ARTICLE

OBC
www.rsc.org/obc

1,N^6-Etheno-7-deaza-2,8-diazaadenosine: syntheses, properties and conversion to 7-deaza-2,8-diazaadenosine†

Wenqing Lin,[a,b] Hong Li,[a,b] Xin Ming[a,b] and Frank Seela*[a,b]

[a] *Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, 49069, Osnabrück, Germany*

[b] *Center for Nanotechnology (CeNTech), Gievenbecker Weg 11, 48149, Münster, Germany. E-mail: Frank.Seela@uni-osnabrueck.de, Seela@uni-muenster.de; Fax: +49-541-9692370; Tel: +49-541-969-2791*

Received 16th December 2004, Accepted 10th March 2005
First published as an Advance Article on the web 30th March 2005

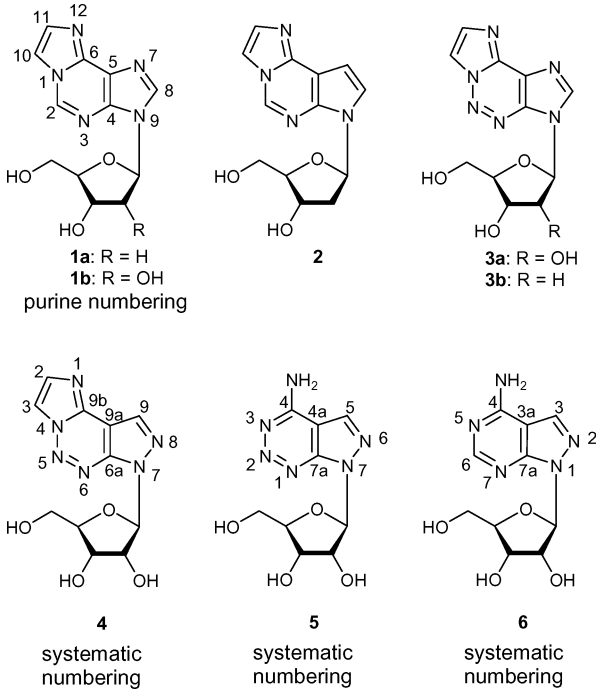
1,N^6-Etheno-7-deaza-2,8-diazaadenosine (**4**) was synthesized from 8-aza-7-deazaadenosine (**6**) in 64% overall yield. The starting material **6** was obtained by the direct glycosylation of 8-aza-7-deazaadenine (**7**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**8**) (NO_2 CH_3, $BF_3 \cdot Et_2O$; 77% yield). Compound **4** was transformed into 7-deaza-2,8-diazaadenosine (**5**). The fluorescence of compound **4** shows an emission maximum at 531 nm (phosphate buffer; pH 7.0), which is bathochromically shifted compared to 1,N^6-etheno-2-azaadenosine (**3a**) (495 nm). A conformational analysis was performed in the solid state and in solution.

Introduction

The etheno derivatives of adenosine and cytidine, as well as their 2′-deoxy congeners, are strongly fluorescent.[1] This behavior is different from the canonical nucleosides which do not show appreciable fluorescence in aqueous solution. The physical properties of etheno nucleosides[2] enable them to be used as structural and functional probes in DNA and RNA. Thus, they are applicable in real-time PCR and have the potential to be used for the detection of nucleic acids in various RNA and DNA formats. The etheno adducts of the 2′-deoxyadenosine and the 2′-deoxycytidine cause mutagenic lesions in DNA.[3] This refers to an unusual base pairing of 1,N^6-etheno-2′-deoxyadenosine (ε-2′-deoxyadenosine, **1a**) and 1,N^6-etheno-adenosine (ε-adenosine, **1b**) (Scheme 1) with guanosine or its 2′-deoxy derivative. Moreover, these nucleosides are rather unstable in acidic and alkaline solution thereby cause problems during oligonucleotide synthesis.[1b] This problem is circumvented by the use of 1,N^6-etheno-7-deaza-2′-deoxyadenosine (**2**) (purine numbering is used in the results and discussion sections, except otherwise stated) being stable under acidic and alkaline conditions due to the replacement of the purine by the pyrrolo[2,3-*d*]pyrimidine system.[4]

Among the various etheno nucleosides, 2-azapurine derivatives, such as 1,N^6-etheno-2-azaadenosine (2-aza-ε-adenosine, **3a**)[5a,b] as well as its 2′-deoxy derivative **3b**[5c] have been incorporated into RNA and DNA chemically or enzymatically.[1b,1c,6] From fluorescence studies performed on those nucleosides it became obvious that the fluorescence emission maximum of compound **3a** (495 nm) is bathochromically shifted compared to **1b** (415 nm)—both measured at pH 7.0 in aq. buffer solution.[7] Nevertheless, the quantum yield of the 2-aza nucleoside **3a** is low compared to that of **1b**.[7] This manuscript reports on the synthesis and properties of 1,N^6-etheno-7-deaza-2,8-diazaadenosine (**4**) being related to the previously published 7-deaza-2,8-diaza-2′-deoxyadenosine.[8] In contrast to compound **1a,b** and **3a,b**, compound **4** cannot form Watson–Crick or Hoogsteen base pairs. Thus, it can act as an universal nucleoside when incorporated in duplex RNA.

