ARTICLE

OBC
www.rsc.org/obc

Synthesis, characterisation and theoretical calculations of 2,6-diaminopurine etheno derivatives†

Piritta Virta,*[a] Andreas Koch,‡[b] Mattias U. Roslund,§[a] Peter Mattjus,¶[c] Erich Kleinpeter,‡[b] Leif Kronberg,[a] Rainer Sjöholm[a] and Karel D. Klika*[d]

[a] *Department of Organic Chemistry, Åbo Akademi University, FI-20500 Turku/Åbo, Finland. E-mail: piritta.virta@abo.fi; Fax: +358 2 2154866; Tel: +358 2 2154502*

[b] *Department of Chemistry, University of Potsdam, P. O. Box 69 15 53, D-14415 Potsdam, Germany*

[c] *Department of Biochemistry and Pharmacy, Åbo Akademi University, FI-20520 Turku/Åbo, Finland*

[d] *Department of Chemistry, University of Turku, FI-20014 Turku, Finland. E-mail: klikakd@yahoo.co.uk; Fax: +358 2 3336700; Tel: +358 2 3336826*

Received 20th April 2005, Accepted 1st June 2005
First published as an Advance Article on the web 6th July 2005

Four derivatives of 2,6-diaminopurine (**1**) were synthesised and characterised. When **1** was reacted with chloroacetaldehyde, 5-aminoimidazo[2,1-*i*]purine (**2**), 9-aminoimidazo[2,1-*b*]purine (**3**), 9-aminoimidazo[1,2-*a*]purine (**4**) and diimidazo[2,1-*b*:2′,1′-*i*]purine (**5**) were formed. The purified products (**3**–**5**) were fully characterised by MS, complete NMR assignments as well as fluorescence and UV spectroscopy. The purified, isolated yields of these products (**3**–**5**) varied from 2.5 to 30%. The relative stability of different tautomers was investigated by theoretical calculations. Fluorescence characteristics are also discussed and compared to the starting material **1** and a reference molecule 2-aminopurine.

Introduction

The reaction of genotoxic chemicals with DNA and RNA, particularly reactions with the base moieties, to form adducts accounts for the detrimental and toxic effects of these compounds. The DNA is a continuous target for endogenous and exogenous damage and some of the most commonly encountered DNA lesions are the exocyclic DNA adducts. In particular, etheno adducts, with an additional five-membered ring fused to the base moiety, have gained much attention since they can result from reactions with a variety of chemicals (*e.g.* chloroacetaldehyde, chloropropanal, bromomalonaldehyde). These etheno compounds formed can be used as models to investigate what may be happening detrimentally on the biomolecular scale (*e.g.* fluorescence markers), as some etheno derivatives of purine are known to be fluorescent.[1]

As mentioned above, haloacetaldehydes have been shown to react with nucleic acid components yielding etheno derivatives of adenosine, guanosine and cytidine.[2] The 1,N^6-ethenodeoxyadenosine derivatives, due to their fluorescence, are proven to be useful compounds, *e.g.* studies of mutagenesis[3] and enzymology.[4]

Our research in the field of nucleoside chemistry originally aimed at synthesising modified fluorescent RNA/DNA bases.[5] However, our present investigations are also directed toward the preparation of nucleoside base analogues, which might also have interesting applications other than those associated with their fluorescence properties.[5,6] To our knowledge 2,6-diaminopurine (**1**) has not previously been used to produce different etheno derivatives.

Owing to the similarity of the expected products and many tautomers (possibly even dimers through hydrogen bonding),[7] theoretical calculations of carbon and nitrogen NMR chemical shifts and the relative stability of the different tautomeric forms of the products were also included into this work.

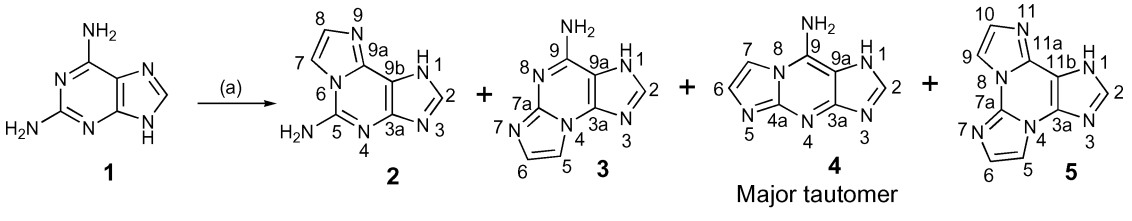
In the current work, we report on the synthesis of four derivatives of 2,6-diaminopurine (**1**). They are formed in the reaction of compound **1** with chloroacetaldehyde (Scheme 1). In this contribution we present the details of the synthetic procedures, the complete structural analysis of the products, which includes MS, and NMR analyses (^{1}H, ^{13}C, ^{15}N NMR data), as well as fluorescence and UV spectroscopic properties for isolated compounds **3**–**5** and NMR analyses for compound **2**. The fluorescence intensities and lifetimes of compounds **3**, **4** and **5** are compared to those of **1** and 2-aminopurine (reference) and are discussed. Also, the theoretical calculations of the NMR chemical shifts are compared to the experimental ones and the relative stabilities of the different tautomers in the gas phase for all compounds are discussed.

