Synthesis of a ^{125}I-labelled Derivative of 25-Hydroxyvitamin D_3

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The facile synthesis of a radioactive 25-hydroxyvitamin D_3 derivative which is labelled with ^{125}I-Bolton–Hunter Reagent is described.

It is known that vitamin D_3 is metabolized to 25-hydroxy vitamin D_3 ($25OHD_3$) in the liver and then to 1,25-dihydroxy vitamin D_3 [$1,25(OH)_2D_3$] in the kidney. The compound $1,25(OH)_2D_3$ is considered to be a hormone-like substance.[1,2]

Radioisotope labelled analogues of active vitamin D_3 [$1,25(OH)_2D_3$, $25OHD_3$] are found to be very useful endocrinologically. However only ^{3}H-labelled derivatives are known,[3] but these have low radioactivity, and so highly radioactive ^{125}I-labelled derivatives have been required for some time.

Chemical conversion of vitamin D_3 is difficult owing to its extreme lability, therefore syntheses of vitamin D_3 derivatives have been mostly from steroidal compounds with many steps.

Therefore we investigated a facile synthesis of a ^{125}I-labelled derivative under mild conditions with $25OHD_3$ (**1**) as the starting material (Scheme 1). Treatment of fluoren-9-ylmethoxycarbonyl (FMOC) protected β-alanine with trimethylacetyl chloride in anhydrous tetrahydrofuran (THF) in the presence of dimethylaminopyridine (DMAP)[4,5] following esterification of $25OHD_3$ (**1**) successfully afforded the derivative (**2**).† The isolated yield of (**2**) was 72.2%. The

† *Selected spectroscopic data* for (**2**): ^{1}H n.m.r. (90 MHz) and u.v.: δ($CDCl_3$) 0.54 (3H, s, CH_3-18), 3.30—3.60 (2H, m, $-CH_2-N-$), 4.10—4.50 (3H, m, FMOC), 4.80—5.60 (4H, m, H-19E, H-3α, H-19Z, -NH-), 5.94—6.29 (2H, m, H-7, H-6), 7.20—7.81 (8H, m, FMOC), λ_{max} (EtOH) nm 300.2, 266.4, 214.4. (**3**): ^{1}H n.m.r. (900 MHz) and u.v.: δ($CDCl_3$) 0.54 (3H, s, CH_3-18), 2.36—2.52 (2H, m, $-CO-CH_2-$), 2.90—3.05 (2H, m, $-CH_2-N-$), 3.50—4.00 (2H, b, $-NH_2$), 4.86—5.08 (3H, m, H-19E, H-3α, H-19Z), 5.90—6.30 (2H, m, H-7, H-6), λ_{max} (EtOH) nm 264.5. (**5**); ^{1}H n.m.r. (400 MHz) and u.v.; δ($CHCl_3$) 0.52 (3H, s, CH_3-18), 0.92 (3H, d, CH_3-21, *J* 6.3 Hz), 1.23 (6H, s, CH_3-26, 27) 2.56—2.20 (8H, m, H-4α, $-NCO-CH_2-$, H-1β, H-4β, $-OCO-CH_2-$, H-1α), 2.85—2.73 (3H, m, $-CH_2-$Ph, H-9β), 3.48 (2H, m, $-CH_2-N-$), 4.86 (1H, d, H-19E, *J* 2.0 Hz), 5.01 (1H, m, H-3α), 5.09 (1H, m, H-19Z), 5.94 (1H, t, -NH-, *J* 5.9 Hz), 6.01 (1H, d, H-7, *J* 11.2 Hz), 6.20 (1H, d, H-6, *J* 11.2 Hz), 6.87 (1H, d, H-Ph, *J* 8.3 Hz), 4.04 (1H, dd, H-Ph, *J* 2.0 and 8.3 Hz), 7.47 (1H, d, H-Ph, *J* 2.0 Hz), λ_{max} (EtOH) nm 268.2.