During our work, it became apparent that compound **4** can be converted efficiently to 7-deaza-2,8-diazaadenosine (**5**) which is otherwise difficult to synthesize.[9] Starting material for compounds **4** and **5** was 8-aza-7-deazaadenosine (**6**). As this nucleoside is not readily accessible,[10] a more efficient synthesis was developed. Apart from the synthetic studies, investigations on the fluorescence and the conformational properties of the nucleosides are performed.



Scheme 1 Structures of nucleosides **1**–**6**.

Results and discussion

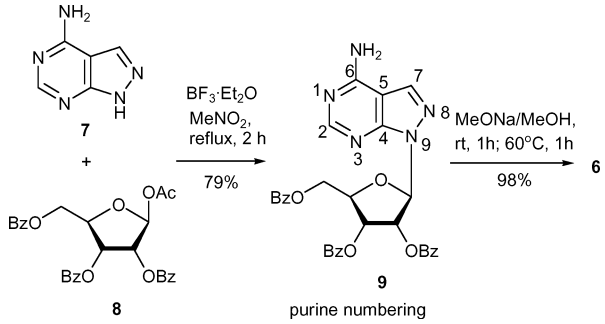
Syntheses of nucleosides

Compound **6** was the starting material for the etheno nucleoside **4** as well as for the 2-aza nucleoside **5**. The first chemical synthesis of **6** was performed by condensation of the chloromercury salt of N^6-benzoyl-8-aza-7-deazaadenine (4-benzamidopyrazolo[3,4-*d*]pyrimidine) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride in boiling toluene giving compound **6** in only 10–20% yield.[10a] Montgomery applied

† In memory of the late Professor John A. Montgomery.

DOI: 10.1039/b418849g

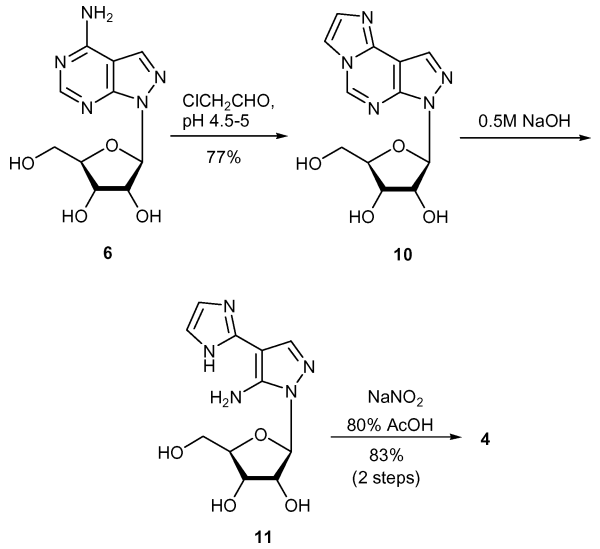
the acid-catalysed fusion procedure employing N^6-benzoyl-8-aza-7-deazaadenine and tetra-*O*-acetyl-*β*-D-ribofuranose in the melt to furnish **6** in 12.5% yield.[10b] Later, Townsend and Revankar synthesized **6** *via* the glycosylation of silylated 4-chloropyrazolo[3,4-*d*]pyrimidine with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in the presence of NaI followed by deprotection and ammonolysis in 30% yield.[10c] A more efficient procedure was reported by Korbukh *et al.*[10d] using the fusion of 4-methylthiopyrazolo[3,4-*d*]pyrimidine with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose at 180 °C (70% yield), which required the additional conversion of the 4-methylthio group into the 4-amino group. Herein, the direct high-temperature glycosylation of 8-aza-7-deazaadenine (**7**)[11] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**8**) in $MeNO_2$ with $BF_3{\cdot}Et_2O$ as catalyst is performed.[12] The glycosylation product, 2′,3′,5′-tribenzoylated 8-aza-7-deazaadenosine (**9**) is isolated in 79% yield. Deblocking with MeONa–MeOH furnished **6** almost quantitatively (Scheme 2). The ^{1}H NMR data of **6** were in accordance with those of Montgomery.[10b] An unambiguous assignment of the glycosylation position was performed on the basis of ^{13}C NMR chemical shifts. According to previous work on 8-aza-7-deazapurines,[13] the ^{13}C NMR signal of C7 is located around 132 ppm when the 8-aza-7-deazapurine is glycosylated at nitrogen-9, while on glycosylation at nitrogen-8, a 10 ppm shift of C-7 towards higher field (122 ppm), is observed.



Scheme 2 The route for the synthesis of nucleoside **6**.

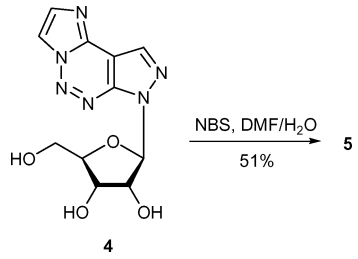
Treatment of compound **6** with 50% aqueous chloroacetaldehyde in 1 M aq. sodium acetate buffer at pH 4.5–5.0 (room temperature) gave 1,N^6-etheno-8-aza-7-deazaadenosine (**10**) in 77% yield.[14] Stirring of compound **10** with 0.5 M aqueous NaOH at ambient temperature overnight furnished the ring-opened compound **11**. It was cyclized afterwards by diazotization with $NaNO_2$ in 80% aqueous AcOH to give 1,N^6-etheno-7-deaza-2,8-diazaadenosine (**4**) in 83% yield (based on **10**) (Scheme 3).

