The Biosynthesis of Spermidine. Part 2:† Preparation and Study by ^{1}H N.m.r. Spectroscopy of Hexahydropyrimidines from Spermidine and Propane-1,3-diamines

Bernard T. Golding *,‡ and Ishaq K. Nassereddin
Department of Chemistry and Molecular Sciences, The University of Warwick, Coventry CV4 7AL

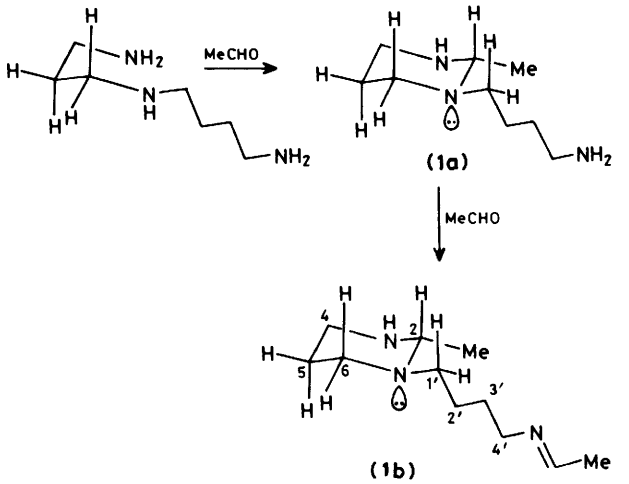
The reaction of spermidine with 1 mol equiv. of ethanal yields 1-(4'-aminobutyl)-2-methylhexahydropyrimidine, whilst spermidine and 2 mol equiv. of ethanal afford 1-[4'-(*N*-ethylidene)aminobutyl]-2-methylhexahydropyrimidine. These assignments are based on the results of ^{1}H n.m.r. spectroscopic experiments and mass spectral analyses, supported by data for a series of model compounds. The 400 MHz ^{1}H n.m.r. spectra of the hexahydropyrimidines from spermidine and of other hexahydropyrimidines (2-methyl-, 2-benzyl- and 1,2-dimethyl-) were analysed with the aid of computer-assisted spectral simulations. The model substances 2-methyl- and 2-benzyl-hexahydropyrimidine were converted into *N,N*-diacetyl derivatives. The ^{1}H n.m.r. spectra of these derivatives show that they possess one acetyl group with its oxygen *syn* to C-2 and the other acetyl with its oxygen *anti* to C-2.

Recent studies on the physiological significance of amine–carbonyl interactions have centred on (i) the design of polyfunctional agents for sequestering metabolically generated ethanal[1] and (ii) reactions between catecholamines and aldehydes.[2] The possible physiological significance of polyamine–aldehyde interactions has been discussed in terms of imines or polyimines as the supposed products of condensation.[3] Recently, the product of chemical and enzymic oxidation of spermine was shown to be 1,5-diazabicyclo[4.3.0]nonane.[4]

Our interest in the reaction of ethanal with polyamines started when we faced the problem of solving the stereochemistry of spermidine synthase.[5] We could not achieve direct analysis of the stereochemistry of deuterium-labelled spermidines either prepared synthetically or obtained biosynthetically from *Escherichia coli* cells. It was decided to approach this problem by converting these spermidines into hexahydropyrimidines. We found that spermidine reacts with ⩾2 mol equiv. of ethanal to give first a hexahydropyrimidine§ (**1a**) and then an iminohexahydropyrimidine (**1b**) (see Scheme). The assignment of the ^{1}H n.m.r. spectra of such derivatives was rather difficult by direct analysis. We found that the preparation and the ^{1}H n.m.r. spectral analysis of model hexahydropyrimidines was essential for understanding the reactions between ethanal and polyamines, and for analysing the hexahydropyrimidines from spermidine.

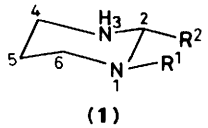
Initially, we attempted the preparation of hexahydropyrimidines by the classical method of Branch,[6] *i.e.* condensation of a monoprotonated 1,3-diamine with a carbonyl compound. However, the maximum yield we achieved by this procedure was 20—25% of the hexahydropyrimidine. We then found that carrying out the condensation of the 1,3-diamine with a carbonyl compound in a neutral organic solvent ($CDCl_3$ or CH_2Cl_2) or in water (D_2O) gave much higher yields (60—70% of the isolated product).

2-Methylhexahydropyrimidine.—The condensation of propane-1,3-diamine with 1.1 mol equiv. of ethanal in water occurred rapidly to give 2-methylhexahydropyrimidine (**1c**) (65% yield),



Scheme. Reaction between spermidine and ethanal leading to hexahydropyrimidine (**1a**) and the iminohexahydropyrimidine (**1b**).

which was purified by distillation. The ^{1}H n.m.r. spectrum of compound (**1c**) showed a resonance at δ ($CDCl_3$, TMS) 2.85, corresponding to the axial protons at C-4 and C-6, and a resonance at δ 3.15 corresponding to the equatorial protons at C-4 and C-6. The difference in the chemical shifts between the axial and equatorial protons in compound (**1c**) is due to the shielding effect of an axial lone-pair of a nitrogen atom on the adjacent axial proton,[7] although the conformations of (**1c**) having at least one equatorial lone pair are probably preferred over the conformation having two axial lone pairs[8,9] (see below on conformational analysis). The spectrum of (**1c**) also showed an AX_3 system for the proton at C-2 [δ 3.64 (1 H, q)], and the methyl group [δ 1.16 (3 H, d)] attached to that position.



