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Bisphosphonate prodrugs: synthesis and in vitro evaluation of novel partial amides of clodronic acid

Riku Niemi [a,*], Heli Pennanen [b], Jouko Vepsäläinen [b], Hannu Taipale [a], Tomi Järvinen [a]

[a] *University of Kuopio, Department of Pharmaceutical Chemistry, P.O. Box 1627, Kuopio, FIN-70211, Finland*
[b] *University of Kuopio, Department of Chemistry, P.O. Box 1627, Kuopio, FIN-70211, Finland*

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Abstract

Novel partial amides of clodronic acid were synthesized and evaluated in vitro for their properties as bioreversible prodrugs of clodronate. The hydrolysis studies indicated that these derivatives release the parent drug via chemical hydrolysis. Monoamides were hydrolysed rapidly ($t_{1/2} = 16–19$ min at pH 7.4) to clodronic acid, which suggests that they are useful intermediates in the design of enzymatically labile double prodrugs of clodronate.

Keywords: Prodrugs; Clodronate; Amides; Bisphosphonates

1. Introduction

Bisphosphonates (BPs), including clodronate, inhibit the formation, aggregation and dissolution of calcium phosphate crystals (Francis, 1969; Fleisch et al., 1970; Russell et al., 1970). BPs are widely used in the treatment of various bone diseases (e.g., osteoporosis and Paget's disease) and the hypercalcemia of malignancy (Fleisch, 1995). However, the therapeutic use of clodronate (like other BPs) is hindered by its very poor oral bioavailability; only 1–2% of the oral dose is absorbed from the gastro-intestinal tract (Yakatan et al., 1982; Pentikäinen et al., 1989) and bioavailability also shows great inter- and intraspecies variation (Fleisch, 1995). The poor oral bioavailability of clodronate is mostly attributed to its very low lipophilicity due to high ionization (Elks and Ganellin, 1990) at physiological pH values (pK_a values of clodronic acid are 1.70, 2.13, 5.66 and 8.30). Also, complexation with divalent cations in the intestinal lumen hinders the absorption of clodronate (Lin, 1996). It is

* Corresponding author. Tel.: + 358 17 163445; fax: + 358 17 162456; e-mail: riku.niemi@uku.fi

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thought that due to low lipophilicities, BPs are absorbed passively by the paracellular route, which is hindered by the relatively large molecular size of BPs (Boulenc et al., 1993; Lin et al., 1994; Twiss et al., 1994).

An oral formulation of clodronate with improved absorption properties would be of significant benefit, and the prodrug technique could be one approach to reach this goal. Masking one or more ionizable groups of clodronate by the prodrug approach would increase the lipophilicity of the drug and could also decrease its complexation with divalent cations. It may be postulated that if the logP-value of a prodrug of clodronic acid is not raised enough to enhance passive transcellular transport, the increased size of the prodrug might even diminish passive paracellular transport, and therefore a significant increase in lipophilicity is necessary to change the absorption from paracellular to transcellular pathway. According to the principles of prodrug technique, a prodrug should release the active drug in the body after absorption (Stella et al., 1985; Waller and George, 1989).

Reports concerning the design of prodrugs of BPs, especially of clodronate, have very rarely been published, which is most probably due to the complicated synthetic chemistry and the problematic chemical structure of BPs. Recently, we have reported that the simple alkyl/aryl esters of clodronic acid do not release the parent drug via chemical or enzymatic hydrolysis and are, therefore, not prodrugs of clodronate (Niemi et al., 1997). Thus, there still exists the need for design of bioreversible clodronate prodrugs.

The aim of the present study was to synthesize novel partial amides of (dichloromethylene)bisphosphonate, and evaluate in vitro their properties as possible bioreversible prodrugs of clodronate.

2. Methods

2.1. HPLC measurements

HPLC determinations were performed with a Merck LaChrom HPLC system consisting of Model L-7250 programmable autosampler, Model L-7100 HPLC pump, Model D-7000 interface module and Model D-7000 HPLC system manager (Hitachi, Tokyo, Japan) and a Sedex 55 evaporative light scattering detector (Sedere, Vitry-Sur-Seine, France). The samples were analyzed by the method described previously (Niemi et al., 1997).