† Electronic supplementary information (ESI) available: modelling details for all tautomers of compounds **1**–**5**. See http://dx.doi.org/10.1039/b505508c

‡ Theoretical calculations.

§ NMR measurements.

¶ Fluorescence measurements.



Scheme 1 *Reagents and conditions*: (a) chloroacetaldehyde ($ClCH_2CHO$), pH 4–5, 80 °C, *ca.* 1 d.

DOI: 10.1039/b505508c

Results and discussion

The products (**2**–**5**) were synthesised according to Scheme 1. The reaction of 2,6-diaminopurine (**1**) with chloroacetaldehyde was carried out in water/DMF solution at pH 4–5 and the progress of the reaction was monitored by HPLC. The products **3**–**5** were isolated by Ca-enriched silica gel (for acid labile compounds) followed by reversed-phase column chromatography and deprotonation with $NaHCO_3$, after which the compounds were fully characterised by ^1H, ^{13}C and ^{15}N NMR spectroscopy, electrospray ionisation mass spectrometry, fluorescence spectroscopy and UV absorption. Compound **2** was only observed and characterised in one of the fractions containing a mixture of all three-ring products **2**–**4**. Owing to the ease of protonation and the many possible tautomers of the products, their absolute characterisation was demanding and during this project one standard magnitude-mode HMBC NMR pulse sequence was also modified in order to identify the major tautomer of compound **4**. The major tautomer was assigned using different 2D techniques, like hetero NOESY (PHOESY) and high resolution HMBC for the determination of heteronuclear coupling constants. Dry DMSO-d_6 was used for NMR measurements as these compounds were not soluble in other solvents better suited for low-temperature NMR to slow down the fast exchange of the NH-protons.[8]

The isolated yields of **3**, **4** and **5** were 9, 2.5 and 30% 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.

Structure determination and modelling

In the reaction, the formation of different regioisomers was possible. Thus, complete NMR spectroscopic analysis was necessary for the determination of which isomers were formed and isolated. Owing to the great similarity of the structures, theoretical calculations of the carbon and nitrogen NMR chemical shifts were also performed as this has been found to be a reliable method for structural determinations, even in the case of distinguishing between tautomeric or other kinds of extremely similar structures.[6b,9]

The formed products were easily protonated and NMR shifts changed considerably upon protonation (*e.g.* even the chemical shifts of the protons adjacent to the site of protonation can be deshielded by as much as 1 ppm[6,10]). Also, different states of protonation had different NMR shifts. Owing to this it was very important to perform the NMR measurements for all of the molecules under similar conditions, after deprotonation with $NaHCO_3$. Structural determinations and signal assignments were accomplished by the application of a comprehensive set of 2D FG ^1H-{^{13}C}-PHOESY,[8] ^1H-{^{13}C}- and ^1H-{^{15}N}-HSQC and ^1H-{^{13}C}- and ^1H-{^{15}N}-HMBC experiments. Unfortunately, homo-NOEs were never observed between labile protons and non-labile protons, leaving structural analysis (in particular) to rely mainly on long-range J hetero-correlations. Other methodologies, when necessary due to ambiguity or lack of correlations, were used on occasion. For example, the assignment of the etheno- and exchangeable-protons was based on the relative magnitude of $^3J_{H,C}$, $^2J_{H,C}$, $^1J_{H,C}$, $^3J_{H,N}$ and $^2J_{H,N}$, and determination of the sign of $^nJ_{H,C}$ to distinguish between 2- and 3-bond couplings.[8]

Compound **5** was readily discernible by NMR from the presence of the additional etheno bridge, further confirmed by MS analysis. Compound **4** has previously been identified by us,[8] thus leaving only the distinction between **2** and **3**. Compound **3** was identified based on the correlation from the amino protons of the NH_2 group to C-9a, a highly distinctive carbon which is outstandingly shielded. This was further confirmed by the correlations from the NH_2-protons to an sp^2-hybridised nitrogen which was not one of the two nitrogens that were correlated to the etheno bridge protons. This left the identification of the structure of **2** by default.

