Bioorganic & Medicinal Chemistry Letters, Vol.3, No.9, pp. 1841-1844, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
Pergamon Press Ltd

1α,24*S*-DIHYDROXY-26,27-CYCLO-22-YNE-VITAMIN D_3: THE SIDE CHAIN TRIPLE BOND ANALOGUE OF MC 903 (CALCIPOTRIOL)

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(*Received* 15 *January* 1993)

Abstract: The side chain propargylic alcohol function (established stereoselectively *via* *S*-Alpine-Borane^R reduction of ynone **8** and correlated with MC 903) in the title compound **1** replaces the metabolically labile allylic alcohol function of MC 903, a selective analogue of the vitamin D hormone used for treating psoriasis. **1** exhibits reduced *in vitro* activity but still shows selectively much lower *in vivo* calcemic effects.

Investigation of the effect of incorporating the (22*E*-) double bond of the vitamin D_2 side chain into compounds of the D_3 series remains a fruitful area of research.[1] The contribution of this feature in concert with the cyclopropane moiety has been shown to be crucial in conferring on the synthetic 1α,25-dihydroxy-vitamin D_3 [1,25-$(OH)_2D_3$] analogue MC 903[2] [calcipotriol (INN), calcipotriene (USAN)] (Fig. 1) a facile metabolic deactivation pathway that dramatically reduces its systemic activity relative to 1,25-$(OH)_2D_3$.[3] Thus, while MC 903 retains the potent cell differentiation inducing / proliferation inhibiting and immunological properties of the natural vitamin D hormone [*i.e.* 1,25-$(OH)_2D_3$] *in vitro*,[4] rapid hepatic metabolism involving initial oxidation to the inactive 24-ketone[5] explains why its *in vivo* calcemic effects in rats are less than 1% of that of 1,25-$(OH)_2D_3$.[6] This unique profile of activity provided a rational basis for the selection of MC 903 as a drug with an advantageous therapeutic index for the topical treatment of psoriasis, a hyperproliferative disease characterised by incomplete terminal differentiation of the epidermal keratinocytes, and the clinical value of MC 903, which reached the market in 1991, is well established.[7]

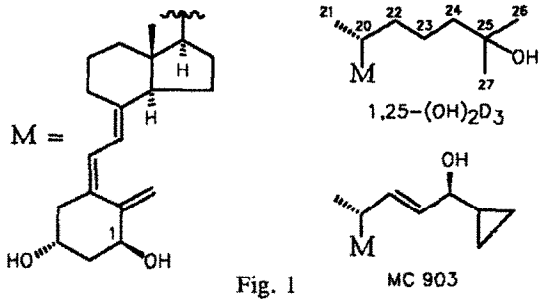
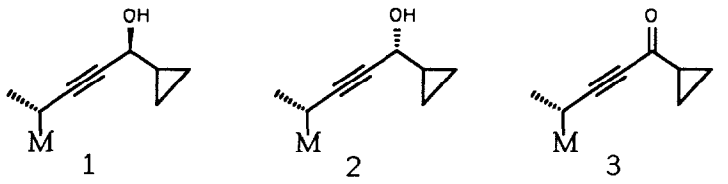
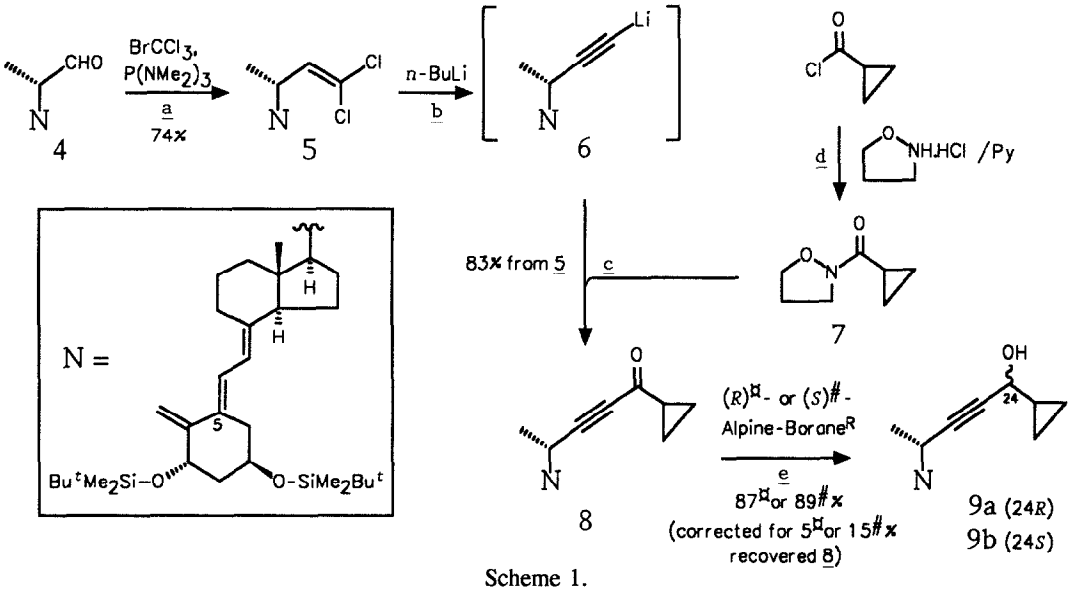