With compound **4** in hand, we became interested to remove the 1,N^6-etheno group selectively, thereby transforming compound **4** into the 2-aza nucleoside **5**. Although compound **5** has been already synthesized from **6** *via* its N-oxide in a 5 step synthesis (9.8% overall yield),[9] little information was given regarding the analytical data. Treatment of **4** with NBS in sodium acetate buffer (pH 4–4.5) formed only trace amounts of **5** as monitored by TLC.[15] As Yamaji *et al.* reported on a



Scheme 3 The synthetic route for the formation of fluorescent nucleoside **4**.

yield increase by pH change[14] we studied this in detail. However, the reaction yield was also not improved when the pH was altered. An increase of the NBS amount or its replacement by ammonium persulfate (phosphonate buffer, pH 7.2) was also unsuccessful.[16] Finally, we observed that the treatment of compound **4** with NBS in DMF–H_2O (1 : 1)[7] furnished the nucleoside **5** in 51% yield (Scheme 4).



Scheme 4 Removal of the etheno residue.

The structures of the nucleosides **4,5** and the intermediates were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy (see Table 1 and Experimental section). The ^{13}C NMR chemical shifts were assigned with the help of gated-decoupled spectra (Table 2). As shown in Table 1, the replacement of C2 by N2 drives the chemical shift of C6 of both etheno and non etheno nucleosides to higher field by 8.5 ppm (**6**: 158.0 ppm, **5**: 149.5 ppm) and 8.3 ppm (**10**: 139.3 ppm, **4**: 131.0 ppm) which is in accordance with findings on related molecules.[5c,8] Smaller effects are observed on the chemical shift of carbon-4. The introduction of nitrogen-2 affects also the chemical shift of C5 of the non etheno nucleosides (**6**: 100.4 ppm, **5**: 95.9 ppm).

Table 1 ^{13}C NMR data of nucleosides[a]

Compound	C(2)[b] C(6)[c]	C(4)[b] C(7a)[c]	C(5)[b] C(3a)[c]	C(6)[b] C(4)[c]	C(7)[b] C(3)[c]	C(10)[b,e]	C(11)[b,e]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
4	—	143.1	104.3	131.0	131.6	132.9	115.5	89.8	73.7	70.7	85.7	61.9
5	—	153.1	95.9	149.5	132.9	—	—	89.2	73.2	70.7	85.4	62.1
6	156.0	153.9	100.4	158.0	133.2	—	—	88.4	73.0	70.8	85.0	62.3
9[d]	156.5	154.2	100.3	158.0	133.4	—	—	85.9	74.0	71.0	78.6	63.2
10	140.3	145.4	103.2	139.3	131.9	132.3	112.8	88.6	73.4	70.8	85.3	62.2
11	—	146.1	94.5	142.3	135.6	126.9	114.1	89.1	73.1	70.8	84.7	62.3

[a] Measured in (D_6) DMSO at 303 K. [b] Purine numbering. [c] Systematic numbering. [d] 165.3, 164.6, 164.5 for three carbonyl in benzoyl group; 134.5, 133.9, 133.8, 129.3, 129.2, 128.8, 128.7, 128.6, 128.5, 128.3 for three phenyl in benzoyl group. [e] Tentative.

Table 2 J(H,C) coupling constants (Hz) of nucleosides[a]

	4	5	6	9	10
^1J(C(2), H–C(2))[b]	—	—	198.8	199.3	215.6
^2J(C(5), H–C(7))[b]	10.9	—	10.8	12.0	10.3
^1J(C(7), H–C(7))[b]	199.5	196.6	193.3	192.3	194.8
^1J(C(10), H–C(10))[b,c]	193.0	—	—	—	190.5
^2J(C(10), H–C(11))[b,c]	9.4	—	—	—	10.3
^1J(C(11), H–C(11))[b,c]	200.2	—	—	—	197.8
^2J(C(11), H–C(10))[b,c]	16.1	—	—	—	16.4
^1J(C(1′), H–C(1′))	166.3	163.7	163.1	168.9	164.4
^1J(C(2′), H–C(2′))	149.6	148.6	145.9	163.5	148.6
^1J(C(3′), H–C(3′))	150.1	147.9	143.8	157.8	146.6
^1J(C(4′), H–C(4′))	147.8	148.2	146.8	149.7	147.6
^1J(C(5′), H–C(5′))	140.3	139.6	139.3	150.0	140.2

[a] Measured in (D_6) DMSO at 303 K. [b] Purine numbering. [c] Tentative.

