An Improved Procedure for the Preparation of 2-Amino-8-alkylpyrido-[2,3-d]pyrimidin-4(3H)-ones (8-Alkyl-N5-deazapterins)

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We report an improved procedure for the preparation of 8-alkyl-N5-deazapterins which allows clean preparation of all ring-methyl substituted compounds, including 5- and 7-methyl substituted compounds. The procedure was also successfully applied to the preparation of N5-deazapterins with improved yield over previous reports. The uv/visible and pKa data confirm the predicted increased basicity of 8-alkyl-N5-deazapterins compared with the N5-deazapterin parents, and indicate that N5-deazapterins protonate on N8 and 8-alkyl-N5-deazapterins protonate on N3.

Introduction.

Dihydrofolate reductase (DHFR) plays an essential part in the metabolism of cells due to its role in the maintenance of adequate cellular levels of thymidine, one of the pyrimidine bases essential for DNA synthesis [1]. Inhibition of DHFR thus results in cell stasis and death [2]. This effect is particularly pronounced in rapidly proliferating cells and on this basis inhibitors of DHFR have been used clinically to treat diseases such as childhood leukemia [3], malaria [4] and bacterial infections [5].

The 8-alkyl-N5-deazapterins are a new class of mechanism-based inhibitors of DHFR we are currently developing [6,7]. These compounds were designed on theoretical grounds [8] to form readily an N3-protonated cation. This cation is intended to mimic the N8-protonated cation of folate, the proposed active form of this natural substrate of DHFR [9]. An initial series of 8-methyl-N5-deazapterins [6] were prepared using procedures adapted from previous studies on the preparation of 8-ribityl-N5-deazalumazines [10,11]. These procedures are based on condensations of 2-amino-6-methylaminopyrimidin-4(3H)-one with either 1,3-dicarbonyl (Scheme 1) or 1,2-unsaturated carbonyl (Scheme 2) compounds. While these procedures were successful in preparing a number of 8-methyl-N5-deazapterins, 7,8-dimethyl-N5-deazapterin could be prepared only as the hydrobromide, 5,8-dimethyl-N5-deazapterin could not be purified, and in general reaction work up was difficult [6].

Scheme 1

	R¹			R¹
1	-CH₃		10	-CH₃
2	-CH₂CH₃		11	-CH₂CH₃
3	-CH₂CH₂CH₃		12	-CH₂CH₂CH₃
4	-CH(CH₃)₂		13	-CH(CH₃)₂
5	-CH₂CH(CH₃)₂		14	-CH₂CH(CH₃)₂
7	-CH₂C₆H₅		15	-CH₂C₆H₅
8	-H		29	-H

Scheme 2

	R¹	R²	R³	R⁴
1	-CH₃			
2	-CH₂CH₃			
3	-CH₂CH₂CH₃			
4	-CH(CH₃)₂			
5	-CH₂CH(CH₃)₂			
6	-CH₂CH₂CH(CH₃)₂			
7	-CH₂C₆H₅			
8	-H			
16		-H	-CH₃	-H
17		-CH₃	-H	-H
18		-H	-H	-CH₃
19	-CH₃	-H	-CH₃	-H
20	-CH₂CH₃	-H	-CH₃	-H
21	-CH₂CH₂CH₃	-H	-CH₃	-H
22	-CH(CH₃)₂	-H	-CH₃	-H
23	-CH₃	-CH₃	-H	-H
24	-CH₂CH₂CH₃	-CH₃	-H	-H
25	-CH₂CH(CH₃)₂	-CH₃	-H	-H
26	-CH₂CH₂CH(CH₃)₂	-CH₃	-H	-H
27	-CH₃	-H	-H	-CH₃
28	-CH₂CH₂CH₃	-H	-H	-CH₃
30	-H	-H	-CH₃	-H
31	-H	-H	-H	-CH₃
32	-H	-CH₃	-H	-H

We report here a new procedure for preparing 8-alkyl-N5-deazapterins and N5-deazapterins, incorporating new reaction and purification conditions for a range of 8-alkyl substituents. Using these new procedures we have prepared cleanly a number of 8-alkyl-N5-deazapterins and N5-deazapterins (Scheme 1) and 5-, 6- and 7-methyl-8-alkyl-N5-deazapterins and -N5-deazapterins (Scheme 2). Electronic spectra and ionization data for both the 8-alkyl-N5-deazapterins and N5-deazapterins are reported.

Results and Discussion.

Initial Studies in the Preparation of 8-Alkyl-N5-deazapterins.

The preparation of 8-propyl-N5-deazapterin was attempted initially by a procedure similar to that reported previously for the synthesis of 8-methyl-N5-deazapterin [6]. This involved the condensation of 2-amino-6-propyl-aminopyrimidin-4(3H)-one (3) with malonaldehyde bis(dimethylacetal) (9) in 0.01 M aqueous hydrochloric acid at moderate temperature. This condensation is similar to the Gabriel-Colman reaction used to prepare 8-alkylpterins in which a 2,5-diamino-6-alkylaminopyrimidin-4(3H)-one is condensed with a 1,2-dicarbonyl such as pyruvic aldehyde [e.g. 12]. Previously we have noted [13] that this type of Gabriel-Colman reaction is characterized by a number of reaction intermediates, including highly conjugated species with long wavelength visible absorption (~470 and ~400 nm). In this deazapterin synthesis we also observed highly coloured reaction intermediates with long wavelength absorption at ~530 and ~470 nm.

This result suggested accumulation of reaction intermediates in the deazapterin synthesis might be a significant problem. This possibility was investigated with a number of reaction-course studies involving condensation of 2-amino-6-propylaminopyrimidin-4(3H)-one (3) with malonaldehyde bis(dimethylacetal) (9), methacrolein (16) and methyl vinyl ketone (18), and 2-amino-6-isoamyl-aminopyrimidin-4(3H)-one (6) with crotonaldehyde (17). The courses of the reactions were followed using SCX hplc. The final hplc traces given in Figure 1(a-d) show a trend of decreasing yield of deazapterin product from 87% for 8-propyl-N5-deazapterin (12) [Figure 1(a)] to only 33% for 5-methyl-8-isoamyl-N5-deazapterin (26) [Figure 1(d)], with 6-methyl-8-propyl-N5-deazapterin (21) [Figure 1(b)] and 7-methyl-8-propyl-N5-deazapterin (28) [Figure 1(c)] intermediate with 72% and 49%, respectively. In each case a major impurity is apparent with retention on the SCX hplc column at ~50% the retention time of the deazapterin product, behavior analogous to that for the intermediate observed in 8-alkylpterin syntheses [12].

Development of New Reaction Conditions for Preparation of 8-Alkyl-N5-deazapterins.
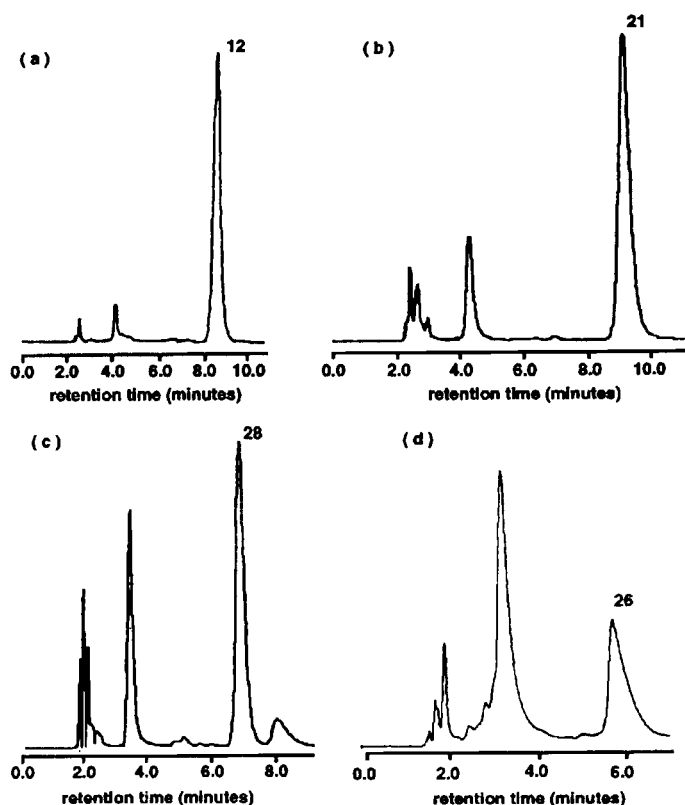
Figure 1. SCX hplc analysis of initial attempted condensations of: (a) 2-amino-6-propylaminopyrimidin-4(3H)-one (3) with malonaldehyde bis(dimethylacetal) (9) to give 8-propyl-N5-deazapterin (12); (b) 3 with methacrolein (16) to give 6-methyl-8-propyl-N5-deazapterin (21); (c) 3 with methyl vinyl ketone (18) to give 7-methyl-8-propyl-N5-deazapterin (28); (d) 2-amino-6-isoamylaminopyrimidin-4(3H)-one (6) with croton-aldehyde (17) to give 5-methyl-8-isoamyl-N5-deazapterin (26). Void peak is at ca. 2 minutes.

In previous syntheses of 8-alkylpterins we noted that the presence of sodium bisulfite completely eliminated these reaction intermediates, leading to clean conversion of reactants to products [12]. Taking account of the similarities between the deazapterin and pterin syntheses discussed above, we examined the usefulness of sodium bisulfite in the synthesis of 8-alkyl-N5-deazapterins.

In preliminary tests reaction conditions were based on those used previously for the preparation of 6-methyl-8-propylpterin [12]. In that synthesis the condensation was performed in mildly acidic solution (pH 4) in the presence of sodium bisulfite, and an initial intermediate was formed which was characterised as a 7,8-bisulfite adduct of 6-methyl-8-propylpterin. The final pterin product was obtained by converting the bisulfite to sulfur dioxide in strongly acidic solution and removing it under reduced pressure. To examine the effectiveness of these conditions the same series of condensations as reported above for time-course studies were performed but with the addition of sodium bisulfite. The reaction courses

were followed using both uv/visible spectroscopy and SCX hplc. For reactions with the 1,2-unsaturated carbonyls [methacrolein (16), methyl vinyl ketone (18) and crotonaldehyde (17)], an initial intermediate was clearly observed as indicated by the pyrimidine peak at 270 nm in the uv/visible spectrum being replaced by a peak at ~280-285 nm. However, for reactions with malonaldehyde bis(dimethylacetal) (9) indications for an initial intermediate were inconclusive. After acidification the intermediates in the 1,2-unsaturated carbonyl reactions were found to decompose relatively rapidly to deazapterin product, particularly when heated and particularly for reactions with methyl vinyl ketone. The reaction with malonaldehyde bis(dimethylacetal) progressed more slowly.