(**1**)

a; R^1 = $(CH_2)_4NH_2$	R^2 = Me
b; R^1 = $(CH_2)_4N{=}CHMe$	R^2 = Me
c; R^1 = H	R^2 = Me
d; R^1 = Me	R^2 = Me
e; R^1 = H	R^2 = $PhCH_2$

† Part 1, preceding paper.

‡ *New address:* Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU.

§ The name hexahydropyrimidine of spermidine is an abbreviation for 1-(4'-aminobutyl)-2-methylhexahydropyrimidine, and the name iminohexahydropyrimidine is an abbreviation for 1-[4'-(*N*-ethylidene)aminobutyl]-2-methylhexahydropyrimidine.

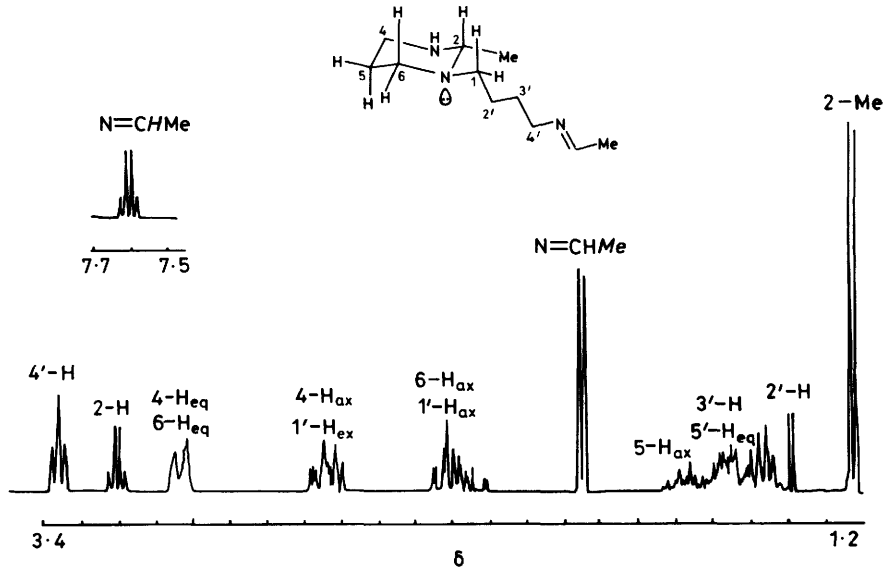


Figure 1. The 400 MHz ^{1}H n.m.r. spectrum ($CDCl_3$, TMS) of 1-[4'-(*N*-ethylidene)aminobutyl]-2-methylhexahydropyrimidine, (**1b**).

1,2-*Dimethylhexahydropyrimidine*.—The preparation of 1,2-dimethylhexahydropyrimidine (**1d**) in 60% yield was achieved by the condensation of *N*-methylpropane-1,3-diamine with 1.1 mol equiv. of ethanal in water. The ^{1}H n.m.r. spectrum of compound (**1d**) showed an AX_3 system at δ 1.21 (3 H, d) and 2.88 (1 H, q) for the proton and methyl group at C-2. The quartet of the C-2 proton was shielded by 0.76 p.p.m. compared to the quartet of the C-2 proton of compound (**1c**). This shielding is suggested to arise from the presence of the methyl group at N-1 in (**1d**), which prefers to occupy the equatorial position and forces the lone pair to take up the axial position. The signal for C-5 protons in the hexahydropyrimidine (**1c**) showed a broad resonance at δ 1.48. The C-5 protons of compound (**1d**) showed a double multiplet at δ 1.53 for 5-$H_{eq.}$ and a quartet of triplets at δ 1.74 for 5-$H_{ax.}$. The 1,3-diaxial interaction between the lone-pair on N-1 and 5-$H_{ax.}$ compared to a predominant 1,3-diaxial H–H interaction in (**1c**), is probably responsible for the separation of 5-$H_{ax.}$ from 5-$H_{eq.}$ in (**1d**). The single *N*-substituent in (**1d**) leads to the appearance of resonances for 4-$H_{ax.}$ and 6-$H_{ax.}$, as well as for 4-$H_{eq.}$ and 6-$H_{eq.}$, at different chemical shifts. The signal for 6-$H_{ax.}$ at δ 2.25 (td, J_{gem} 13 Hz, J_{vic} 3 and 13 Hz) was shielded by 0.6 p.p.m. [in comparison to the signal of 6-$H_{ax.}$ (δ 2.85) in compound (**1c**)] due to the presence of the axial lone-pair at N-1. To a lesser extent, the lone-pair also shielded the 6-$H_{eq.}$ at δ 2.95 (dm J_{gem} 13 Hz) by 0.2 p.p.m. [in comparison to the signal of 6-H_{eq} (δ 3.15) in compound (**1c**)]. The N-3 lone-pair probably prefers an equatorial orientation as in (**1c**),[9] and so there are only small chemical shift differences between 4-$H_{ax.}$/4-$H_{eq.}$ in (**1d**) and the corresponding protons in (**1c**). The assignments of the C-4 to C-6 protons were confirmed by a series of decoupling experiments in which each proton was irradiated in turn.

