Synthesis and X-Ray Structure Analysis of 13,17-Bis(2-methoxycarbonylethyl)-12,18-bis(methoxycarbonylmethyl)-2,2,8,8,20-pentamethylisobacteriochlorin

Colin L. Gibson, Michael J. Doyle, Paul R. Raithby and Alan R. Battersby*
University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK

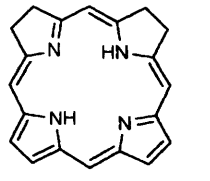
An improved synthesis is developed for the crystalline isobacteriochlorin **5** which carries a 20-methyl group. X-Ray analysis shows that the molecule **5** is substantially puckered in contrast to a close relative lacking the 20-methyl group which is planar.

The isobacteriochlorin macrocycle **1** changed in status during the mid-70's from being something of a curiosity to one attracting strong interest. This happened in part because a co-factor for the enzyme sulfite reductase was found to be an isobacteriochlorin[1] but also because dihydroisobacteriochlorins are important for the biosynthesis of vitamin B_{12}.[2,3] One of the isobacteriochlorins on which attention focused in the researches on vitamin B_{12} was the system **2** which, surprisingly at the time, carries a 20-methyl group.[4–6] This interest in 20-methylated systems has recently been reinforced by the detection[7] of a new biosynthetic intermediate for vitamin B_{12}, precorrin-3B, **3**, which is formed by enzymic oxidation of precorrin-3A, the dihydro derivative of structure **2**. All these developments stimulated a considerable effort on the synthesis of isobacteriochlorins and one of the synthetic products[8] **4** crystallised suitably for X-ray analysis[9] which showed the molecule to be planar. We therefore wished to find out what effect the 20-methyl group of **5** has on the shape of the molecule.

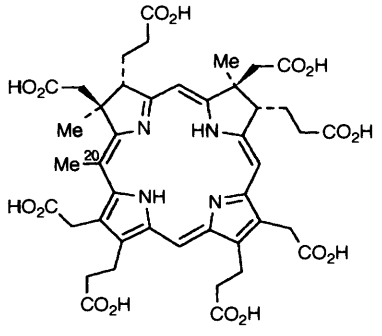
The 20-methyl system **5** had been synthesised earlier[10] but two stages gave modest yields; one was that which generated the 20-methyl group of **5** and the other was the final assembly of the macrocycle. Accordingly, the earlier route[10] was followed up to the lactam **6** but now the amine generated by Raney nickel reduction was formylated to yield the formamide **7**. This was dehydrated using phosphorus oxychloride and 'proton sponge' to yield the isonitrile **8**. Radical cleavage[11] of the C–N bond initiated by azoisobutyronitrile (AIBN) with reduction using tributyltin hydride then afforded the required C-methyl group of lactam **9** in 63% overall yield from the nitrile **6** as a 1:1.9 mixture, respectively, of the *Z*:*E* isomers. This product, without separation of the *Z*/*E*-forms in preparative runs, was carried forward as earlier[10] to a 4:1 mixture of the *Z*:*E* aldehydes **11**.

The conditions for the condensation of the foregoing building block **11** with the pyrrole[10] **12** were modified (see Experimental section) so that the overall yield of this step to form **13** together with its photochemical cyclisation to **5** was raised to 35–40%. The product **5** was identical with that synthesised earlier[10] and yielded crystals suitable for X-ray analysis. The structure so revealed is shown in Fig. 1; the full details of the crystallographic work are held in the Cambridge Crystallographic Data Centre† and the essential data are reported in the Experimental section. In contrast to the macrocycle **4** lacking the C-20 methyl group which was planar,[9] the 20-methylated molecule is substantially puckered, no doubt due to steric pressures in its western segment around rings A and D and C-20. This puckering is best

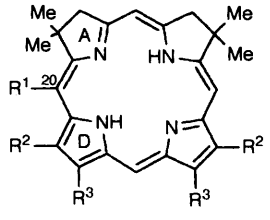
† For details, see 'Instructions for Authors (1994)', *J. Chem. Soc., Perkin Trans 1*, 1994, Issue 1.



