Synthesis and Study of Alendronate Derivatives as Potential Prodrugs of Alendronate Sodium for the Treatment of Low Bone Density and Osteoporosis

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Abstract: Alendronate derivatives were evaluated as potential prodrugs for the osteoporosis drug alendronate sodium in an attempt to enhance the systemic exposure after oral administration. An investigation of the chemical behavior of alendronate derivatives led to development of practical synthetic strategies and prediction of each structural class's prodrug potential. Pharmacokinetic studies of *N*-myristoylalendronic acid revealed that 25% have been converted in vivo after iv administration in rat, providing an important proof-of-concept for this strategy.

Low bone density and osteoporosis represent a major health threat for millions of people worldwide. According to the U.S. Surgeon General, by 2020 half of all Americans over the age of 50 could be at risk for fractures from low bone mass or osteoporosis.[1] Significant efforts devoted to prevention, treatment, and impediment of osteoporosis yielded a class of highly effective structurally related nonhormonal bisphosphonate drugs including the FDA-approved alendronate sodium, ibandronate sodium, and risedronate sodium. The enormous benefit of this class could be enhanced by addressing two major downfalls common to all three drugs: virtually identical low oral bioavailability ($F = 0.6\%$) and incidents of upper gastrointestinal tract irritation. Both shortcomings may be theoretically dealt with concurrently by administrating the active compound as a more orally bioavailable derivative (a prodrug) that would release the active form of the bisphosphonate in vivo along with innocuous byproducts, translating in a higher systemic exposure of the parent drug. This report outlines the results of our investigation of the potential of different classes of alendronate derivatives to serve as such prodrugs by studying their chemical behavior, in vitro and in vivo stability, and their ability to release the parent alendronic acid.

Figure 2 outlines the proposed designs of alendronate prodrugs. Derivatization of any of the four functional groups of alendronic acid may theoretically provide compounds of the desired properties: (1) Tetraalkyl alendronates (Figure 2, design 1) no longer have a zwitterionic character of aminobisphosphonic acid, which in turn has a pronounced effect on the overall polarity of the molecule because of the lack of acidic protons. The polarity change of the molecule and increased lipophilicity may improve the pharmacokinetic profile.[2] (2) Similar to tetraalkyl alendronates, *N*-acylalendronates (design 2) also abolish the zwitterionic nature of the parent drug and increase the lipophilicity of the molecule. We primarily focused on *N*-acylalendronates derived from fatty acids, contemplating the possibility of an in vivo lipase-based deacylation (design 2, RCO = fatty acid residue). Simple *N*-alkylalendronates were not considered as a viable prodrug option because they are unlikely to significantly alter the pharmacokinetic properties (Figure 1; alendronate and ibandronate have identical oral bioavailability).[3] (3) *O*-Acylalendronates are anticipated to have a limited stability in phase I metabolism and thus are only considered in the context of derivatives of the first two designs. In this paper, our findings for each of the alendronate prodrug designs are discussed in detail.

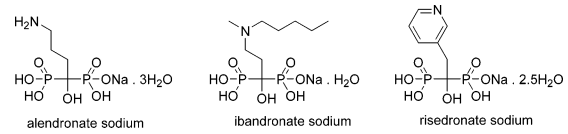

Figure 1. Bisphosphonate drugs approved by the FDA for osteoporosis.

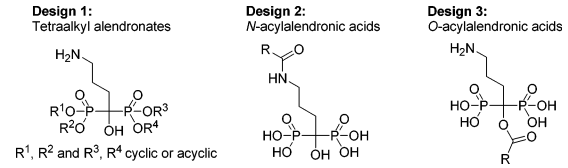

Figure 2. Design of potential alendronate prodrugs.

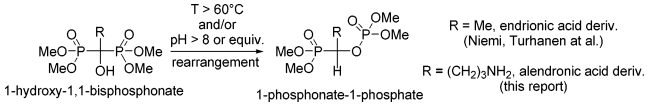

Figure 3. Rearrangement of the 1-hydroxy-1,1-bisphosphonate to 1-phosphonate-1-phosphate observed for etindronates[4c,d,l] and alendronates.