The final SCX hplc traces for each reaction shown in Figure 2(a-d) indicate significant improvement in yields of deazapterin product for the reactions with methacrolein [Figure 1(b); 90% 6-methyl-8-propyl-N5-deazapterin (21)], methyl vinyl ketone [Figure 1(c); 80% 7-methyl-8-propyl-N5-deazapterin (28)] and crotonaldehyde [Figure 1(d); 55% 5-methyl-8-*iso*amyl-N5-deazapterin (26)] when compared with the reactions without bisulfite shown in Figure 1. For the reaction with malonaldehyde bis-(dimethylacetal) [Figure 2(a); 8-propyl-N5-deazapterin (12)] the yield is marginally lower than for the reaction without bisulfite [Figure 1(a)]. The chromatograms in Figure 2 also show that, for all reactions, the impurities are eluted predominantly in the void volume suggesting that purification of these reaction mixtures should be possible using cation exchange column chromatography. These results were very encouraging and consequently sodium bisulfite was incorportated in the preparation of all deazapterins shown in Schemes 1 and 2.

Compound Purification.

Typically pterins and N5-deazapterins have been found to be very difficult to work with, primarily due to their extreme insolubility in a range of solvents. This low solubility has been attributed to the preference for ring stacking and intermolecular hydrogen bonding interactions between the pteridine rings rather than interactions with solvent [17]. For 8-alkylpterins a remarkable increase in solubility in polar solvents has been observed [12] due probably to the substituent preventing pteridine-ring aggregation. At least for small alkyl substituents this solubility effect increases with the size of the 8-substituent such that 8-propyl-N5-deazapterin is significantly more soluble in water than 8-methyl-N5-deazapterin. This effect is so pronounced that while 8-methyl-N5-deazapterins can be recrystallized readily from ethanol [6] as their hydrochloride salts, 8-propyl-N5-deazapterin is too soluble in this solvent. Our attempts to find suitable recrystallization solvents for N5-deazapterins with the larger 8-substituents reported here have been unsuccessful, but some
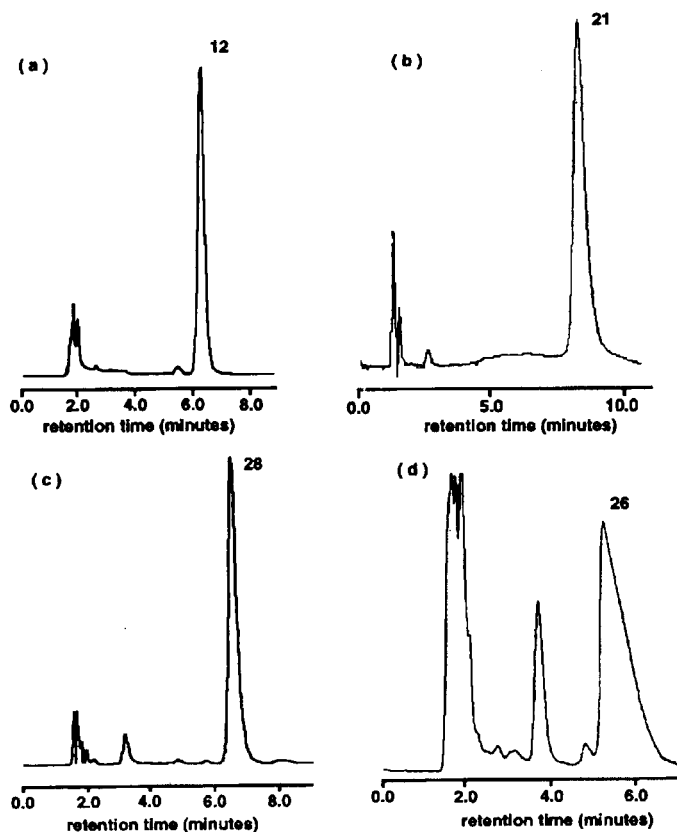
Figure 2. SCX hplc analysis of initial studies of the effect of bisulfite on condensations of: (a) 2-amino-6-propylaminopyrimidin-4(3*H*)-one (3) with malonaldehyde bis(dimethylacetal) (9) to give 8-propyl-N5-deazapterin (12); (b) 3 with methacrolein (16) to give 6-methyl-8-propyl-N5-deazapterin (21); (c) 3 with methyl vinyl ketone (18) to give 7-methyl-8-propyl-N5-deazapterin (28); (d) 2-amino-6-*iso*amylaminopyrimidin-4(3*H*)-one (6) with crotonaldehyde (17) to give 5-methyl-8-*iso*amyl-N5-deazapterin (26). Void peak is at *ca.* 2 minutes.

compounds with large alkyl sidechains have been recrystallized as hydrochloride salts from methanol/ethyl acetate mixes (Wang, Liu and Gready, unpublished results).

Consequently we developed chromatographic procedures for purification involving firstly weak cation exchange (wcx) followed by reverse phase (rp) silica chromatography. In the initial step reaction mixture is applied to a wcx column after *p*H adjustment to 4. As product is cationic at this *p*H it is retained. Elution of the column with water does not elute the product, which is visible under a uv lamp as a strongly blue-fluorescent band at the top of the column, but does elute the major impurity, as might be expected from the SCX hplc results [Figure 2], and also salt. The elution of the impurity is marked by the passage of a yellow, pale-purple fluorescent band which has a uv/visible spectrum suggesting it to be the intermediate formed in the first stage of the reaction with bisulfite. After the impurity and salt have been completely removed, the product is eluted using weak

acid (0.01 M hydrochloric acid). The solution is freeze dried to give crude product, which for 8-substituted, and 6-methyl- and 7-methyl-8-substituted compounds is generally greater than 95% pure. Final purification is achieved using rp silica chromatography with sample applied to the column dissolved in very dilute acid (pH 4). On elution with water the product moves rapidly down the column and impurities are retained. Product was obtained by freeze drying the collected fraction. This procedure was particularly useful for removing color from the compounds to give cream or pure white products of >99% purity. Microanalysis results for most compounds indicated some water and extra hydrochloric acid was present even after prolonged drying of the sample under vacuum at room temperature.

Purification of 5-methyl-substituted compounds proved to be significantly more difficult than for other compounds. This could be anticipated from the hplc trace shown in Figure 2(d) which shows that while most impurities elute in the void there is still a substantial proportion of impurity (~10-15%) with significant retention on the SCX hplc column. Thus, it was found that purification to ~90% purity was readily achieved using wcx chromatography, but further purification was extremely difficult requiring multiple passages through rp silica or wcx columns and careful monitoring of fractions by SCX hplc. This resulted in very significant losses of compound and very low yields. For 5,8-dimethyl-N5-deazapterin this problem was overcome by recrystallizing the crude solid obtained from the wcx column in ethanol to give a relatively good yield. This could be a solution for other 5,8-disubstituted compounds if suitable solvents can be found.

Preparation of N5-Deazapterins

These improved reaction conditions and purification procedures were successfully applied to the preparation of N5-deazapterin (29), 6-methyl-N5-deazapterin (30),7-methyl-N5-deazapterin (31) and 5-methyl-N5-deazapterin (32). For condensation of 2,6-diaminopyrimidin-4(3H)-one 8 with the 1,2-unsaturated carbonyls, 16 and 18, to form 30 and 31, similar conditions as for the 6-methyl- and 7-methyl-8-alkyl-N5-deazapterins were used with very similar results. For the preparation of N5-deazapterin conditions were modified to improve the procedure. A problem with this condensation is that while we wish to have bisulfite present initially in the reaction the strong acid required to hydrolyze the acetal to start the reaction also destroys the bisulfite. Hence, in the synthesis of N5-deazapterin we altered the procedure such that acid was added initially to hydrolyze the acetal and start the condensation, and then sufficient solid sodium bisulfite was added to remove the colored reaction intermediates. As this process raises the solution pH and tends to stop the reaction, more acid was added. This cycle was repeated until reaction was com-

plete. The result was very clean conversion to N5-deazapterin which was collected by filtration after adjusting the pH of the reaction mixture. This product without further purification had uv/visible spectral properties very similar to those previously reported [14]. The yield for conversion of pyrimidine to deazapterin by our procedure was substantially higher than that previously reported [15] for a synthesis involving isolation of a pyrimidine dimer intermediate which is subsequently converted to deazapterin with concomitant loss of one pyrimidine molecule.

Isomer Characterization and Spectroscopic Properties of 8-Alkyl-N5-deazapterins.

The synthesis of isomeric 6- or 7-substituted-pterins has classically been complicated by the non-regioselectivity of the synthetic procedures [16], usually the Gabriel-Colman reaction in which unsymmetrical dicarbonyls are used in the condensation. Resolution of this problem has been further complicated by difficulty in correctly identifying the different isomers. The similarity between the Gabriel-Colman synthesis and the condensation reactions used here for the preparation of N5-deazapterins and 8-alkyl-N5-deazapterins suggest that similar non-regioselectivity in synthetic outcomes for these reactions could be expected when unsymmetrical dicarbonyls or 1,2-unsaturated carbonyls are used as the 3-carbon unit. However, both in our previous report [6] and in the present studies we have not observed significant mixtures of isomers but rather that condensations with both crotonaldehyde (17) and methyl vinyl ketone (18) result in only one N5-deazapterin product.

The remaining problem is to assign unambiguously the structures of the products. Condensations could reasonably form either 5-methyl or 7-methyl-substituted compounds depending on the order of reactivity of the condensing groups. For the identification of isomeric products in the preparation of 6- or 7-methyl-8-alkylpterins use has been made previously of the existence of different tautomeric forms of the two isomers in neutral solution. 7-isomer forms a proportion of *exo*methylene compound, which is not possible for the 6-isomer, and this can be observed easily in ^{1}H nmr spectra from the exchangeability of the 7-CH_3 protons in neutral deuterium oxide solution [12]. These absolute assignments could then be correlated with characteristic uv/visible spectra for the two isomers to provide convenient methods for isomer identification. Unfortunately for the identification of 5-methyl and 7-methyl-8-alkyl-N5-deazapterins both compounds can theoretically form an *exo*methylene tautomer and this was confirmed by monitoring of the ^{1}H nmr spectra of 5,8-dimethyl- and 7,8-dimethyl-N5-deazapterins which indicated deuterium oxide exchange of the 5- and 7-methyl group protons over time in neutral to basic solution. This phenomenon was observed more readily for the 5-methyl

isomer as it was considerably more soluble in base than the 7-methyl isomer.