Fig. 1

For our systematic investigation of structure-function relationships in the MC 903 series, in particular the effect of side chain structure on the selective biological actions,[3] we have prepared analogues in which the ring size is changed, or the ring is opened; which have halogen substitution at C-25; which are modified at C-24 or the 24-OH; which have the inverted configuration at C-20;[8] or which differ in the nature of the 22,23-bond. With regard to the latter, while we have reported on the 22*Z*-isomer of MC 903[9] and the 22,23-dihydro-derivative,[3] the analogue incorporating a 22,23-triple bond is conspicuously missing.[10] We now report the synthesis and preliminary biological evaluation of this compound (**1**), together with the synthesis of its 24-epimer (**2**), and also the corresponding 24-ketone (**3**), an anticipated metabolite of **1** (and **2**) (*cf.* MC 903 and its 24-epimer[3]).

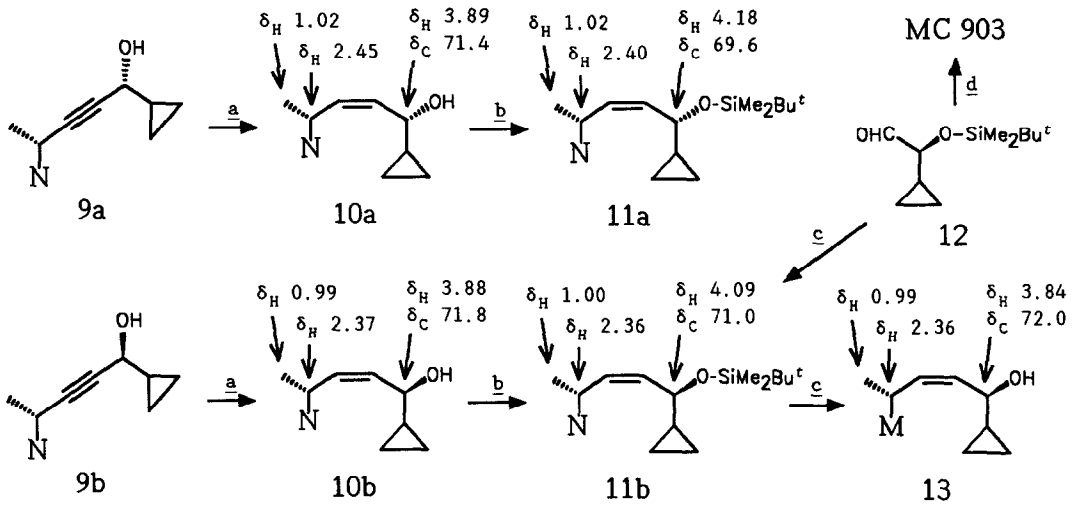


The retro-synthetic disconnection of the 23,24-bond of **1** finds its precedence in the partial syntheses of several 22-yne steroids,[11,12] including a synthesis of 24(ξ)-hydroxy-22-yne-cholesterol,[12] and conversion of the alcohol-protected 1α-hydroxy-(5*E*)-vitamin D C_{22}-aldehyde (**4**)[2] to the acetylene precursor **5** (mp 107-109 °C), and thence to **6**, was performed analogously to the literature reactions[11,13] (Scheme 1). Instead of the coupling of the acetylenic anion with an aldehyde followed by oxidation of the resulting propargylic alcohol[12,14] we elected to use the less commonly employed coupling with an activated carboxylic acid for the synthesis of the acetylenic ketone, and the acid isoxazolidide method[15] was successful. Thus, treatment of **6** *in situ* with a slight excess of cyclopropane carbonyl isoxazolidide **7** [bp 112-115 °C/15 mmHg, IR ν_{max} ($CHCl_3$) 1638 cm^{-1}] gave the desired intermediate **8** [mp 76-77 °C, IR ν_{max} (KBr) 1665, 2200 cm^{-1}] cleanly. Stereoselective reduction of the acetylenic ketone function in **8** using Midland's method[14] was performed using the commercially available Alpine-Borane^R (Aldrich) reagents. According to Midland's rule, the reaction of **8** with the reagent derived from *R*-pinene is predicted to give mainly **9a**, while *S*-Alpine-Borane^R should afford mainly the intermediate having the 24*S*-configuration of **1**, *viz.* **9b**. We were unable to determine directly the diastereoisomeric ratios in the respective reduction products since **9a** and **9b** had very



