Selectively ^{13}C-Enriched 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (Trimethoprim) and 2,4-Diaminopyrimidine

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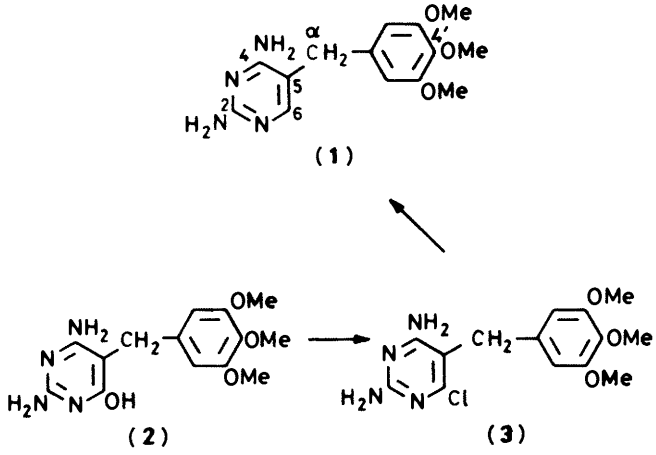
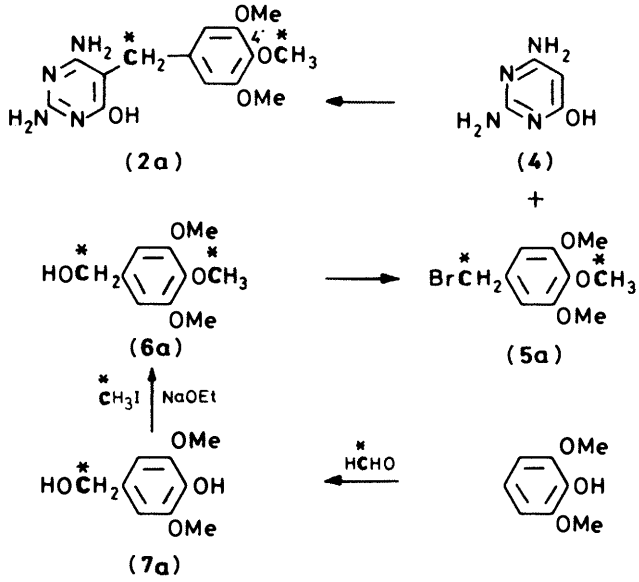
Samples of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (Trimethoprim) were synthesized, enriched with ^{13}C at the following positions: 2 and 5; 4 and 6; α and 4-methoxy. For the first two series (having ^{13}C-enrichment at the pyrimidine), appropriately labelled cyanoacetic acid was converted into labelled methyl 2-(3,4,5-trimethoxybenzyl)cyanoacetate and thence to the labelled 6-hydroxy derivative of Trimethoprim. For the last series (having ^{13}C-enrichment at the trimethoxybenzyl moiety), the key reaction was the nucleophilic attack of C–5 of 2,4-diamino-6-hydroxypyrimidine on labelled 3,4,5-trimethoxybenzyl bromide, again yielding the labelled 6-hydroxy derivative of Trimethoprim. The conversion of the 6-hydroxy derivatives into ^{13}C-enriched Trimethoprim was *via* the 6-chloride. Also synthesized was [6-^{13}C]-2,4-diaminopyrimidine by cyclization of guanidine and [3-^{13}C]-3-anilinoacrylonitrile. The identity, and the site(s) of the ^{13}C label(s) of each final product and intermediate, were established on the basis of ^1H and ^{13}C n.m.r. spectral evidence.

A number of antitumour, antibacterial, and antiparasitic agents exert their therapeutic effects by strongly inhibiting the enzyme dihydrofolate reductase, thereby leading to depletion of thymidylate, on which DNA synthesis vitally depends.[1] As part of a programme to study by n.m.r. the binding of such anti-folate drugs to dihydrofolate reductase,[2] and in particular the origin of the high selectivity of some of these agents for dihydrofolate reductases from different sources,[3] a property responsible to a considerable extent for the therapeutic effect, we have synthesized the antibacterial agent Trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] (**1**)[4] selectively enriched with ^{13}C (*ca.* 90% isotope purity) at the following sets of positions: 2 and 5; 4 and 6; and α and 4-methoxy. In connection with the n.m.r. work, we have also synthesized [6-^{13}C]-2,4-diaminopyrimidine.



