Synthesis of a Photoaffinity-labelled Analogue of 1,25-Dihydroxyvitamin D_3

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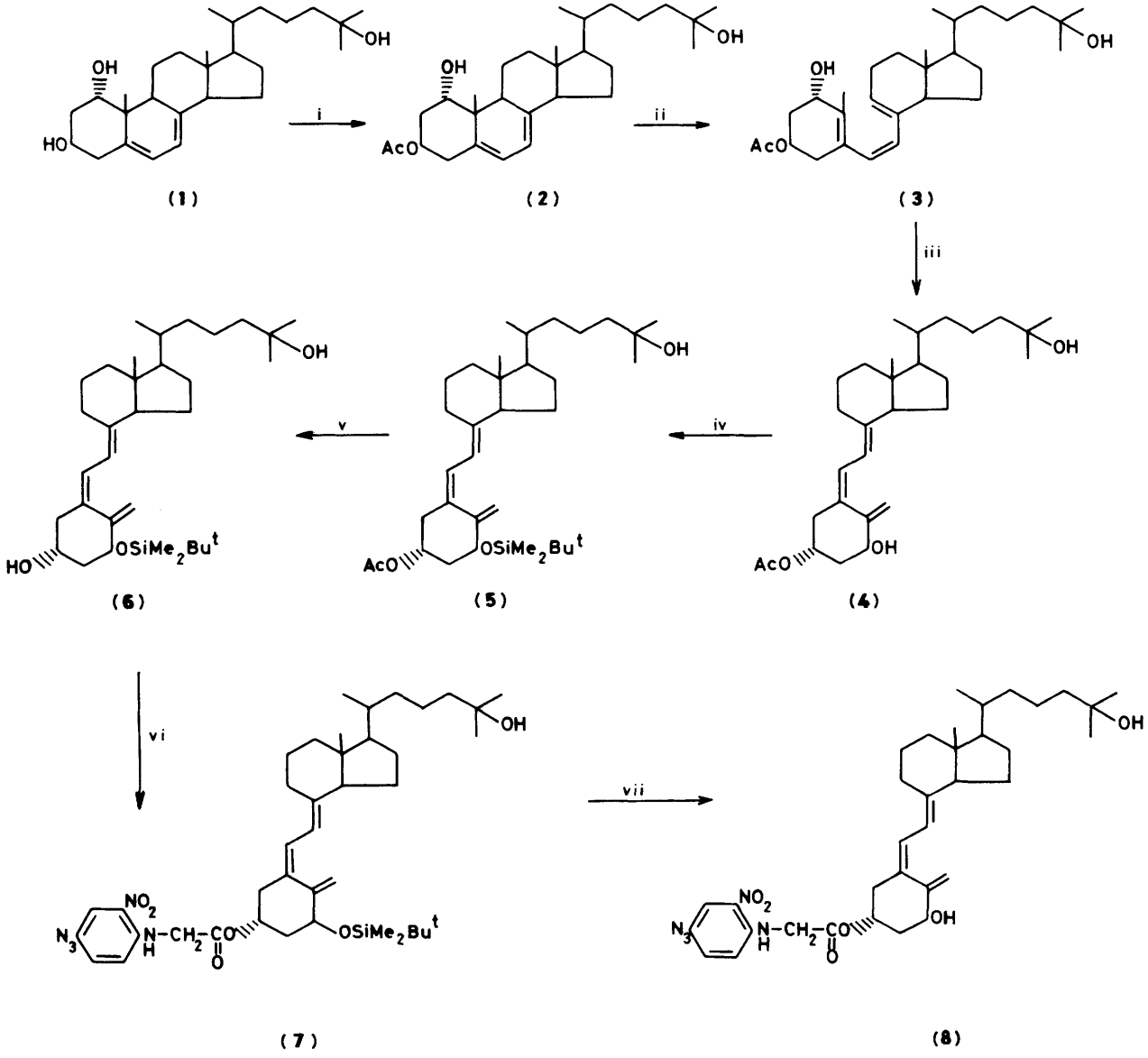
The synthesis of a biologically active analogue of 1,25-dihydroxyvitamin D_3 containing a photolabile azidonitrophenyl group is described.

It is well established that vitamin D must be metabolized sequentially in the liver to 25-hydroxyvitamin D_3 (25-OH-D_3) and then in the kidney to 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2$-D_3] before it can carry out its physiological functions.[1,2] Photoaffinity labelling of peptide and steroid hormone receptors has been effectively used as a molecular probe for mapping of ligand binding sites.[3,4] However, to date, no attempt has been made to use this powerful tool to investigate ligand–receptor interactions for vitamin D_3 and its biologically important metabolites, 25-OH-D_3 and 1,25-$(OH)_2$-D_3. In devizing a successful scheme for synthesizing photoaffinity derivatives of 1,25-$(OH)_2$-D_3, it was necessary to take into consideration the inherent photolability of the parent compound. In addition, it was necessary to derivatize selectively only the 3β-OH because this position was least likely to interfere with binding properties.



