Synthesis of Queuine, the Base of Naturally Occurring Hypermodified Nucleoside (Queuosine), and Its Analogues

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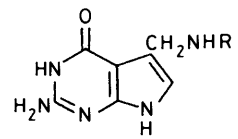
A convenient new method for synthesizing queuine (**1**) {2-amino-5-[(1*S*,2*R*,3*S*)-2,3-dihydroxycyclopent-4-enylaminomethyl]pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one}, the base of the naturally occurring hypermodified nucleoside, queuosine, present in certain transfer RNAs, and its biosynthetic precursor, 2-amino-5-aminomethylpyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**2**) (Pre Q1 base), was successfully exploited. This method involved two critical reactions: the Mannich reaction using dibenzylamine–formaldehyde of 2-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**7**), which resulted in the selective introduction of the dibenzylaminomethyl group into the 5-position of (**7**), and an amine exchange reaction of the 5-dibenzylamino function in the resulting Mannich base (**17**) with (1*S*,2*R*,3*S*)-2,3-isopropylidenedioxycyclopent-4-enylamine, which yielded the desired queuine (**1**). Similar reaction of (**17**) with ammonia gave the biosynthetic precursor of queuine (**2**) (Pre Q1 base). Thus, a series of queuine analogues with structural variations in their 5-aminomethyl side-chains was synthesized by the amine exchange reaction of (**17**) with appropriate amines or by acylation of (**2**) with appropriate acylating agents.

Queuine (**1**) {2-amino-5-[(1*S*,2*R*,3*S*)-2,3-dihydroxycyclopent-4-enylaminomethyl]pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one} is the base of a hypermodified nucleoside, queuosine, located in the first position of the anticodon of tRNA molecules from various organisms, including mammalian tissues. Because of its unique structure and particular biosynthesis, much attention has been focussed on its biological function in cells, tissues, and whole mammals.[1] Its functional role, however, remains to be clarified. The present study was initiated in order to obtain a sample, sufficient in amount to allow further extensive biological studies of (**1**).[1] The first total syntheses of queuosine, queuine, and its biosynthetic precursor,[2] pre Q1 base (**2**) {2-amino-5-aminomethylpyrrolo[2,3-*d*]-pyrimidin-4(3*H*)-one}, were achieved by Goto *et al.*[3] However, their methods of synthesis, although elegant, require extremely lengthy and multistep procedures which are inefficient when synthesizing large quantities of (**1**) and its analogues for extensive biological studies.



(**1**) R =

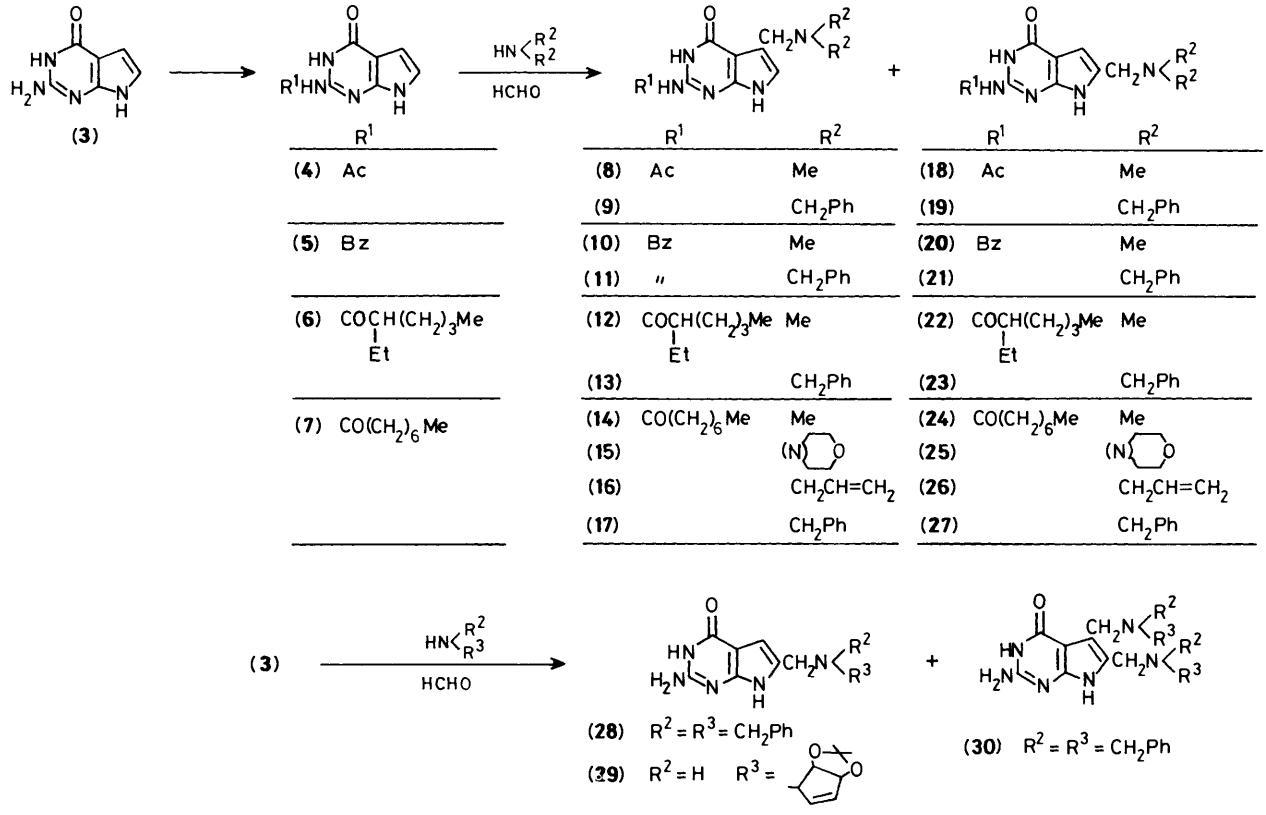
(**2**) R = –H

Structures of Q Base (**1**) and Pre Q1 Base (**2**)

In the present paper, we report an improved method for synthesizing queuine and its analogues with natural and unnatural 5-substituted aminomethyl side-chains, starting from the 2-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones. Our method involves two critical steps, the direct introduction of a substituted aminomethyl function into the 5-position of 2-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones by the Mannich reaction and an amine exchange reaction between the 5-substituted aminomethyl group of the resulting Mannich base (**17**) and an amine suitable for obtaining the desired products. The present method enabled us to synthesize sufficient amounts of (**1**) and a wide variety of its analogues (**2**) and (**35**)—(**48**) to allow extensive biological testing.[1,4]

