

## Analysis of Vitamin D and its Metabolites: Derivatization and Detection by Electrospray Ionization Mass Spectrometry

Stephen R. Wilson,\* Qingyi Lu, Michael L. Tulchinsky and Yunhui Wu

*Department of Chemistry, New York University, Washington Square, New York, NY 10003, USA*

*N*-(Benzo-15-crown-5)-1,2,4-triazol-3,5-dione is used to prepare an 'electrospray active' derivative of vitamin D; this general method could be applied to electrospray ionisation mass spectrometry of biomedically important products, which are not normally observable.

Vitamin D **1** is synthesized in the skin and is metabolized in the liver and kidneys to its hormonal form calcitriol **2** (1,25-dihydroxyvitamin D).<sup>1</sup> Further metabolism to a major degradation product, calcitroic acid **3**,<sup>2</sup> has also been established. Understanding the critical role of vitamin D and its active metabolites in biology however, requires effective and reliable

methods for determination of the identity and levels of the compounds in serum. To the best of our knowledge, there is no general method to determine directly vitamin D and its metabolites under near-physiological (sub-micromolar) concentrations.<sup>3</sup>

Electrospray ionization mass spectrometry (ESIMS) is a

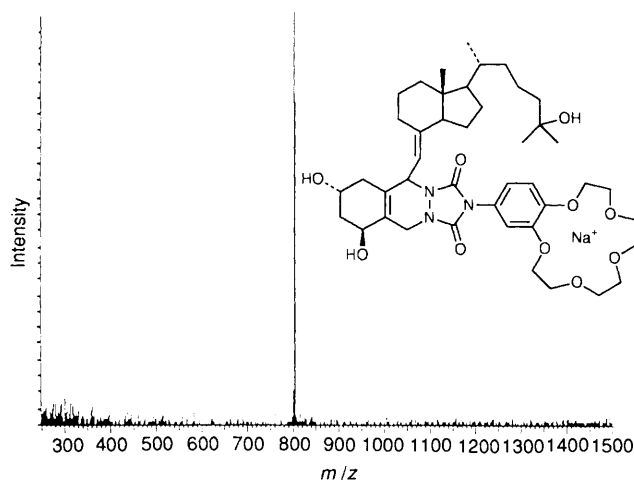
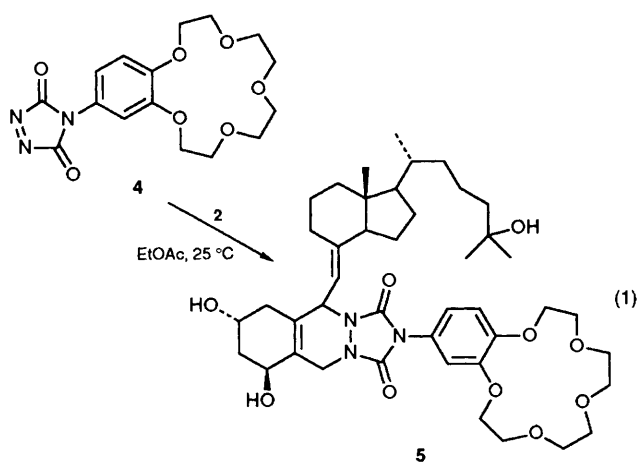
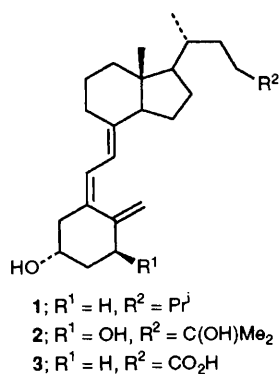


Fig. 1 Diels-Alder adduct **5** at ca.  $10^{-5}$  mol dm<sup>-3</sup> in methanol containing 0.5% NaClO<sub>4</sub> ( $[M + Na]^+$ ,  $m/z = 805$ )

new MS technique, which has revolutionized analysis of large biopolymers.<sup>4</sup> Studies of small organic molecules are less frequent.<sup>5,6</sup> Recent reports of subfemtogram sensitivity<sup>7</sup> for ESIMS suggest that selective online derivatization methods combined with ESIMS would provide a powerful new technique for monitoring biological systems. In order to observe signals by ESIMS, the molecules must be charged, so vitamin D compounds **1** and **2** do not show up in ESIMS. We have developed several tagging reagents that (i) are selective for derivitization of the vitamin D triene system, (ii) vastly increase their ESIMS signal and (iii) are suitable for online analysis at physiologically relevant concentrations.<sup>8</sup>

