Pergamon

Tetrahedron, Vol. 53, No. 37, pp. 12595-12604, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00528-0

Novel Heterocyclic Dyes as DNA Markers. Part I. Synthesis and Characterization.

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Abstract: A range of new cyanine dyes has been prepared and characterised. These dyes are more conveniently synthesised by the reaction of two heterocyclic quaternary salts. The dyes contain a purine heterocycle coupled to an aro-(thia-, selena-, oxa-, imida)zole and are in the form of iodide or *p*-toluenesulphonate salts. Characterisation were done by FABMS and NMR spectroscopy. These dyes can be used as fluorescent dyes for DNA marking in the diagnosis of malaria parasites.

INTRODUCTION

Cyanine dyes are characterized by a chain of conjugated methine (–CH=) groups, which are normally in the E (trans) configuration. The carbon atoms of the methine groups may be attached to groups other than hydrogen, or they may be part of carbocyclic or heterocyclic ring systems.[1]

Most cyanine dyes are fluorescent and have replaced conventional dyes for some staining techniques because fluorescence methods provide far greater sensitivity as shown by enhanced signal-to-noise ratio and better colour differentiation of stained cells.[2] The study of the structure and distribution of cellular nucleic acids by fluorescence microscopy is now common practice.[3,4] These dyes when complexed with double stranded DNA show intense fluorescence.[5] An application of this technique is the detection of blood stream malaria parasites. Malaria has been the most important disease affecting man, particularly in the tropical and sub-tropical regions of the world. The global incidence of malaria is estimated to involve 1230 million clinical cases each year.

Conventional microscopic examination of blood films for malaria parasites detects one parasite in 10^6 red blood cells. It is also species and stage specific. This provides information about the viability of any parasites present in peripheral blood and such information is useful when response to treatment is being assessed. A further development of this technique is the use of fluorescent stains,[6] such as Acridine Orange,[7,8] DAPI[9], PI[6] and BCP[10]. These systems[11] are about eight fold more sensitive than Giemsa-stained thick smears for detecting malaria parasites.[12] Therefore the synthesis of even more sensitive fluorescent dyes with different specificities and which allow the detection of parasitic disease other than malaria, is highly desirable.

RESULT AND DISCUSSION

Polymethine dyes are synthesised by the reaction of two heterocyclic quaternary salts, one bearing a methyl group in the 2-position (**1**), such as indoline, aro-thiazole, -oxazole, -selenazole, and the other a good nucleofugic leaving group Y in the 2-position (**2**). Deprotonation by a tertiary aliphatic amine gives the methylene derivative

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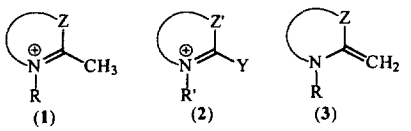
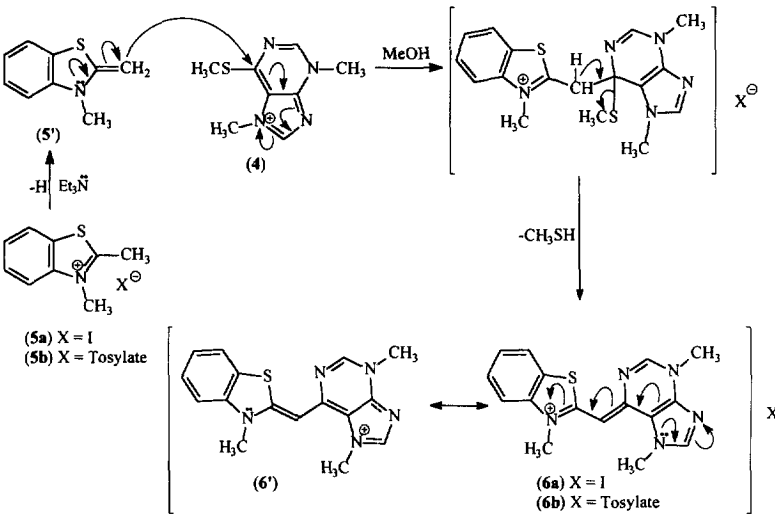


Figure 1

(3), which acts as the nucleophilic reagent (**Figure 1**). Thioalkyl groups are generally used as the leaving groups Y. Using this general method the cyanine dyes (**6-12**) were prepared (**Figure 2**).

According to a previous study[13] 6-methyl-mercaptopurine is a mixture of two tautomers. The 6-methylmercapto-purine when treated with methyl iodide in an aprotic solvent in the absence of acid or base is methylated at position 3.[14] That only one N-methyl derivative is formed, indicates that the exact position of the NH-group in the imidazole moiety is irrelevant in the alkylation process. When methyl-*p*-toluenesulphonate was used as methylating agent the thiopurinium *p*-toluene-sulphonate quaternary salt (**4**) was obtained which was used as an acceptor in the synthesis of analogues of cyanine type dyes.[15]



Scheme 1

The quaternary salts of heterocyclic bases containing a reactive methyl group e.g. (**1**) readily lose a proton to give a "methylene base".[16] In general, a methylene base will be liberated from the corresponding cation more readily the lower the basicity of the nuclei in the ring.[17] The greater the basicity of the nuclei, the lower is the concentration of "methylene base" generated under the conditions of the reaction and therefore, the yields decrease in proportion to the basicity of the nuclei.[17]

The synthesis of PUR-1 (**6a**) requires the condensation of the heterocyclic base (**5a**) with the 6-methylthiopurinium salt (**4**). The base is formed from the benzothiazolium iodide (**5a**) and triethylamine, shown in **Scheme 1**.