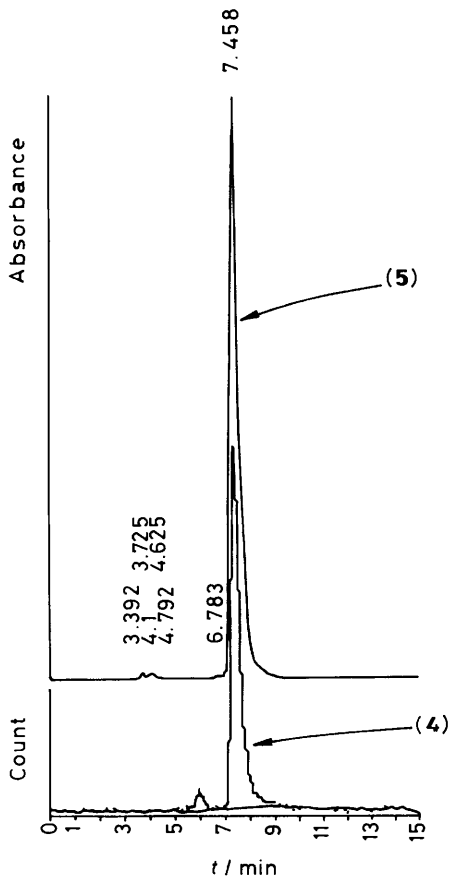
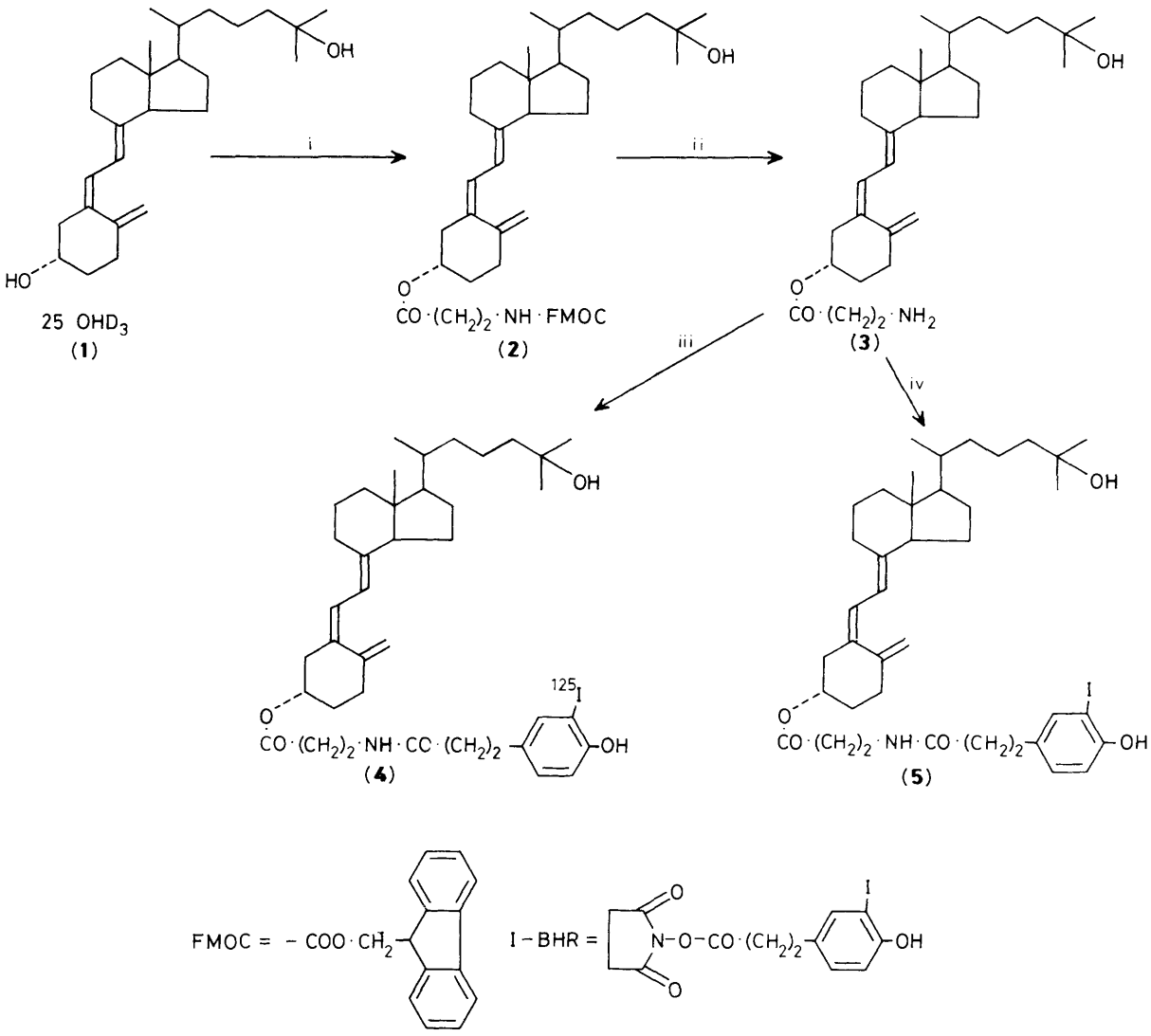