Conformation of compounds 4 and 5 in solid and in solution‡

The conformation of compounds **4** and **5** was determined in the solid state and in solution. The crystal structures of both compounds **4**[17] and **5** are shown in Fig. 2. In the solid state, both compounds **4** and **5** adopt the high-anti conformation with torsion angles χ being at 83.0(3) and −103.5(3)°, respectively. A similar high-anti conformation was also observed for compound **6** (χ −77.6°).[18] The ribofuranose moieties of compounds **4**–**6** adopts the C2′-endo conformation (S-type sugar). Usually the exocyclic C4′-C5′ bond of several purine and 8-azapurine nucleosides prefers to adopt the *trans* conformation, *e.g.* in compound **6**, which has been correlated with the C2′-endo ribose pucker and the high-anti glycosylic bond conformation.[18] However, in the case of the N2-modified nucleosides, the conformation of this side chain –CH_2OH group changed to the *syn* (*gauche–gauche*, + sc) conformation (compounds **4,5** and 2-azaadenosine). The etheno group does not significantly alter the conformational properties of the nucleosides.

In solution, the conformational analysis of the sugar moiety of compounds **4**–**6** was performed with the aid of the PSEUROT program (version 6.3).[19] A minimization of the differences between the experimental and calculated couplings is accomplished by a nonlinear Newton–Raphson minimization; the quality of the fit is expressed by the root-mean-square (rms) difference. This procedure presupposes the existence of a two state N/S equilibrium. The input contained the following coupling constants: J(H1′,H2′), J(H2′,H3′), J(H3′,H4′), J(H4′,H5′), J(H4′,H5″). During the iterations either the puckering parameters (P, Ψ_{max}) of the minor conformer (N) or the puckering amplitudes of both conformers were constrained. The coupling constants and the pseudo rotational parameters are shown in Table 3.

Compound **5** shows a nearly equal population of N- and S-conformers (51 *vs.* 49%), while compound **4** and **6** are biased toward S (63 and 64% S) (Table 3). The conformation of the exocyclic C(4′)–C(5′) bond of compounds **4**–**6** were calculated based on J(4′,5′) and J(4′,5″) according to Westhof *et al.*[20]

‡ CCDC reference numbers 262698. See http://www.rsc.org/suppdata/ob/b4/b418849g/ for crystallographic data in CIF format.

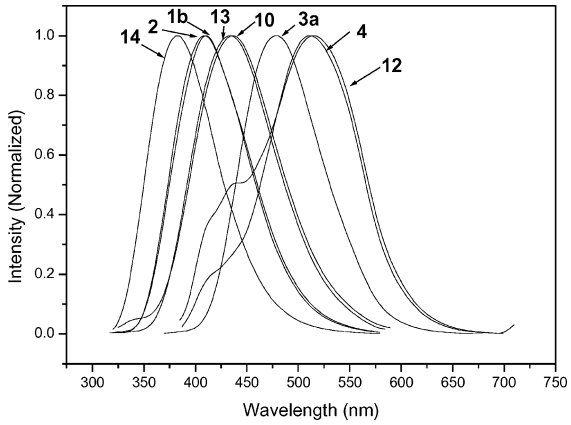


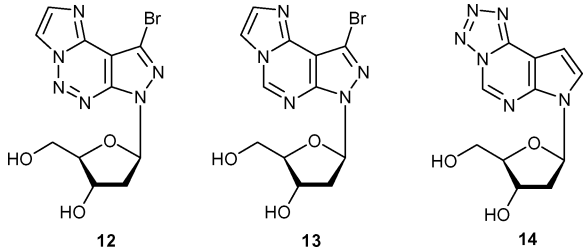
Fig. 1 The fluorescence emission spectra of compound **4** and related nucleosides measured in MeOH at room temperature. Intensities are normalized.

Both compounds, **4** and **5**, measured in D_2O show the preferred *gauche–gauche* conformation of 51 and 46% population, respectively, similar to that in the crystalline state. In solution, compound **6** exhibits a *gauche–gauche* conformer population of 51%, which is different from that in the crystal.

UV and fluorescence properties of the etheno nucleoside 4

As observed for the ^{13}C NMR spectra, the replacement of carbon-2 by nitrogen-2 also affects the UV-spectra and the pK_a values. The UV maxima of compounds with nitrogen at position 2 show a strong red shift compared to those with a carbon at that position: **6** (λ_{max} = 260 nm) to **5** (λ_{max} = 309 nm); **10** (λ_{max} = 278 nm) to **4** (λ_{max} = 295 nm), **1a** (λ_{max} = 275 nm) to **3a** (λ_{max} = 282 nm), and 2′-deoxyadenosine (λ_{max} = 260 nm) to 2-aza-2′-deoxyadenosine (λ_{max} = 293 nm). The presence of nitrogen-2 also lowers the pK_a values of protonation, which results from the higher electronegativity of nitrogen compared to carbon: **6** (pK_a = 4.0) to **5** (pK_a = 1.7), **10** (pK_a = 3.7) to **4** (pK_a = 2.2) and 2′-deoxyadenosine (pK_a = 3.8) to 2-aza-2′-deoxyadenosine (pK_a = 1.9).

The fluorescence emission spectra of compound **4** and the related nucleosides (Scheme 5) are shown in Fig. 2, and the corresponding data are summarized in Table 4. Compound **4** shows an emission maximum at 511 nm in MeOH (Fig. 2 and Table 4) which is further shifted to 531 nm in aq. phosphate buffer (pH 7.0). This relative long wavelengths emission is rather unusual for the etheno nucleosides. The data of this nucleoside



Scheme 5 Structures of the fluorescent nucleosides **12**–**14**.

