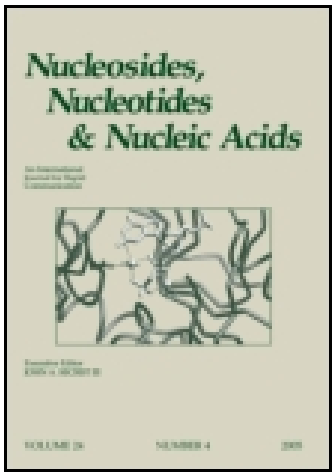

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Fluorescent 7- and 8-Methyl Etheno Derivatives of Adenosine and 6-Amino-9-ethylpurine: Syntheses and Fluorescence Properties

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Published online: 24 Jun 2011.

To cite this article: Piritta Virta, Toni Holmström, Tony Munter, Thomas Nyholm, Leif Kronberg & Rainer Sjöholm (2003) Fluorescent 7- and 8-Methyl Etheno Derivatives of Adenosine and 6-Amino-9-ethylpurine: Syntheses and Fluorescence Properties, Nucleosides, Nucleotides and Nucleic Acids, 22:1, 85-98, DOI: 10.1081/NCN-120018625

To link to this article: http://dx.doi.org/10.1081/NCN-120018625

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, No. 1, pp. 85–98, 2003

Fluorescent 7- and 8-Methyl Etheno Derivatives of Adenosine and 6-Amino-9-ethylpurine: Syntheses and Fluorescence Properties

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ABSTRACT

Two fluorescent adenosine derivatives (**5** and **7**) (Sch. 1) and two 6-amino-9-ethylpurine derivatives (**6** and **8**) (Sch. 1), were synthesised using 2-chloropropanal and 3-chloropropyne as reagents. The structures of the products were determined by spectroscopic and spectrometric methods (^{1}H-, ^{13}C- and 2D NMR, MS, UV and fluorescence spectrometry). Their fluorescence properties were determined and found to be similar to those of ethenoadenosine. Also, the stabilites of **5** and **7** in aqueous solutions were determined and found to be higher than that of the etheno derivative of adenosine.

Key Words: Nucleosides; Fluorescence.

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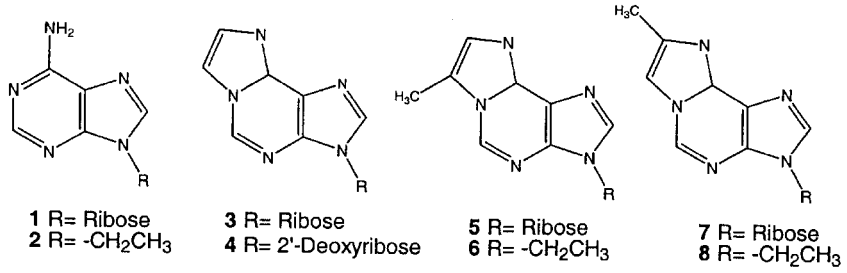
#Fluorescence measurements.

DOI: 10.1081/NCN-120018625
1525-7770 (Print); 1532-2335 (Online)



Scheme 1.

INTRODUCTION

Flourescence based techniques are powerful tools for investigation of biological material. The sensitivity of fluorescence spectroscopy is several orders of magnitude better than that of UV detection and in some cases even superior to radioactivity based techniques. Fluorescence can provide information on the structure, distance, orientation, complexation and location of biomolecules. In addition, time-resolved methods are used in measurements of molecular dynamics and kinetics. Fluorescent oligonucleotides and nucleic acids are important tools in molecular biology, diagnostics and structural studies.[1] In order to make oligonucleotides and nucleic acids fluorescent several methods have been developed. Most of the methods are based on the covalent attachment of a fluorescent dye molecule to the nucleoside either at the base or the sugar moiety.[2]

Unlike radioisotopes, dyes may have a negative influence on the structure and mobility of oligonucleotides and nucleic acids. Even small changes in the conformation of a nucleic acid can lead to reduced specificity of hybridisation with its target. One way to minimise this problem is to use only slightly modified fluorescent nucleoside analogs as starting materials for the synthesis of oligonucleotides and nucleic acids with fluorescent properties. Ideally, a fluorescent nucleoside analog should closely resemble the naturally occurring purine or pyrimidine base structure, especially at hydrogen bonding sites critical to base pairing. It should also have reasonable fluorescence under physiological conditions. That is, the developed fluorescent structures should as far as possible chemically and physically resemble their natural counterparts.[3]

The most attractive approach is the use of nucleosides which have been made fluorescent by only slightly modifying their structure. However, in the literature there are only a few examples of such compounds.[4] The ''etheno-nucleosides'', represent fluorescent nucleosides synthesised from non-fluorescent starting materials. They are formed when adenosine, cytidine or guanosine reacts with chloroacetaldehyde.[4a,5] The etheno derivatives are commercially available as phosphoramidites. The major drawback of the etheno derivatives is their gradual degradation upon long-term storage in aqueous media.[6] However, we found that methyl substituted ethenoderivatives posses more favourable stability properties than the unsubstituted ones.

