Synthesis of a Photoaffinity-labelled Analogue of 1,25-Dihydroxyvitamin D₃

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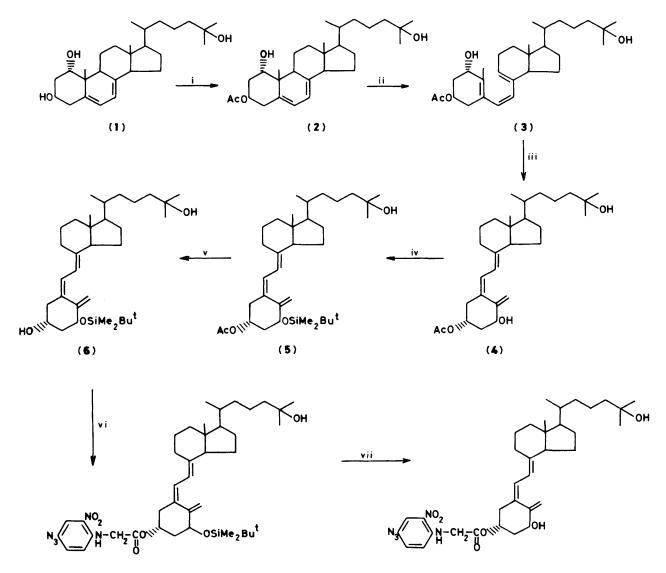
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The synthesis of a biologically active analogue of 1,25-dihydroxyvitamin D₃ containing a photolabile azidonitrophenyl group is described.

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 3 β -OH because this position was least likely to interfere with binding properties.

(8)



Scheme 1. Reagents and conditions: i, acetic anhydride, pyridine, 4 °C; ii, hv, Et₂O; iii, EtOH, 60 °C; iv, Bu^tMe₂SiSO₂CF₃, 4-N,N-dimethylaminopyridine (DMAP), CH₂Cl₂; v, 10% KOH in EtOH; vi, ANP-glycine, DCC, DMAP, CH₂Cl₂; vii, 5% HF, MeCN.

(7)

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 1,25dihydroxycholesta-5,7-diene-3 β -ol (1) furnished the 3-acetate (2). Photolysis⁶ of (2) produced the previtamin derivative (3). Preparative t.l.c. separation and thermal isomerization of (3) furnished 1,25-(OH)₂-D₃ acetate (4). Silylation of (4) with t-butyldimethylsilyl trifluoromethanesulphonate⁷ produced (5), which in turn, was deacetylated with 10% KOH in ethanol to produce 1-t-butyldimethylsilyloxy-25-hydroxyvitamin D₃ (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 ¹H n.m.r. spectrum (CDCl₃) of (8) was as follows: $\delta 0.55$ (s, 3H, 18-Me), 0.95 (d, 3H, J 6.02 Hz, 21-Me), 4.09 (d, 2H, J 5.44 Hz, CH₂CO), 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 λ_{max} at 265 nm of vitamin D was masked by the aromatic absorption in this region (λ_{max} . 258 nm and a broad peak at 450 nm). The i.r. spectrum (CHCl₃) 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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