Results and Discussion

Mannich Reaction of 2-Acylaminopyrrolo[2,3-d]*pyrimidin-4*(3H)*-ones.*—The strategy employed in synthesizing (**1**) and its analogues was the development of a straightforward method for introducing a single-carbon unit into the 5-position of the pyrrolo[2,3-*d*]pyrimidine ring. The Mannich reaction seemed to be favourable for this purpose. When used for 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) with an amine and formaldehyde, the reaction leads to a substitution, not in the desired 5-position, but in the 6-position.[5] Careful examination of this reaction in our laboratories under different reaction conditions, including changing the structures of both pyrrolo-[2,3-*d*]pyrimidines and the secondary amines, led to the finding that differences in the structures of the 2-substituent of the pyrrolo[2,3-*d*]pyrimidines (**3**) or (**4**)—(**7**) and of the amines markedly affected the reaction products (Table 1). The reaction of the 2-aminopyrrolo[2,3-*d*]pyrimidines (**3**) with more than 2 mole equivalents of the reagent pair, amine/formaldehyde, failed to afford the desired 5-substituted product (**1**) and (**42**), as reported previously,[5] but instead gave 2-amino-6-substituted aminomethylpyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**28**) and (**29**) and the corresponding 5,6-bis(substituted aminomethyl) compound (**30**) (Table 1, Scheme 1). However, we found that, contrary to previous belief, the reaction using 2-acylamino-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**4**)—(**7**) prepared from (**3**) by direct acylation provided predominantly the desired 5-substituted aminomethyl compounds (**9**), (**11**), (**13**), and (**17**), under certain reaction conditions, together with a small amount of the C-6 isomer (**19**), (**21**), (**23**), and (**27**) (Table 1). On high performance liquid chromatography (h.p.l.c.) analysis, the ratio of 5- and 6-substituted aminomethyl compounds was found to depend more on the structure of the amine used than



Scheme 1. Mannich reaction of pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones

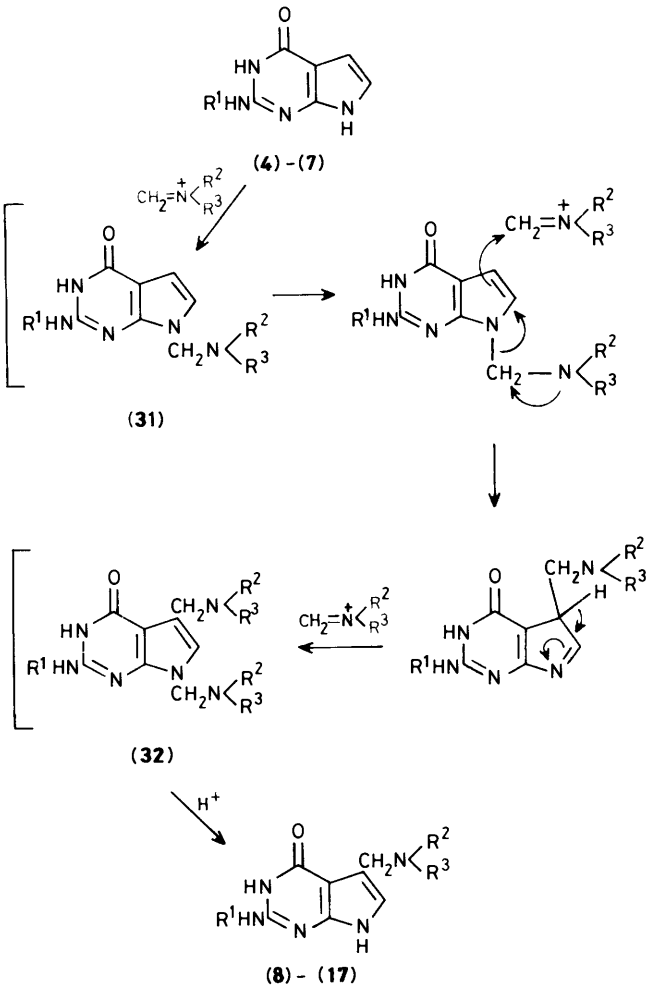
Table 1. Mannich reaction of 2-amino- or 2-acylamino-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**3**)—(**7**)[a]

Pyrrolo[2,3-*d*]pyrimidine	Disubstd. amine	Product/Aminomethyl Mannich base[b] 5-substd.:6-substd. (ratio)	Total yield (%)[c]
(**3**)	$HN(CH_2Ph)_2$	(**42**):(**28**) (<0.01:1)	23[d]
(**4**)	$HNMe_2$	(**8**):(**18**) (0.1:1)	82
(**4**)	$HN(CH_2Ph)_2$	(**9**):(**19**) (12.8:1)	65
(**5**)	$HNMe_2$	(**10**):(**20**) (0.12:1)	86
(**5**)	$HN(CH_2Ph)_2$	(**11**):(**21**) (14.0:1)	73
(**6**)	$HNMe_2$	(**12**):(**22**) (0.1:1)	90
(**6**)	$HN(CH_2PH)_2$	(**13**):(**23**) (14.3:1)	70
(**7**)	$HNMe_2$	(**14**):(**24**) (0.12:1)	92
(**7**)	$HN(CH_2)_2O(CH_2)_2$	(**15**):(**25**) (0.40:1)	75
(**7**)	$HN(CH_2CHMe_2)_2$	(**16**):(**26**) (15.8:1)	53
(**7**)	$HN(CH_2Ph)_2$	(**17**):(**27**) (14.2:1)	75

[a] Reaction conditions are described in the Experimental section. [b] Isomer ratios are given in parentheses, determined by h.p.l.c. (relative percent intensities of the peaks). [c] The number indicates the sum of the individual yields of 5- and 6-aminomethyl Mannich bases, unless otherwise indicated. Yields were not optimized. [d] The sum of the yields of 6-dibenzylaminomethyl (**28**) and the corresponding 5,6-disubstituted compound (**30**) (*ca.* 7%) is given here. No 5-isomer was detected in the reaction mixture.

on the structure of the acyl of the pyrrolo[2,3-*d*]pyrimidines (**4**)—(**7**) (see Table 1). The use of an amine with a bulky substituent favoured the introduction of the Mannich reagent into the 5-position, regardless of the difference in the spatial size of the 2-acyl group of (**4**)—(**7**). Thus, upon the reaction of 2-acylaminopyrrolo[2,3-*d*]pyrimidines with a less hindered amine, *e.g.* dimethylamine, the major product was a 6-substituted Mannich base (**18**), (**20**), (**22**), and (**24**), despite the difference in the size of the 2-acyl function (Table 1). In contrast, the reaction using bulky secondary amines, *e.g.* dibenzylamine and di-isobutylamine, mainly gave the desired 2-acylamino-5-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**9**), (**11**), (**13**), (**16**), and (**17**). These 5-substituted aminomethyl compounds are useful as key intermediates for the synthesis of queuine and its analogues.