Many methods have been devised for the synthesis of Trimethoprim and related 2,4-diamino-5-benzylpyrimidines.[5] With consideration for the availability of appropriate ^{13}C-enriched starting materials, we have chosen two methods for the incorporation of ^{13}C into various selected positions of Trimethoprim, both leading to the 6-hydroxy derivative of the relevant ^{13}C-enriched Trimethoprim, *viz.* 2,4-diamino-6-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (**2**). Conversion into labelled Trimethoprim was *via* the 6-chloro product (**3**) formed from the reaction with phosphorus pentachloride and phosphorus oxychloride, followed by hydrogenolysis using palladium–charcoal.[6–8]

In the first method, the nucleophilicity of 2,4-diamino-6-hydroxypyrimidine at C–5 was exploited. Thus, treatment of 2,4-diamino-6-hydroxypyrimidine (**4**) (2.6 mol equiv.) with



[α,4-*methoxy*-$^{13}C_2$]-3,4,5-trimethoxybenzyl bromide (**5a**)† in dimethyl sulphoxide yielded [α,4-*methoxy*-$^{13}C_2$]-2,4-diamino-6-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (**2a**). The corresponding reaction starting with unlabelled 3,4,5-trimethoxybenzyl chloride was described by Menzl.[6] We prepared the labelled 3,4,5-trimethoxybenzyl bromide (**5a**) by the action of hydrobromic acid on the corresponding benzyl alcohol (**6a**), *viz.*

† In this paper, the letters **a**, **b**, and **c**, used in conjunction with a number in the designation of structures [*e.g.* (**5a**)] refer to ^{13}C-enriched species.

[α,4-*methoxy*-$^{13}C_2$]-3,4,5-trimethoxybenzyl alcohol; this was in turn prepared by the reaction of 2,6-dimethoxyphenol[9,10] with [^{13}C]formalin solution, followed by alkylation[11] of the intermediate [α-^{13}C]syringyl alcohol (**7a**) using [^{13}C]methyl iodide and sodium ethoxide.

The above procedure was not suitable for the synthesis of Trimethoprim with ^{13}C-enrichment at the pyrimidine ring since, in the reaction between 2,4-diamino-6-hydroxypyrimidine (**4**) and 3,4,5-trimethoxybenzyl halide, the former had to be used in considerable excess to minimize the formation of the 5-bis-(3,4,5-trimethoxybenzyl) product.[6]

The alternative route to 2,4-diamino-6-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (**2**), for labelling at the pyrimidine ring, is based on the Traube synthesis[12] as carried out by Plantex Ltd.[7] Thus condensation of guanidine with the methyl ester of 2-(3,4,5-trimethoxybenzyl) [1,3-$^{13}C_2$]cyanoacetic acid (**8b**) produced product (**2b**) labelled at carbons 4 and 6, while starting with [^{13}C]guanidine and the acid (**8c**) labelled at position 2, one obtained product (**2c**) enriched at carbons 2 and 5. In each case, the literature procedure was modified with regard to the ratio of reactants in order to conserve expensive ^{13}C-enriched reagents. The labelled substituted cyanoacetic acids (**8b**/**8c**) were synthesized as follows. [2-^{13}C]- and [1,3-$^{13}C_2$]-Cyanoacetic acid were prepared as described by Cheung and Gray.[13] These were separately condensed with 3,4,5-trimethoxybenzaldehyde, and the resultant ^{13}C-enriched 2-cyano-3,4,5-trimethoxycinnamic acid intermediate was hydrogenated to yield the required labelled product (**8b**/**8c**).

In our synthesis of 2,4-diamino[6-^{13}C]pyrimidine (**9**), we adopted a variant of Stenbuck's method[14] for the synthesis of 2,4-diaminopyrimidines having 5-(substituted benzyl) groups, *viz.* (**10**) wherein guanidine is condensed with 3-anilino-2-benzylacrylonitriles (**11**).[15] For our work, 3-anilino[3-^{13}C]acrylonitrile (**12**) required for cyclization to 2,4-diamino[6-^{13}C]pyrimidine (**9**) was prepared from ethyl [^{13}C]formate by condensation with acetonitrile, followed by reaction of the intermediate alkoxide (**13**) of 3-hydroxy-[3-^{13}C]acrylonitrile with aniline (*cf.* ref. 16).