The presence of the 8-alkyl-substituent, however, provides a spectroscopic probe which can be used in a ^{1}H nmr NOE experiment to determine the position of the methyl substituent. This approach was used previously [6] to characterize unambiguously 7,8-dimethyl-N5-deazapterin as the product from condensation of 2-amino-6-methylaminopyrimidin-4(3*H*)-one (1) with methyl vinyl ketone (18).

We used an NOE ^{1}H nmr experiment to confirm that the product from the condensation of 2-amino-6-methylaminopyrimidin-4(3*H*)-one (1) with crotonaldehyde (17) in the presence of sodium bisulfite is the expected 5,8-dimethyl-N5-deazapterin: separate irradiations of the 8-methyl and 5-methyl groups induces a 2% increase in intensity of the 7-*H* and 6-*H* proton resonances, respectively. Comparison of the uv/visible and ^{1}H nmr data for the product of the condensation of 2-amino-6-methylaminopyrimidin-4(3*H*)-one (1) with methyl vinyl ketone (18) in the presence of sodium bisulfite with previous data [6] for 7,8-dimethyl-N5-deazapterin confirms them to be the same compound, thus indicating that the presence of sodium bisulfite does not affect the orientation of the condensation reaction.

Once the primary assignment of structures was made, examination of the uv/visible spectra of the compound series 8-methyl-, 5,8-dimethyl-, 6,8-dimethyl- and 7,8-dimethyl-N5-deazapterin [Figure 3] indicated a strong dependence on the position of the methyl substituent and the tautomeric state of the compound. Hence, uv/visible spectra can be used readily to identify the 5-, 6- and 7-methyl isomers. The spectra for the cations of each compound [Figure 3(a)] exhibit three strong bands with two in the uv region (215-230 and 276-278 nm) and a third in the blue/violet region (337-355 nm). The position of the long wavelength band shows considerable variability in position moving to shorter wavelength in going from 6,8-dimethyl-(355 nm) to 8-methyl-(348 nm) to 7,8-dimethyl-(347 nm) to 5,8-dimethyl-N5-deazapterin (337 nm). The band at ~277 nm shows little wavelength variation but varies significantly in intensity compared with the longer wavelength band. In particular, for the 7-methyl isomer this middle band has less intensity relative to the longer wavelength band, a pattern analogous to similar bands in 7,8-dimethylpterin [12]. While the shorter wavelength band (~220 nm) for the 8-methyl, 6,8-dimethyl and 7,8-dimethyl-N5-deazapterin compounds are very similar, the 5-methyl isomer band exhibits a bathochromic shift of between 13 and 15 nm and also significant fine structure. The spectra of the neutral species shown in Figure 3(b) are again characterized by three intense bands. The main effect of charge neutralization is a general bathochromic shift in the long wavelength band of ~10 nm and a hypsochromic shift in the central band of ~22 nm. The relative differences between the different isomers are in line with those observed in the cationic spectra [Figure 3(a)].
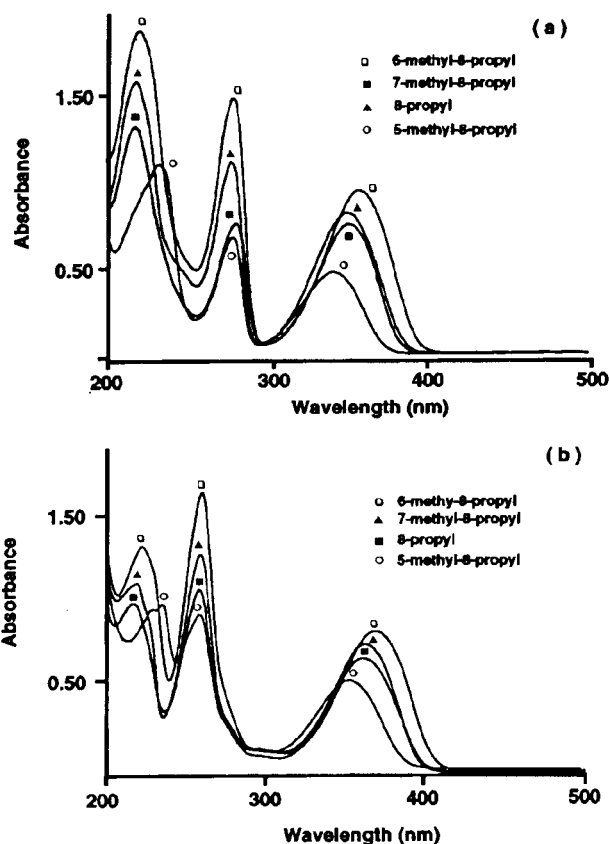

Figure 3. Ultra-violet/visible spectra of 8-propyl- (12), 6-methyl-8-propyl- (21), 7-methyl-8-propyl- (28) and 5-methyl-8-propyl-N5-deazapterin (24) in: (a) 0.01 *M* hydrochloric acid (cation); (b) *p*H 10 (butylamine/hydrochloric acid buffer) (neutral form).

Table 1 summarizes both the uv/visible (λ_{max}, ε) and ionization data (p*K*a) for all 8-alkyl-N5-deazapterins. It is apparent that the trends in uv/visible spectra discussed for the 8-methyl compounds are also present for the other 8-substituents. The ionization data show that the basic p*K*a for 5-methyl-substituted compounds is surprisingly increased by between 0.7-1 units compared with the other isomers. This phenomenon has been investigated by quantum mechanical calculations and shown to be due primarily to solvation effects [18]. These distinguishing features have allowed us to confirm that, in all cases investigated, condensation with crotonaldehyde (17) results in formation of 5-methyl-substituted compounds while condensation with methyl vinyl ketone (18) results in 7-methyl isomers.

Ionization Behavior and Site of Protonation of N5-Deazapterins.

The uv/visible spectra of N5-deazapterin and 8-propyl-N5-deazapterin in cationic and neutral forms are shown in Figure 4. The spectra of the cationic species [Figure 4(a)] for the two compounds show remarkable similarity with

Table 1

Ultra-violet/visible Spectral and Ionisation Data (pKa) for 2-Amino-8-alkylaminopyrido[2,3-d]pyrimidin-4(3H)-ones (8-Alkyl-N5-deazapterins)

Compound	pKa	pH	Species	λ max (nm)	log ε
10 (8-methyl)	6.40 ± 0.03	2	+	348, 277, 217	4.02, 4.16, 4.31
		9	0	358, 258, 217	4.03, 4.32, 4.24
26 (5,8-dimethyl)	7.32 ± 0.06	2	+	337, 276, 230	3.98, 4.14, 4.34
		10	0	348, 256, 232, 227 (sh)	4.04, 4.24, 4.26, (4.24)
19 (6,8-dimethyl)	6.60 ± 0.03	2	+	355, 277, 217	4.04, 4.23, 4.32
		9	0	365, 257, 219	4.05, 4.36, 4.25
25 (7,8-dimethyl)	6.66 ± 0.03	2	+	347, 278, 215	4.14, 4.12, 4.33
		9	0	357, 255, 214	4.14, 4.29, 4.24
11 (8-ethyl)	6.57 ± 0.02	2	+	349, 276, 216	4.03, 4.17, 4.35
		9	0	359, 257, 216	4.02, 4.30, 4.24
20 (6-methyl-8-ethyl)	6.81 ± 0.02	2	+	356, 278, 217	4.00, 4.21, 4.33
		9	0	366, 257, 219	4.02, 4.33, 4.24
12 (8-propyl)	6.51 ± 0.03	2	+	349, 276, 217	3.99, 4.13, 4.30
		9	0	359, 257, 217	4.00, 4.28, 4.22
21 (6-methyl-8-propyl)	6.78 ± 0.03	2	+	357, 278, 218	4.00, 4.20, 4.32
		9	0	368, 257, 220	4.02, 4.34, 4.25
28 (7-methyl-8-propyl)	6.86 ± 0.03	2	+	350, 279, 216	4.06, 4.06, 4.31
		9	0	361, 256, 214	4.06, 4.21, 4.14
24 (5-methyl-8-propyl)	7.52 ± 0.02	2	+	342, 279, 232	3.93, 4.09, 4.30
		10	0	353, 259, 235, 230 (sh)	3.94, 4.18, 4.20, (4.18)
13 (8-isopropyl)	6.42 ± 0.06	2	+	348, 277, 216	4.07, 4.22, 4.38
		9	0	360, 257, 216	4.08, 4.36, 4.32
22 (6-methyl-8-isopropyl)	7.12 + 0.03	2	+	355, 278, 217	4.01, 4.22, 4.35
		9	0	367, 257, 220	4.03, 4.33, 4.23
14 (8-isobutyl)	6.71 ± 0.07	2	+	351, 279, 219	4.00, 4.13, 4.28
		9	0	363, 259, 220	4.00, 4.26, 4.19
25 (5-methyl-8-isobutyl)	7.62 + 0.04	2	+	343, 279, 233	3.99, 4.14, 4.35
		10.5	0	354, 259, 236, 231 (sh)	4.01, 4.24, 4.26, (4.24)
15 (8-benzyl)	6.69 ± 0.06	2	+	353, 279, 212	4.01, 4.11, 4.38
		9	0	365, 260, 209 (sh)	4.00, 4.26, (4.38)
26 (5-methyl-8-isoamyl)	7.65 ± 0.03	2	+	342, 279, 233	3.99, 4.14, 4.36
		10.5	0	353, 259, 236, 231 (sh)	4.00, 4.24, 4.27, (4.25)

Table 2

Ultra-violet/visible Spectral and Ionisation Data (pKa) for 2-Aminopyrido[2,3-d]pyrimidin-4(3H)-ones (N5-Deazapterins)

Compound	pKa	pH	Species	λ max	log ε
29	3.99 ± 0.05	1	+	343, 312 (sh), 274, 242 (sh), 212	3.79, (3.48), 4.00, (3.80), 4.31
	8.93 ± 0.08	7	0	333 (sh), 315, 308 (sh), 267, 217	(3.46), 3.65, (3.64), 3.93, 4.30
		11	-	328, 268, 239	3.73, 3.79, 4.18
30 (6-methyl)	4.32 ± 0.06	1	+	351, 325 (sh), 276, 247 (sh), 215	3.85, (3.61), 4.11, (3.87), 4.36
	9.10 ± 0.04	7	0	341 (sh), 323, 265, 217	(3.18), 3.44, 3.75, 4.16
		13	-	338, 267, 241	3.79, 4.00, 4.31
31 (7-methyl)	4.68 ± 0.02	1	+	345, 277, 214	4.05, 4.11, 4.35
	9.09 ± 0.10	7	0	328 (sh), 316, 308, 268, 218	(3.64), 3.86, 3.84, 3.97, 4.44
		13	-	328, 268, 239	3.79, 4.00, 4.31
32 (5-methyl)	4.98 ± 0.08	1	+	336, 274, 227	3.88, 4.09, 4.32
	9.69 ± 0.05	7	0	329 (sh), 312, 266, 224	(3.52), 3.69, 3.99, 4.43
		12	-	327, 267, 241 (sh), 228	3.76, 3.90, (4.20), 4.38

all three bands in very similar positions for both compounds. However, the spectra of the neutral species are significantly different with the long wavelength band showing a strong hypsochromic shift for the N5-deazapterin. These results suggest that N5-deazapterins are protonated on N8 and in the neutral form are not quinonoid in nature but are probably in the N3(H) form.