2.2. Syntheses

Syntheses of the title compounds (1–4) were accomplished according to Scheme 1. The starting materials, compounds 5a–d, were prepared from the desired monophosphorus species according to the reported procedure (Vepsäläinen et al., 1997).

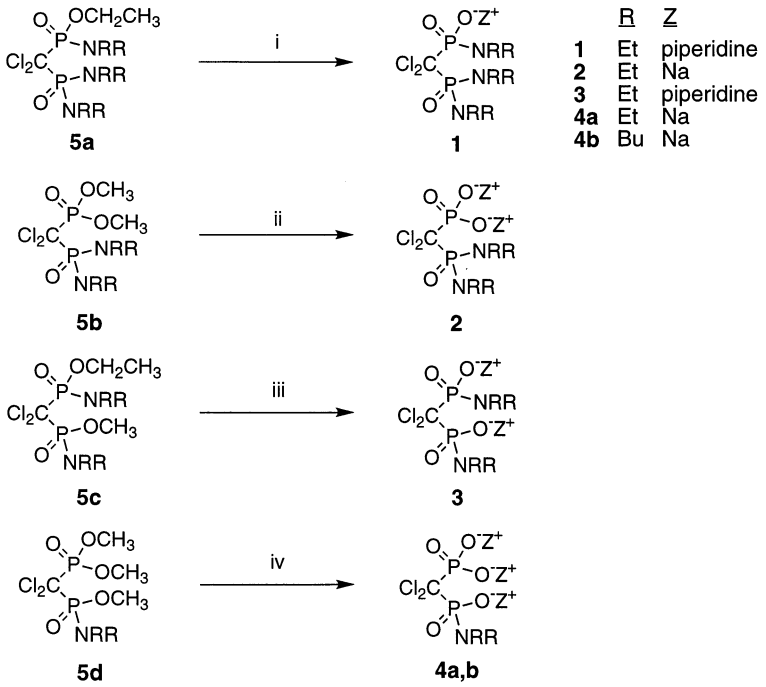
Compounds 5a and 5c were allowed to react with piperidine at elevated temperature to give compounds 1 and 3, respectively. The excess of piperidine was evaporated at reduced pressure, and the residues were crystallized from ether to obtain 1 and 3 as white crystals. Compounds 2 and 4 were prepared by silylating ester bonds of 5b and 5d. After reaction the residue was evaporated to dryness, and stirred for 30 min with methanol. After stirring the mixture was cooled to 0°C and pH was adjusted to 9 with 1 N NaOH solution. The product was crystallized from water/methanol-mixture. The NMR data of the target compounds are given in Table 1.

2.3. Hydrolysis in aqueous solution

The hydrolysis of compounds 1–4 was studied in phosphate buffer solutions (50 mM, $\mu = 0.15$, pH 7.4 or 5.0) at 37°C. Compounds were dissolved in pre-heated phosphate buffer (initial concentrations 1.9–2.8 mM). The solutions were placed in a thermostated water-bath and at suitable intervals 200-μl samples were withdrawn and analyzed by HPLC.

2.4. Hydrolysis in human serum

Compounds 1–4 were dissolved in one volume of phosphate buffer (50 mM, $\mu = 0.15$, pH 7.4) at 37°C. Four volumes of pre-heated human serum were added and the solutions were kept in a water-bath at 37°C. The initial concentration of



Scheme 1. Synthetic scheme for partial amides of clodronic acid. (i) Piperidine, 100°C 1 h. (ii) Me_3SiBr (2 equiv), NaI (two equiv), CH_2Cl_2, N_2-atmosphere, 20°C 5 h, MeOH/NaOH. (iii) Piperidine, reflux 30 min. (iv) Me_3SiCl (8.9 equiv), Et_3N (3 equiv), CH_3CN, 100°C 2 h, MeOH/NaOH.

the compounds was 3.7–7.2 mM. At suitable intervals 200-μl samples were withdrawn and added to 200 μl of methanol to 'deproteinize' the serum. After mixing and centrifugation 300 μl of the supernatant was evaporated to dryness under a stream of air at 40°C, redissolved with 300 μl of the mobile phase buffer and analyzed by HPLC. In case of rapid degradation the supernatant was analyzed without evaporation and redissolution.