Recently, we have reported that crown ether complexes are excellent substrates for detection by ESIMS. The new compound *N*-(benzo-15-crown-5)-1,2,4-triazole-3,5-dione **4** has been prepared by modification of a known route.<sup>9</sup> Reagent **4** is a powerful dienophile<sup>10</sup> and smoothly introduces a crown ether binding site into a diene such as vitamin D. (The adduct of **1** and **4** was isolated in 45% yield after alumina chromatography and completely characterized.) When a red solution of **4** was allowed to contact 1,25-dihydroxyvitamin D **2**, instantaneous decolourization indicated formation of the Diels-Alder adduct **5**. ESIMS analysis of adduct **5** ( $m/z = 805$ ) in the presence of an excess of NaClO<sub>4</sub> is shown in Fig. 1. Detection limits with our current system are less than ca.  $10^{-6}$  mol dm<sup>-3</sup> for **5**, close to physiological concentrations. Similar reactions with vitamin D **2** and metabolite **3** gave adducts showing  $[M + Na]^+$  peaks at  $m/z$  773 and 761, respectively.<sup>†‡</sup><sup>11</sup>

Received, 16th December 1992; Com. 2/06666A

## References

- 1 *Proceedings of the 8th Vitamin D Workshop*, ed. A. Norman, De Gruyter, Berlin, 1991.
- 2 G. S. Reddy and K.-Y. Tserng, *Biochemistry*, 1989, **28**, 1763.
- 3 M. Shimizu, T. Takahashi, S. Uratsuka and S. Yamada, *J. Chem. Soc., Chem. Commun.*, 1990, 1416; M. Shimizu, S. Kamachi, Y. Nishi and S. Yamada, *Anal. Biochem.*, 1991, **194**, 77.
- 4 Reviews, J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science*, 1989, **246**, 64; M. Mann, C. K. Meng and J. B. Fenn, *Anal. Chem.*, 1989, **61**, 1702; J. A. Loo, R. R. O. Loo, K. J. Light, C. G. Edmonds and R. D. Smith, *Anal. Chem.*, 1992, **64**, 81.
- 5 R. D. Voyksner, *Nature*, 1992, **345**, 74.
- 6 (a) S. R. Wilson, J. Perez and Y. Wu, *Nat. Prod. Lett.*, 1992, **1**, 104; (b) S. R. Wilson, A. Yasmin and Y. Wu, *J. Org. Chem.*, 1992, **57**, 6941; (c) S. R. Wilson and M. L. Tulchinsky, *J. Org. Chem.*, 1993, **58**, 1407; (d) S. R. Wilson, J. Perez and A. Pasternak, *J. Am. Chem. Soc.*, in the press; (e) S. R. Wilson and Y. Wu, *J. Am. Soc. Mass Spectrom.*, in the press.
- 7 C. G. Edwards, J. A. Loo, S. M. Fields, C. J. Barinaga, H. R. Udseth and R. D. Smith, in *Biol. Mass Spectrom. Proc. Int. Symp.*, ed. A. L. Burlingame, Elsevier, 1990, pp. 77-100.
- 8 This work was reported in preliminary form: S. R. Wilson, Y. Wu and M. Tulchinsky, *Abstr. Proc. 40th Am. Soc. Mass Spectrom. Conf. on Mass Spectrom. & Allied Topics*, Washington, DC, June 1992, pp. 1641-1642. ESI derivatization has also been applied in other special cases: R. Abersold, E. J. Bures, M. Namchuck, M. H. Goghari, B. Shushan and T. C. Covey, *Protein Sci.*, 1992, **1**, 494; Z. Lam, B. Reinhold and V. Reinhold, *Abstr. Proc. 39th Am. Soc. Mass Spectrom. Conf. on Mass Spectrom. & Allied Topics*, Nashville, TN, June 1991, pp. 282-284; K. D. Henry, E. R. Williams, B. H. Wang, F. W. McLafferty, J. Shabanowitz and D. F. Hunt, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 9075.
- 9 T. J. Gilbertson and T. Ryan, *Synthesis*, 1982, 159; R. E. Berson, *Org. Synth.*, 1974, **51**, 121-127.
- 10 R. C. Cookson, S. S. H. Gilani and I. D. R. Stevens, *Tetrahedron Lett.*, 1962, 615; D. J. Aberhart and A. C. Hsu, *J. Org. Chem.*, 1976, **41**, 2098.
- 11 M. H. Allen and M. L. Vestal, *J. Am. Soc. Mass Spectrom.*, 1992, **3**, 18.

† Positive ion ESIMS spectra were obtained on a Vestec Model M-200 quadrupole ESIMS instrument<sup>11</sup> by infusing a ca.  $10^{-4}$  mol dm<sup>-3</sup> methanol solution at 3-5  $\mu$ l min<sup>-1</sup> with typical settings [ref. 6(b)].

‡ Additionally, we have prepared 4-(*N*-maleimido)benzo-15-crown-5, a stable easily handled compound. Although this reagent showed much less reactivity in the Diels-Alder reaction, it proved to be an excellent Michael acceptor. For example, derivatization of glutathione (GSH) affords an adduct which displays a peak at  $m/z = 693$  in the presence of Na<sup>+</sup> ions. Derivatization both enhances signals of GSH and makes them pH-independent.