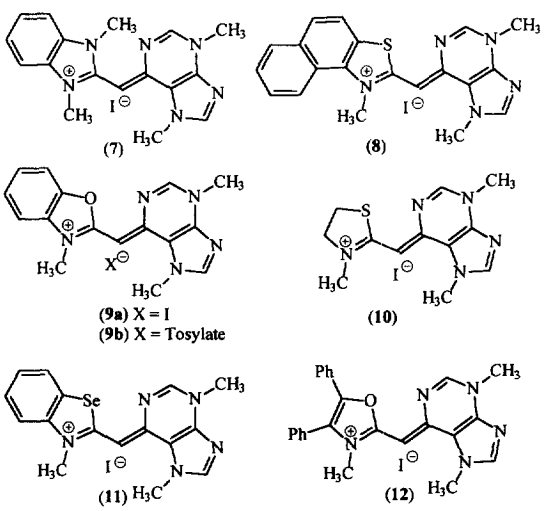


Figure 2

Characterization of the Dyes

UV-vis Spectra

The colour of cyanine dyes depends primarily on the terminal groups and the length of the conjugated system. Dyes having unsaturated terminal groups show greater bathochromic shifts than those with saturated terminal groups [e.g. (**10**) and (**12**)] and additional

Table 1 Light absorption of some unsymmetrical cyanines in methanol.

Compound	λ_{max}(nm)	ϵ_{max}
(6a)	455	21000
(6b)	449	22000
(7)	397	24000
(8)	470	36000
(9a)	427	13000
(9b)	428	25000
(10)	441	25000
(11)	452	34000
(12)	440	25000

conjugation in the terminal group results in further shifts [e.g. (**6a**) and (**8**)][1] (**Table 1**). The distortion caused by the N-Me group in the imidazole portion in (**7**) reduces the bathochromic shift. In contrast, if the terminal heterocycles in an unsymmetrical dye have widely different basicities, then the dye absorbs at an unexpectedly short wavelength. The dye (**7**), derived from a strongly basic benzimidazole and a less basic purine gives absorption at a shorter wavelength than dyes with terminal groups in which basicities are similar. Another important feature of cyanine dyes is the narrowness of their absorption bands. Thus, cyanine dyes are exceptionally bright. The width of the absorption band depends on how closely the geometry of the molecule in the first excited state resembles that in the ground state. All synthesised cyanine dyes showed prominent shoulders on the short wavelength side of the intense absorption band. Steric inhibition effects which result in non-planarity always cause a reduction in ϵ_{max} [e.g. (**7**)]. Thus, in addition to the decrease in ϵ_{max} the absorption shifts to shorter wavelengths.

Fluorescence

Dye fluorescence occurs at relatively long wavelengths. Many cyanine dyes possess more than one dissociable proton so a number of partially-ionized species exist in different pH ranges each characterized by different absorption and fluorescence spectra. When studying fluorescence it is desirable to have only one ground-state species in the solution. However cyanine dyes exhibit a pronounced tendency to aggregate[18] into dimeric or higher polymeric forms at concentrations greater than 10^{-4} M due to the high polarizability of the dyes. A feature of importance, is the existence of geometric isomers which can exhibit quite different fluorescence characteristics, as observed for sterically hindered cyanine dye (**7**). These have low efficiency fluorescence, while those not possessing crowed structures such as (**6a**) are intensely fluorescent (**Table 2**). Highly fluorescent dyes are characterized by having a rigid, planar structure. For example (**9a**) exhibits intense fluorescence in solution, whereas (**12**) shows only weak fluorescence, although it is structurally similar. The reason for this difference is that in (**12**) the rotation and vibrations of the aromatic rings enables the electronic excitation to be easily dissipated internally without leading to emission. The vibrational dissipation of electronic energy is considerably more difficult in a rigid structure such as (**9a**). The internal dissipation of excitation energy is also facilitated by steric crowding leading to decreased fluorescence intensity, as in (**6a**) and (**7**) in **Table 2**. The most intensely fluorescent dyes are planar.

Table 2 Fluorescence data of some cyanine dyes.

Compound	Absorption λ_{max}(nm)	Emission λ_{max}(nm)	Relative Intensity
(6a)	455	470	87
(7)	397	440	53
(8)	470	500	195
(9a)	427	450	93
(10)	402	430	180
(11)	452	475	102
(12)	440	468	33

Solubility

Cyanine dyes have low solubility in most solvents (**Table 3**). The synthesis of more soluble dyes containing a bigger organic anion, such as *p*-toluenesulphonate, as in (**6b**) makes purification possible and enables elemental analysis to be performed.

