·**26** opposite to them. A single incorporation of abasic site replacing dT gives a T_m of 33 °C against dA and 36 °C against dT. Similar values were observed for the nucleosides **4** (**27**·**26**) and **6** (**25**·**28**) when they are placed at same position as that of abasic site. When an abasic site is placed opposite to the 1,2,3-triazole nucleosides, even a two times higher duplex destabilization was observed. These results demonstrate that tethered 1,2,3-triazole nucleosides behave like abasic sites. On the basis of this information, the energy minimized molecular models of DNA duplexes containing triazole nucleosides **4** and **6** were constructed using MM+ force field by Hyperchem 7.0/8.0 program (Hypercube Inc., Gainesville, FL, USA, 2001). The duplexes **27**·**26**, **27**·**28**, and **35**·**28** were built as B-type DNA and are shown in Figure 4. According to the models, the nucleobases of **4** and **6** are arranged perpendicular to the triazole moiety and the methylene bridge forces the base moiety apart, disrupting hydrogen bond formation with the complementary bases leading to the destabilization (Figs. 5A–C). The 1,2,3-triazole moiety cannot take part in stacking interactions with adjacent bases even though they are well accommodated in the double helix. The stacking of triazole moieties was recently reported to be important for the thermal stabilization of duplexes.[41]

3. Conclusions

Various alkynyl nucleobases of pyrimidines, purines, or 7-deazapurines were synthesized and utilized as starting materials in the 'click' reaction to attach azido residues. We accomplished a convenient methodology for the synthesis of novel 1,2,3-triazole nucleosides using the Cu(I)-catalyzed Huisgen–Sharpless–Meldal alkyne-azide cycloaddition reaction. This reaction has an advantage over high temperature cycloaddition, which resulted in the formation of a mixture of regioisomers. The antiviral activity of such 1,2,3-triazole compounds was studied. Moreover, alkynyl bases were conjugated with azido coumarin yielding strongly fluorescent 1,2,3-triazole conjugates. These rigid short chain 1,2,3-triazolyl compounds are considered as useful fluorescent probes because of the absence of interactions between the dye and nucleobase. The nucleosides **4** and **6** were converted into their phosphoramidite building blocks and incorporated into DNA using solid-phase oligonucleotide synthesis. These nucleosides are almost behaving as an abasic site, with a destabilizing effect on the DNA duplexes; when they are placed at the terminus of a duplex stability is retained.

4. Experimental

4.1. General

4.1.1. Monomers

All chemicals were purchased from Acros, Aldrich, Sigma, or Fluka (Sigma–Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. Thin-layer chromatography (TLC): aluminum sheets, silica gel 60 F_{254}, 0.2 mm layer (VWR International, Darmstadt, Germany). Column flash chromatography (FC): silica gel 60 (VWR International, Darmstadt, Germany) at 0.4 bar; Sample collection with an UltroRac II fractions collector (LKB Instruments, Sweden). UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); λ_{max} (ε) in nm. NMR Spectra: Avance-250 or Avance-300 spectrometers (Bruker, Karlsruhe, Germany), at 250.13 or 300.15 MHz for ^1H and ^{13}C; δ in ppm relative to Me_4Si as internal standard or external 85% H_3PO_4 for ^{31}P. The J values in Hz. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). Electron spray ionization (ESI) MS for the nucleosides: Bruker-Daltonics-MicroTOF spectrometer with loop injection (Bremen, Germany).

4.1.2. Fluorescence measurements

All measurements were done in double-distilled water at 20 °C. Absorption spectra were measured with a Cary 100 Bio UV–visible spectrophotometer. In order to avoid inner filter effects the sample was not allowed to exceed 0.1 at the excitation wavelength using standard quartz cuvettes with a pathlength of 1 cm. Fluorescence spectra were recorded in the wavelength range between 320 and 600 nm using the Fluorescence Spectrophotometer F-2500 (Hitachi, Tokyo, Japan). For all calculations the water background was subtracted from the sample. The fluorescence quantum yields were determined using quinine sulfate in 0.1 N H_2SO_4 (fluorescence quantum yield 0.53)[42] as a standard with the following relation: $\Phi_{f.sample} = \Phi_{f.standard} \times (F_{sample}/F_{standard}) \times (A_{standard}/A_{sample})$, where $\Phi_{f.sample}$ is the unknown fluorescence quantum yield of the fluorophore, F is the integrated fluorescence intensity; A is the absorbance in 1 cm cuvettes and always not exceeds 0.1 at and above the excitation wavelength.