2.5. Hydrolysis in rabbit liver homogenate

The hydrolysis of compounds 1–4 was studied at 37°C in 10% (m/V) rabbit liver homogenate. The rabbit liver was homogenized with four volumes of isotonic phosphate buffer pH 7.4 using an X-1020 homogenizer (Ystral, Germany). The homogenate was centrifuged for 90 min at 9000 × g at 4°C with Biofuge 28 RS-centrifuge (Heraeus Instruments, Germany). The supernatant was stored at − 80°C. Before use 20% liver homogenate was diluted to 10% with isotonic phosphate buffer pH 7.4. An appropriate amount of compound was dissolved in pre-heated 10% liver homogenate (initial concentrations 3.5–5.2 mM). The solution was incubated at 37°C. The samples were withdrawn and pretreated as described for serum samples.

2.6. Apparent partition coefficients

The apparent partition coefficients (log P_{app}) were determined between (pre-equilibrated) 1-octanol and phosphate buffer (0.16 M, pH 7.4) using the traditional shake-flask technique.

3. Results and discussion

Log P_{app} value of compound 1 (most lipophilic) was 1.2. The result suggests that this type of prodrug can be useful in the design of a prodrug of clodronate with increased lipophilicity. The log P_{app} value of clodronate can be estimated to be less than − 5.4 (Björkroth et al., 1991).

Table 1
^{1}H (400 MHz), ^{13}C (101 MHz) and ^{31}P (161 MHz) NMR data of the target compounds

Compound	NMR data
1	^{1}H NMR ($CDCl_3$): d 3.36 (8H, m), 3.29 (4H, m), 3.10 (4H, m), 1.82 (4H, m), 1.61 (2H, m), 1.14 (12H, t, $J = 7.1$ Hz), 1.12 (6H, t); ^{13}C: d 84.00 ($^{1}J_{CP} = 100.9$ and 96.4 Hz), 44.16 t, 40.58 td ($^{2}J_{CP} = 3.3$ Hz), 39.93 td ($^{2}J_{CP} = 3.2$ Hz), 22.72 t, 14.49 qd ($^{3}J_{CP} = 1.5$ Hz), 13.86 qd ($^{3}J_{CP} = 2.8$ Hz); ^{31}P: d 30.91 (P, d, $^{2}J_{PP'} = 16.5$ Hz), 12.26 (P′, d).
2	^{1}H NMR ($CDCl_3$): d 3.32 (4H, m), 3.24 (4H, m), 1.14 (12H, t, $J = 6.5$ Hz); ^{13}C: d 85.56 ($^{1}J_{CP} = 102.9$ and 103.0 Hz), 42.64 td ($^{2}J_{CP} = 3.7$ Hz), 15.99 qd ($^{3}J_{CP} = 6.9$ Hz); ^{31}P: d 34.71 (P, d, $^{2}J_{PP'} = 15.8$ Hz), 11.27 (P′, d).
3	^{1}H NMR (D_2O): d 3.21 (8H, t, $J = 7.4$ Hz), 3.17 (8H, m), 1.79 (8H, m), 1.68 (4H, m), 1.10 (12 H, t, $J = 7.1$ Hz); ^{13}C: d 84.20 t*, 47.40 t, 43.94 td ($^{2}J_{CP} = 3.2$ Hz), 25.08 t, 24.35 t, 17.42 q; ^{31}P: d 13.94.
4a	^{1}H NMR (D_2O): d 3.24 (4H, m), 1.11 (6H, t, $J = 7.1$ Hz); ^{13}C: d 87.56 ($^{1}J_{CP} = 118.2$ and 114.5 Hz), 43.86 td ($^{2}J_{CP} = 3.8$ Hz), 17.39 t; ^{31}P: d 15.72 (P, d, $^{2}J_{PP'} = 15.2$ Hz), 10.23 (P′, d).
4b	^{1}H NMR (D_2O): d 3.02 (4H, t, $J = 7.7$ Hz), 1.64 (4H, m), 1.37 (4H, m), 0.91 (6H, t, $J = 7.3$ Hz); ^{13}C: d 78.89 dd*, 51.32 t**, 31.39 t, 22.49 t, 15.96 q; ^{31}P: d 18.22 (P, d, $^{2}J_{PP'} = 15.3$ Hz), 12.90 (P′, d).