Table 3 Solubility of the cyanine dye (**6a**) in different solvents and compared with other cyanines.

Ether < THF < Py < 2-propanol < AcOH < Acetone < EtOH < MeOH < DMF < DMSO (10 mg/ml)
(**10**) < (**8**) < (**11**) < (**6a**)

The phenomenon that influences their solubilities is their state of aggregation.[18] The dyes in solution exist as loosely bonded dimers or complexes, which explains the non-reversible recrystallization process of the iodide derivatives (**6-12**, **Figure 2**). These derivatives are water insoluble which is a disadvantage in the study of their redox properties. The presence of a surfactant such as SDS (sodium dodecyl sulphate) improves their solubilities in water[19].

Stereochemistry

Cyanine dyes in solution are considered to exist largely in all-trans (*E*) forms. In the case of cyanine dye (**6a**) the *Z* form seems from solid state NMR measurements to be the more stable because the *E* form is sterically congested. Furthermore, the ^{1}H-NMR spectrum in solution is simple, showing that over a range of temperatures there is only one stereoisomer present. This result has been confirmed by theoretical calculations (PM3 method) showing (**6a**) to be in the *Z* conformation which is the most stable (Moreda *et al*, Novel Heterocyclic Dyes as DNA Markers. Part II. Structure and Biological Activity).

NMR Spectroscopy The dyes are easily distinguished by their characteristic N$^{\oplus}$Me, CH=N, and CH=CH chemical shifts in the 3.8 - 4.3, 7.8 - 8.3, and 6.0 - 6.5 regions, respectively. Although the aromatic protons form an ABCD system, the aromatic signals appear remarkably simple. First order treatment of the coupling, while not precise, leads to the chemical shifts reported. The acidity of the 2-methyl group in the heterocyclic nuclei plays an important role in the synthesis of the cyanine dyes but the chemical shift of the methyl protons in the ^{1}H-NMR cannot be successfully correlated with the acidity of such protons. The ^{13}C chemical shift of the C-2 atom proved to be an excellent indicator of the acidity of these protons. The acidity was found to decrease in the order:[20] for Se > S > O > NR compounds. The ^{15}N-CPMAS spectrum at 30.4-MHz of the cyanine dye (**7**) shows six clear signals in which N_1, N_9 signals are at -136.9 and -148.1 ppm and those for N_3, N_7 are at -219.2 and -231.2 ppm in the purine residue, and those for the imidazole, $N_{1'}$, $N_{3'}$ are at -238.5 and -244.3 ppm.

Mass Spectrometry

Cyanine dyes, isolated as iodides do not give accurate elemental analysis because of the interference of the iodine. The MS of cyanine dyes frequently does not show a molecular ion in the mass spectrum. In many cases the ion detected is the result of the loss of a methyl group. Thus the mass spectrum (EI) of the cyanine dye (**8**) shows a weak ion at m/z 360 and a more abundant ion at m/z 359 corresponding to the dye cation and to the loss of HI from the dye respectively. When FABMS was used in the elucidation of the structure the dye cation is the base peak of the spectrum.

Cyclic-Voltammetry

The ideal medium in which to perform a cyclic voltammetry experiment is water but PUR-1 and its analogues are poorly soluble in aqueous media. Their solubility was improved with the use of a 0.1 M solution of the anionic surfactant, sodium dodecylsulphate (SDS) which forms a micelle around each of the dye molecules, isolating one from another. It would be expected that a reduction voltammogram would be observed in a water-SDS solution but surprisingly the oxidation voltammogram was obtained. This shows a clear two-step one-electron reversible oxidation-reduction process. The reversibility of the process is confirmed by the difference between the oxidation and reduction potentials being ≈ 0.059 V. In this voltammogram the two step one-electron changes correspond to the abstraction of one electron and then a second from the sulphur or selenium atoms in PUR-1 and its analogues. The detection of a voltammogram indicating an oxidation instead of a reduction is a consequence of the surfactant's inhibition of the process of reduction.[21] That the oxidation process involves the formation of radicals was confirmed by the results obtained in a cyclic voltammetry experiment in which a spin trap, phenyl *tert*-butyl nitrone (PBN), was added. This voltammogram shows the same oxidation-reduction sequence but has peaks shifted to higher voltage. Despite this result, no ESR signal was observed when cyclic voltammetry was conducted in a ESR cavity. **Table 4** shows the potentials, current intensities and the halfwidth potentials of the peaks characteristic of the oxidation voltammogram of PUR-1 and its analogues containing sulphur or selenium. The cyclic voltammogram for the compound (**9a**), which has oxygen instead of sulphur shows no oxidation or reduction peaks. DMSO was examined as a non-aqueous solvent for PUR-1 and its analogues in the expectation that a reduction process would be observed. The resulting cyclic voltammograms showed as expected, irreversible reduction processes occurring. This voltammogram shows two reduction steps and only one oxidation step. As the process is irreversible it is impossible to assess the number of electrons involved. It is possible that dimerisation[22] takes place after the addition of two electrons, and the resulting carbanion couples with an unreduced molecule.

