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New nucleoside analogs from 2-amino-9-(β-D-ribofuranosyl)purine

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Four novel derivatives of 2-amino-9-(β-D-ribofuranosyl)purine (**1**) were synthesised and fully characterised. When **1** was reacted with chloroacetaldehyde (**a**), 2-chloropropanal (**b**), bromomalonaldehyde (**c**) and a mixture of chloroacetaldehyde + malonaldehyde (**d**), 3-(β-D-ribofuranosyl)-imidazo-[1,2a]purine (**2**), 3-(β-D-ribofuranosyl)-5-methylimidazo-[1,2a]purine (**3**), 3-(β-D-ribofuranosyl)-5-formylimidazo-[1,2a]purine (**4**) and 9-(β-D-ribofuranosyl)-2-(3,5-diformyl-4-methyl-1,4-dihydro-1-pyridyl)purine (**5**) were formed, respectively. The products were isolated, purified by chromatography and characterised by MS, complete NMR assignment as well as fluorescence and UV spectroscopy. The yields of these reactions were moderate (14–20%). The fluorescence properties differed from those of the starting compound and the quantum yields were considerably lower.

Introduction

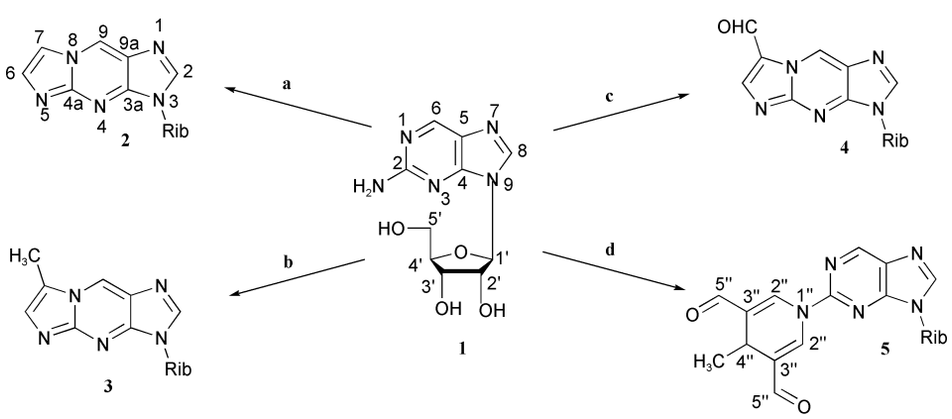
Haloacetaldehydes have been shown to react with nucleic acid components yielding etheno derivatives of adenosine, guanosine and cytidine.[1] The 1,N^6-ethenodeoxyadenosine derivatives, due to their fluorescence, are useful compounds for *e.g.* the studies of mutagenesis[2] and enzymology.[3] Fluorescence in general is an extremely useful tool for the investigation of biological material. The incorporation of fluorescent nucleosides into oligonucleotides will greatly facilitate studies of the structure–function of various RNAs, protein–RNA structures, and DNA-RNA based diagnostic applications.[4] That is why a large variety of fluorescent molecules have been incorporated into DNA/RNA and proteins *e.g.* 3-methyl-isoxanthopterin and 2-aminopurine, 1,N^6-ethenoadenosine.[1f,5] Fluorescent dihydropyridine derivatives of natural nucleosides and proteins have also been previously reported.[6]

Our research in the field of nucleoside chemistry originally aimed at synthesising modified fluorescent RNA bases.[7] However, our present investigations are also directed toward the preparation of RNA base analogues, which might have other interesting applications than only those associated with their fluorescence properties. To our knowledge 2-amino-9-(β-D-ribofuranosyl)purine (**1**) has not been used to produce different etheno or dihydropyridine derivatives and it was expected that the reactivity of the unnatural nucleobase would be higher than that of natural nucleobases, which are generally classified as quite unreactive. The compound **1** was prepared in two steps starting from guanosine.[8] Other methods are also available.[9]

In the current work, we report on the synthesis of four novel derivatives of 2-amino-9-(β-D-ribofuranosyl)purine (**1**). They are formed in the reaction of compound **1** with chloroacetaldehyde (**a**), 2-chloropropanal (**b**), bromomalonaldehyde (**c**) and chloroacetaldehyde + malonaldehyde (**d**), respectively (Scheme 1). We present in this contribution, the details of the synthetic procedures, the complete structural analysis, which includes MS, NMR analyses (^{1}H-, ^{13}C-, ^{15}N- chemical shifts and coupling constants), as well as fluorescence and UV spectral properties. The fluorescence intensities and lifetimes compared to those of **1** are discussed.

Results and discussion

The products (**2**–**5**) were synthesised using the reagents shown in Scheme 1. The reactions of 2-amino-9-(β-D-ribofuranosyl)purine (**1**) with the aldehydes **a–d** were carried out in aqueous solution at pH 4.5. The progress of the reactions was monitored by HPLC analysis on a reversed phase (C 18) column and the molecular weights of formed products were



a. $ClCH_2CHO$, **b.** $CH_3CHClCHO$, **c.** $BrCH(CHO)_2$, **d.** $CH_3CHO + CH_2(CHO)_2$

Scheme 1

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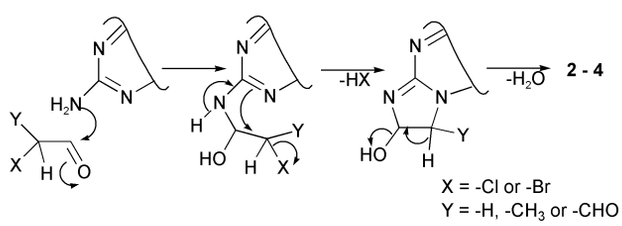
obtained from LC-MS analyses of the reaction mixture. The products were isolated by preparative reversed phase chromatography and were fully characterised by ^{1}H-, ^{13}C- and ^{15}N NMR spectroscopy, electrospray mass spectrometry, HRMS, fluorescence spectroscopy and UV absorption.

