Bisphosphonate prodrugs: unusual dimerisation of clodronic acid trimethyl ester to a cyclic bis(bisphosphonate)

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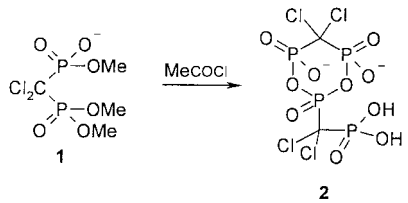
$Cl_2C[P(O)(OMe)_2P(O)(OMe)(O^-Z^+)]$ selectively reacts with acetyl chloride to provide a new enzymatically stable heterocyclic bis(bisphosphonate); the structure is confirmed by X-ray crystallography.

Methylenebisphosphonates (MBP), such as clodronate (Cl_2MBP), are an important class of drugs which have proven to be effective in the treatment of various diseases of bone and calcium metabolism including Paget's disease, non tumor-induced hypercalcaemia, and osteoporosis.[1] Recently, we reported[2] the synthesis and *in vitro* evaluation of clodronic acid dianhydrides as bioreversible prodrugs of clodronate. Exploration[3] of new strategies to prepare clodronate anhydrides lead us to a discovery of the selective synthesis of a new cyclic bis(bisphosphonate) anion **2**. The prepared dimer is the first approach to the self-prodrug of clodronate in which the number of promoities are minimised. We report here the selective synthesis and the X-ray structure of a stable cyclic dimer of clodronate, and its stability in aqueous buffer and human plasma.

Triester **1** (0.206 mmol), prepared by known method,[4] reacted selectively with acetyl chloride (2.196 mmol) in dry acetonitrile (4.0 ml) under reflux for 2 h to give a cyclic bis(bisphosphonate). The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 and extracted once with cold water to give $[NMeBu_3]_2$**2**† as a colourless oil in 92% yield after evaporation of the aqueous phase (Scheme 1). The backbone structure was assigned by ^{31}P NMR spectroscopy, where peaks with intensities of 1:2:1 appeared as two doublets at δ 1.78 ($^2J_{PP}$ 16.9 Hz) and −2.17 ($^2J_{PP}$ 42.6 Hz), and a triplet of doublets at δ −17.58 (ring phosphorus). This structure was confirmed by X-ray crystallography‡ (Fig. 1).

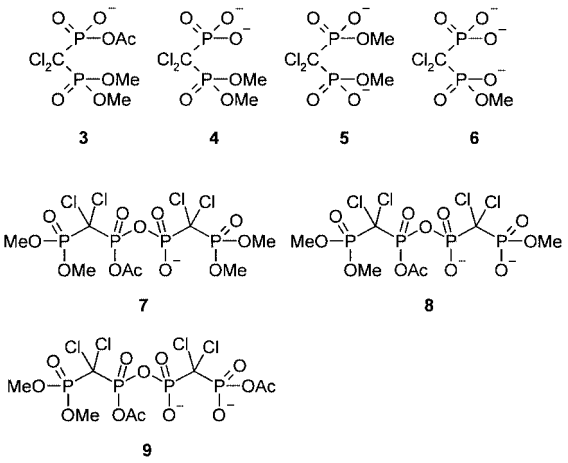
This type of selective and quantitative cyclisation reaction is rather unusual.[5–8] According to NMR studies, the reaction starts with the removal of the methyl group (first step) as MeCl from the anionic phosphorus **1** to form monoacetyl compound **3**. The formation of anionic bisphosphonates **4**–**9** was also detected during the reaction by 2D ^{31}P NMR P,P-COSY. However, all these species led to selective formation of **2**.

X-Ray diffraction study of $[NMeBu_3]_2$**2** showed a strained six-membered ring as a consequence of the O13–P1–C1 and O23–P2–C1 angles of 98.8°, 6° smaller than for acyclic derivatives.[9] The bond angle distortion of P1–O13–P3 is *ca.* 15° wider than in non-cyclic derivatives.[9] The stability of the cyclic structure is likely due to the short hydrogen bonds between



1 **2**

Scheme 1 Preparation of **2**.



3 **4** **5** **6**

7 **8**

9

O12–O43 and O21–O42. Moreover, the two oxygens bonds, O11(O22) and O12(O21) at P1(P2), are short (1.466 and 1.490 Å) indicating a strong double bond character for both bonds. Other bond lengths are within normal ranges.