Table 4 Characteristics of the oxidation cyclic voltammogram of PUR-1 and analogues.

Compound	Potential/(V)	Current Intensity/10^{-4} (A)	Halfwidth Potentials/10^{-1} (V)
PUR-1 (6a)	0.482	2.308	1.1581
	0.759	2.390	0.9567
	0.688	-1.690	1.0071
	0.417	-2.799	1.4099
(8)	0.648	0.720	0.9064
	0.598	-1.025	1.4099
(10)	0.683	2.101	1.0071
	0.623	-2.582	1.3596
(11)	0.668	0.995	0.9567
	0.613	-1.207	1.2085

EXPERIMENTAL

All melting points were determined on a Reichart hot stage apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using aluminium sheets coated with silica gel 60 GF_{254} (0.2 mm thickness). All solvents were dried by general procedures. Evaporation refers to rotary evaporation under reduced pressure. All the electronic spectra were recorded on a Perkin Elmer 402 spectrophotometer in methanol in a range of concentration of 10^{-4} to 10^{-5} mol l^{-1}. The fluorescence spectra were recorded in methanol (1μg/ml) using a Perkin Elmer LS-50 spectrofluorimeter equipped with a 1 cm quartz cuvette. The excitation and emission monochromator

slitwidths were set at 10 and 15 nm respectively. The IR spectra were obtained in potassium bromide. The NMR spectra were recorded on a Bruker AC-F 250 spectrometer at 250 MHz (^{1}H) and 62.9 MHz (^{13}C). Other spectra were recorded on a Bruker 300 AMX at 300 MHz (^{1}H) and 75.5 MHz (^{13}C) and on a Bruker 500 AMX at 500 MHz (^{1}H) and 125.7 MHz (^{13}C). The solid state NMR spectra were recorded on a Bruker 300 AMX at 75.5 MHz (^{13}C) and 30.5 MHz (^{15}N) using ammonium nitrate as internal reference for chemical shift values. The mass spectra were measured using a KRATOS MS-80RFA spectrometer.

A cyclic voltammetry cell was set up using a gold wire as counter electrode, a platinum wire as a working electrode, and a Ag/AgCl electrode as a reference; the Ag/AgCl electrode was prepared by the method reported by Brown.[23] The cyclic voltammograms were obtained using an approximately 10^{-4} M solution of PUR-1 and its analogues in DMSO and water-SDS, which contains 0.1 M of *tert*-butyl-ammonium bromide and 0.1 M of sodium chloride respectively as background electrolyte. The voltammograms were recorded on an Eco Chemie AUTOLAB/GPES PGSTAT20 using a scan rate of 0.01 V s^{-1}.

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzothiazolium Iodide **(6a)**

A mixture of 2,3-dimethylbenzothiazolium iodide (**5a**) (1.28 g, 4.4 mmol), 3,7-dimethyl-6-(methylthio)purinium p-toluenesulphonate (**4**) (1.5 g, 4.1 mmol), methanol (20 ml) and triethylamine (0.5 ml) was refluxed for 45 minutes. The red solution formed contained a yellow solid. The latter was collected, washed with methanol and ether to give 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzothiazolium iodide (**6a**) as a yellow-orange solid, (1.3 g, 67%), m.p.>300° C (Literature[24] m.p. 340-5° C).

UV data: (MeOH; [c] 1.4 10^{-4} M); λ_{max} = 455 (log ϵ 4.32) and 220 nm (4.33). ^{1}H-NMR data: δ(DMSO-D_6) 4.0 (s, 3H, NCH_3), 4.01 (s, 3H, NCH_3), 4.28 (s, 3H, N^+CH_3), 6.51 (s, 1H, H-6a), 7.37 (t, 1H, H-5'), 7.54 (t, 1H, H-6'), 7.77 (d, 1H, H-4'), 8.04 (d, 1H, H-7'), 8.54 (s, 1H, H-8), 8.85, (s, 1H, H-2); $J_{H\text{-}4',H\text{-}5'} = J_{H\text{-}6',H\text{-}7'} = 8.0$, $J_{H\text{-}5',H\text{-}6'} = 7.2$ Hz. ^{13}C-NMR data: δ(DMSO-D_6) 35.5 (NCH_3), 36.4 (NCH_3), 37.4 (N^+CH_3), 88.0 (C-6a), 114.4 (C-7'), 119.7 (C-5), 124.2 (C-4'), 126.0 (C-5'), 129.1 (C-6'), 129.2 (C-8'), 141.8 (C-9'), 147.5 (C-8), 148.4 (C-4), 148.7 (C-2), 151.3 (C-6), 163.6 (C-2'). Mass spectrum (H.R.E.I.): m/z 437 ($[M]^+$, $C_{16}H_{16}IN_5S$ requires 437.0171 Found: 437.0129) (0.5), 309 ($[M-IH]^+$, $C_{16}H_{15}N_5S$ requires 309.1048 Found: 309.1052) (3), 295 (75), 280 (9), 253 (4), 186 (5), 163 (5), 148 (10), 142 (100), 127 (35), 91 (4). Mass spectrum (F.A.B.): m/z 437 (($[M]^+$) (1), 311 ($[M - I] + H^+$) (25), 310 ($[M - I]^+$) (100).

