7-Substituted 7-Deaza-2′-deoxyadenosines and 8-Aza-7-deaza-2′-deoxyadenosines: Fluorescence of DNA-Base Analogues Induced by the 7-Alkynyl Side Chain

by **Frank Seela***[a]), **Matthias Zulauf**[a]), **Markus Sauer**[b]), and **Michael Deimel**[b])

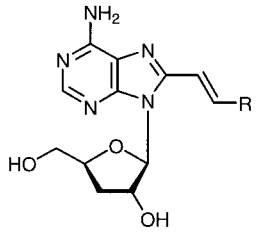
[a]) Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D-49069 Osnabrück

[b]) Institut für Physikalische Chemie, Universität Heidelberg, Im Neuenheimer Feld 253, D-69120 Heidelberg

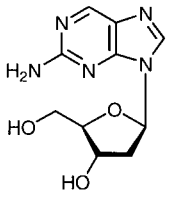
7-Alkynylated 7-deazaadenine (pyrrolo[2,3-*d*]pyrimidin-4-amine) 2′-deoxyribonucleosides show strong fluorescence which is induced by the 7-alkynyl side chain (*Table 3*). A large *Stokes* shift with an emission around 400 nm is observed when the compound is irradiated at 280 nm. The solvent dependence indicates the formation of a charged transition state. The fluorescence appears when the triple bond is in conjugation with the heterocyclic base. Electron-donating substituents at the triple bond increase the fluorescence, while electron-withdrawing residues reduce it. In comparison, the 7-alkynylated 8-aza-7-deazaadenine (pyrazolo[3,4-*d*]pyrimidin-4-amine) 2′-deoxyribonucleosides are rather weakly fluorescent (*Table 4*). Quantum yields and fluorescence decay times are measured. The synthesis of the 7-alkynylated 7-deaza-2′-deoxyadenosines and 8-aza-7-deaza-2′-deoxyadenosines was performed with 7-deaza-2′-deoxy-7-iodoadenosine (**6**) or 8-aza-7-deaza-2′-deoxy-7-iodoadenosine (**22**) as starting materials and employing the Pd^0-catalyzed cross-coupling reaction with the corresponding alkynes (*Schemes 1*, *4*, and *5*). Catalytic hydrogenation of the side chain of the unsaturated nucleosides **5** and **17** afforded the 7-alkyl derivatives **18** and **19**, respectively, which do not show significant fluorescence (*Scheme 2*).

Introduction. – The use of short-lived radioisotopes such as ^{32}P in DNA sequencing is problematic from the health and safety point of view [1][2]. The use of nonradioactive fluorescent labels circumvents this problem and provides also ready detectability [3][4]. Oligonucleotides bearing either fluorescent reporter groups, bioaffinity groups, or additional functional groups have been proven to be effective tools in molecular biology that find application in hybridization diagnostics, sequencing, chemical tailoring, and molecular recognition studies of nucleic acids [5–8]. Fluorophore-labeled nucleosides can be incorporated enzymatically in the form of their triphosphates on a nucleic-acid template [9–11]. Solid-phase oligonucleotide synthesis with phosphoramidites [12–15] or phosphonates [16] carrying suitably protected fluorescent dyes represents another technique to incorporate fluorophores in any position of a DNA or an RNA chain. The most currently used procedures for non-isotopic labeling of nucleic acids involve the incorporation of fluorescent dyes at the 5′-terminus of an oligonucleotide [17][18], the phosphate backbone [19][20], the sugar moiety [21], or on nucleobase spacer arms [5][22][23]. However, problems arise due to the bulkiness of the reporter groups, which are not well-tolerated by the DNA polymerases [11], or which interfere with the DNA molecule sterically. Consequently, the search for base-modified nucleosides that are self-fluorescent, thereby showing the normal *Watson-Crick* base-pairing properties of a natural nucleic-acid component, is of considerable interest.

Naturally occurring purine bases (*e.g.*, 2′-deoxyadenosine) do not exhibit appreciable fluorescence in aqueous solution at room temperature. However, some modified derivatives, such as 7-methylguanosine are strongly fluorescent [24][25]. Also deoxyadenosine derivatives, such as **1** or **2**, bearing alkenyl side chains at C(8) are fluorescent [25][26]. Other purine nucleoside derivatives that exhibit fluorescence are the purin-2-ones [27] or the purin-2-amine nucleoside **3**, as well as the so-called etheno-nucleosides (ribosides or 2′-deoxyribosides) [26][28–31]. Moreover, 8-azapurine nucleosides of type **4**, carrying an additional N-atom in the five-membered ring, show intensive fluorescence [30][32].



1 R = CN
2 R = COOEt



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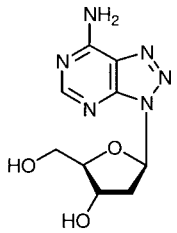

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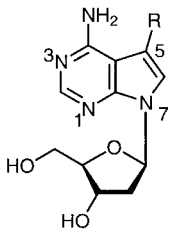
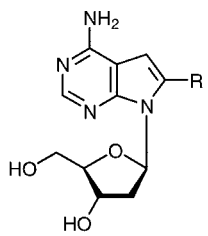
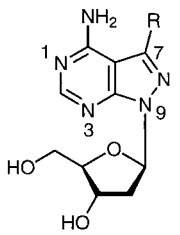


5 R = $Me(CH_2)_3C{\equiv}C$
7 - 14, **16 - 20**, **47 - 62** for R, see *Table 3*

(systematic numbering)



63 R = $Me(CH_2)_3C{\equiv}C$
64 R = Ph–C≡C



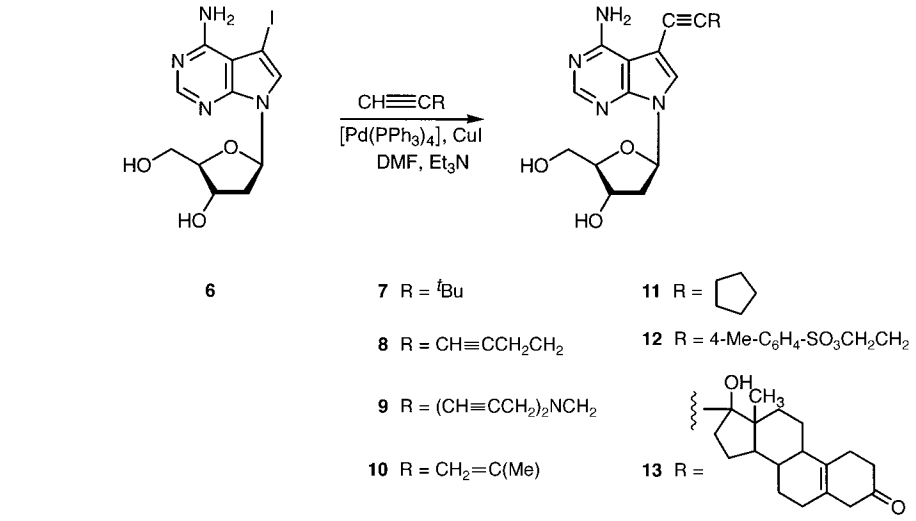
65 R = H
23 - 34 for R, see *Scheme 4*
66 - 73 for R, see *Table 4*

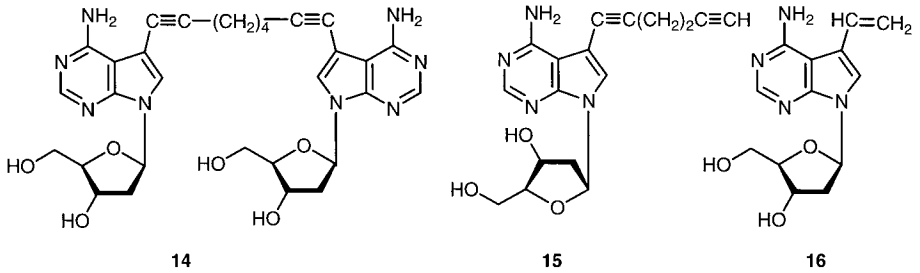
(purine numbering)

Recently, it was demonstrated that 7-deaza-2′-deoxy-7-(hex-1-ynyl)adenosine (**5**) is strongly fluorescent [30][33]. Therefore, it was of interest to synthesize a series of 7-alkynyl-, 7-alkenyl-, and 7-alkyl-7-deaza-2′-deoxyadenosines (see **5**, **7**–**14**, **16**–**20**, and **47**–**62**) and to correlate the fluorescence quantum yields and fluorescence life times with the structure of the side chain. Also 8-substituted derivatives are investigated for comparison (see **63** and **64**). Moreover, the related pyrazolo[3,4-*d*]pyrimidine analogs (see **23**–**34** and **66**–**73**), namely the 7-alkynylated derivatives of 8-aza-7-deaza-2′-deoxyadenosine (**65**) are the subject of this investigation. These compounds contain an unaltered *Watson-Crick* recognition site for the pyrimidine moiety and are modified with the rather bulky substituents at a position that is well-accommodated in duplex DNA and accepted by DNA polymerases [11][33][34].

Results and Discussion. – 1. *Syntheses.* 1.1. *Pyrrolo[2,3-*d*]pyrimidine* (= 7-Deazapurine) *Nucleosides.* Earlier, the synthesis of a series of 7-alkynyl-7-deaza-2′-deoxyadenosines was reported [35]. For the preparation of the new 7-alkynyl derivatives **7**–**14**, the palladium(0)-catalyzed cross-coupling reaction of 7-deaza-2′-deoxy-7-iodoadenosine (**6**) [35] with various alkynes and alkenes was performed (*Scheme 1*). The α-D-nucleoside **15** was prepared by the same route from the α-D-anomer [36] of the iodonucleoside **6**. The use of vinyltributylstannane [37] in the Pd^0-catalyzed reaction of **6** furnished the 7-alkenyl nucleoside **16**. Catalytic hydrogenation of compounds **5** and **17**, afforded the alkylated nucleosides **18** and **19**, respectively (*Scheme 2*).

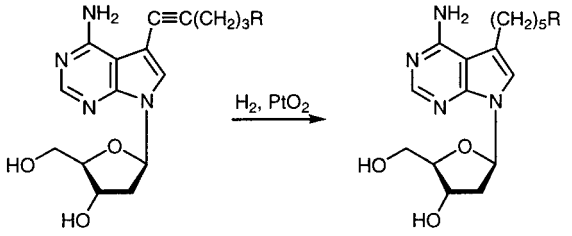
Scheme 1





The treatment of compound **20** [35] with NaOMe/MeOH resulted in an intramolecular cyclization, yielding the tricyclic compound **21** (*Scheme 3*). In the case of the ring-closure product **21**, the olefinic protons have *cis*-configuration (^3J(H,H) = 11.8 Hz), compared to a *trans*-configuration of the C=C bond in the case of the educt **20** (^3J(H,H) = 15.6 Hz). A similar ring-closure reaction and the configurational change at the C=C bond has been already observed in 5-[2-(ethoxycarbonyl)ethenyl]pyrimidines [38] and in corresponding 8-aza-7-deazaadenine 2′-deoxyribonucleosides [39].