4.1.3. Synthesis, purification, and characterization of oligonucleotides

The oligonucleotide synthesis was performed on a DNA synthesizer, model ABI 392-08 (Applied Biosystems, Weiterstadt, Germany) at 1-μmol scale using the phosphoramidites **21** and **24** following the synthesis protocol for 3′-cyanoethyl phosphoramidites (user's manual for the 392 DNA sythesizer, Applied Biosystems, Weiterstadt, Germany). The coupling efficiency was always higher than 95%. After cleavage from the solid support, the oligonucleotides were deprotected with 25% aqueous NH_3 for 14–16 h at 60 °C (during this process the amine protecting groups of the adenine with tBPA (4-*tert*-butylphenoxy)acetyl protecting group were removed. The purification of the 5′-*O*-(dimethoxytrityl)oligomers was carried out on reversed-phase HPLC (Merck-Hitachi-HPLC: 250 × 4 mm RP-18 column with the following gradient system [A, 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B, MeCN]; gradient: 3 min 20% B in A, 12 min 20–50% B in A, and 25 min 20% B in A with a flow rate of 1 mL/min. The purified 'trityl-on' oligonucleotides were treated with 2.5% dichloroacetic acid in CH_2Cl_2 for 5 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC (gradient: 0–20 min 0–20% B in A, flow rate 1 mL/min). The oligomers were desalted on a short column (RP-18, silica gel) and lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at −24 °C.

Melting curves were measured with a Cary-1/3 UV/vis spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program. Cary 100 Bio UV–vis spectrophotometer was used for the UV-melting curves of oligonucleotides (Table 6) with a heating rate of 1 °C per min.

The molecular masses of the oligonucleotides were determined in negative mode by Applied Biosystems Voyager DE PRO spectrometer with 3-hydroxypicolinic acid (3-HPA) as a matrix. The detected masses were identical to the calculated values (Table 7). Extinction coefficients ε_{260} of the nucleosides: dA 15400 (H_2O), dT 8800 (H_2O), **4** 10,200 (MeOH), **6** 15,000 (MeOH).

4.1.4. 2-[(2-Deoxy-3,5-di-*O*-*p*-toluyl-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl- methyl]uracil (3a)

To a mixture of *N*-1-propargyluracil **1a** (0.22 g, 1.46 mmol) and β-azido-2-deoxyribose **2** (0.5 g,1.26 mmol) in THF/H_2O/*t*-BuOH, 3:1:1, (16 mL) was added sodium ascorbate (0.68 mL, 0.67 mmol) of a freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (0.54 mL, 0.162 mmol). The heterogeneous reaction mixture was stirred for 15 h at rt and then evaporated and applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH, 98:2), which gave compound **3a** (0.61 g, 76%) as a colorless foam. TLC: R_f (CH_2Cl_2/MeOH, 98:2): 0.25. UV (MeOH): λ_{max} 241 (34,000), 271 (11,000). ^{1}H NMR (DMSO-d_6): 2.36–2.39 (m, 6H, $2CH_3$); 2.77–2.87 (m, 1H, H_α-C(2′)); 3.10–3.21 (m, 1H, H_β-C(2′)); 4.37–4.63 (m, 3H, H-C(5′), H-C(4′)); 4.94 (s, 2H, CH_2); 5.56 (d, *J* = 7.8 Hz, 1H, H-C(5)); 5.75 (m, 1H, H-C(3′)); 6.59 ('t', *J* = 6.2 Hz, 1H, H-C(1′)); 7.72 (d, *J* = 7.9 Hz, 1H, H-C(6)); 7.27–7.99 (m, 8H, arom. H); 8.33 (s, 1H, C═CH); 11.33 (s, 1 H, NH). Anal. Calcd for $C_{28}H_{27}N_5O_7$ (545.19): C, 61.64; H, 4.99; N, 12.84. Found: C, 61.76; H, 5.10; N, 12.76.

4.1.5. 2-[(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]uracil (4)

A solution of compound **3a** (450 mg, 0.82 mmol) in 0.2 M NaOMe/MeOH (30 mL) was stirred at rt overnight. The solution was evaporated, and the residue was applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 85:15). After evaporation, the main zone yielded **4** as a colorless solid (220 mg, 86%). TLC: R_f (CH_2Cl_2/MeOH, 9:1): 0.31. UV (MeOH): λ_{max} 264 (10,300). ^{1}H NMR (DMSO-d_6): 2.30–2.39 (m, 1H, H_α-C(2′)); 2.54–2.64 (m, 1H, H_β-C(2′)); 3.46 (m, 2H, H-C(5′)); 3.85 (m, 1H, H-C(4′)); 4.36 (m, 1H, H-C(3′)); 4.89 (t, *J* = 5.3 Hz, 1H, OH-C(5′)); 4.94 (s, 2H, CH_2); 5.34 (d, *J* = 4.17 Hz, 1H, OH-C(3′)); 5.57 (d, *J* = 7.7 Hz, 1H, H-C(5)); 6.34 (t, *J* = 6.1 Hz, 1H, H-C(1′)); 7.73 (d, *J* = 7.8 Hz, 1H, H-C(6)); 8.25 (s, 1H, C═CH); 11.31 (s, 1H, NH). Anal. Calcd for $C_{12}H_{15}N_5O_5$ (309.11): C, 46.60; H, 4.89; N, 22.64. Found: C, 46.71; H, 4.83; N, 22.70.