1,3-Dimethyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzimidazolium Iodide **(7)**

A mixture of 1,2,3-trimethylbenzimidazolium iodide (0.42 g, 1.46 mmol), 3,7-dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol), dimethylformamide (6 ml) and triethylamine (0.3 ml) was refluxed for 45 minutes, producing a red solution containing a yellow solid. The latter was cooled to 20° C for 3 hours and washed with methanol and ether to give 1,3-dimethyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzimidazolium iodide (**7**) as a yellow-orange solid (0.38 g, 60%), m.p.>300° C.

UV data: (MeOH; [c] 0.7 10^{-5} M); λ_{max} = 397 (log ϵ 4.37), 269 (3.98), 216 (4.20) and 202 nm (4.27). ^{1}H-NMR data: δ(DMSO-D_6) 3.71 (s, 3H, NCH_3), 3.87 (s, 3H, NCH_3), 4.14 (s, 3H, N^+CH_3), 5.59 (s, 1H, H-6a), 7.5 - 7.6 (m, 2H, H-5', H-6'), 7.76 - 7.8 (m, 2H, H-4', H-7'), 8.06 (s, 1H, H-8), 8.14 (s, 1H, H-2). ^{13}C-NMR data: δ(DMSO-D_6) 20.9 (NCH_3), 33.0 (NCH_3), 34.5 (NCH_3), 34.6 (N^+CH_3), 74.2 (C-6a), 113.2 (C-7'), 116.6 (C-5), 126.5 (C-4'), 127.2 (C-5'), 129.4 (C-6'), 133.8 (C-8'), 138.8 (C-9'), 145.2 (C-8), 145.9 (C-4), 147.3 (C-2), 149.2 (C-6), 153.6 (C-2'). Mass spectrum (H.R.E.I.): m/z 434($[M]^+$) (0.6), 307 ($[M - I]^+$, $C_{17}H_{19}N_6$ requires 307.1672 Found: 307.1676) (3), 292 (53),

277 (8), 250 (7), 186 (61), 155 (55), 107 (20), 91 (100). Mass Spectrum (F.A.B.): m/z 308 ([M - I] + H^+) (58), 307 ([M - I]$^+$) (100).

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]naphtho[1,2-d]thiazolium Iodide **(8)**

This compound was prepared by the method described previously for compound (**6a**). 2,3-Dimethylnaphtho[1,2-d]thiazolium iodide (0.4 g, 1.2 mmol), 3,7-dimethyl-6-(methylthio)purinium p-toluenesulphonate (**4**) (0.4 g, 1.1 mmol), methanol (5.5 ml) and triethylamine (0.2 ml) gave 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl] naphtho[1,2-d]thiazolium iodide (**8**) (0.51 g, 70%), m.p. >300° C.

UV data: (MeOH; [c] 3.25 10^{-5} M); λ_{max} = 470 (log ϵ 4.56), 452 (4.48), 257 (4.32) and 222 nm (4.58). ^{1}H-NMR data: δ(DMSO-D_6) 3.98 (s, 3H, NCH_3), 4.28 (s, 3H, NCH_3), 4.47 (s, 3H, N^+CH_3), 6.61 (s, 1H, H-6a), 7.65 (t, 1H, H-5'), 7.72 (t, 1H, H-6'), 7.89 (d, 1H, H-4'), 8.07 (d, 1H, H-7'), 8.14 (d, 1H, H-8'), 8.49 (s, 1H, H-8), 8.71 (d, 1H, H-9'), 8.76 (s, 1H, H-2); $J_{H\text{-}4',\,H\text{-}5'} = J_{H\text{-}6',\,H\text{-}7'} = J_{H\text{-}8',\,H\text{-}9'}$ = 8.0, $J_{H\text{-}5',\,H\text{-}6'}$ = 7.2 Hz. ^{13}C-NMR data: δ(DMSO-D_6) 6.3 (NCH_3), 36.4 (NCH_3), 37.3 (N^+CH_3), 86.5 (C-6a), 119.7 (C-5), 121.0 (C-9'), 123.3 (C-7'a), 123.8 (C-4'), 127.7 (C-6'), 128.0 (C-7'), 128.8 (C-8'), 130.4 (C-3'b), 131.1 (C-5'), 135.1 (C-9'a), 137.1 (C-3'a), 147.4 (C-8), 148.0 (C-4), 148.3 (C-2), 150.6 (C-6), 162.2 (C-2'). Mass spectrum (H.R.E.I.): m/z 360 ([M-I]$^+$, $C_{20}H_{18}N_5S$ requires 360.1282 Found: 360.1215) (4), 359 ([M-IH]$^+$, $C_{20}H_{17}N_5S$ requires 359.1205 Found: 359.1213) (20), 345 (90), 330 (10), 212 (15), 198 (40), 142 (100), 127 (25), 91 (10). Mass spectrum (F.A.B.): m/z 487 ([M]$^+$) (1), 361 ([M - I] + H^+) (30), 360 ([M - I]$^+$) (100).