Owing to the lack of a sugar moiety attached to N-3 (*cf.* ref. 5), many tautomeric forms of the products were possible. The only product for which distinct tautomers could be observed directly (18.5 °C in DMSO) was compound **4** where two tautomers were present. The major tautomer was identified[8] as 1*H*-9-aminoimidazo[1,2-*a*]purine after modifying a magnitude-mode HMBC NMR pulse sequence. The assignment of the major tautomer was based on the sign of the proton–carbon coupling constants [between H-1 to C-3a (^3J positive), between H-1 to C-2 (^2J negative) and between H-1 to C-9a (^2J negative)].[8] For the other products, distinct tautomers were not seen under similar conditions. For the compounds **3** and **5**, additional indirect evidence for the identity of the tautomers present was forthcoming by the correlations of H-2 to two sp^2-hybridised nitrogens. This could be interpreted as evidence for either the H-7 or H-8 amino tautomers in the case of **3** (*i.e.* disregarding imino tautomers for which there was a lack of evidence in all cases based on the chemical shifts of the NH_2 groups) or the H-11 tautomer in the case of **5**. However, in the former case (**3**) the chemical shifts of −162 and −191 ppm for N-7 and N-8 (an appropriate value with an exocyclic double bond at position 7a), respectively, clearly indicate the predominance of either the H-1 or the H-3 amino tautomer, or possibly both, together with the presence of the H-7 and/or H-8 amino tautomer(s) based on the comparison of the calculated and experimental chemical shifts. It is not possible to be more precise than this due to an insufficient number of constraints. In the case of **5**, the situation is less complex as only three tautomers are possible. Again, the chemical shift of −153 ppm for N-11 discounts the predominance of the H-11 tautomer. Comparison of the calculated and experimental chemical shifts revealed that the tautomeric composition is dominated by H-1 and H-3 together with a minor contribution of H-11. Compound **2** was clearly dominated by only either the H-1 or H-3 tautomers or a mixture of both, but no clear indication was provided as to which was the dominate one.

To obtain information of the different tautomers present, theoretical tautomeric equilibrium calculations for all products in the gas phase were performed, with additional calculations considering DMSO solvation employing the polar-continuum model. The results at the different levels of theory (HF/6-311G**, B3LYP/6-311G**, MP2/6-31G**) are presented in the ESI† though it is known that it is notoriously difficult to accurately replicate tautomeric energies, even with inclusion of the solvent (hence the multiple calculations).[11] These aberrations can have a variety of origins – correct representation of the solvent, the formation of H-bonds with the molecules and, for these kinds of compounds, the possibility for dimer formation (Fig. 1) – thus moving to higher levels of theory does not necessarily improve matters.[12]

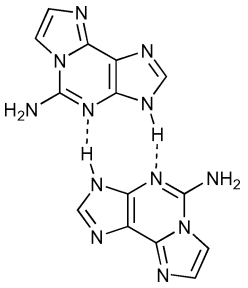
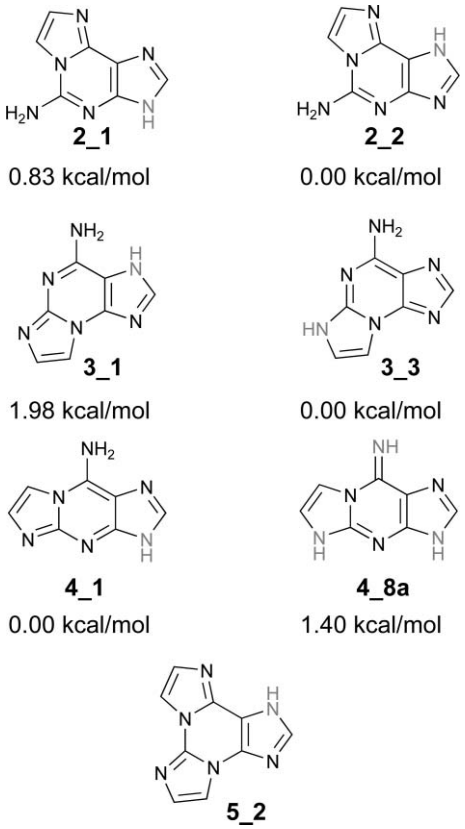


Fig. 1 An example of possible dimer formation for product **2**.

For compound **4**, two favourable tautomers in the gas phase with an energy difference of 1.40 kcal mol^{-1} were found: tautomers **4_1** (3*H*) and **4_8a** in Scheme 2. However, at no time was there any evidence for imine tautomers for any



Scheme 2

of the compounds, and generally it has been shown that purines are a mixture of 1*H* and 3*H* tautomers with the 1*H* tautomer dominating.[11,13] (Indeed, this was found to be the case for the calculations on **1** where little variance amongst the levels of theory was evident.) Experimentally, it was the 1*H* tautomer that was found to be the most stable with the minor tautomer presumed to be the 3*H* tautomer (the 4*H* could not be excluded).[8] But comparison of the chemical shifts does in fact correlate to these two structures (*i.e.* the 1*H* and 3*H* tautomers) and thus confirms our earlier findings. Moreover, the 4*H* tautomer can now be categorically discounted based on the calculated ^{13}C and ^{15}N chemical shifts.

Also for compound **3**, two major tautomers were found with an energy difference of 1.98 kcal mol^{-1} (**3_1** and **3_3**, Scheme 2). However, no sense could be made of the calculated chemical shifts in comparison to the experimental values (as individual tautomers or as combinations of two tautomers in fast exchange) leading to the conclusion that at least one other tautomer in addition to the known 1*H* and 3*H* tautomers must be involved in a fast exchange equilibrium. This third tautomer could well be structure **3_3** (7*H* tautomer), but with too many unknowns (the number of participating tautomers and mole fractions thereof) it is not possible to describe the system definitively. Although **3_3** always remained the most stable species, significant alterations in the relative energies of **3_1** and **3_2** (3*H* tautomer) occurred, particularly with higher levels and upon inclusion of the solvent.

