Dyes and Pigments **12** (1990) 21–26

Synthesis of Pyrimido[5,4-*d*]pyrimidine Derivatives and their Ultraviolet Absorption and Fluorescence Spectral Properties

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(Received 26 September 1988; accepted 31 October 1988)

ABSTRACT

*This paper describes the synthesis of some tri- and tetra-substituted pyrimido[5,4-*d*]pyrimidine derivatives and their absorption and fluorescence spectral properties, in the expectation of such compounds having application as fluorescence reagents in the peroxyoxalate-chemiluminescence reaction system. Of the 18 derivatives investigated, the tetra-substituted* m*-methoxyanilino compound showed the strongest relative fluorescence intensity.*

1 INTRODUCTION

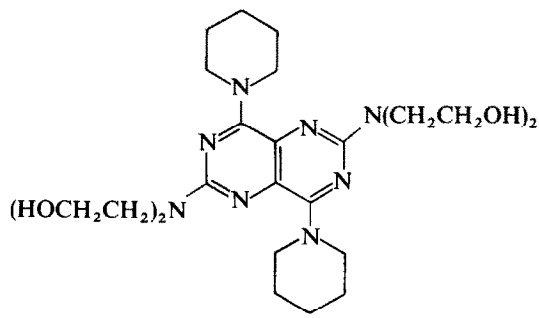
Pyrimidopyrimidines having coronary vasodilatory and diuretic effects were initially synthesized by Fischer *et al.*[1,2] Later, they and Iida *et al.* reported the fluorescence spectra of some of these compounds[3,4] and [2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine] (Dipyridamole) was found to show a particularly intense blue–green fluorescence.[5,6] We have recently reported that Dipyridamole shows the lowest lower detection limit in a study on the estimation of the detection limits of fluorescent compounds when using the peroxyoxalate-chemiluminescence (CL) reaction system, in which the presence of a coexisting fluorescent

Dyes and Pigments 0143-7208/89/$03·50

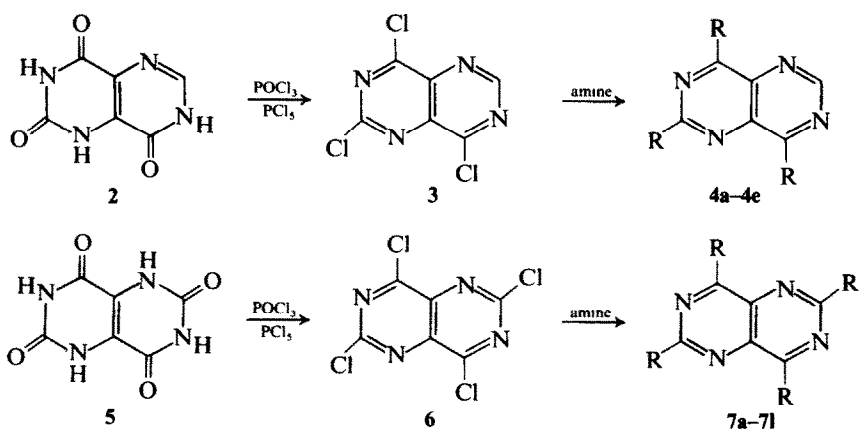
substance having an intensive fluorescence intensity is very important.[7] We now report the synthesis of further fluorescent compounds having the same pyrimido[5,4-*d*]pyrimidopyrimidine skeleton as Dipyridamole and an evaluation of their ultraviolet absorption and fluorescence spectra with respect to their possible use as CL reagents.

2 RESULTS AND DISCUSSION

As shown in Scheme 1, the reactions of **3** or **6** with amines were carried out using Fischer's[1,8] or Iida's[4] method to give the corresponding tri- or tetra-substituted pyrimidopyrimidines (**4a–4e** or **7a–7e**) in good yields. Melting



Dipyridamole, **1**



2 **3** **4a–4e**

5 **6** **7a–7l**

R			
4a	anilino	**7a** anilino	**7g** piperidino
4b	*m*-methoxyanilino	**7b** *m*-methoxyanilino	**7h** morpholino
4c	*p*-methoxyanilino	**7c** *p*-methoxyanilino	**7i** thiomorpholino
4d	pyrrolidino	**7d** diphenylamino	**7j** thiazolidino
4e	morpholino	**7e** benzylamino	**7k** ethanolamino
		7f pyrrolidino	**7l** diethanolamino