The protonation of N5-deazapterin on N8 rather than N1 as in pterins [17] is consistent with the known increased basicity of the pyridine ring and has been implicated in the binding of N5-deazafolate to DHFR [19].

Table 2 summarizes the spectral and ionization data for the N5-deazapterins. These indicate that the cationic spectrum of each methyl isomer is very similar to its 8-alkyl-
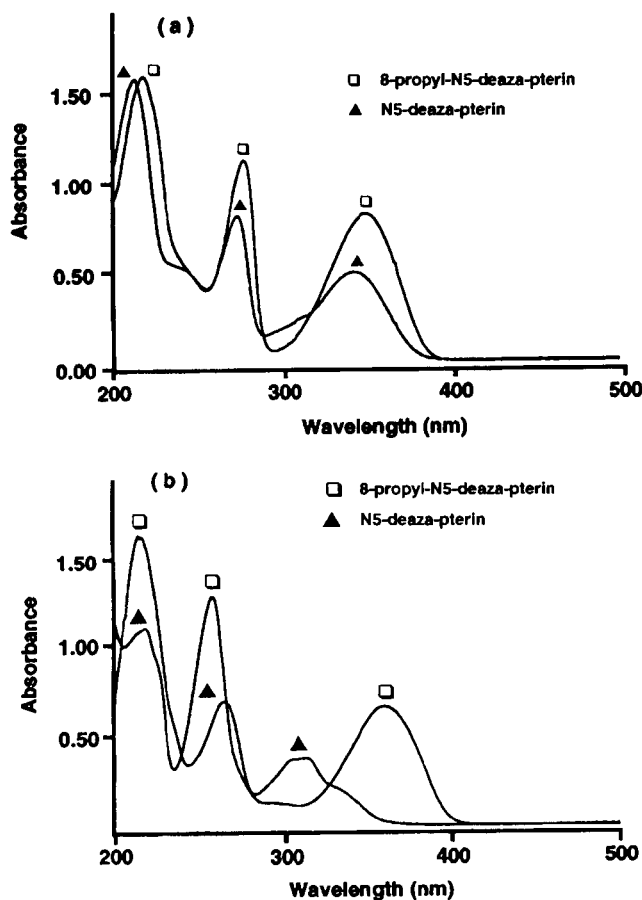
Figure 4. Comparison of uv/visible spectra of 8-propyl-N5-deazapterin (12) and N5-deazapterin (29): (a) cations (*p*H 2); (b) neutral forms (*p*H 9 for 12 and *p*H 7 for 29).

analogue but with the long wavelength band blue-shifted by ~5-6 nm, thus suggesting that all these compounds are protonated on N8. Also, the cationic spectra of these isomeric N5-deazapterins show spectral differences between isomers similar to those discussed for the different methyl-substituted 8-alkyl-N5-deazapterins. The spectra for the neutral and anionic forms are very similar for all the isomers, and analogous to those for the parent N5-deazapterin. The p*K*a data for the N5-deazapterins show significant differences compared with values for the 8-alkyl-N5-deazapterins. The basic p*K*a values are ~2-2.5 units lower than their 8-substituted analogues as predicted on theoretical grounds [20]. The basic p*K*a value for 7-methyl-N5-deazapterin appears anomalously high, although it is still lower than that for the 5-methyl isomer. The acidic ionization, which is not possible for the 8-alkyl-N5-deazapterins, probably involves the N3 proton, as has been established for pterins [17]. Similar to the basic p*K*a values, 5-methyl-N5-deazapterin also has an elevated acidic p*K*a. In contrast to the behavior for 8-alkyl-N5-deazapterins with 7-methyl or 5-methyl substituents, nmr experiments at *p*D 13 show

no evidence for deuterium exchange of the methyl protons of 7-methyl- and 5-methyl-N5-deazapterins.

EXPERIMENTAL

Reagents and Instrumental Analysis.

All reagents were of AR grade or better. 2,6-Diamino-pyrimidin-4(3*H*)-one, 2-amino-4-chloropyrimidin-4(3*H*)-one, methacrolein, malonaldehyde bis(dimethylacetal), methyl vinyl ketone and crotonaldehyde were from Aldrich. Sodium bisulfite was obtained from Sigma. Amberlite CG-50 was obtained from May and Baker and ICN. Reverse phase silica was initially prepared according to ref [21], and later obtained from Sigma. The uv/visible and nmr spectra were recorded using Cary 3 and Bruker AS200 spectrometers. All ^{1}H nmr spectra were recorded in either deuterium oxide or D_6-DMSO with spectra referenced to water at δ 4.76 and DMSO at δ 2.50, respectively. The uv/visible spectral measurements were in 0.01 *M* hydrochloric acid unless otherwise indicated. The p*K*a values were determined by spectrophotometric analysis [22]. Microanalyses were performed by the Australian National University Microanalysis Service. The hplc analysis [23] was performed with an LKB system equipped with LKB UV detector set at 278 nm, Waters fluorescence detector (λ_{ex} 338 nm / λ_{em} 455 nm), Waters analysis software (Maxima and Millenium) and an Activon Exsil SCX 10 μm analytical column (from Activon Scientific Products, Australia) with a flow rate of 2 ml/minute. The mobile phase was 0.1-0.2 *M* ammonium formate/acetonitrile (80/20) with *p*H adjusted to 3.3 with formic acid.

Preparative Chromatography.

Weak cation exchange (wcx) column chromatography was used for primary purification of reaction mixtures. The column was ~10 cm long and 2 cm in diameter and contained ~10 g of Amberlite CG-50 weak cation exchange resin. After *p*H adjustment to 4 with 5 *M* sodium hydroxide followed by filtration, the reaction mixture was applied directly to the column. Impurities and residual salt were removed by initial elution with water. The passage of the impurities was marked by a pale-purple fluorescent band. The product, indicated by a strongly blue-fluorescing band, was eluted using 0.01 *M* hydrochloric acid and the solution freeze dried to give crude solid product.

Reverse phase (rp) silica [21] chromatography was used for final purification of the freeze-dried crude product from wcx chromatography. Typically, a plug of packing material approximately 5-10 cm long and 1 cm diameter was used. The deazapterin as the hydrochloride salt was dissolved in water, applied to the column, and eluted using either water or dilute acid (0.01-0.0001 *M* hydrochloric acid). Under these conditions impurities are retained and deazapterins as the water-soluble cation are rapidly eluted. The collected fraction was then acidified with a few drops of 0.01 *M* hydrochloric acid and freeze-dried to give the final product.

Pyrimidine Precursors.

The pyrimidine starting materials 1-4 were prepared as outlined previously [12]. Pyrimidines 5-7 were prepared by the same procedure and characterized as follows:

2-Amino-6-*iso*butylaminopyrimidin-4(3*H*)-one (**5**).

This compound had uv (0.01 *M* hydrochloric acid): λ max 270 nm; ¹H nmr (D₆-DMSO): δ 9.68 (broad s, 1H, pyrimido-N*H*), δ 6.36 (t, 1H, 6-N*H*), δ 6.10 (s, 2H, 2-N*H₂*), δ 4.41 (s, 1H, 5-C*H*), δ 2.84 (t, 2H, N-C*H₂*), δ 1.75 (m, 1H, N-CH₂C*H*), δ 0.84 (d, 6H, (C*H₃*)₂).

2-Amino-6-*iso*amylpyrimidin-4(3*H*)-one (**6**).

This compound had uv (0.01 *M* hydrochloric acid): λ max 269 nm; ¹H nmr (D₆-DMSO): δ 9.54 (broad s, 1H, pyrimido-N*H*), δ 6.27 (t, 1H, 6-N*H*), δ 6.08 (s, 2H, 2-N*H₂*), δ 4.39 (s, 1H, 5-C*H*), δ 3.01 (qrt, 2H, N-C*H₂*), δ 1.59 (m, 1H, N-CH₂CH₂C*H*), δ 1.32 (m, 2H, N-CH₂C*H₂*), δ 0.86 (d, 6H, (C*H₃*)₂).

2-Amino-6-benzylaminopyrimidin-4(3*H*)-one (**7**).

This compound had uv (0.01 *M* hydrochloric acid): λ max 270 nm; ¹H nmr (D₆-DMSO): δ 9.67 (broad s, 1H, pyrimido-N*H*), δ 7.23 (m, 5H, phenyl protons), δ 6.92 (t, 1H, peak width 12.41 Hz, 6-N*H*), δ 6.16 (s, 2H, 2-N*H₂*), δ 4.40 (s, 1H, 5-C*H*), δ 4.27 (d, 2H, J = 6.0 Hz, N-CH₂).

All pyrimidines were used in subsequent syntheses without further purification.

Attempted Synthesis of 2-Amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (8-Propyl-N5-deazapterin) (**12**).