$^{1}J_{CP}$ couplings not observed due to poor S/N ratio (relaxation time to Cl_2C typically >60 s and no nOe).
$^{2}J_{CP}$ and $^{3}J_{CP}$ not observed because of broad lines.

Amides of clodronic acid hydrolyzed chemically to clodronic acid at pH 5.0 and 7.4 (Fig. 1). The rate of hydrolysis decreased with an increased number of substituents (mono > di > tri) and was faster at pH 5.0 than pH 7.4 (Table 2). The rate of hydrolysis in 10% rabbit liver homogenate (pH 7.4) was approximately the same as in the buffer solution at pH 7.4, which suggests that chemical hydrolysis dominates the degradation in rabbit liver homogenate. However, in 80% human serum (pH 7.4) the degradation was significantly slower compared to liver homogenate, which may be due to abundant protein binding of the drug in the serum.

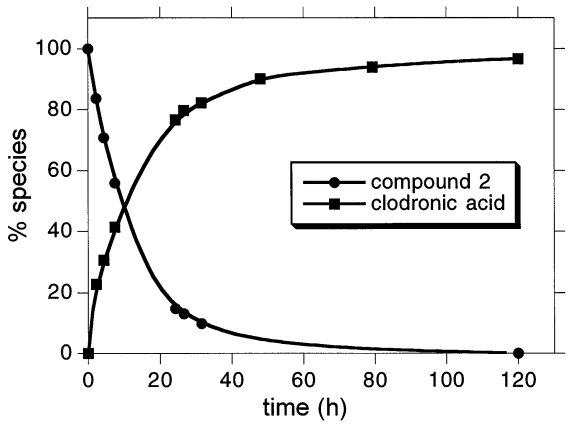


Fig. 1. The degradation of compound **2** to clodronic acid in phosphate buffer (pH 5.0) at 37°C.

In conclusion, because the release of clodronic acid is based on chemical hydrolysis of the amide-bond, the clinical usefulness of these amides of clodronic acid as bioreversible prodrugs is limited. However, monoamides (4a and 4b) may be useful intermediates for the design of enzymatically labile double prodrugs, which after enzymatic hydrolysis release the monoamide that is chemically hydrolyzed to the parent compound. Chemical cleavage of the last pro-moiety of a clodronate prodrug would be favorable because enzymatic susceptibility of the highly charged mono-substituted bisphosphonate is usually very weak. Studies are in progress to examine the utility of the present amides as intermediates for the preparation of bioreversible double prodrugs of clodronate.

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Table 2
Half-lives ($t_{1/2}$) of compounds 1–4 in 50 mM phosphate buffer, 80% human serum and 10% rabbit liver homogenate

Compound	Hydrolysis in phosphate buffer		Hydrolysis in	
	pH 5.0	pH 7.4	10% Rabbit liver homogenate	80% Human serum
1 Tris(*N*,*N*-diethyl)	87 h	No degradation[a]	Slow degradation[b]	No degradation[c]
2 P,P-bis(*N*,*N*-diethyl)	6.6 h	75 h	18 h	Slow degradation[b]
3 P,P′-bis(*N*,*N*-diethyl)	24 min	13 h	11 h	Slow degradation[b]
4a *N*,*N*-diethyl	12 min	19 min	21 min	2.2 h
4b *N*,*N*-dibutyl	10 min	16 min	13 min	8.8 h

[a] No degradation was observed during 17 days.
[b] Less than 5% of the amide was converted to clodronic acid during 24 h.
[c] No degradation was observed during 24 h.

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