Strategies for the Preparation of (1-Acetyloxyethylidene)-1,1-bisphosphonic Acid Derivatives

Petri A. Turhanen,* Jouko J. Vepsäläinen

University of Kuopio, Department of Chemistry, P.O. Box 1627, 70211 Kuopio, Finland
Fax +358(17)163259; E-mail: Petri.Turhanen@uku.fi

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Abstract: The novel strategies for the synthesis of acetylated etidronic acid derivatives were investigated. (1-Acetyloxyethylidene)-1,1-bisphosphonic acid and its P,P′-dimethyl, trimethyl and tetramethyl esters were prepared in high 81–100% yields.

Key words: bisphosphonate, etidronate, partial esters, synthesis, prodrug

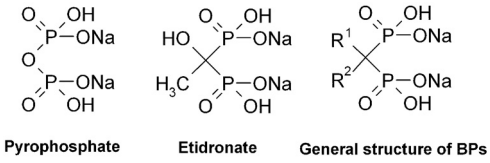
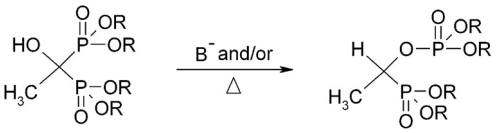


Figure 1

Chemically and enzymatically stable bisphosphonates (BPs), characterized by a P–C–P bridge, are analogs of naturally occurring pyrophosphate and used as drugs in treatment of various bone diseases, like osteoporosis (Figure 1).[1] They are tetra acidic compounds, unfortunately very hydrophilic and their bioavailabilities are very poor.[2] It is of great interest to prepare more lipophilic biodegradable derivatives of BPs. A rather straightforward method to improve lipophilicity is to mask one or more acidic P(O)–OH groups with ester functionality by using the prodrug approach.[3] Most of the clinically used BPs are so called hydroxybisphosphonates where the bridging carbon contains the OH group, which can be also derivatized with, e.g., ester function to improve lipophilicity. (1-Hydroxyethylidene)-1,1-bisphosphonic acid (HEBPA) disodium salt (etidronate) is an example of this kind of compound (Figure 1).

Our group has designed, synthesized and studied in vitro several different etidronic acid ester and amide derivatives to act as biodegradable prodrugs of etidronate.[4] We have previously reported that simple HEBPA tetra- and partial methyl esters do not act as prodrugs of etidronate, but acetylated pivaloyloxymethyl derivatives of HEBPA released etidronate in vitro.[4b] There are two main limitations in the synthesis of etidronate derivatives: rearrangement and poor solubility. The rearrangement of the P–C(OH)–P structure to a P–C–O–P structure occurs in the reaction mixture at temperatures over 60 °C and in basic conditions, even in phosphate buffer at pH 7.4 (physiological pH) (Scheme 1).[4b,d] Due to the high hydrophilicity of the BP tetraacids, their reactivity and solubility in organic solvents is very low.

Here we report that acetylated compounds are solutions to both of these problems. These compounds are used as model molecules and starting materials for our ongoing study to prepare new bioreversible derivatives of BPs. Tri- and dimethyl esters of HEBPA were chosen because of easy remove of methyl groups after molecule modification using alkalimetal salts or silylation method.[4b,d]



Scheme 1 Rearrangement of HEBPA tetraesters to tetraalkyl phosphono phosphate

The preparation of **1**–**4** was straightforward and the products were easily separated and purified. Synthesis of compounds **1**–**4** with observed yields is presented in Scheme 2. We have previously reported the preparation of **1** from **6** by stirring in excess acetyl chloride at 55 °C, but only with 46% yield after isolation.[4b] We examined milder reaction conditions to obtain better yields. The tetramethyl ester **6** does not react in excess Ac_2O or Ac_2O/HOAc (1:1) at 60 °C. If one equivalent of sodium acetate or an even milder base such as trichloroacetic acid sodium salt (0.1 equivalents) was used as the catalyst in Ac_2O, the product mixture contains in both cases 82% of **1**, but unfortunately also rearranged **6** (18%). The optimal conditions to quantitative formation of **1** were found when ca. five equivalents of acetyl chloride (AcCl) were used in acetic anhydride at 55 °C. Synthesis of compound **3** from **6** via **1** has also been reported previously by us, but the difficulty in this route is the formation of a gelatinous reaction mixture, making evaporation of solvent difficult. The alternative route to **3** from **6** via **8** was found. Product **3** was easily separated and the purity of the compound was better than compared to other methods.