Table 3 ^{1}H NMR coupling constants (^{3}J(H, H)) and conformation of the nucleosides **4**–**6**[a]

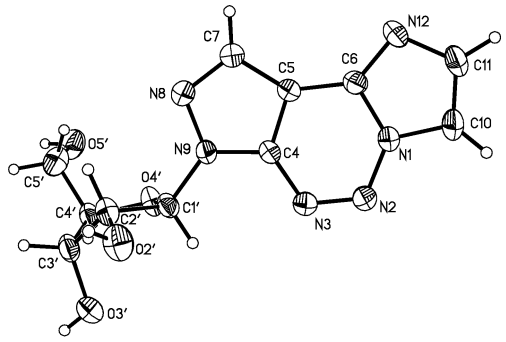
	^3J(H,H)/Hz					Pseudorotational parameters					Conformations				
	J(1′,2′)	J(2′,3′)	J(3′,4′)	J(4′,5′)	J(4′,5″)	P_N	$\psi_{m(N)}$	P_S	$\psi_{m(S)}$	Rms	%N	%S	γ^{g+}	γ^{t}	γ^{g-}
4	4.34	4.87	5.05	3.33	5.53	−1.6	32.0	193.7	35.0	0.256	36	64	0.46	0.14	0.39
5	4.69	4.88	4.57	3.20	5.25	10.4	32.0	163.3	35.0	0.033	51	49	0.51	0.13	0.36
6	5.20	4.79	4.50	3.38	5.08	−1.2	32	170.6	35.0	0.193	37	63	0.51	0.15	0.34

[a] Measured in D_2O at 303 K.

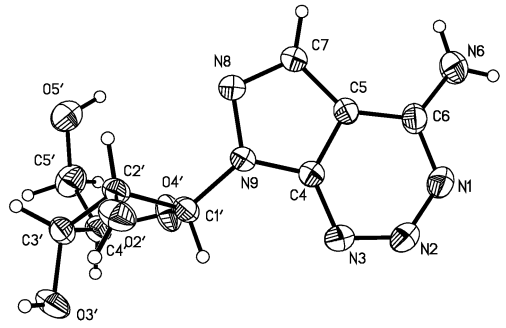
Table 4 Fluorescence data of compound **4** and related nucleosides measured in MeOH at room temperature[a]

Compound	Excitation max/nm	Emission max/nm	Quantum yield[b]	Compound	Excitation max/nm	Emission max/nm	Quantum yield[b]
4	367	511	0.01	**3a**	349	481	0.05
10	300	441	0.14	**1b**	298	410	0.2
12	365	514	0.003	**2**	297	409	0.14
13	303	438	0.04	**14**	301	381	0.08

[a] The concentration of the nucleosides were in about 1×10^{-5} M. [b] Fluorescence quantum yield were determined using quinine sulfate dihydrate in 0.1 M $HClO_4$ ($\phi_s = 0.59$)[23] according to Onidas *et al.*[24]



4



5

Fig. 2 Single crystal X-ray structures of compounds **4**[17a] and **5**.[17b]

are compared with that of the related etheno nucleosides in Fig. 1 and Table 3. From the comparison of the fluorescence data, one can conclude that, (i) the replacement of C(2)-H by nitrogen-2 shifts the emission maxima bathochromically from 441 nm for **10** to 511 nm for **4**, from 438 nm for **13**[21] to 514 nm for **12**[21] and, 410 nm for **1b** to 481 nm for **3a**, (ii) the shift of nitrogen-7 in the imidazole to the position-8 in the pyrazole moiety also causes a bathochromic shift, albeit to a smaller degree than that of nitrogen-2 (see Table 4), (iii) the displacement of nitrogen-7 within the imidazole moiety by C(7)-H in the pyrrole system does not change the emission maxima as seen for compound **1b** and **2**, (iv) the replacement of the HC=CH moiety of the etheno group by an N=N residues causes an hypsochromic shift (compound **2** and **14**[22]), (v) the bromination has a small effect on the emission maxima (compound **10** and **13**, compound **4** and **12**), (vi) both the presence of a nitrogen in the 2-position and the bromo sustituent at position-7 lower the quantum yield strongly.

Conclusions

An efficient synthesis of 1,N^6-etheno-7-deaza-2,8-diazaadenosine (**4**) is described. The synthesis of 7-deaza-2,8-diazaadenosine (**5**) and the precursor molecule 8-aza-7-deazaadenosine (**6**) were significantly improved. Both compounds **4** and **5** show a high-anti conformation in the crystalline state, with the ribose in the unusual S-conformation. In solution, the population of the S-conformers population is 63% for **4** and 49% for **5**. Compound **4** possesses unique fluorescent properties with regard to the emission at 511 nm (MeOH) and 531 nm (sodium phosphate buffer; pH 7.0). This relatively long wavelength emission suggests that compound **4** will broaden the application of etheno nucleosides to be used as a fluorescent probes. As compound **4** is not expected to form base pairs with canonical DNA constituents it can be considered as an universal nucleoside.