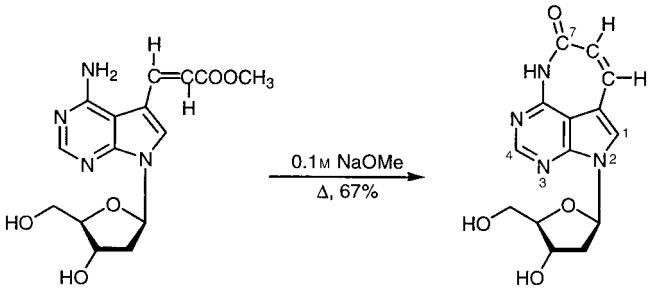
Scheme 2



5 R = Me
17 R = CF_3CONH

18 R = Me; 89%
19 R = CF_3CONH; 69%

Scheme 3



20 **21**

All new compounds were characterized by ^{1}H-, or ^{13}C-NMR spectroscopy (*Table 1* and *Exper. Part*), as well as by elemental analyses or mass spectra. The ^{13}C-NMR chemical shifts of the 7-substituted 7-deaza-2′-deoxyadenosine derivatives were assigned

Table 1. *^{13}C-NMR Chemical Shifts of 7-Alkynylated 7-Deazaadenine 2′-Deoxyribofuranosides, Measured in (D_6)DMSO at 303 K*[a])

	C(2)[b]) C(2)[c])	C(4)[b]) C(6)[c])	C(4a)[b]) C(5)[c])	C(5)[b]) C(7)[c])	C(6)[b]) C(8)[c])	C(7a)[b]) C(4)[c])	C≡C	C(1′)	C(3′)	C(4′)	C(5′)
7	152.5	157.6	102.4	95.2	125.1	149.0	100.3, 72.5	83.0	70.9	87.4	61.8
8	152.7	157.6	102.4	95.2	125.7	149.1	91.1, 83.6, 74.6, 72.3	83.3	71.1	87.6	62.0
9	152.6	157.5	102.2	94.4	126.5	149.1	86.9, 78.9, 78.1, 76.0	83.1	70.9	87.5	61.8
10	152.9	157.6	102.2	94.7	126.3	149.4	92.7, 82.2	83.3	71.0	87.6	61.9
11	152.5	157.5	102.3	95.4	125.2	149.0	96.3, 73.3	83.0	70.9	87.4	61.8
12	152.5	157.4	102.1	94.8	127.5	149.1	87.5, 74.5	83.1	70.9	87.5	61.8
13	152.6	157.5	102.3	95.0	125.4	149.1	96.5, 77.6	83.1	70.9	87.5	61.8
15	152.7	157.6	102.1	94.9	126.5	149.1	90.4, 83.6, 74.6, 72.3	83.1	70.9	88.3	61.8
16	151.5	157.6	100.6	114.1	118.6	151.5	129.0, 113.0[d])	82.9	71.0	87.3	62.0
18	151.3	157.7	102.2	115.5	118.6	150.4		82.9	71.2	87.2	62.2
19	151.2	157.6	102.1	115.2	118.5	150.3		82.8	71.1	87.1	62.1
21	152.7	154.0	105.6	112.3	123.4	151.5	133.5, 120.9[d])	83.3	70.8	87.6	61.7
48	152.5	157.5	102.3	95.6	125.3	149.0	88.3, 72.6	83.1	70.9	87.4	61.8

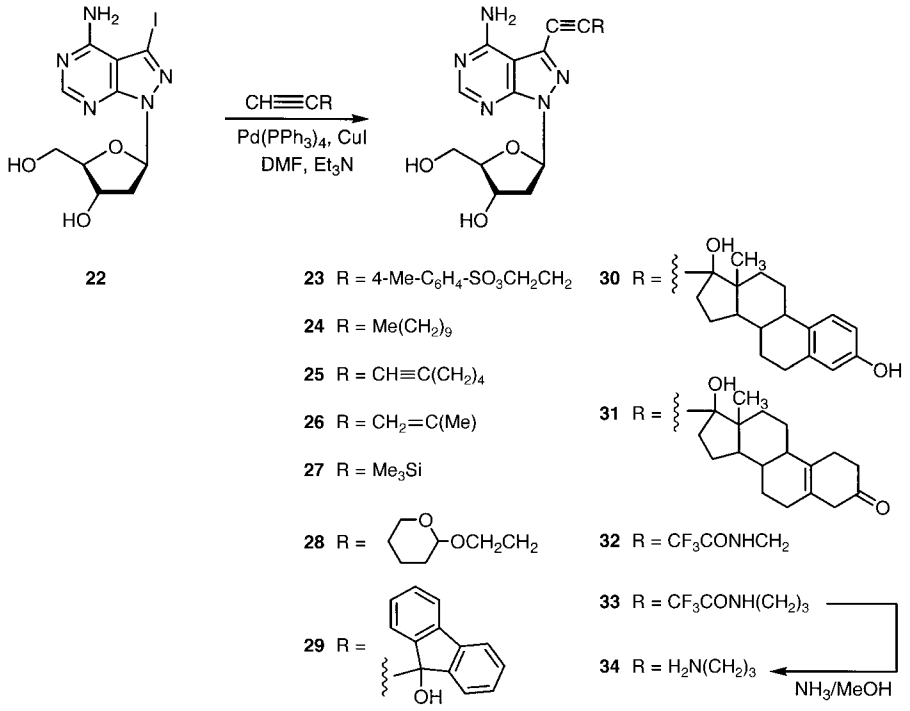
[a]) C(2′) is superimposed by DMSO. [b]) Systematic numbering. [c]) Purine numbering. [d]) Olefinic protons.

on the basis of the coupling pattern taken from the gated-decoupled $^1H/^{13}C$-NMR spectra. Also heteronuclear correlation spectra confirmed the assignments.

1.2. *Pyrazolo[3,4-*d*]pyrimidine* (= 8-Aza-7-deazapurine) *Nucleosides.* The synthesis of some 7-alkynylated 8-aza-7-deaza-2′-deoxy-β-D-adenosines with compound **22** [39] as precursor has already been reported [39]. The iodo compound **22** [39][40] served also as starting material for the synthesis of a series of 7-substituted 8-aza-7-deaza-2′-deoxyadenosines (**23**–**33**) by the Pd^0-catalyzed cross-coupling; they carry alkynyl, silylalkynyl, oxyalkynyl, (acylamino)alkynyl, arylalkynyl, as well as steroid-derived residues (*Scheme 4*). Compound **34**, which contains a free exocyclic amino function, was synthesized in 63% yield by treatment of **33** with NH_3/MeOH (*Scheme 4*).

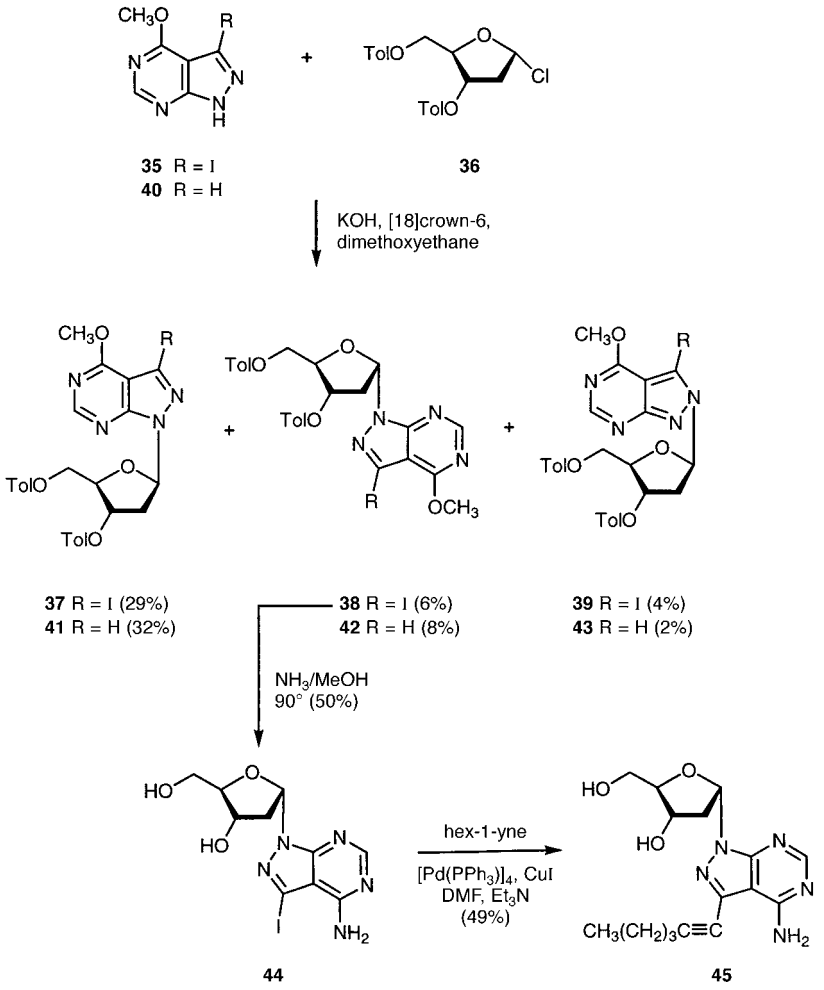
For the synthesis of 7-hexynyl-8-aza-7-deaza-2′-deoxy-α-D-adenosine (**45**) *via* the α-D-configured 7-iodo compound **44**, glycosylation of the nucleobase **35** [39] in dimethoxyethane with 2-deoxy-3,5-di-*O*-(4-toluoyl)-β-D-*erythro*-pentofuranosyl chloride (**36**) [41] was first performed. Three reaction products **37**–**39** were formed (TLC monitoring) when KOH and [18]crown-6 were used as catalysts (*Scheme 5*); they were isolated after flash chromatography (see *Exper. Part*). An analogous formation of the α-D-anomer **42** was observed on glycosylation of 6-methoxy-8-aza-7-deazapurine (**40**) [42][43] with **36** under the same conditions: the retention factors of the three formed nucleosides were identical to those reported for the β-D- or α-D-nucleosides **41**–**43** [42]. Also the comparison of the NMR data with the data published earlier supports the

Scheme 4



structural assignment [42]. However, it is not clear whether the use of [18]crown-6 instead of TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) during the glycosylation reaction or/and a partial anomerization of the halogenose **36** before glycosylation is responsible for the α-D-anomer formation. The glycosylation product **38** was then treated with ammonia/MeOH leading to **44**. When the Pd^0-catalyzed cross-coupling reaction was performed with the α-D-anomer **44** and hex-1-yne, the desired **45** was obtained in 49% yield (*Scheme 5*).