The C-5 orientation of the Mannich substitution on 2-acylaminopyrrolo[2,3-*d*]pyrimidines (**4**)—(**7**) is postulated as that set out in Scheme 2. The existence of the 2-acylamino group appears to affect the electronic property of the pyrrolo[2,3-*d*]pyrimidine ring, *i.e.* it increases the nucleophilicity of the β-carbon of the pyrrole ring in quite a different way from that for (**3**). In addition, the C-5 orientation can be further explained from a stereochemical point of view (Scheme 2). When a 2-acylaminopyrrolo[2,3-*d*]pyrimidine and a

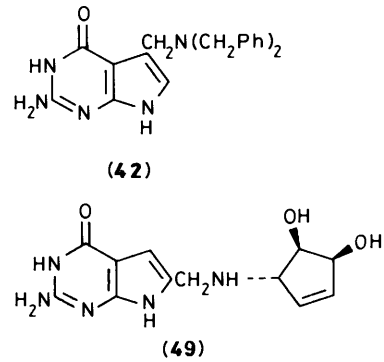


Scheme 2. Reaction mechanism of Mannich reaction

hindered amine are used, the first attack by the Mannich reagent, formed from formaldehyde and an amine, appears to be on the pyrrole nitrogen in the pyrrolo[2,3-*d*]pyrimidine (**4**)—(**7**), providing a 7(*N*)-Mannich base (**31**). This means that the second attack must occur at the 5-position of (**31**), which is less hindered, to give a 5,7-bis(substituted aminomethyl) compound (**32**). High performance thin-layer chromatography (h.p.t.l.c.) analysis showed the existence of the proposed Mannich bases (**31**) and (**32**) in the corresponding reaction mixture, but, due to the lability of the 7-*N*-substituent, they could not be isolated in pure forms. Upon very mild acid hydrolysis, compound (**32**) gave the desired 2-acylamino-5-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**9**), (**11**), (**13**), (**16**), and (**17**).

Structural assignments for the 5- and 6-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**8**)—(**17**) and (**18**)—(**27**) were supported by elemental analyses and spectral data. The n.m.r. spectra of each pair of C-5 and C-6 isomers were very similar. A comparison of the chemical shifts of each pair of isomers, however, showed that the signal due to the pyrrole ring proton (6-H) in the former was generally shifted downfield [0.41—0.60 p.p.m.; $(CD_3)_2SO$] from that of the corresponding proton (5-H) in the latter (Table 5). The finding that the signal due to 6-H of the C-5 isomer generally exists as a doublet, in contrast to the singlet due to 5-H for the C-6 isomer, provided further support for the proposed structure of the C-5 and C-6 isomers.

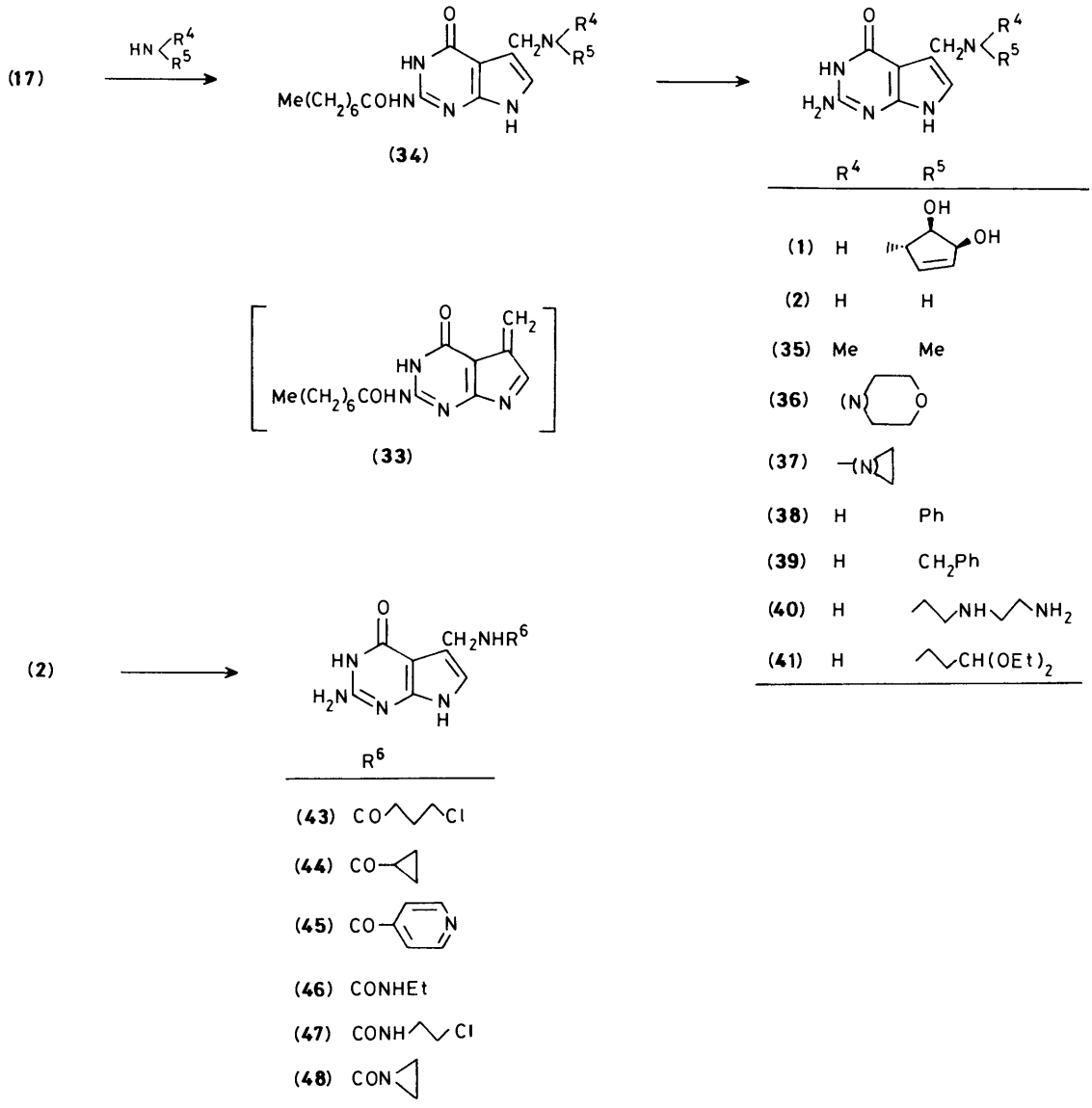
The structure of (**17**), as a representative of the 5-substituted Mannich bases, was confirmed by its conversion into queuine (**1**), which was shown to be identical by direct comparison with an authentic sample. A similar reaction using the 6-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidines (**29**) failed to give the geometrical isomer (**49**) as mentioned in the following section; however, (**49**) was synthesized smoothly *via* the isopropylidene derivative starting from (**3**) [(**29**) Scheme 1].