Experimental

General

Solvents: technical grade, distilled before use. Flash chromatography (FC): 0.4 bar, silica gel 60 H (VWR, Darmstadt, Germany). TLC: Aluminium sheet, silica gel 60 F_{254} (0.2 mm, VWR, Germany). UV spectra were recorded on a U3200 spectrophotometer (Hitachi, Japan). NMR Spectra were measured on an Avance-DPX-250 spectrometer or AMX-500 spectrometer (Bruker, Rheinstetten, Germany), at 250.13 MHz and 500 MHz for ^{1}H and 62.90 and 125.13 MHz for ^{13}C, δ values are in ppm relative to internal $SiMe_4$ (^{1}H, ^{13}C). Fluorescence spectra were determined on Fluorescence Spectrophotometer F-4500 (Hitachi, Japan). Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). Chemicals were commercial products of ACROS, Fluka or Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany).

4-Amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (9). 4-Aminopyrazolo[3,4-*d*]pyrimidine **7**[11] (1.5 g, 11.1 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose **8** (8.1 g, 16.1 mmol) were suspended in $MeNO_2$ (150 mL). The mixture was heated under reflux. $BF_3 \cdot Et_2O$ (2 mL) was added in one portion resulting in a clear solution. After refluxing for 2 h, the reaction mixture was cooled to room temperature and evaporated *in vacuo*. The residue was applied to flash chromatography (FC) (silica gel, EtOAc–petroleum ether, 2 : 1) to give **9** (5.1 g, 79%) as a colourless foam (found: C, 64.23; H, 4.40; N, 12.03%. $C_{31}H_{25}N_5O_7$ requires C, 64.24; H, 4.35; N, 12.08%); TLC (silica gel, CH_2Cl_2–MeOH, 20 : 1): R_f 0.15; δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 4.51–4.67 (2H, m, 5′-H), 4.87 (1H, m, 4′-H), 6.20–6.31 (2H, m, 3′-H, 2′-H), 6.70 (1H, d, *J* 2.13, 1′-H), 7.42–8.02 (17H, m, NH_2, 3 × Ph), 8.22, 8.27 (2H, 2s, 3-H, 6-H).

4-Amino-1-(*β*-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (6). To a solution of compound **9** (5.1 g, 8.8 mmol) in MeOH (250 mL), MeONa (0.6 g, 11.1 mmol) was added until the pH reached 11.0. The mixture was stirred at room temperature for 1 h, and then heated at 60 °C for an additional 1 h. The reaction volume was reduced to *ca.* 150 mL *in vacuo*, and THF (100 mL) was added. The solution was stored in a refrigerator for 1 h. The precipitate was filtered off and washed with MeOH–THF (1 : 1.5, 3 × 5 mL). The solid material was dried *in vacuo* at 70 °C to give **6** (1.5 g) as colourless crystals; mp 250–252 °C (lit[10c]: 255 °C). The mother liquid was evaporated and purified by FC (silica gel, CH_2Cl_2–MeOH, 6 : 1→4 : 1) to give another 0.81 g

of **6**. In total, 2.31 g (98%) of **6** was obtained; TLC (silica gel, CH_2Cl_2–MeOH, 4 : 1): R_f 0.38; δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.44, 3.57 (2H, 2m, 5′-H), 3.88 (1H, m, 4′-H), 4.21 (1H, m, 3′-H), 4.58 (1H, m, 2′-H), 4.89 (1H, t, *J* 6.0, 5′-OH), 5.14 (1H, d, *J* 5.5, 3′-OH), 5.36 (1H, d, *J* 5.9, 2′-OH), 6.08 (1H, d, *J* 4.7, 1′-H), 7.77 (2H, bs, NH_2), 8.16, 8.19 (2H, 2s, 3-H, 6-H).

7-(β-D-Ribofuranosyl)-imidazo[1,2-*c*]-7*H*-pyrazolo[4,3-*e*]pyrimidine (10). Compound **6** (0.76 g, 2.8 mmol) was dissolved in aqueous AcONa (1 M, pH 4.5–5.0, 43 mL) at 40–50 °C. After addition of chloroacetaldehyde (50% aqueous solution, 9 mL), the solution was stirred at room temperature for 24 h. The reaction mixture was evaporated, and the residue was applied to FC (silica gel, CH_2Cl_2–MeOH, 6 : 1) to give **10** (0.64 g, 77%) as colourless needles; mp 211–213 °C from methanol (lit[14]: 179–181 °C); TLC (silica gel, CH_2Cl_2–MeOH, 4 : 1): R_f 0.74; δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.45, 3.59 (2H, 2m, 5′-H), 3.94 (1H, m, 4′-H), 4.26 (1H, m, 3′-H), 4.64 (1H, m, 2′-H), 4.78(1H, s, 5′-OH), 5.21 (1H, m, 3′-OH), 5.47 (1H, m, 2′-OH), 6.26 (1H, s, H-1′), 7.53 (1H, s, 2-H (tentative)), 8.07(1H, s, 3-H (tentative)), 8.43 (1H, s, 9-H), 9.33 (1H, s, 2-H).