The yields of **2**, **3**, **4** and **5** were 14%, 17%, 20% and 16% respectively. The NMR spectroscopic data, the mass spectrometric and UV properties are given in the Experimental section. The fluorescence properties are presented in Tables 1 and 2 and Fig. 2.

Mechanisms

The formation of ethenoadenosine from adenosine on treatment with chloroacetaldehyde has earlier been described in the literature.[10] The formation of compounds **2**–**4** from reactions **a–c** can be expected to follow a similar mechanism. That is, the reaction is initiated by attack of the exocyclic amino group of the nucleobase on the carbonyl carbon of the different aldehydes used in **a–c**. Subsequently, the halogen atom is displaced by intramolecular nucleophilic attack of the ring nitrogen. Finally, the products are obtained through dehydration of the cyclic intermediates (Scheme 2). LC-MS analysis of the reaction mixture of aldehyde **c** and starting material **1**, showed the presence of a molecule with mass *m/z* 338 and according to ^{1}H NMR the compound was the carbinolamine intermediate (Fig. 1), which supports the described mechanism.



Scheme 2

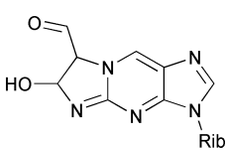
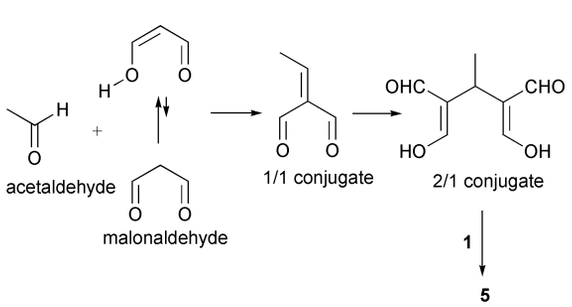


Fig. 1 The carbinolamine intermediate observed by NMR and LC-MS.

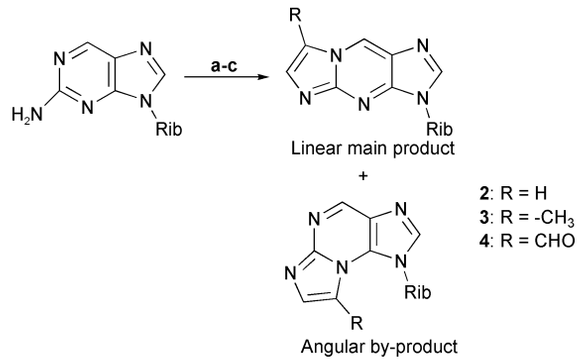
Malonaldehyde–acetaldehyde conjugates have previously been reacted with 2′-deoxyadenosine, cytidine and proteins.[6] There have been different suggestions for the mechanism involved.[11] In the work of Gómez-Sánchez *et. al.* it was presented that condensation of aliphatic aldehydes with malonaldehyde is a general reaction giving first a 1 : 1 conjugate and with an excess of a malonaldehyde, a 2 : 1 conjugate. (Scheme 3).[12] In the present reaction the 2 : 1 conjugate would then react with the amino group of compound **1**, to form product **5** (Scheme 3).



Scheme 3

NMR parameters

In the reactions **a–c** the formation of two different regioisomers was possible. Thus complete NMR spectroscopic analysis was necessary for the determination of which isomer was preferentially formed (Scheme 4).



Scheme 4

3-(β-D-Ribofuranosyl)-imidazo-[1,2a]purine (2). In the ^{1}H NMR spectrum of compound **2**, two singlet and two doublet resonance signals from the protons in the base unit were observed. The signal at δ 8.79 ppm was assigned to H-2 based on the observed long-range H–H (long-range COSY) and HMBC correlation to the sugar unit. The other singlet at δ 9.56 ppm was assigned to H-9. The large downfield shift of H-9 can be explained by the high electron density at C-9 (δ 126.67 ppm) due to resonance of the non-aromatic double-bond system with the non-bonding electrons on N-3 This resonance places a partial negative charge on C-9 which repels the electron cloud around H-9 causing a downfield shift of the proton. This indicates that **2** has the linear (1,N^2-etheno) rather than the angular (N^2,3-etheno) tricyclic structure. The doublets at δ 7.95 (J 1.5 Hz) and 7.68 ppm (J 1.5 Hz) were assigned to H-7 and H-6, respectively. Both doublets showed a strong H–H correlation with each other. The linear tricyclic structure was definitely assigned based on the 2D NOESY spectrum and the ^{1}H–^{15}N HMBC data. The signal at δ −191 ppm was assigned to N-8 based on its chemical shift and its correlations to the signals of H-6, H-7 and H-9. Correlations between the signals of H-6, H-7 and the nitrogen signal at δ −155 ppm assigned the signal to N-5 confirming that the etheno bridge was situated between N-5 and N-8. The nitrogen signal at δ −216 ppm was assigned to N-3 based on its chemical shift and its correlation with the signals of H-1′ and H-2′. The nitrogen signal at δ −142 ppm showed correlation to the signal from H-2 and was assigned to N-1. The nitrogen signal at δ −161 ppm correlated with the signal of H-9 and was assigned to N-4. No amino group nitrogen could be detected by ^{15}N NMR spectroscopy. In the ^{13}C NMR spectrum seven signals from the base and five signals from the ribosyl unit were observed. The signals of carbons bonded to hydrogen atoms were assigned from the one-bond C–H correlation spectra (HMQC or HETCOR). Thus the signals at δ 126.67 ppm and 148.68 ppm were assigned to C-9 and C-2, respectively. The latter signal also displayed a long-range correlation to H-1′ and H-2′ in the HMBC spectrum. The signal at δ 146.51 ppm displayed H–C long-range correlation with the signals of H-6 and H-7 and was assigned to C-4a. The carbon signal at δ 127.57 ppm was assigned to C-9a based on its correlation with the signal of H-2 and its large *trans*-vicinal coupling constant to H-2 (J 13 Hz), which has been observed previously for similar compounds.[13] The signal at δ 149.72 ppm displayed H–C long-range correlation with the signals of H-2, H-9 and H-1′ and was assigned to C-3a. The signals of the ribosyl moiety were assigned using correlation spectroscopy.