Scheme 1. *Reagents and conditions*: i, acetic anhydride, pyridine, 4 °C; ii, *hν*, Et_2O; iii, EtOH, 60 °C; iv, $Bu^tMe_2SiSO_2CF_3$, 4-*N*,*N*-dimethylaminopyridine (DMAP), CH_2Cl_2; v, 10% KOH in EtOH; vi, ANP-glycine, DCC, DMAP, CH_2Cl_2; vii, 5% HF, MeCN.

N-(4-Azido-2-nitrophenyl)glycine (ANP-glycine),[5] containing a photolabile nitroaryl azide group, has a suitably protected carboxylic acid group and could be coupled selectively to the 3β-hydroxy group of the vitamin D skeleton *via* an ester linkage. Furthermore, photolysis of the coupled product could be effected at λ 400—450 nm without affecting the sensitive triene system of vitamin D.

Low temperature regiospecific acetylation of 1,25-dihydroxycholesta-5,7-diene-3β-ol (**1**) furnished the 3-acetate (**2**). Photolysis[6] of (**2**) produced the previtamin derivative (**3**). Preparative t.l.c. separation and thermal isomerization of (**3**) furnished 1,25-$(OH)_2$-D_3 acetate (**4**). Silylation of (**4**) with t-butyldimethylsilyl trifluoromethanesulphonate[7] produced (**5**), which in turn, was deacetylated with 10% KOH in ethanol to produce 1-t-butyldimethylsilyloxy-25-hydroxyvitamin D_3 (**6**). Dicyclohexylcarbodiimide (DCC) coupling of (**6**) with ANP-glycine gave (**7**). Finally, desilylation of (**7**) with 5% HF in acetonitrile provided the desired product (**8**) (Scheme 1).

The 250 MHz ^{1}H n.m.r. spectrum ($CDCl_3$) of (**8**) was as follows: δ 0.55 (s, 3H, 18-Me), 0.95 (d, 3H, *J* 6.02 Hz, 21-Me), 4.09 (d, 2H, *J* 5.44 Hz, CH_2CO), 4.39 (m, 1H, 1-H), 5.04 and 5.37 (broad s, 2H, 19-H), 5.33 (m, 1H, 3-H), 5.99 and 6.31 (ABq, 2H, *J* 11.31 Hz, 6,7-H), 6.71 (d, 1H, aromatic H), 7.12 and 7.16 (dd, 1H, NH), 7.9 (narrow d, 1H, aromatic H), and 8.36 (m, 1H, aromatic H). In the u.v. spectrum (EtOH) of (**8**), the characteristic $\lambda_{max.}$ at 265 nm of vitamin D was masked by the aromatic absorption in this region ($\lambda_{max.}$ 258 nm and a broad peak at 450 nm). The i.r. spectrum ($CHCl_3$) of (**8**) has strong absorptions at 2130 (azide) and 1745 cm^{-1} (ester) along with broad absorptions between 3300 and 3600 cm^{-1}.

Bioassay of the synthetic analogue (**8**) with calcium and rats deficient in vitamin D indicated that this compound stimulated intestinal calcium transport and bone calcium mobilization. A binding study of (**8**) with chick intestinal cytosolic preparation showed that (**8**) was, indeed, capable of competing with 1,25-$(OH)_2$-D_3 for binding sites.

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References

1 H. F. DeLuca, *Nutr. Rev.*, 1979, **37**, 161.
2 M. F. Holick and J. T. Potts, Jr., in 'Harrisons Principles of Internal Medicine,' eds. R. G. Petersdorf, R. D. Adams, E. Braunwald, K. J. Isselbacher, J. B. Martin, and J. D. Wilson, McGraw-Hill, New York, 10th edn., 1983, p. 1944.
3 M. D. Coltrera, J. T. Potts, Jr., and M. Rosenblatt, *J. Biol. Chem.*, 1981, **256**, 10555.
4 H. Bayley and J. R. Knowles, *Methods Enzymol.*, 1977, **48**, 69; M. Das and C. F. Fox, *Am. Rev. Biophys. Bioeng.*, 1979, **8**, 165; J. A. Katzenellenbogen, H. J. Johnson, K. E. Carlson, and H. N. Meyers, *Biochemistry*, 1974, **16**, 1970; 1974, **13**, 2, 986.
5 D. Levy, *Biochim. Biophys. Acta*, 1973, **322**, 329; G. W. J. Fleet, J. R. Knowles, and R. R. Porter, *Biochem. J.*, 1972, **128**, 499.
6 J. A. MacLaughlin, R. R. Anderson, and M. F. Holick, *Science*, 1982, **216**, 1001.
7 E. J. Corey, H. Cho, C. Rucker, and D. Hua, *Tetrahedron Lett.*, 1981, **22**, 3455.