Scheme 5



The 7-substituted 8-aza-7-deaza-2′-deoxyadenosines were characterized by ^{1}H- or ^{13}C-NMR spectra (*Table 2* and *Exper. Part*) as well as by microanalyses or FAB mass spectra. The ^{13}C-NMR chemical shifts of these derivatives were assigned on the basis of the coupling pattern taken from the gated-decoupled ^{1}H,^{13}C-NMR spectra. Also, heteronuclear correlation spectra confirmed the assignments. The assignments of the

structures of **37**, **39**, and **41**–**43** have been already performed [39][42]. The configuration of the α-D-anomer **44** followed from that of the already established configuration of the starting material. Furthermore, the configurational assignment for compounds **38** and **44** was confirmed by the chemical shifts of the two H–C(2′) signals which nearly coincide in the case of β-D-anomers but are well-separated for the α-D-anomers [42][44]. Contrary to other anomeric purine-nucleoside analogues [43], the α-D-anomer **44** migrated faster on TLC than the β-D-anomer **22**.

Table 2. *^{13}C-NMR Chemical Shifts of 7-Substituted 8-Aza-7-deazaadenine 2′-Deoxyribofuranosides, Measured in (D_6)DMSO at 303 K*

	C(3)[a]) C(7)[b])	C(3a)[a]) C(5)[b])	C(4)[a]) C(6)[b])	C(6)[a]) C(2)[b])	C(7a)[a]) C(4)[b])	C≡C	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
22	91.0	103.5	157.6	156.2	154.0		84.0	37.9	70.9	87.7	62.3
23	126.7	100.8	157.6	156.6	153.6	91.6, 73.3	84.0	37.7	70.9	87.7	62.3
24	127.2	100.8	157.7	156.5	153.6	96.6, 72.1	84.0	37.9	70.9	87.7	62.3
25	127.2	100.8	157.7	156.6	153.6	96.3, 84.2, 72.2, 71.3	84.0	37.9	70.9	87.7	62.3
26	126.5	100.8	157.7	156.6	153.7	94.9, 79.7	84.1	37.9	70.9	87.7	62.3
27	126.3	101.0	157.7	156.7	153.5	101.1, 95.9	84.1	37.9	70.8	87.7	62.2
28	127.1	100.9	157.7	156.6	153.6	94.4, 72.7	84.0	37.9	71.0	87.7	62.3
29	125.9	101.2	157.6	156.8	153.6	96.3, 73.2	84.1	37.8	70.8	87.7	62.1
30	126.7	101.0	157.7	156.6	153.6	99.9, 76.3	84.3	37.9	70.8	87.7	62.2
31	126.7	101.0	157.7	156.7	153.6	99.9, 76.2	84.3	37.9	70.8	87.7	62.2
32	126.1	101.0	157.6	156.7	153.7	90.2, 74.3	84.1	37.9	71.0	87.7	62.3
33	127.1	100.8	157.7	156.6	153.6	95.5, 72.3	84.0	37.9	70.9	87.7	62.3
38	91.0	106.4	163.3	155.9	155.2		83.7	35.9	74.1	82.3	64.1
44	91.2	103.4	157.7	156.2	153.8		83.1	37.8	70.0	86.0	61.1
45	127.2	100.7	157.8	156.6	153.4		83.3	37.9	70.2	86.2	61.2

[a]) Systematic numbering. [b]) Purine numbering.

2. *Fluorescence Properties.* As mentioned above, the naturally occurring purine or pyrimidine bases show almost no fluorescence at room temperature. Adenine, *e.g.*, exhibits a fluorescence quantum yield between 0.004 and 0.006 in various glasses at 77 K [45]. The emission spectrum of adenine in EtOH at 77 K shows two bands, one with maxima at 310 and 322 nm and another structured band with maxima at 368, 385, 405, and 423 nm. The higher- and lower-energy bands are attributed to fluorescence and phosphorescence, respectively. In contrast to the poor spectroscopic characteristics of the canonical nucleosides at room temperature, the 7-alkynylated derivatives of 7-deaza-2′-deoxyadenosine are strongly fluorescent. In addition, the fluorescence emission maxima are shifted by *ca.* 40–100 nm towards longer wavelengths. To investigate the spectroscopic influence of substituents at the 7-position, various 7-deazaadenine nucleosides were studied by steady-state and time-resolved fluorescence spectroscopy. As demonstrated in *Fig. 1*, the excitation spectra of 7-deazaadenine derivatives are very similar to their absorption spectra, indicating that the samples are sufficiently pure and that the excited singlet state is the origin of the observed emission. As expected, the absorption and emission bands of all derivatives in H_2O are more or less unstructured at room temperature.

As can be seen in *Tables 3* and *4*, most of the decays can be described satisfactorily by a mono-exponential model. However, some 7-deazaadenine nucleoside derivatives

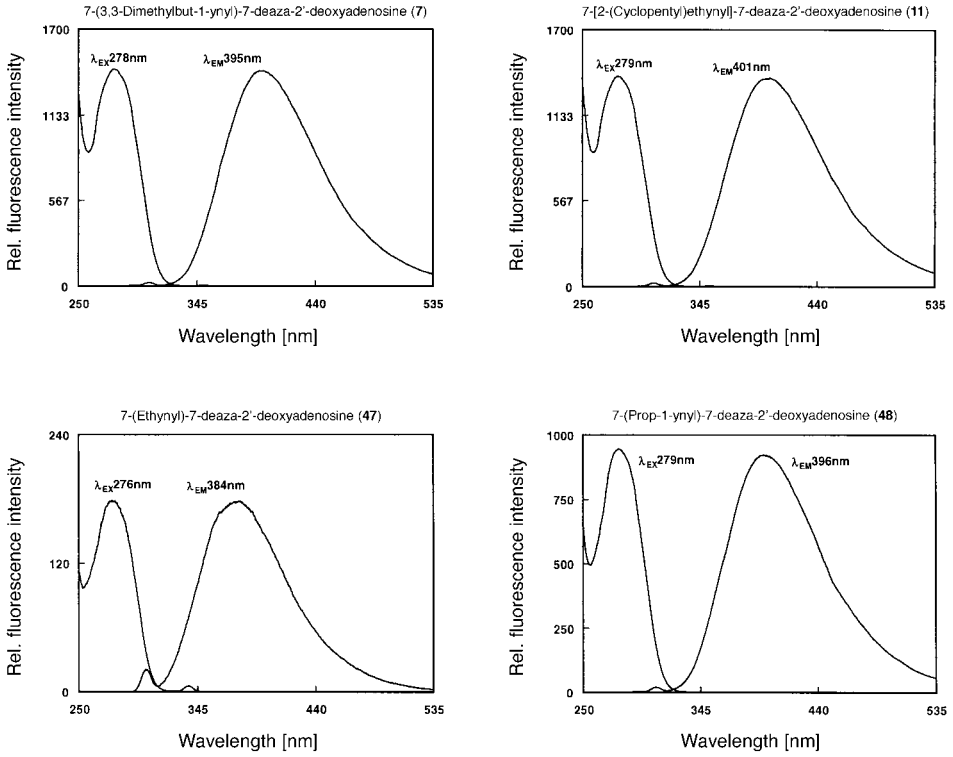


Fig. 1. *Fluorescence spectra of 7-deaza-2′-deoxyadenosines* **7**, **11**, **47**, *and* **48**. Measured in double-distilled H_2O with 10^{-5} M nucleoside concentration.

exhibit multi-exponential fluorescence kinetics (*Fig. 2*) which can be described at least by a bi-exponential model with a short main component ($t_1 < 1$ ns, $a_1 > 95\%$) and a longer component ($4\text{ ns} < t_2 < 10\text{ ns}$). To get more insight into the properties of the first excited singlet state, we studied the spectroscopic characteristics of compound **49** in various solvents of different polarity (*Fig. 3*). While the emission maximum shifts to longer wavelengths with increasing solvent polarity [46], the absorption maximum remains nearly unchanged (*Fig. 3,a*). In addition, the fluorescence quantum yield (*Fig. 3,b*) and decay time (*Fig. 3,c*) increase drastically with increasing solvent polarity. The observed behavior leads to the following conclusion: upon excitation of 7-deaza-2′-deoxyadenosines, an excited state with an increased dipole moment is formed. Thus, the emission in 7-deaza-2′-deoxyadenosines occurs from an excited singlet state that exhibits a more or less pronounced charge-transfer character. A more polar solvent stabilizes the excited state and causes a bathochromic shift of the emission maximum, an increased fluorescence quantum yield, and an increased decay time.

As can be seen in *Table 3*, with increasing size of the alkyl substituent, there is a rise in the fluorescence quantum yield and fluorescence decay time of the 7-deaza-2′-deoxyadenosines (*e.g.*, $\Phi_f = 0.04$ and $\tau = 0.81$ for **47**, $\Phi_f = 0.06$ and $\tau = 1.82$ for **48**, and

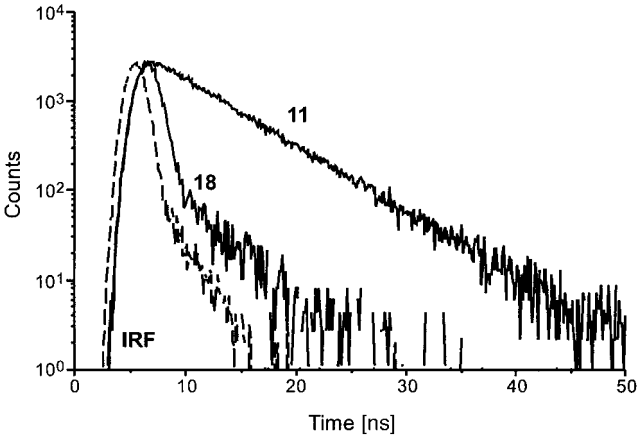


Fig. 2. *Fluorescence decay curves of the alkynylated 7-deaza-2′-deoxyadenosine* **11** (mono-exponential) *and of the alkylated* **18** (bi-exponential). Recorded in H_2O and lamp profile (512 channels, 100 ps/channel) measured with a H_2-filled flash lamp using the time-correlated single-photon counting technique.

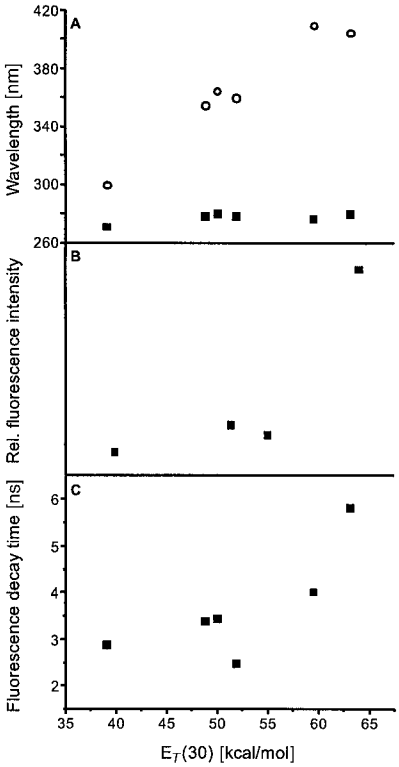


Fig. 3. *Spectroscopic properties of the alkynylated 7-deaza-2′-deoxyadenosine* **49** *in solvents of different polarity using* E_T (30) *values in kcal/mol* ($CHCl_3$ 39.1, hexan-1-ol 48.8, benzonitrile 50.0, EtOH 51.9, CF_3CH_2OH 59.5, H_2O 63.1): a) *Absorption* (■) *and emission maxima* (○), b) *relative fluorescence intensity* (■), *and* c) *fluorescence decay time* (■)

$\Phi_f = 0.21$ and $\tau = 5.83$ for **49**). Once a certain size of the alkyl substituent is reached, a further increase of the chain length shows no significant effect on the quantum yield and decay time. For example nucleosides **5**, **7**, **8**, **49**, **50**, and **53** show all quantum yields Φ_f of 0.21 – 0.27 and decay times τ of 5.30 – 6.81 ns. As the 7-alkynyl-7-deaza-2′-deoxyadenosines containing nucleobases related to adenine or guanine are accepted by DNA polymerases in form of their triphosphates [11] and are well-accommodated in the DNA duplex [33], these compounds can serve as potent labeling reagents.