Scheme 1.

a. $P(NMe_2)_3$ (2.2 mol. equiv.) added dropwise at -20 °C to a CH_2Cl_2 solution of **4** and $BrCCl_3$ (1 mol. equiv.), whereafter the reaction is run for 2 h at r.t.; b. *n*-BuLi (2 mol. equiv.) added to a solution of **5** in THF at -78 °C. After 30 min the reaction solution was warmed momentarily to -10 °C and then recooled to -78 °C, whereupon: c. 1.2 mol. equiv. **7** was added, and the solution allowed to warm to about -40 °C over 20 min before quenching with wet ether. d. 1.1 mol. equiv. isoxazolidine hydrochloride; 2.2 mol. equiv. pyridine, CH_2Cl_2, 30 min at -10 °C. e. **8** was dissolved in Alpine-Borane^R (0.5 M solution in THF, 2 mol. equiv.) and the solution concentrated to a syrup *in vacuo*; the reaction was quenched after 72 h at r.t. by the addition of acetaldehyde and reconcentrated *in vacuo* before a work-up that involved dissolving in petroleum ether, precipitation with ethanolamine (2.5 mol. equiv.) and isolation by direct chromatographic purification of the filtrate.



Scheme 2.

a. H_2 (1 atm), Lindlar catalyst, quinoline, hexane, r.t., 1 h; b. *t*-BuMe$_2$SiCl, imidazole, DMF, r.t., 1 h; c. Ref. 9; d. Ref. 3.

similar retention times on analytical HPLC and moreover had superimposable NMR spectra. The derived (+)-MTPA esters[16] were however resolvable, and both reduction products were after derivatisation found to consist of *ca.* 90:10 mixtures (HPLC) of 24-epimers, the ratios being complementary.

Correlation of the 24*S*-isomer **9b** with a reference compound of established 24-configuration was achieved as shown in Scheme 2. Lindlar hydrogenation of each Alpine-Borane reduction product was rapid, quantitative, and gave rise to one major and one minor 22*Z*-allylic alcohol (**10**). These compounds, which were distinguishable on TLC, were shown by analytical HPLC to be produced in a *ca.* 90:10 (or 10:90) ratio, confirming the diastereoisomeric ratio deduced from analysis of the (+)-MTPA esters. NMR comparison with the 22*Z*-isomer of MC 903[9] (**13**) already suggested that the characteristic side chain signals observed for the major product in the *S*-Alpine-Borane series could be correlated with compound **10b** (as indicated), but in order to provide a direct comparison with a known compound, both alcohols **10** were converted to their silyl ethers **11**. These compounds showed significant differences in their ^{1}H- and ^{13}C-NMR spectra (notably *C*-24), and, as anticipated, the *S*-Alpine-Borane series major compound **11** was found to be identical to the described intermediate **11b** used in the synthesis of 22*Z*-MC 903 (**13**). In that synthesis,[9] the aldehyde **12** was used to build up the side chain, and since **12** has also been used in a stereoselective synthesis of MC 903,[3] the 24*S*-configuration is confirmed. The observed stereoselectivities for the ynone reductions, **8** → **9**, are thus in accord with the predictions based on Midland's rule.[14]

The amount of 24*R*-isomer **9a** contaminating the 24*S*-isomer **9b** in the *S*-Alpine-Borane reduction product was reduced to <5% by recycle chromatography (analysis of fractions after Lindlar reduction of an aliquot) prior to the next step in the synthesis, while the *R*-Alpine-Borane reduction product (**9a** contaminated with 10% **9b**) was used without further purification. The standard sequence ("N" → "M")[2,9] of triplet-sensitised 5*E* to 5*Z* photo-isomerisation (hν, anthracene, Et_3N, toluene, r.t., 1 h) followed by desilylation with *n*-$Bu_4N^+F^-$ (4 mol. equiv., THF, 55 °C, 1 h) converted the intermediates **9b** and **9a** to the target compounds **1** and **2**,[17] in *ca.* 60% yields respectively. The intermediate **8** was similarly converted to target compound **3**,[17] except that an alternative method (HF, H_2O, MeCN, r.t., 1 h) (*cf.*[5]) was employed for the desilylation step.