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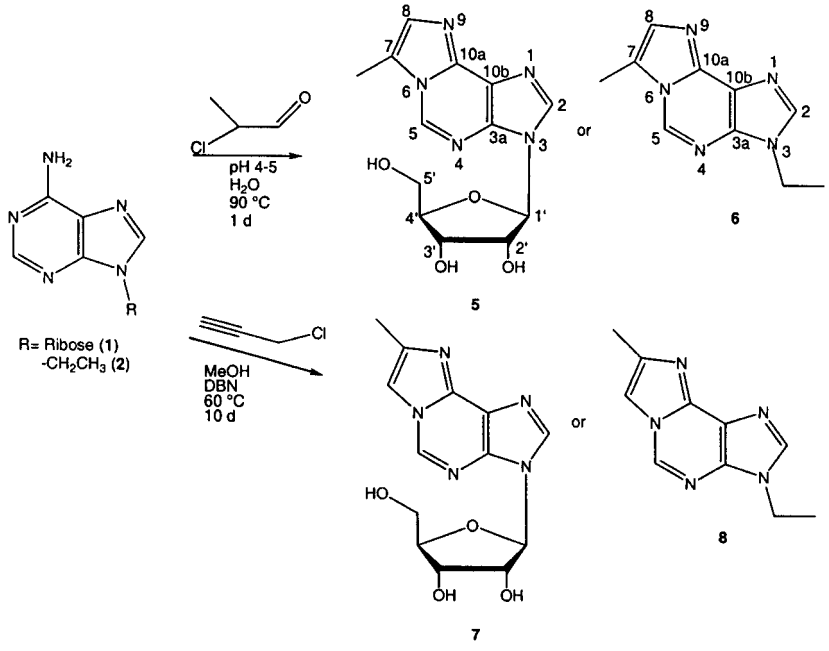
The synthesis of methyletheno derivatives using 2-bromopropanal was reported 20 years ago by Krzyzosiak *et al*. However, the fluorescence properties and the stability of the products were not studied. Complete ^{1}H- and ^{13}C NMR data were not reported either.[5c] The reaction with 2-chloropropanal and adenosine has also been performed previously but the authors did not determine the structure of the product.[7] In this paper, we report the synthesis and complete characterisation of two fluorescent adenosine derivatives (**5** and **7**) (Sch. 1) and the corresponding 6-amino-9-ethylpurine derivatives (**6** and **8**) (Sch. 1). Their fluorescence properties and stability data for compounds **5** and **7** are also given.

RESULTS AND DISCUSSION

The compounds were synthesised according to the reactions shown in Sch. 2.

The yields of the products (**5**–**8**) from 2-chloropropanal and 3-chloropropyne reactions with **1** and **2** (Sch. 1) were about 10%. Most of the starting material was recovered unchanged. It was found in both reactions, that besides the main product the other isomer (7-methyl etheno for **7** and **8** and 8-methyl etheno for **5** and **6**) was formed in very small amounts. It has been shown that in the corresponding derivatives of 2-aminopyrimidine a Dimroth rearrangement takes place leading to isomerisation.[5d] However, the methyletheno derivatives of adenine (**5** and **6**) were found



Scheme 2.

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no to undergo isomerisation to **7** and **8** upon storage for two months in aqueous solutions at room temperature.

The reactions were first performed with **2** as the starting material and after its successful reaction, the same reaction was performed with **1**.

The reactions were studied and tested in many different reaction conditions applying different reaction times, temperatures (e.g., room temperature, 50°C, 100°C) and solvents (e.g., water, methanol, ethanol, DMF, 2-methoxyethanol, acetate buffer pH 4.5). The best method for the synthesis of **5** and **6**, was to use water (pH 4-5) as solvent, a temperature of 90°C and 1d reaction time. The best method for the synthesis of **7** and **8** was to use methanol as solvent, a temperature of 60°C and 10 d reaction time. The same product was also obtained using chloroacetone as reagent (water solution pH 4-5), but the yield was not any higher. The details of the procedures are described in the Experimental Section.

The progress of the reactions was monitored by HPLC analysis on a reversed phase (C 18) column. The products were isolated by preparative reversed phase chromatography and characterised by UV absorption (etheno derivatives of adenosine have a characteristic UV absorption spectrum[8a]), ^{1}H-, ^{13}C-, and 2D NMR spectroscopy, electrospray mass spectrometry and fluorescence spectroscopy. The fluorescence spectroscopic data are presented in Fig. 1 and Tables 1 and 2. The NMR spectroscopic and the mass spectrometric data are given in the Experimental Section.

The formation of these products may be rationalised by the mechanisms discussed.

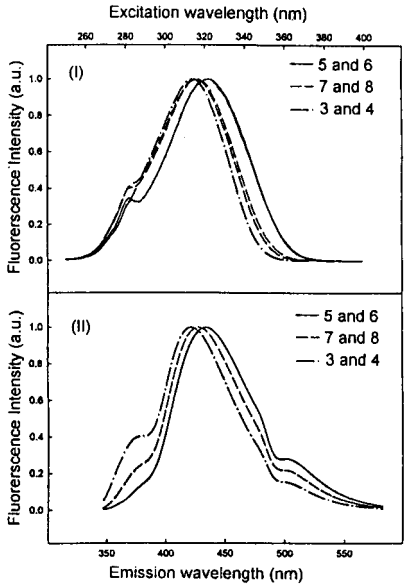

Figure 1. Normalised excitation (I) and emission (II) spectra of the **5**–**8** in water at 23°C. **3** and **4** were used as reference molecules.

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

Sample	Excitation max (nm)	Emission max (nm)	Quantum yield ϕ
3	314 ± 0.5	422 ± 0.8	0.540 ± 0.028
4	313 ± 1.0	422 ± 0.5	0.553 ± 0.041
5	322 ± 0.1	432 ± 0.7	0.319 ± 0.020
7	316 ± 0.3	428 ± 0.5	0.438 ± 0.024
6	322 ± 0.1	433 ± 0.8	0.329 ± 0.013
8	316 ± 0.5	427 ± 0.8	0.474 ± 0.035

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

Mechanisms

The formation of etheno adenosine from adenosine on treatment with chloroacetaldehyde has been described in the literature.[5a,5b,8] The formation of **5** and **6** from 2-chloropropanal follows a similar mechanism.[5c]

The reaction is initiated by attack of the exocyclic amino group of the nucleobase on the carbonyl carbon of 2-chloropropanal. Subsequently, the chlorine is displaced by intramolecular nucleophilic attack of the ring nitrogen. Finally, the product is obtained through dehydration of the cyclic intermediate (Sch. 3).