There is a strong difference in fluorescence properties of 7-deaza-2′-deoxyadenosines and 8-aza-7-deaza-2′-deoxyadenosines (see *Tables 3* and *4*, and *Figs. 3* and *4*) for substituents with large alkyl substituents (for comparison, see nucleosides **5** *vs.* **68**, **49** *vs.* **67**, and **53** *vs.* **69**). However, if the nucleosides are substituted with conjugated systems containing C=C bonds or a single Me group, the 7-deaza-2′-deoxyadenosines and 8-aza-7-deaza-2′-deoxyadenosines exhibit almost the same fluorescence quantum yield and decay time (see **48** *vs.* **66**, **10** *vs.* **26**, and **55** *vs.* **70**).

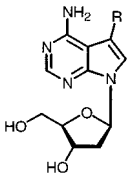


Table 3. *Absorption Maxima* (λ_{abs}), *Excitation Maxima* (λ_{exc}), *Emission Maxima* (λ_{em}), *Fluorescence Quantum Yields* (ϕ_f), *and Fluorescence Decay Times* τ *of 7-Substituted 7-Deaza-2′-deoxyadenosines* (= Pyrrolo[2,3-*d*]pyrimidin-4-amine 2′-Deoxyribofuranosides) *in Double-Distilled Water*

	R	λ_{abs} [nm]	λ_{exc} [nm]	λ_{em} [nm]	ϕ_f	τ [ns]
6 [35]	I ($c^7I^7A_d$)	281	282	367	0.002	0.28
46	H (c^7A_d)	270	270	[a])	[a])	[a])
47 [35]	CH≡C	278	278	366	0.04	0.81
48 [47]	MeC≡C	280	286	374	0.06	1.82
49 [35]	$Me(CH_2)_2C\equiv C$	280	280	404	0.21	5.83
5 [35]	$Me(CH_2)_3C\equiv C$	280	279	400	0.27	5.78
50 [35]	$Me(CH_2)_4C\equiv C$	280	280	402	0.26	6.03
7	$Me_3CC\equiv C$	280	281	404	0.24	7.98
8	$CH\equiv C(CH_2)_2C\equiv C$	280	280	403	0.24	5.30
10	$CH_2{=}C(Me)C\equiv C$	289	287	415	0.05	2.86
9	$CH\equiv C(CH_2)_2NCH_2C\equiv C$	280	279	384	0.05	2.20
51 [49]	$CF_3CONHCH_2C\equiv C$	279	280	375	0.07	1.32
17 [49]	$CF_3CONH(CH_2)_3C\equiv C$	280	280	397	0.15	4.07
52 [49][b])	$H_2N(CH_2)_3C\equiv C$	280	279	392	0.17	3.88
11	(cyclopentyl)C≡C	280	280	405	0.31	6.87
53 [35]	(cyclohexyl)C≡C	280	289	404	0.26	6.81
54 [48]	(1-hydroxycyclooctyl)C≡C	280	278	383	0.13	2.73
55 [35]	PhC≡C	296	297	412	0.02	0.69
56 [48]	3-(CH≡C)-C_6H_4−C≡C	300	293	460	0.02	0.82
57 [48]	4-Me−C_6H_4−C≡C	295	296	421	0.06	1.78
12	4-Me−C_6H_4−$SO_3CH_2CH_2C\equiv C$	280	280	375	0.07	1.32
58 [35]	(9-hydroxy-9*H*-fluoren-9-yl)C≡C	279	280	350	0.01	< 0.20
59 [35]	$Me_3SiCH_2C\equiv C$	280	281	423	0.08	2.83
60 [35]	$Me_3SiC\equiv C$	281	284	379	0.12	3.52
61 [48]	$Ph_3SiC\equiv C$	284	283	362	0.02	1.92
13	17β-hydroxy-3-oxo-19-norpregn-5(10)-en-20-yn-21-yl[c])	280	278	387	0.11	4.55
62 [35]	17β-hydroxy-3-oxopregn-4-en-20-yn-21-yl	280	280	380	0.10	3.84
14 [48]	$C\equiv C(CH_2)_4C\equiv C-(7\text{-}c^7A_d)$[c])	280	281	403	0.03	1.51
16	$CH_2{=}CH$	284	284	430	0.16	2.29
20 [35]	MeOOCCH=CH	268, 325	[a])			
21[b])	ring product[d])	283	282	361	0.03	0.48
18[b])	$Me(CH_2)_5$	279	282	363	0.01	0.37
19[b])	$CF_3CONH(CH_2)_5$	279	280	363	0.01	0.28
63 [50]	($Hxy^8c^7A_d$)	299	297	[a])	[a])	[a])
64 [50]	($Phy^8c^7A_d$)	320	317	359	0.01	0.2

[a]) No fluorescence. [b]) A double-exponential fit was necessary to describe the decay. The given fluorescence decay time represents the main component (amplitude > 95%) of the decay. [c]) For formula, see *Scheme 1*). [d]) Compound **21** is the tricyclic 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,6-dihydro-7*H*-2,3,5,6-tetraazabenz[*cd*]azulen-7-one (see *Scheme 3*).

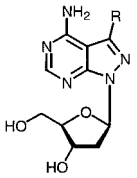


Table 4. *Absorption Maxima* (λ_{abs}), *Excitation Maxima* (λ_{exc}), *Emission Maxima* (λ_{em}), *Fluorescence Quantum Yields* (ϕ_f), *and Fluorescence Decay Times* τ *of 7-Substituted 8-Aza-7-deaza-2′-deoxyadenosines* (= Pyrazolo[3,4-*d*]pyrimidin-4-amine 2′-Deoxyribofuranosides) *in Double-Distilled Water*

	R	λ_{abs} [nm]	λ_{exc} [nm]	λ_{em} [nM]	ϕ_f	τ [ns]
65	H ($c^7z^8A_d$)	270	272	–	–	–
22 [39]	I ($c^7z^8I^7A_d$)	283	283	–	–	–
32	$CF_3CONHCH_2C\equiv C$	286	285	334	0.01	0.24
33	$CF_3CONH(CH_2)_3C\equiv C$	286	286	349	0.06	0.56
66 [39]	$MeC\equiv C$	286	285	348	0.06	2.05
67 [39]	$Me(CH_2)_2C\equiv C$	286	286	351	0.08	0.68
68 [34]	$Me(CH_2)_3C\equiv C$	286	280	350	0.10	0.85
26	$CH_2=C(Me)C\equiv C$	288	287	360	0.04	0.35
69 [39]	(cyclohexyl)$C\equiv C$	287	280	350	0.14	1.32
70 [39]	$PhC\equiv C$	294	295	360	0.08	0.55
71 [39]	4-Me$-C_6H_4-C\equiv C$	297	297	372	0.07	0.53
29	(9-hydroxy-9*H*-fluoren-9-yl)$C\equiv C$	287	278	339	0.05	2.2
23	4-Me$-C_6H_4-SO_3(CH_2)_2C\equiv C$	286	287	332	0.03	0.27
30	3,17β-dihydroxy-19-norpregna-1,3,5(10)-trien-20-yn-21-yl[a])	287	286	337	0.03	0.36
31	17β-hydroxy-3-oxo-19-norpregn-5(10)-en-20-yn-21-yl[a])	287	276	339	0.04	0.56
72 [39]	$CH_2=CH$	277	280	364	0.16	2.52
73 [39]	$MeOCOCH=CH_2$	289	289	474	0.05	1.07
74 [39]	ring product[b])	285	284	498	0.04	1.65

[a]) For formula see *Scheme 4*. [b]) Compound **74** is the tricyclic 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,6-dihydro-7*H*-1,2,3,5,6-pentaazabenzo[*cd*]azulen-7-one [39] (*cf.* **21**).

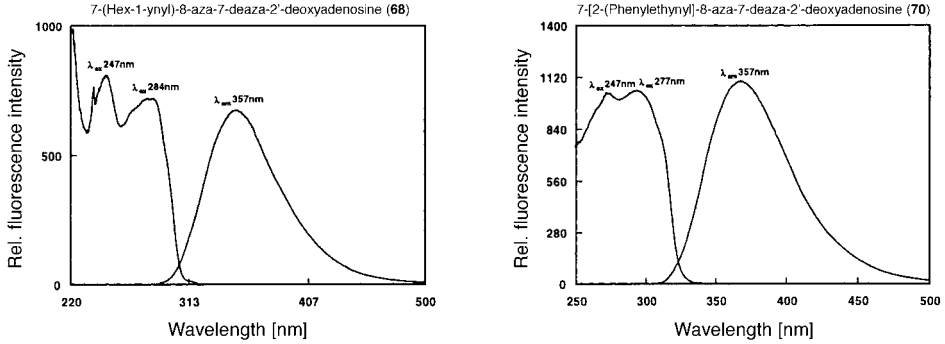


Fig. 4. *Fluorescence spectra of 8-aza-7-deaza-2′-deoxyadenosines* **68** *and* **70**. Measured in double-distilled H_2O with 10^{-5} M nucleoside concentration.

Experimental Part

1. *General*. All chemicals were purchased from *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 (*Merck*, Germany). Column flash chromatography (FC): silica gel *60* (*Merck*, Germany) at 0.4 bar ($4 \cdot 10^4$ Pa); solvent systems *A* (CH_2Cl_2/MeOH 9:1), *B* (CH_2Cl_2/MeOH 95:5), *C* (petroleum ether/AcOEt 1:1), sample collection with an *UltroRac-II* fraction collector (*LKB Instruments*, Sweden). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland); uncorrected. UV Spectra *U-3200* spectrometer (*Hitachi*, Japan); λ_{max} (ε) in nm. NMR Spectra: *AC-250*, *Avance-DPX-250*, or *AMX-500* spectrometers (*Bruker*, Karlsruhe, Germany); at 250.13 and 500.14 MHz (^{1}H) or 125.13 MHz (^{13}C); δ in ppm rel. to $SiMe_4$ as internal standard; *J* in Hz; st. = steroid. Positive-ion fast-atom-bombardment (FAB) mass spectra: 3-nitrobenzyl alcohol (3-NOBA) as matrix. Elemental analyses were performed by *Mikroanalytisches Labor Beller*, Göttingen, Germany.