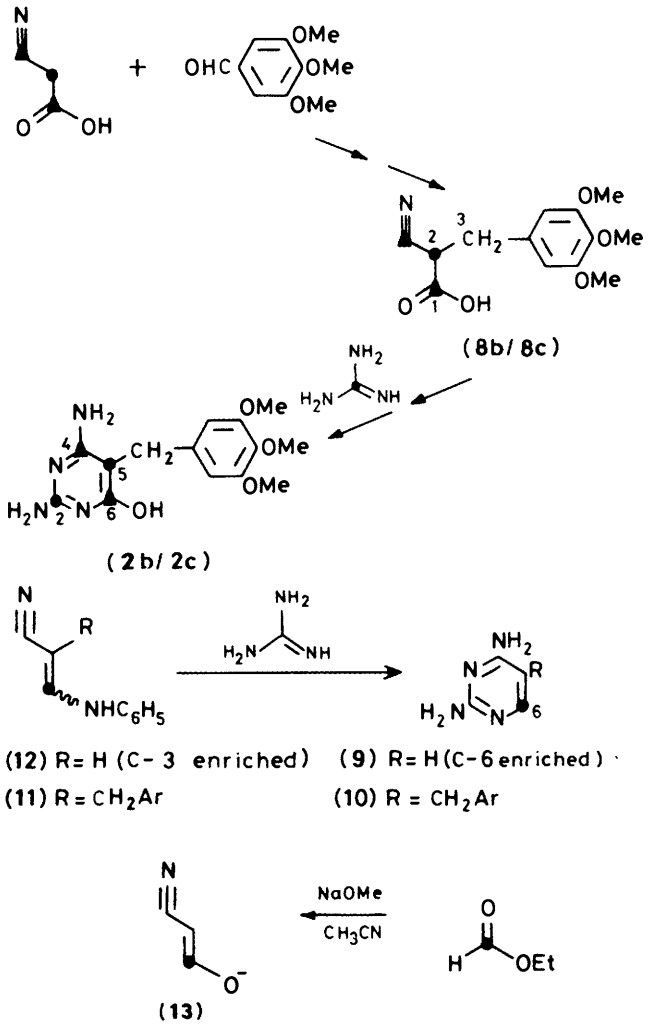


Table 1. ^{1}H and ^{13}C N.m.r. data[a] of ^{13}C-enriched 2,4-diaminopyrimidines

Compd.	5-H	6-H	7-H	2′-H	3′-OC*H*$_3$	4′-OC*H*$_3$	C-2	C-4	C-5	C-6	C-7	4′-OCH$_3$
(**9**)[b]	5.90 dd (3.0, 6.0)	7.68 dd (6.3, 176)								155.0[h]		
(**2a**)[d]			3.62 d (129)	6.60	3.82	3.74 d (145)					28.9	60.4
(**2b**)[d]			3.62 t (*ca.* 5)[g]	6.60	3.82	3.74		163.2		160.5		
(**2c**)[e]			3.65 d (*ca.* 6)[g]	6.58	3.85	3.80	153.8		105.7			
(**1a**)[c]		7.79 d (3.5)	3.66 d (129)	6.39 d (4.4)	3.81	3.82 d (144)					34.9	60.8
(**1a**)[f]		7.36 br s	3.70 d (129)	6.61 d (4)	3.80	3.73 d (146)					33.2	61.6
(**1b**)[c]		7.79 dd (6.3, 173)	3.66 t (4.9)	6.39	3.81	3.81		162.7		156.8		
(**1b**)[f]		7.37 dd (8, 183)	3.70 br s	6.61	3.79	3.73		165.0		141.9		
(**1c**)[c]		7.79 dd (6.6, 12.2)	3.66 d (6.3)	6.39	3.81	3.81	162.2 d (12.5)		106.5 d (12.5)			
(**1c**)[f]							156.0 d (7.8)		109.5 d (7.8)			

[a] Chemical shifts δ in p.p.m. downfield from $SiMe_4$, and in brackets coupling constants *J* in Hz; for ^{13}C spectra, δ_c refer to ^{13}C-enriched carbons and *J* refers to carbon–carbon couplings. [b] In 1:1 (v/v) $CDCl_3$–CD_3OD with $\delta_c(CD_3OD)$ 49.5 p.p.m. [c] In $CDCl_3$ with $\delta_c(CDCl_3)$ 77.0 p.p.m. [d] Protonated species (see Experimental section) measured in 4:1 (v/v) $CDCl_3$–$(CD_3)_2SO$ with $\delta_c(CDCl_3)$ 78.1 p.p.m. [e] As for footnote *d*, but 10:1 v/v and 77.8 p.p.m., respectively. [f] At pH 6.5 in D_2O containing 0.05M-K_2HPO_4 and 0.5M-KCl, with δ_H and δ_C of 1,4-dioxane internal standard 3.71 and 67.4 p.p.m. respectively; species measured is mostly protonated, the pK_a of Trimethoprim being 7.7 (G. C. K. Roberts, J. Feeney, A. S. V. Burgen, and S. Deluge, *FEBS Lett.*, 1981, **131**, 85). [g] Overlaps with other signal. [h] J_{CH} 178 Hz from gated spectrum.