2-*Benzylhexahydropyrimidine*.—Preparation of 2-benzylhexahydropyrimidine (**1e**) was achieved in 73% yield by the condensation of 1.1 mol equiv. of phenylethanal with 1 mol equiv. of propane-1,3-diamine in water. The ^{1}H n.m.r. spectrum of compound (**1e**) showed resonances at δ 2.26 (d, $CHCH_2Ph$) overlapping with resonances of 4-$H_{ax.}$ and 6-$H_{ax.}$ at δ 2.27. The signals for 6-$H_{eq.}$ and 4-$H_{eq.}$ resonated at δ 3.15 (d, J_{gem} 13.6 Hz, with additional fine splitting from the vicinal couplings). Signals for the C-5 protons appeared as a broad multiplet at δ 1.46 and signals for 2-H appeared at δ 3.7.

Preparation of 1-(4'-*Aminobutyl*)-2-*methylhexahydropyrimidine* (**1a**).—The hexahydropyrimidine derivative (**1a**) of spermidine was prepared by dropwise addition of 1 mol equiv. of ethanal into a solution of spermidine in chloroform. This reaction was monitored by ^{1}H n.m.r. spectroscopy which showed after 5 min the disappearance of free spermidine and the complete formation of the cyclic derivative (**1a**). In this reaction, spermidine is reacting like the propane-1,3-diamines described above. We found that under the reaction conditions described for 1,3-diamines, ethane-1,2-diamine reacted with ethanal to give 2-methylimidazolidine (according to monitoring by ^{1}H n.m.r. spectroscopy), whereas butane-1,4-diamine and ethanal (1.1 mol equiv.) gave a mono-imine and a bis-imine (with ≥2 mol equiv. of ethanal). The ^{1}H n.m.r. spectrum of compound (**1a**) is very similar to that of 1-[4'-(*N*-ethylidene)aminobutyl]-2-methylhexahydropyrimidine (**1b**) (see below), the only differences being the absence of the resonance for the N=CHMe group, and the appearance of the 4'-H_2 signals partly overlapping the resonances of the 1'-$H_{eq.}$* and 4-$H_{ax.}$ at δ *ca.* 2.67. Compound (**1a**) was analysed by c.i. and e.i. mass spectrometry. The c.i. mass spectrum showed a major peak for the protonated molecular ion $(M + 1)^+$ at *m/z* 172. The e.i. mass spectrum showed a major peak at *m/z* 156 corresponding to the ion $(M - 15)^+$.

Preparation of 1-[4'-(N-*Ethylidene*)*aminobutyl*]-2-*methylhexahydropyrimidine* (**1b**).—The iminohexahydropyrimidine derivative (**1b**) of spermidine was prepared by dropwise addition of ethanal (≥2 mol equiv.) to a solution of spermidine in chloroform. For analysis by ^{1}H n.m.r. spectroscopy this reaction was carried out directly in the n.m.r. tube. The cyclisation and imine formation to give compound (**1b**) was complete within 5 min at room temperature (see Scheme). Compound (**1b**) was analysed by c.i. and e.i. mass spectrometry. The c.i. mass spectrum showed a major peak for $(M + 1)^+$ at *m/z* 198. The e.i. mass spectrum showed a peak at 182 corresponding to the $(M - 15)^+$ ion.

The assignment of the 400 MHz ^{1}H n.m.r. spectrum of

* Although the protons on C-1' to C-4' are not attached to a cyclic system, the designations ax. and eq. are used for convenience to denote different orientations of these protons on a chain of defined geometry (see the Scheme).