2. *Fluorescence Measurements.* Absorption spectra were recorded either on a *U-3200* spectrometer (*Hitachi*, Japan) or on a *Lambda-18-UV/Vis* spectrophotometer (*Perkin Elmer*, Germany). Unless otherwise stated, all measurements were performed in double-distilled H_2O at 20° in standard quartz cuvettes with a path length of 1 cm. Polarity parameters of all solvents were obtained from the literature. To avoid inner-filter effects, the sample was not allowed to exceed 0.05 absorbance units at the absorption maximum. Fluorescence spectra were recorded in the wavelength range 320–600 nm using the fluorescence spectrometer *LS 100* (*PTI*, Canada). All samples were excited at 280 nm. Solvent background was subtracted when necessary. Fluorescence quantum yields were determined using quinine sulfate in 0.1N H_2SO_4 with a fluorescence quantum yield Φ_f of 0.70 [51] as a standard in the following relationship:

$$\Phi_{f,nucleoside} = \Phi_{f,standard} \cdot (F_{nucleoside}/F_{standard}) \cdot (A_{standard\ at\ 280\ nm}/A_{nucleoside\ at\ 280\ nm})$$

where $\Phi_{f,nucleoside}$ is the unknown fluorescence quantum yield of the nucleoside, *F* is the integrated fluorescence intensity between 300 and 600 nm after excitation at 280 nm, and *A* is the absorbance at 280 nm in 1-cm cuvettes. Fluorescence decay times were measured with a H_2-filled flash lamp by the time-correlated single-photon counting (TCSPC) technique with the *LS-100* instrument. Samples were excited at 280 nm. The fluorescence decay was monitored at the emission maximum. The instrument response function of the system needed for deconvolution was obtained from a scattering solution. The quality of the decay fits was controlled by the reduced χ-squared statistical parameter. Most of the decays could be described satisfactorily by a mono-exponential model ($\chi^2 < 1.2$). In the cases where a second component was necessary, a bi-exponential model was used to fit the decay, *i.e.*, $I(t) = I(0) \cdot [a_1 \cdot \exp(-t/\tau_1) + a_2 \cdot \exp(-t/\tau_2)]$, a_1 and a_2 being pre-exponential factors that describe the ratio of the excited species ($a_1 + a_2 = 1$), and τ_1 and τ_2 being the corresponding decay times.

3. *Nucleosides*. 3.1. *Pyrrolo[2,3-*d*]pyrimidine (= 7-Deazapurine) Nucleosides: General Procedure I (G.P. I) for the Pd⁰-Catalyzed Cross Coupling*. A suspension of 7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**6**) [35] (100 mg, 0.27 mmol) and CuI (10.1 mg, 0.05 mmol) in anh. DMF (3 ml) was treated with the alkyne (10–20 equiv.), anh. Et_3N (54 mg, 0.53 mmol), and [$Pd(PPh_3)_4$] (31 mg, 0.027 mmol). The mixture was stirred under Ar at r.t. After the reaction was complete (TLC monitoring), the mixture was diluted with MeOH/CH_2Cl_2 1:1 (20 ml), and *Dowex 1 × 8* (100–200 mesh; 500 mg, hydrogen carbonate form) was added. After stirring for 45 min, the mixture was filtered, the resin washed with MeOH/CH_2Cl_2 1:1 (100 ml), the combined filtrate evaporated, and the residue purified by FC (silica gel, 2–10% MeOH/CH_2Cl_2). The main zone afforded the nucleoside derivative upon evaporation.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(prop-1-ynyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**48**) [47]. According to *G.P. I*, with a propyne-saturated DMF soln. (8 h): **48** (45 mg, 58%). Colorless foam. TLC (*A*): R_f 0.40. UV (MeOH): 280 (10100), 237 (13000). ^{1}H-NMR ((D$_6$)DMSO): 2.07 (*s*, Me); 2.16 (*m*, H_α–C(2′)); 2.48 (*m*, H_β–C(2′)); 3.51 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.31 (*m*, H–C(3′)); 5.07 (*t*, *J* = 5.4, OH–C(5′)); 5.26 (*d*, *J* = 3.9, OH–C(3′)); 6.45 (‘*t*’, *J* = 6.9, H–C(1′)); 6.70 (br. *s*, NH_2); 7.63 (*s*, H–C(6)); 8.08 (*s*, H–C(2)).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(3,3-dimethylbut-1-ynyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**7**). According to *G.P. I* with 3,3-dimethylbut-1-yne (440 mg, 5.36 mmol) (4 h): **7** (55 mg, 62%). Colorless foam. TLC (*A*): R_f 0.44. UV (MeOH): 280 (10500), 240 (14400). ^{1}H-NMR ((D$_6$)DMSO): 1.32 (*s*, Me); 2.18 (*m*, H_α–C(2′)); 2.46 (*m*, H_β–C(2′)); 3.57 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.2, OH–C(5′)); 5.24 (*d*, *J* = 3.7, OH–C(3′)); 6.48 (‘*t*’, *J* = 6.9, H–C(1′)); 6.62 (br. *s*, NH_2); 7.64 (*s*, H–C(6)); 8.12 (*s*, H–C(2)). Anal. calc. for $C_{17}H_{22}N_4O_3$ (330.39): C 61.80, H 6.71, N 16.96; found: C 61.86, H 6.81, N 16.11.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(hexa-1,5-diynyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**8**). According to *G.P. I*, with hexa-1,5-diyne (415 mg, 5.31 mmol) (5 h): **8** (40 mg, 45%). Slightly yellow foam. TLC

(*A*): R_f 0.41. UV (MeOH): 280 (10400), 238 (12200). ^{1}H-NMR ((D$_6$)DMSO): 2.18 (*m*, H$_\alpha$–C(2′)); 2.48 (*m*, H$_\beta$–C(2′), CH$_2$); 2.66 (*t*, *J* = 6.6, CH$_2$); 2.86 (*s*, C≡CH); 3.53 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.32 (*m*, H–C(3′)); 5.06 (*t*, *J* = 5.5, OH–C(5′)); 5.25 (*d*, *J* = 4.0, OH–C(3′)); 6.46 (‘*t*’, *J* = 6.9, H–C(1′)); 6.71 (br. *s*, NH$_2$); 7.66 (*s*, H–C(6)); 8.09 (*s*, H–C(2)). FAB-MS (3-NOBA): 327.2 ([*M* + H]$^+$, [C$_{17}$H$_{18}$N$_4$O$_3$ + H]$^+$; calc. 327.36).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-[3-[di(prop-2-ynyl)amino]prop-1-ynyl]-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**9**). According to *G.P. I*, with tri(prop-2-ynyl)amine (1.4 g, 10.7 mmol) for 4 h: **9** (51 mg, 50%). Yellowish foam. TLC (*A*): R_f 0.42. UV (MeOH): 280 (11400), 238 (11600). ^{1}H-NMR ((D$_6$)DMSO): 2.17 (*m*, H$_\alpha$–C(2′)); 2.48 (*m*, H$_\beta$–C(2′)); 3.23 (*s*, 2 C≡CH); 3.45 (*s*, 2 CH_2C≡CH); 3.53 (*m*, 2 H–C(5′)); 3.68 (*s*, 2 C≡CCH$_2$N); 3.81 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.06 (*t*, *J* = 5.5, OH–C(5′)); 5.26 (*d*, *J* = 3.9, OH–C(3′)); 6.47 (‘*t*’, *J* = 6.8, H–C(1′)); 6.69 (br. *s*, NH$_2$); 7.76 (*s*, H–C(6)); 8.11 (*s*, H–C(2)). FAB-MS (3-NOBA): 380.1 ([*M* + H]$^+$, [C$_{20}$H$_{21}$N$_5$O$_3$ + H]$^+$; calc. 380.43).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(3-methylbut-3-en-1-ynyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**10**). According to *G.P. I*, with 2-methylbut-1-en-3-yne (350 mg, 5.30 mmol) (6 h): **10** (40 mg, 47%). Slightly yellow foam. TLC (*A*): R_f 0.45. UV (MeOH): 289 (15400), 262 (11600). ^{1}H-NMR ((D$_6$)DMSO): 1.98 (*s*, Me); 2.20 (*m*, H$_\alpha$–C(2′)); 2.47 (*m*, H$_\beta$–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.4, OH–C(5′)); 5.25 (*d*, *J* = 3.9, OH–C(3′)); 5.38 (*s*, 1 H, CH$_2$); 5.39 (*s*, 1 H, CH$_2$); 6.50 (‘*t*’, *J* = 6.9, H–C(1′)); 6.67 (br. *s*, NH$_2$); 7.80 (*s*, H–C(6)); 8.14 (*s*, H–C(2)). Anal. calc. for C$_{16}$H$_{18}$N$_4$O$_3$ (314.34): C 61.14, H 5.77, N 17.82; found: C 60.85, H 5.81, N 17.52.