4.1.6. 2-[(2-Deoxy-3,5-di-*O*-*p*-toluyl-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]cytosine (3b)

To a mixture of *N*-1-propargylcytosine **1b** (0.4 g, 2.68 mmol) and β-azido-2-deoxyribose **2** (0.83 g, 2.1 mmol) in THF/H_2O/*t*-BuOH, 3:1:1, (36 mL) was added sodium ascorbate (1.1 mL, 1.09 mmol) of freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (0.8 mL, 0.24 mmol). The heterogeneous reaction mixture was stirred for 6 h at rt and then evaporated and applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH 9:1), which gave compound **3b** (1.14 g, 78%) as a colorless foam. TLC: R_f (CH_2Cl_2/MeOH, 94:6): 0.34. UV (MeOH): λ_{max} 240 (38,000), 270 (10,000). ^{1}H NMR (DMSO-d_6): 2.37–2.39 (m, 6H, $2CH_3$); 2.75–2.85 (m, 1H, H_α-C(2′)); 3.09–3.20 (m, 1H, H_β-C(2′)); 4.38–4.59 (m, 3H, H-C(5′), H-C(4′)); 4.89 (s, 2H, CH_2); 5.65 (d, *J* = 6.7 Hz, 1H, H-C(5)); 5.75 (m, 1H, H-C(3′)); 6.59 ('t', *J* = 6.4 Hz, 1H, H-C(1′)); 7.04 (br s, 2H, NH_2); 7.70 (d, *J* = 7.2 Hz, 1H, H-C(6)); 7.20–7.94 (m, 8H, arom. H); 8.25 (s, 1H, C═CH). Anal. Calcd for $C_{28}H_{28}N_6O_6$ (544.21): C, 61.76; H, 5.18; N, 15.43. Found: C, 61.68; H, 5.26; N, 15.35.

4.1.7. 2-[(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]cytosine (5)

A solution of compound **3b** (550 mg, 1.01 mmol) in 0.2 M NaOMe/MeOH (40 mL) was stirred at rt overnight. The clear solution was evaporated, and the residue was applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 85:15). After evaporation, the main zone yielded **5** as a colorless solid (270 mg, 87%). TLC: R_f (CH_2Cl_2/MeOH, 85:15): 0.14. UV (MeOH): λ_{max} 274 (8000). ^{1}H NMR (DMSO-d_6): 2.30–2.38 (m, 1H, H_α-C(2′)); 2.54–2.64 (m, 1H, H_β-C(2′)); 3.46 (m, 2H, H-C(5′)); 3.85 (m, 1H, H-C(4′)); 4.36 (m, 1H, H-C(3′)); 4.88–4.91 (m, 3H, OH-C(5′), CH_2); 5.34 (d, *J* = 4.3 Hz, 1H, OH-C(3′)); 5.68 (d, *J* = 7.2 Hz, 1H, H-C(5)); 6.35 ('t', *J* = 6.3 Hz, 1H, H-C(1′)); 7.03 (br s, 2H, NH_2); 7.69 (d, *J* = 7.2 Hz, 1H, H-C(6)); 8.17 (s, 1H, C═CH). Anal. Calcd for $C_{12}H_{16}N_6O_4$ (308.12): C, 46.75; H, 5.23; N, 27.26. Found: C, 46.78; H, 5.20; N, 27.09.

4.1.8. 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]adenine (6)

To a mixture of *N*-9-propargyladenine **1c** (0.17 g, 1.01 mmol) and β-azido-2-deoxyribose **2** (0.5 g,1.26 mmol) in THF/H_2O/*t*-BuOH, 3:1:1, (16 mL) was added sodium ascorbate (680 μL, 0.67 mmol) of freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (540 μL, 0.16 mmol). The heterogeneous reaction mixture was stirred for 48 h at rt, then evaporated and the product was used directly without further purification. A solution of above crude compound in 0.2 M NaOMe/MeOH was stirred at rt overnight. The clear solution was evaporated, and the residue was applied onto FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 85:15). After evaporation, the main zone yielded **6** as a colorless solid (220 mg, 67%). TLC: R_f (CH_2Cl_2/MeOH, 85:15): 0.12. UV (MeOH): λ_{max} 261 (15,000). ^{1}H NMR (DMSO-d_6): 2.28–2.38 (m, 1H, H_α-C(2′)); 2.52–2.62 (m, 1H, H_β-C(2′)); 3.45 (m, 2H, H-C(5′)); 3.85 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.86 (t, *J* = 5.4 Hz, 1H, OH-C(5′)); 5.34 (d, *J* = 4.32 Hz, 1H, OH-C(3′)); 5.43 (s, 2H, CH_2); 6.32

('t', J = 6.15 Hz, 1H, H-C(1′)); 7.24 (br s, 2H, NH_2); 8.14 and 8.19 (2s, 2H, H-C(2), H-C(8)); 8.29 (s, 1H, C═CH). Anal. Calcd for $C_{13}H_{16}N_8O_3$ (332.13): C, 46.98; H, 4.85; N, 33.72. Found: C, 46.79; H, 4.90; N, 33.80.