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzoxazolium Iodide **(9a)**

To a mixture of 3,7-dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol) and 2,3-dimethylbenzoxazolium iodide (0.41 g, 1.49 mmol) was added triethylamine (0.3 ml) followed by dimethylformamide (5 ml). The mixture was refluxed for 45 minutes, producing a red solution containing a yellow solid. The latter was washed with methanol and ether to give 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzoxazolium iodide (**9a**) as a yellow-orange solid (0.15 g, 24%), m.p. >300° C.

UV data: (MeOH; [c] 2 10^{-4} M); λ_{max} = 427 (log ϵ 4.11), 262 (3.48), 215 (3.65) and 202 nm (3.78). ^{1}H-NMR data: δ(DMSO-D_6) 3.80 (s, 3H, NCH_3), 3.94 (s, 3H, NCH_3), 4.23 (s, 3H, N^+CH_3), 5.71 (s, 1H, H-6a), 7.36 (t, 1H, H-6'), 7.46 (t, 1H, H-5'), 7.64 (d, 1H, H-7'), 7.77 (d, 1H, H-4'), 8.53 (d, 1H, H-8), 8.71 (s, 1H, H-2); $J_{H\text{-}5',\,H\text{-}6'}$ = 7.2, $J_{H\text{-}4',\,H\text{-}5'} = J_{H\text{-}6',\,H\text{-}7'}$ = 8.0 Hz. ^{13}C-NMR data: δ(DMSO-D_6) 32.4 (NCH_3), 36.6 (NCH_3), 37.2 (N^+CH_3), 74.6 (C-6a), 112.5 (C-4'), 119.8 (C-5), 122.4 (C-7'), 126.3 (C-5'), 127.5 (C-6'), 132.9 (C-9'), 147.9 (C-8), 148.1 (C-4), 149.1 (C-8'), 149.3 (C-2), 153.5 (C-6), 163.2 (C-2'). Mass spectrum (H.R.E.I.): m/z 294 ([M - I]$^+$, $C_{16}H_{16}N_5O$ requires 294.1355 Found: 294.1374) (0.6), 293 ([M - IH]$^+$, $C_{16}H_{15}N_5O$ requires 293.1277 Found: 293.1282) (0.9), 279 (93), 264 (11), 186 (49), 161 (27), 155 (56), 107 (13) 91 (100). Mass Spectrum (F.A.B.): m/z 295 ([M - I] + H^+) (29), 294 ([M - I]$^+$) (100).

4,5-Dihydro-3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]thiazolium Iodide **(10)**

This compound was prepared by the method described previously for compound (**6a**). 4,5-Dihydro-2,3-dimethylthiazolium iodide (0.36 g, 1.47 mmol), 3,7-dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol), methanol (7 ml) and triethylamine (0.3 ml) gave 4,5-dihydro-3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]thiazolium iodide (**10**) (0.22 g, 40%), m.p.>300° C.

UV data: (MeOH; [c] 1 10^{-4} M); λ_{max} = 441 (log ϵ 4.40), 425 (4.37) and 202 nm (4.31). ^{1}H-NMR data: δ(DMSO-D_6) 3.26 (s, 3H, NCH_3), 3.26 (t, 2H, H-5'), 3.89 (s, 3H, NCH_3), 3.98 (t, 2H, H-4'), 4.17 (s, 3H, N^+CH_3), 5.97 (s, 1H, H-6a), 8.49 (s, 1H, H-8), 8.66 (s, 1H, H-2); $J_{H\text{-}4', H\text{-}5'}$ = 7.9 Hz. ^{13}C-NMR data: δ(DMSO-D_6) 27.6 (NCH_3), 34.9 (NCH_3), 36.7 (N^+CH_3), 56.9 (C-4'), 57.0 (C-5'), 85.7 (C-6a), 117.6 (C-5), 145.6 (C-4), 147.0 (C-8), 147.1 (C-2), 151.4 (C-6), 168.6 (C-2'). Mass spectrum (H.R.E.I.): m/z 261 ([M - IH]$^+$, $C_{12}H_{15}N_5S$ requires 261.1048 Found: 261.1048) (29), 247 (40), 232 (13), 186 (56), 175 (24), 161 (25), 148 (13), 142 (100), 127 (24). Mass Spectrum (F.A.B.): m/z 263 ([M - I] + H$^+$) (21), 262 ([M - I]$^+$) (100). Analysis: Calculated for ($C_{12}H_{16}IN_5S$) (%): C, 37.02; H, 4.14; N, 17.99; Found (%): C, 36.87; H, 4.08; N, 17.54.