Table 2. ^1H and ^{13}C N.m.r. data[a] of ^{13}C-enriched intermediates, R-C_6H_2-2,4-OMe-3-OR[1]

Compd.	R	R[1]	RCH_2	4'-OCH_3	3'-OCH_3	2'-H	CH(CN)CO_2H	CH_2	4'-OCH_3	CH	CN	CO_2H
(**7a**)[b]	$\overset{*}{C}H_2OH$	H	4.64 d (J_{CH} 143)	3.92	3.92	6.65 d (J_{HCCC} 4.7)		65.8				
(**6a**)[b]	$\overset{*}{C}H_2OH$	$O\overset{*}{C}H_3$	*ca.* 4.65 d[d] (J_{HC} *ca.* 149)	3.87[d] (J_{CH} 145)	3.90	6.66 d (J_{HCCC} 4.7)		65.5	60.7			
(**8b**)[c]	$CH_2CH(\overset{*}{C}N)\overset{*}{C}O_2H$	OCH_3	3.20 m ($W_{h/2}$ 18)	3.85	3.89	6.60	*ca.* 3.75 m[d]				117.5	168.0 (J_{CCC} 4.8)
(**8c**)[c]	$CH_2\overset{*}{C}H(CN)CO_2H$	OCH_3	3.20 m[d]	3.85	3.90	6.60	3.77 dt (J_{HC} 134, J_{HCCH} 7.0)			40.1		

[a] Thickened $\overset{*}{C}$ designates ^{13}C-enriched carbon. [b] In $CDCl_3$ with $\delta_C(CDCl_3)$ 77.0 p.p.m. [c] In 10:1 v/v $CDCl_3$–$(CD_3)_2SO$ with $\delta_C(CDCl_3)$ 77.8 p.p.m. Natural abundance C-2' and OMe observed at 107.0 and 56.7 p.p.m., respectively. [d] Partly masked.

Table 3. C–H Coupling constants (in Hz) of 2,4-diaminopyrimidines (**1**), (**2**), and (**9**) in organic solvents

	C-2	C-4	C-5	C-6	C-7	4'-OMe
5-H			184[c]	3[c]		
6-H	*e*	6.5[a,d]	6.5[a]	*e*	3.5[a]	
7-H		5[a,b]	6.5[a,b]	5[a,b,d]	129[a,b]	
2'-H					4.5[a,b]	
4'-OMe						145[a,b]

[a] Derived from ^{13}C-enriched Trimethoprim (**1a**—**c**) in $CDCl_3$. [b] Derived from ^{13}C-enriched *protonated* 6-hydroxy derivative of Trimethoprim (**2a**—**c**) in $CDCl_3$–$(CD_3)_2SO$. [c] Derived from [6-^{13}C]-2,4-diaminopyrimidine (**9**) in $CDCl_3$–CD_3OD. [d] Little changed on protonation (addition of 10% CF_3CO_2H to 10:1 $CDCl_3$–CD_3OD solution). [e] Affected by protonation, see Table 4.

carbon–proton coupling constants for the three 2,4-diaminopyrimidine systems (**1**), (**2**), and (**9**) given in Table 1 turn out to be insensitive to substitution at positions 5 and 6, and these coupling data are summarized in Table 3. However, for labelled 2,4-diaminopyrimidines wherein C–6 is unsubstituted, $^1J_{C-6,6-H}$ increases while $^3J_{C-2,6-H}$ and $^3J_{C-2,C-5}$ decrease upon protonation at N–1 (*cf.* ref. 17), and the relevant data are shown in Table 4.