1

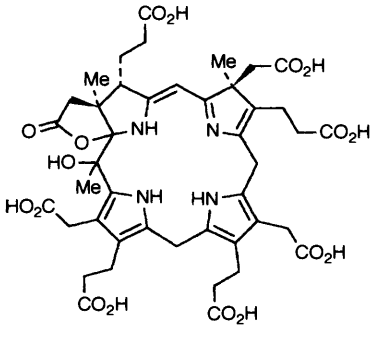


2

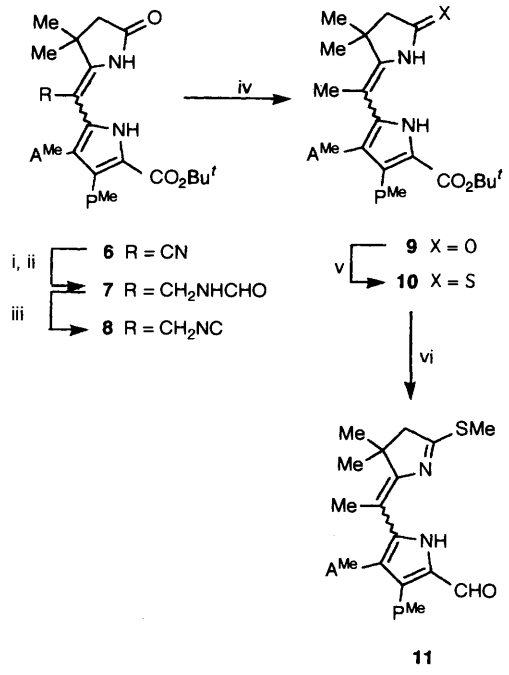


4 R^1 = H, R^2 = R^3 = Me

5 R^1 = Me, R^2 = CH_2CO_2Me
R^3 = $CH_2CH_2CO_2Me$



3



Scheme 1 *Reagents and conditions:* i, Raney Ni in MeOH–H_2O–HCO_2H; ii, pivalic formic anhydride in CH_2Cl_2 (78%); iii, 1,8-bis(dimethylamino)naphthalene (100 equiv.), $POCl_3$ (45 equiv.) in CH_2Cl_2; iv, Bu_3SnH, AIBN, toluene, heat (58%, $Z:E$ = 1:1.9); v, Lawesson's reagent in toluene at 100 °C (91%, $Z:E$ = 4:1); vi, TFA, trimethyl orthoformate (99%)

illustrated by an edge-on view of the molecule which is shown in Fig. 2; the individual atomic deviations from the mean plane through the central chromophore lie in the range −0.45 to −0.42 Å compared to a range of −0.09 to −0.08 Å in the macrocycle **4**.[9] It was pleasing to see this effect because the differences we have observed between isobacteriochlorins with and without a methyl group at C-20 are probably dependent at least to some extent on this puckering. For example, the macrocycle **2** differs from its relative without the 20-methyl group in chromatographic behaviour, basicity and in its UV–VIS spectrum. Also we found in our synthetic work that conditions for the final steps, which were successful for the analogue of **5** lacking the 20-methyl group, failed or gave poor yields of **5** itself.

It has not yet been possible to crystallise the octamethyl ester of the isobacteriochlorin **2** isolated from B_{12}-producing organisms but there can be little doubt that the puckering effect found for **5** is also present for **2** and for the dihydro derivative of **2**, precorrin-3A, which is the biosynthetic intermediate.

Experimental

tert-Butyl 5-[1-(3,3-Dimethyl-5-oxopyrrolidin-2-ylidene)-ethyl]-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-pyrrole-2-carboxylate **9**.—A solution of the nitrile **6** (0.787 g) in methanol–water–formic acid (80:19:1, 250 cm^3) was stirred with Raney nickel (W-2, *ca.* 3.5 g) under nitrogen for 27 h. The mixture was filtered, the catalyst washed with 1% formic acid in methanol (1 dm^3) and the filtrate was evaporated at 0.5 mmHg. The residue was dissolved in toluene (100 cm^3) and evaporated to an oil which in dry dichloromethane (100 cm^3) was stirred under argon with pivalic formic anhydride (5 cm^3) for 10 min and then evaporated finally at 0.1 mmHg. The residue was partitioned between dichloromethane (100 cm^3) and water (50 cm^3) and the aqueous layer was extracted with dichloromethane

† 1 mm^3 = 1 μl.