To a suspension of 0.25 g (1.47 mmoles) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**5**) in a mixture of 10 ml of methanol, 40 ml of water and fifteen drops of methanolic hydrochloric acid (11 *M*) was added 0.25 g (1.52 mmoles) of malonaldehyde bis(dimethylacetal) (**9**) and the mixture was heated at 70°. After 10 minutes the solution had turned a dark red/orange color and exhibited visible absorbance at ~530 nm. After heating the mixture for a further 46 hours the uv/visible spectrum of an aliquot exhibited the characteristic 3 peaks (350, 278 and 217 nm) of an 8-alkyl-N5-deazapterin. The reaction mixture was then taken to dryness to give 0.15 g of orange/brown solid. Attempts to purify this crude solid by recrystallization were unsuccessful as no suitable solvent could be found. Attempted purification by reverse phase silica column chromatography also proved unsuccessful.

Reaction Course Studies into the Preparation of 2-Amino-8-alkylpyrido[2,3-*d*]pyrimidin-4(3*H*)-ones.

2-Amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (8-Propyl-N5-deazapterin) (**12**).

To a suspension of 0.06 g (0.35 mmole) of 2-amino-6-propy-laminopyrimidin-4(3*H*)-one (**3**) in 20 ml of 0.01 *M* hydrochloric acid was added 5 drops of 11 *M* hydrochloric acid and 0.10 ml (0.61 mmole) of malonaldehyde bis(dimethylacetal) (**9**). After refluxing for 10 minutes the solution had turned a dark red/amber color. The uv/visible spectrum showed absorbance at ~530 nm, which was still present after refluxing for a further 2 hours when the solution was a dark amber color; uv (0.01 *M* hydrochloric acid): λ max 530 (ε relative 0.07), 353 (ε relative 1.00), 278 (ε relative 1.63), 219 nm (ε relative 2.13); SCX hplc analysis [Figure 1(a)] indicated 87% 2-amino-8-propylpyrido-[2,3-*d*]pyrimidin-4(3*H*)-one (**12**).

2-Amino-6-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (6-Methyl-8-propyl-N5-deazapterin) (**21**).

To a suspension of 0.06 g (0.35 mmole) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) in 20 ml of 0.01 *M* hydrochloric

acid was added 5 drops of 11 *M* hydrochloric acid and 0.10 ml (1.21 mmoles) of methacrolein (**16**), and the mixture was refluxed for 4 hours; uv (0.01 *M* hydrochloric acid): λ max 357 (ε relative 1.00), 280 (ε relative 2.20), 214 nm (ε relative 2.60);. SCX hplc analysis [Figure 1(b)] indicated 72% 2-amino-6-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**21**).

2-Amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (7-Methyl-8-propyl-N5-deazapterin) (**28**).

To a suspension of 0.02 g (0.12 mmole) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) in 10 ml of 0.01 *M* hydrochloric acid and 5 drops 11 *M* hydrochloric acid was added 0.10 ml (1.20 mmoles) of methyl vinyl ketone (**18**), and the mixture was refluxed for 6 hours; SCX hplc analysis [Figure 1(c)] indicated 49% 2-amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**28**).

2-Amino-5-methyl-8-*iso*amylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (5-Methyl-8-*iso*amyl-N5-deazapterin) (**26**).

To a suspension of 0.25 g (1.28 mmoles) of 2-amino-6-*iso*amylaminopyrimidin-4(3*H*)-one (**6**) in 20 ml of 0.01 *M* hydrochloric acid and 5 drops 11 *M* hydrochloric acid was added 0.25 ml (3.0 mmoles) of crotonaldehyde (**17**), and the mixture heated at 80° for 9 hours; uv (0.01 *M* hydrochloric acid): λ max 345 (ε relative 1.00), 279 (ε relative 3.49), 216 nm (ε relative 3.59); SCX hplc analysis [Figure 1(d)] indicated 33% 2-amino-5-methyl-8-*iso*amylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**26**).

Effect of Sodium Bisulfite on the Preparation of 2-Amino-8-alkylpyrido[2,3-*d*]pyrimidin-4(3*H*)-ones (8-Alkyl-N5-deazapterins).

2-Amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (8-Propyl-N5-deazapterin) (**12**).

To a suspension of 0.02 g (0.12 mmole) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) and 0.1 g (0.97 mmole) of sodium bisulfite in 10 ml of 0.01 *M* hydrochloric acid and 5 drops 11 *M* hydrochloric acid was added 0.10 ml (0.61 mmole) of malonaldehyde bis(dimethylacetal) (**9**), and the mixture was stirred at room temperature for 2 hours; uv (0.01 *M* hydrochloric acid): λ max 349 (ε relative 1.0), 270 nm (ε relative 5.28). A further 5 drops of 11 *M* hydrochloric acid was then added and the mixture stirred under reflux for 3 hours; SCX hplc analysis of the reaction mixture [Figure 2(a)] indicated 80% 2-amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**12**).

2-Amino-6-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (6-Methyl-8-propyl-N5-deazapterin) (**21**).

To a suspension of 0.25 g (1.46 mmoles) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) and 0.25 g (2.5 mmoles) of sodium bisulfite in 10 ml of water was added 0.5 ml (6.0 mmoles) of methacrolein (**16**). The *p*H of the mixture was adjusted to 4 and the suspension was stirred at room temperature for 3 days; uv (0.01 *M* hydrochloric acid): λ max 285 (ε relative 1.27), 209 nm (ε relative 1.0); (*p*H 7): λ max 285 (ε relative 1.0), 227 nm (ε relative 1.58). The mixture was acidified to *p*H 1 and stirred under vacuum at room temperature for 7 days; uv (0.01 *M* hydrochloric acid): λ max 358 (ε relative 1.00), 278 (ε relative 1.77), 219 nm (ε relative 2.11); SCX hplc analysis of the mixture [Figure 2(b)] indicated 90% 2-amino-6-methyl-8-propylpyrido[2,3-*d*]pyrim-idin-4(3*H*)-one (**21**).

2-Amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (7-Methyl-8-propyl-N5-deazapterin) (**28**).

To a suspension of 0.02 g (0.12 mmole) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) and 0.1 g (0.97 mmole) of sodium bisulfite in 10 ml 0.01 *M* hydrochloric acid was added 0.1 ml (1.20 mmoles) of methyl vinyl ketone (**18**), and the mixture was stirred at room temperature for 45 minutes; uv (0.01 *M* hydrochloric acid): 284 nm. Ten drops of 11 *M* hydrochloric acid was added and the mixture heated under reflux for 5 hours; SCX hplc analysis of the mixture [Figure 2(c)] indicated 80% 2-amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**28**).

2-Amino-5-methyl-8-*iso*amylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (5-Methyl-8-*iso*amyl-N5-deazapterin) (26).

To a suspension of 0.25 g (1.28 mmoles) of 2-amino-6-*iso*amylaminopyrimidin-4(3*H*)-one (**6**) and 0.25 g (2.5 mmoles) of sodium bisulfite in 20 ml 0.01 *M* hydrochloric acid was added 0.25 ml (3.0 mmoles) of crotonaldehyde (**17**), and the mixture was stirred at room temperature for 3 hours; uv (0.01 *M* hydrochloric acid): 280 nm. Ten drops of 11 *M* hydrochloric acid was added and the mixture stirred at 80° for 4 hours; uv (0.01 *M* hydrochloric acid): λ max 344 (ε relative 1.00), 279 (ε relative 2.09), 233 nm (ε relative 2.81); SCX hplc analysis of the mixture [Figure 2(d)] indicated 55% 2-amino-5-methyl-8-*iso*amylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**26**).

Preparation of 2-Amino-8-alkylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochlorides (8-Alkyl-N5-deazapterin Hydrochlorides).

2-Amino-8-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (8-Methyl-N5-deazapterin Hydrochloride) (10).

To a suspension of 0.15 g (1.06 mmoles) of 2-amino-6-methyl-aminopyrimidin-4(3*H*)-one (**1**) in 5 ml of water was added 0.2 ml (1.16 mmoles) of malonaldehyde bis(dimethylacetal) (**9**) and 0.15 g (1.44 mmoles) of sodium bisulfite in 3 ml of water. After the *p*H of the suspension was adjusted to 4, the mixture was stirred at 50° for 2 hours, acidified to *p*H 1, and heated for a further 2 hours. The crude product was obtained using wcx chromatography as 0.044 g (20%) of pale yellow solid. The solid was purified using rp silica chromatography to give 0.033 g (15%, SCX hplc purity 99.4%) of 2-amino-8-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**10**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.78 (lit [6] δ 8.82) (dd, 2H, J = 1.6, 7.6 Hz, 5-*H* or 7-*H*), δ 8.64 (lit [6] δ 8.62) (dd, 2H, J = 1.6, 7.6 Hz, 5-*H* or 7-*H*), δ 7.39 (lit [6] 7.41) (dd, 1H, J = 6.46, 6.51 Hz, 6-*H*), δ 4.10 (lit [6] δ 4.13) (s, 3H, N-C*H*₃).

Anal. Calcd. for C₈H₉N₄OCl•1.0H₂O•0.15 HCl (236.13): C, 40.69; H, 4.76; N, 23.73; Cl, 17.27. Found: C, 41.04; H, 4.88; N, 23.36; Cl, 17.51.

2-Amino-8-ethylpyrido[2,3-*d*]pyrimidin-4[3*H*]-one Hydrochloride (8-Ethyl-N5-deazapterin Hydrochloride) (11).

To a suspension of 0.15 g (0.97 mmole) of 2-amino-6-ethyl-aminopyrimidin-4(3*H*)-one (**2**) in 5 ml of water was added 0.2 ml (1.16 mmoles) of malonaldehyde bis(dimethylacetal) (**9**) and 0.15 g (1.44 mmoles) of sodium bisulfite in 2 ml of water. After the *p*H of the resulting suspension was adjusted to 4, the mixture was stirred at 40° for 2 hours, acidified to *p*H 1, and heated at 60° for a further 2 hours. The crude product was obtained using wcx chromatography as 0.092 g (40%) of pale yellow solid. The solid was purified using rp silica chromatography to give 0.08 g (35%, SCX hplc purity >99.5%) of 2-amino-8-ethylpyrido[2,3-

d]pyrimidin-4(3*H*)-one hydrochloride (**11**) as a cream solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.77 (dd, 2H, J = 1.7, 7.6 Hz, 5-*H* or 7-*H*), δ 8.68 (dd, 2H, J = 1.7, 7.6 Hz, 5-*H* or 7-*H*), δ 7.41 (t, 1H, J = 7.6 Hz, 6-*H*), δ 4.60 (qrt, 2H, N-C*H*₂), δ 1.48 (t, 3H, J = 7.3, 7.3 Hz, N-CH₂C*H*₃).

