Biosynthesis of Vitamin B_{12}: Use of a Single ^{13}C Label in the Macrocycle to Confirm C-11 Methylation in Precorrin-6x

Francis Blanche,*[a] Masahito Kodera,[b] Michel Couder,[a] Finian J. Leeper,[b] Denis Thibaut[a] and Alan R. Battersby*[b]

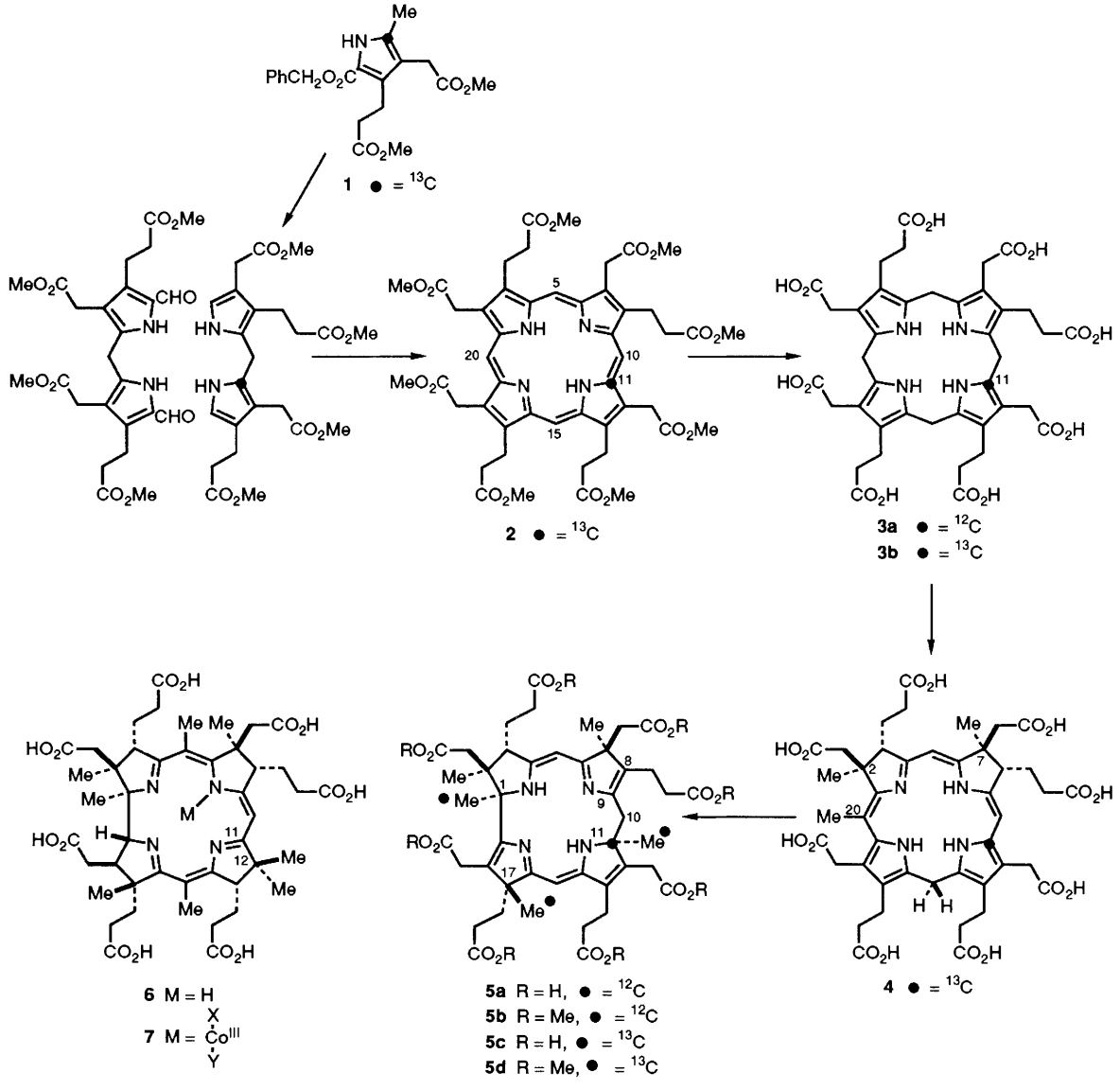
[a] *Department Analyse, Centre de Recherche de Vitry-Alfortville, Rhône-Poulenc Rorer, BP14, F-94403 Vitry-sur-Seine Cedex, France*

[b] *University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK*

[11-^{13}C]Uro'gen III is unambiguously synthesised for enzymic conversion into precorrin-6x which as its octamethyl ester gives a ^{13}C NMR spectrum confirming the presence of a C-11 methyl group.