3-(β-D-Ribofuranosyl)-5-methylimidazo-[1,2a]purine (3). In the ^{1}H NMR spectrum of **3**, besides the ribosyl protons four signals from the base moiety were observed. The singlet at δ 2.51 ppm was assigned to CH_3 on the basis of the area integral (3H, doublet J 0.3 Hz) and the large upfield shift. Further, it had NOESY correlations to H-9 (δ 9.29 ppm) and H-6 (δ 7.43 ppm, d, J 0.3 Hz). The proton giving a singlet at δ 8.76 ppm displayed a NOESY interaction and a long-range correlation with H-1′ and H-2′ in the ribose unit, and the signal was assigned to H-2. All ^{15}N signal shifts were determined from the ^{1}H–^{15}N HMBC spectrum as described for compound **2** and had similar chemical shifts to those of compound **2**. The ^{13}C NMR spectrum displayed eight signals that originated from the modified base moiety. The proton binding carbons were identified from C–H correlation spectra. The carbon signal for the methyl group was observed at δ 8.89. The carbons C-6 (one proton attached) and C-7 in the etheno bridge gave resonance signals at δ 131.44 and 117.68 ppm, respectively. The methyl group carbon and C-7 showed also long-range C–H couplings to the H-9 in the base unit. The signals at δ 124.27 ppm and 148.38 ppm were assigned to be C-9 and C-2, respectively. These assignments were supported by the 2D HMBC correlation data. The carbon signals at δ 127.37 ppm, 146.07 ppm and 148.83 ppm were assigned to C-9a, C-4a and C-3a, respectively, based on the long-range H–C correlations in HMBC as described for compound **2**. The signals of the ribosyl moiety were assigned using correlation spectroscopy.

3-(β-D-Ribofuranosyl)-5-formylimidazo-[1,2a]purine (4). In addition to the ribosyl protons the ^{1}H NMR spectrum of compound **4** also displayed four singlets. The singlet at δ 9.89 ppm was assigned to the formyl proton on the basis of its correlation with the carbon signal at δ 178.13 ppm and its H–H correlation to the proton signal at δ 8.67 ppm assigned to H-6 and the HMBC correlation to C-7 at δ 127.46 ppm identified from the large geminal C–H_{CHO} coupling (J 31 Hz). The large downfield shift of H-9 (δ 10.10 ppm) can be explained by the anisotropic effect of the formyl group.[14] The proton signal at δ 9.00 ppm was assigned to H-2 in the purine ring on the basis of its COSY correlation to H-1′ in the ribosyl moiety. An eight-bond H–H correlation was also observed between the signals of H-2 and H-6 suggesting a highly planar zig-zag arrangement of the bonds and this supported the linear structure of the three-ring system. All ^{15}N signal shifts were determined from the ^{1}H–^{15}N HMBC spectra. Eight carbon signals from the modified base moiety were detected. The methine carbons at δ 149.46 and 127.90 ppm were assigned to C-2 and C-9, respectively, C-2 displayed a long-range H–C correlation to H-1′. The signals at δ 148.77 and 122.46 ppm were assigned to the etheno bridge carbons C-6 and C-7 based on the HMBC correlations to the formyl group. In the coupled carbon spectrum the signal of C-7 was split into two doublets due to the coupling to the formyl carbon (δ 178.13, J 31 Hz) and the geminal coupling to H-6 (J 15 Hz). The high value of this coupling may be explained by the electronegativity of the nitrogens and the carbonyl group.[13] The carbon signals at δ 129.03, 150.20 and 151.14 ppm were assigned to C-9a, C-4a and C-3a, respectively, from the long-range H–C correlations. All nitrogen shifts could be assigned from the inverse detection experiment and the signals of the ribosyl moiety were assigned using correlation spectroscopy.

9-(β-D-Ribofuranosyl)-2-(3,5-diformyl-4-methyl-1,4-dihydro-1-pyridyl)purine (5). The ^{1}H NMR spectrum of compound **5** showed four singlets and one doublet in addition to the ribosyl moiety signals. The singlet at δ 8.81 ppm was assigned to H-8, due to the long-range H–H correlation to H-1′. The other proton (H-6) from the base moiety was detected at δ 9.08 ppm (long-range COSY correlation to H-8). The methyl group in the dihydropyridyl ring displayed a doublet at δ 1.04 ppm (J 6.7 Hz). The slightly broadened signal at δ 8.55 ppm was assigned to proton H-2″ and the signal at 3.66 ppm (d, J 6.7 Hz) to H-4″. The singlets at δ 9.57 and 9.56 ppm respectively, were assigned to the formyl protons (C-5″) due to their long-range COSY correlations to the H-4″ and methyl group signals. The broadening of the δ 8.55 (H-2″) and the separate signals of the formyl protons are most probably due to the presence of a mixture of two diastereomers. All ^{15}N signal shifts were determined from the ^{1}H–^{15}N HMBC spectrum. In addition to the ribosyl carbons the ^{13}C NMR spectrum displayed 12 signals from the modified base moiety. The methine carbon signals at δ 145.60 and 149.02 ppm were assigned to C-8 and C-6, respectively, from heteroatom correlations. The signal at δ 21.14 ppm was assigned to the methyl group from the dihydropyridyl moiety and the signal at δ 22.65 ppm was assigned to C-4″ due to its heteroatom correlation to H-4″. The signals at δ 140.81 and 140.76 ppm were assigned to C-2″ according to heteroatom correlations. H-2″ displayed long-range H–C HMBC correlations to C-2 at δ 150.07 ppm. The signal at δ 125.17 ppm was assigned from long-range H–C HMBC correlations to both C-3″ and the signals detected at δ 190.92 and 190.87 ppm were assigned to C-5″ due to their heteroatom correlation to H-5″. The signals of C-2″ as well as those of the formyl carbons appeared as pairs of peaks, most probably due to the presence of a mixture of two diastereomers. This could, however, not be confirmed by HPLC as only one peak was shown in the chromatogram. The carbon signals at δ 132.05, 152.18 and 150.07 ppm were assigned to C-5, C-4 and C-2, respectively, from the long-range H–C correlations in HMBC. All nitrogen shifts could be assigned from the inverse detection experiment and the signals of the ribosyl moiety were easily assigned using correlation spectroscopy.