Anal. Calcd. for C₉H₁₁N₄OCl•0.25H₂O (231.17): C, 46.76; H, 5.01; N, 24.24; Cl, 15.33. Found: C, 46.87; H, 5.15; N, 23.92; Cl, 15.48.

2-Amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (8-Propyl-N5-deazapterin Hydrochloride) (12).

To a suspension of 0.25 g (1.48 mmoles) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) in 10 ml of water was added 0.3 ml (1.76 mmoles) of malonaldehyde bis(dimethylacetal) (**9**) and 0.16 g (1.55 mmoles) of sodium bisulfite in 2 ml of water. After the *p*H of the mixture was adjusted to 3-4 it was stirred at room temperature for 24 hours, acidified to *p*H 1-2, and heated at 50° for 5 hours. The crude product was obtained using wcx chromatography as 0.16 g (45%, SCX hplc purity 96%) of pale cream solid. The solid was purified using rp silica chromatography to give 0.132 g (37%, SCX hplc purity 99.5%) 2-amino-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**12**) as a pale cream solid; ^{1}H nmr (*p*D 3 deuterium oxide/deuterium chloride): δ 8.77 (dd, 1H, J = 1.8, 7.6 Hz, 5-*H* or 7-*H*), δ 8.65 (dd, 1H, J = 1.8, 7.6 Hz, 5-*H* or 7-*H*), δ 7.41 (t, 1H, J = 7.6, 7.6 Hz, 6-*H*), δ 4.52 (t, 2H, J = 7.2, 7.2 Hz, N-CH₂), δ 1.90 (m, 2H, N-CH₂C*H*₂), δ 0.92 (t, 3H, J = 7.4, 7.6 Hz, N-CH₂CH₂C*H*₃).

Anal. Calcd. for C₁₀H₁₃N₄OCl•1.5H₂O•0.22HCl (275.73): C, 43.55; H, 5.93; N, 20.32; Cl, 15.68. Found: C, 43.32; H, 5.67; N, 20.15; Cl, 15.46.

2-Amino-8-*iso*propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (8-*Iso*propyl-N5-deazapterin Hydrochloride) (13).

To a suspension of 0.15 g (0.89 mmole) of 2-amino-6-*iso*propyl-aminopyrimidin-4(3*H*)-one (**4**) in 7 ml of water was added 0.16 ml (0.94 mmole) of malonaldehyde bis(dimethylacetal) (**9**) and 0.10 g (0.97 mmole) of sodium bisulfite in 2 ml of water. After the *p*H of the mixture was adjusted to 4, it was stirred at 50° for 24 hours. The crude product was obtained using wcx chromatography as 0.060 g (28%) of pale yellow solid. The solid was purified using rp silica chromatography to give 0.050 g (23%, SCX hplc purity 99.1%) of 2-amino-8-*iso*propylpyrido[2,3-*d*]pyrimidin-4[3*H*]-one hydrochloride (**13**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.76 (d, 2H, J = 6.9 Hz, 5-*H* & 7-*H*), δ 7.46 (t, 1H, J = 7.2, 7.2 Hz, 6-*H*), δ 5.83 (m, 1H, N-C*H*), δ 1.53 (d, 6H, J = 7.0 Hz, N-CH(C*H*₃)₂).

Anal. Calcd. for C₁₀H₁₃N₄OCl•1.05H₂O•0.05HCl (261.43): C, 45.94; H, 5.84; N, 21.44; Cl, 14.24. Found: C, 45.66; H, 6.14; N, 21.79; Cl, 14.38.

2-Amino-8-*iso*butylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (8-*Iso*butyl-N5-deazapterin Hydrochloride) (14).

To 0.25 g (1.37 mmoles) of 2-amino-6-*iso*butylaminopyrim-idin-4(3*H*)-one (**5**) and 0.375 g (3.64 mmoles) of sodium bisul-fite in 20 ml of 0.01 *M* hydrochloric acid was added 0.40 ml (2.33 mmoles) of malonaldehyde bis(dimethylacetal) (**9**) and the mixture was heated at 50° for 2 hours. The crude product was obtained using wcx chromatography as 0.20 g (57%, SCX hplc purity 98%) of cream solid. The solid was purified using rp silica chromatography to give 0.14 g (40%, SCX hplc purity 99.4%) of 2-amino-8-*iso*butylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one

hydrochloride (**14**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.79 (dd, 1H, J = 7.75, 1.74 Hz, 5-*H* or 7-*H*), δ 8.61 (dd, 1H, J = 6.4, 1.8 Hz, 5-*H* or 7-*H*)), δ 7.40 (dd, 1H, J = 7.7, 6.4 Hz, 6-*H*), δ 4.38 (d, 2H, J = 7.4 Hz, N-C*H*$_2$), δ 2.32 (m, 1H, NCH$_2$C*H*), δ 0.91 (d, 6H, J = 6.72 Hz, N-CH$_2$CH(C*H*$_3$)$_2$).

Anal. Calcd. for C$_{11}$H$_{15}$N$_4$OCl (254.72): C, 51.86; H, 5.94; N, 22.00; Cl, 13.92. Found: C, 51.65; H, 5.99; N, 22.01; Cl, 14.04.

2-Amino-8-benzylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (8-Benzyl-N5-deazapterin Hydrochloride) (**15**).

To 0.25 g (1.15 mmoles) of 2-amino-6-benzylaminopyrimidin-4(3*H*)-one (**7**) and 0.375 g (3.64 mmoles) of sodium bisulfite in 20 ml of 0.01 *M* hydrochloric acid was added 0.35 ml (2.04 mmoles) of malonaldehyde bis(dimethylacetal) (**9**). The solution was heated at 80° for 30 minutes. After addition of sufficient solid sodium bisulfite to remove the red color developing in the reaction mixture, the solution was heated for a further 30 minutes, acidified with 5 drops 11 *M* hydrochloric acid, and heated for a further 1 hour. The crude product was obtained using wcx chromatography as two very pale yellow blue fluorescent fractions which hplc analysis indicated were 97 and 99.4% pure. Freeze drying these two fractions gave 0.08 and 0.027 g, respectively (total 0.107 g, 32%) of 2-amino-8-benzylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**15**). The second solid had ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.71 (m, 2H, 5-*H* and 7-*H*), δ 7.35 (m, 6H, phenyl and 6-*H*), δ 5.72 (s, 2H, C*H*$_2$)

Anal. Calcd. for C$_{14}$H$_{13}$N$_4$OCl•1.25H$_2$O•0.25HCl (320.37): C, 52.48; H, 4.96; N, 17.49; Cl, 13.83. Found: C, 52.25; H, 4.74; N, 17.16; Cl, 13.68.

2-Amino-6,8-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (6,8-Dimethyl-N5-deazapterin Hydrochloride) (**19**).

To a suspension of 0.5 g (2.96 mmoles) of 2-amino-6-methylaminopyrimidin-4(3*H*)-one (**1**) in 50 ml of water was added a solution of 0.80 ml (9.6 mmoles) of methacrolein (**16**) and 0.75 g (7.28 mmoles) of sodium bisulfite in 10 ml of water. The *p*H of the resulting suspension was adjusted to 2-3 and the mixture was stirred at room temperature for 2 hours, acidified to *p*H 1 and heated at 75° for 4 hours. The crude product was obtained using wcx chromatography as two blue fluorescent fractions, the first yellow and the second colorless. Freeze drying the two fractions gave 0.50 g and 0.257 g of yellow and cream solid, respectively. The yellow solid was repurified using wcx chromatography to give a colorless blue fluorescent fraction, which yielded 0.15 g of cream solid after freeze drying. The combined cream solids (crude yield 0.4 g, 50%) were ~98% by SCX hplc. Further purification by both rp and wcx chromatography was unsuccessful and the sample was finally purified by recrystallization from ethanol/methanolic hydrogen chloride (3 drops) to give 0.08 g (10%, SCX hplc purity 99.2%) of 2-amino-6,8-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**19**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.64 (lit [6] δ 8.65), (d, 1H, J = 2.0 Hz, 5-*H* or 7-*H*), δ 8.51 (lit [6] δ 8.53), (d, 1H, J = 1.7 Hz, 5-*H* or 7-*H*), δ 4.06 (lit [6] 4.07), (s, 3H, N-C*H*$_3$), δ 2.40 (lit [6] δ 2.40), (s, 3H, 6-C*H*$_3$).

Anal. Calcd. for C$_9$H$_{11}$N$_4$OCl•1.1H$_2$O (246.48): C, 43.85; H, 5.40; N, 22.73; Cl, 14.38. Found: C, 44.22; H, 5.35; N, 22.44; Cl, 14.39.

2-Amino-6-methyl-8-ethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (6-Methyl-8-ethyl-N5-deazapterin Hydrochloride) (**20**).

To a suspension of 0.13 g (0.84 mmole) of 2-amino-6-ethylaminopyrimidin-4(3*H*)-one (**2**) in 10 ml of water was added a solution of 0.20 ml (2.42 mmoles) of methacrolein (**16**) and 0.10 g (0.97 mmole) of sodium bisulfite in 5 ml of water, and the *p*H of the mixture was adjusted to 4. The suspension was stirred at room temperature for 24 hours, acidified to *p*H 1, and stirred under vacuum at room temperature for 6 days. The product was obtained using wcx chromatography as 0.03 g (15%, SCX hplc purity >99.5%) of 2-amino-6-methyl-8-ethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**20**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.64 (s, 1H, 5-*H* or 7-*H*), δ 8.56 (s, 1H, 5-*H* or 7-*H*), δ 4.57 (qrt, 2H, N-C*H*$_2$), δ 2.41 (s, 3H, 6-C*H*$_3$), δ 1.47 (t, 3H, J = 7.3, 7.3 Hz, N-CH$_2$C*H*$_3$).

Anal. Calcd. for C$_{10}$H$_{13}$N$_4$OCl•0.25H$_2$O (245.20): C, 48.98; H, 5.55; N, 22.85; Cl, 14.46. Found: C, 49.25; H, 5.53; N, 22.64; Cl, 14.57.

2-Amino-6-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (6-Methyl-8-propyl-N5-deazpterin Hydrochloride) (**21**).