5-Amino-1-(β-D-ribofuranosyl)-4-(imidazol-2-yl)pyrazole (11). Compound **10** (0.79 g, 2.7 mmol) was stirred in aqueous NaOH (0.5 M, 19 mL) overnight at room temperature. The solution was neutralized with aq. HCl (2 M), and concentrated *in vacuo* to afford **11** as a syrup. This was applied directly to the next reaction step. For analytical purposes, the syrup was purified by FC (silica gel, CH_2Cl_2–MeOH, 6 : 1) to give a yellowish foam (Found: C, 46.85; H, 5.42; N, 24.80%. $C_{11}H_{15}N_5O_4$ requires C, 46.97; H, 5.38; N, 24.90%); TLC (silica gel, CH_2Cl_2–MeOH, 6 : 1): R_f 0.41; λ_{max}(MeOH)/nm 319 (ε/dm^3 mol^{-1} cm^{-1} 6 900) and 256 (5 700); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.42, 3.55 (2H, 2m, 5′-H), 3.86 (1H, m, 4′-H), 4.15 (1H, t, *J* 4.81, 3′-H), 4.47 (1H, t, *J* 4.41, 2′-H), 4.94–5.23 (3H, 3br, 2′-OH), 3′-OH, 5′-OH), 5.67 (1H, d, *J* 2.01, 1′-H), 6.39 (2H, s, 3′-H, 4′-H in imidazole), 6.91 (2H, br, NH_2), 7.65 (1H, s, 3-H), 11.92 (1H, s, NH).

7-(β-D-Ribofuranosyl)-imidazo[1,2-*c*]-7*H*-pyrazolo[4,3-*e*]-[1,2,3]triazine (4). Compound **11** (prepared from **10** (0.79 g, 2.7 mmol)) was dissolved in 80% aqueous HOAc (30 mL) and treated with $NaNO_2$ (0.19 g, 2.8 mmol) for 0.5 h at room temperature. The reaction mixture was evaporated. The residue was applied to FC (silica gel, CH_2Cl_2–MeOH, 15 : 1) to give **4** (0.66 g, 83% based on **10**) as yellow needles from methanol (Found: C, 45.25; H, 4.04; N, 28.56%. $C_{11}H_{12}N_6O_4$ requires C, 45.21; H, 4.14; N, 28.76%); mp 209–210 °C; TLC (silica gel, CH_2Cl_2–MeOH, 15 : 1): R_f 0.26; λ_{max}(MeOH)/nm 294 (ε/dm^3 mol^{-1} cm^{-1} 5 300) and 232 (34 300); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.48, 3.62 (2H, 2m, 5′-H), 4.01 (1H, m, 4′-H), 4.31 (1H, m, 3′-H), 4.75 (2H, m, 2′-H, 5′-OH), 5.33 (1H, d, *J* 5.7, 3′-OH), 5.62 (1H, d, *J* 5.78, 2′-OH), 6.53 (1H, d, *J* 4.36, 1′-H), 7.82 (1H, s, 3-H (tentative)), 8.76 (1H, s, 9-H), 8.80 (1H, d, *J* 1.57, 2-H (tentative)).

4-Amino-7-(β-D-ribofuranosyl)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (7-deaza-2,8-diazaadenosine) (5). To a solution of compound **4** (200 mg, 0.7 mmol) in DMF (20 mL)–H_2O (20 mL) was added *N*-bromosuccinimide (1.3 g, 7.3 mmol). The mixture was stirred at room temperature overnight, diluted with H_2O (50 mL) and purified by Dowex 1 × 2(OH^-) to give 0.13 g of a yellowish solid, which was crystallized from H_2O to yield **5** (93 mg, 51%) as colourless crystals; mp 208–209 °C (lit[9]: 207–208 °C); TLC (silica gel, CH_2Cl_2–MeOH, 4 : 1): R_f 0.43; λ_{max}(MeOH)/nm 309 (ε/dm^3 mol^{-1} cm^{-1} 9 500) and 238 (6 000); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.44, 3.57 (2H, 2m, 5′-H), 3.96 (1H, m, 4′-H), 4.25(1H, m, 3′-H), 4.67(1H, m, 2′-H), 4.82 (s1H, 5′-OH), 5.23 (1H, d, *J* 4.8, 3′-OH), 5.48 (1H, d, *J* 5.5, 2′-OH), 6.30 (1H, d, *J* 3.8, 1′-H), 8.31 (3H, s, NH_2 and 5-H).

Acknowledgements

We are grateful to Dr Helmut Rosemeyer and Dr Yang He for the measurement of NMR spectra. Financial support by the European Community (grant no. QLRT-2001-00506, "Flavitherapeutics") is gratefully acknowledged.