*5-(2-Cyclopentylethynyl)-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**11**). According to *G.P. I*, with cyclopentylacetylene (500 mg, 5.31 mmol) (4 h): **11** (59 mg, 64%). Colorless foam. TLC (*A*): R_f 0.46. UV (MeOH): 280 (10500), 242 (13900). ^{1}H-NMR ((D$_6$)DMSO): 1.60–2.02 (several *m*, 4 CH$_2$); 2.18 (*m*, H$_\alpha$–C(2′)); 2.46 (*m*, H$_\beta$–C(2′)); 2.93 (*quint.*, *J* = 7.1, CH); 3.54 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.0, OH–C(5′)); 5.24 (*d*, *J* = 3.7, OH–C(3′)); 6.48 (‘*t*’, *J* = 6.9, H–C(1′)); 6.70 (br. *s*, NH$_2$); 7.64 (*s*, H–C(6)); 8.11 (*s*, H–C(2)). Anal. calc. for C$_{18}$H$_{22}$N$_4$O$_3$ (342.40): C 63.14, H 6.48, N 16.36; found: C 62.74, H 6.37, N 16.77.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-{4-[[(4-methylphenyl)sulfonyl]oxy}but-1-ynyl]-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**12**). According to *G.P. I*, with but-3-ynyl-4-methylbenzenesulfonate (1.2 g, 5.35 mmol) (8 h): **12** (68 mg, 54%). Colorless foam. TLC (*A*): R_f 0.51. UV (MeOH): 280 (10600). ^{1}H-NMR ((D$_6$)DMSO): 2.19 (*m*, H$_\alpha$–C(2′)); 2.33 (*s*, Me); 2.47 (*m*, H$_\beta$–C(2′)); 2.85 (*t*, *J* = 6.0, CH$_2$); 3.57 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.20 (*t*, *J* = 6.1, CH$_2$); 4.36 (*m*, H–C(3′)); 5.05 (*t*, *J* = 5.1, OH–C(5′)); 5.25 (*d*, *J* = 3.9, OH–C(3′)); 6.49 (‘*t*’, *J* = 7.0, H–C(1′)); 6.60 (br. *s*, NH$_2$); 7.41 (*d*, *J* = 8.0, 2 arom. H); 7.66 (*s*, H–C(6)); 7.82 (*d*, *J* = 8.0, 2 arom. H); 8.12 (*s*, H–C(2)). Anal. calc. for C$_{22}$H$_{24}$N$_4$O$_6$S (472.51): C 55.92, H 5.12, N 11.86; found: C 55.80, H 5.50, N 11.82.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(17β-hydroxy-3-oxo-19-norpregn-5(10)-en-20-yn-21-yl)-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**13**). According to *G.P. I*, with norethynodrel (= 17*β*-hydroxy-19-norpregn-5(10)-en-20-yn-3-one; 790 mg, 2.6 mmol) (8 h): **13** (67 mg, 46%). Colorless solid. TLC (*A*): R_f 0.33. UV (MeOH): 280 (10400), 237 (15600). ^{1}H-NMR ((D$_6$)DMSO): 0.80 (*s*, Me); 1.30–2.49 (several *m*, H$_\alpha$–C(2′), H$_\beta$–C(2′), CH$_2$(1)(st.), CH$_2$(2)(st.), CH$_2$(4)(st.), CH$_2$(6)(st.), CH$_2$(7)(st.), CH$_2$(11)(st.), CH$_2$(12)(st.), CH$_2$(15)(st.), CH$_2$(16)(st.), H–C(8)(st.), H–C(9)(st.), H–C(14)(st.)); 3.55 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.06 (*t*, *J* = 5.3, OH–C(5′)); 5.25 (*d*, *J* = 3.7, OH–C(3′)); 5.63 (*s*, OH–C(17)(st.)); 6.47 (‘*t*’, *J* = 6.9, H–C(1′)); 6.72 (br. *s*, NH$_2$); 7.67 (*s*, H–C(6)); 8.10 (*s*, H–C(2)). Anal. calc. for C$_{31}$H$_{38}$N$_4$O$_5$ (546.67): C 68.11, H 7.01, N 10.25; found: C 67.78, H 6.68, N 10.90.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-ethenyl-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**16**). According to *G.P. I* at 40° with **6** and tributyl(vinyl)stannane [37] (1.5 g, 4.73 mmol) (12 h): **16** (50 mg, 67%). Colorless foam. M.p. 169–174° (dec.). TLC (*A*): R_f 0.33. UV (MeOH): 284 (7900), 248 (9100). ^{1}H-NMR ((D$_6$)DMSO): 2.17 (*m*, H$_\alpha$–C(2′)); 2.48 (*m*, H$_\beta$–C(2′)); 3.53 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 5.06 (*t*, *J* = 4.9, OH–C(5′)); 5.14 (*d*, *J* = 10.8, 1 H, CH$_2$); 5.24 (*d*, *J* = 3.6, OH–C(3′)); 5.58 (*d*, *J* = 17.2, 1 H, CH$_2$); 6.52 (‘*t*’, *J* = 6.5, H–C(1′)); 6.69 (br. *s*, NH$_2$); 7.11 (*dd*, *J* = 11.1, 5.8, CH=C); 7.65 (*s*, H–C(6)); 8.06 (*s*, H–C(2)). FAB-MS (3-NOBA): 277.2 ([*M* + H]$^+$). Anal. calc. for C$_{13}$H$_{16}$N$_4$O$_3$ (276.29): C 56.51, H 5.84; found: C 56.26, H 6.00.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-[5-(trifluoroacetamido)pentyl]-7*H-*pyrrolo[2,3-*d*]*pyrimidin-4-amine* (**19**). 7-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-5-[5-(trifluoroacetamido)pent-1-ynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**17**) [49] (50 mg, 0.12 mmol) in MeOH (20 ml) was hydrogenated over PtO$_2$. After 1 h, the mixture was diluted with MeOH (50 ml), filtered, and washed twice with MeOH (50 ml, each). The combined filtrates were evaporated and the residue purified by FC (silica gel, column 8 × 1 cm, 5–10% MeOH/

CH_2Cl_2): **19** (35 mg, 69%). Colorless foam. TLC (*A*): R_f 0.29. UV (MeOH): 279 (8500). ^{1}H-NMR ((D$_6$)DMSO): 1.37 (*quint.*, J = 6.5, CH_2); 1.55 (*m*, 2 CH_2); 2.10 (*m*, H_α–C(2′)); 2.47 (*m*, H_β–C(2′), superimposed by DMSO); 2.72 (*m*, CH_2); 3.19 (*m*, CH_2); 3.50 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.05 (br., OH–C(5′)); 5.20 (*d*, J = 3.5, OH–C(3′)); 6.47 (‘*t*’, J = 6.9, H–C(1′)); 6.49 (br. *s*, NH_2); 7.08 (*s*, H–C(6)); 8.02 (*s*, H–C(2)); 9.38 (*s*, NH). FAB-MS (3-NOBA): 432.3 ([M + H]$^+$, [$C_{18}H_{24}F_3N_5O_4$ + H]$^+$; calc. 432.42).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-hexyl-7*H-*pyrrolo[2,3-*d*]pyrimidin-4-amine* (**18**). 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(hex-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**5**) [35] (25 mg, 0.08 mmol) was hydrogenated as described for **19**: **18** (22 mg, 89%). Colorless foam. TLC (*A*): R_f 0.33. UV (MeOH): 279 (8200). ^{1}H-NMR ((D$_6$)DMSO): 0.85 (*t*, J = 6.6, Me); 1.28 (*m*, 3 CH_2); 1.54 (*m*, CH_2); 2.09 (*m*, H_α–C(2′)); 2.46 (*m*, H_β–C(2′), superimposed by DMSO); 2.71 (*m*, CH_2); 3.50 (*m*, 2 H–C(5′)); 3.78 (*m*, H–C(4′)); 4.31 (*m*, H–C(3′)); 5.09 (br., OH–C(5′)); 5.21 (*d*, J = 3.9, OH–C(3′)); 6.45 (*dd*, J = 7.5, 6.0, H–C(1′)); 6.52 (*s*, NH_2); 7.07 (*s*, H–C(6)); 8.00 (*s*, H–C(2)). FAB-MS (3-NOBA): 335.3 ([M + H]$^+$, [$C_{17}H_{26}N_4O_3$ + H]$^+$; calc. 335.43).

*7-(2-Deoxy-α-*D-erythro-*pentofuranosyl)-5-(hexa-1,5-diynyl)-7*H-*pyrrolo[2,3-*d*]pyrimidin-4-amine* (**15**). According to *G.P. I* with 7-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [36] (100 mg, 0.27 mmol; α-D-anomer of **6**) and hexa-1,5-diyne (415 mg, 5.31 mmol) for 5 h: **15** (41 mg, 47%). Yellowish foam. TLC (*A*): R_f 0.43. UV (MeOH): 280 (10500), 238 (12900). ^{1}H-NMR ((D$_6$)DMSO): 2.48 (*m*, H_β–C(2′), CH_2); 2.66 (*t*, J = 6.6, CH_2); 2.74 (*m*, H_α–C(2′)); 2.86 (*s*, C≡CH); 3.41 (*m*, 2 H–C(5′)); 4.04 (*m*, H–C(4′)); 4.27 (*m*, H–C(3′)); 4.81 (*t*, J = 5.6, OH–C(5′)); 5.58 (*d*, J = 4.0, OH–C(3′)); 6.48 (*dd*, J = 5.8, 3.0, H–C(1′)); 6.70 (br. *s*, NH_2); 7.79 (*s*, H–C(6)); 8.10 (*s*, H–C(2)). FAB-MS (3-NOBA): 327.7 ([M + H]$^+$, [$C_{17}H_{18}N_4O_3$ + H]$^+$; calc. 327.36).

*2-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-2,6-dihydro-7*H-*2,3,5,6-tetraazabenz[*cd*]*azulen-7-one* (**21**). For 4 h, methyl 3-[4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]prop-2-enoate (**20**) [35] (50 mg, 0.15 mmol) was heated under reflux with 0.1M NaOMe/MeOH (30 ml) for 4 h. The soln. was evaporated and the residue purified by FC (silica gel, column 8 × 2 cm, solvent *A*): **21** (30 mg, 67%). Colorless solid. TLC (*A*): R_f 0.36. UV (MeOH): 283 (9700), 236 (23000). ^{1}H-NMR ((D$_6$)DMSO): 2.25 (*m*, H_α–C(2′)); 2.48 (*m*, H_β–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 4.96 (*t*, J = 4.9, OH–C(5′)); 5.29 (*d*, J = 3.6, OH–C(3′)); 5.68 (*d*, J = 11.7, C=CH); 6.49 (‘*t*’, J = 6.6, H–C(1′)); 7.05 (*d*, J = 11.8, CH=C); 7.78 (*s*, H–C(1)); 8.35 (*s*, H–C(4)); 10.65 (*s*, NH). Anal. calc. for $C_{14}H_{14}N_4O_4$ (302.29): C 55.63, H 4.67, N 18.53; found: C 55.64, H 4.86, N 18.59.

3.2. *Pyrazolo[3,4-*d*]pyrimidine* (= 8-Aza-7-deazapurine) *Nucleosides: General Procedure II (G.P. II) for the Pd*^{0}*-Catalyzed Cross Coupling. Method 1.* A suspension of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**22**) [39] [40] (200 mg, 0.53 mmol) and CuI (20.2 mg, 0.106 mmol) in anh. DMF (3 ml) was treated with the alkyne (10–20 equiv.), anh. Et_3N (108 mg, 1.07 mmol), and [$Pd(PPh_3)_4$] (62 mg, 0.054 mmol). The mixture was stirred under Ar at r.t. After the reaction was complete (TLC monitoring), the mixture was diluted with MeOH/CH_2Cl_2 1:1 (10 ml), and *Dowex 1* × *8* (100–200 mesh; 500 mg, hydrogen carbonate form) was added. After 45 min stirring, the mixture was filtered, the resin washed twice with MeOH/CH_2Cl_2 1:1 (100 ml), the combined filtrate evaporated, and the residue subjected to FC (column 15 × 3 cm, 2–10% MeOH/CH_2Cl_2). The main zone afforded the nucleoside derivative upon evaporation.

Method 2. As described in *Method 1*, but with **22** (100 mg, 0.265 mmol), CuI (10.1 mg, 0.053 mmol), anh. DMF (1.5 ml), alkyne (10–20 eq.), anh. Et_3N (54 mg, 0.53 mmol), and [$Pd(PPh_3)_4$] (31 mg, 0.027 mmol). Workup as described in *Method 1* (FC: silica gel, column 15 × 2 cm).

Method 3. As described in *Method 1*, with 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**44**) (35 mg, 0.093 mmol) and hex-1-yne as coupling reagents, CuI (5.0 mg, 0.026 mmol), anh. DMF (1.0 ml), anh. Et_3N (30 mg, 0.29 mmol), and [$Pd(PPh_3)_4$] (15 mg, 0.013 mmol). Workup as described in *Method 1* (FC: silica gel, column 10 × 1 cm).

