Facile, General and Productive Syntheses of the Fluorescent Wye (4,9-Dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine) in Phenylalanine *t*RNA, its 2-Substituted Derivatives and 7-Aza Analogues

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Novel syntheses of 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine (Y base skeleton), its 2-substituted derivatives and 7-aza analogues and their fluorescence characteristics in relation to their structures are described.

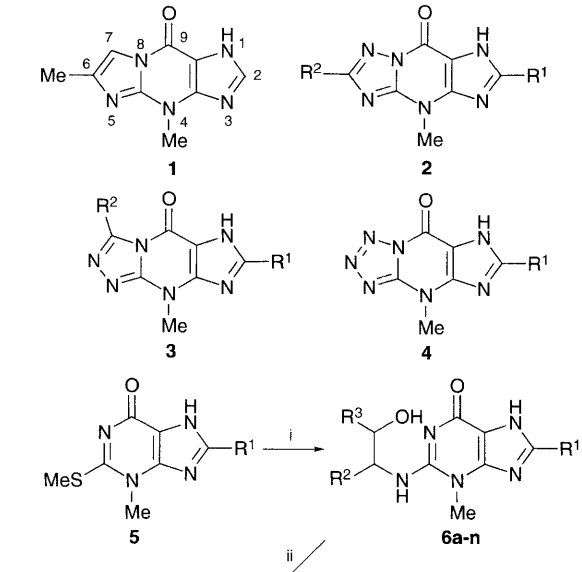
The hypermodified nucleic acid base wye (Y_t-base) **1**, a tricyclic fluorescent base adjacent to the 3′-end of the anticodon loop of yeast phenylalanine transfer ribonucleic acid (*t*RNA^{phe}),[1] has been isolated from *Torulopsis utilis* *t*RNA^{phe} and the structure elucidated as 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine.[2] Its 7-substituted congeners, wybutine and wybutoxine,[3] and its 3-β-D-ribofuranosyl congeners, wyosine, wybutosine and wybutoxosine,[4] have also been found in various eukaryotic species and are believed to play an important role in codon–anticodon recognition.[1,5] Since the recent discovery of the antiallergic and bronchodilator activities[6] of the 6,7-dihydro analogues of wye and the anti-herpetic and -viral activities[7] of the 4-dealkyl analogues they have received much attention. We prepared wye and its analogues because of their biological activity and distinctive chemical properties. Here we report a new, convenient and general preparation of the tricyclic purine compounds (**2** and **8**) in connection with our earlier work (compounds **3** and **4**)[8] and also their fluorescence properties in relation to their structures.

Wye **8b**, 6-demethylwye **8a** and its 7-methyl isomer **8c** have previously been prepared in low yields by the condensation of 3-methylguanine with bromoacetone, chloroacetaldehyde and α-bromopropionaldehyde.[2] Later the yield of wye **8b** was improved by using bromoacetone and 3-methylguanine protected by a benzyl group at the 7-position.[9] However, 2-substituted wye derivatives (**8**, R^1 = alkyl or aryl) are little known.[6] Although 3-methylguanines appear to be a good synthetic intermediates for the synthesis of the tricyclic purine derivatives, the preparation of 8-substituted 3-methylguanines, to our knowledge has not yet been reported. The availability of 3-methyl-2-methylsulfanyl-7*H*-purine-6(3*H*)-one and its 8-substituted derivatives **5**[10] in our laboratory enabled us to synthesise the wye derivatives **8a–c** and their 8-substituted derivatives **8d–n** starting from compounds **5** as shown in Scheme 1.