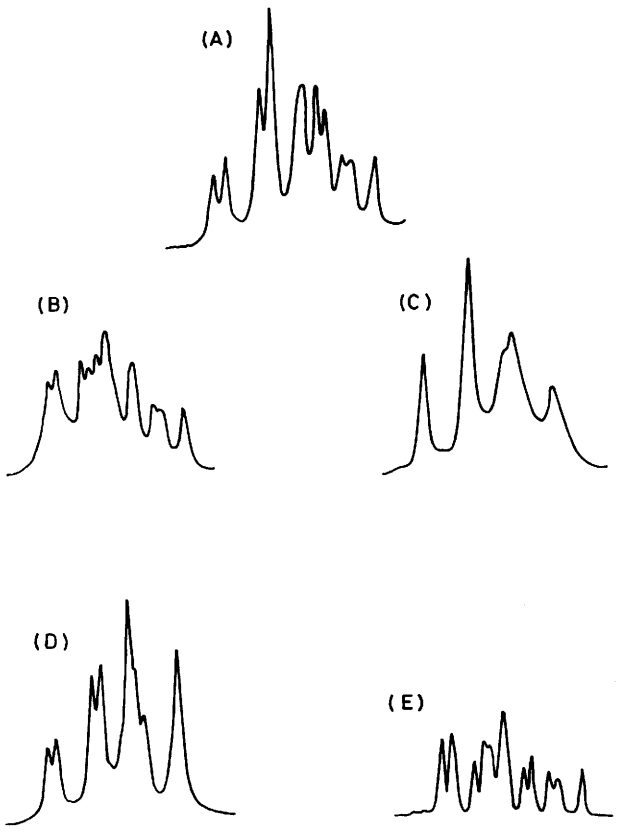


Figure 2. (A) The resonance for 6-$H_{ax.}$ and 1′-$H_{ax.}$ (*cf.* Figure 1). The resonance after decoupling (B) 5-$H_{ax.}$, (C) 5-$H_{eq.}$, (D) 2′-H, and (E) 6-$H_{eq.}$

compound (**1b**) (Figure 1), was achieved with the aid of data for the model compounds described above. The 4′-(*N*-ethylideneaminobutyl) chain of compound (**1b**) is expected to take up an equatorial position on the hexahydropyrimidine ring and so the lone-pair at N-1 is axial. It is expected on steric grounds that the butyl chain takes up a conformation which extends away from the ring. We made a first-order analysis of the 400 MHz ^{1}H n.m.r. spectrum of compound (**1b**) (Figure 1) as follows: δ 1.22 (3 H, d, *J* 6 Hz, 2-Me), 1.95 (3 H, d, *J* 4.5 Hz, N=CH*Me*), 3.20 (1 H, q, *J* 5.8 Hz, 2-H), 3.36 (2 H, t, *J* 6.9 Hz, 4′-H), and 7.60 (1 H, q, *J* 4.5 Hz, N=C*H*Me). In agreement with Booth and Lemieux,[9] we did not observe coupling between 2-H and the adjacent NH for measurements at *ca.* 20 °C. Similarly, 4-$H_{ax.}$ and 4-$H_{eq.}$ did not couple with this NH. The signals for 5-$H_{eq.}$, 2′-H and 3′-H were all in the region 1.35—1.65 p.p.m., which appears as a complicated multiplet. Although the position of each signal for 5-$H_{eq.}$, 2′-H and 3′-H can be roughly assigned, the exact shape and position of these signals is impossible to judge because of overlap. The 5-$H_{ax.}$ signal appeared at δ 1.67 (qt, J_{gem} 12 Hz, J_{vic} 4, 4, 12 and 12 Hz). This assignment was confirmed by decoupling experiments. Irradiation at δ 3.04 (6-$H_{eq.}$ and 4-$H_{eq.}$), resulted in the appearance of the 5-$H_{ax.}$ signal as a quartet (J_{gem} 12 Hz, J_{vic} 12 and 12 Hz) due to the loss of the small J_{vic} couplings with 4-$H_{eq.}$ and 6-$H_{eq.}$. Irradiation at δ 2.33 (6-$H_{ax.}$) changed the signal from 5-$H_{ax.}$ into a triple triplet (J_{gem} 12 Hz, J_{vic} 4, 4 and 12 Hz) owing to the loss of the big J_{vic} coupling with 6′-$H_{ax.}$. A similar result (*i.e.* to the effect from 6-$H_{ax.}$ decoupling) was obtained when the 4-$H_{ax.}$ proton was decoupled. Signals at δ 3.04 were assigned to 6-$H_{eq.}$ and 4-$H_{eq.}$ (d, J_{gem} 12 Hz, with additional fine splitting). The assignment was confirmed by decoupling 5-$H_{ax.}$ at δ 1.67. This caused the signals from 6-$H_{eq.}$ and 4-$H_{eq.}$ to lose the fine splitting [due to coupling with 5-$H_{ax.}$ (J_{vic} *ca.* 4 Hz)]. It remains to justify the assignments for 6-$H_{ax.}$, 1′-$H_{ax.}$, 4-$H_{ax.}$, and 1′-$H_{eq.}$.