4.1.9. 7-[(2-Deoxy-β-D-*erythro*-pentofuranosyl)-imidazo[1,2-*c*]-1,2,3-triazol-4-yl-methyl]adenine (7)

To a stirred solution of compound **6** (300 mg, 0.90 mmol) in aqueous sodium acetate (1 M, pH 4.5–5.0, 33 mL) chloroacetaldehyde (50% aqueous solution, 5.6 mL) was added at 40–50 °C and stirring was continued for 2 days. The reaction mixture was evaporated, and the residue was applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 90:10) to give compound **7** as colorless foam (0.25 g, 78%). TLC: R_f (CH_2Cl_2/MeOH, 85:15): 0.15. UV (MeOH): λ_{max} 229 (29,000), 275 (5200), 293 (3000), 266 (5100). ^{1}H NMR (DMSO-d_6): 2.28–2.37 (m, 1H, H_α-C(2′)); 2.56–2.64 (m, 1H, H_β-C(2′)); 3.42 (m, 2H, H-C(5′)); 3.84 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.88 (t, 1H, OH-C(5′)); 5.32 (d, 1H, OH-C(3′)); 5.61 (s, 2H, CH_2); 6.31 ('t', J = 6.15 Hz, 1H, H-C(1′)); 7.54 (s, 1H, H-C(11)); 8.07 (s, 1H, H-C(10)); 8.32 (s, 1H, H-C(8)); 8.37 (s, 1H, C═CH); 9.28 (s, 1H, H-C(2)). Anal. Calcd for $C_{15}H_{16}N_8O_3$ (356.13): C, 50.56; H, 4.53; N, 31.45. Found: C, 50.52; H, 4.61; N, 31.30.

4.1.10. 4-Chloro-7-prop-1-ynyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (1d)

4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8**) (1.5 g, 9.8 mmol), potassium carbonate (2.02 g, 14.66 mmol), and propargyl bromide (1.74 g,14.7 mmol) were stirred in anhydrous DMF (27 mL) at rt for 24 h. The solvent was evaporated under reduced pressure and applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 98:2). The resulting solid was recrystallised from methanol to give **1d** as colorless needles (1.33 g, 71%). TLC: R_f (CH_2Cl_2/MeOH, 98:2): 0.8. UV (MeOH): λ_{max} 271 (5000). ^{1}H NMR (DMSO-d_6): 3.47 (t, 1H, J = 2.5 Hz, C≡C–H); 5.17 (d, 1H, J = 2.5 Hz, CH_2C≡C); 6.70 (d, 1H, J = 3.8 Hz, H-C(7)); 7.82 (d, J = 3.8 Hz, 1H, H-C(8)); 8.67 (s, 1H, H-C(2)). Anal. Calcd for $C_9H_6ClN_3$ (191.03): C, 56.41; H, 3.16; N, 21.93. Found: C, 56.36; H, 3.20; N, 22.01.

4.1.11. 4-Chloro-7-[(2-deoxy-3,5-di-*O*-*p*-toluyl-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3d)

To a mixture of **1d** (0.21 g, 1.09 mmol) and β-azido-2-deoxyribose **2** (0.5 g, 1.26 mmol in THF/H_2O/*t*-BuOH, 3:1:1, (16 mL) was added sodium ascorbate (0.63 mL, 0.62 mmol) of freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (0.5 mL, 0.15 mmol). The heterogeneous reaction mixture was stirred for 24 h at rt and then evaporated and applied to FC (silica gel, column 3 × 10 cm, PE/EtOAc, 1:1), which gave compound **3d** (0.55 g, 85%) as a colorless solid. TLC: R_f (PE/EtOAc, 1:1): 0.62. UV (MeOH): λ_{max} 242 (32,000), 271 (7000). ^{1}H NMR (DMSO-d_6): 2.35–2.38 (m, 6H, 2CH_3); 2.79–2.83 (m, 1H, H_α-C(2′)); 3.10–3.15 (m, 1H, H_β-C(2′)); 4.37–4.59 (m, 3H, H-C(5′), H-C(4′)); 5.57 (s, 2H, CH_2); 5.73 (m, 1H, H-C(3′)); 6.56 ('t', 1H, H-C(1′)); 6.65 (d, 1H, J = 3.2 Hz, H-C(7)); 7.22–7.92 (m, 8H, arom. H); 7.74 (d, 1H, H-C(8)); 8.32 (s, 1H, C═CH); 8.64 (s,1H, H-C(2)). Anal. Calcd for $C_{30}H_{27}ClN_6O_5$ (586.17): C, 61.38; H, 4.64; N, 14.32. Found: C, 61.40; H, 4.74; N, 14.26.

4.1.12. 4-Amino-7-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (9)

A suspension of **3d** (0.4 g, 0.68 mmol) in dioxane (40 mL) and 25% aq NH_3 (50 mL) was introduced into an autoclave and stirred at 90 °C for 24 h. The above reaction mixture was evaporated, and the residue was applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 9:1). After evaporation, the main zone yielded **9** as colorless solid (187 mg, 83%). TLC: R_f (CH_2Cl_2/MeOH 85:15): 0.2. UV (MeOH): λ_{max} 273 (8500). ^{1}H NMR (DMSO-d_6): 2.27–2.37 (m, 1H, H_α-C(2′)); 2.52–2.60 (m, 1H, H_β-C(2′)); 3.29–3.56 (m, 2H, H-C(5′)); 3.84 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.88 (t, 1H, OH-C(5′)); 5.39 (m, 3H, OH-C(3′), H-CH_2); 6.30 ('t', J = 6.25 Hz, 1H, H-C(1′)); 6.64 (d, 1H, J = 3.5 Hz, H-C(7)); 7.22 (d, J = 3.4 Hz, 1H, H-C(8)); 7.36 (br s, 2H, NH_2); 8.13 (s,1H, H-C(2)); 8.22 (s, 1H, C═CH). Found: ESI-HR-MS: 332.14, $C_{14}H_{17}N_7O_3$; requires 331.14.