A mixture of compound **5** and the appropriate aminoalcohols (5 equiv.), *e.g.* 2-aminoethanol, DL-2-amino-propan-1-ol, and 1-amino-propan-2-ol, was heated at 110 °C and then diluted with ethanol to afford the corresponding 2-(2-hydroxyethylamino)-3-methyl-7*H*-purin-6(3*H*)-ones **6a–n** in good yields.† Heating the products **6a–n** with polyphosphoric acid (PPA) (6 equiv.) at 140 °C or refluxing with excess phosphoryl chloride then gave the corresponding 6,7-dihydro tricyclic purine derivatives, 4,6,7,9-tetrahydro-4-methyl-9-oxo-1*H*-imidazo[1,2-*a*]purines **7a–n** in good yields (Table 1). Finally, the oxidation of compounds **7a–n** with manganese dioxide (10 equiv.) in DMF at 140 °C led to the desired 2-, 6- or 7-substituted wye derivatives, 4,9-dihydro-4-methyl-9-oxo-1*H*-imidazo[1,2-*a*]purines **8a–n** in good yields. The structures of all the new compounds were verified by satisfactory spectral and analytical data. The method described constitutes a very general and useful method for the preparation of not only wye **1** but also the 2-, 6- and/or 7-substituted wye derivatives **8**.

Compounds **5** are also good starting key compounds for the synthesis of the 7-aza analogue ring system **2**, Scheme 2. When compounds **5a–c** were treated with liquid ammonia at 150 °C in an autoclave for 2 d, 8-substituted 3-methylguanine derivatives **9a–c** were obtained in high yield.† Subsequent treatment of compounds **9a–c** with hydroxylamine-*O*-sulfonic acid (HAOS) (3 equiv.) in NaOH (1 mol dm^{-3}) at room temperature afforded the corresponding diamino purine derivatives **10a–c**.† Heating compounds **10a–c** thus obtained with the appropriate *ortho* esters (6 equiv.) under reflux or with aldehydes and diethyl azodicarboxylate (DEAD) in DMF at 50–100 °C gave the desired 7-aza analogues, 4-methyl-1*H*-[1,2,4]triazolo[1,5-*a*]purin-9(4*H*)-ones **2a–h**, in good yields (Table 2).

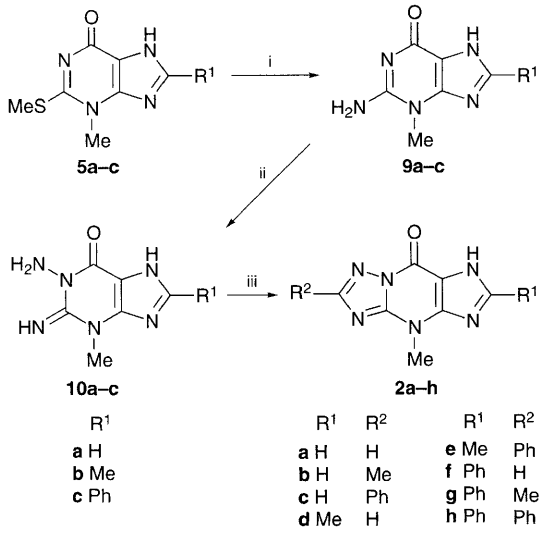
The fluorescence characteristics of the above tricyclic purine derivatives were examined in relation to their ring systems. The fluorescence quantum yields were determined according to the method developed by Paker and Rees[12] and the value (0.55) for quinine sulfate solution in H_2SO_4 (0.05 mol dm^{-3}) was used as a standard. The fluorescence of nucleic acid bases is very weak in neutral aqueous solution, while that of modified bases in natural nucleic acids such as wye and Y-nucleosides is sufficiently strong.[2,13] The fluorescence quantum yield (ϕ_f) of wye has been reported as 0.32 (in methanol) and 0.21 (in water).[14] Table 1 summarises the positions of absorption,



	R^1	R^2	R^3		R^1	R^2	R^3
a	H	H	H	**h**	4-F-C_6H_4	H	H
b	H	Me	H	**i**	4-Cl-C_6H_4	H	H
c	H	H	Me	**j**	2-HO-C_6H_4	H	H
d	Me	H	H	**k**	4-Me-C_6H_4	H	H
e	Ph	H	H	**l**	4-MeO-C_6H_4	H	H
f	2-F-C_6H_4	H	H	**m**	4-O_2N-C_6H_4	H	H
g	3-F-C_6H_4	H	H	**n**	3,4-CH_2O_2-C_6H_3	H	H