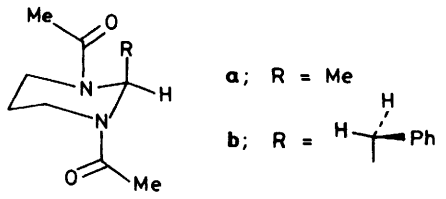
The signal from 6-$H_{ax.}$ appeared at δ 2.33 (td, J_{gem} 12 Hz, J_{vic} 3 and 12 Hz), partly overlapping with the signals of 1′-$H_{ax.}$ at δ 2.28 (ddd, J_{gem} 13 Hz, J_{vic} 6 and 8.5 Hz) [see Figure 2 (A)]. Confirmation of these assignments was derived from a series of decoupling experiments involving 5-$H_{ax.}$, 5-$H_{eq.}$, 6-$H_{eq.}$, and 2′-H and spectral simulation with the aid of the instrument's computer. Irradiation at 5-$H_{ax.}$ (δ 1.67) resulted in the formation of a double doublet in place of the original signal of 6-$H_{ax.}$ (J_{gem} 12 Hz and J_{vic} 3 Hz). The signal of 1′-$H_{ax.}$ was essentially unaffected [*cf.* Figure 2 (B)]. The decoupling of 5-$H_{eq.}$ (*ca.* δ 1.52) from 6-$H_{ax.}$ resulted in the collapse of the signal of 6-$H_{ax.}$ into a triplet with $J_{gem} = J_{vic} = 12$ Hz. The power of the irradiation also affected the signal of 1′-$H_{ax.}$ [N.B. because the signals of 5-$H_{eq.}$ and 2′-H are very close together, the power of the irradiation also decoupled 2′-H protons from 1′-$H_{ax.}$, which collapsed into a broad doublet (*cf.* Figure 2(C)]. This doublet was sharpened when the irradiation was centred at the protons of C-2′ (*ca.* δ 1.47). This irradiation did not affect the triple doublet of 6-$H_{ax.}$ which became easier to discern [*cf.* Figure 2(D)]. Irradiation at δ 3.04 (6-$H_{eq.}$) resulted in the collapse of the triple doublet of 6-$H_{ax.}$ into a double doublet. The double double doublet of 1′-$H_{ax.}$ was essentially unaffected [*cf.* Figure 2(E)].

The assignments for 6-$H_{ax.}$ and 1′-$H_{ax.}$ were further supported by simulation of their signals on a Bruker WH-400 computer ('Panic' Programme) and independently by a SIMEQ-II programme (Varian).

The signals for 4-$H_{ax.}$ at δ 2.66 appeared as a triple doublet (J_{gem} *ca.* 13 Hz, J_{vic} 3 and *ca.* 12 Hz), partially overlapping with the signal of 1′-$H_{eq.}$ at δ 2.63 ddd, observed couplings J_{gem} 13 Hz, J_{vic} *ca.* 7 and 8 Hz). The assignments were confirmed by a sequence of decoupling experiments at 4-$H_{eq.}$, 5-$H_{ax.}$, 5-$H_{eq.}$, and 2′-H, and were further supported by simulation of the overlapping 4-$H_{ax.}$, 1′-$H_{eq.}$ signals using a Bruker WH-400 computer ('Panic' Programme).

Conformational Analysis of Hexahydropyrimidines.—Hexahydropyrimidines prefer a chair conformation with the maximum number of substituents equatorial.[8] Nitrogen inversion is a lower energy process ($\Delta G^{\ddagger} \ll 45$ kJ mol^{-1}) than ring inversion ($\Delta G^{\ddagger}$ *ca.* 45 kJ mol^{-1}).[8] A lone pair on nitrogen has a greater preference for an equatorial position than NH.[8,9] Hence, the preferred conformations for hexahydropyrimidines (**1a**)—(**1e**) are as shown. At *ca.* 300 K these conformers will be the dominant contributors to the time-averaged n.m.r. spectrum. Some hexahydropyrimidines (*e.g.* 2,2-dimethylhexahydropyrimidine) exhibit ring-chain tautomerism (*i.e.* equilibration of the cyclic structure with an acyclic amino imine).[6b] However, none of the hexahydropyrimidines described herein showed evidence (*e.g.* $\nu_{max.}$ 1 670 cm^{-1} for C=N) for any significant contribution of an acyclic tautomer.

Bis-N-*Acetylhexahydropyrimidines.*—For further characterisation of hexahydropyrimidines we examined their *N*-acetyl derivatives. It was also hoped that these derivatives would show even better separation of ring proton resonances than the parent hexahydropyrimidines and be of use for stereochemical analyses of dideuteriated spermidines. Both 2-methyl-(**1c**) and 2-benzylhexahydropyrimidine (**1e**) gave crystalline bis(*N*-acetyl) derivatives (**2a**) and (**2b**), respectively on acetylation with acetic anhydride in aqueous sodium hydroxide. Attempted acetylation of 1,2-dimethylhexahydropyrimidine (**1d**) with this reagent gave di-*N*,*N*′-acetyl-*N*-methylpropane-1,3-diamine. We also attempted to acetylate the hexahydropyrimidine (**1a**) of spermidine but failed to isolate any product from this reaction.