4.1.13. 2-Amino-4-chloro-7-prop-1-ynyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (1e)

2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**10**) (0.63 g, 3.75 mmol), potassium carbonate (0.51 g, 3.69 mmol) and propargyl bromide (0.43 g, 3.61 mmol) were stirred in anhydrous DMF (16 mL) at rt for 48 h. The solvent was evaporated under reduced pressure and applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 98:2) to give **1e** as a colorless solid (0.57 g, 74%). TLC: R_f (CH_2Cl_2/MeOH 97:3): 0.50. UV (MeOH): λ_{max} 261 (4000), 318 (5500). ^{1}H NMR (DMSO-d_6): 3.38 (t, 1H, J = 2.5 Hz, C≡C–H); 4.87 (d, 1H, J = 2.5 Hz, CH_2C≡C); 6.34 (d, 1H, J = 3.8 Hz, H-C(7)); 6.76 (br s, 2H, NH_2); 7.21 (d, J = 3.5 Hz, 1H, H-C(8)). Anal. Calcd for $C_9H_7ClN_4$ (206.04): C, 52.31; H, 3.41; N, 27.11. Found: C, 52.46; H, 3.48; N, 26.95.

4.1.14. 2-Amino-4-chloro-7-[(2-deoxy-3,5-di-*O*-*p*-toluyl-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl] 7*H*-pyrrolo[2,3-*d*]pyrimidine (3e)

To a mixture of **1e** (0.94 g, 4.55 mmol) and β-azido-2-deoxyribose **2** (2.0 g, 5.05 mmol) in THF/H_2O/*t*-BuOH, 3:1:1, (43 mL) was added sodium ascorbate (2.6 mL, 2.58 mmol) of freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (2.0 mL, 0.6 mmol). The heterogeneous reaction mixture was stirred for 24 h at rt and then evaporated and applied to FC (silica gel, column 3 × 10 cm, PE/EtOAc, 1:1), gave compound **3e** (2.32 g, 85%) as a colorless solid. TLC: R_f (CH_2Cl_2/MeOH, 9:1): 0.33. UV (MeOH): λ_{max} 317 (5700). ^{1}H NMR (DMSO-d_6): 2.36–2.38 (m, 6H, 2 CH_3); 2.75–2.85 (m, 1H, H_α-C(2′)); 3.08–3.19 (m, 1H, H_β-C(2′)); 4.38–4.61 (m, 3H, H-C(5′), H-C(4′)); 5.31 (s, 2H, CH_2); 5.73 (m, 1H, H-C(3′)); 6.30 (d, 1H, J = 3.7 Hz, H-C(7)); 6.56 ('t', J = 6.5 Hz, 1H, H-C(1′)); 6.70 (br s, 2H, NH_2); 7.15 (d, 1H, J = 3.5 Hz, H-C(8)); 7.25–7.93 (m, 8H, arom. H); 8.22 (s, 1H, C═CH). Anal. Calcd for $C_{30}H_{28}ClN_7O_5$ (601.18): C, 59.85; H, 4.69; N, 16.29. Found: C, 59.69; H, 4.79; N, 16.15.

4.1.15. 2-Amino-4-chloro-7-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]7*H*-pyrrolo[2,3-*d*]pyrimidine (13)

A solution of compound **3e** (0.9 g, 1.49 mmol) in NH_3/MeOH (saturated at 0 °C, 60 mL) was stirred overnight at rt. The clear solution was evaporated, and the residue was applied to FC (silica gel, column 4 × 16 cm, CH_2Cl_2/MeOH, 85:15). After evaporation, the main zone yielded **13** as a colorless solid (470 mg, 86%). TLC: R_f (CH_2Cl_2/MeOH, 9:1): 0.35. UV (MeOH): λ_{max} 260 (4000), 317 (5600). ^{1}H NMR (DMSO-d_6): 2.32–2.37 (m, 1H, H_α-C(2′)); 2.55–2.60 (m, 1H, H_β-C(2′)); 3.37–3.49 (m, 2H, H-C(5′); 3.81–3.85 (m, 1H, H-C(4′)); 4.35 (m, H-C(3′)); 4.85 (t, 1H, J = 5.5 Hz, OH-C(5′)); 5.30–5.38 (m, 3H, OH-C(3′), H-CH_2); 6.29–6.40 (m, 2H, H-C(7), H-C(1′)); 6.69 (br s, 2H, NH_2); 7.18 (d, 1H, J = 3.75 Hz, H-C(8)); 8.19 (s, 1H, C═CH). Anal. Calcd for $C_{14}H_{16}ClN_7O_3$ (365.10): C, 45.97; H, 4.41; N, 26.81. Found: C, 46.03; H, 4.50; N, 26.70.