A route for the preparation of new triester compound **2** was also examined. There was no selectivity in the preparation of **2** from **1** as there was for the synthesis of **7** from **6**, which we have reported previously, indicating that a free hydroxyl group is needed for the selective mono-

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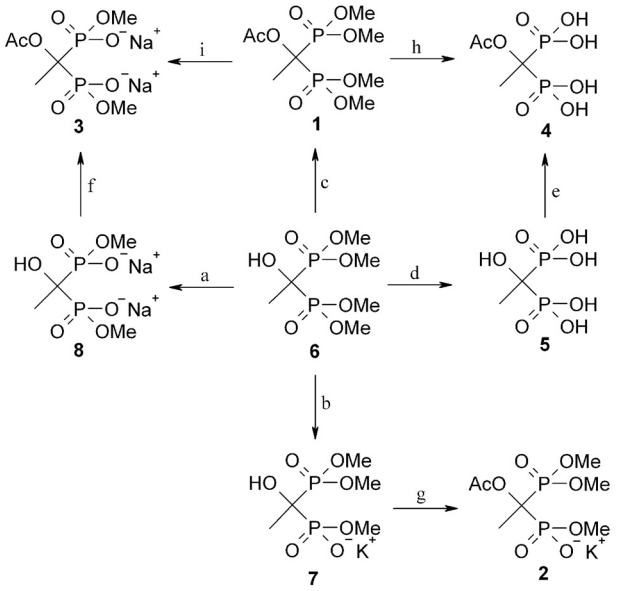

Scheme 2 *Reaction conditions:* a) 2 equiv NaI in acetone, 94%; b) 1 equiv KI in acetone, 88%; c) 5 equiv AcCl in Ac_2O, 100% or AcCl, 46%; d) concd HCl, 100%; e) Ac_2O, 84%; f) Ac_2O–HOAc (1:1), 83%; g) Ac_2O, 81%; h) ca. 4 equiv $(CH_3)_3SiCl$/NaI in CH_3CN, then MeOH, 100%; i) 2 equiv NaI in acetone, 98%

dealkylation of tetraesters.[4d] Selective acetylation of **7** to target compound **2** was observed in excess Ac_2O.

Acetylated etidronic acid **4** has been reported to be one of the products described by Prentice et al.[5] in their experiments, but its synthesis and separation required several steps and two recrystallizations for purification. Our strategy to obtain **4** was more straightforward. The synthesis started from tetramethyl ester **6**, which was first hydrolyzed by concentrated HCl and crystallized from acetic acid to give a solid etidronic acid (**5**) in quantitative yield. Compound **5** was then stirred in excess Ac_2O at 60 °C for 46 hours to give **4** in 84% yield after isolation. (1-Acetyloxyethylidene)-1,1-bisphosphonic acid (**4**) can also be prepared by a silylation method from **1** as described earlier.[4b]

Formation of the acetylated products **1**–**4** during the reactions was easily detected from ^1H and ^{31}P NMR spectra. For example, the methyl protons on the P–C–P backbone for compound **8** give rise to a characteristic triplet at 1.53 ppm, which shifts to a higher ppm value after acetylation (1.73 ppm for **3**), like for the other acetylated derivatives. In the ^{31}P NMR spectra the signal shifts to a lower ppm value after acetylation [e.g. for **6** from 22.85 ppm to 20.15 ppm (**1**)]. Another characteristic finding was the lower $^2J_{PP}$ coupling constant obtained for the new compound **2** (23.6 Hz as compared to 34.4 Hz for the starting material **7**).

In conclusion, an alternative route for the selective preparation of (1-acetyloxyethylidene)-1,1-bisphosphonic acid P,P′-dimethyl ester (**3**) was found. Improvement in the yield from 46% to 100% in the preparation of the acetylated tetramethyl ester of etidronate (**1**) was observed. Synthesis of (1-acetyloxyethylidene)-1,1-bisphosphonic acid trimethyl ester (**2**) in 83% yield is reported for the first time. The selective synthesis of (1-acetyloxyethylidene)-1,1-bisphosphonic acid (**4**) in 84% yield from the etidronic acid tetramethyl ester (**6**) is also described. The acetylated derivatives **1**–**4** are used as starting materials and model compounds in our ongoing study of the preparation of new BP prodrugs.