References

1 (*a*) J. A. Secrist, III, J. R. Barrio, N. J. Leonard and G. Weber, *Biochemistry*, 1972, **11**, 3499–3506; (*b*) S. C. Srivastava, S. K. Raza and R. Misra, *Nucleic Acids Res.*, 1994, **22**, 1296–1304; (*c*) W. Zhang, R. Rieger, C. Iden and F. Johnson, *Chem. Res. Toxicol.*, 1995, **8**, 148–156.
2 G. A. Leonard, K. E. McAuley-Hecht, N. J. Gibson, T. Brown, W. P. Watson and W. N. Hunter, *Biochemistry*, 1994, **33**, 4755–4761.
3 A. K. Basu, M. L. Wood, L. J. Niedernhofer, L. A. Ramos and J. M. Essigmann, *Biochemistry*, 1993, **32**, 12793–12801.
4 F. Seela, H. Rosemeyer, E. Schweinberger and E.-M. Becker, 2005, in preparation.
5 (*a*) K.-F. Yip and K.-C. Tsou, *Tetrahedron Lett.*, 1973, **33**, 3087–3090; (*b*) K.-C. Tsou, K.-F. Yip, E. E. Miller and K. W. Lo, *Nucleic Acids Res.*, 1974, **1**, 1800; (*c*) T. Sugiyama, E. Schweinberger, Z. Kazimierczuk, N. Ramzaeva, H. Rosemeyer and F. Seela, *Chem. Eur. J.*, 2000, **6**, 369–378.
6 H. Paulsen and W. Wintermeyer, *Eur. J. Biochem.*, 1984, **138**, 117–123.
7 K.-F. Yip and K.-C. Tsou, *J. Org. Chem.*, 1975, **40**, 1066–1070.
8 F. Seela, M. Lindner, V. Glaçon and W. Lin, *J. Org. Chem.*, 2004, **69**, 4695–4700.
9 J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, 1972, **15**, 182–187.
10 (*a*) J. Davoll and K. A. Kerridge, *J. Chem. Soc.*, 1961, 2589–2591; (*b*) J. A. Montgomery, S. J. Clayton and W. E. Fitzgibbon, Jr., *J. Heterocycl. Chem.*, 1964, **1**, 215–216; (*c*) G. R. Revankar and L. B. Townsend, *J. Chem. Soc., Perkin Trans. I*, 1971, 2440–2442; (*d*) I. A. Korbukh, N. G. Yakunina and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1980, **6**, 1632–1638.
11 R. K. Robins, *J. Am. Chem. Soc.*, 1956, **78**, 784–790.
12 (*a*) G. A. Bhat, J.-L. G. Montero, R. P. Panzica, L. L. Wotring and L. B. Townsend, *J. Med. Chem.*, 1981, **24**, 1165–1172; (*b*) H. B. Cottam, G. R. Revankar and R. K. Robins, *Nucleic Acids Res.*, 1983, **11**, 871–882; (*c*) J. D. Anderson, H. B. Cottam, S. B. Larson, L. D. Nord, G. R. Revankar and R. K. Robins, *J. Heterocycl. Chem.*, 1990, **27**, 439–453; (*d*) J. D. Anderson, N. K. Dalley, G. R. Revankar and R. K. Robins, *J. Heterocycl. Chem.*, 1986, **23**, 1869–1878; (*e*) B. G. Ugarkar, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, 1984, **27**, 1026–1030; (*f*) H. B. Cottam, C. R. Petrie, P. A. McKernan, R. J. Goebel, N. K. Dalley, R. B. Davidson, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, 1984, **27**, 1119–1127.
13 F. Seela and H. Steker, *Helv. Chim. Acta*, 1985, **68**, 563–570.
14 G. A. Bhat and L. B. Townsend, *J. Chem. Soc., Perkin Trans. I*, 1981, 2387–2392.
15 (*a*) N. Yamaji and M. Kato, *Chem. Lett.*, 1975, 311–314; (*b*) N. Yamaji, K. Suda, Y. Onoue and M. Kato, *Chem. Pharm. Bull.*, 1977, **25**, 3239–3246.
16 M. Sako, T. Hayashi, K. Hirota and Y. Maki, *Chem. Pharm. Bull.*, 1992, **40**, 1656–1658.
17 (*a*) W. Lin, F. Seela, H. Eickmeier and H. Reuter, *Acta Crystallogr., Sect. C*, 2004, **60**, 566–568; (*b*) W. Lin, X. Ming, H. Eickmeier and F. Seela, *Acta Crystallogr.*, 2005, in preparation.
18 S. Sprang, R. Scheller, D. Rohrer and M. Sundaralingam, *J. Am. Chem. Soc.*, 1978, **100**, 2867–2872.
19 L. van Wijk, C. A. G. Haasnoot, F. A. A. M. de Leeuw, B. D. Huckriede, A. J. A. Westra Hoekzema and C. Altona, *PSEUROT6.3, Leiden Institute of Chemistry*, Leiden University, The Netherlands, 1999.
20 E. Westhof, O. Röder, I. Croneiss and H.-D. Lüdemann, *Z. Naturforsch., C: Biosci.*, 1975, **30**, 131–140.
21 W. Lin and F. Seela, 2005 submitted.
22 M. Zulauf, PhD Thesis, University of Osnabrück, 1999.
23 R. A. Verapoldi and K. D. Mielenz, *A fluorescence standard reference material: quinine sulfate dihydrate*, NBS special publication SP 260–64, National Bereau of Standards, Washington DC, 1980, p. 139.
24 D. Onidas, D. Markovitsi, S. Marguet, A. Sharonov and T. Gustavsson, *J. Phys. Chem. B*, 2002, **106**, 11367–11374.