Fluorescence properties

Unlike natural DNA bases, 2-amino-9-(β-D-ribofuranosyl)-purine (**1**), is fluorescent at neutral pH and this native fluorescence has been demonstrated to be an extremely useful probe of *e.g.* DNA conformational changes and DNA base flipping.[5c–d] On the other hand, the linear 1,N^2-ethenoguanosine, which resembles the products **2**–**4**, does not exhibit fluorescence at all.[1a] This indicates that the conjugated systems in the products **2**–**4** and 1,N^2-ethenoguanosine are different.

We were interested in comparing the fluorescence characteristics of the products with those of 2-amino-9-(β-D-ribofuranosyl)purine (**1**). It is well known that an etheno bridge between N-1 and N^6 of adenosine enhances the fluorescence intensity considerably in comparison to unmodified adenosine and therefore it was of interest to investigate the fluorescence properties of the similar products formed from **1**. The results of the fluorescence measurements are shown in Tables 1 and 2 and Fig. 2. All in all, the fluorescence properties of the products differed quite a lot from those of compound **1**. The quantum yields were considerably lower for the products, but the fluorescence lifetime of molecule **3** was equal and of molecule **4** it was more than five ns longer than for the starting material. When comparing the properties of the etheno derivatives **2**–**4** it was obvious that a methyl group in the five membered-ring slightly increased the fluorescence lifetime but did not affect the quantum yield, while an aldehyde group increased both the lifetime and the quantum yield considerably. Product **3** had the same emission maximum as **1**, but all other compounds had their emission maxima at a longer wavelength. The excitation maximum for compound **3** was also the same as for **1**, for compound **2** at a slightly shorter wavelength (298 nm), for compound **4** at a 31 nm shorter (276 nm) and for the product **5** at a slightly longer (313 nm) wavelength. Therefore the Stokes shifts for all other products than for **3** were also larger. For example, the Stokes shift for compound **4** was 167 nm, but only 56 nm for the reference molecule **1**.

Table 1 Fluorescence parameters of the molecules in water at 23 °C[a]

Molecule	Excitation $\lambda_{max}(H_2O)$/nm	Emission $\lambda_{max}(H_2O)$/nm	Quantum Yield
1	307 ± 0.5	363 ± 0.8	0.607 ± 0.110
2	298 ± 0.5	465 ± 0.9	0.073 ± 0.013
3	306 ± 0.9	363 ± 0.9	0.068 ± 0.021
4	276 ± 0.1	437 ± 0.1	0.168 ± 0.048
5	313 ± 0.1	442 ± 0.5	0.178 ± 0.013

[a] The parameters are an average of at least three sets of experimental data. All experiments were performed using samples with an optical density < 0.05

Table 2 Fluorescence decay parameters of the molecules in water at 23 °C[a]

Molecule	Lifetime τ/ns	χ^2	DW
1	10.42 ± 0.21	1.120	1.60
2	7.94 ± 0.42	0.903	2.18
3	10.15 ± 0.47	1.081	0.99
4	16.07 ± 0.71	0.906	1.96
5	2.78 ± 0.06	1.068	1.83

[a] The parameters were calculated using non-linear squared analysis, and the goodness of fit was characterized using chi-squared (χ^2) and the Durbin–Watson parameter (DW)

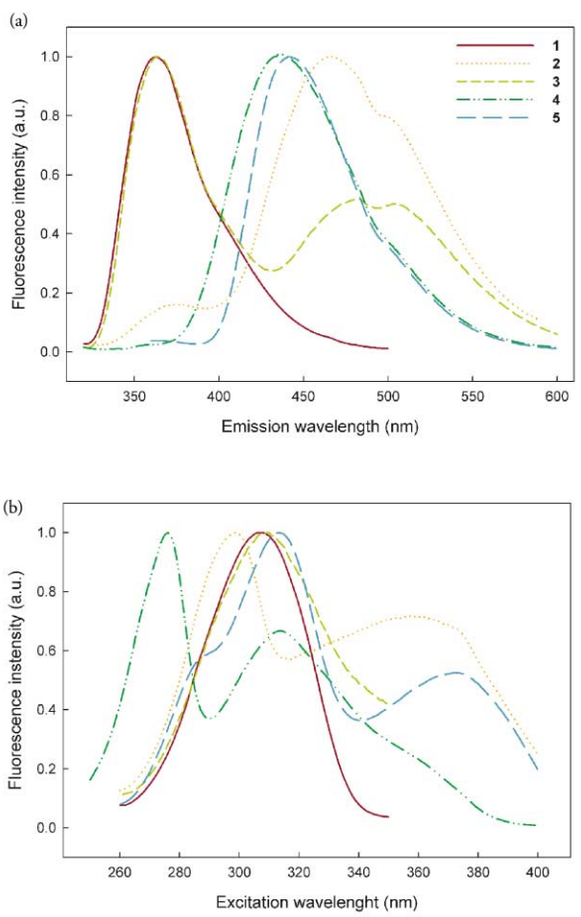


Fig. 2 Normalised emission (a) and excitation (b) spectra of the molecules **1**–**5** in water at 23 °C.