Structures of 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**42**) and (**49**)

Amine Exchange Reaction of 5-(*Substituted aminomethyl*)-*pyrrolo*[2,3-d]*pyrimidines*.—The amine exchange reaction of 3-dimethylaminomethylindole and its related compounds with an amine (primary or secondary) has been shown to proceed by an elimination-addition mechanism to afford indoles with a variety of aminomethyl side chains at the 3-position (gramine degradation).[6] This reaction, however, has not yet been reported with respect to 5-(substituted aminomethyl)pyrrolo-[2,3-*d*]pyrimidines. We found that these compounds smoothly underwent the amine exchange reaction. Warming a solution of compound (**17**) with an excess of ammonia or primary or secondary amine, and the subsequent deprotection of the 2-acyl moiety with alkali, led to good yield of the corresponding 5-aminomethylpyrrolo[2,3-*d*]pyrimidines (**1**), (**2**), and (**35**)—(**41**) (Table 2). In contrast, the less active 6-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidines (**18**), (**20**), (**22**), and (**24**) failed to react under similar conditions. A change in the nature of the added ammonia, or primary and secondary alkyl or aralkyl amines appeared to have little effect upon the progress of the reaction (Table 2). An unprotected 2-amino compound (**42**), although showing a high reactivity in this reaction, appeared to be less useful than the corresponding 2-acylamino compound (**17**) owing to its lability. Among several 2-acyl functions of 2-acylaminopyrrolo[2,3-*d*]pyrimidines (**4**)—(**7**), octanoyl was selected as the optimal choice because of its better solubility and ease of deprotection after this reaction. Thus, the amine exchange reaction of 5-(dibenzylaminomethyl)-2-octanoylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**17**) with a variety of amines, followed by alkaline deprotection, efficiently gave a variety of the 2-aminopyrrolo[2,3-*d*]pyrimidine-4(3*H*)-ones having 5-aminomethyl and/or 5-substituted aminomethyl side-chains of natural and unnatural structure [(**1**), (**2**), and (**35**)—(**41**); Table 2]. The introduction of a new amino function into the pyrrolo[2,3-*d*]pyrimidine ring was presumed to have progressed *via* the formation of a conjugated unsaturated ring system (**33**) by amine elimination from (**17**), followed by the addition of an added amine (HNR^3R^4, Scheme 3).

Synthesis of Queuine (**1**), *Pre Q1 Base* (**2**), *and Their Analogues*.—The Mannich reaction of the 2-acylaminopyrrolo-[2,3-*d*]pyrimidines and the subsequent amine exchange re-



Scheme 3. Amine exchange reaction of compound (**17**) and acylation reaction of compound (**2**)

action opened up an efficient new route to synthesizing the naturally occurring hypermodified bases, queuine (**1**) and Pre Q1 base (**2**). For the total synthesis of (**1**) and (**2**), 2-octanoylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**7**) was allowed to react with dibenzylamine/formaldehyde in 80% acetic acid at 70 °C for 20 h, to yield 5-dibenzylaminomethyl-2-octanoylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one [(**17**) 70% yield] which, on warming with (1*S*,2*R*,3*S*)-2,3-isopropylidenedioxycyclopent-4-enylamine[3c] in MeOH–THF at 75 °C for 24 h, gave the pyrrolo[2,3-*d*]pyrimidine having a (2,3-isopropylidenedioxycyclopent-4-enyl)aminomethyl function at the 2-position. Deprotection with alkali, followed by mild acid hydrolysis and subsequent purification by chromatography, gave the desired 2-amino-5-[1*S*,2*R*,3*S*)-2,3-dihydroxycyclopent-4-enylaminomethyl]pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**1**) in 72% yield from (**17**). In a similar way, treatment of compound (**17**) with methanolic ammonia in a sealed tube gave 2-amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**2**) (Pre Q1 base), a biosynthetic precursor of queuine, in 87% yield. The structures of the synthetic products, (**1**) and (**2**), were confirmed as identical with the respective authentic samples obtained from natural sources on the basis of their physicochemical and biochemical properties. A variety of the pyrrolo-[2,3-*d*]pyrimidin-2(3*H*)-ones having a 5-substituted aminomethyl side-chain of unnatural structure (**43**)—(**48**) was also synthesized by acylation starting from compound (**2**) (Table 3). All of the analogues were purified by column chromatography and their purities were checked by h.p.t.l.c. and h.p.l.c. analyses. Each structure was assigned on the basis of elemental analysis and spectral data.

The present method proved very advantageous in actual practice from the viewpoints of yields and simplicity of synthesizing queuine (**1**) and its analogues (**2**), (**35**)—(**41**), and (**43**)—(**48**). This allowed us to examine the structure-activity relationships of these compounds (some results have already been reported[4]).