4.1.16. 2-Amino-7-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-1,2,3-triazol-4-yl-methyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (11)

Compound **3e** (1.6 g, 2.65 mmol) was suspended in 0.2 N NaOMe in MeOH (100 mL). The mixture was kept stirring at rt

for 15 h. The mixture was evaporated and applied to FC (silica gel, column 2 × 10 cm), and eluted with CH_2Cl_2/MeOH (9:1). After evaporation, the main zone yielded **11** as a colorless solid (0.8 g, 83%). TLC: R_f (CH_2Cl_2/MeOH, 97:3): 0.37. UV (MeOH): λ_{max} 262 (9000), 287 (8000). ^{1}H NMR (DMSO-d_6): 2.27–2.37 (m, 1H, H_α-C(2′)); 2.52–2.62 (m, 1H, H_β-C(2′)); 3.39–3.51 (m, 2H, H-C(5′); 3.81–3.99 (m, 3H, H-C(4′), H-OMe); 4.35 (m, 1H, H-C(3′)); 4.86 (t, 1H, J = 5.25 Hz, OH-C(5′)); 5.25 (s, 2H, H-CH_2); 5.33(d, 1H, J = 4.37 Hz, OH-C(3′)); 6.20–6.23 (m, 3H, H-C(7), NH_2); 6.31 (‘t’, 1H, J = 6.25 Hz, H-C(1′)); 6.91 (d, 1H, J = 3.50 Hz, H-C(8)); 8.15 (s, 1H, C═CH). Anal. Calcd for $C_{15}H_{19}N_7O_4$ (361.15): C, 49.86; H, 5.30; N, 27.13. Found: C, 49.92; H, 5.22; N, 27.10.

4.1.17. [(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (12)

A suspension of **3e** (0.6 g, 1.0 mmol) in dioxane (30 mL) and 25% aq NH_3 (50 mL) was introduced into an autoclave and stirred at 120 °C for 24 h. The solution was evaporated and the residue was applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH, 20:1), which gave compound **12** (238 mg, 69%) as a colorless solid. TLC: R_f (CH_2Cl_2/MeOH, 9:1): 0.26. UV (MeOH): λ_{max} 266 (7000), 286 (6000), ^{1}H NMR (DMSO-d_6): 2.27–2.61 (m, 2H, H_α-C(2′), H_β-C(2′)); 3.41–3.47 (m, 2H, H-C(5′); 3.83 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.92 (t, 1H, J = 5.2 Hz, OH-C(5′)); 5.19 (s, 2H, H-CH_2); 5.38 (d, 1H, OH-C(3′)); 6.03 (br s, 2H, NH_2); 6.31 (‘t’, 1H, J = 6.47 Hz, H-C(1′)); 6.43 (d, 1H, J = 3.5 Hz, H-C(7)); 6.80 (d, 1H, J = 3.7 Hz, H-C(8)); 7.06 (br s, 2H, NH_2); 8.16 (s, 1H, C═CH). Found: ESI-HR-MS: 347.15, $C_{14}H_{18}N_8O_3$; requires 346.15.

4.1.18. 2-Amino-7-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidine-4-one (14)

Compound **11** (150 mg, 0.41 mmol) was dissolved in 2 N NaOH (36 mL) and 1,4-dioxane (6 mL). The mixture was stirred under reflux for 6 h. After neutralization with 1 N HCl, The solution was evaporated and the residue was applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH 20:1), which gave compound **14** (80 mg, 55%) as a colorless solid. TLC: R_f (CH_2Cl_2/MeOH, 85:15): 0.4. UV (MeOH): λ_{max} 262 (12,000). ^{1}H NMR (DMSO-d_6): 2.28–2.37 (m, 1H, H_α-C(2′)); 2.53–2.66 (m, 1H, H_β-C(2′)); 3.39–3.54 (m, 2H, H-C(5′); 3.85 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.86 (t, 1H, J = 5.25 Hz, OH-C(5′)); 5.18 (s, 2H, H-CH_2); 5.34 (m, 1H, OH-C(3′)); 6.22–6.31 (m, 4H, NH_2, H-C(1′); H-C(7)); 6.72 (d, 1H, J = 3.50 Hz, H-C(8)); 8.14 (s, 1H, C═CH); 10.31(s, 1H, NH) Anal. Calcd for $C_{14}H_{17}N_7O_4$ (347.13): C, 48.41; H, 4.93; N, 28.23. Found: C, 48.57; H, 5.04; N, 28.01.

4.1.19. 2-[(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]uracil (20)