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-3-{4-[(4-methylphenyl)sulfonyl]oxy}but-1-ynyl]-1*H-*pyrazolo[3,4-*d*]*pyrimidin-4-amine* (**23**). According to *G.P. II* (*Method 1*), with but-3-ynyl 4-methylbenzenesulfonate (250 mg, 1.1 mmol) (7 h): **23** (135 mg, 54%). Colorless foam. TLC (*A*): R_f 0.52. UV (MeOH): 286 (10300), 248 (10000). ^{1}H-NMR ((D$_6$)DMSO): 2.25 (*m*, H_α–C(2′)); 2.31 (*s*, Me); 2.78 (*m*, H_β–C(2′)); 2.93 (*t*, J = 6.0, 2 CH_2); 3.46 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.27 (*t*, J = 6.1, CH_2); 4.44 (*m*, H–C(3′)); 4.75 (*t*, J = 5.1, OH–C(5′)); 5.25 (*d*, J = 4.3, OH–C(3′)); 6.55 (‘*t*’, J = 6.4, H–C(1′)); 6.60, 7.90 (2*s*, NH_2); 7.39 (*d*, J = 8.0, 2 arom. H); 7.82 (*d*, J = 8.0, 2 arom. H); 8.25 (*s*, H–C(6)). Anal. calc. for $C_{21}H_{23}N_5O_6S$ (473.51): C 53.27, H 4.90, N 14.79; found: C 53.09, H 5.39, N 14.89.

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-(dodec-1-ynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**24**). According to *G.P. II* (*Method 2*), with dodec-1-yne (450 mg, 2.71 mmol) (4 h): **24** (48 mg, 44%). Colorless solid. TLC (*A*): R_f 0.49. UV (MeOH): 287 (10800), 249 (10300). ^{1}H-NMR ((D$_6$)DMSO): 0.82 (*t*, *J* = 7.1, Me); 1.23 (br. *m*, several CH$_2$); 1.39 (*quint.*, *J* = 7.0, CH$_2$); 1.57 (*quint.*, *J* = 7.1, CH$_2$); 2.23 (*m*, H$_\alpha$–C(2′)); 2.53 (*t*, *J* = 7.1, CH$_2$); 2.74 (*m*, H$_\beta$–C(2′)); 3.43 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.40 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.7, OH–C(5′)); 5.25 (*d*, *J* = 4.5, OH–C(3′)); 6.52 (‘*t*’, *J* = 6.4, H–C(1′)); 6.58, 7.95 (2 br. *s*, NH$_2$); 8.21 (*s*, H–C(6)). FAB-MS (3-NOBA): 416.4 ([*M* + H]$^+$, [C$_{22}$H$_{33}$N$_5$O$_3$ + H]$^+$; calc. 416.54).

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-(octa-1,7-diynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**25**). According to *G.P. II* (*Method 2*), with octa-1,7-diyne (570 mg, 5.37 mmol) (4 h): **25** (39 mg, 41%). Yellowish foam. TLC (*A*): R_f 0.51. UV (MeOH): 286 (11300), 249 (11000). ^{1}H-NMR ((D$_6$)DMSO): 1.63 (*m*, C*H*$_2$C*H*$_2$CH$_2$C≡CH); 2.23 (*m*, H$_\alpha$–C(2′), C*H*$_2$C≡CH); 2.53 (CH$_2$C≡C, superimposed by DMSO); 2.76 (*m*, H$_\beta$–C(2′), C≡CH); 3.50 (*m*, H–C(5′)); 3.79 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.5, OH–C(5′)); 5.25 (*d*, *J* = 4.2, OH–C(3′)); 6.52 (‘*t*’, *J* = 6.2, H–C(1′)); 6.59, 7.94 (2 br. *s*, NH$_2$); 8.22 (*s*, H–C(6)).

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-(3-methylbut-3-en-1-ynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**26**). According to *G.P. II* (*Method 1*), with 2-methylbut-1-en-3-yne (700 mg, 10.6 mmol) (4 h): **26** (87 mg, 52%). Colorless foam. TLC (*A*): R_f 0.50. UV (MeOH): 288 (13500), 266 (12400). ^{1}H-NMR ((D$_6$)DMSO): 2.03 (*s*, Me); 2.25 (*m*, H$_\alpha$–C(2′)); 2.79 (*m*, H$_\beta$–C(2′)); 3.45 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.40 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.6, OH–C(5′)); 5.26 (*d*, *J* = 4.3, OH–C(3′)); 5.56 (*s*, 1 H, CH$_2$); 5.65 (*s*, 1 H, CH$_2$); 6.57 (‘*t*’, *J* = 6.3, H–C(1′)); 6.65, 7.92 (2 br. *s*, NH$_2$); 8.27 (*s*, H–C(6)). Anal. calc. for C$_{15}$H$_{17}$N$_5$O$_3$ (315.33): C 57.14, H 5.43, N 22.21; found: C 57.27, H 5.55, N 22.32. FAB-MS (3-NOBA): 316.2 ([*M* + H]$^+$).

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-[2-(trimethylsilyl)ethynyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**27**). According to *G.P. II* (*Method 2*), with (trimethylsilyl)acetylene (520 mg, 5.30 mmol) (4 h): **27** (37 mg, 40%). Colorless solid. TLC (*A*): R_f 0.52. UV (MeOH): 285 (10100), 246 (8900). ^{1}H-NMR ((D$_6$)DMSO): 0.29 (*s*, Me); 2.26 (*m*, H$_\alpha$–C(2′)); 2.75 (*m*, H$_\beta$–C(2′)); 3.48 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.7, OH–C(5′)); 5.27 (*d*, *J* = 4.5, OH–C(3′)); 6.53 (‘*t*’, *J* = 6.3, H–C(1′)); 6.48, 7.90 (2 br. *s*, NH$_2$); 8.25 (*s*, H–C(6)). FAB-MS (3-NOBA): 348.1 ([*M* + H]$^+$, [C$_{15}$H$_{21}$N$_5$O$_3$Si + H]$^+$; calc. 348.46).

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-[4-(tetrahydro-2*H*-pyran-2-yl)but-1-ynyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**28**). According to *G.P.II* (*Method 2*), with 4-(tetrahydro-2*H*-pyran-2-yl)but-1-yne (800 mg, 5.19 mmol) (5 h): **28** (48 mg, 45%). Colorless oil. TLC (*A*): R_f 0.51. UV (MeOH): 286 (11200), 248 (10500). ^{1}H-NMR ((D$_6$)DMSO): 1.43–1.69 (several *m*, 4 H(thp), CH$_2$C≡C); 2.20 (*m*, H$_\alpha$–C(2′)); 2.74 (*m*, H$_\beta$–C(2′), C*H*$_2$CH); 3.26–3.63 (several *m*, C≡CCH$_2$C*H*$_2$O, 2 H–C(5′)); 3.77 (*m*, H–C(4′), CH$_2$O of thp); 4.38 (*m*, H–C(3′)); 4.62 (*m*, CH); 4.69 (br., OH–C(5′)); 5.20 (br., OH–C(3′)); 6.49 (br., H–C(1′)); 6.56, 7.95 (2 br. *s*, NH$_2$); 8.19 (*s*, H–C(6)). FAB-MS (3-NOBA): 404.3 ([*M* + H$^+$], [C$_{19}$H$_{25}$N$_5$O$_5$ + H]$^+$; calc. 404.45).

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-[2(9-)hydroxy-9*H*-fluoren-9-yl)ethynyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**29**). According to *G.P.II* (*Method 1*), with 9-ethynyl-9*H*-fluoren-9-ol (1.1 g, 5.33 mmol) (4 h): **29** (135 mg, 56%). Colorless solid. M.p. 237–240° (dec.). TLC (*A*): R_f 0.35. UV (MeOH): 287 (21200), 280 (21500), 253 (20700), 236 (27100), 231 (26900). ^{1}H-NMR ((D$_6$)DMSO): 2.20 (*m*, H$_\alpha$–C(2′)); 2.72 (*m*, H$_\beta$–C(2′)); 3.41 (*m*, 2 H–C(5′)); 3.78 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 4.67 (*t*, *J* = 5.4, OH–C(5′)); 5.21 (*d*, *J* = 4.0, OH–C(3′)); 6.35, 8.10 (2 br. *s*, NH$_2$); 6.51 (‘*t*’, *J* = 6.4, H–C(1′)); 7.22 (*s*, OH–C(9″)); 7.47, 7.79 (2 *m*, arom. H); 8.27 (*s*, H–C(6)). Anal. calc. for C$_{25}$H$_{21}$N$_5$O$_4$ (455.47): C 65.93, H 4.65, N 15.38; found: C 66.05, H 4.80, N 15.25.

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-(3,17β-dihydroxy-19-norpregna-1,3,5(10)-trien-20-yn-21-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**30**). According to *G.P. II* (*Method 1*), with 19-norpregna-1,3,5(10)-trien-20-yne-3,17β-diol (1.57 g, 5.30 mmol) (4 h): **30** (165 mg, 57%). Colorless crystals. M.p. 255–257° (dec.). TLC (*A*): R_f 0.29. UV (MeOH): 287 (14600), 281 (14300), 251 (13900). ^{1}H-NMR ((D$_6$)DMSO): 0.86 (*s*, Me); 1.30–2.80; 1.30–2.49 (several *m*, H$_\alpha$–C(2′), H$_\beta$–C(2′), CH$_2$(6)(st.), CH$_2$(7)(st.), CH$_2$(11)(st.), CH$_2$(12)(st.), CH$_2$(15)(st.), CH$_2$(16)(st.), H–C(8)(st.), H–C(9)(st.), H–C(14)(st.)); 3.45 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 4.75 (br., OH–C(5′)); 5.24 (br., OH–C(3′)); 5.91 (*s*, OH–C(17)(st.)); 6.39, 8.10 (2 br. *s*, NH$_2$); 6.45 (br. *s*, H–C(4)(st.)); 6.54 (*m*, H–C(1′), H–C(2)(st.)); 7.05 (*d*, *J* = 8.4, H–C(1)(st.)); 8.27 (*s*, H–C(6)); 8.96 (*s*, OH–C(3)(st.)). Anal. calc. for C$_{30}$H$_{35}$N$_5$O$_5$ (545.64): C 66.04, H 6.47, N 12.84; found: C 66.20, H 6.46, N 12.89.