(2)

The ^{1}H n.m.r. spectrum of diamide (**2a**) showed several interesting features. There were two separate *N*-acetyl resonances (at δ 2.08 and 2.21) and consequently the signals for 4-$H_{eq.}$ and 6-$H_{eq.}$ appeared at very different shifts (δ 3.74 and 4.59). The preferred conformation of this diamide in $CDCl_3$ at 293 K is therefore as shown. Hence, one of adjacent equatorial hydrogen atoms (4-H or 6-H) lies in the deshielding region of an adjacent carbonyl group. Furthermore, the *N*-acetyl groups will cause the C-2 methyl to prefer an axial configuration.[10] The benzyldiamide (**2b**) showed similar effects in its ^{1}H n.m.r. spectrum, *i.e.* two *N*-acetyl resonances and separate resonances for 4-$H_{eq.}$ and 6-$H_{eq.}$. The vicinal coupling constants (3.6 and 14 Hz) between 2-H and the benzylic protons are consistent with this diamide having a dihedral angle of *ca.* 60° between the C-2/2-H bond and the C–Ph bond.

Experimental

For general directions see ref. 5*b*.

Hexahydropyrimidines: General Procedure for 2-Methyl-, 2-Benzyl-, and 1,2-Dimethylhexahydropyrimidine.—A pre-cooled solution of ethanal (6.7M) or phenylethanal (5M) in water was added dropwise to a stirred 4M solution of propane-1,3-diamine or *N*-methylpropane-1,3-diamine in water, the temperature of the reaction being kept at 5—10 °C (ice-bath). A 10% excess of aldehyde was used. The reaction mixture was sealed and stirred overnight. Crude product was brought out of the solution by addition of an excess of aqueous NaOH with cooling. The oily product was separated, dried, and fractionally distilled to give: 2-methylhexahydropyrimidine (**1c**) (65%), b.p. 139—142 °C (lit.,[6b] b.p. 143—145 °C), δ_H 1.16 (3 H, d, *J* 6 Hz, CH*Me*), 1.48 (2 H, m, 5-H), 2.85 (2 H, m, 4-$H_{ax.}$ and 6-$H_{ax.}$), 3.15 (2 H, d, with additional fine structure, J_{gem} 14 Hz, 4-$H_{eq.}$ and 6-$H_{eq.}$), and 3.64 (1 H, q, *J* 6 Hz, 2-H); this compound was further characterised by its *N*,*N*-diacetyl derivative (**2a**) (see below); 2-benzylhexahydropyrimidine (**1e**) (73%), b.p. 80 °C at 0.02 mmHg, δ_H 1.96 (2 H, m, 5-H), 2.26 (2 H, d, *J* 6 Hz, CH_2Ph), 2.27 (2 H, m, 4-$H_{ax.}$ and 6-$H_{ax.}$), 3.15 (2 H, d, with additional fine splitting, J_{gem} 13.6 Hz, 4-$H_{eq.}$ and 6-$H_{eq.}$), 3.7 (1 H, t, *J* 6 Hz, 2-H), and 7.25 (5 H, m, ArH). This compound was further characterised by its *N*,*N*-diacetyl derivative (**2b**) (see below); 1,2-dimethylhexahydropyrimidine (**1d**) (60%), b.p. 35 °C at 12 mmHg, δ_H (400 MHz) 1.21 (3 H, d, *J* 6 Hz, CH*Me*), 1.53 (1 H, dm J_{gem} 13 Hz, 5-$H_{eq.}$), 1.74 (1 H, qt, J_{gem} 13 Hz, J_{vic} 3, 3, 13 and 13 Hz, 5-$H_{ax.}$), 2.21 (3 H s, N*Me*), 2.25 (1 H, td, J_{gem} 13 Hz, J_{vic} 3 and 13 Hz, 6-$H_{ax.}$), 2.65 (1 H, td, J_{gem} 13 Hz, J_{vic} 3 and 13 Hz, 4-$H_{ax.}$), 2.88 (1 H, q, *J* 6 Hz, 2-H), 2.95 (1 H, dm, J_{gem} 13 Hz, 6-$H_{eq.}$), and 3.06 (1 H, dm, J_{gem} 13 Hz, 4-$H_{eq.}$); *m*/*z*, M^+ (5%), $(M-1)^+$ (27), $(M-2)^+$ (88), and $(M-15)^+$ (100).