While the reactivity of tetraalkyl 1-hydroxy-1,1-bisphosphonates has been extensively studied,[4] a general methodology for the preparation of 1-hydroxy-1,1-bisphosphonates has not yet been developed. Most of the published synthetic strategies are limited to a handful of simple unfunctionalized 1-hydroxy-1,1-bisphosphonates and not applicable to derivatives bearing any functional groups either unprotected or protected in a manner that would allow for further selective derivatization. It is noted that no synthesis of a simple tetraalkyl alendronate has ever been reported.[5] To evaluate the potential of tetraalkyl alendronates to be used as prodrugs for the parent alendronic acid, we first devised a practical and scalable synthetic route for their preparation.

Attempts to directly esterify the phosphonic acid functionality of a readily available[5] alendronic acid could not be carried out because of rapid rearrangement of the desired tetraalkyl 1-hydroxy-1,1-bisphosphonate product to the 1-phosphonate-1-phosphate byproduct under various reaction conditions (Figure 3). The rearrangement has been discovered by Fitch and Moedritzer[6] and recently studied in detail by Niemi, Turhanen, and co-workers in the context of etidronate derivatives.[4c,d,l] Niemi and Turhanen not only proposed the mechanism of the rearrangement but also defined conditions under which it takes place: at greater than pH 8 and/or at 60 °C and higher. Our observations for alendronate derivatives are generally consistent with their results for etidronates. The relatively broad scope of

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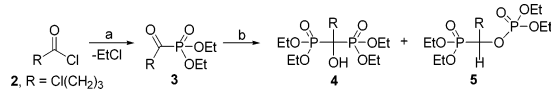
Scheme 1. One-pot Two-Stage Reaction Sequence for the Preparation of 1-Hydroxy-1,1-bisphosphonate **4**[a]



[a] Conditions: (a) $P(OEt)_3$, 0 °C; (b) $HOP(OEt)_2$, base, solvent (see Table 1), room temp, 1 h.

Table 1. Optimization of the Pudovik Reaction (Scheme 1, **3** → **4**): Conversion and Selectivity as Functions of the Solvent Polarity and Base Strength[a]

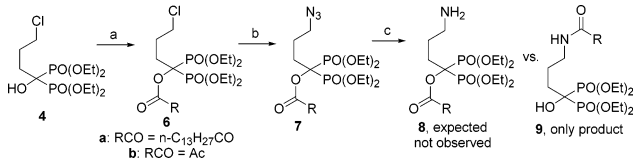
base B (pK_a of BH^+ in H_2O)[14]	ratio **4**:**5** @ 90% conversion of **3** → **4** + **5** in various solvents						
	DMF	THF	CH_2Cl_2	toluene	hexane	cyclohexane	none
imidazole (7.0)	1.5:1	3:1	5:1 (41%[b])	7:1	11:1	13:1 (38%[b])	7:1
DMAP (9.2)	1:1	1.5:1	4:1 (37%[b])	5:1	9:1	10:1	6:1
DIPEA (11)	1:1	1:1	3:1	5:1	7:1	6:1	7:1
DBU (12[c])	na	0:1	0.5:1	1:1	1:1	1.5:1	1:1
KHMDS (26)	0:1	0:1	0:1	0:1	0.05:1	0.05:1	0.1:1

[a] Ratio **4**:**5** was determined by ^{31}P NMR of the crude reaction mixture. [b] Isolated yield after column chromatography. [c] pK_a in DMSO.

the conditions favorable for the rearrangement caused a failure of all investigated methods for a direct conversion of *N*-(carbobenzyloxy)alendronic acid (*N*-Cbz-alendronic acid, **1**) to its tetraalkyl ester.[7] The presence of the highly reactive primary amine functionality in the alendronate structure precluded the use of a previous preparative method designed specifically to prevent the rearrangement of related 1-hydroxybisphosphonates.[8] Additionally, the position of the 4-amino group caused formation of complex mixtures of intra- and intermolecular phosphoramides under many synthetic conditions. Because of these challenges, it was determined that an alternative well-defined synthetic strategy was needed for the preparation and isolation of tetraalkyl alendronates.