To a suspension of 0.25 g (1.48 mmoles) of 2-amino-6-propylaminopyrimidin-4(3*H*)-one (**3**) in 10 ml of water was added a solution of 0.5 ml (6.0 mmoles) of methacrolein (**16**) and 0.25 g (2.43 mmoles) of sodium bisulfite in 5 ml of water. After adjusting the *p*H of the suspension to 4, the mixture was stirred at room temperature for 24 hours, acidified to *p*H 1 and stirred under vacuum for a further 7 days. The product was obtained using wcx chromatography as 0.030 g (8%, SCX hplc purity >99.5%) of 2-amino-6-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**21**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.64 (s, 1H, 5-*H* or 7-*H*), δ 8.54 (s, 1H, 5-*H* or 7-*H*), δ 4.50 (t, 2H, J = 7.2, 7.2 Hz, N-C*H*$_2$), δ 2.41 (s, 3H, 6-C*H*$_3$), δ 1.90 (m, 2H, N-CH$_2$C*H*$_2$), δ 0.90 (t, 3H, J = 7.4, 7.6 Hz, N-CH$_2$CH$_2$C*H*$_3$).

Anal. Calcd. for C$_{11}$H$_{15}$N$_4$OCl•1.60H$_2$O•0.29HCl (294.12): C, 44.92; H, 6.34; N, 19.05; Cl, 15.55. Found: C, 45.33; H, 5.86; N, 18.58; Cl, 15.15.

2-Amino-6-methyl-8-*iso*propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (6-Methyl-8-*iso*propyl-N5-deazapterin Hydrochloride) (**22**).

To a suspension of 0.13 g (0.77 mmole) of 2-amino-6-*iso*propylaminopyrimidin-4(3*H*)-one (**4**) in 5 ml of water was added a solution of 0.20 ml (2.29 mmoles) of methacrolein (**16**) and 0.15 g (1.46 mmoles) of sodium bisulfite in 3 ml of water. The *p*H of the suspension was adjusted to *p*H 4, the mixture was stirred at 50° for 1 hour, then acidified to *p*H 1 and heated for a further 4 hours. The crude product was obtained using wcx chromatography as 0.080 g (41%, SCX hplc purity ~97%) of a pale yellow solid. The crude solid was purified using rp silica chromatography to give 0.043 g (18%, SCX hplc purity >99.5%) of 2-amino-6-methyl-8-*iso*propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**22**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.63 (s, 2H, 5- & 7-*H*), δ 5.83 (m, 1H, N-C*H*), δ 2.44 (s, 3H, 6-C*H*$_3$), δ 1.52 (d, 6H, J = 6.7 Hz, N-CH(C*H*$_3$)$_2$).

Anal. Calcd. for C$_{11}$H$_{15}$N$_4$OCl•0.4H$_2$O•0.02HCl (262.66): C, 50.30; H, 6.07; N, 21.34; Cl, 13.77. Found: C, 50.56; H, 6.43; N, 21.00; Cl, 14.03.

2-Amino-5,8-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (5,8-Dimethyl-N5-deazapterin Hydrochloride) (23).

To a suspension of 0.25 g (1.78 mmoles) of 2-amino-6-methyl-aminopyrimidin-4(3*H*)-one (1) in 25 ml of water was added a solution of 0.4 ml (4.89 mmoles) of crotonaldehyde and 0.375 g (3.64 mmoles) of sodium bisulfite in 10 ml of water. After adjustment of the *p*H to 2-3, the mixture was stirred at room temperature for 1.5 hours, then acidified to *p*H 1 and the solution heated at 60° for a further 4 hours. The crude product was obtained using wcx chromatography as 0.282 g (70%) of a pale cream solid. Attempted purification of this solid by rp and wcx chromatography was unsuccessful. Recrystallization of the remaining solid from ethanol/5 drops methanolic hydrogen chloride gave 0.06 g (15% yield, SCX hplc purity 99.3%) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.37 (d, 1H, J = 6.6 Hz, 7-*H*), δ 7.20 (d, 1H, J = 6.6 Hz, 6-*H*), δ 3.99 (s, 3H, N-C*H*$_3$), δ 2.82 (s, 3H, 5-C*H*$_3$). NOE experiments: irradiation of 5-C*H*$_3$ gave enhancement of 6-*H* of 2.0%; irradiation of 8-C*H*$_3$ gave enhancement of 7-*H* of 2.2%.

Anal. Calcd. for $C_9H_{11}N_4OCl\cdot1.1H_2O$ (246.49): C, 43.85; H, 5.40; N, 22.74; Cl, 14.38. Found: C, 44.09; H, 5.67; N, 22.62; Cl, 14.30.

2-Amino-5-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (5-Methyl-8-propyl-N5-deazapterin Hydrochloride) (24).

To a suspension of 0.25 g (1.48 mmoles) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (3) in 40 ml of water was added 0.25 ml (3.06 mmoles) of crotonaldehyde (17) and 0.25 g (2.43 mmoles) of sodium bisulfite. The mixture was stirred at room temperature for 2 hours, then acidified to *p*H 1 and stirred at room temperature overnight, and finally heated at 90° for 3 hours. The crude product was obtained using wcx chromatography as 0.14 g (37%, SCX hplc purity ~90%) of a pale yellow solid. Several attempts at purification of this solid by both rp silica and wcx chromatography were unsuccessful. Pure product was achieved by rp silica chromatography coupled with careful monitoring (SCX hplc) of the column eluant to give 0.014 g (3.7%, SCX hplc purity 99.3%) of 2-amino-5-methyl-8-propyl-pyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (24) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.37 (d, 1H, J = 6.7 Hz, 7-*H*), δ 7.16 (d, 1H, J = 6.7 Hz, 6-*H*), δ 4.43 (t, 2H, J = 7.0, 7.4 Hz, N-C*H*$_2$), δ 2.81 (s, 3H, 5-C*H*$_3$), δ 1.85 (m, 2H, N-CH$_2$C*H*$_2$), δ 0.88 (t, 3H, J = 7.4, 7.4 Hz, N-CH$_2$CH$_2$C*H*$_3$).

Anal. Calcd. for $C_{11}H_{15}N_4OCl\cdot1.1H_2O\cdot0.1HCl$ (278.18): C, 47.49; H, 6.27; N, 20.14; Cl, 14.02. Found: C, 47.47; H, 6.41; N, 20.27; Cl, 13.76.

2-Amino-5-methyl-8-*iso*butylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (5-Methyl-8-*iso*butyl-N5-deazapterin Hydrochloride) (25).

To a suspension of 0.25 g (1.37 mmoles) of 2-amino-6-*iso*butylaminopyrimidin-4(3*H*)-one (5) in 40 ml of 0.01 *M* hydrochloric acid was added 0.25 g (2.43 mmoles) of sodium bisulfite and 0.25 ml (3.06 mmoles) of crotonaldehyde (17). The mixture was stirred at room temperature for 3 hours and a white precipitate formed; uv (*p*H 2): λ max 281 nm. The precipitate dissolved on acidification to *p*H 0-1, and the solution was stirred for 2 hours and then heated at 60-70° for a further 2 hours. The crude product was obtained using wcx chromatography as 0.11 g (30%, SCX hplc purity ~95%) of a pale yellow solid. The solid

was purified by rp chromatography collecting seven fractions of ~2 ml. Fraction 1 was freeze dried to give 0.02 g (5.5%, SCX hplc purity 99.3%) of 2-amino-5-methyl-8-*iso*butylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (25) as a white solid. Fractions 2 and 3 were combined and freeze dried to give 0.033 g (8%, SCX hplc purity 98.7%); ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.35 (d, 1H, J = 6.7 Hz, 7-*H*), δ 7.17 (d, 1H, J = 6.8 Hz, 6-*H*), δ 4.29 (d, 2H, J = 7.4 Hz, N-C*H*$_2$), δ 2.83 (s, 3H, 5-C*H*$_3$), δ 2.28 (m, 1H, N-CH$_2$C*H*), δ 0.89 (d, 6H, J =6.7 Hz (C*H*$_3$)$_2$).

Anal. Calcd. for $C_{12}H_{17}N_4OCl\cdot1.0H_2O\cdot0.05HCl$ (288.58): C, 49.94; H, 6.65; N, 19.42; Cl, 12.90. Found: C, 49.81; H, 6.73; N, 19.34; Cl, 12.51.

2-Amino-5-methyl-8-*iso*amylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (5-Methyl-8-*iso*amyl-N5-deazapterin Hydrochloride) (26).

To a suspension of 0.25 g (1.28 mmoles) of 2-amino-6-*iso*amylaminopyrimidin-4(3*H*)-one (6) and 0.25 g (2.43 mmoles) of sodium bisulfite in 20 ml of 0.01 *M* hydrochloric acid was added 0.25 ml (3.06 mmoles) of crotonaldehyde (17). The mixture was stirred at room temperature for 3 hours; uv (0.01 *M* hydrochloric acid): 280 nm. After acidification to *p*H 0-1, the mixture was stirred at room temperature for 30 minutes and then heated at 80° for 4 hours. The crude product was obtained using wcx chromatography as 0.143 g (39%, SCX hplc purity ~90%) as a cream solid. Several attempts to purify this product using wcx and rp silica chromatography were unsuccessful. Pure product was obtained using wcx chromatography eluting with water then 0.0001 *M* hydrochloric acid and finally 0.01 *M* hydrochloric acid with careful monitoring of eluant with SCX hplc to select fractions of highest purity (100 ml). Freeze drying gave 0.02 g (5.5%, SCX hplc purity 99.2%) as a cream solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.39 (d, 1H, J = 6.6 Hz, 7-*H*), δ 7.16 (d, 1H, J = 6.6 Hz, 6-*H*), δ 4.42 (t, 2H, J =7.3, 7.8 Hz, N-C*H*$_2$), δ 2.80 (s, 3H, 5-C*H*$_3$), δ 1.68 (m, 3H, N-CH$_2$C*H*$_2$C*H*), δ 0.90 (d, 6H, J = 6.4 Hz, N-(CH$_2$)$_2$CH(C*H*$_3$)$_2$).

Anal. Calcd. for $C_{13}H_{19}N_4OCl\cdot1.25H_2O$ (305.29): C, 51.14; H, 7.10; N, 18.36; Cl, 11.61. Found: C, 51.16; H, 7.47; N, 18.18; Cl, 11.89.

2-Amino-7,8-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (7,8-Dimethyl-N5-deazapterin Hydrochloride) (27).