The fluorescence measurement results showed, that the formation of a five membered ring to the 2-amino-9-(β-D-ribofuranosyl)purine changed the fluorescence properties dramatically and instead of enhancing the fluorescence the newly introduced ring-structure quenched it. It can also be noted that while in ethenoadenosine the fluorescence is decreased by the presence of an aldehyde group on the etheno bridge,[15] the aldehyde group in **4** increased the quantum yield. This suggests that the conjugation is more efficient in **4** than in the corresponding adenosine adduct.

For adenosine, the formation of a dihydropyridine ring-system, has been reported to enhance fluorescence intensity significantly.[6a] Instead in **1**, the dihydropyridine ring quenched the fluorescence. Both quantum yield and lifetime were lower than for the starting material **1**.

Conclusions

Four 2-amino-9-(β-D-ribofuranosyl)purine (**1**) derivatives have been synthesised. The compounds **2**–**5** were formed in the reaction of compound **1** with chloroacetaldehyde (**a**), 2-chloropropanal (**b**), bromomalonaldehyde (**c**) and chloroacetaldehyde + malonaldehyde (**d**) (Scheme 1). The syntheses were straightforward but the yields were moderate and most of the starting materials were recovered unchanged in the reaction mixture. A 95% yield of etheno deoxyadenosine has been reported in the reaction of chloroacetaldehyde with deoxyadenosine.[1a] However, with 2-amino-9-(β-D-ribofuranosyl)purine (**1**) the yield was considerably lower, only 14%. For the other reactions **b–d**, the yields were slightly higher than for the adenosine reactions with the same reagents.[6a,7,10c] Consequently it seems that the reactivity of 2-amino-9-(β-D-ribofuranosyl)purine (**1**) towards these reagents, is much lower than that of adenosine. The reactions were studied and tested in many different reaction conditions applying different reaction times, temperatures, reactant concentrations and solvents but no increase of the yields could be achieved. No definitive explanation to the unreactivity of the starting material (**1**) can yet be given. However, this topic is currently under investigation in our laboratory.

The fluorescence properties of the products were determined and compared with the starting material 2-amino-9-(β-D-ribofuranosyl)purine. The fluorescence properties of the products **2**–**5** differed considerably from those of compound **1** and it can be concluded that the "etheno"- or dihydropyridine ring systems do not enhance the fluorescence characteristics of 2-amino-9-(β-D-ribofuranosyl)purine in the same way they do in the similar adenosine products. Therefore it must be concluded that the conjugated double bond system of these product molecules is critically different.

The NMR data were consistent with the structures. The linear (1,N^2-etheno) rather than the angular (N^2, 3-etheno) tricyclic structure was determined for compounds **2**–**4** from the ^{15}N NMR measurements and the NOESY spectra.

LC-MS analysis of the reaction mixtures revealed the presence of low concentrations of products with the same masses as **2**–**4** indicating the formation of small amounts of the angular derivatives (Scheme 4).

Experimental

Chemicals

Chloroacetaldehyde diethyl acetal (99%) was obtained from Acros Organics, 2-chloropropionaldehyde dimethyl acetal (>96%) was obtained from Fluka AG and 1,1,3,3-tetra-

methoxypropane (99%) was obtained from Aldrich Chemical Co. 2-Amino-9-(β-D-ribofuranosyl)purine was synthesised according to known methods. The solvents for the synthesis were of analytical grade and for HPLC of commercial HPLC grade.

Spectroscopic and spectrometric methods

The ^{1}H-, ^{13}C- ^{15}N- and 2D NMR spectra were recorded in DMSO at 30 °C on a JEOL JNM A 500 Fourier transform NMR spectrometer at 500.16, 125.78 and 50.69 MHz, respectively. ^{1}H and ^{13}C shifts were referenced against DMSO-d_5 solvent signal 2.50 ppm and 39.51 ppm, respectively. The ^{1}H NMR signal assignments were based on chemical shifts from the 2D H–H, H–C and H–N correlation spectroscopy data. The assignment of carbon signals was based on the same techniques and carbon–proton coupling constants. All nitrogen shifts were from HMBC spectra and were referenced externally to 90% nitromethane in CD_3NO_2 (0.00 ppm). All inverse detected HMBC- and HMQC experiments were recorded with an inverse 5 mm probe with z-axis pulse-field gradient capability. The ^{1}H NMR spectra were analysed by PERCH software[16] to perform complete spectral analyses.

The mass spectrometric analyses were performed on an Agilent 1100 Series LC/MSD Trap system equipped with an electrospray source and operated in the positive mode. Nitrogen was used as nebulizer gas (40 psi) and as drying gas (12 mL min^{-1}). The drying gas was heated to 350 °C. The capillary exit offset had a value of 71.2 V and skim 1 was set at 29.9 V. The maximum ion accumulation time was 50 ms and the target value was 50000. Scanning from *m*/*z* 100–500 was applied for the recording of the full mass spectrum.

The electron impact high-resolution mass spectra (EI) were recorded on Fisons ZABSpec-oaTOF instrument. The spectra were acquired using a direct insert probe scanning from 50 to 1500 amu and using electrons energised to 70 eV. Accurate mass measurements were performed using a peak matching technique with PFK as a reference substance at a resolution of 8000–10000 (at 10% peak height).

The UV spectra of the compounds were recorded with a Shimadzu UV-160A spectrophotometer.

The fluorimetric properties of the compounds were studied at 23 °C. Steady state fluorescence measurements were performed on a Photon Technology International (PTI) Quantamaster 1 spectrofluorimeter operating in the T-format. The emission wavelength scans were performed with the excitation wavelength set at 320 nm. Excitation wavelength scans were made with the emission monochromator set at 430 nm. In the steady-state measurements, the slit widths were kept at 5 nm. The water used in the experiments was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system, to yield a product with a resistance of at least 18.2 MΩcm^{-1}.