Scheme 1 *Reagents and conditions.* i, $H_2NCH(R^2)CH(R^3)OH$, 110 °C, 3–10 h; ii, PPA, 140 °C, 10–24 h, or $POCl_3$, reflux, 4–8 h; iii, MnO_2, DMF, 140 °C, 10–24 h

fluorescence excitation and fluorescence emission maximum, and the fluorescence quantum yields for the wye derivatives **8a–n** and their dihydro derivatives **7a–n** in ethanol. Ethanol was used as the solvent on the basis of the good solubility of all compounds. The fluorescence intensities of wye and its 2-alkyl derivatives **8a–d** (ϕ_f = *ca.* 0.2–0.3) were stronger than those of the 2-aryl derivatives **8e–n** (ϕ_f = *ca.* 0.1–0.2). Although 6,7-dihydro wye and its 2-alkyl derivatives **7a–d** (ϕ_f = *ca.* 0.02–0.03) showed a very weak fluorescence intensity, the 2-aryl derivatives (**7e**, **h–n**) (ϕ_f = *ca.* 0.3–0.6) showed a stronger fluorescence intensity than the wye derivatives **8a–d** without a 2-aryl group but with a 2- or 3-fluoro group **7f**,**g**. On the other hand, the fluorescence intensity of the 7-aza analogues **2a–h** were greatly enhanced with a phenyl substituent at the 2-position (ϕ_f: **2f** = 0.67, **2g** = 0.36, **2h** = 0.63), but reduced with a phenyl substituent at the 6-position (ϕ_f: **2c** = none, **2e** = 0.02) (Table 2). Moreover, some of the 6-aza **3a–e** and 6,7-diaza analogues **4a–c** also showed strong fluorescence intensity (ϕ_f: **3c** = 0.49, **4b** = 0.23, **4c** = 0.36). Other derivatives indicated had no significant fluorescence.



Scheme 2 *Reagents and conditions*: i, liq. NH_3, 150 °C, 50 kg cm^{-2}, 2 d; ii, H_2NOSO_3H, 1M NaOH, room temp., 4 d; iii, $R^2C(OEt)_3$, reflux, 10–15 h, or R^2-CHO, DMF, DEAD, 50–100 °C, 10 h

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Table 1 Analytical data and absorption and fluorescence spectral data for the compounds **7a–n** and **8a–n**

Compound **7**[a]	Yield (%)	Mp/°C[b]	Absorption and fluorescence (λ_{max}/nm) UVλ; Fλ$_{ex}$; Fλ$_{em}$ (ϕ_f)[c]	Compound **8**[a]	Yield (%)	Mp/°C[b]	Absorption and fluorescence (λ_{max}/nm) UVλ; Fλ$_{ex}$; Fλ$_{em}$ (ϕ_f)[c]
a	65	326–328	299; 303; 424 (0.02)	**a**[d]	85	> 300	303; 305; 418 (0.31)[e]
b	66	292–294	298; 301; 423 (0.02)	**b**[d]	82	> 300	304; 307; 435 (0.30)[f]
c	69	293–295	298; 303; 424 (0.02)	**c**[d]	79	> 300	311; 313; 449 (0.23)
d	71	305–307	298; 303; 416 (0.03)	**d**	75	> 300	300; 303; 410 (0.21)
e	78	304 (decomp.)	333; 336; 432 (0.32)	**e**	84	> 300	328; 328; 455 (0.12)
f	69	302 (decomp.)	334; 341; 438 (0.17)	**f**	82	> 300	332; 291; 471 (0.08)
g	83	264 (decomp.)	336; 340; 436 (0.07)	**g**	81	> 300	327; 289; 471 (0.09)
h	85	297 (decomp.)	331; 336; 438 (0.29)	**h**	83	> 300	325; 326; 449 (0.12)
i	79	257 (decomp.)	337; 342; 441 (0.29)	**i**	78	> 300	316; 320; 478 (0.10)
j	71	> 300	348; 344; 425 (0.41)	**j**	83	> 300	344; 331; 463 (0.13)
k	72	301 (decomp.)	333; 337; 434 (0.34)	**k**	84	> 300	329; 329; 437 (0.16)
l	73	266 (decomp.)	333; 336; 424 (0.38)	**l**	75	> 300	326; 326; 440 (0.17)
m	69	> 300	345; 348; 419 (0.38)	**m**	58	> 300	344; 345; 427 (0.14)
n	82	> 300	340; 342; 427 (0.59)	**n**	75	> 300	334; 322; 446 (0.17)