Table 2. Analytical data for the 2-amino-5-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**1**), (**2**), and (**35**)—(**41**)

Compound (Formula)	Yield (%)[a]	Solvent	M.p. (°C) (decomp.)	$\nu_{max.}$(KBr)/cm^{-1}	δ_H[b]	Found (%) (Required) C	H	N
(**1**)[c] ($C_{12}H_{15}N_5O_3$·2HCl·½H_2O)	72	MeOH	230—235	1 675, 1 660	4.28—4.60 (2H, m), 4.50 (2 H, br s), 6.13 (1H, dd), 6.35 (1 H, m), 7.12 (1 H, s)	40.4 (40.12	5.1 5.05	19.4 19.50)
(**2**) ($C_7H_9N_5O$·2HCl·2H_2O)	87	MeOH–Et_2O	220—225	1 670, 1 605	4.32 (2 H, s), 7.12 (1 H, s)	29.45 (29.18	5.2 5.25	24.4 24.31)
(**35**) ($C_9H_{13}N_5O$)	73	MeOH–H_2O	>250	1 640	3.05 (3 H, s), 3.10 (3 H, s), 4.58 (2 H, d), 7.25 (1 H, d)	52.05 (52.16	6.2 6.32	33.55 33.79)
(**36**) ($C_{11}H_{15}N_5O_2$)	78	MeOH	>250	1 635, 1 560	2.40—2.53 (4 H, m), 3.43—3.60 (4 H, m), 3.87 (2 H s), 6.63 (1 H, d)	53.2 (53.00	6.0 6.07	27.9 28.09)
(**37**) ($C_9H_{11}N_5O$)	30	MeOH	>250	1 640	1.65 (3 H, s), 4.43 (2 H, d), 7.20 (1 H, s)	52.5 (52.67	5.6 5.40	34.05 34.13)
(**38**) ($C_{13}H_{13}N_5O$)	87	EtOH–Et_2O	>250	1 675, 1 645, 1 600	4.77 (2 H, br s), 7.00 (1 H, br s), 7.50 (5 H, m)	60.9 (61.17	5.3 5.13	27.45 27.43)
(**39**) ($C_{14}H_{15}N_5O$)	72	MeOH	>250	1 660, 1 645, 1 605	4.20 (4 H, br s), 6.70 (1 H, d), 7.40 (5 H, br s)	62.4 (62.44	5.7 5.61	26.15 26.01)
(**40**) ($C_{11}H_{19}N_7O$·3HCl·H_2O)	58	EtOH	176—178	1 680, 1 625	3.47—3.57 (8 H, m), 4.43 (2 H, s),	33.95 (33.64	6.05 6.16	25.15 24.47)
(**41**) ($C_{13}H_{21}N_5O_3$)	68	EtOH–Et_2O	162—169	1 670, 1 615	7.07 (1 H, s) 1.33 (6 H, t), 2.77 (2 H, d), 3.33—3.83 (6 H, m), 4.27 (2 H, br s), 6.70 (1 H, d)	52.9 (52.87	7.2 7.17	23.55 23.71)

[a] Yields are based on (**17**) and in no case optimised. [b] Solvent: CD_3OD for (**1a**), (**2**), (**40**); CF_3CO_2H for (**35**), (**37**), (**38**); [$(CD_3)_2SO$] for (**36**), (**39**), (**41**). [c] The intermediary isopropylidene derivative of (**1**), prepared by the reaction of (**17**) with (1*S*,2*R*,3*S*)-2,3-isopropylidenedioxycyclopent-4-enylamine was subsequently treated with acid to remove the isopropylidene protecting group.

Table 3. Synthesis of the 5-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**43**)—(**48**)

Acylating agent XR^5[a]	Product (Formula)	Yield (%)	Solvent	M.p. (°C) (decomp.)	$\nu_{max.}$(KBr)/cm^{-1}	δ_H in [$(CD_3)_2SO$]	Found (%) (Required) C	H	N
$ClCO(CH_2)_3Cl$	(**43**) ($C_{11}H_{14}ClN_5O_2$)	73	MeOH–CH_2Cl_2	>250	1 685, 1 625, 1 605, 1 540	1.80(2H,q),2.23(2H,t), 3.60(2H,t),4.27(2H,d), 6.46 (1 H, d)	46.35 (46.57	4.8 4.97	24.7 24.68)
PNPhCOCH$_2$CH$_2$CH$_2$	(**44**) ($C_{11}H_{13}N_5O_2$)	97	MeOH–CH_2Cl_2	248—251	1 640, 1 530	0.70 (4 H, br s), 1.53 (1H,m),4.30(2H,s),6.52 (1 H, s)	53.5 (53.43	5.15 5.30	28.5 28.32)
ClCO-pyridyl-4	(**45**) ($C_{13}H_{12}N_6O_2$)	20	MeOH–CH_2Cl_2	>250	1 650, 1 625, 1 560	4.53(2H,d),6.59(1H,d), 7.77(2H,d),8.71(2H,d)	54.7 (54.93	4.35 4.25	29.4 29.56)
$OCNCH_2Me$	(**46**) ($C_{10}H_{14}N_6O_2$)	56	DMF–MeOH	>250	1 690, 1 660, 1 625, 1 605	1.05(3H,t),3.05(2H,q), 4.23 (2 H, s), 6.53 (1 H, s)	48.05 (47.99	5.8 5.64	33.4 33.58)
$OCNCH_2CH_2Cl$	(**47**) ($C_{10}H_{13}ClN_6O_2$)	61	MeOH–CH_2Cl_2	>250	1 650, 1 565	3.39(2H,t),3.63(2H,t), 4.30 (2 H, s), 6.60 (1 H, s)	42.5 (42.19	4.55 4.60	29.55 29.52)
PNPhCONCH$_2$CH$_2$	(**48**) ($C_{10}H_{12}N_6O_2$)	63	$CHCl_3$–Et_2O	216—221	1 650, 1 530	2.10 (4 H, s), 4.33 (2 H, br s), 6.63 (1 H, s)	48.35 (48.38	4.9 4.87	33.75 33.85)

[a] X = Cl, OCN, and PNPh (= *p*-nitrophenoxy).

Experimental

M.p.s were measured on a Yanagimoto hot-plate apparatus (Model MP-3S) and are uncorrected. I.r. spectra were determined in KBr disks on a Hitachi 215 spectrometer. N.m.r. spectra were obtained using Varian XL-100-12 and Varian EM-360 instruments: chemical shifts (δ) are reported in p.p.m. downfield from internal tetramethylsilane (TMS). Thin-layer chromatography was carried out on h.p.t.l.c. pre-coated Kieselgel 60F_{254} analytical plates (E. Merck, Art. 5642) or on 1.00 mm silica gel preparative plates (E. Merck, Art. 13895) with u.v. and I_2 development. For simple column chromatography, E. Merck's Kieselgel 60 (70—230 mesh; Art. 7734) was used. Reversed-phase h.p.l.c. analysis was performed on a Waters ALC/PGPC 204 instrument using a C_{18} μ-Bondapak column (Waters Associates, No. 27324).