Experimental

^{13}C-Enriched materials (90% enrichment) were from Merck, Sharp, and Dohme (Canada) except for guanidine nitrate which was from Stohler Isotopes (U.S.A.). ^1H and ^{13}C Fourier-

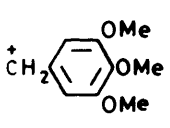
Table 4. C–C and C–H Coupling constants (in Hz)[a] of ^{13}C-enriched 2,4-diaminopyrimidines. Effect of N-1 protonation

	$^2J(C_6H_6)$		$^3J(C_2NCH_6)$	$^3J(C_2NCC_5)$		
Conditions	(**1b**)	(**9**)	(**1c**)	(**1c**)	(**14**)[b]	(**15**)[b]
(a) Free base						
$CDCl_3$	173		12.2	12.5		
$CDCl_3$–CD_3OD (10:1)	174		12.0	11.7		
(1:1)		176				
(b) N-1 Protonated						
$CDCl_3$–CD_3OD–CF_3CO_2H (10:1:1)	183		7.7	7.7		
0.05M K_2HPO_4, 0.5M KCl in D_2O, pH 6.5[c]	183			7.8		
D_2O (bisulphite salt)					7.0	
D_2O (pH 0—1)						7.0

[a] $J_{C,H}$ are from ^1H and/or gated ^{13}C spectra. [b] Our data given in ref. 13. [c] See footnote *f* to Table 1.

The ^1H and ^{13}C n.m.r. chemical shifts and ^1H-^1H,^{13}C-^{13}C and ^1H-^{13}C coupling constant data of ^{13}C-enriched 2,4-diaminopyrimidines and intermediates are given in Tables 1—4.

These data, together with the methane chemical ionization data (with ions *a* and *b* indicating the sites of ^{13}C-enrichment) given in the Experimental section, serve to show the identity of the labelled species synthesized. The 1-, 2-, and 3-bond



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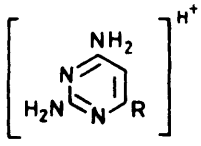
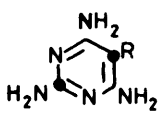


b, R = H or Cl



(**14**) R = NH_2

(**15**) R = $\overset{+}{N}H_3$

transform n.m.r. data for ^{13}C-enriched compounds described below are collated in Tables 1—4. They were obtained using a JEOL FX-90Q instrument operating at 89.6 and 22.5 MHz, or (for D_2O solutions) a Bruker WH-270 spectrometer operating at 270 and 67.9 MHz. Chemical ionization (c.i.) mass spectral data were obtained using a Finnigan 3200E quadrupole mass spectrometer, with CH_4 as ionizing gas. While the $M + C_2H_5$ and $M + C_3H_5$ ions were observed in most cases, they are not listed below. Unless otherwise stated, washing, drying, and evaporation of organic solutions were done with saturated aqueous sodium chloride, anhydrous magnesium sulphate, and under reduced pressure, respectively. M.p.s were uncorrected.

The synthetic procedures given were adopted with consideration of the necessity of conserving costly ^{13}C-enriched materials, and after repeated trial experiments on unlabelled reagents. Where more than one labelled species of the same

compound are described in succession, the lit. m.p. of the unlabelled analogue is referred to only once.

(4-*Hydroxy*-3,5-*dimethoxyphenyl*)[^{13}C]*methanol* (**7a**).—2,6-Dimethoxyphenol (463 mg, 3.0 mmol) was dissolved in water (2 ml) and 1M sodium hydroxide (3 ml), and [^{13}C]formalin solution (17.5% w/v) (0.5 ml, 2.8 mmol) was added. The stoppered reaction flask was kept in the dark for 4 days. 10M-Hydrochloric acid was added until the solution changed from green to red. The solution, made saturated with sodium chloride, was extracted with ethyl acetate (4 × 6 ml), and the combined organic extracts were washed, dried, and evaporated. The resultant oil when set aside formed needles of [1-^{13}C]-syringyl alcohol (**7a**) (506 mg, 91%); m.p. 128—130 °C (lit.,[9,10] 135—136 °C), *m/z* (CH_4 c.i.) 186 (95%, MH^+) and 168 (100, $MH - H_2O$); ^{1}H and ^{13}C n.m.r., see Table 2 (for comparison, see ref. 10 for ^{1}H n.m.r. data of unlabelled syringyl alcohol).