To a suspension of 0.25 g (1.78 mmoles) of 2-amino-6-methyl-aminopyrimidin-4(3*H*)-one (1) in 15 ml of water was added a solution of 0.40 ml (4.83 mmoles) of methyl vinyl ketone (18) and 0.375 g (3.64 mmoles) of sodium bisulfite in 10 ml of water. After adjustment of the *p*H to 2-3, the mixture was stirred at 50° for 30 minutes, acidified to *p*H 1 and stirred for a further 2 hours. The crude product was obtained using wcx chromatography as 0.206 g (52%, SCX hplc purity ~98%) of a pale cream solid. Further purification using rp and wcx chromatography was unsuccessful. Final purification was by recrystallization from ethanol/5 drops methanolic hydrogen chloride to give 0.07 g (18%, SCX hplc purity 99.4%) of 2-amino-7,8-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (27) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.59 (d, 1H, J = 8.1 Hz, 5-*H*), δ 7.33 (d, 1H, J = 8.1 Hz, 6-*H*), δ 4.05 (s, 3H, N-C*H*$_3$), δ 2.77 (s, 3H, 7-C*H*$_3$).

Anal. Calcd. for $C_9H_{11}N_4OCl$ (226.67): C, 47.69; H, 4.89; N, 24.72; Cl, 15.64. Found: C, 47.46; H, 5.04; N, 24.44; Cl, 15.72.

2-Amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (7-Methyl-8-propyl-N5-deazapterin Hydrochloride) (**28**).

To a suspension of 0.19 g (1.11 mmoles) of 2-amino-6-propyl-aminopyrimidin-4(3*H*)-one (**3**) in 7 ml of water was added a solution of 0.16 g (1.55 mmoles) of sodium bisulfite and 0.20 ml (2.42 mmoles) of methyl vinyl ketone (**18**) in 3 ml of water. After adjustment of the *p*H to 3-4, the mixture was allowed to stand at room temperature for 24 hours, then acidified to *p*H 0-1 and heated at ~50° for 12 hours. The crude product was obtained using wcx chromatography as 0.07 g (25%) of a pale yellow solid. The solid was purified using rp silica chromatography to give 0.06 g (21%, SCX hplc purity 99.5%) of 2-amino-7-methyl-8-propylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**28**) as a white solid; ^{1}H nmr (*p*D 2 deuterium oxide/deuterium chloride): δ 8.57 (d, 1H, J = 8.2 Hz, 5-*H*), δ 7.31 (d, 1H, J = 8.2 Hz, 6-*H*), δ 4.54 (dd, 2H, J = 5.9, 7.9 Hz, N-C*H*$_2$), δ 2.81 (s, 3H, 7-C*H*$_3$), δ 1.80 (m, 2H, N-CH$_2$C*H*$_2$), δ 1.00 (dd, 3H, J = 7.4, 10.6 Hz, N-CH$_2$CH$_2$C*H*$_3$).

Anal. Calcd. for C$_{11}$H$_{15}$N$_4$OCl•0.5H$_2$O (263.73): C, 50.09; H, 6.12; N, 21.25; Cl, 13.44. Found: C, 50.04; H, 6.22; N, 21.35; Cl, 13.78.

Preparation of 8-Unsubstituted N5-Deazapterins.

2-Aminopyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (N5-Deazapterin Hydrochloride) (**29**).

To a suspension of 0.2 g (1.56 mmoles) of 2,6-diaminopy-rimidin-4(3*H*)-one in 20 ml of 0.01 *M* hydrochloric acid was added 0.26 g (1.58 mmoles) of malonaldehyde bis(dimethylac-etal) (**9**) and the mixture was stirred at 70°. Over five minutes the solution turned orange then pink. Solid sodium bisulfite was added to discharge the color, the solution re-acidified and further heated resulting again in a pink solution. Four cycles of bisulfite addition, re-acidification and heating were completed over *ca.* 2 hours with reaction progress being followed by uv/visible spectral measurements in 0.01 *M* hydrochloric acid. After adjustment of the *p*H to 1-2, the solution was cooled to yield 0.187 g (73%, SCX hplc purity 98.3%) of 2-aminopyri-do[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**29**) as a very light pink-cream solid; uv (*p*H 1 hydrochloric acid): 343 (log ε 3.79) (lit [24] 342 log ε 3.83), 312 (log ε 3.48) (lit [24] 308 log ε 3.47), 274 (log ε 4.00) (lit [24] 273 log ε 4.04), 242 (log ε 3.80) (lit [24] 238 log ε 3.85), 212 (log ε 4.31) (lit [24] 212 log ε 4.31); ^{1}H nmr (*p*D 1 deuterium oxide/deuterium chloride): δ 8.46 (dd, 1H, J = 1.9, 7.8 Hz, 5-*H* or 7-*H*), δ 8.26 (dd, 1H, J = 1.8, 5.8 Hz, 5-*H* or 7-*H*), δ 7.17 (dd, 1H, J = 7.8, 5.8 Hz, 6-*H*); (D$_6$-DMSO): δ 11.46 (bs, 1H, pyrimido-N*H*), δ 8.60 (dd, 1H, J = 1.7, 7.3 Hz, 5-*H* or 7-*H*) (lit [24] δ 8.62, dd), δ 8.21 (dd, 1H, J = 1.7, 7.8 Hz, 5-*H* or 7-*H*), (lit [24] δ 8.21, dd), δ 7.12 (dd, 1H, J = 7.3, 7.8 Hz, 6-*H*) (lit [24] δ 7.12, dd), δ 6.97 (bs, 2H, 2-N*H*$_2$) (lit [24] δ 6.75, bs).

2-Amino-6-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (6-Methyl-N5-deazapterin Hydrochloride) (**30**).

To a suspension of 0.25 g (1.98 mmoles) of 2,6-diaminopy-rimidin-4(3*H*)-one (**1**) and 0.375 g (3.64 mmoles) of sodium bisulfite in 20 ml of 0.01 *M* hydrochloric acid was added 0.40 ml (4.8 mmoles) of methacrolein (**16**) and the mixture was stirred at room temperature for 1 hour. A white solid was filtered off and re-suspended in 20 ml of 0.01 *M* hydrochloric acid to which was added 11 *M* hydrochloric acid until the solid dis-

solved. The solution was heated at 70-80° for 4 hours, neutralized with 5 *M* sodium hydroxide and filtered to give 120 mg (35%) of cream solid. Purification was by repeated precipitation from strong base; ^{1}H nmr (*p*D 0 deuterium oxide/deuterium chloride): δ 8.36 (d, 1H, J = 2.2 Hz, 5-*H* or 7-*H*), δ 8.16 (d, 1H, J = 2.7 Hz, 5-*H* or 7-*H*), δ 2.14 (s, 3H, 6-C*H*$_3$).

Anal. Calcd. for C$_8$H$_9$N$_4$OCl•1.3H$_2$O•0.05HCl (237.88): C, 40.39; H, 4.94; N, 23.56; Cl, 15.65. Found: C, 40.75; H, 4.84; N, 23.20; Cl, 15.43.

2-Amino-7-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (7-Methyl-N5-deazapterin Hydrochloride) (**31**).

To a suspension of 0.25 g (1.98 mmoles) of 2,6-diaminopy-rimidin-4(3*H*)-one and 0.30 g (2.91 mmoles) of sodium bisulfite in 20 ml of 0.01 *M* hydrochloric acid was added 0.20 ml (2.42 mmoles) of methyl vinyl ketone (**18**). This solution was stirred at room temperature for 2 hours and a white precipitate formed; uv (0.01 *M* hydrochloric acid): 272 nm. After acidification with 11 *M* hydrochloric acid to dissolve the precipitate, the solution was heated at 80° for 4 hours. After adjustment of the *p*H to 6 with 5 *M* sodium hydroxide, 0.132 g (38%) of a tan solid was recovered. Purification was by rp silica chromatography to give 0.06 g (14%, SCX hplc purity 99.5%) of 2-amino-7-methylpyri-do[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride (**31**) as a cream solid; ^{1}H nmr (*p*D 0 deuterium oxide/deuterium chloride): δ 8.33 (d, 1H, J = 8.0 Hz, 5-*H*), δ 7.01 (d, 1H, J = 8.0 Hz, 6-*H*), δ 2.41 (s, 3H, 7-C*H*$_3$).

Anal. Calcd. for C$_8$H$_9$N$_4$OCl•0.1H$_2$O•0.02HCl (215.173): C, 44.65; H, 4.32; N, 26.04; Cl, 16.80. Found: C, 44.70; H, 4.26; N, 25.78; Cl, 16.95.

2-Amino-5-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one Hydrochloride (5-Methyl-N5-deazapterin Hydrochloride) (**32**).

To a suspension of 0.25 g (1.98 mmoles) of 2,6-diaminopyrim-idin-4(3*H*)-one (**1**) and 0.25 g (2.43 mmoles) of sodium bisulfite in 20 ml of 0.01 *M* hydrochloric acid was added 0.25 ml (3.06 mmoles) of crotonaldehyde (**17**) and the mixture was stirred at room temperature for 1 hour; uv (0.01 *M* hydrochloric acid) 275 nm. The mixture was acidified to *p*H 1 and heated at 80° for 1 hour. Solid sodium bisulfite was added until the *p*H was 3 and the mixture was heated with stirring for a few minutes, then acidified to *p*H 1 and heated at 80° for another hour. This procedure was repeated a further three times and the mixture finally heated for three hours. After cooling, the mixture was neutralized and allowed to stand overnight at 4°. After filtering, 0.275 g of yellow solid (SCX hplc purity 50%, yield 33%) was recovered and redissolved in 0.01 *M* hydrochloric acid. After adjusting the *p*H to 3, the solution was purified using wcx chromatography to give 25 ml of pale yellow solution (SCX hplc purity 82%). A portion was purified using rp silica chromatography to give 20 ml of a colorless blue-fluorescent eluant which was freeze dried to give 0.007 g (SCX hplc purity >99.5%) of white solid; ^{1}H nmr (*p*D 0 deuterium oxide/deuterium chloride): δ 8.04 (d, 1H, J = 6.3 Hz, 7-*H*), δ 6.98 (d, 1H, J = 6.3 Hz, 6-*H*), δ 2.54 (s, 3H, 5-C*H*$_3$). hrms Calcd. for C$_8$H$_8$N$_4$O: 176.0697. Found: 176.0682.

Anal. Calcd. for C$_8$H$_9$N$_4$OCl•0.5H$_2$O•0.2HCl (228.94): C, 41.97; H, 4.49; N, 24.47. Found: C, 42.17; H, 4.13; N, 24.22.

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