2-Acylaminopyrrolo[2,3-d]*pyrimidin*-4(3H)-*ones* (**4**)—(**7**): *General Procedure.*—A vigorously stirred and chilled suspension of 2-aminopyrrolo[2,3-*d*]pyrimidin-4-one[7] (**3**) (1.0 mol) in dry pyridine (1.0 l) was treated with an appropriate acyl chloride (3.0 mol) at 85 °C for 30 min. After cooling, the mixture was neutralized with 6.5% NH_3 in EtOH (3.0 l) in an ice-bath. The resulting dark red solution, when left at room temperature overnight, gave a crystalline precipitate, which was collected and washed successively with EtOH and ether to give the desired product (Table 4).

2-Acylamino-5-(substituted aminomethyl)pyrrolo[2,3-d]*pyrimidin*-4(3H)-*ones* (**8**)—(**17**): *General Procedure.*—A solution of 2-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3H)-ones (**4**)—(**7**) (0.1 mol), 37% formalin solution (0.2 mol), and an appropriate

Table 4. Analytical data for the 2-acylaminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**4**)—(**7**)

Compound (Formula)	Yield (%)	Solvent	M.p. (°C) (decomp.)	$\nu_{max.}$(KBr)/cm^{-1}	δ_H in [$(CD_3)_2SO$]	Found (%) (Required) C	H	N
(**4**) ($C_8H_8N_4O_2$)	65	DMF–MeOH	>250	1 675, 1 660, 1 645	2.16 (3 H, s), 6.33—6.43 (1 H, m), 6.86—6.96 (1 H, m)	49.8 (50.00)	4.15 (4.20)	28.95 (29.15)
(**5**) ($C_{13}H_{10}N_4O_2$)	67	EtOH	>250	1 695, 1 650, 1 640	6.38—6.50 (1 H, m), 6.90—7.02 (1 H, m), 7.43—7.73 (3 H, m), 7.97—8.13 (2 H, m)	61.6 (61.41)	3.95 (3.96)	21.8 (22.04)
(**6**) ($C_{14}H_{20}N_4O_2$)	88	EtOH	249—251	1 685, 1 665	0.85 (6 H, t × 2), 1.04—1.67 (8 H, m), 2.37—2.63 (1 H, m), 6.38 (1 H, d), 6.92 (1 H, d)	60.75 (60.85)	7.35 (7.30)	20.05 (20.27)
(**7**) ($C_{14}H_{20}N_4O_2$)	78	EtOH	>250	1 670, 1 660, 1 645	0.87 (3 H, t), 1.27 (8 H, s), 1.47—1.80 (2 H, m), 2.43 (2 H, t), 6.33—6.43 (1 H, m), 6.87—6.97 (1 H, m)	61.1 (60.85)	7.2 (7.30)	20.35 (20.27)

Table 5 (1). Analytical data for the 2-acylamino-5-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**8**)—(**17**)

Compound (Formula)	Solvent	M.p. (°C) (decomp.)	$\nu_{max.}$(KBr)/cm^{-1}	δ_H in [$(CD_3)_2SO$]	Found (%) (Required) C	H	N
(**8**) ($C_{11}H_{15}N_5O_2$)	DMF–H_2O	>250	1 700, 1 670, 1 645, 1 630	2.13 (9 H, s), 3.57 (2 H, s), 6.73 (1 H, d)	53.1 (53.00)	6.2 (6.07)	27.95 (28.09)
(**9**) ($C_{23}H_{23}N_5O_2$)	DMF-H_2O	>250	1 670, 1 660, 1 640	2.17 (3 H, s), 3.60 (4 H, br s), 3.80 (2 H, s), 6.90 (1 H, d), 7.07—7.56 (10 H, m)	68.65 (68.81)	5.9 (5.78)	17.25 (17.44)
(**10**) ($C_{16}H_{17}N_5O_2$)	DMF-MeOH	>250	1 670, 1 645, 1 610	2.22 (6H, s), 3.67 (2H, s), 6.73 (1 H, d), 7.45—7.65 (3 H, m), 8.03 (2 H, dd)	61.5 (61.73)	5.35 (5.50)	22.5 (22.49)
(**11**) ($C_{28}H_{25}N_5O_2$)	DMF–EtOH	>250	1 670, 1 650, 1 610	3.60 (4H, s), 3.80 (2H, s), 6.90 (1 H, d), 7.10—7.70 (13 H, m), 8.03 (2 H, dd)	72.35 (72.55)	5.23 (5.44)	15.0 (15.11)
(**12**) ($C_{17}H_{27}N_5O_2$)	DMF–EtOH	>250	1 685, 1 670, 1 660	0.85 (6 H, t × 2), 1.13—1.83 (8 H, m), 2.17 (6 H, s), 2.40—2.73 (1 H, m), 3.62 (2 H, s), 6.77 (1 H, d)	61.1 (61.24)	8.05 (8.16)	20.95 (21.00)
(**13**) ($C_{29}H_{35}N_5O_2$)	EtOH	225—226	1 670, 1 645, 1 615	0.85 (6H, t × 2), 1.10—1.70 (8H, m), 2.33—2.66 (1 H, m), 3.57 (4 H, s), 3.77 (2 H, s), 6.85 (1 H, d), 7.17—7.50 (10 H, m)	71.85 (71.73)	7.3 (7.26)	14.45 (14.42)
(**14**) ($C_{17}H_{27}N_5O_2$)	DMF–EtOH	>250	1 645	0.87 (3 H, t), 1.27 (8 H, br s), 1.60 (2 H, m), 2.15 (6 H, s), 2.43 (2 H, t), 3.57 (2 H, s), 6.73 (1 H, d)	61.1 (61.24)	8.0 (8.16)	21.15 (21.00)
(**15**) ($C_{19}H_{29}N_5O_2$)	EtOH	>250	1 640, 1 640	0.85 (3 H, t), 1.27 (8 H, br s), 1.30—1.75 (2 H, m), 2.25—2.60 (6 H, m), 3.10—3.50 (4 H, m), 3.65 (2 H, s), 6.73 (1 H, d)	63.25 (63.49)	8.0 (8.13)	19.3 (19.48)
(**16**) ($C_{21}H_{31}N_5O_2$)	MeOH	>250	1 645, 1 615	0.83 (12 H, d and 3 H, t), 1.27 (8 H, br s), 1.30—1.90 (4H, m), 2.10 (4H, d), 2.37 (2H, t), 3.70 (2H, s), 6.68 (1 H, d)	65.3 (65.43)	8.0 (8.11)	18.0 (18.17)
(**17**) ($C_{29}H_{35}N_5O_2$)	DMF–EtOH	225—228	1 640, 1 615	0.86 (3 H, t), 1.27 (8 H, br s), 1.47—1.76 (2 H, m), 2.43 (2 H, m), 3.60 (4 H, s), 3.80 (2 H, s), 6.86 (1 H d), 7.16—7.53 (10 H, m)	71.7 (71.73)	7.25 (7.26)	14.45 (14.42)