The direction of research on the biosynthesis of vitamin B_{12} has been sharply changed by the isolation of a new intermediate, precorrin-6x[1] whose octamethyl ester was assigned[1,2] the structure **5b**. This leads to **5a**, used in this paper, as the structure of precorrin-6x itself but it is possible that the natural octa-acid has a tautomeric structure, *e.g.* with a 9,10 double-bond rather than 8,9, and that migration of the double-bond occurs during isolation and esterification. Precorrin-6x is biosynthesised from uro'gen III **3a** by many steps including six *C*-methylations. One of these introduced methyl groups was placed at C-11 and this was one of several unexpected features[1,2] of structure **5a**: the C-11 methyl presumably migrates[2] to its final C-12 position at a later stage on the biosynthetic pathway to hydrogenobyrinic acid **6** and cobyrinic acid **7**.

The structure **5b** was deduced[1,2] from the results of many ^{3}H, ^{14}C and ^{13}C labelling experiments using the early precursors 5-aminolaevulinic acid and *S*-adenosylmethionine (SAM). In particular, the positioning of a *C*-methyl group at C-11 depended on a set of three ^{13}C labelling experiments[2] based on the biosynthetic incorporation into precorrin-6x of, respectively, [3-^{13}C], [4-^{13}C] and [5-^{13}C]-5-aminolaevulinic acid together with [*methyl*-^{13}C]SAM. The various NMR spectra of these samples of precorrin-6x ester showing one-bond ^{13}C–^{13}C couplings and 2–3 bond ^{1}H–^{13}C couplings together with ^{1}H NOE-difference spectra gave an interlocking



set of data supporting C-11 methylation. Nevertheless, when studying a molecule of this complexity and for a feature as unexpected as the C-11 methylation, it was important to provide confirmation by labelling only C-11 of the macrocycle. The results are now outlined.

The [5-^{13}C]pyrrole[3] **1** (90 atom % ^{13}C) was built into [11-^{13}C]uro'gen III **3b** *via* octamethyl [11-^{13}C]uroporphyrin III **2** by an established synthesis[4] as indicated in Scheme 1. Enzymic methylation of **3b** using unlabelled SAM, SAM: uro'gen III methyltransferase[5] and SAM: precorrin-2 methyltransferase[6] afforded the trimethylated intermediate [11-^{13}C]precorrin-3 **4**. The further biosynthetic steps through to precorrin-6x **5c** were carried out by the protein preparation from *Pseudomonas denitrificans* including [*methyl*-^{13}C]SAM but omitting the reducing cofactor NADPH; this omission[1] causes precorrin-6x **5c** to accumulate.

It should be noted that by the foregoing procedure, the first three *C*-methyl groups introduced to form precorrin-3 **4** are unlabelled and that two of these, at C-2 and C-7, carry forward into precorrin-6x **5c**; that at C-20 is lost as acetic acid during the ring-contraction process.[7,8] The next three *C*-methylations needed to reach precorrin-6x **5c** insert ^{13}C-labelled methyl groups.

The proton-decoupled ^{13}C NMR spectrum of the resultant precorrin-6x octamethyl ester **5d** showed two sharp singlets at δ 23.5 and 29.5 corresponding to the 17-methyl and 1-methyl groups respectively. Importantly, the third methyl signal was a doublet (*J* 37.9 Hz) at δ 21.5. This methyl group must be attached to C-11 since the macrocycle is only labelled at this site; the signal for C-11 itself was a doublet (*J* 38.2 Hz) at δ 71.1. All these chemical shifts and *J* values match exactly those found earlier[2] when the macrocycle carried eight ^{13}C-labels from 5-amino[4-^{13}C]laevulinic acid.

The C-11 methyl group of precorrin-6x **5a** is thus confirmed and the structures of later intermediates[9] which in part build on structure **5a** also gain additional strength.

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References

1 D. Thibaut, L. Debussche and F. Blanche, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8795.
2 D. Thibaut, F. Blanche, L. Debussche, F. J. Leeper and A. R. Battersby, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8800.
3 A. R. Battersby, C. J. R. Fookes, M. J. Meegan, E. McDonald and H. K. W. Wurziger, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2786.
4 A. R. Battersby, M. Ihara, E. McDonald, J. Saunders and R. J. Wells, *J. Chem. Soc., Perkin Trans. 1*, 1976, 283.
5 F. Blanche, L. Debussche, D. Thibaut, J. Crouzet and B. Cameron, *J. Bacteriol.*, 1989, **171**, 4222.
6 D. Thibaut, M. Couder, J. Crouzet, L. Debussche, B. Cameron and F. Blanche, *J. Bacteriol.*, 1990, **172**, 6245.
7 L. Mombelli, C. Nussbaumer, H. Weber, G. Müller and D. Arigoni, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 11.
8 A. R. Battersby, M. J. Bushell, C. Jones, N. G. Lewis and A. Pfenninger, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 13.
9 *E.g.* D. Thibaut, F. Kiuchi, L. Debussche, F. J. Leeper, F. Blanche and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1992, following communication.