The fluorescence quantum yield of the compounds was estimated by comparison with the known quantum yield of a standard.[17] The quantum yield standard was quinine sulfate (Fluka AG, Switzerland) in 0.1 M H_2SO_4 which is known to have a quantum yield of 0.53 ± 0.023.[17–18]

In order to avoid inner filter effects the optical density was kept below 0.05 in all measurements. The quantum yield was calculated according to eqn (1):

$$Q = Q_R\,(I/I_R)\,(OD_R/OD)\,(n^2/n_R^2) \qquad (1)$$

where Q is the quantum yield, I is the integrated intensity OD is the optical density, and n is the refractive index.[17] The subscript R refers to the reference fluorophore of known quantum yield.

The fluorescence decay parameters of the reaction products were determined using a PTI Timemaster instrument (N_2 laser). In these experiments, the excitation wavelength was set to 337 nm, and the emission wavelength to 430 nm. The slit width was set to 5 nm. Analyses of the data were performed with the software supplied by PTI (Time Master 1.2).

Chromatographic methods

The HPLC analyses were made on a Kontron Instruments liquid chromatographic system consisting of a model 322 pump, a 440 diode array detector (UV), a Jasco FP-920 fluorescence detector, and a Kromasystem 2000 data handling program. The chromatographic separations were performed on an analytical 5 μm, 4 mm × 125 mm reversed phase C 18 column (Hypersil BDS-C18, Hewlett Packard/Agilent). The column was eluted isocratically for 5 min with 0.01 M phosphate buffer (pH 7.1) and then with a gradient from 0 to 40% acetonitrile over the course of 25 min at a flow rate of 1 mL min^{-1}.

The products were isolated from the reaction mixtures on flash chromatography columns, packed with 40 μm C 18 reversed-phase silica gel, preparative column. The products were eluted with an acetonitrile–water gradient.

Syntheses

Chloroacetaldehyde (a). A mixture of chloroacetaldehyde diethyl acetal (5.0 mL, 32 mmol), 1 M HCl (15 mL) and ethanol (5.0 mL) was stirred for 2 hours at 70 °C. The solution was stored at 20 °C and used for reactions without further treatment.

2-Chloropropanal (b). A mixture of 2-chloropropionaldehyde dimethyl acetal (5.0 mL, 34 mmol), 1.0 M HCl (15 mL) and ethanol (5.0 mL) was stirred for 2 hours at 70 °C. The solution was stored at 20 °C and used for reactions without further treatment.

Bromomalonaldehyde (c). This reagent was prepared by the method of Trofimenko.[19]

2-Amino-9-(β-D-ribofuranosyl)purine (1). The preparation of the compound **1** was performed in two steps starting from guanosine. In the first step 6-thioguanosine was prepared according to a method by Kung and Jones.[8a] In the second step 6-thioguanosine was reduced with Raney nickel according to a method developed by Fox *et al.* to yield the starting material **1**.[8b]

3-(β-D-Ribofuranosyl)-imidazo-[1,2a]purine (2). Chloroacetaldehyde (8.5 mL, 11 mmol) was added to 1.0 g (3.7 mmol) of **1** dissolved in 0.5 M KH_2PO_4 buffer (15 mL, pH 4.5) and ethanol (10 mL). The reaction mixture was stirred at 37 °C for 22 h. The pH was kept at 4.5 by addition of 0.5 M Na_2HPO_4 (pH 9.0) buffer solution. The reaction was stopped and the reaction mixture was neutralised with $NaHCO_3$ (aq.). The mixture was filtered and the product was isolated from the solid by preparative reversed phase chromatography. The fractions containing the product were combined and evaporated to give oil. The residue was precipitated from dry ethanol (151 mg, 14%) of pure **2** as yellow powder. λ_{max}(H_2O)/nm 229 and 294 (ε/dm^3 mol^{-1} cm^{-1} 26600 and 5700); λ_{min}(H_2O)/nm 204 and 261 (ε/dm^3 mol^{-1} cm^{-1} 10000 and 2820); *m*/*z* (EI) 291.0966 (M^+, $C_{12}H_{13}N_5O_4$ requires 291.0967), 159 (100%). δ_H(500.16 MHz; DMSO) 3.61 (1 H, ddd[a], $J_{5'b,5'a}$ −11.9, $J_{5'b,5'\text{-OH}}$ 4.8, H-5′b), 3.73 (1 H, ddd[a], $J_{5'a,5'\text{-OH}}$ 3.6, H-5′a), 4.00 (1 H, ddd[a], $J_{4',5'a}$ 3.9, $J_{4',5'b}$ 3.7, H-4′), 4.22 (1 H, ddd[a], $J_{3',4'}$ 4.1, $J_{3',3'\text{-OH}}$ 4.8, H-3′), 4.65 (1 H, ddd[a], $J_{2',3'}$ 3.3, $J_{2',2'\text{-OH}}$ 5.3, H-2′), 5.11 (1 H, t, 5′-OH), 5.24 (1 H, d, 3′-OH), 5.56 (1 H, d, 2′-OH), 6.00 (1 H, d, $J_{1',2'}$ 5.5, H-1′), 7.68 (1 H, d, $J_{6,7}$ 1.5, H-6), 7.95 (1 H, d, $J_{7,6}$ 1.5, H-7), 8.79 (1 H, s, H-2), 9.56 (1 H, s, H-9). δ_C(125.78 MHz; DMSO) 61.20 (t, $^{1}J_{C,H}$ 139, C-5′), 70.17 (d, $^{1}J_{C,H}$ 151, C-3′), 73.13 (d, $^{1}J_{C,H}$ 148, C-2′), 85.31 (d, $^{1}J_{C,H}$ 148, C-4′), 87.15 (d, $^{1}J_{C,H}$ 166, C-1′),

110.65 (dd, $^1J_{C,H}$ 196, $^{>1}J_{C,H}$ 15, C-7), 126.67 (d, $^1J_{C,H}$ 190, C-9), 127.57 (dd, $^{>1}J_{C,H}$ 13 and 2, C-9a), 133.90 (dd, $^1J_{C,H}$ 188, $^{>1}J_{C,H}$ 9, C-6), 146.51 (ddd, $^{>1}J_{C,H}$14; 7 and 4, C-4a), 148.68 (dd, $^1J_{C,H}$ 214, $^{>1}J_{C,H}$ 4, C-2), 149.72 (m, $^{>1}J_{C,H}$ 7; 4 and 2, C-3a). δ_N(50.69 MHz; DMSO) −216 (N-3), −191 (N-8), −161 (N-4), −155 (N-5), −142 (N-1). [a]The signals appear as broad triplets/quartets ($J_{H,H}$ ± 0.3 Hz).