Compound **4** (100 mg, 0,32 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2 × 3 mL) and dissolved in anhydrous pyridine (4 mL). Then, 4,4′-dimethoxytrityl chloride (217 mg, 0.64 mmol) was added at rt and stirring was continued for another 4 h. Thereupon, MeOH (1 mL) was added and the stirring was continued for 10 min. The mixture was poured into a 5% aq $NaHCO_3$ soln (30 mL) and extracted with CH_2Cl_2 (2× 30 mL). The combined organic layer was dried (Na_2SO_4) and evaporated and the resulting residue subjected to FC (silica gel, column 3 × 12 cm, pre wet with CH_2Cl_2/TEA, 98:2, v/v, until basic and eluted with CH_2Cl_2/MeOH/TEA, 96:4:1) to yield a colorless foam **20** (160 mg, 81%). TLC: R_f (CH_2Cl_2/MeOH, 93:7): 0.46. UV (MeOH): λ_{max} 233 (22,000), 265 (11,000). ^{1}H NMR (DMSO-d_6): 2.32–2.42 (m, 1H, H_α-C(2′)); 2.66–2.73 (m, 1H, H_β-C(2′)); 3.06 (m, 2H, H-C(5′)); 3.72 (s, 6H, 3MeO); 3.95 (m, 1H, H-C(4′)); 4.41 (m, 1H, H-C(3′)); 4.89 (s, 2H, CH_2); 5.40 (d, J = 4.8 Hz, 1H, OH-C(3′)); 5.47 (d, J = 7.8 Hz, 1H, H-C(5)); 6.37 (‘t’, J = 5.3 Hz, 1H, H-C(1′)); 6.82–7.31 (m, 13H, arom. H); 7.61 (d, J = 7.8 Hz, 1H, H-C(6)); 8.22 (s, 1H, C═CH); 11.30 (s, 1H, NH). Anal. Calcd for $C_{33}H_{33}N_5O_7$ (611.24): C, 64.80; H, 5.44; N, 11.45. Found: C, 48.57; H, 5.04; N, 28.01.

4.1.20. 2-[(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosy)-1,2,3-triazol-4-yl-methyl]uracil 3′-(2-Cyanoethyl diisopropylphosphoramidite) (21)

To a solution of **20** (100 mg, 0.16 mmol) in anhyd CH_2Cl_2 (4 mL) were added (i-Pr)$_2$EtN (0.05 μL, 0.28 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.08 mL, 0.35 mmol) while stirring under Ar at rt stirring was continued for another 30 min, and then CH_2Cl_2 (15 mL) was added. The soln. was then washed with 5% aq $NaHCO_3$ soln. (15 mL), the aq layer extracted with CH_2Cl_2 (2× 30 mL), the combined org. layer dried over Na_2SO_4 and evaporated, the resulting residue subjected to FC (silica gel, column 3 × 12 cm, pre wet with CH_2Cl_2/TE A, 98:2, v/v, until basic and eluted with CH_2Cl_2/Me_2CO/TEA, 96:4:1) to give compound **21** as a colorless foam (100 mg, 75%). TLC: R_f (CH_2Cl_2/MeOH, 98:2): 0.63. ^{31}P NMR ($CDCl_3$): 150.41, 150.23.

4.1.21. 4-[(Benzoylamino)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-ylmethyl]adenine (22)

Me_3SiCl (1.2 mL, 9.41 mmol) was added to a solution of compound **6** (300 mg, 0.9 mmol) in anhydrous pyridine (5.4 mL), and was stirred at rt. After 30 min, the benzoyl chloride (4.5 mmol) was introduced and the solution was kept at rt for another 2 h. The mixture was cooled to 0 °C, diluted with H_2O (3.6 mL), and stirred for 10 min. After the addition of 25% aq NH_3 (3.6 mL), stirring was continued for 2 h at rt. The solution was evaporated and the residue was applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH, 9:1), which gave compound **22** (0.29 g, 74%) as a colorless solid. TLC: R_f (CH_2Cl_2/MeOH, 9:1): 0.24. UV (MeOH): λ_{max} 263 (13,000). ^{1}H NMR (DMSO-d_6): 2.29–2.39 (m, 1H, H_α-C(2′)); 2.53–2.68 (m, 1H, H_β-C(2′)); 3.47 (m, 2H, H-C(5′)); 3.86 (m, 1H, H-C(4′)); 4.35 (m, 1H, H-C(3′)); 4.87 (t, J = 5.4 Hz, OH-C(5′)); 5.34 (d, J = 4.32 Hz, 1H, OH-C(3′)); 5.59 (s, 2H, CH_2); 6.34 (‘t’, J = 6.2 Hz, 1H, H-C(1′)); 7.43–8.05 (m, 7H, arom.H); 8.37 and 8.56 (2s, 2H, H-C(2), H-C(8)); 8.74 (s, 1H, C═CH); 11.11 (s, NH). Anal. Calcd for $C_{20}H_{20}N_8O_4$ (436.16): C, 55.04; H, 4.62; N, 25.68. Found: C, 55.09; H, 4.74; N, 25.49.

4.1.22. 4-[(Benzoylamino)-7-(2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-methyl]adenine (23)