[a] Satisfactory elemental combustion analyses and mass, IR and ^{1}H NMR spectral data were obtained for all new compounds. [b] Compounds **7a–n** and **8a–n** were recrystallised from DMF and EtOH respectively. [c] The absorption and fluorescence spectra of all compounds were measured in EtOH and parentheses stand for the quantum yield. [d] See reference 2. [e] In H_2O; UVλ: 306; Fλ$_{ex}$: 308; Fλ$_{em}$: 430 (0.16). [f] In H_2O; UVλ: 306; Fλ$_{ex}$: 308; Fλ$_{em}$: 440 (0.15).

Table 2 Analytical data and absorption and fluorescence spectral data for the compounds **2a–h**, **3a–e** and **4a–c**

Compound **2**[a]	Yield (%)	Mp/°C[b]	Absorption and fluorescence (λ_{max}/nm) UVλ; Fλ$_{ex}$; Fλ$_{em}$ (ϕ_f)[c]	Compound[d]	R^1	R^2	Absorption and fluorescence (λ_{max}/nm) UVλ; Fλ$_{ex}$; Fλ$_{em}$ (ϕ_f)[c]
a	63	> 300	288; 314; 421 (0.08)	**3a**	H	H	286; 325; 423 (N.d.)
b	65	> 300	288; 323; 422 (N.d.)	**3b**	Me	H	302; 349; 423 (N.d.)
c	72	> 300	296; 320; 424 (N.d.)	**3c**	Ph	H	328; 328; 382 (0.49)
d	73	> 300	286; 325; 420 (N.d.)	**3d**	H	Me	306; 318; 415 (0.01)
e	67	> 300	292; 295; 375 (0.02)	**3e**	H	Ph	296; 328; 421 (N.d.)
f	61	> 300	316; 315; 364 (0.67)	**4a**	H	—	294; 305; 435 (N.d.)
g	69	> 300	308; 307; 371 (0.36)	**4b**	Me	—	294; 313; 468 (0.23)
h	53	> 300	334; 292; 366 (0.63)	**4c**	Ph	—	322; 320; 364 (0.36)

[a] Satisfactory elemental combustion analyses and mass, IR and ^{1}H NMR spectral data were obtained for all new compounds. [b] Compounds **2a–h** were recrystallised from DMF. [c] The absorption and fluorescence spectra of all compounds were measured in EtOH and parentheses stand for the quantum yield. N.d. means not detected. [d] See reference 8.

Footnote

† *Physical data* (mp; yield). For **6a**: 273–275 °C; 70%. For **6b**: 236–238 °C; 70%. For **6c**: 253–255 °C; 78%. For **6d**: 301–303 °C; 67%. For **6e**: 296–298 °C; 67%. For **6f**: 301–303 °C; 69%. For **6g**: 291 °C (decomp.); 76%. For **6h**: 286 °C (decomp.); 75%. For **6i**: 264–266 °C; 71%. For **6j**: 277–279 °C; 66%. For **6k**: 291–293 °C; 74%. For **6l**: 269–271 °C; 74%. For **6m**: 284–286 °C; 68%. For **6n**: 285 °C (decomp.); 67%. For **9a** (lit.,[11] >300 °C): 338 °C (decomp.); 82%. For **9b**: >330 °C; 81%. For **9c**: >300 °C; 73%. For **10a**: 304–306 °C; 33%. For **10b**: 270–272 °C; 31%. For **10c**: 277–279 °C; 34%. All new compounds gave satisfactory elemental combustion analyses and mass, IR and ^{1}H NMR spectral data.

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