Based on the structures of the reaction products a mechanism for the formation of compounds **7** and **8** from 3-chloropropyne is suggested in Sch. 4.

A reaction involving an allylic rearrangement is probable and the first step results in an allene derivative, which then forms the product by cyclisation.

Fluorescence Properties

The fluorescence properties for the four products (**5**–**8**) were measured and compared with commercially available ethenoadenosine (**3**) (Sch. 1) and ethendeoxyo-

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

Molecule	Lifetimes (ns) τ	χ^2	DW
3	23.77 ± 0.17	1.01	1.98
4	23.17 ± 0.16	1.06	1.59
5	17.03 ± 0.08	0.944	1.78
7	20.34 ± 0.17	0.758	1.62
6	16.84 ± 0.08	0.954	2.67
8	21.27 ± 0.01	0.895	2.09

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

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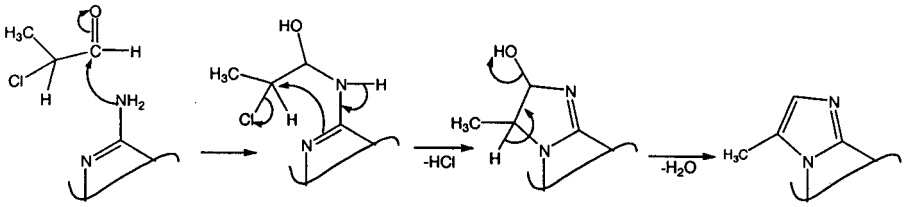
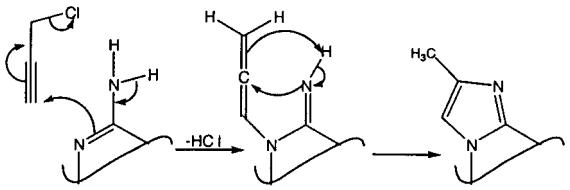


Scheme 3.



Scheme 4.

adenosine (**4**) (Sch. 1) (Fig. 1, Tables 1 and 2). The fluorescence excitation maxima of **3** and **4** were observed at 314 and 313 nm, respectively and fluorescence emission maxima at 422 nm (Table 1). The maxima were the same as those reported on the ethenoadenosine.[8a] The methyl-etheno derivatives have fluorescence excitation maxima at 322 (**5** and **6**) and 316 nm (**7** and **8**) and fluorescence emission maxima at 432 (**5**), 433 (**6**), 428 (**7**) and 427 nm (**8**) (Table 1). This shows that the Stokes shift in methyl-etheno derivatives (**5**–**8**) is slightly larger than in the etheno derivatives (**3** and **4**). That is, Stokes shifts are from 10 to 12 nm in **5** to **8** and, 8 and 9 nm in **3** and **4**.

The methyl-etheno derivatives (**5** to **8**) have lower fluorescence quantum yields (ϕ 0.319–0.474) and shorter lifetimes (16.84–21.27 ns) than ethenoadenosine and ethenodeoxyadenosine (ϕ 0.540–0.553 and 23.17–23.77 ns) (Tables 1 and 2). The results show that the type of sugar moiety does not have much effect on the fluorescence lifetimes (Table 2). On the other hand, molecules with an ethyl group instead of the sugar group (**6** and **8**) have higher fluorescence quantum yield (Table 1).

Stability Tests of 5 and 7

The stability of **5** and **7** was determined and compared with the stability of ethenoadenosine (**3**). It was found that **5** and **7** were most stable at basic (pH 9.0) conditions than the unsubstituted etheno derivatives. At these conditions **7** was found to be the most stable compound. At neutral conditions (pH 7.4), all tested compounds were quite stable, but again **7** was the most stable one. At acidic conditions (pH 4.6) all compounds were more stable than at basic conditions after 8 d, but less stable than at neutral conditions after 32 d. It can be concluded that both **5** and **7** have

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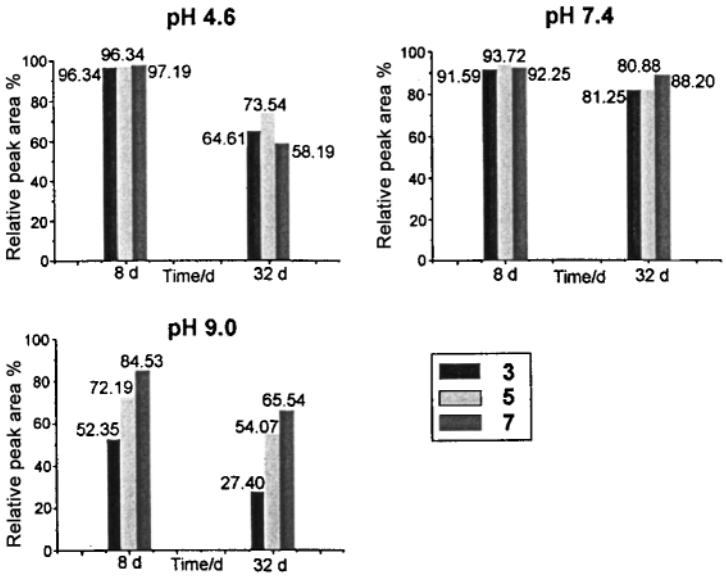

Figure 2. Relative amounts of the tested compounds **3**, **5**, and **7** after 8 d and 32 d. The Y-axis shows the relative area of the peak observed in the UV-chromatogram at the wavelength 254 nm, and the X-axis shows the time.

comparable or higher stability than **3** and that **7** is the most stable one at neutral or alkaline conditions (Fig. 2).