The usefulness of the cyclic structure as a prodrug was investigated in aqueous buffer and human plasma. Ring **2** was cleaved to an acyclic dimer **10** (Scheme 2) in 50 mM aqueous

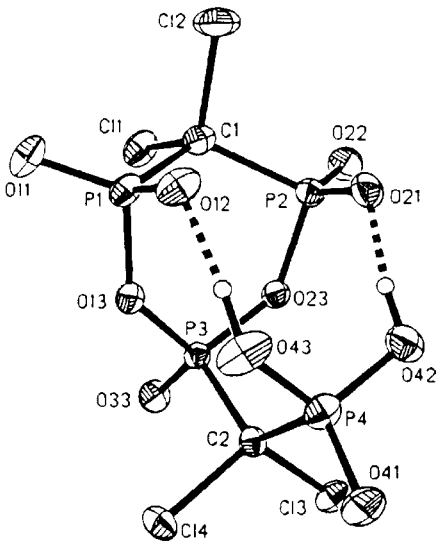
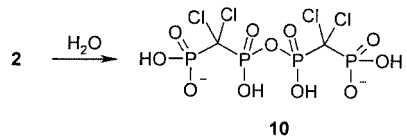


Fig. 1 Crystal structure of the anion **2**.



10

Scheme 2

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phosphate buffer solution at 37 °C. The half-lives for chemical degradation of **2** were 60 min (pH 5.0) and 63 min (pH 7.4). Further hydrolysis of dimer **10** to clodronate was not observed during 9 h at pH 7.4. Compounds **2** and **10** are resistant to enzymatic hydrolysis, probably because the bridging carbon prevents[10] stepwise hydrolysis, which is generally observed for terminal phosphates.

Notes and references

† *Spectroscopic data* for 1,1-dichloro-1-(5,5-dichloro-4,6-dihydroxy-2,4,6-trioxo-2λ^5,4λ^{5}6λ^5-[1,3,2,4,6]dioxatriphosphinan-2-yl)methylphosphonic acid bis(tributyl(methyl)ammonium) salt $[NMeBu_3]_2$**2**: δ_H(400.1 MHz, CD_3COCD_3): 3.48 (m, 12H, NCH_2), 3.24 (s, 6H, NCH_3), 1.83 (m, 12H, NCH_2CH_2), 1.44 (m, 12H, CH_2CH_3), 0.99 (t, 18H, *J* 7.2 Hz, CH_2CH_3); δ_P(162.0 MHz, CD_3COCD_3): 1.78 (d, $^2J_{PP}$ 16.9 Hz), −2.17 (d, $^2J_{PP}$ 42.6 Hz), −17.58 (td, $^2J_{PP}$ 16.9, $^2J_{PP}$ 42.6 Hz); δ_C(100.6 MHz, CD_3COCD_3): 62.16 (CH_2), 48.95 (CH_3) 24.84 (CH_2), 20.39 (CH_2) 14.00 (CH_3); ES-MS *m/z* 435.1 (M − 2MeN$^+$Bu$_3$ − H_2O + 2H$^+$). Anal. calc. for $C_{28}H_{62}Cl_4N_2O_{10}P_4 \cdot 0.25H_2O$: C, 39.62; H, 7.41; N 3.24. Found: C, 39.84; H, 7.46; N 3.26%.

‡ *Crystal data* for $C_{28}H_{62}Cl_4N_2O_{10}P_4 \cdot 0.25H_2O$, $[NMeBu_3]_2$**2**$\cdot 0.25H_2O$: colorless single crystals were obtained by slow air evaporation of ethyl acetate–acetone solution, M = 856.98, triclinic, space group $P\bar{1}$, a = 11.3054(4), b = 11.5193(4), c = 17.8036(7) Å, U = 2067.68(13) Å^3, T = 120 K, Z = 2, λ = 0.71073 Å, μ(Mo-Kα) = 0.492 mm^{-1}, 17 094 reflections measured, 8667 unique (R_{int} = 0.0326). Final R_1 = 0.0371, wR_2 = 0.0828 (for 8667 data). CCDC 182/1575. See http://www.rsc.org/suppdata/cc/b0/b000558o/ for crystallographic data in .cif format.

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