For compound **2**, the two most stable tautomers were found to differ in energy by 0.83 kcal mol^{-1} (**2_1** and **2_2**, Scheme 2) and these two tautomers were always close in energy regardless of the level or upon inclusion of the solvent (in fact, both the B3LYP and MP2 methods reversed their preference upon inclusion of the solvent, *i.e.* **2_1** became favoured).

For the tetracyclic product **5**, only one major tautomer in the gas phase was found, tautomer **5_2**, and this did not change upon inclusion of the solvent. For both these compounds (**2** and **5**) it was not possible to evaluate the experimental chemical shifts in terms of the calculated chemical shifts to discern the state of the system and thus it must be concluded that both systems are comprised of multiple tautomers in fast exchange. Thus, the clear preference of two tautomers (in the case of **2**) and one tautomer (for **5**) provided by the energy calculations is at odds with the chemical shifts which are known to be reliable.[6b,9]

Fluorescence

Unlike natural DNA bases, 2-aminopurine is fluorescent at neutral pH and this native fluorescence has been demonstrated to be an extremely useful probe of, for example, DNA conformational changes and DNA base flipping.[14] On the other hand, the linear 1,N^2-ethenoguanosine, which resembles the product **4**, does not exhibit fluorescence at all.[2d]

We were interested in comparing the fluorescence characteristics of the isolated products **3**–**5** with those of 2,6-diaminopurine (**1**), and 2-aminopurine as references. 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 etheno products formed from compound **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 significantly from those of the reference compound 2-aminopurine but not much from the starting material **1**. The quantum yields of the products were considerably lower and the fluorescence lifetimes were also much shorter than for 2-aminopurine. The presence of etheno ring(s) did not have drastic effect on quantum yields or fluorescence lifetimes compared to the starting material **1**. For compound **3**, two lifetimes were

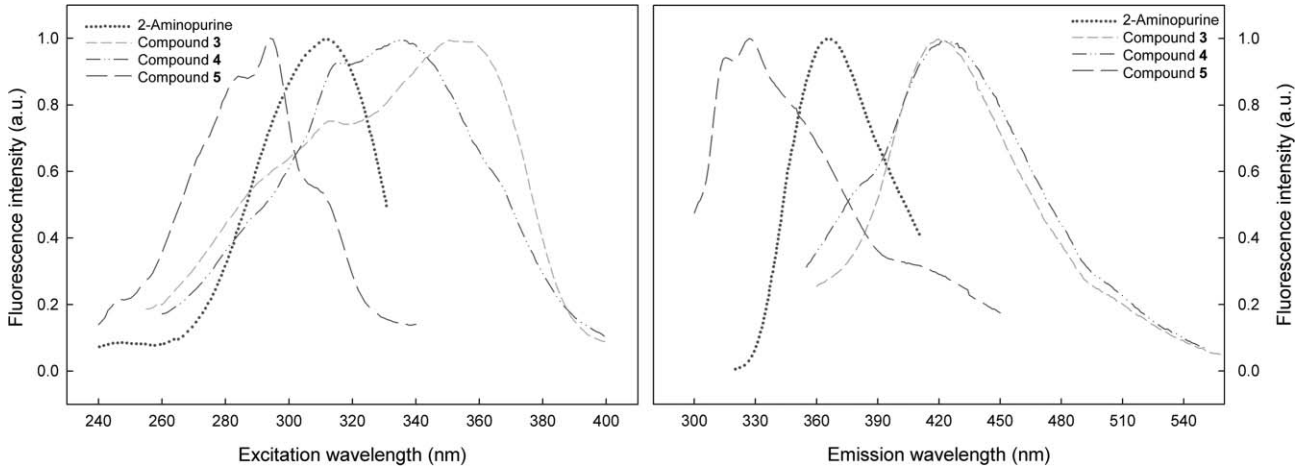


Fig. 2 Normalised excitation (left) and emission (right) spectra of the compounds **3**–**5** in water at 25 °C.

Table 1 Fluorescence parameters of the compounds in water at 25 °C[a]

Compound	Absorbance, $\lambda_{max}(H_2O)$/nm	Excitation, $\lambda_{max}(H_2O)$/nm	Emission, $\lambda_{max}(H_2O)$/nm	Quantum yield
1	280.3 ± 0.5	295.7 ± 0.9	349.7 ± 0.9	0.02 ± 0.01
2-Aminopurine	303.7 ± 2.6	312.0 ± 0.8	366.0 ± 1.0	0.50 ± 0.03
3	278.3 ± 1.2	353.3 ± 3.3	418.3 ± 1.7	0.02 ± 0.00
4	282.7 ± 1.2	334.7 ± 3.7	419.5 ± 2.5	0.01 ± 0.01
5	283.3 ± 0.5	294.0 ± 0.0	327.0 ± 0.0	0.03 ± 0.00

[a] The parameters are an average of at least three sets of experimental data. All experiments were performed using samples with an optical density < 0.1.