It is known that the etheno derivatives of adenosine are not "fully" stable in water solutions. The major degradation products have been identified as bi-imidazoles.[9]

NMR Parameters of 5 and 7

The ^{1}H NMR spectrum of **5** displayed besides the signals from the protons of the ribose moiety, one-proton singlets at $\delta = 8.56$ (H-2) and 9.06 ppm (H-5) and a one-proton doublet at $\delta = 7.31$ ppm (H-8). The ^{1}H NMR spectrum of **7** displayed besides the ribose moiety signals one-proton singlets at $\delta = 8.57$ (H-2) and 9.20 ppm (H-5) and a one-proton quartet at $\delta = 7.80$ ppm (H-7). The three-proton doublets for the methyl group appeared at $\delta = 2.57$ ppm in **5** and at δ 2.38 ppm in **7**. The ^{1}H NMR spectra of the two isomers differed in many respects. In the ^{1}H NMR spectrum of **5** the signal of H-5 was shifted upfield (in comparison to **7**), possibly due to the inductive effect of the methyl group at C-7. Moreover, the signals of the protons of the methyl group were shifted downfield, probably due to steric compression.

The ^{13}C NMR spectrum of **5** showed besides the signals from purine and ribose moieties, carbon signals at $\delta = 120.16$ (C-7) and 130.19 ppm (C-8). In the spectrum of **7**, the same carbons were observed at 108.41 and 142.17 ppm, respectively. The steric

compression observed in the ^{1}H NMR spectrum is also observed in the relative ^{13}C NMR shifts of the methyl carbons of the two isomers ($\delta = 8.81$ ppm for **5** and $\delta = 14.12$ ppm for **7**).

In the H-H correlation spectra both isomers displayed connection between the methyl protons and H-5. Conclusive evidence for the structures was obtained from the NOESY spectra. A strong methyl – H-5 interaction was observed in the spectrum of **5** while this interaction was absent in the spectrum of **7**.

CONCLUSIONS

In conclusion, two adenosine and two 3-amino-9-ethylpurine derivatives have been synthesised. The compounds **5** and **6** were formed in the reaction of **1** and **2** with 2-chloropropanal, and **7** and **8** were formed in the reaction of **1** and **2** with 3-chloropropyne. The syntheses were straightforward but the yields were quite poor and most of the starting materials were recovered unchanged in the reaction mixture. The products have been identified and their structures determined. The fluorescent properties and relative stabilities of the products were determined and compared with commercially available ethenoadenosine (**3**) and ethenodeoxyadenosine (**4**). The fluorescence properties (quantum yields and lifetimes) are somewhat lower for the methyl-etheno derivatives than for the corresponding etheno derivatives (**3** and **4**), but otherwise quite similar to those of **3** and **4**. The results show that the methyl-etheno derivatives are more stable at basic conditions and that **5** is more stable at acidic conditions than the unsubstituted ethenoadenosine (**3**).

EXPERIMENTAL

Chemicals

Adenosine (**1**), 2-chloropropionaldehyde dimethyl acetal and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) were obtained from Fluka AG (Buchs, Switzerland). 6-Amino-9-ethylpurine (**2**) was synthesised according to a known method.[10] 3-Chloropropyne (propargyl chloride) was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA). The solvents were of analytical grade. The solvents for HPLC were of commercial HPLC grade.

Spectroscopic and Spectrometric Methods

The ^{1}H-,^{13}C- and 2D NMR spectra were recorded in DMSO or $CDCl_3$ at 30°C on a JEOL JNM A 500 Fourier transform NMR spectrometer (JEOL, Tokyo, Japan) at 500.16 and 125.78 MHz, respectively. The ^{1}H NMR signal assignments were based on chemical shifts and H-H and C-H correlation data. The assignment of carbon signals was based on chemical shifts, C-H correlations, and carbon-proton coupling constants.

The electrospray ionisation mass spectra (EI-MS) and the high resolution mass spectra (HRMS) were recorded on Fisons ZABSpec-oaTOF instrument

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(Manchester, U.K.). Ionisation was carried out using nitrogen as both the nebulizing and bath gas. A potential of 8.0 kV was applied to the ESI needle. The temperature of the pepperpot counter electrode was 90°C. The isolated component was introduced by loop injection at a flow rate of 20 μL/min (80/20/1 H_2O/CH_3CN/acetic acid). Poly(ethyleneglycol) (PEG) was used as the standard for the exact mass determination. The mass spectrometer was working at a resolution of 7000. The UV spectra of the isolated compounds were recorded with a diode-array detector as the peaks eluted from the HPLC column.

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 resistivity of 18.2 MΩcm.

The fluorescence quantum yield of the compounds was estimated by comparison with the known quantum yield of a standard.[11] 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.[11,12]

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,

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

where Q is the quantum yield, I is the integrated intensity, OD is the optical density, and n is the refractive index.[11] 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. Analysis 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) (Kontron Instruments S. P. A., Milan, Italy), a Jasco FP-920 fluorescence detector (Jasco Corporation, Tokyo, Japan), and a Kromasystem 2000 data handling program (Kontron Instruments S. P. A., Milan, Italy). The chromatographic separations were performed on an analytical 5 μm, 4 mm × 125 mm reversed phase C 18 column (Hypersil BDS-C18, Hewlett Packard, Espoo/Esbo, Finland). 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 50% acetonitrile over the course of 25 min at a flow rate of 1 mL/min.

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The products were isolated from the reaction mixtures by chromatography on a manually packed 40 μm, 4 cm × 8 cm C 18 preparative column (Bondesil, Analytichem International, Harbor City, California, USA). The column was eluted with 100 ml of water and then with 100 mL of 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 25% and 40% acetonitrile solutions in water.

The Stability Determinations

The stability determinations were done at 37°C at three different pH conditions (4.6, 7.4 and 9.0), using 0.1 M phosphate buffer as the solvent. The degradation of methyl-etheno products of adenosine (**5** and **7**) and ethenoadenosine (**3**) were monitored by HPLC using the analytical RP C 18 column.