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzselenazolium Iodide **(11)**

This compound was prepared by the method described previously for compound (**6a**). 2,3-Dimethyl-benzselenazolium iodide (0.43 g, 1.26 mmol), 3,7-dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.43 g, 1.16 mmol), methanol (6 ml) and triethylamine (0.26 ml) gave 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl] benzselenazolium iodide (**11**) (0.25 g, 40%), m.p.>300° C.

UV data: (MeOH; [c]: 4.1 10^{-5} M); λ_{max} = 452 (log ϵ 4.53), 438 (4.37), 221 (4.07) and 202 nm (4.06). ^{1}H-NMR data: δ(DMSO-D_6) 4.01 (s, 3H, NCH_3), 4.03 (s, 3H, NCH_3), 4.29 (s, 3H, N^+CH_3), 6.84 (s, 1H, H-6a), 7.35 (t, 1H, H-5'), 7.57 (t, 1H, H-6'), 7.77 (d, 1H, H-4'), 8.07 (d, 1H, H-7'), 8.53 (d, 1H, H-8), 8.91 (s, 1H, H-2); $J_{H\text{-}4', H\text{-}5'} = J_{H\text{-}6', H\text{-}7'}$ = 8.0, $J_{H\text{-}5', H\text{-}6'}$ = 7.2 Hz. ^{13}C-NMR data: δ(DMSO-D_6) 35.1 (NCH_3), 35.1 (NCH_3), 35.9 (N^+CH_3), 88.8 (C-6a), 115.2 (C-7'), 118.4 (C-5), 124.2 (C-4'), 126.0 (C-8'), 127.5 (C-5'), 128.6 (C-6'), 141.8 (C-9'), 146.1 (C-8), 146.6 (C-4), 146.9 (C-2), 149.1 (C-6), 164.3 (C-2'). Mass spectrum (H.R.E.I.): m/z 357 ([M - IH]$^+$, $C_{16}H_{15}N_5Se$ requires 357.0493 Found: 357.0496) (2), 343 (63), 328 (7), 316 (6), 301 (3), 262 (12), 210 (5), 161 (16), 142 (100), 127 (19), 77 (4).

4,5-Diphenyl-3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]oxazolium Iodide **(12)**

This compound was prepared by the method described previously for compound (**8**). 2,3-Dimethyl-4,5-diphenyloxazolium iodide (0.56 g, 1.5 mmol), 3,7-dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol), dimethylformamide (5 ml) and triethylamine (0.3 ml) gave 4,5-diphenyl-3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]oxazolium iodide (**12**) (0.35 g, 45%), m.p. >300° C.

UV data: (MeOH; [c] 6 10^{-4} M); λ_{max} = 441 (log ϵ 4.40), 421 (4.42) and 202 nm (4.31). ^{1}H-NMR data: δ(DMSO-D_6) 3.19 (s, 3H, NCH_3), 3.43 (s, 3H, NCH_3), 4.21 (s, 3H, N^+CH_3), 5.70 (s, 1H, H-6a), 7.4 - 7.7 (m, 10H, 2Ph), 8.37 (s, 1H, H-8), 8.60 (s, 1H, H-2). ^{13}C-NMR data: δ(DMSO-D_6) 31.5 (NCH_3), 34.8 (NCH_3), 35.4 (N^+CH_3), 86.8 (C-6a), 116.7 (C-5), 124.2 (C-4", C-4'"), 126.3 (C-1'"), 126.6 (C-1"), 128.1 (C-5'), 129.2 (C-5'", C-6'"), 129.3 (C-5", C-6"), 129.9 (C-2'", C-3'"), 130.9 (C-2", C-3"), 131.2 (C-4'), 137.6 (C-6), 141.1 (C-8), 146.4 (C-2), 150.1 (C-4), 159.2 (C-2'). Mass spectrum (H.R.E.I.): m/z 396 ([M - I]$^+$, $C_{24}H_{22}N_5O$ requires 396.1825 Found: 396.1843) (0.4), 395 ([M - IH]$^+$) (3), 381 (40), 366 (3), 352 (2), 235 (3), 186 (3), 148 (3), 142 (100), 127 (20), 77 (6). Mass spectrum (F.A.B.): m/z 397 ([M - I] + H$^+$) (47), 396 ([M - I]$^+$) (100).

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzothiazolium p-Toluenesulphonate **(6b)**

This compound was prepared by the method described previously for compound (**9a**). 3,7-Dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol), 2,3-dimethylbenzothiazolium *p*-toluenesulphonate (0.5 g, 1.47 mmol), triethylamine (0.3 ml) and dimethylformamide (5.2 ml) gave 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzothiazolium *p*-toluensulphonate (**6b**) (0.5 g, 74%), m.p.>300° C.