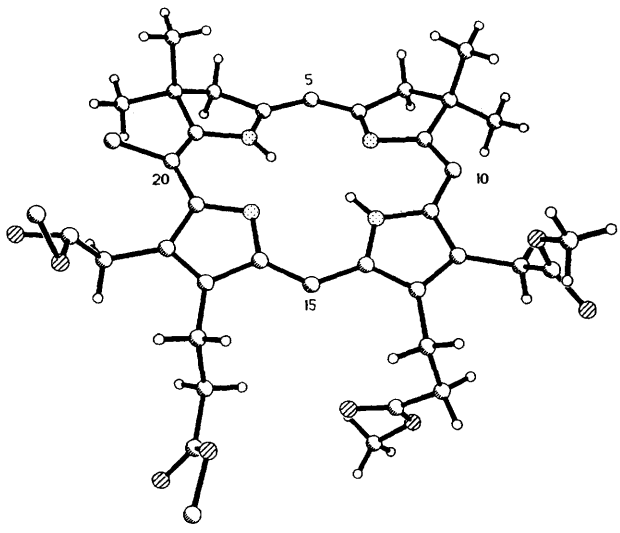
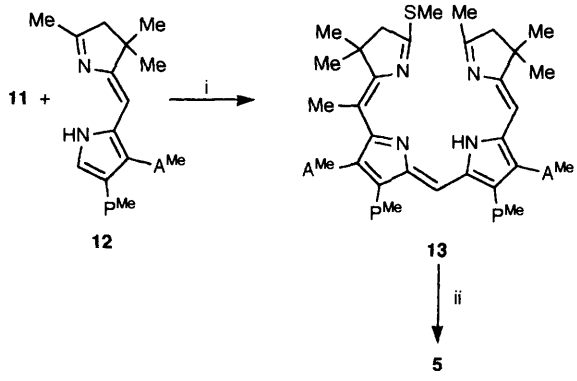


Fig. 1 The molecular structure of **5** showing the numbering of the key carbon atoms. Only one orientation of the disordered atoms has been shown and hydrogen atoms have been omitted from the 20-methyl group for clarity.



Scheme 2 *Reagents and conditions:* i, TFA, MeOH; ii, *N,N*-diisopropylethylamine, THF, *hv* (40%)

(3 × 100 cm^3) and methyl acetate (75 cm^3). The product from the combined organic phases by flash column chromatography using methyl acetate–dichloromethane (97:3) yielded the *E*/*Z* isomeric formamides **7** (0.65 g, 78% or 92% on unrecovered starting material), m.p. 72–76 °C (M^+, 505). Starting material **6** (0.12 g) was also isolated.

To a stirred solution of the formamides **7** (266 mg, 0.53 mmol) and 1,8-bis(dimethylamino)naphthalene (11.33 g, 52.9 mmol) in dry dichloromethane (100 cm^3) under argon was added phosphorus oxychloride (490 mm^3†, 5.28 mmol). Further aliquots of phosphorus oxychloride were added after 30 min (490 mm^3), 75 min (490 mm^3), 120 min (490 mm^3) and 150 min (240 mm^3) and after a further 1 h the mixture was poured into iced aqueous sodium hydrogen carbonate (100 cm^3) and stirred vigorously for 5 min. The aqueous phase was extracted with dichloromethane (2 × 100 cm^3) and the combined organic extracts were evaporated. The residue in toluene (50 cm^3) was filtered and the solution was evaporated at 0.1 mmHg, this process being repeated twice. To a solution of the resulting oil in dry toluene (50 cm^3) was added tributyltin hydride (290 mm^3, 1.1 mmol) and AIBN (5 mg) in toluene (75 cm^3) under argon. The mixture was stirred at 18 °C for 30 min, then refluxed for 45 min, cooled and evaporated (0.1 mmHg). Chromatography of the residue on silica using dichloromethane–methanol (95:5) afforded the *Z* lactam **9** as a white solid (49.9 mg, 20%) together with the crude *E* isomer. Purification of the latter by PLC using ether gave the *E* lactam **9** from the faster band as a white solid