Preparation of 7-Methyl-3-(β-D-ribofuranosyl) imidazo[2,1-*i*]purine (5). 2-Chloropropionaldehyde dimethyl acetal (6.3 mL, 48 mmol) was hydrolysed with 0.1 M HCl (150 mL) at 90°C for 30 min. The pH was adjusted to 4–5 with 4 M sodiumhydroxide and adenosine (**1**) (6.5 g, 24 mmol) was added to solution. The reaction was allowed to proceed for 1 d at 90°C. The solution was cooled down to room temperature and then evaporated to dryness at 40°C in a rotary evaporator. The product was isolated from the reaction mixture by preparative reversed phase chromatography. The fractions containing the product were combined and evaporated to dryness. The product was obtained as a white solid. The yield was ca. 10%. The purified product had the following spectral characteristics: UV_{max} 230 nm, 268 nm, 278 nm and 301 nm, UV_{min} 250 nm and 289 nm (HPLC eluent, 26% acetonitrile in 0.01 M phosphate buffer, pH 7.1). The fluorescence excitation and emission maxima were observed at 322 nm and 432 nm, respectively.

In the positive ion electrospray mass spectra (EI-MS), the following ions were observed [IP 70 eV; m/z (% r. a.)]: 305 (10, M^+), 173 (100, M^+ -Rib). High resolution mass spectrometry (HRMS) gave the protonated molecular formula as $C_{13}H_{15}N_5O_4$ (MH^+ 305.1124 $gmol^{-1}$, calculated mass 305.1124 $gmol^{-1}$). The fluorescence characteristic data of **5** are presented in Tables 1 and 2 and Fig. 2.

^{1}H NMR (500.16 MHz, DMSO) : δ = 9.06 (s, 1H, H-5), 8.56 (s, 1H, H-2), 7.31 (d, 1H, H-8, J = 1.0 Hz), 6.07 (d, 1H, H-1′, J = 5.6 Hz), 5.55 (d, 1H, 2′-OH, J = 5.7 Hz), 5.26 (d, 1H, 3′-OH, J = 5.0 Hz), 5.10 (t, 1H, 5′-OH, J = 5.5 Hz), 4.61 (t, 1H, H-2′, J = 5.3 Hz), 4.21 (q, 1H, H-3′, J = 4.1 Hz), 4.00 (q, 1H, H-4′, J = 4.0 Hz), 3.72 (dd, 1H, H-5′, J = 11.9 and 4.6 Hz), 3.61 (ddd, 1H, H-5″, J = 11.9; 5.5 and 4.3 Hz), 2.57 (d, 3H, -C$\underline{H}_3$, J = 1.0 Hz).

^{13}C NMR (125.78 MHz, DMSO) : δ = 140.14 (ddd, C-10a, $^{>1}J_{C,H}$ 11; 5 and 1 Hz), 139.81 (dd, C-2, $^1J_{C,H}$ 214 Hz, $^{>1}J_{C,H}$ 4 Hz), 138.02 (ddd, C-3a, $^{>1}J_{C,H}$ 12; 6 and 3 Hz), 135.42 (d, C-5, $^{>1}J_{C,H}$ 214 Hz, $^{>1}J_{C,H}$ 1 Hz), 130.19 (dq, C-8, $^1J_{C,H}$ 187 Hz, $^{>1}J_{C,H}$ 4 Hz), 122.92 (d, C-10b, $^{>1}J_{C,H}$ 11 Hz), 120.16 (dq, C-7, $^{>1}J_{C,H}$ 16 and 7 Hz), 87.80 (d, C-1′, $^1J_{C,H}$ 166 Hz), 85.56 (d, C-4′, $^1J_{C,H}$ 149 Hz), 74.11 (d, C-2′, $^1J_{C,H}$ 147 Hz), 70.31 (d, C-3′, $^1J_{C,H}$ 148 Hz), 61.32 (t, C-5′, $^1J_{C,H}$ 140 Hz), 8.81 (q, -$\underline{C}H_3$, $^1J_{C,H}$ 129 Hz).

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Preparation of 3-Ethyl-3*H*-7-methyl imidazole[2,1-*i*]purine (6). Compound **6** was prepared as described for **5**. 2-Chloropropionaldehyde dimethylacetal (2.4 mL, 18 mmol), 0.1 M HCl (60 mL) and 6-amino-9-ethylpurine (**2**) (1.5 g, 9.1 mmol). The product was obtained as a white solid. The yield was ca. 10%. The purified product had the following spectral characteristics: UV_{max} 232 nm, 270 nm, 280 nm and 301 nm, UV_{min} 252 nm and 289 nm (HPLC eluent, 32% acetonitrile in 0.01 M phosphate buffer, pH 7.1). Fluorescence excitation and emission maxima were observed at 322 nm and 433 nm, respectively.

In EI-MS the following ions were observed [IP 70 eV; m/z (% r. a.)]: 201 (100, M^+), 186 (4, M^+-Me), 172 (20, M^+-Et). HRMS gave the protonated molecular formula as $C_{10}H_{11}N_5$ (MH^+ 201.1016 $gmol^{-1}$, calculated mass 201.1014 $gmol^{-1}$).

^{1}H NMR (500.16 MHz, DMSO) : $\delta = 9.00$ (s, 1H, H-5), 8.31 (s, 1H, H-2), 7.28 (d, 1H, H-8, $J = 1.2$ Hz), 4.37 (q, 2H, -C$\underline{H}_2$CH$_3$ $J = 7.3$ Hz), 2.56 (d, 3H, -C$\underline{H}_3$, $J = 0.9$ Hz), 1.48 (t, 3H, -CH$_2$C$\underline{H}_3$, $J = 7.3$ Hz).