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3-(17β-hydroxy-3-oxo-19-norpregn-5(10)-en-20-yn-21-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**31**). According to *G.P. II* (*Method 1*), with norethynodrel (= 17β-hydroxy-19-norpregn-5(10)-en-20-yn-3-one; 1.6 g, 5.36 mmol) (4 h): **31** (145 mg, 50%). Colorless solid. TLC (*A*): R_f 0.38. UV (MeOH): 287 (11000), 281 (10600), 250 (11800). ^{1}H-NMR ((D$_6$)DMSO): 0.83 (*s*, Me); 1.30–2.84 (several *m*, H$_\alpha$–C(2′), H$_\beta$–C(2′), CH$_2$(1)(st.), CH$_2$(2)(st.), CH$_2$(4)(st.), CH$_2$(6)(st.), CH$_2$(7)(st.), CH$_2$(11)(st.),

CH_2(12)(st.), CH_2(15)(st.), CH_2(16)(st.), H–C(8)(st.), H–C(9)(st.), H–C(14)(st.)); 3.45 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.40 (*m*, H–C(3′)); 4.78 (br., OH–C(5′)); 5.26 (br., OH–C(3′)); 5.89 (*s*, OH–((17)(st.)); 6.45, 8.10 (2 br. *s*, NH_2); 6.52 (‘*t*’, *J* = 6.4, H–C(1′)); 8.26 (*s*, H–C(6)). Anal. calc. for $C_{30}H_{37}N_5O_5$ (547.65): C 65.80, H 6.81, N 12.79; found: C 65.40, H 6.42, N 12.99.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-3-[3-(trifluoroacetamido)prop-1-ynyl]-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**32**). According to *G.P. II* (*Method 1*), with 2,2,2-trifluoro-*N*-(prop-2-ynyl)acetamide [52][53] (1.6 g, 10.59 mmol) (4 h): **32** (104 mg, 49%). Colorless solid. M.p. 184–187° (dec.). TLC (*A*): R_f 0.30. UV (MeOH): 286 (11900), 249 (9900). ^{1}H-NMR ((D$_6$)DMSO): 2.26 (*m*, H_α–C(2′)); 2.78 (*m*, H_β–C(2′)); 3.46 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.43 (*m*, H–C(3′), CH_2NH); 4.73 (*t*, *J* = 5.7, OH–C(5′)); 5.26 (*d*, *J* = 4.5, OH–C(3′)); 6.56 (‘*t*’, *J* = 6.3, H–C(1′)); 6.70, 8.13 (2 br. *s*, NH_2); 8.26 (*s*, H–C(6)); 10.15 (*s*, NH). Anal. calc. for $C_{15}H_{15}F_3N_6O_4$ (400.32): C 45.01, H 3.78, N 20.99; found: C 45.10, H 3.82, N 21.07.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-3-[5-(trifluoroacetamido)pent-1-ynyl]-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**33**). According to *G.P. II* (*Method 1*), with 2,2,2-trifluoro-*N*-(pent-4-ynyl)acetamide [52][53] (1.9 g, 10.61 mmol) (4 h): **33** (107 mg, 47%). Colorless foam. TLC (*A*): R_f 0.35. UV (MeOH): 286 (10300), 249 (9800). ^{1}H-NMR ((D$_6$)DMSO): 1.84 (*quint.*, *J* = 6.9, $CH_2CH_2CH_2$); 2.26 (*m*, H_α–C(2′)); 2.59 (*t*, *J* = 7.0, $CH_2C{\equiv}C$); 2.77 (*m*, H_β–C(2′)); 3.36 (*m*, CH_2NH, superimposed by H_2O); 3.45 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 4.74 (*t*, *J* = 5.6, OH–C(5′)); 5.25 (*d*, *J* = 4.4, OH–C(3′)); 6.54 (‘*t*’, *J* = 6.3, H–C(1′)); 6.60, 7.98 (2 br. *s*, NH_2); 8.23 (*s*, H–C(6)); 9.50 (*s*, NH). Anal. calc. for $C_{17}H_{19}F_3N_6O_4$ (428.37): C 47.67, H 4.47, N 19.62; found: C 47.65, H 4.45, N 19.10.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-3-(5-aminopent-1-ynyl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**34**). Compound **33** (50 mg, 0.12 mmol) was stirred in a MeOH/aq. NH_3 soln. 1:4 (30 ml) for 5 h at r.t. After evaporation, the residue was purified by FC (column 10 × 2 cm, CH_2Cl_2/MeOH/NH_3 soln. 75:20:5): **34** (25 mg, 63%). Yellowish foam. TLC (CH_2Cl_2/MeOH/NH_3 soln. 65:30:5): R_f 0.36. UV (MeOH): 279 (8700), 249 (8100). ^{1}H-NMR ((D$_6$)DMSO): 1.87 (*quint.*, *J* = 6.9, $CH_2CH_2CH_2$); 2.25 (*m*, H_α–C(2′)); 2.69 (*t*, *J* = 7.0, $CH_2C{\equiv}C$); 2.77 (*m*, H_β–C(2′)); 2.91 (*t*, *J* = 7.3, CH_2NH_2); 3.45 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 4.76 (br., OH–C(5′)); 5.27 (br., OH–C(3′)); 6.53 (‘*t*’, *J* = 6.3, H–C(1′)); 6.65, 7.98 (2 br. *s*, NH_2); 8.23 (*s*, H–C(6)). FAB-MS (3-NOBA): 333.2 ($[M+H]^+$, $[C_{15}H_{20}N_6O_3+H]^+$; calc. 333.37).

*1-(2-Deoxy-α-*D-erythro-*pentofuranosyl)-3-(hex-1-ynyl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**45**). According to *G.P. II* (*Method 3*), with hex-1-yne (150 mg, 1.83 mmol) (4 h). FC (column 10 × 2 cm, 2–10% MeOH/CH_2Cl_2) and crystallization from MeCN afforded **45** (15 mg, 49%). Colorless crystals. M.p. 145–146° (dec.). TLC (*A*): R_f 0.52. UV (MeOH): 288 (11100), 282 (10700), 250 (10300). ^{1}H-NMR ((D$_6$)DMSO): 0.93 (*t*, *J* = 7.3, Me); 1.45 (*sext.*, *J* = 7.5, CH_2CH_3); 1.60 (*quint.*, *J* = 7.4, $CH_2CH_2CH_3$); 2.60 (*t*, *J* = 7.0, $CH_2C{\equiv}C$); 2.61 (*m*, H_α–C(2′)); 2.73 (*m*, H_β–C(2′)); 3.50 (*m*, 2 H–C(5′)); 3.93 (*m*, H–C(4′)); 4.15 (*m*, H–C(3′)); 4.71 (*t*, *J* = 5.6, OH–C(5′)); 5.48 (*d*, *J* = 6.9, OH–C(3′)); 6.47 (‘*t*’, *J* = 6.5, H–C(1′)); 6.80, 8.00 (2*s*, NH_2); 8.25 (*s*, H–C(6)). Anal. calc. for $C_{16}H_{21}N_5O_3$ (331.37): C 57.99, H 6.39, N 21.13; found: C 58.08, H 6.28, N 21.06.

*Glycosylation of 4-Methoxypyrazolo[3,4-*d*]pyrimidine* (**40**) *with 2-Deoxy-3,5-di-*O*-toluoyl-α-*D*-erythro-pentofuranosyl Chloride* (**36**). To a suspension of **40** [42] (1.5 g, 10 mmol) in dimethoxyethane (200 ml), KOH (1.32 g, 85% KOH, 20 mmol) and [18]crown-6 (100 mg, 0.38 mmol) were added. After stirring for 10 min, 2-deoxy-3,5-di-*O*-toluoyl-α-D-erythro-pentofuranosyl chloride (**36**) [41] (4.7 g, 12 mmol) was introduced, and stirring was continued for another 50 min. The insoluble material was filtered off, the solvent evaporated, and the residue subjected to FC (column 20 × 4 cm, 25–66%. AcOEt/petroleum ether) to furnish sequentially 1.6 g (32%) of *1-[2-deoxy-3,5-di-*O*-(p-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**41**) [42] as colorless crystals (from i-PrOH), 402 mg (8%) of *1-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-*D*-erythro-pentofuranosyl]-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**42**) [42] as a colorless solid, and 100 mg (2%) of *2-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-methoxy-2*H-*pyrazolo[3,4-*d*]pyrimidine* (**43**) [42] as colorless foam. Compounds **41**–**43** showed identical R_f values and UV and NMR data as those reported for authentic samples [42].

*Glycosylation of 3-Iodo-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**35**) with **36**. As described for **40** + **36**, with **35** [39] (1.0 g, 3.6 mmol), dimethoxyethane (150 ml), KOH (0.48 g, 85% KOH, 7.3 mmol), [18]crown-6 (20 mg), and **36** (1.66 g, 4.3 mmol): 656 mg (29%) of **37** and 136 mg (6%) of **38**, both after recrystallization from ^{i}PrOH, and 90 mg (4%) of **39**.

*Data of 1-[2-Deoxy-*3,5-*di-*O*-(*p*-toluoyl)-β-*D*-erythro-pentofuranosyl]-3-iodo-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**37**) [39]: Colorless crystals. M.p. 149–151°. TLC (*C*): R_f 0.70.

*Data of 1-[2-Deoxy-*3,5-*di-*O*-(*p*-toluoyl)-α-*D*-erythro-pentofuranosyl]-3-iodo-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**38**). Colorless crystals. M.p. 151–152° (i-PrOH; dec.). TLC (*C*): R_f 0.63. UV (MeOH): 272 (7800), 239 (3800). ^{1}H-NMR ((D$_6$)DMSO): 2.38 (*s*, Me); 2.41 (*s*, Me); 3.14 (*m*, H_β–C(2′), H_α–C(2′)); 4.13

(*s*, MeO); 4.49 (*m*, 2 H–C(5′)); 4.70 (*m*, H–C(4′)); 5.64 (*m*, H–C(3′)); 6.81 (*dd*, *J* = 7.7, 7.8, H–C(1′)); 7.36, 7.95 (4*d*, *J* = 7.9, 2 C_6H_4); 8.65 (*s*, H–C(6)).

Data of 2-[2-Deoxy-3,5-di-O-*(*p-*toluoyl)-β-*D-erythro-*pentofuranosyl]-3-iodo-4-methoxy-2*H-*pyrazolo[3,4-*d*]pyrimidine* (**39**) [39]: Colorless foam.

*1-(2-Deoxy-α-*D-erythro-*pentofuranosyl)-3-iodo-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**44**). Compound **38** (100 mg, 0.16 mmol) was stirred at 90° for 3 h with sat. (0°) NH_3/MeOH soln. (100 ml) in an autoclave. Then the soln. was evaporated and the residue subjected to FC (silica gel, column 8 × 2 cm, *A*). Crystallization from MeOH afforded **44** (30 mg, 50%). Colorless crystals. M.p. 191–193° (dec.). TLC (*A*): R_f 0.42. UV (MeOH): 285 (8000), 257 (6600), 241 (7300). ^{1}H-NMR ((D_6)DMSO): 2.68 (*m*, H_β–C(2′), H_α–C(2′)); 3.45 (*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.12 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.7, OH–C(5′)); 5.48 (*d*, *J* = 6.7, OH–C(3′)); 6.42 (*t*, *J* = 6.7, H–C(1′)); 6.80, 7.75 (2*s*, NH_2); 8.23 (*s*, H–C(6)). Anal. calc. for $C_{10}H_{12}IN_5O_3$ (377.14): C 31.85, H 3.21, N 18.57; found: C 32.25, H 3.12, N 18.23.

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