Table 2 Fluorescence decay parameters of the compounds in water at 25 °C[a]

Compound	Lifetime, τ/ns	χ^2	DW
1	1.73 ± 0.01	1.10 ± 0.12	1.95 ± 0.00
2-Aminopurine	10.85 ± 0.31	0.10 ± 0.05	1.98 ± 0.00
3	1.04 ± 0.05 5.81 ± 0.37	1.07 ± 0.05	1.85 ± 0.10
4	2.85 ± 0.37	1.06 ± 0.05	1.76 ± 0.02
5	1.19 ± 0.02	1.44 ± 0.27	1.93 ± 0.05

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

found, one of which was three times longer than the lifetime for the starting material 2,6-diaminopurine. These two lifetimes could be explained, for example, by two different tautomers of the compound **3**. When comparing the lifetimes and quantum yields of the etheno derivatives **3**–**5** it can be concluded that they are quite similar.

The emission maxima for compounds **3** and **4** were at longer wavelength, and for compound **5** at shorter wavelength, than the emission maximum for the starting material **1**. The excitation maxima for compounds **3** and **4** were also at longer wavelength, and for compound **5** close to the excitation maximum of the starting material. Therefore the Stokes shifts for products **3** and **4** were also slightly larger, and for product **5** slightly smaller than for the starting material 2,6-diaminopurine (**1**). For example, the Stokes shift for compound **4** was 85 nm, and for compound **5** it was 33 nm in comparison to the Stokes shift of 54 nm for the starting material.

The fluorescence measurement results showed that the formation of additional five-membered ring(s) to the 2,6-diaminopurine structure did not change the fluorescence properties dramatically and therefore the formation of a more conjugated double bond system did not enhance the fluorescence properties for these molecules. This could be explained by the fact that all of the products contain many tautomers in rapid exchange. Different tautomers have been shown to have different fluorescent charasteristics.[13,15]

Comparison of the data to those of 2-aminopurine shows that these molecules **3**–**5** are not fluorescent enough to be used as fluorescent probes.

Conclusions

The isolated yield for compound **4** (2.5%) was low and for compounds **3** (9%) and **5** (30%) moderate. Part of the starting material (**1**) was recovered unchanged from the reaction mixture. Yields of the products **3** and **4** in the reaction mixture were higher (*ca.* 15–20%), but isolation of the pure products was difficult. The yield of the product **5** increased as the reaction time increased to *ca.* 24 h, but essentially not after that. The separation of this tetracyclic product from the other products and starting material on Ca-enriched silica column, using dichloromethane/methanol as eluent, was good. The reactions were studied and tested under many different reaction conditions applying different reaction times, temperatures, reactant concentrations and solvents but no increase of the yields could be achieved.

One reason for the moderate yields and difficult purification could be the protonation of the starting material and products. Protonation of the products was first noticed when NMR proton shifts of the samples changed after normal silica column purification. Therefore all of the products had to be deprotonated with $NaHCO_3$ (to the MeOH solution of the product, $NaHCO_3$ powder was added and the mixture stirred for 5 min before the $NaHCO_3$ was filtered off) before characterisation, and for purification 0.1% Ca-enriched silica was used.

The fluorescence properties of the products were determined and compared with those of the starting material **1** and 2-aminopurine. The fluorescence properties of the products **3**–**5** did not differ considerably from those of compound **1** and were much lower than for 2-aminopurine. It can be concluded that the 'etheno' ring(s) system does not enhance the fluorescence characteristics of 2,6-diaminopurine products in the same way as they do in similar adenosine products.[1] Therefore it must be concluded that the conjugated double bond system and substituent effects of these product molecules are critically different compared to 2-aminopurine or ethenoadenosine.

Theoretical tautomeric equilibrium calculations for all products in the gas phase were performed in order to obtain information on the different tautomers present. It has to be noted that tautomers in the gas phase can differ from those present in DMSO as DMSO can form H-bonds with the molecules, and also for these kinds of compounds there is also the possibility for dimer formation.[12a]

Regarding the product distribution it may be concluded that the NH_2 group at position 2 in the purine ring system is more reactive than the NH_2 group at position 6 in the starting material 2,6-diaminopurine **1**. It might be then assumed that the four-ring product **5** is formed from product **3** as the reaction proceeds.

Experimental

Chemicals

2,6-Diaminopurine (**1**, 98%) was obtained from Adrich. Chloroacetaldehyde diethyl acetal (99%) was obtained from Acros Organics. The solvents for the synthesis were of analytical grade and for HPLC of commercial HPLC grade. 2-Aminopurine (98%) was obtained from MP Biomedicals (formerly ICN Biochemicals, Inc.).