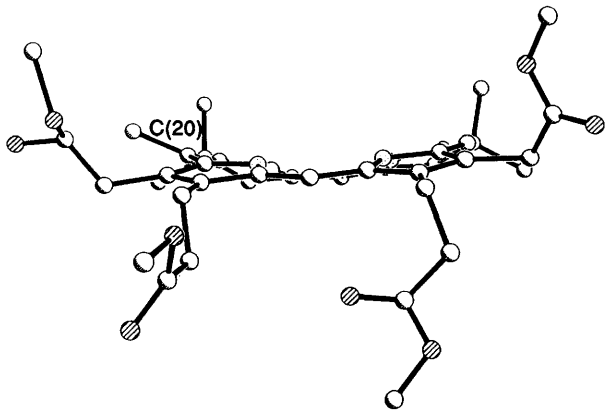


Fig. 2 An edge-on view of the structure of **5** showing the puckering of the ring system

(93.2 mg, 38%). The slower band gave starting amide **7** (21.3 mg, 8%).

Z *Isomer* **9**: m.p. 149.5–151 °C (dichloromethane–hexane) (lit.,[10] m.p. 148–150 °C) (Found: M^+, 462.2399. $C_{24}H_{34}N_2O_7$ requires M^+, 462.2366).

E *Isomer* **9**: m.p. 211–214.5 °C (dichloromethane–hexane) (lit.,[10] m.p. 201–205 °C) (Found: M^+, 462.2390. $C_{24}H_{34}N_2O_7$ requires M^+, 462.2366). Both isomers were identical by NMR and TLC with earlier samples.[10]

13,17-*Bis*(2-*methoxycarbonylethyl*)-12,18-*bis*(*methoxycarbonylmethyl*)-2,2,8,8,20-*pentamethylisobacteriochlorin* **5**.—To the imine **12** (41.7 mg, 0.12 mmol) prepared as previously,[8] was added dry methanol (3 cm^3) and trimethyl orthoformate (70 mm^3) and the mixture was added to the aldehyde **11** (40 mg, 0.95 mmol) all under argon. The resulting blue–black pigment was treated with trifluoroacetic acid (0.1 cm^3) to give a royal blue solution which was stirred for 45 min and then diluted with dry THF (20 cm^3) and treated with *N*,*N*-diisopropylethylamine (*ca.* 0.3 cm^3) until the colour was maroon. *N*,*N*-Diisopropylethylamine trifluoroacetate salt (0.259 g) was added to this mixture which was then transferred to a glass tube; this, after four freeze–pump–thaw cycles, was sealed. It was irradiated for 64 h as previously[10] and the solution was evaporated. Purification of the residue by PLC using 9:1 dichloromethane–methyl acetate afforded the 20-methylisobacteriochlorin **5** as purple needles (26.7 mg, 0.038 mmol, 40%), m.p. 159.5–162 °C (from dichloromethane–methanol) (lit.,[10] m.p. 152–155 °C) identical (NMR and TLC) with that produced previously.[10]

X-Ray Crystallographic Analysis of **5**.—Purple crystals of **5** were obtained by slow evaporation of dichloromethane solution, and a single crystal was sealed in a Lindemann capillary tube containing a small volume of mother liquor. The crystal was then transferred to a Siemens R3m/V diffractometer.

Crystal data. $C_{39}H_{48}N_4O_8$, M = 700.81, triclinic, space group $P\bar{1}$ (No. 2), a = 9.647(1), b = 13.705(6), c = 15.672(6) Å, α = 112.14(3), β = 103.02(2), γ = 93.61(1)°, U = 1845.6(11) $Å^3$ (by least-squares refinement of diffractometer angles from 25 automatically centred reflections in the range $40 < 2\theta < 60°$, λ = 1.541 78 Å), D_m = not measured, Z = 2, D_c = 1.262 g cm^{-3}, $F(000)$ = 748, μ(Cu-Kα) = 7.22 cm^{-1}. Dark purple needles. Crystal dimensions 0.28 × 0.17 × 0.15 mm, μR = 0.72.