[4-methoxy-^{13}C]-3,4,5-*Trimethoxyphenyl*[^{13}C]*methanol* (**6a**).—[α-^{13}C]Syringyl alcohol (**7a**) (371 mg, 2.0 mmol) was, without prior purification, treated with sodium ethoxide [prepared from sodium (50 mg, 2.2 mmol) and ethanol (7 ml)]. [^{13}C]Methyl iodide (0.15 ml, 2.4 mmol) was added, and the mixture was stirred for 3 h at 35 °C. The residue obtained on evaporation was dissolved in water (5 ml) and 4M-sodium hydroxide (0.1 ml), and the product was extracted into chloroform (3 × 8 ml). Upon evaporation of the washed and dried chloroform solution, the labelled trimethoxybenzyl alcohol (**6a**) was obtained as a yellow oil (236 mg, 59%), *m/z* (CH_4 c.i.) 201 (85%, MH^+) and 183 (100, *a*). After acidification of the aqueous phase and similar extraction with chloroform, [α-^{13}C]syringyl alcohol (**7a**) was recovered as a red oil (102 mg, 28% recovery).

[4-methoxy-^{13}C]-3,4,5-*Trimethoxyphenylbromo*[^{13}C]-*methane* (**5a**).—The labelled trimethoxybenzyl alcohol (**6a**) (226 mg, 1.13 mmol) in benzene (3 ml) was stirred with hydrobromic acid (48%, w/v) (0.3 ml) for 17 h. The layers were separated, and the aqueous layer was further extracted with benzene (3 × 2 ml). The combined organic extracts were washed, dried, and evaporated to yield the labelled trimethoxybenzyl bromide (**5a**) as a yellow oil (253 mg, 85%), *m/z* (CH_4 c.i.) 265/263 (20%, MH^+) and 183 (100, *a*).

2-[^{13}C]*cyano*-3-(3,4,5-*trimethoxyphenyl*)[1-^{13}C]-*propanoic Acid* (**8b**).—To [1-^{13}C]bromoacetic acid (418 mg, 3.0 mmol) in water (3 ml) brought to neutral pH with sodium hydrogen carbonate (257 mg, 3.1 mmol) was added sodium [^{13}C]cyanide (150 mg, 3.0 mmol) in water (1 ml), and the mixture was heated at 100 °C for 7 min. The solution was cooled to 50 °C, and 3,4,5-trimethoxybenzaldehyde (604 mg, 3.1 mmol), water (5 ml), and 1M-NaOH (0.4 ml) were added. The mixture was stirred at 50 °C for 1 l until the aldehyde dissolved, and the pH was adjusted to *ca.* 6 with 2M hydrochloric acid. Water (3 ml) and 5% palladium-on-charcoal (95 mg) were added, and the mixture was hydrogenated at room temperature and atmospheric pressure until the uptake of hydrogen ceased. The catalyst was filtered off and washed with a little water. The combined filtrate was acidified with 10M-hydrochloric acid. The mixture was kept at 5 °C overnight, and the white precipitate formed was collected and washed with ice–water to yield the 1,3-labelled benzylcyanoacetic acid (**8b**) (483 mg, 61%), m.p. 75—80 °C, [lit.,[7] unlabelled analogue, dried at 50 °C for 10 h., *m/z* (CH_4 c.i.) 268 (100%, MH^+), 181 (20, *a*), and 88 (15, $NCCH_2COOH_2^+$)].

2-*Cyano*-3-(3,4,5-*trimethoxyphenyl*)[2-$^{13}C_1$]*propanoic Acid* (**8c**).—This was synthesized as described above from [2-^{13}C]bromoacetic acid (417 mg, 3.0 mmol), sodium cyanide (164 mg, 3.3 mmol), and 3,4,5-trimethoxybenzaldehyde (612 mg, 3.1 mmol). The [2-^{13}C]-labelled benzylcyanoacetic acid (**8c**) obtained (524 mg, 66%) had m.p. 70—73 °C, *m/z* (CH_4 c.i.) 267 (100%, MH^+), 181 (10, *a*), and 87 (20, $NCCH_2\dot{C}OOH_2$).

2,4-*Diamino*-6-*hydroxy*-5-(3,4,5-*trimethoxybenzyl*)[4,6-$^{13}C_2$]*pyrimidine* (**2b**).—2-([^{13}C]Cyano)-3-(3,4,5-trimethoxyphenyl)[1-^{13}C]propanoic acid (**8b**) (483 mg, 1.8 mmol) was suspended in diethyl ether at 0 °C and an excess of diazomethane in diethyl ether was added. After the acid had dissolved, the solution was evaporated to yield the methyl ester. A solution of guanidine prepared by adding guanidine nitrate (1.0 g, 8.2 mmol) to a solution of sodium (240 mg, 10.4 mmol) in methanol (10 ml) was added to the methyl ester, and the mixture was refluxed for 16 h. The solvent was evaporated and the yellow residue dissolved in boiling water (4 ml). The solution was cooled and adjusted to pH 5 to yield a white precipitate. The mixture was kept at 0 °C for 4 h, after which the solid was filtered off, and washed with ice–water to give 4,6-labelled 6-hydroxy derivative of Trimethoprim (**2b**) (492 mg, 89%), m.p. 275 °C (lit.,[7] m.p. of unlabelled analogue, 275—276 °C), *m/z* (CH_4 c.i.) 309 (35%, MH^+), 181 (20, *a*), and 141 (100, *b*).