In the preliminary biological screening (performed using the methods previously described[6]), **1** was found to be only about 1/10 as potent as 1,25-$(OH)_2D_3$ (or MC 903[6]) in inducing cancer cell (U 937) differentiation and inhibiting cell proliferation *in vitro* and had similarly reduced binding affinity for the hormone receptor [as measured by its ability to displace radiolabelled 1,25-$(OH)_2D_3$ bound to the chicken intestinal receptor]. *In vivo*, **1** had no effects on calcium homeostasis in rats dosed with up to 100 μg/kg daily for 7 days [1,25-$(OH)_2D_3$ produces marked hypercalciuria at 0.5 μg/kg]. Metabolism studies are in progress.

Acknowledgements

We appreciate the contribution of Ms. L. Poteur for enthusiastically helping with much of the experimental work while on an IAESTE student exchange visit. We thank Dr. L. Binderup for the biological screening, Mr. N.R. Andersen for spectroscopic services, and Ms. P.A.M. Madsen for excellent technical assistance.

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13. An excess of *n*-BuLi should be avoided since it converts **6** into its 19-butyl-isovitamin D derivative during the warming-up, diminishing the yield of **8**; *cf.* Calverley, M.J. *Tetrahedron Lett.* **1986**, *27*, 4903.
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17. **1, 2**: UV (EtOH): λ_{max} 264 nm (ϵ 17500), λ_{min} 228 nm (ϵ 10100); NMR: ($CDCl_3$, $SiMe_4$) δ_H (300 MHz) (*J* in Hz) 0.35-0.58 [m, 7 H, including 0.56 (s, 3 H, 18-H_3), 26-H_2, 27-H_2], 1.19 (d, *J* = 6.9, 3 H, 21-H_3), 2.31 (dd, *J* = 7 + 13, 1H, 4β-*H*), 2.48 (m, 1 H, 20-*H*), 2.59 (dd, *J* = 3 + 13, 1 H, 4α-*H*), 2.84 (bd, *J* = 11, 1 H, 9β-*H*), 4.23 (m, 1 H, 3-*H*), 4.28 (m, 1 H, 24-*H*), 4.43 (m, 1 H, 1-*H*), 5.00 (br s, 1 H, 19*E*-*H*), 5.33 (br s, 1 H, 19*Z*-*H*), 6.02 and 6.37 (each: d, *J* = 11.3, 1 H, 7-*H* and 6-*H*) ppm; δ_C (75.5 MHz) 1.0, 2.9 (*C*-26, 27), 12.2 (*C*-18), 17.0 (*C*-25), 21.3 (*C*-21), 22.0 (*C*-15), 23.1 (*C*-11), 26.2 (*C*-16), 27.5 (*C*-20), 28.8 (*C*-9), 39.4 (*C*-12), 42.6 (*C*-2), 45.0 (*C*-4), 45.6 (*C*-13), 55.7, 55.8 (*C*-14, 17), 65.6 (*C*-24), 66.6 (*C*-3), 70.6 (*C*-1), 79.0, 90.0 (*C*-22, 23), 111.7 (*C*-19), 117.1 (*C*-7), 124.6 (*C*-6), 133.0 (*C*-5), 142.4 (*C*-8) and 147.4 (*C*-10). **3**: UV (EtOH): λ_{max} 264 nm (ϵ 18100), λ_{min} 245 nm (ϵ 16300); NMR: data exactly as quoted above, except: δ_H 0.58 (18-H_3), 1.00 and 1.20 (each: m, 2 H, 26-H_2 and 27-H_2), 1.27 (21-H_3), 2.64 (m, 1 H, 20-*H*); δ_C 10.4, 10.4 (*C*-26, 27), 20.3 (*C*-21), 22.0 (*C*-15), 23.0 (*C*-11), 24.2 (*C*-25), 26.1 (*C*-16), 27.8 (*C*-20), 28.7 (*C*-9), 39.3 (*C*-12), 55.1, 55.5 (*C*-14, 17), 79.3, 97.7 (*C*-22, 23), 111.6 (*C*-19), 117.2 (*C*-7), 124.5 (*C*-6), 133.2 (*C*-5), 141.8 (*C*-8), and 188.6 (*C*-24).