Data collection and processing. Siemens R3m/V diffractometer, 96-step $\omega/2\theta$ scan mode from 0.9° below $K_{\alpha 1}$ to 0.9° above $K_{\alpha 2}$, with a variable scan speed in the range 2.00–29.3° min^{-1}, graphite-monochromated Cu-Kα radiation; 5488 reflections measured ($5.0 < 2q < 116.0°$, $-h$, $\pm k$, $\pm l$), 5000 unique [merging R = 0.035 after semi-empirical absorption correction (maximum, minimum transmission factors 0.834, 0.572)]. Three check reflections showed no significant variation during data collection.

Structure analysis and refinement. Centrosymmetric direct methods (SHELXTL-PLUS)[12] followed by Fourier difference techniques for the non-hydrogen atoms. Full-matrix least-squares refinement on F^2 (SHELXL-93).[13] The side chains on the central unit showed severe positional disorder, and where two positions could be located for each atom they were refined with partial occupancies so that the total occupancy corresponded to unity. The non-hydrogen atoms that did not show disorder were refined with anisotropic displacement parameters, and hydrogen atoms were placed in idealised positions and allowed to ride on the relevant C or N atoms; these hydrogen atoms were assigned displacement parameters corresponding to 1.2 times the value of the relevant non-hydrogen atom. In the final stages of refinement a weighting scheme of the form $w = 1/[\sigma^2(F_o)^2 + (xP)^2 + yP]$, where $P = (F_o^2 + 2F_c^2)/3$, was introduced and this gave a satisfactory agreement analysis. The final converged residuals were R_1 = 0.118 [3462 reflections with $I > 2\sigma(I)$] and wR_2 = 0.353 on all data; goodness-of-fit on F^2 = 1.043. The highest residual peak in a final Fourier difference map was 1.13 $eÅ^{-3}$, and this lay in the region of the disordered side chains.

Acknowledgements

Grateful acknowledgement is made to Christ's College, Cambridge, for the award of the Dow Senior Research Fellowship (to C. L. G.) and to SERC, Zeneca, F. Hoffman La Roche and Roche Products for financial support. Dr. S. Handa is also thanked for providing quantities of the precursor to imine **12**.

References

1 M. J. Murphy, L. M. Siegel, H. Kamin and D. Rosenthal, *J. Biol. Chem.*, 1973, **243**, 2801 and references therein.
2 F. J. Leeper, *Nat. Prod. Rep.*, 1989, **6**, 171 and earlier reviews there cited.
3 A. R. Battersby, *Acc. Chem. Res.*, 1993, **26**, 15.
4 A. R. Battersby, G. W. J. Matcham, E. McDonald, R. Neier, M. Thompson, W.-D. Woggon, V. Bykhovsky and R. H. Morris, *J. Chem. Soc., Chem. Commun.*, 1979, 185.
5 N. G. Lewis, R. Neier, E. McDonald and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1979, 541.
6 G. Müller, K. D. Gneuss, H. P. Kriemler, A. I. Scott and A. J. Irwin, *J. Am. Chem. Soc.*, 1979, **101**, 3655.
7 L. Debussche, D. Thibaut, M. Danzer, F. Debu, D. Fréchet, F. Herman, F. Blanche and M. Vuilhorgne, *J. Chem. Soc., Chem. Commun.*, 1993, 1100; L. Debussche, D. Thibaut, B. Cameron, J. Crouzet and F. Blanche, *J. Bacteriol.*, 1993, **175**, 7430; *cf.* A. I. Scott, C. A. Roessner, N. J. Stolowich, J. B. Spencer, C. Min and S.-I. Ozaki, *FEBS Lett.*, 1993, **331**, 105.
8 P. J. Harrison, C. J. R. Fookes and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1981, 797.
9 W. B. T. Cruse, P. J. Harrison and O. Kennard, *J. Am. Chem. Soc.*, 1982, **104**, 2376.
10 D. M. Arnott, P. J. Harrison, G. B. Henderson, Z.-C. Sheng, F. J. Leeper and A. R. Battersby, *J. Chem. Soc., Perkin Trans. 1*, 1989, 265.
11 D. H. R. Barton, G. Bringmann and W. B. Motherwell, *Synthesis*, 1980, 68.
12 SHELXTL-PLUS, G. M. Sheldrick, program package for structure solution, Göttingen, 1986.
13 SHELXL-93, G. M. Sheldrick, program package for structure refinement, Göttingen, 1993.

Paper 4/02304H
Received 19*th April* 1994
Accepted 16*th May* 1994