Solvents were HPLC grade and dried before use. Tubes filled with anhydrous $CaCl_2$ were used to protect reactions from humidity unless otherwise stated. ^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.1, 202.5 and 125.8 MHz, respectively. TMS or TSP (for D_2O solutions) was used as an internal standard for ^1H and ^{13}C measurements, and 85% H_3PO_4 was used as an external standard for ^{31}P measurements. The $^nJ_{HP}$ coupling constants were calculated from proton spectra and all J values are given in Hz. The number of protons on each carbon were detected from DEPT-135 experiments and marked after each carbon by the letters d (doublet), t (triplet), q (quartet) or qt (quintet). The $^nJ_{CP}$ coupling constants were calculated from carbon spectra with the coupling constants given in Hz. In the case of symmetric structure only the sums of the J_{CP} couplings (ΣJ_{CP}, the width of the virtual triplet) are given, since the satellite lines were unambiguous to detect from the background. The purity of products was determined from ^1H and ^{31}P NMR spectra and was 95%.

(1-Acetyloxyethylidene)-1,1-bisphosphonic Acid Tetramethyl Ester (1)

Compound **6** (212 mg, 0.81 mmol) was dissolved in Ac_2O (2 mL) and AcCl (300 μL, 4.22 mmol) was added. The mixture was stirred at 55 °C for 26 h and evaporated in vacuo to give **1** (247 mg, 100%) as a colorless syrup.

^1H NMR ($CDCl_3$): δ = 3.91–3.86 (m, 12 H), 2.14 (s, 3 H), 1.91 (t, 3 H, $^3J_{HP}$ = 15.7 Hz).

^{13}C NMR ($CDCl_3$): δ = 168.75 (t; $^3J_{CP}$ = 7.9 Hz), 79.13 (t; $^1J_{CP}$ = 155.4 Hz), 54.73 (qt; J_{CP} = 6.6 Hz), 54.33 (qt; J_{CP} = 6.9 Hz), 21.28 (q), 18.57 (qt; $^2J_{CP}$ = 2.5 Hz).

^{31}P NMR ($CDCl_3$): δ = 20.15.

[1-(Dimethoxyphosphoryl)-1-acetyloxyethyl]-1-phosphonic Acid Monomethyl Ester Monopotassium Salt (2)

Compound **7** (305 mg, 1.07 mmol) and Ac_2O (5 mL) were stirred at 60 °C for 5 h and evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 (2 mL), Et_2O (5 mL) and hexanes (10 mL) was added with vigorous stirring. The solution was removed and the remaining syrup was washed with hexanes and Et_2O. The residue was dried for 6 h in vacuo to give **2** (283 mg, 81%) as a white solid.

^1H NMR ($CDCl_3$): δ = 3.83 (d, 3 H, $^3J_{HP}$ = 10.6 Hz), 3.79 (d, 3 H, $^3J_{HP}$ = 10.7 Hz), 3.68 (d, 3 H, $^3J_{HP}$ = 10.0 Hz), 2.09 (s, 3 H), 1.81 (dd, 3 H, $^3J_{HP}$ = 13.1 Hz, $^3J_{HP'}$ = 16.4 Hz).

^{13}C NMR ($CDCl_3$): δ = 169.66 (dd; $^3J_{CP}$ = 6.7 Hz, $^3J_{CP'}$ = 6.4 Hz), 81.43 (dd; $^1J_{CP}$ = 135.7 Hz, $^1J_{CP'}$ = 135.7 Hz), 53.97 (qd; $^2J_{CP}$ = 7.2 Hz), 53.92 (qd; $^2J_{CP}$ = 7.3 Hz), 53.62 (qd; $^2J_{CP}$ = 6.4 Hz), 21.68 (q), 20.03 (q).

^{31}P NMR ($CDCl_3$): δ = 24.70 (d, $^2J_{PP}$ = 23.6 Hz), 11.15 (d).

(1-Acetyloxyethylidene)-1,1-bisphosphonic Acid P,P′-Dimethyl Ester (3)

Compound **8** (800 mg, 2.88 mmol), AcOH (3 mL) and Ac_2O (3 mL) were stirred at 55 °C for 19 h and evaporated to dryness in vacuo. The residue was dissolved in MeOH (5 mL), then *i*-PrOH (5 mL) was added and the mixture was heated to reflux. After the addition

of H_2O (600 μL), reflux was stopped, *i*-PrOH (1 mL) was added, and the mixture was placed in the freezer overnight. The resulting precipitate was filtered, washed with *i*-PrOH and dried in vacuo to give **3** (763 mg, 83%) as a slightly yellow powder.