Preparation of 1,3-Diacetyl-2-methylhexahydropyrimidine (**2a**).—2-Methylhexahydropyrimidine (1 g, 0.01 mol) in water (5 cm^3) was added at 10 °C to a stirred solution of aqueous sodium hydroxide (2.4 g, in 12 cm^3 water). To the resulting solution was added acetic anhydride (6.12 g, 0.06 mol) over 1 h. The reaction mixture was stirred for a further 2 h. Evaporation gave a viscous yellow residue which was extracted into dichloromethane. Decolourising charcoal was added and the mixture was stirred at room temperature for 30 min. The charcoal was removed by filtration through Celite, followed by the removal of dichloromethane to give the crude product (**2a**) as an oil. Crystallisation was achieved by dissolving the product in the minimum volume of dichloromethane (5 cm^3), followed by addition of diethyl ether (120 cm^3). Cooling to −20 °C for 3 h precipitated clean white crystals. The precipitate was filtered off and washed with diethyl ether (20 cm^3). Drying at 40 °C gave *1,3-diacetyl-2-methylhexahydropyrimidine* (1.35 g, 73%), m.p. 85 °C, *m*/*z* (e.i.) 284 $(M)^+$, (1%), 283 $(M-1)^+$ (5), and 269 $(M-15)^+$ (100); δ_H 1.45 (3 H, d, *J* 6 Hz, CH*Me*), 1.69 (2 H, m, 2 × 5-H), 2.08 [3 H, s, N(3)-COMe], 2.21 (3 H, s, 1-COMe), 2.97 (1 H, td, J_{gem} 15 Hz, J_{vic} 3 and 15 Hz, 6-$H_{ax.}$), 3.49 (1 H, td, J_{gem} 15 Hz, J_{vic} 3 and 15 Hz, 4-$H_{ax.}$), 3.74 (1 H, dt, J_{gem} 15 Hz, J_{vic} 2.5 and 2.5 Hz, 4-$H_{eq.}$), 4.59 (1 H, dt, J_{gem} 15 Hz, J_{vic} 2.5 and 2.5 Hz, 6-$H_{eq.}$), and 6.65 (1 H, q, *J* 6 Hz, 2-H); $\nu_{max.}$(Nujol) 1 630s cm^{-1} (Found: C, 58.8; H, 8.7; N, 15.3. $C_9H_{16}N_2O_2$ requires C, 58.65; H, 8.75; N, 15.2).

Preparation of 1,3-Diacetyl-2-benzylhexahydropyrimidine (**2b**).—2-Benzylhexahydropyrimidine (1.3 g, 7.4 mmol) was dissolved in water (5 cm^3). To the aqueous solution was added aqueous sodium hydroxide (3 g, 12 cm^3) with cooling to 10 °C. Acetic anhydride (3.5 g, 35 mmol) was added dropwise over 1 h. The reaction was brought to room temperature and stirred overnight. The reaction mixture was worked up as described for the preparation of compound (**2a**) to give a colourless oil. This was dissolved in dichloromethane (3 cm^3) and to the resulting solution, diethyl ether (30 cm^3) was added. The mixture was kept at −20 °C overnight. The precipitated crystals were collected, washed with diethyl ether (20 cm^3), and dried, to give pure *1,3-diacetyl-2-benzylhexahydropyrimidine* (1.01 g, 52%), m.p. 113—115 °C, *m*/*z* (e.i.) M^+ (5%), $(M-1)^+$ (3), $(M-91)^+$ (100), $(M-133)^+$ (97), and $(M-175)^+$ (92); δ_H 1.64 (3 H, s, 3-COMe), 1.78 (2 H, m, 2 × 5-H), 2.1 (3 H, s, 1-COMe), 2.88 (1 H, dd, J_{gem} 12 Hz, J_{vic} 3.6 Hz, 1′-H_B), 3.12 (1 H, td, J_{gem} 12 Hz, J_{vic} 3.4 and 12 Hz, 6-$H_{ax.}$), 3.32 (1 H, dd, J_{gem} 12 Hz, J_{vic} 14 Hz, 1′-H_A), 3.57 (1 H, td, J_{gem} 12 Hz, J_{vic} 3.4 and 12 Hz, 4-$H_{ax.}$), 3.79 (1 H, dt, J_{gem} 2 Hz, J_{vic} 3.4 and 3.4 Hz, 4-$H_{eq.}$), 4.7 (1 H, dt, J_{gem} 12 Hz, 6-$H_{eq.}$), 6.57 (1 H, dd, J_{vic} 3.6 and 14 Hz, 2-H), and 7.25 (5 H, m, ArH); $\nu_{max.}$(Nujol) 1 639s and 1 645s cm^{-1} (Found: C, 69.2; H, 7.75; N, 10.75. $C_{15}H_{20}N_2O_2$ requires C, 69.2; H, 7.75; N, 10.75%).

Preparation of Hexahydropyrimidines from Spermidine. Preparation of 1-(4′-Aminobutyl)-2-methylhexahydropyrimidine (Hexahydropyrimidine derivative of Spermidine. (**1a**).—The hexahydropyrimidine derivative (**1a**) of spermidine was prepared by the dropwise addition of ethanal (0.1 g, 2.2 mmol) to a stirred solution of spermidine (0.145 g, 1 mmol) in chloroform (5 cm^3), with cooling to 5—10 °C. The reaction was stirred for 5 min. The solvent was removed under reduced pressure to give the oily compound (**1a**). This was pure by ^{1}H n.m.r. analysis: δ_H ($CDCl_3$, TMS) 1.22 (3 H, d, –CH*Me*), 135—1.65 (5 H, complex, 5-$H_{eq.}$, 2′-H and 3′-H), 1.67 (1 H, qt, 5-$H_{ax.}$), 2.28 (1 H, ddd, 1′-$H_{ax.}$), 2.33 (1 H, td, 6-$H_{ax.}$), 2.63 (1 H, m, 1′-$H_{eq.}$), 2.66 (3 H, td, 4-$H_{ax.}$), 2.67 (2 H, m, 4′-H), 3.04 (2 H, d, with additional splitting, 4-$H_{eq.}$ and 6-$H_{eq.}$), and 3.20 (1 H, q, 2-H); *m*/*z* (c.i.) 172 $(M+1)^+$ (major peak); *m*/*z* (e.i.) 156 $(M-15)^+$. (Found: 156.1494. $C_8H_{18}N_3$ requires 156.149).