^{13}C NMR (125.78 MHz, DMSO) : $\delta = 140.55$ (dt, C-2, $^1J_{C,H}$ 211 Hz, $^{>1}J_{C,H}$ 4 Hz), 140.40 (ddd, C-10a, $^{>1}J_{C,H}$ 12; 5 and 1 Hz), 138.17 (ddd, C-3a, $^{>1}J_{C,H}$ 12; 6 and 3 Hz), 134.89 (dd, C-5, $^1J_{C,H}$ 213 Hz, $^{>1}J_{C,H}$ 1 Hz), 129.93 (dq, C-8, $^1J_{C,H}$ 187 Hz, $^{>1}J_{C,H}$ 5 Hz), 122.53 (d, C-10b, $^{>1}J_{C,H}$ 11 Hz), 119.77 (dq, C-7, $^{>1}J_{C,H}$ 15 and 7 Hz), 38.72 (tq, -$\underline{C}$H$_2$CH$_3$, $^1J_{C,H}$ 141 Hz, $^{>1}J_{C,H}$ 5 Hz), 15.49 (qt, -CH$_2\underline{C}$H$_3$, $^1J_{C,H}$ 128 Hz, $^{>1}J_{C,H}$ 3 Hz), 8.79 (q,-CH$_3$).

Preparation of 8-Methyl-3-(β-D-ribofuranosyl) imidazo[2,1-*i*]purine (7). Adenosine (**1**) (6.5 g, 24 mmol) was dissolved in methanol and 3-chloropropyne (14 mL, 195 mmol) and DBN (6.0 mL, 49 mmol) were added to the solution. The mixture was stirred 10 days at 60 °C. The solution was cooled down to room temperature and then evaporated to dryness at 40°C in a rotary evaporator. The product was isolated from the reaction mixture by preparative reversed phase chromatography. The fractions containing product were combined and evaporated to dryness. The product was obtained as a white solid. The yield was ca. 10%.

The purified product had the following spectral characteristics: UV_{max} 230 nm, 268 nm, 278 nm and 301 nm, UV_{min} 250 nm and 289 nm (HPLC eluent, 26% acetonitrile in 0.01 M phosphate buffer, pH 7.1). Fluorescence excitation and emission maxima were observed at 316 nm and 428 nm, respectively.

In EI-MS the following ions were observed [IP 70 eV; m/z (% r. a.)]: 305 (7, M^+), 173(21, M^+, -Rib).). HRMS gave the protonated molecular formula as $C_{13}H_{15}N_5O_4$ (MH^+ 305.1122 $gmol^{-1}$, calculated mass 305.1124 $gmol^{-1}$).

The fluorescence characteristic data of **7** are presented in Tables 12 and Figure 2.

^{1}H NMR (500.16 MHz, DMSO) : $\delta = 9.20$ (s, 1H, H-5), 8.57 (s, 1H, H-2), 7.80 (q, 1H, H-7, $J = 1.0$ Hz), 6.07 (d, H-1', $J = 5.6$ Hz), 5.60 (br, 1H, 2'-OH), 5.30 (br, 1H, 3′-OH), 5.13 (br, 1H, 5′-OH), 4.63 (t, 1H, H-2′, $J = 5.3$ Hz), 4.24 (dd, 1H, H-3′, $J = 3.8$ and 4.6 Hz), 4.03 (q, 1H, H-4′, $J = 3.9$ Hz), 3.74 (dd, 1H, H-5′, $J = 12.1$ and 3.8 Hz), 3.63 (dd, 1H, H-5″, $J = 12.1$ and 4.1 Hz), 2.38 (d, 3H, -C$\underline{H}_3$, $J = 1.0$ Hz)

^{13}C NMR (125.78 MHz, DMSO) : $\delta = 142.17$ (dq, C-8, $^1J_{C,H}$ 9 and 7 Hz), 140.06 (t, C-10a, $^{>1}J_{C,H}$ 6 Hz), 139.83 (dd, C-2, $^1J_{C,H}$ 214 Hz, 4 Hz), 138.43 (ddd, C-3a, $^1J_{C,H}$ 13; 5 and 3 Hz), 136.35 (d, C-5, $^1J_{C,H}$ 214 Hz, $^{>1}J_{C,H}$ 1 Hz), 122.48 (d, C-10b, $^{>1}J_{C,H}$ 12 Hz), 108.41 (dq, C-7, $^1J_{C,H}$ 195 Hz, $^{>1}J_{C,H}$ 5 Hz), 87.78 (d C-1′,

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$^1J_{C,H}$ 166 Hz), 85.56 (d C-4′, $^1J_{C,H}$ 148 Hz), 74.11 (d C-2′, $^1J_{C,H}$ 148 Hz), 70.31 (d C-3′, $^1J_{C,H}$ 149 Hz), 61.31 (t C-5′, $^1J_{C,H}$ 141 Hz), 14.12 (q -$\underline{C}H_3$, $^1J_{C,H}$ 127 Hz).

Preparation of 3-Ethyl-3*H*-8-methyl imidazo[2,1-*i*]purine (8). Compound **8** was prepared as described for **7**. 6-amino-9-ethylpurine (**2**) (1.5 g, 9.1 mmol), 3-chloropropyne (5.3 mL, 74 mmol) and DBN (2.3 mL, 49 mmol). The product was obtained as a white solid. The yield was ca. 10%.

The purified product had the following spectral characteristics: UV_{max} 232 nm, 270 nm, 280 nm and 301 nm, UV_{min} 252 nm and 289 nm (HPLC eluent, 32% acetonitrile in 0.01 M phosphate buffer). Fluorescence excitation and emission maxima were observed at 316 and 427 nm, respectively.

In EI-MS the following ions were observed [IP 70 eV; m/z (% r. a.)]: 201 (100, M^+), 186 (13, M^+-Me), 173 (35, M^+-Et). HRMS gave the protonated molecular formula as $C_{10}H_{11}N_5$ (MH^+ 201.1015 $gmol^{-1}$, calculated mass 201.1014 $gmol^{-1}$).