amine (0.2 mol) in 80% aqueous acetic acid was warmed at 60 °C for 20 h. The reaction mixture was cooled, diluted with 0.5M HCl (500 ml), and left at room temperature for 30 min. After neutralization with aqueous ammonia, the mixture was thoroughly extracted with $CHCl_3$ and the combined extracts were evaporated to dryness. The residue was chromatographed on a silica gel column using 5—20% MeOH in $CHCl_3$ (a stepwise gradient of increasing MeOH in $CHCl_3$) as eluant. Fractions containing the desired product, identified by t.l.c. analysis, were collected, and the desired product was obtained from the combined solution after work-up. The yield of each congener is given in Table 1. Elemental analyses and i.r. and ^{1}H n.m.r. spectral results are shown in Table 5.

2-Acylamino-6-(substituted aminomethyl)pyrrolo[2,3-d]*pyrimidin-4*(3H)*-ones* (**18**)—(**27**): *General Procedure.*—In the chromatographic separation of the reaction mixture described in the preceding section, the C-6 isomers (**18**)—(**27**) were always eluted faster than the corresponding C-5 isomers (**8**)—(**17**), regardless of whether or not the side-chain structure of the pyrrolo[2,3-*d*]pyrimidines was altered. The fractions containing the 6-isomers in the chromatography mentioned in the preceding section were collected and combined, and this was worked up to yield the desired product (**18**)—(**27**). Under the same conditions, except that compound (**3**) (0.1 mol) was used instead of the acyl derivatives (**4**)—(**7**), the Mannich reaction and the subsequent work-up gave 6-(substituted aminomethyl)-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**28**) and (**29**) exclusively. The yields and the physical data are given in Tables 1 and 5, respectively.

H.p.l.c. Analysis of the Product Ratios by the Mannich Reaction of Pyrrolo[2,3-d]*pyrimidines* (**3**) *and* (**4**)—(**7**).—A mixture of 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) (1.0 mmol) or its 2-acyl derivative (**4**)—(**7**) (1.0 mmol), 37% formalin solution (2.0 mmol), and disubstituted amine (2.0 mmol) in 80% aqueous acetic acid (10 ml) was stirred and warmed at 60 °C for 20 h. The reaction mixture was cooled, diluted with 0.5M HCl

Table 5 (2). Analytical data for the 2-acylamino-6-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**8**)—(**17**)

Compound (Formula)	Solvent	M.p. (°C) (decomp.)	$\nu_{max.}$(KBr)/cm^{-1}	δ_H in [$(CD_3)_2SO$]	Found (%) (Required) C	H	N
(**18**) ($C_{11}H_{15}N_5O_2$)	DMF–H_2O	246—248	1 695, 1 670, 1 655	2.15 (3 H, s), 2.18 (6 H, s), 3.42 (2 H, s), 6.23 (1 H, s)	52.85 (53.00	6.05 6.07	28.0 28.09)
(**19**) ($C_{23}H_{23}N_5O_2$)	DMF-Et_2O	>250	1 650, 1 640, 1 620	2.15 (3 H, s), 3.53 (4 H, br s), 3.58 (2 H, br s), 6.30 (1 H, d), 7.16—7.50 (10 H, m)	68.9 (68.81	5.9 5.78	17.3 17.44)
(**20**) ($C_{16}H_{17}N_5O_2$)	EtOH	>250	1 675, 1 635, 1 605	2.23 (6 H, s), 3.50 (2 H, s), 6.32 (1 H, s), 7.20—7.70 (13 H, m), 8.07 (1 H, dd)	61.6 (61.73	5.4 5.50	22.35 22.49)
(**21**) ($C_{28}H_{25}N_5O_2$)	EtOH	209—212	1 670, 1 650, 1 610	3.55 (4 H, s), 3.62 (2 H, s), 7.20—7.70 (13 H, m), 8.02 (2 H, dd)	72.5 (72.55	5.65 5.44	15.35 15.11)
(**22**) ($C_{17}H_{27}N_5O_2$)	EtOH	203—204	1 695, 1 670, 1 660	0.85 (6 H, t × 2), 1.10—1.67 (8 H, m), 2.13 (6 H, s), 2.36—2.70 (1 H, m), 3.36 (2 H, s), 6.20 (1 H, s)	61.3 (61.24	8.25 8.16	21.05 21.00)
(**23**) ($C_{29}H_{35}N_5O_2$)	EtOH	201—203	1 665, 1 645, 1 610	0.85 (6 H, t × 2), 1.07—1.77 (8 H, m), 2.36—2.67 (1 H, m), 3.53 (4 H, s), 3.60 (2 H, s), 6.32 (1 H, d), 7.16—7.50 (10 H, m)	71.6 (71.73	7.3 7.26	14.4 14.42)
(**24**) ($C_{17}H_{27}N_5O_2$)	EtOH	>250	1 645	0.87 (3 H, t), 1.27 (8 H, br s), 1.60 (2 H, m), 2.13 (6 H, s), 2.43 (2 H, t), 3.37 (2 H, s), 6.20 (1 H, s)	61.4 (61.24	7.95 8.16	20.95 21.00)
(**25**) ($C_{19}H_{29}N_5O_2$)	EtOH	234—237	1 645, 1 610	0.85 (3 H, br t), 1.28 (8 H, br s), 1.30—1.75 (2 H, m), 2.30—2.60 (6 H, m), 3.20—3.60 (4 H, m), 3.50 (2 H, s), 6.25 (1 H, s)	63.3 (63.49	8.25 8.13	19.25 19.48)
(**26**) ($C_{21}H_{31}N_5O_2$)	EtOH	>250	1 645, 1 615	0.87 (12 H, d and 3 H, t), 1.26 (8 H, br s), 1.60—1.90 (4 H, m), 2.10 (2 H, d), 2.42 (2 H, t), 3.52 (2 H, s), 6.20 (1 H, s)	65.5 (65.43	8.25 8.11	18.05 18.17)
(**27**) ($C_{29}H_{35}N_5O_2$)	EtOH	192—195	1 650, 1 610	0.85 (3 H, t), 1.27 (8 H, br s), 1.47—1.80 (2 H, m), 2.43 (2 H, t), 3.55 (4 H, s), 3.58 (2 H, s), 6.33 (1 H, s), 7.15—7.55 (10 H, m)	71.6 (71.73	7.35 7.26	14.35 14.42)
(**28**) ($C_{21}H_{21}N_5O\cdot\frac{1}{2}H_2O$)	MeOH–H_2O	>250	1 660, 1 615	3.40 (6 H, br s), 6.13 (1 H, d), 7.20 (10 H, m)	68.15 (68.46	5.9 6.02	18.95 19.01)
(**29**) ($C_{15}H_{19}N_5O_3$)	MeOH	156—160	1 660, 1 620	1.33 (3 H, s), 1.37 (3 H, s), 3.80 (2 H, s), 4.23—4.53 (2 H, m), 5.20 (1 H, d), 5.87 (2 H, s), 6.30 (1 H, s)	75.9 (76.03	6.2 6.38	15.05 14.78)