3-(β-D-Ribofuranosyl)-5-methylimidazo-[1,2a]purine (3). 2-Chloropropanal (8.5 mL, 12 mmol) was added to the solution of **1** (1.0 g, 3.7 mmol) in 0.5 M KH_2PO_4 (25 mL, pH 4.5) buffer and ethanol (10 mL). The reaction was allowed to proceed at 37 °C for 29 h. The pH was adjusted to 4.5. The reaction was stopped and the mixture was neutralised with aqueous $NaHCO_3$. The mixture was filtered and the product was isolated from the solid by preparative reversed phase chromatography. The fractions containing the product were combined and evaporated. The oily product was crystallised from dry ethanol at +8 °C to give **3** (195 mg, 17%) as a crystalline light yellow powder. $\lambda_{max}(H_2O)$/nm 231, 296 and 343 (ε/dm^3 mol^{-1} cm^{-1} 35600, 6530 and 2880); $\lambda_{min}(H_2O)$/nm 265 and 320 (ε/dm^3 mol^{-1} cm^{-1} 1890 and 2500); *m/z* (EI) 305.1128 (M^+, $C_{13}H_{15}N_5O_4$ requires 305.1124), 173 (100%). δ_H(500.16 MHz; DMSO) 2.51 (3 H, $J_{CH3,6}$ 0.3, s, – CH_3), 3.59 (1 H,ddd[a], $J_{5'b,\ 5'a}$ −12.0, $J_{5'b,5'\text{-OH}}$ 5.8, H-5′b), 3.71 (1 H, ddd[a], $J_{5'a,5'\text{-OH}}$ 5.1, H-5′a), 3.98 (1 H, ddd[a], $J_{4',5'a}$ 4.00, $J_{4',5'b}$ 4.2, H-4′), 4.21 (1 H, ddd[a], $J_{3',4'}$ 3.7, $J_{3',3'\text{-OH}}$ 5.1, H-3′), 4.64 (1 H, ddd[a], $J_{2',3'}$ 4.7, $J_{2',2'\text{-OH}}$ 5.9, H-2′), 5.10 (1 H, t, 5′-OH), 5.22 (1 H, d, 3′-OH), 5.56 (1 H, d, 2′-OH), 5.98 (1 H, d, $J_{1',2'}$ 5.6, H-1′), 7.44 (1 H, d, $J_{6,CH3}$ 0.3, H-6), 8.76 (1 H, s, H-2), 9.29 (1 H, s, H-9). δ_C(125.78 MHz; DMSO) 8.89 (q, $^1J_{C,H}$ 129,-CH_3), 61.23 (t, $^1J_{C,H}$ 140, C-5′), 70.20 (d, $^1J_{C,H}$ 149, C-3′), 73.11 (d, $^1J_{C,H}$ 148, C-2′), 85.30 (d, $^1J_{C,H}$ 149, C-4′), 87.14 (d, $^1J_{C,H}$ 164, C-1′), 117.68 (ddd, $^{>1}J_{C,H}$ 14; 14 and 7, C-7), 124.27 (d, $^1J_{C,H}$ 189, C-9), 127.37 (d, $^{>1}J_{C,H}$ 12, C-9a), 131.44 (ddd, $^1J_{C,H}$ 186, $^{>1}J_{C,H}$ 9 and 4, C-6), 146.07 (dd, $^{>1}J_{C,H}$ 13 and 4, C-4a), 148.38 (dd, $^1J_{C,H}$ 214, $^{>1}J_{C,H}$ 4, C-2), 148.83 (dd, $^{>1}J_{C,H}$ 8 and 6, C-3a). δ_N(50.69 MHz; DMSO) −217 (N-3), −190 (N-8), −161 (N-4), −156 (N-5), −141 (N-1). [a]The signals appear as broad triplets/quartets ($J_{H,H}$ ± 0.3 Hz).