^{1}H NMR (500.16 MHz, $CDCl_3$) : $\delta = 8.71$ (s, 1H, H-5), 7.93 (s, 1H, H-2), 7.42 (q, 1H, H-7, $J = 1.0$ Hz), 4.33 (q, 2H, -C$\underline{H}_2$CH$_3$, $J =$ 7.3 Hz), 2.48 (d, 3H, -C$\underline{H}_3$, $J = 1.0$ Hz), 1.57 (t, 3H, -CH$_2$C$\underline{H}_3$, $J = 7.3$ Hz).

^{13}C NMR (125.78 MHz, $\underline{C}DCl_3$) : $\delta = 143.74$ (dq, C-8, $^{>1}J_{C,H}$ 9 and 7 Hz), 141.28 (t, C-10a, $^{>1}J_{C,H}$ 6 Hz), 139.79 (dt, C-2, $^1J_{C,H}$ 209 Hz , $^{>1}J_{C,H}$ 4 Hz), 139.05 (C-3a), 134.44 (dd, C-5, $^1J_{C,H}$ 210 Hz, $^{>1}J_{C,H}$ 1 Hz), 123.39 (d, C-10b, $^{>1}J_{C,H}$ 11 Hz), 107.14 (dq, C-7, $^1J_{C,H}$ 192 Hz, $^{>1}J_{C,H}$ 5 Hz), 39.38 (tq, -$\underline{C}H_2CH_3$, $^1J_{C,H}$ 141 Hz, $^{>1}J_{C,H}$ 5 Hz), 15.75 (qt, -CH$_2\underline{C}H_3$, $^1J_{C,H}$ 128 Hz, $^{>1}J_{C,H}$ 3 Hz), 14.40 (q, -$\underline{C}H_3$, $^1J_{C,H}$ 127 Hz).

ACKNOWLEDGMENTS

This project was financed by TEKES (The National Technology Agency). We are grateful to Mr. Markku Reunanen for the mass spectra.

REFERENCES

1. (a) Ranasinghe, R.T.; Brown, L.J.; Brown, T. Linear fluorescent oligonucleotide probes with an acridine quencher generate a signal upon hydridization. Chem. Comm. **2001**, *16*, 1480–1481. (b) Singleton, S.F.; Shan, F.; Kanan, M.W.; McIntosh, C.M.; Stearman, C.J.; Helm, J.S.; Webb, K.J. Facile synthesis of a fluorescent deoxycytidine analogue suitable for probing the RecA nucleoprotein filament. Org. Lett. **2001**, *3* (24), 3919–3922. (c) Godde, F.; Toulmé, J.J.; Moreau, S. 4-amino-1H-benzo[g]quinazoline-2-one: a fluorescent analog of cytosine to probe protonation sites in triplex forming oligonucleotides. Nucleic Acids Res. **2000**, *28* (15), 2977–2985. (d) Pandey, R.K.; Tripatni, S.; Misra, K. Synthesis and hybridization studies of oligonucleotide sequences with modified fluorescent nucleoside analogs. Nucleos. Nucleot. **1998**, *17* (9–11), 1937–1948. (e) Pelicano, H.; Maury, G.; Elalaoui, A.; Shafiee, M.; Imbach, J.L., Goody, R.S.; Divita, G. Study of the substrate-binding properties of bovine liver adenosine kinase and inhibition by fluorescent nucleoside analogs.

Eur. J. Biochem. **1997**, *248*, 930–937. (f) Allan, B.W.; Reich, N.O. Targeted base stacking disruption by the EcoRI DNA methyltransferase. Biochemistry **1996**, *35*, 14,757–14,762. (g) Ren, J.; Goss, D.J. Synthesis of a fluorescent 7-methylguanosine analog and a fluorescence spectroscopic study of its reaction with wheatgerm cap binding proteins. Nucleic Acids Res. **1996**, *24* (18), 3629–3634. (h) Frey, M.W.; Sowers, L.C.; Millar, D.P.; Benkovic, S.J. The nucleotide analog 2-aminopurine as a spectroscopic probe of nucleotide incorporation by the Klenow fragment of Escherichia coli polymerase I and bacteriophage T4 DNA polymerase. Biochemistry **1995**, *34*, 9185–9192. (i) Hawkins, M.E.; Pfleiderer, W.; Mazumder, A.; Pommier, Y.G.; Balis, F.M. Incorporation of a fluorescent guanosine analog into oligonucleotides and its application to a real time assay for the HIV-1 integrase 3′-processing reaction. Nucleic Acids Res. **1995**, *23* (15), 2872–2880. (j) Nordlund, T.M.; Andersson, S.; Nilsson, L.; Rigler, R. Structure and dynamics of a fluorescent DNA oligomer containing the EcoRI recognition sequence: fluorescence, molecular dynamics, and NMR studies. Biochemistry **1989**, *28*, 9095–9103.