Spectroscopic and spectrometric methods

The ^{1}H-, ^{13}C- ^{15}N- and 2D NMR spectra were recorded in 100% DMSO-d_6 at 18.5 and 25 °C. The NMR experiments were performed at 14.1 T using an NMR spectrometer equipped with a *z*-axis field gradient 5 mm inverse broadband probe operating at 600.13, 150.92 and 60.81 MHz for ^{1}H, ^{13}C and ^{15}N, respectively. Spectral widths for the 2D experiments were optimised from the 1D spectra and acquired with an appropriate level of resolution. Both ^{1}H and ^{13}C spectra were referenced to the solvent signal (2.50 and 39.51 ppm, respectively). All nitrogen shifts for the products were from HMBC spectra

and referenced externally to 90% nitromethane in CD_3NO_2 (0.00 ppm). The ^1H NMR signal assignments were based on chemical shifts and correlations from 2D ^1H–^1H, ^1H–^{13}C and ^1H–^{15}N correlation spectra. The assignment of carbon signals was based on the same techniques and carbon–proton coupling constants.

The electron impact high-resolution mass spectra (EI) were recorded on a 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 8000–10 000 (at 10% peak height).

The UV spectra of the compounds were recorded with a Shimadzu UV-160A spectrophotometer. The absorbance measurements (Table 1) for the compounds were obtained using a Varian Cary 50 Conc UV-visible spectrophotometer.

The fluorimetric properties of the compounds were studied at 25 °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 MΩ cm^{-1}.

The fluorescence quantum yield of the compounds was estimated by comparison with the known quantum yield of a standard.[16] 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 ± 0.02.[16,17]

In order to avoid inner filter effects the optical density was kept below 0.1 in all measurements. The quantum yield was calculated according to eqn. (1),

$$Q = Q_R\ (I/I_R)(OD_R/OD)(n^2/n_R{}^2) \quad (1)$$

where Q is the quantum yield, I is the integrated intensity OD is the optical density, and n is the refractive index.[16] 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).

Modelling

Geometry optimisations were performed using the Gaussian 98 program package[18] and either *ab initio* (at either the HF/6-311G** level of theory[19] or the MP2/6-31G** level of theory[20]) or density functional theory methods (at the B3LYP/6-311G** level of theory[21]). Additional calculations were also made including the effect of the solvent (SCIPCM method, dielectric constant = 46.7 for DMSO). The calculation of the energies and chemical shifts were both performed at the same level of theory in each case. The isotropic magnetic shieldings (σ) so obtained were computed as chemical shifts (δ) based on their difference from the shieldings of TMS (for ^{13}C) or nitromethane (for ^{15}N) and then calibrated based on the results for **1**. This has been found to be a reliable method for the calculation of chemical shifts for these nuclei.[6b,9] Various platforms were used for the calculations, *e.g.* an SGI Octane workstation or Linux cluster.

Chromatographic methods

HPLC analyses were carried out using an Agilent Technologies 1100 series liquid chromatographic system consisting of an autosampler, degasser, quaternary pump, diode array detector (UV), a Jasco FP-920 fluorescence detector (Jasco), and a ChemStation data handling program. The chromatographic separations were performed on an analytical 5 μm, 4.6 mm × 125 mm Zorbax SB-CN column (Agilent Technologies). The column was eluted isocratically for 5 min with 0.01 M acetate buffer (pH 7.1) and then with a gradient from 1 to 30% acetonitrile over the course of 15 min at a flow rate of 1.0 mL min^{-1}.

Compounds **3**–**5** were isolated from the reaction mixtures by flash column chromatography, initially with a Ca-enriched silica gel 60 (Fluka) column and afterwards with a 40 μm C-18 reversed-phase column. The products were eluted over silica using a dichloromethane/methanol gradient containing 2% triethylamine and over reversed-phase silica using a water/acetonitrile gradient.

Synthesis

5-Aminoimidazo[2,1-*i*]purine (2), 9-aminoimidazo[2,1-*b*]purine (3), 9-aminoimidazo[1,2-*a*]purine (4) and diimidazo[2,1-*b*:2′,1′-*i*]purine (5). Chloroacetaldehyde diethyl acetal (1.0 mL, 6.7 mmol) was added to 0.1 M HCl (5.0 mL) and stirred at 80 °C for *ca.* 1 h. The clear solution containing the chloroacetaldehyde was then added to a solution of 2,6-diaminopurine (0.50 g, 3.3 mmol) in DMF (*ca.* 10 mL). The pH was adjusted to 4–5 and the reaction mixture was stirred at 80 °C for *ca.* 24 h. The pH was maintained at 4–5 by the appropriate addition of either 1.0 M NaOH or 1.0 M HCl. Upon completion of the reaction, the solution was neutralised with $NaHCO_3$ (aq.). The mixture was evaporated to dryness and crude purified using a Ca-enriched silica column and then further purified using a reversed-phase C-18 column to yield pure **3** (52 mg, 9%), **4** (10 mg, 2,5%) and **5** (200 mg, 30%) as white powders.

5-Aminoimidazo[2,1-*i*]purine (2). δ_H(600.13 MHz; DMSO) 7.36 (2 H, s, NH_2), 7.38 (1 H, s, $J_{8,7}$ = 1.6 Hz, H-8), 7.90 (1 H, d, $J_{7,8}$ = 1.6 Hz, H-7), 8.02 (1 H, s, H-2), H-1/H-3 not observed; δ_C(150.92 MHz; DMSO) 104.3 (C-7), 108.9 (C-10b), 131.8 (C-8), 140.5 (C-5), 141.6 (C-2), 149.3 (C-10a), 151.8 (C-3a); δ_N(60.81 MHz; DMSO) −307 (*N*H_2), −220 (N-1/N-3), −209 (N-6), −163 (N-9), −148 (N-1/N-3).