3-(β-D-Ribofuranosyl)-5-formylimidazo-[1,2a]purine (4). Bromomalonaldehyde (1.7 g, 11 mmol) was added to the solution of **1** (0.78 g, 3.0 mmol) in 0.5 M KH_2PO_4 (35 ml, pH 4.5) buffer and ethanol (15 mL). The reaction was allowed to proceed at 37 °C for 28 h and the pH was adjusted to 4.5. The reaction was stopped and the mixture was filtered. The product was isolated from the solid by preparative reversed phase chromatography. Methanol was added to the syrup and evaporated. The product was crystallised from ethanol (185 mg, 20%) as yellow crystalline **4**. $\lambda_{max}(H_2O)$/nm 214, 277 and 313 (ε/dm^3 mol^{-1} cm^{-1} 15900, 35300 and 10600); $\lambda_{min}(H_2O)$/nm 233 and 292 (ε/dm^3 mol^{-1} cm^{-1} 5650 and 7120); *m/z* (EI) 319.0913 (M^+, $C_{13}H_{13}N_5O_5$ requires 319.0916), 187 (100%). δ_H(500.16 MHz; DMSO) 3.61 (1 H, dd[a], $J_{5'b,5'a}$ −12.0, $J_{5'b,5\text{-OH}}$ 5.0, H-5′b), 3.73 (1 H, dd[a], $J_{5'a,5\text{-OH}}$ 4.8, H-5′a), 4.01 (1 H, dd[a], $J_{4',5'a}$ 3.7, $J_{4'5'b}$ 3.7, H-4′), 4.22 (1 H, dd[a], $J_{3',4'}$ 4.3, $J_{3',3'\text{-OH}}$ 5.2, H-3′), 4.62 (1 H, dd[a], $J_{2',3'}$ 3.7, $J_{2',2'\text{-OH}}$ 5.7, H-2′), 5.11 (1 H, s, 5′-OH), 5.26 (1 H, s, 3′-OH), 5.60 (1 H, s, 2′-OH), 6.07 (1 H, d, $J_{1',2'}$ 5.2, H-1′), 8.67 (1 H, s, H-6), 9.00 (1 H, s, H-2), 9.89 (1 H, s, C*H*O), 10.10 (1 H, s, H-9). δ_C(125.78 MHz; DMSO) 60.98 (t, $^1J_{C,H}$ 140, C-5′), 70.03 (d, $^1J_{C,H}$ 150, C-3′), 73.64 (d, $^1J_{C,H}$ 148, C-2′), 85.48 (d, $^1J_{C,H}$ 148, C-4′), 87.44 (d, $^1J_{C,H}$ 166, C-1′), 122.46 (dd, $^{>1}J_{C,H}$ 31 and 15, C-7), 127.90 (d, $^1J_{C,H}$ 193, C-9), 129.03 (dd, $^{>1}J_{C,H}$ 12 and 2, C-9a), 148.77 (dm, $^1J_{C,H}$ 189, C-6), 149.46 (dd, $^1J_{C,H}$ 216, $^{>1}J_{C,H}$4, C-2), 150.20 (dd, $^{>1}J_{C,H}$ 15 and 5, C-4a), 151.14 (ddd, $^{>1}J_{C,H}$ 6; 5 and 3, C-3a), 178.13 (d, $^1J_{C,H}$ 178, *C*HO). δ_N(50.69 MHz; DMSO) −211 (N-3), −195 (N-8), −158 (N-4), −141 (N-5), −139 (N-1). [a]The signals appear as broad triplets/quartets ($J_{H,H}$ ± 0.3 Hz).

9-(β-D-Ribofuranosyl)-2-(3,5-diformyl-4-methyl-1,4-dihydro-1-pyridyl)purine (5). 1,1,3,3-Tetramethoxypropane (3.74 g, 17 mmol) was hydrolysed with HCl (50 mL, 0.1 M) and the pH was adjusted to 4.5. Chloroacetaldehyde diethyl acetal (0.75 g, 5.0 mmol) was added and the mixture stirred for 10 min. The pH was adjusted again to 4.5. The starting material (1.0 g, 3.7 mmol) was dissolved in 35 mL of water. The solution was added to the first made mixture of hydrolysed 1,1,3,3-tetramethoxypropane and chloroacetaldehyde diethyl acetal and the pH was adjusted to 4.5. The reaction mixture was stirred at 37 °C for 45 h. The reaction was stopped and the reaction mixture was filtered. The filtrate was evaporated and methanol added. The product crystallised to give (247 mg, 16%) **5** as orange crystals. $\lambda_{max}(H_2O)$/nm 235, 287, 313 and 369 (ε/dm^3 mol^{-1} cm^{-1} 17600, 16900, 22900 and 5860); $\lambda_{min}(H_2O)$/nm 213, 262, 294 and 349 (ε/dm^3 mol^{-1} cm^{-1} 12000, 7780, 15700 and 5170); *m/z* (EI): 401.1337 (M^+, $C_{18}H_{19}N_5O_6$ requires 401.1335), 254 (100%), 269 (7). δ_H(500.16 MHz; DMSO) 1.04 (3 H, d, $J_{CH3,4''}$ 6.7, – CH_3), 3.66 (1 H, d, H-4″), 3.67 (1 H, dd[a], $J_{5'b,5'a}$ −12.2, H-5′b), 3.74 (1 H, dd[a], H-5a′), 4.04 (1 H, dd[a], $J_{4',5'a}$ 3.8, $J_{4',5'b}$ 3.4, H-4′), 4.29 (1 H, dd[a], $J_{3',4'}$ 3.8, H-3′), 4.67 (1 H, dd[a], $J_{2',3'}$ 5.0, H-2′), 6.10 (1 H, dd[a], $J_{1',2'}$ 5.4, H-1′), 8.55 (2 H, s, H-2″), 8.81 (1 H, s, H-8), 9.08 (1 H, s, H-6), 9.56 (1 H, s, H-5″) and 9.57 (1H, s, H-5″). δ_C(125.78 MHz; DMSO) 21.14 (dd, $^1J_{C,H}$ 128, $^{>1}J_{C,H}$ 6, -CH_3), 22.65 (dm, $^1J_{C,H}$ 133, C-4″), 61.07 (t, $^1J_{C,H}$ 142, C-5′), 70.07 (d, $^1J_{C,H}$ 148, C-3′), 73.70 (d, $^1J_{C,H}$ 150, C-2′), 85.70 (d, $^1J_{C,H}$ 150, C-4′), 87.48 (d, $^1J_{C,H}$ 166, C-1′), 125.17 (2C, dm, $^{>1}J_{C,H}$ 26, C-3″), 132.05 (dd, $^{>1}J_{C,H}$ 12 and 6, C-5), 140.76 and 140.81 (2C, dm, $^1J_{C,H}$ 184, $^{>1}J_{C,H}$ 5, C-2″), 145.60 (dd, $^1J_{C,H}$ 216, $^{>1}J_{C,H}$ 4, C-8), 149.02 (d, $^1J_{C,H}$ 186, C-6), 150.07 (dt, $^{>1}J_{C,H}$ 12 and 3, C-2), 152.18 (ddd, $^{>1}J_{C,H}$ 8; 3 and 3, C-4), 190.87 and 190.92 (2C, dt, $^1J_{C,H}$ 176, $^{>1}J_{C,H}$ 7, C-5″). δ_N(50.69 MHz; DMSO) −236 (N-1″), −208 (N-3), −164 (N-4), −136 (N-6), −135 (N-1). [a]The signals appear as broad triplets/quartets ($J_{H,H}$ ± 0.3 Hz).

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