Figure 1. H.p.l.c. of compounds (**4**) and (**5**). Detection of (**4**): (ALOKA) Radioanalyzer RLC-551, (ALOKA) γ-rayflow detector FGD-101. Detection of (**5**): (SHIMADZU) u.v. spectrophotometric detector SPD-6A, 265 nm. H.p.l.c. conditions: (MISTUITOASTU) Zorbax Pro10 (silica), 4.6 × 250 mm, (SHIMADZU) Guardcolumn, 4.6 × 50 mm, 20% isopropanol–n-hexane 1 ml/min.



Scheme 1. *Reagents and conditions*: i, β-Ala–FMOC, trimethylacetylchloride, DMAP, in anhydrous THF, at 5 °C for 1 h, and then at room temp. for 2 h; ii, morpholine, at room temp. for 2 h; iii, ^{125}I–BHR(NEN, NEX-120-10), Et_2N, in anhydrous THF, at 25 °C for 18 h; iv, I-BHR synthesized in our laboratory, Et_3N, in anhydrous THF, at 5 °C for 16 h.

deprotection of the derivative (**2**) with morpholine gave compound (**3**),† with an isolated yield of 83.9%.

In the presence of a base, treatment of compound (**3**) with ^{125}I-Bolton–Hunter Reagent {*N*-succinimidyl 3-[4-hydroxy-3-(^{125}I) iodophenyl] propionate: NEN NEX-120-10 2200Ci/mmol (1Ci = 3.7 × 10^{10} Bq)},[6] a protein labelling reagent, gave the ^{125}I-labelled derivative (**4**) with an isolated radioactive yield of 50—60% which was purified by h.p.l.c. The same treatment of (**3**) with non-radioactive I-Bolton–Hunter Reagent (I-BHR) [*N*-succinimidyl 3-(4-hydroxy-3-iodophenyl)propionate] gave the corresponding compound (**5**).† The isolated yield, purified by h.p.l.c., of (**5**) was 47.2%. Compounds (**4**) and (**5**) had an identical retention time for h.p.l.c. (Figure 1).

The ethanol solution of (**4**) was stable for 60 days at −20 °C. The radioactivity of (**4**) can be calculated at 2200Ci/mmol as the radioactivity of the ^{125}I-BHR is known. This radioactivity of (**4**) is ten times greater than that of ^{3}H-labelled 25OHD$_3$ which has been used previously as a tracer. These results indicate that it is possible to use ^{125}I-labelled compound (**4**) as a tracer with high sensitivity in radiobioassay.

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