Scheme 1

TABLE 1
Pyrimido[5,4-*d*]pyrimidine Derivatives (**4** and **7**)

Compound	*M.p.* (°C)	*Molecular formula*	*Elemental analyses (%) Calc. (Found)*			
			C	*H*	*N*	*S*
4a	195–199 (203–204)[a]	$C_{24}H_{19}N_7 \cdot \frac{1}{2}H_2O$	69·6 (70·0)	4·9 (4·9)	23·7 (23·6)	
4b	173–136	$C_{27}H_{25}O_3N_7$	65·4 (65·2)	5·1 (5·2)	19·8 (19·5)	
4c	205–206	$C_{27}H_{25}O_3N_7$	65·4 (65·0)	5·1 (5·2)	19·8 (19·6)	
4d	169–173	$C_{18}H_{25}N_7$	63·7 (63·9)	7·4 (7·4)	28·9 (28·9)	
4e	184–186 (182–184)[a]	$C_{18}H_{25}O_3N_7$	55·8 (55·8)	6·5 (6·5)	25·3 (25·3)	
7a	>300 (>310)[b]	$C_{30}H_{24}N_8$	72·6 (72·4)	4·9 (5·2)	22·6 (22·2)	
7b	221–223 (228–230)[b]	$C_{34}H_{32}N_8O_4$	66·2 (65·5)	5·2 (5·2)	18·2 (18·0)	
7c	256–261 (264–266)[b]	$C_{34}H_{32}N_8O_4 \cdot C_4H_8O_2$[d]	64·8 (64·7)	5·7 (5·7)	15·9 (16·1)	
7d	>300	$C_{54}H_{40}N_8 \cdot HCl$	77·5 (76·8)	4.9 (4·9)	13.4 (13·2)	
7e	175–178 (176–178)[c]	$C_{34}H_{32}N_8$	73.9 (73·8)	5.8 (5·9)	20.3 (20·1)	
7f	>300	$C_{22}H_{32}N_8$	64·7 (64·7)	7·9 (7·9)	27·4 (27·5)	
7g	163–164 (163–165)[a]	$C_{26}H_{40}N_8$	67·2 (67·2)	8·7 (8·6)	24·1 (24·0)	
7h	269–271 (266–268)[a]	$C_{22}H_{32}O_4N_8$	55·9 (55·9)	6·8 (6·7)	23·7 (23·7)	
7i	237–239	$C_{22}H_{32}N_8S_4$	49·2 (49·3)	6·0 (5·9)	20·9 (20·8)	24·0 (24·1)
7j	265–267	$C_{18}H_{24}N_8S_4 \cdot \frac{1}{2}2H_2O$	44·1 (44·2)	5·2 (4·9)	22·9 (22·6)	26·2 (26·0)
7k	190–192 (180–182)[a]	$C_{14}H_{24}O_4N_8$	45·6 (45·9)	6·6 (6·4)	30·4 (29·8)	
7l	208–210	$C_{22}H_{40}O_8N_8$	48·5 (48·6)	7·4 (7·4)	20·6 (20·4)	

[a] Ref. 9.
[b] Ref. 4.
[c] Ref. 10.
[d] Dioxane.

points and analytical data of the compounds thus prepared are listed in Table 1.

Ultraviolet spectral data in dioxane or methanol are shown in Table 2. All the compounds showed intense absorption bands in the 280–340 nm region and molar absorptivities of the tetra-substituted compounds were generally larger than those of the tri-substituted compounds. Compounds **7a–7d**, which have aromatic substituents and compounds **7i–7j**, which have alicyclic thiosubstituents, had strong absorption intensities.

TABLE 2
Absorption Spectral Data of Pyrimido[5,4-*d*]pyrimidine Derivatives (Solvent: Dioxane)