Compound **22** (200 mg, 0.45 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2× 3 mL) and dissolved in anhydrous pyridine (6 mL). Then, 4,4′-dimethoxytrityl chloride (0.35 g, 1.03 mmol) was added at rt while stirring, and stirring was continued for another 4 h. Thereupon, MeOH (2 mL) was added and the stirring was continued for 10 min. The mixture was poured into a 5% aq $NaHCO_3$ soln (40 mL) and extracted with CH_2Cl_2 (2× 40 mL). The combined organic layer was dried (Na_2SO_4) and evaporated and the resulting residue subjected to FC (silica gel, column 3 × 12 cm, pre wet with CH_2Cl_2/TEA, 98:2, v/v, until basic and eluted with CH_2Cl_2/MeOH/TEA, 98:2:1) to yield a colorless foam **23** (0.27 g, 80%). TLC: R_f (CH_2Cl_2/MeOH 93:7): 0.45. UV (MeOH): λ_{max} 273 (15,000), 231 (33,000). ^{1}H NMR (DMSO-d_6): 2.31–2.41 (m, 1H, H_α-C(2′)); 2.62–2.71 (m, 1H, H_β-C(2′)); 3.06 (m, 2H, H-C(5′)); 3.72 (s, 6H, 3MeO); 3.95 (m, 1H, H-C(4′)); 4.39 (m, 1H, H-C(3′)); 5.39 (d, J = 4.8 Hz, 1H, OH-C(3′)); 5.55 (s, 2H, CH_2); 6.37 (t, J = 5.5 Hz, 1H, H-C(1′)); 6.82–8.05 (m, 19H, arom. H); 8.32 and 8.49 (s, 2H, H-C(2), H-(8)); 8.69 (s, 1H, C═CH); 11.18 (s, 1H, NH). Anal. Calcd for $C_{41}H_{38}N_8O_6$ (738.29): C, 66.65; H, 5.18; N, 15.17. Found: C, 66.75; H, 5.30; N, 15.07.

4.1.23. 4-[(Benzoylamino)-7-(2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-1,2,3-triazol-4-yl-ethyl]adenine 3′-(2-cyanoethyl diisopropylphosphoramidite) (24)

To a solution of **23** (200 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (6 mL) were added $(i\text{-Pr})_2$EtN (80 μL, 0.44 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.13 mL, 0.59 mmol) while stirring under Ar at rt. Stirring was continued for another 30 min, and then CH_2Cl_2 (20 mL) was added. The soln was then washed with 5% aq $NaHCO_3$ soln (20 mL), the aq layer extracted with CH_2Cl_2 (2× 30 mL), the combined organic layer dried (Na_2SO_4) and evaporated, the resulting residue subjected to FC (silica gel, column 3 × 12 cm, pre wet with CH_2Cl_2/TEA, 98:2, v/v, until basic and eluted with CH_2Cl_2/Me_2CO/TEA, 96:4:1) to give compound **24** as a colorless foam (200 mg, 79%). TLC: R_f (CH_2Cl_2/MeOH, 93:7): 0.64. ^{31}P NMR ($CDCl_3$): 150.43, 150.23.

4.1.24. Preparation of the conjugate 16 from 1a and 15 by the Cu(I)-catalyzed cycloaddition

To a solution of compound **1a** (120 mg, 0.8 mmol) and 3-azido-7-hydroxycoumarin (**15**; 160 mg, 0.79 mmol) in THF/H_2O/*t*-BuOH, 2:1:1, (8 mL), was added sodium ascorbate (330 μL, 0.32 mmol) of a freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (0.26 mL, 0.08 mmol). The emulsion was stirred for 18 h at rt evaporated, and applied to FC (silica gel, column 3 × 10 cm, CH_2Cl_2/MeOH, 95:5). From the main zone compound **16** (230 mg, 81%) was isolated as a yellowish solid. TLC: R_f (CH_2Cl_2/MeOH, 95:5): 0.25. UV (MeOH): λ_{max} 258 (8200), 346 (12,100). ^{1}H NMR (DMSO-d_6): 5.04 (s, 2H, CH_2); 5.62 (d, J = 7.8, 1H, H-C(5)); 6.83–6.92 (m, 2H, H-arom.); 7.75 (d, 1H, J = 8.5, H-arom.); 7.83 (d, J = 7.9, 1H, H-C(6)); 8.54 (s, 1H, triazole C═CH); 8.59 (s, 1H, H-arom.); 10.90 (s, 1H, H-OH); 11.34 (s, 1H, NH). Found: ESI-HR-MS: 376.06 $(M+Na)^+$, $C_{16}H_{11}N_5O_5$; requires 353.08.

4.1.25. Preparation of the conjugate 17 from 1c and 15 by the Cu(I)-catalyzed cycloaddition

To a solution of compound **1c** (100 mg, 0.58 mmol) and 3-azido-7-hydroxycoumarin (**15**; 117 mg, 0.58 mmol) in THF/H_2O/*t*-BuOH, 2:1:1, (8 mL), was added sodium ascorbate (0.29 mL, 0.28 mmol) of a freshly prepared 1 M solution in water, followed by the addition of copper (II) sulfate pentahydrate 7.5% in water (230 μL, 0.07 mmol). The emulsion was stirred for 24 h at rt evaporated, and applied to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH, 90:10). From the main zone compound **17** (170 mg, 78%) was isolated as a yellowish solid. TLC: R_f (CH_2Cl_2/MeOH 90:10): 0.34. UV (MeOH): λ_{max} 258 (10,400), 346 (12,700). ^{1}H NMR (DMSO-d_6): 5.55 (s, 2H, CH_2); 6.84–6.90 (m, 2H, H-arom.); 7.25 (s, 2H, NH_2); 7.73 (d, 1H, J = 8.5, H-arom.); 8.16 (s, 1H, H-C(8)), 8.26 (s, 1H, H-C(2)); 8.56 (s, 1H, C═CH); 8.58 (s, 1H, H-arom.); 10.90 (s, 1H, H-OH). Found: ESI-HR-MS: 377.11, $C_{17}H_{12}N_8O_3$; requires 376.33.

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