2. (a) Davies, M.J.; Shah, A.; Bruce, I.J. Synthesis of fluorescently labelled oligonucleotides and nucleic acids. Chem. Soc. Rev. **2000**, *29*, 97–107. (b) Proudnikov, D.; Mirzabekov, A. Chemical methods of DNA and RNA fluorescent labeling. Nucleic Acids Res. **1996**, *24* (22), 4535–4542. (c) Wojczewski, C.; Stolze, K.; Engels, J.W. Fluorescent oligonucleotides versatile tools as probes and primers for DNA and RNA analysis. Synlett **1999**, *10*, 1667–1678. (d) Strāssler, C.; Davis, N.E.; Kool, E.T. Novel nucleoside analogues with fluorophores replacing the DNA base. Helv. Chim. Acta **1999**, *82*, 2160–2171.
3. Hemmilä, I.A. Photoluminescent probes. In *Applications of Fluorescence in Immunoassays*; John Wiley & Sons: New York, 1991; Vol. 117, 108–109.
4. (a) Leonard, N.J. Etheno-substituted nucleotides and coenzymes fluorescence and biological activity. CRC Crit. Rev. Biochem. **1984**, *15* (2), 125–199. (b) Zagorowska, I.; Adamiak, R.W. 2-Aminopurine labeled RNA bulge loops Synthesis and thermodynamics. Biochemie **1996**, *78* (2), 123–130. (c) Odijk, W.M.; Koomen, G.J. Fluorescent nucleoside analogs. Synthesis of fluorescent pyrido[2,1-i]purines, and their corresponding ribosides. Tetrahedron **1985**, *41* (10), 1893–1904. (d) Driscoll, S.L.; Hawkins, M.E.; Balis, F.M.; Pfleiderer, W.; Laws, W.R., Fluorescence properties of a new guanosine analog incorporated into small oligonucleotides. Biophys. J. **1997**, *73*, 3277–3286.
5. (a) Biernat, J.; Ciesiolka, J.; Gornicki, P.; Adamiak, R.W.; Krzyzosiak, W.J.; Wiewiorowski, M. New observations concerning the chloroacetaldehyde reaction with some RNA constituents. Stable intermediates, kinetics and selectivity of the reaction. Nucleic Acids Res. **1978**, *5* (3), 789–804. (b) Biernat, J.; Ciesiolka, J.; Gornicki, P.; Krzyzosiak, W.J.; Wiewiorowski, M. Further studies on the reaction of adenine, cytosine and corresponding nucleosides with chloroacetaldehyde. Nucleic Acids Res. Spec. Publ. **1978**, *4*, 203–208. (c) Krzyzosiak, W.J.; Biernat, J.; Ciesiolka, J.; Gornicki, P.; Wiewiorowski, M. Comparative studies on reactions of adenosine and cytidine with chloroacetaldehyde, α-bromopropionaldehyde and chloroacetone. Synthesis of 1, N6-ethenoadenosine and 3,N4-ethenocytidine derivatives methylated at

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the etheno bridge. Pol. J. Chem. **1983**, *57*, 779–787. (d) Kost, A.A.; Ivanov, M.V. Etheno derivatives of adenine and cytosine. (Review). Chem. Heterocycl. Comp. **1980**, *16* (3), 209–221. (e) Barrio, J.R.; Secrist III, J.A.; Leonard, N.J. Fluorescent adenosine and cytidine derivatives. Biochem. Bioph. Res. Co. **1972**, *46* (2), 597–604. (f) Boryski, J. 1,N2-Ethenoguanosine: three methods of synthesis. Nucleos. Nucleot. **1990**, *9* (6), 803–813.

6. Srisvastava, S.; Raza, S.K.; Misra, R. 1,N6-etheno deoxy and ribo adenosine and 3,N4-etheno deoxy and ribo cytidine phosphoramidites. Strongly fluorescent structures for selective introduction in defined sequence DNA and RNA molecules. Nucleic Acids Res. **1994**, *22* (7), 1296–1304.
7. Kochetkov, N.K.; Shibaev, V.N.; Kost, A.A. Modification of nucleic acid components with α-halo aldehydes. Dokl. Akad. Nauk. SSSR **1972**, *205* (1), 100–103.
8. (a) Secrist, J.A.; Barrio, J.R.; Leonard, N.J.; Weber, G. Fluorescent modification of adenosine containing coenzymes. Biological activities and spectroscopic properties. Biochemistry **1972**, *11* (19), 3499–3506, (b) Mikkola, S.; Koissi, N.; Ketomäki, K.; Rauvala, S.; Neuvonen, K. Reactions of adenosine with bromo- and chloromalonaldehydes in aqueous solution kinetics and mechanism. Eur. J. Org. Chem. **2000**, *12*, 2315–2323. (c) Nair, V.; Offerman, R.J.; Turner, G.A. Structural alteration of nucleic acid bases by bromomalonaldehyde. J. Org. Chem. **1984**, *49* (21), 4021–4025. (d) Kochetkov, N.K.; Shibaev, V.N.; Kost, A.A.; Razzhivin, A.P.; Ermolin, S.V. Mechanism of the reaction of adenosine 5′-phosphate with chloroacetaldehyde. Dokl. Akad. Nauk SSSR **1977**, *234* (6), 1339–1342.
9. (a) Shaw, G.; Smallwood, B.M. Purines pyrimidines, and imidazoles XXXIV. Synthesis and some reactions of N(6)-(formylmethyl)adenine. J. Chem. Soc. C **1970**, *16*, 2206–2208, (b) Sattsangi, P.D.; Barrio, J.R.; Leonard, N.J. 1,N6-Etheno-bridged adenines and adenosines. Alkyl substitution, fluorescence properties, and synthetic applications. J. Am. Chem. Soc. **1980**, *102* (2), 770–774. (c) Yip, K.F.; Tsou, K.C. Synthesis of fluorescent adenosine derivatives. Tetrahedron Lett. **1973**, *33*, 3087–3090.
10. Nair, V.; Richardson, S.G. Utility of purinyl radicals in the synthesis of base-modified nucleosides and alkylpurines: 6-amino group replacement by hydrogen, chlorine, bromine, and iodine. J. Org. Chem. **1980**, *45*, 3969–3974.
11. Lakowicz, J.R. In *Principles of Fluorescence Spectroscopy*; 2nd Ed.; Kluvert Ed.; Academic/Plenum Publishers: New York, 1999.
12. Adams, M.J.; Highfield, J.G.; Kirkbright, G.F. Determination of absolute fluorescence quantum efficiency of quinine bisulfate in aqueous medium by optoacoustic spectrometry. Anal. Chem. **1977**, *49*, 1850–1852.

Received June 11, 2002
Accepted November 11, 2002