Compound	λ_{max} (*nm*), (log ε)
1	238 (4·14) 295 (4·49) 307 (4·48) 413 (3·94) 432 (3·89)
4a	239 (4·17) 265 (4·13) 312 (4·37) 378 (4·13) 397 (4·02)
4b	238 (4·40) 260 (4·33) 314 (4·55) 380 (4·35) 398 (4·25)
4c	241 (4·42) 264 (4·30) 319 (4·53) 386 (4·34)
4d	242 (4·29) 269 (4·12) 306 (4·40) 375 (4·15) 395 (4·07)
4e	238 (4·30) 304 (4·39) 375 (4·12)
7a	237 (4·40) 304 (4·64) 403 (4·03) 425 (3·98)
7b	240 (4·46) 320 (4·83) 405 (4·15) 426 (4·09)
7c	247 (4·40) 318 (4·70) 410 (4·06)
7d	254 (4·71) 290 (4·67) 337 (4·73) 455 (4·10)
7e	258 (4·39) 287 (4·40) 297 (4·37) 378 (3·89)
7f	238 (4·05) 299 (4·33) 310 (4·33) 383 (3·80) 403 (3·88) 426 (3·64)
7g	239 (4·23) 288 (4·49) 297 (4·50) 310 (4·50) 411 (3·92)
7h	236 (4·20) 295 (4·48) 308 (4·47) 405 (3·92)
7i	240 (4·42) 295 (4·64) 309 (4·62) 413 (4·03)
7j	235 (4·39) 279 (4·55) 296 (4·60) 307 (4·59) 400 (4·10)
7k[a]	255 (4·35) 282 (4·41) 376 (3·88)
71[a]	226 (3·96) 293 (3·98) 305 (3·97) 406 (4·46) 427 (3·40)

[a] Solvent: Methanol.

The fluorescence spectral data of **1**, **4** and **7** are shown in Table 3. Emission maxima are observed in the 435–547 nm region and the order of decreasing fluorescence intensity (RFI) is **7b** ≫ **1** > **7a** > **7i** > **4d**.

In the CL system for determining peroxides, the development of fluorescence reagents is particularly desirable in order to obtain a more intense fluorescence intensity. From this point of view, compounds **7a–7b** and **7i** appear to be particularly useful. A study on further derivatives of pyrimidopyrimidines is in progress in attempt to produce a stronger fluorescence.

TABLE 3
Fluorescence Spectral Data of Pyrimido[5,4-*d*]pyrimidine Derivatives (Solvent: Dioxane)

Compound	λ_{ex}	λ_{em}	*RFI*[a]
1	310	472	100
4a	322	445	11
4b	325	450	18
4c	330	470	4
4d	317	435	63
4e	312	445	40
7a	327	470	88
7b	332	472	149
7c	332	485	40
7d	340	547	31
7e	298	445	46
7f	312	455	42
7g	312	475	50
7h	310	475	42
7i	310	472	75
7j	310	467	45
7k[b]	294	452	26
71[b]	307	480	13

[a] Relative fluorescence intensity; fluorescence intensity of **1** was taken as 100.
[b] Solvent: methanol.

3 EXPERIMENTAL

3.1 General

All melting points are uncorrected. Ultraviolet spectra were recorded on a Hitachi 200-10 spectrophotometer and fluorescence spectra on a Hitachi 650-10S fluorescence spectrophotometer using 10 mm × 10 mm quartz cells. The concentrations of the solutions used for these measurements were $(2–5) \times 10^{-5}$ and 1×10^{-6} M, respectively. Dipyridamole was purchased from Sigma Chemical Co. Ltd, USA.

3.2 Synthesis

*3.2.1 2,4,8-Trichloropyrimido[5,4-d]pyrimidine (**3**) and 2,4,6,8-tetrachloropyrimido[5,4-d]pyrimidine (**6**)*

These compounds were prepared according to the literature.[8]

3: Yield 46%, m.p. 178–180°C (ether) (lit.[8] 181°C).
Found: C, 30·6; H, 0·4; N, 23·8; Cl, 45·1%.
$C_6H_4Cl_3$ requires: C, 31·0; H, 0·7; N, 24·0; Cl, 44·2%.

6: Yield 50%, m.p. 252–256°C ($CHCl_3$) (lit.[8] 254–258°C).
Found: C, 26·7; N, 20·8; Cl, 52·6%.
$C_6N_4Cl_4$ requires: C, 26·6; N, 20·7; Cl, 52·8%.

*3.2.2 Pyrimidopyrimidines (**4** and **7**): general procedure*
A mixture of **6i** (0·37 mmol) and thiomorpholine (9·3 mmol) was heated at *ca* 180°C for 1·5 h. After cooling the mixture was diluted with water (20 ml) to give dark yellow crystals (**7i**), which were filtered and recrystallized from acetone (56%).

Data for **7i**, and other compounds similarly prepared, are given in Tables 1, 2 and 3.

ACKNOWLEDGEMENTS

This research was part of a study supported by a Grant-in-Aid for Scientific Research (No. 63571023) from the Ministry of Education, Science and Culture of Japan.

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