UV data: (MeOH; [c] 8 10^{-5} M); λ_{max} = 449 (log ϵ 4.35), 435 (4.18), 219 (3.93) and 202 nm (3.90). ^{1}H-NMR data: δ(DMSO-D_6) 2.29 (s, 3H, $ArCH_3$), 4.00 (s, 3H, NCH_3), 4.02 (s, 3H, NCH_3), 4.28 (s, 3H, N^+CH_3), 6.58 (s, 1H, H-6a), 7.08 (d, 2H, H-3", H-5"), 7.41 (t, 1H, H-5'), 7.53 (d, 2H, H-2", H-6"), 7.58 (t, 1H, H-6'), 7.64 (d, 1H, H-4'), 8.0 (d, 1H, H-7'), 8.49 (s, 1H, H-8), 8.82 (s, 1H, H-2); $J_{H\text{-}4',\,H\text{-}5'} = J_{H\text{-}6',\,H\text{-}7'}$ = 8.0 $J_{H\text{-}5',\,H\text{-}6'}$ = 7.0 Hz. The appearance of the signals of the aromatic protons of the *p*-toluensulphonate is typical of an AA'BB' system. ^{13}C-NMR data: δ(DMSO-D_6) ($ArCH_3$), 35.4 (NCH_3), 36.3 (NCH_3), 37.4 (N^+CH_3), 87.9 (C-6a), 114.4 (C-7'), 119.7 (C-5), 124.2 (C-4'), 126.0 (C-5'), 127.2 (C-3", C-5"), 129.1 (C-8'), 129.2 (C-6'), 129.4 (C-2", C-6"), 138.9 (C-4"), 141.8 (C-1"), 147.5 (C-8), 148.0 (C-9'), 148.7 (C-2), 151.2 (C-4), 163.5 (C-6), 207.5 (C-2'). Mass spectrum (F.A.B.): m/z 481 ([M]$^+$) (4), 311 ([M - $C_7H_7SO_3$] + H^+) (30), 310 ([M - $C_7H_7SO_3$]$^+$) (100). Analysis: Calculated for ($C_{23}H_{23}N_5O_3S_2$) (%): C, 57.37; H, 4.82; N, 14.55; S, 13.29; Found (%): C, 57.13; H, 4.59; N, 14.32; S, 13.46.

3-Methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzoxazolium p-Toluenesulphonate **(9b)**

This compound was prepared by the method described previously for compound (**9a**). 3,7-Dimethyl-6-(methylthio)purinium *p*-toluenesulphonate (**4**) (0.5 g, 1.36 mmol), 2,3-dimethylbenzoxazolium *p*-toluenesulphonate (0.5 g, 1.55 mmol), triethylamine (0.3 ml) and dimethylformamide (5.2 ml) gave 3-methyl-2-[(3,7-dimethyl-6-purinylidene)-methyl]benzoxazolium *p*-toluenesulphonate (**9b**) (0.3 g, 40%), m.p.>300° C.

UV data: (MeOH; [c] 6.9 10^{-5} M); λ_{max} = 428 (log ϵ 4.40), 260 (3.41) and 204 nm (3.90). ^{1}H-NMR data: δ(DMSO-D_6) 2.26 (s, 3H, $ArCH_3$), 3.80 (s, 3H, NCH_3), 3.94 (s, 3H, NCH_3), 4.24 (s, 3H, N^+CH_3), 5.72 (s, 1H, H-6a), 7.09 (d, 2H, H-3", H-5"), 7.36 (t, 1H, H-5'), 7.45 (d, 2H, H-2", H-6"), 7.47 (t, 1H, H-6'), 7.64 (d, 1H, H-4'), 7.76 (d, 1H, H-7'), 8.53 (s, 1H, H-8), 8.72 (s, 1H, H-2); $J_{H\text{-}4',\,H\text{-}5'} = J_{H\text{-}6',\,H\text{-}7'}$ = 8.0, $J_{H\text{-}5',\,H\text{-}6'}$ = 7.2 Hz. The appearance of the signals of the aromatic protons of the *p*-toluensulphonate is typical of an AA'BB' system. ^{13}C-NMR data: δ(DMSO-D_6) 21.2 ($ArCH_3$), 31.4 (NCH_3), 34.9 (NCH_3), 35.3 (N^+CH_3), 73.2 (C-6a), 110.9 (C-4', C-7'), 118.0 (C-5), 124.6 (C-5'), 125.6 (C-3", C-5"), 125.9 (C-6'), 128.1 (C-2", C-6"), 129.3 (C-9'), 131.3 (C-8'), 137.7 (C-4"), 145.8 (C-1"), 146.4 (C-8), 147.3 (C-2), 147.8 (C-4), 151.6 (C-6), 161.3 (C-2'). Mass spectrum (F.A.B.): m/z 465 ([M]$^+$) (1), 295 ([M - $C_7H_7SO_3$] + H^+) (28), 294 ([M - $C_7H_7SO_3$]$^+$) (100). Analysis: Calculated for ($C_{23}H_{23}N_5O_4S$) (%): C, 59.39; H, 4.98; N, 15.12; S, 6.89; Found (%): C, 59.20; H, 4.77; N, 15.02; S, 6.79.

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(*Received in UK* 31 *December* 1996; *accepted* 15 *May* 1997)