2,4-*Diamino*-6-*hydroxy*-5-(3,4,5-*trimethoxybenzyl*)[2,5-$^{13}C_2$]*pyrimidine* (**2c**).—This was prepared as described above from the methyl ester of 2-cyano-3-(3,4,5-trimethoxyphenyl)[2-^{13}C]propanoic acid (**8c**) (514 mg, 1.95 mmol), sodium (85 mg, 3.7 mmol), methanol (7 ml) and [^{13}C]guanidine nitrate (224 mg, 1.82 mmol). The 2,5-Labelled 6-hydroxy derivative of trimethoprim (**2c**) obtained (295 mg, 52%) had m.p. 272—274 °C, *m/z* (CH_4c.i.) 309 (40%, MH^+), 181 (20, *a*), and 141 (100, *b*). Acidification of the filtrate to pH 1 yielded the starting acid (**8c**) (182 mg, 37% recovery), m.p. 68—70 °C.

[α,4-methoxy-$^{13}C_2$]-2,4-*Diamino*-6-*hydroxy*-5-(3,4,5-*trimethoxybenzyl*)*pyrimidine* (**2a**).—The labelled trimethoxybenzyl bromide (**5a**) (245 mg, 0.93 mmol) was stirred with 2,4-diamino-6-hydroxypyrimidine (**4**) (300 mg, 2.38 mmol) in dimethyl sulphoxide (2 ml) for 3 days. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (Merck 60H). On elution with methanol–chloroform (1:9) the labelled derivative (**2a**) was obtained as colourless crystals (142 mg, 43%), m.p. 260—270 °C, *m/z* (CH_4c.i.) 309 (25%, MH), 183 (15, *a*), and 140 (100, *b*).

2,4-*Diamino*-5-(3,4,5-*trimethoxybenzyl*)[2,5-$^{13}C_2$]*pyrimidine* (**1c**).—2,4-Diamino-6-hydroxy-5-(3,4,5-trimethoxybenzyl) [2,5-$^{13}C_2$]pyrimidine (**2c**) (153 mg, 0.50 mmol) was heated with phosphorus pentachloride (20 mg) (sublimed before use) and phosphorus oxychloride (1.5 ml) (freshly distilled from sodium) for 6 h at 103 °C. The solution was evaporated under reduced pressure, and water (2 ml) was added to the resultant orange syrup. The mixture was heated to 100 °C, and then cooled in ice. Saturated aqueous sodium hydroxide was added until the solution was basic to yield a yellow precipitate which was filtered off and washed with ice–water. The chloro compound (**3b**) so obtained [*m/z* (CH_4 c.i.) 327/329 (60/20%, MH^+), 291 (20, MH – HCl), 181 (30, *a*), and 159/161 (100/30, *b*)] was dissolved in 1:1 acetic acid–water (4 ml). 5% Palladium-on-charcoal (22 mg) was added and the mixture was hydrogenated under atmospheric conditions until the uptake of hydrogen ceased (*ca.* 1.5 days). The catalyst was filtered off and washed with acetic acid. The crystalline residue obtained on evaporation of the combined filtrates was dissolved in boiling 0.3M-hydrochloric acid (1.2 ml). The solution was cooled and basified to pH 10 with 4M-aqueous sodium hydroxide. After several hours at 5 °C the solid so formed was filtered off, washed with water, and so purified by preparative t.l.c. (silica GF254; with

elution by 8% methanol in chloroform) and crystallization from ethanol to yield colourless crystals of 2,5-labelled Trimethoprim (**1c**) (18 mg, 12%), m.p. 197—199 °C (lit.,[18] unlabelled Trimethoprim, 197—199 °C), *m/z* (CH_4 c.i.) 293 (70%, MH^+), 183 (10, *a*), and 125 (100, *b*).