9-Aminoimidazo[2,1-*b*]purine (3). δ_H(600.13 MHz; DMSO) 6.13 (2 H, s, NH_2), 7.00 (1 H, d, $J_{6,5}$ = 1.5 Hz, H-6), 7.34 (1 H, s, H-2), 7.39 (1 H, d, $J_{5,6}$ = 1.5 Hz, H-5), H-1/H-3 not observed; δ_C(150.92 MHz; DMSO) 104.5 (C-5), 115.6 (C-9a), 127.3 (C-6), 144.0 (C-3a), 145.7 (C-2), 148.5 (C-7a), 151.9 (C-9); δ_N(60.81 MHz; DMSO) −308 (*N*H_2), −212 (N-4), −191 (N-8), −162 (N-7), −151, −143 (sp^2-hybridised N-1/N-3), sp^3-hybridised N-3/N-1 not observed. *m/z* (EI) 174.0654 (M^+, $C_7H_6N_6$ requires 174.0654). UV: $\lambda_{max}(H_2O)$/nm 220 and 276 (ε/dm^3 mol^{-1} cm^{-1} 2260 and 1150); $\lambda_{min}(H_2O)$/nm 252 (ε/dm^3 mol^{-1} cm^{-1} 770).

9-Aminoimidazo[1,2-*a*]purine (4).

Major tautomer. δ_H(600.13 MHz; DMSO) 7.38 (1 H, s, H-6), 7.41 (2 H, s, NH_2), 7.81 (1 H, s, H-2), 7.92 (1 H, s, H-7), 12.68 (1 H, s, H-1); δ_C(150.92 MHz; DMSO) 109.4 (C-7), 115.5 (C-9a), 131.2 (C-6), 135.1 (C-2), 141.2 (C-3a), 142.2 (C-4a), 144.7 (C-9); δ_N(60.81 MHz; DMSO) −306 (*N*H_2), −223 (N-1), −202 (N-8), −150 (N-5), −138 (N-3).

Minor tautomer. δ_H(600.13 MHz; DMSO) 7.19 (2 H, s, NH_2), 7.44 (1 H, s, H-6), 7.94 (1 H, s, H-7), 7.97 (1 H, s, H-2), 13.20* (1 H, s, H-3); δ_C(150.92 MHz; DMSO) 109.8 (C-7), 115.5 (C-9a), 131.2 (C-6), 137.9 (C-2), 138.7 (C-4a), 143.9 (C-9), 149.4 (C-3a); δ_N(60.81 MHz; DMSO) −309 (*N*H_2), −231 (N-3)*, −226 (N-8), −180 (N-5). *m/z* (EI) 174.0648 (M^+, $C_7H_6N_6$ requires 174.0654). UV: $\lambda_{max}(H_2O)$/nm 214 and 277 (ε/dm^3 mol^{-1} cm^{-1} 2180 and 1010); $\lambda_{min}(H_2O)$/nm 247 (ε/dm^3 mol^{-1} cm^{-1} 590). *Assuming the minor tautomer to be the N-3.

Diimidazo[2,1-*b*:2′,1′-*i*]purine (5). δ_H(600.13 MHz; DMSO) 7.15 (1 H, d, $J_{6,5} = 1.5$ Hz, H-6), 7.23 (1 H, d, $J_{10,9} = 1.4$ Hz, H-10), 7.30 (1 H, s, H-2), 7.70 (1 H, d, $J_{5,6} = 1.5$ Hz, H-5), 7.81 (1 H, d, $J_{9,10} = 1.4$ Hz, H-9), H-1/H-3 not observed; δ_C(150.92 MHz; DMSO) 109.1 (C-9), 109.4 (C-5), 116.0 (C-12b), 126.4 (C-6), 129.8 (C-10), 136.2 (C-7a), 137.6 (C-3a), 141.4 (C-12a), 144.9 (C-2); δ_N(60.81 MHz; DMSO) −215 (N-4), −213 (N-8), −164 (N-7), −153 (N-11), −150, −137 (sp^2-hybridised N-1/N-3), sp^3-hybridised N-3/N-1 not observed. *m/z* (EI) 198.0658 (M^+, $C_9H_6N_6$ requires 198.0654). UV: $\lambda_{max}(H_2O)$/nm 234, 283 and 295 (ε/dm^3 mol^{-1} cm^{-1} 7840, 3680 and 2690); $\lambda_{min}(H_2O)$/nm 252 (ε/dm^3 mol^{-1} cm^{-1}1840).