^{1}H NMR (CD_3OD): δ = 3.68–3.65 (m, 6 H), 2.06 (s, 3 H), 1.73 (t, 3 H, $^3J_{HP}$ = 13.7 Hz).

^{13}C NMR (D_2O): δ = 175.31 (t; $^3J_{CP}$ = 7.1 Hz), 85.27 (t; $^1J_{CP}$ = 144.7 Hz), 54.73 (qt; J_{CP} = 6.3 Hz), 24.54 (q), 22.00 (t).

^{31}P NMR (D_2O): δ = 17.30.

(1-Acetyloxyethylidene)-1,1-bisphosphonic Acid (4)

Etidronic acid (**5**, 4.0 g, 19.80 mmol) and Ac_2O (15 mL) were stirred at 60 °C for 46 h; Et_2O (50 mL) was added and the mixture was placed in the freezer for 1 h. The precipitate was filtered, washed with Et_2O and dried in vacuo to give **4** (4.05 g, 84%) as a white powder.

^{1}H NMR (D_2O): δ = 2.15 (s, 3 H), 1.84 (t, 3 H, $^3J_{HP}$ = 15.3 Hz).

^{13}C NMR (D_2O): δ = 175.41 (t; $^3J_{CP}$ = 7.5 Hz), 81.76 (t; $^1J_{CP}$ = 143.3), 23.87 (q), 20.84 (t).

^{31}P NMR (D_2O): δ = 16.87.

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References

(1) (a) Fleisch, H. *Bisphosphonates in Bone Disease: From the Laboratory to the Patient*; The Parthenon Publishing Group Inc.: New York, **1995**. (b) Papapoulos, S. E.; Landman, J. O.; Bijvoet, O. L. M.; Löwik, C. W. G. M.; Valkema, R.; Pauwels, E. K. J.; Vermeij, P. *Bone* **1992**, *13*, S41. (c) Yates, A. J.; Rodan, G. A. *Drug Discovery Today* **1998**, *3*, 69. (d) Socrates, E.; Papapoulos, M. D. *Am. J. Med.* **1993**, *95*, 48S. (e) Giannini, S.; D'Angelo, A.; Sartori, L.; Passeri, G.; Garbonare, L. D.; Crebaldi, C. *Obstet. Gynecol.* **1996**, *88*, 431.

(2) (a) Vepsäläinen, J. J. *Curr. Med. Chem.* **2002**, *9*, 1075. (b) Lin, J. H. *Bone* **1996**, *18*, 75. (c) Recker, R. R.; Saville, P. D. *Toxicol. Appl. Pharmacol.* **1973**, *24*, 580.

(3) (a) Sinkkula, A. A.; Yalkowsky, S. H. *J. Pharm. Sci.* **1975**, *64*, 181. (b) Stella, V. J.; Charman, W. N. A.; Naringregar, V. H. *Drugs* **1985**, *29*, 455. (c) Balant, L. P.; Doelker, E.; Buri, P. *Eur. J. Drug Metab. Pharmacokinet.* **1990**, *15*, 143. (d) Waller, D. G.; George, C. F. *Br. J. Clin. Pharmacol.* **1989**, *28*, 497.

(4) (a) Turhanen, P. A.; Niemi, R.; Peräkylä, M.; Järvinen, T.; Vepsäläinen, J. J. *Org. Biomol. Chem.* **2003**, *1*, 3223. (b) Niemi, R.; Turhanen, P.; Vepsäläinen, J.; Taipale, H.; Järvinen, T. *Eur. J. Pharm. Sci.* **2000**, *11*, 173. (c) Turhanen, P. A.; Ahlgren, M. J.; Järvinen, T.; Vepsäläinen, J. J. *Phosphorus, Sulfur Silicon Relat. Elem.* **2001**, *170*, 115. (d) Turhanen, P. A.; Ahlgren, M. J.; Järvinen, T.; Vepsäläinen, J. J. *Synthesis* **2001**, *4*, 633.

(5) Prentice, J. B.; Quimby, O. T.; Grabenstetter, R. J.; Nicholson, D. A. *J. Am. Chem. Soc.* **1972**, *94*, 6119.