Preparation of 1-[4′-(N-Ethylidene)aminobutyl]-2-methylhexahydropyrimidine. [*Iminohexahydropyrimidine Derivative of Spermidine* (**1b**)].—The iminohexahydropyrimidine derivative (**1b**) of spermidine was prepared by dropwise addition of ethanal (0.2 g, 4.5 mmol) to a stirred solution of spermidine (0.29 g, 2 mmol) in chloroform (6 cm^3), with cooling to 5—10 °C. The

reaction was stirred and monitored by ^{1}H n.m.r. spectroscopy. The cyclisation of spermidine and imine formation at the terminal nitrogen was complete within 5 min. The solvent was evaporated to leave a colourless oily residue of (**1b**) (0.38 g, 97%), pure by ^{1}H n.m.r. analysis, δ_H ($CDCl_3$, TMS) 1.22 (3 H, d, *J* 6 Hz, CH*Me*), 1.35—1.65 (5 H, complex m, 5-$H_{eq.}$, 2′-H and 3′-H), 1.67 (1 H, qt, J_{gem} 12 Hz, J_{vic} 4, 4, 12 and 12 Hz, 5-$H_{ax.}$), 1.95 (3 H, d, *J* 4.5 Hz, N=CH*Me*), 2.28 (1 H, ddd, J_{gem} 13 Hz, J_{vic} 6 and 8.5 Hz, 1′-$H_{ax.}$), 2.33 (1 H, td, J_{gem} 12 Hz, J_{vic} 3 and 12 Hz, 6-$H_{ax.}$), 2.63 (1 H, m, including J_{gem} 13 Hz, J_{vic} *ca.* 7 and 8 Hz, 1′-$H_{eq.}$), 2.66 (1 H, td, J_{gem} *ca.* 13 Hz, J_{vic} 3 and *ca.* 12 Hz, 4-$H_{ax.}$), 3.04 (2 H, d, J_{gem} 12 Hz with additional fine splitting, 4-$H_{eq.}$ and 6-$H_{eq.}$), 3.20 (1 H, q, *J* 5.8 Hz, 2-H), 3.36 (2 H, t, *J* 6.9 Hz, 4′-H), and 7.6 (1 H, q, *J* 4.5 Hz–N=C*H*Me). These assignments were supported by a sequence of decoupling experiments of 4-H, 5-H, 6-H, and 1′-H (see text) and by simulation of signals by a Bruker WH-400 computer ('Panic' Programme) and independently by a SIMEQ-II programme (Varian); *m/z* (c.i.) 198 $(M + 1)^+$ (major peak); *m/z* (e.i.) 182 $(M - 15)^+$ (Found: 182.1657. $C_{18}H_{20}N_3$ requires 182.1657).

References

1 H. T. Nagasawa, J. A. Elberling, and E. G. DeMaster, *J. Med. Chem.*, 1980, **23**, 140.
2 R. Deitrich and V. Erwin, *Ann. Rev. Pharm. Tox.*, 1980, **20**, 55.
3 D. R. Morris, 'Advances in Polyamine Research,' eds. R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves, and F. Bartos, Raven Press, New York, 1978, vol. 2.
4 S. J. Croker, R. S. T. Loeffler, T. A. Smith, and R. B. Sessions, *Tetrahedron Lett.*, 1983, 1559.
5 (*a*) B. T. Golding and I. K. Nassereddin, *J. Am. Chem. Soc.*, 1982, **104**, 5815; (*b*) *J. Chem. Soc., Perkin Trans 1*, 1985, preceding paper.
6 (*a*) G. E. K. Branch, *J. Am. Chem. Soc.*, 1916, **38**, 2466; (*b*) R. F. Evans, *Aust. J. Chem.*, 1967, **20**, 1643.
7 J. B. Lambert and R. G. Keske, *J. Am. Chem. Soc.*, 1966, **88**, 620.
8 F. G. Riddell, 'The Conformational Analysis of Heterocyclic Compounds,' Academic Press, London, 1980, pp. 89—91.
9 H. Booth and R. U. Lemieux, *Can. J. Chem.*, 1971, **49**, 777.
10 B. T. Golding and A. J. Smith, *J. Chem. Soc., Chem. Commun.*, 1980, 702.

Received 6th August 1984; *Paper* 4/1383