Acknowledgements

We are grateful to Mr Markku Reunanen for recording the mass spectra. Professor J. Peter Slotte is acknowledged for access to the fluorescence facility, and Dr Thomas Nyholm is acknowledged for assistance. Financial support from the Graduate School of Bioorganic and Medicinal Chemistry (P. V.), Academy of Finland, Sigrid Jusélius Foundation, Magnus Ehrnrooth Foundation, Medicinska understödsföreningen Liv och Hälsa, K. Albin Johansson Foundation (P. M.) is gratefully acknowledged.

References

1 J. R. Barrio, J. A. Secrist III and N. J. Leonard, *Biochem. Biophys. Res. Commun.*, 1972, **46**(2), 597–604; S. C. Srivastava, S. K. Raza and R. Misra, *Nucleic Acid Res.*, 1994, **22**, 1296–1304.
2 (*a*) S. Mikkola, N. Koissi, K. Ketomäki, S. Rauvala, K. Neuvonen and H. Lönnberg, *Eur. J. Org. Chem.*, 2000, 2315–2323; (*b*) R. Khazanchi, P.-L. Yu and F. Johnson, *J. Org. Chem.*, 1993, **58**(9), 2552–2556; (*c*) J. T. Kusmierek, D. E. Jensen, S. J. Spengler, R. Stolarski and B. Singer, *J. Org. Chem.*, 1987, **52**(12), 2374–2378; (*d*) P. D. Sattsangi, N. J. Leonard and C. R. Frihart, *J. Org. Chem.*, 1977, **42**(20), 3292–3296; (*e*) P. D. Sattsangi, J. R. Barrio and N. J. Leonard, *J. Am. Chem. Soc.*, 1980, **102**, 770–774.
3 G. A. Pandya and M. Moriya, *Biochemistry*, 1996, **35**, 11 487–11 492.
4 J. A. Secrist, J. R. Barrio, N. J. Leonard and G. Weber, *Biochemistry*, 1972, **11**(19), 3499–3506.
5 P. Virta, T. Holmström, T. Munter, T. Nyholm, L. Kronberg and R. Sjöholm, *Nucleosides Nucleotides Nucl. Acids*, 2003, **22**(1), 85–97; P. Virta, T. Holmström, M. U. Roslund, P. Mattjus, L. Kronberg and R. Sjöholm, *Org. Biomol. Chem.*, 2004, **2**(6), 821–827.
6 (*a*) J. Mäki, K. D. Klika, R. Sjöholm and L. Kronberg, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1216–1219; (*b*) J. Mäki, P. Tähtinen, L. Kronberg and K. D. Klika, *J. Phys. Org. Chem.*, 2005, **18**, 240–249.
7 R. J. Sijbesma and E. W. Meijer, *Chem. Commun.*, 2003, 5–16.
8 M. U. Roslund, P. Virta and K. D. Klika, *Org. Lett.*, 2004, **6**(16), 2673–2676.
9 P. Tähtinen, A. Bagno, K. D. Klika and K. Pihlaja, *J. Am. Chem. Soc.*, 2003, **125**, 4609–4618; K. D. Klika, J. Imrich, I. Danihel, S. Böhm, P. Kristian, S. Hamul'aková, K. Pihlaja, A. Koch and E. Kleinpeter, *Magn. Reson. Chem.*, 2005, **43**, 380–388; E. Kleinpeter and A. Koch, *J. Phys. Org. Chem.*, 2001, **14**, 566–576.
10 J. Arpalahti and K. D. Klika, *Eur. J. Inorg. Chem.*, 1999, 1199–1201.
11 A. Laxer, D. T. Major, H. E. Gottlieb and B. Fischer, *J. Org. Chem.*, 2001, **66**, 5463–5481.
12 (*a*) R. Marek, personal communication; (*b*) I. Alkorta and J. Elguero, *Struct. Chem.*, 2003, **14**(4), 377–389.
13 S. K. Mishra, M. K. Shukla and P. C. Mishra, *Spectrochim. Acta, Part A*, 2000, **56**, 1355–1384.
14 J. Fujimoto, Z. Nuesca, M. Mazurek and L. C. Sowers, *Nucleic Acids Res.*, 1996, **24**(4), 754–759; B. Holz, S. Klimasauskas, S. Serva and E. Weinhold, *Nucleic Acids Res.*, 1998, **26**(4), 1076–1083.
15 R. W. Wilson and P. R. Callis, *Photochem. Photobiol.*, 1980, **31**, 323–327.
16 *Principles of Fluorescence Spectroscopy*, J. R. Lakowicz, ed., Kluwer Academic/Plenum Publishers, New York, 1999, 2nd edn.
17 M. J. Adams, J. G. Highfield and G. F. Kirkbright, *Anal. Chem.*, 1977, **49**(12), 1850–1852.
18 Gaussian 98 (Revision A.12), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andrés, C. Gonzalez, M. Head-Gordon, E. S. Replogle and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 2002.
19 W. J. Hehre, L. Random, P. V. R. Schleyer and J. A. Pople, *Ab Initio Molecular Orbital Theory*, John Wiley & Sons Inc., New York, 1986.
20 C. Møller and M. S. Plesset, *Phys. Rev.*, 1934, **46**, 618–622.
21 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.