(5.0 ml), and left at room temperature for 30 min. Following this, aliquots of the solution were analysed to find the ratio of the 5- and 6-(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**8**)—(**17**) and (**18**)—(**27**) by using h.p.l.c. (μ-Bondapak C_{18} column, 3.9 mm × 30 cm, MeOH–H_2O–acetic acid = 60:39:1). Authentic samples were used as reference standards. The analytical results are summarized in Table 1. In the case of the reaction using compound (**3**), no C-5 isomers were detected, and the 6-isomers (**28**) and (**29**) were found to be main products along with a small amount of 5,6-bis(substituted aminomethyl)pyrrolo[2,3-*d*]pyrimidine (**30**).

2-Amino-5-(substituted aminomethyl)pyrrolo[2,3-d]*pyrimidin-4*(3H)*-ones* (**1**), (**2**), *and* (**35**)—(**41**): *Preparation by Amine Exchange Reaction of 5-(Dibenzylaminomethyl)-2-octanoylaminopyrrolo*[2,3-d]*pyrimidin-4*(3H)*-one* (**17**): *General Procedure.*—Compound (**17**) (0.01 mol) was added to a solution of an appropriate amine (0.05 mol) in MeOH–THF (1:1; 50 ml), and the resulting mixture was allowed to react in a sealed tube at 75 °C for 24 h. The reaction mixture was cooled to room temperature and 5M KOH (0.5 ml) was added. It was then stirred for 60 h at room temperature and evaporated to dryness. The residue was purified by chromatography on a silica gel column using 5—20% MeOH in $CHCl_3$ as eluant and, after work-up, the desired product was obtained pure. The yields and physical data are given in Table 2.

5-Acylaminomethyl-2-aminopyrrolo[2,3-d]*pyrimidin-4*(3H)*-ones* (**43**)—(**47**): *Preparation by Acylation of 2-Amino-5-aminomethylpyrrolo*[2,3-d]*pyrimidin-4*(3H)*-one* (**2**): *General Procedure.*—To a solution of compound (**2**) (1.0 mmol) in dry DMF (10 ml), an active acylating agent (1.5 mmol), listed in Table 3, and triethylamine (2.0 mmol) were added. The mixture was left at 0 °C for 2 h and then evaporated to dryness under reduced pressure to provide a residue which was purified by chromatography on a silica gel column using $CHCl_3$–EtOH (9:1) as eluant. After work-up, the desired product was obtained. The yields and physical data are given in Table 3.

2-Amino-5-(aziridinocarbonylaminomethyl)pyrrolo[2,3-d]*pyrimidin-4*(3H)*-one* (**48**).—Compound (**2**) (2.69 g) dissolved in DMF (125 ml) was treated with *p*-nitrophenyl aziridine-1-carboxylate (2.57 g) in the presence of triethylamine (3.42 ml) at room temperature for 30 min. The reaction mixture was evaporated to dryness under reduced pressure and the residue was subjected to chromatography on a silica gel column using $CHCl_3$–EtOH (9:1) as eluant. Fractions containing the desired product were combined and evaporated under reduced pressure and the residue crystallized from $CHCl_3$–Et_2O to give the desired product as a white powder (1.73 g). The physical data are given in Table 3.

2-Amino-6-[(1S,2R,3S)-2,3-dihydroxycyclopent-4-enylaminomethyl]pyrrolo[2,3-d]*pyrimidin-4*(3H)*-one* (**49**).—A stirred suspension of compound (**3**) (150 mg), 37% formalin solution (86 mg), and 2,3-isopropylidenedioxycyclopent-4-enylamine (155 mg) in 80% acetic acid (7.0 ml) was left at room temperature for 19 h. The reaction mixture was diluted with 1M HCl (3.0 ml), left for a further 1 h at room temperature, and then neutralized with 25% aqueous ammonia. After evaporation of the solvent under reduced pressure, the residue was chromatographed on silica gel using $CHCl_3$–EtOH (4:1) as eluant to give the title compound (**49**) (62 mg, 28%); $\nu_{max.}$ 1 680, 1 660, 1 620, and 1 540 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 1.33 (3 H, s), 1.37 (3 H, s),

3.80 (3 H, s), 4.23—4.53 (2 H, m), 5.20 (1 H, d), 5.87 (2 H, s), and 6.30 (1 H, s). A stirred solution of this compound (35 mg) in 1M HCl (1.1 ml) was set aside at room temperature for 16 h after which it was evaporated and the residue crystallized from MeOH–Et_2O to give the desired product as colourless prisms (40 mg, 95%), m.p. 189—192 °C; (Found: C, 38.2; H, 5.15; N, 18.85. $C_{12}H_{15}N_5O_3$·2HCl·1.5H_2O requires C, 38.21; H, 5.34; N, 18.57%); $\nu_{max.}$ 1 690, 1 620, 1 605, and 1 580 cm^{-1}; δ_H(90 MHz; D_2O) 4.43—4.60 (2 H, m), 4.63 (2 H, s), 5.30—5.40 (1 H, m), 6.17—6.30 (1 H, m), 6.37—6.63 (1 H, m), and 6.90 (1 H, s).

Acknowledgements

We are grateful to Dr. Yukio Sugino, General Manager of this Division and to Drs. Masao Nishikawa and Masahiko Fujino for their continuous encouragement throughout this work. We are also indebted to Drs. Toshio Goto and Tadao Kondo for their helpful advice.

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Received 27*th April* 1987; *Paper* 7/751