2,4-*Diamino*-5-(3,4,5-*trimethoxybenzyl*)[4,6-$^{13}C_2$]-*pyrimidine* (**1b**).—2,4-Diamino-6-hydroxy-5[(3,4,5-trimethoxyphenyl)methyl][4,6-$^{13}C_2$]pyrimidine (**2b**) (153 mg) was converted as described above, *via* the 4,6-labelled chloro-compound (**3b**) [*m/z* (CH_4 c.i.) 327/329 (75/25%, MH^+), 291 (30, MH − HCl), 181 (50, *a*), and 159/161 (100/35, *b*)], into the 4,6-labelled Trimethoprim (**1b**) (40 mg, 27%), m.p. 190—195 °C; *m/z* (CH_4 c.i. 293 (55%, MH^+), 181 (10, *a*), and 125 (100, *b*).

[α, 4-methoxy-$^{13}C_2$]-2,4-*Diamino*-5-(3,4,5-*trimethoxybenzyl*) *pyrimidine* (**1a**).—[α,4-*methoxy*-$^{13}C_2$]-2,4-Diamino-6-hydroxy-5-[(3,4,5-trimethoxybenzyl)pyrimidine (**2a**) (125 mg) was likewise converted, *via* the corresponding chloro-compound (**3a**) [*m/z* (CH_4 c.i.) 327/329 (55/20%, MH^+), 291 (20, MH − HCl), 183 (25, *a*), and 158/160 (100/35, *b*)], into the corresponding labelled Trimethoprim (**1a**) (30 mg, 25%), m.p. 190—194 °C, *m/z* (CH_4 c.i.) 293 (50%, MH^+), 183 (10, *a*), and 124 (100, *b*).

3-*Anilino*[3-^{13}C]acrylonitrile (**12**).—To sodium [^{13}C]formate (0.50 g) in absolute ethanol (2.5 ml) was added concentrated sulphuric acid (0.85 ml). The solution was refluxed for 7 h and then distilled. The combined fractions boiling at 55—65 °C (0.42 g) consisted of (by ^{1}H n.m.r. analysis) *ca.* 3 parts of ethyl [^{13}C]formate [δ($CDCl_3$) 4.2 (dq, J_{HH} 7, J_{HCOC} 3.5 Hz, OCH_2), and 8.05 (dq, J_{HC} 226, CHO)] to one part of ethanol. Anhydrous diethyl ether (15 ml) was added followed by, after cooling to −10 °C, sodium (0.15 g). After being stirred for 20 min, dry acetonitrile (0.25 ml) was added, and stirring at −10 °C was continued for 1.5 h. The mixture was refluxed for 8 h, and the solvent removed under reduced pressure. To the resulting sodium salt of [3-^{13}C]-3-hydroxyacrylonitrile was added dry acetonitrile (10 ml) and dry aniline hydrochloride (0.55 g), and the mixture was refluxed with stirring for 4 h. The residue obtained on removal of solvent was distributed between dichloromethane (20 ml) and 7M-hydrochloric acid (30 ml). The dichloromethane solution was washed with pH 7.1 phosphate buffer, dried (sodium sulphate), and evaporated to give a crude mixture of *cis*- and *trans*-[3-^{13}C]-3-anilinoacrylonitrile (**12**) as a gum (50 mg); δ($CDCl_3$) 4.1 (d, J_{HH} 7.5 Hz, 2-H of *cis* isomer), 4.5 (dd, J_{HH} 13.5, J_{HCC} 5, 2-H of *trans*), 7.1 (dd, J_{HH} 7.5, J_{HC} 174, 3-H of *cis*), 7.35 (dd, J_{HH} 13.5, J_{HC} 173, 3-H of *trans*), and 6.7—7.5 (m, Ph); *m/z* (CH_4 c.i.) 146 (100%, MH^+). A mixture of the *cis* and *trans* isomers of the unlabelled analogue, prepared in the same way, showed δ 4.1 (d, *J* 7.5), 4.5 (d, *J* 12), 7.2 (d, *J* 7.5), 7.4 (d, *J* 12), and 6.6—7.5 (m); *m/z* (CH_4 c.i.) 145 (100%, MH^+).

2,4-*Diamino*[6-^{13}C]*pyrimidine* (**9**).—Crude labelled intermediate (**12**) above (50 mg) was stirred at reflux with sodium methoxide (100 mg) and guanidine hydrochloride (35 mg) for 7 h. The residue obtained after evaporation of the filtered solution was purified by preparative t.l.c. over silica to give [6-^{13}C]-2,4-diaminopyrimidine (**9**) (7 mg). For ^{1}H and ^{13}C n.m.r. data see Table 1.

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