The most commonly utilized strategies for the preparation of 1-hydroxy-1,1-bisphosphonates[4] involve some variation of a sequence consisting of trialkyl phosphite addition to an acyl chloride, called the Arbuzov or Michaelis−Arbuzov reaction[9] (Scheme 1, **2** → **3**), followed by dialkyl phosphite addition to the α-ketophosphonate **3**, commonly referred to as the Pudovik reaction (**3** → **4**).[10] In many cases, low overall yield and/or yield variability was observed mainly because of the rearrangement depicted in Figure 3. In some cases, the rearranged products were even incorrectly reported as being the desired 1-hydroxy-1,1-bisphosphonates.[11] We conducted a thorough investigation of the reaction sequence and found that the Michaelis−Arbuzov reaction proceeded smoothly under solvent-free conditions at 0 °C with no excess of reagents and no catalyst or base needed. The only reaction byproduct, alkyl chloride, does not interfere with the majority of the subsequent reactions. For many examples it can be removed under reduced pressure. A more extensive optimization was needed for the second stage of the sequence, the Pudovik reaction, which requires the use of a stoichiometric amount of base.[12] The base not only promotes the desired transformation of **3** to **4** but also simultaneously catalyzes the undesired rearrangement of **4** to **5**. The relative rates of both transformations are functions of two factors: the strength of the base and the polarity of the reaction media.[13] Table 1 demonstrates that as long as a base is strong enough to effectively promote the Pudovik reaction, the weaker the base and the less polar the solvent, the higher is the ratio of **4** to **5** and thus the higher is the overall isolated yield of **4**. In addition, combining both steps into a single one-pot reaction sequence not only simplified the overall synthetic process but also circumvented the decomposition of **3** observed upon its prolonged storage. Analytically pure 1-hydroxy-1,1-bisphosphate **4** was isolated in modest but fully reproducible overall yield (Scheme 1).

Scheme 2. Use of *O*-Acyl-1-hydroxy-1,1-bisphosphonates as Intermediates for the Synthesis of Other Alendronate Derivatives: Potential and Limitations[a]



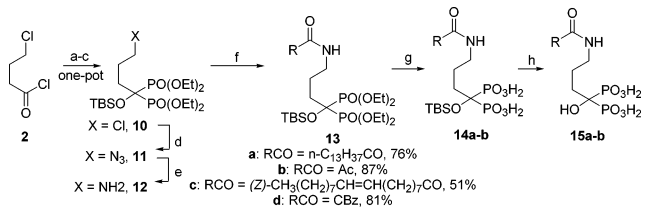
[a] Conditions: (a) RCOCl, 60 °C, 36 h, 15%; (b) NaN_3, DMF, 60 °C, 15 h, 17%; (c) Pd−C, DCM, H_2, 2 h, >99%.

Once isolated in pure form, 1-hydroxy-1,1-bisphosphonates **4** are bench-stable for extended periods of time. Unfortunately, their good stability in neat form ($t_{1/2,\mathbf{4}\rightarrow\mathbf{5}}$ > 1 year)[15] does not translate into stability in organic solvents ($t_{1/2,\mathbf{4}\rightarrow\mathbf{5}}$ = 72 and 48 h for solutions at room temperature in cyclohexane and dichloromethane, respectively). The rearrangement is even more rapid in aqueous solutions; at biological pH 7.4 and 37 °C, the rearrangement is >95% complete in less than 2 h ($t_{1/2,\mathbf{4}\rightarrow\mathbf{5}}$ < 30 min). The in vitro stability studies clearly indicate that tetraalkyl alendronates **4** are unlikely to significantly improve the systemic exposure of the parent drugs after oral dosing because they are more rapidly rearranged then hydrolyzed to the parent drug. These observations may represent a fundamental obstacle to the application of tetraalkyl alendronates as prodrugs for alendronate sodium.

1-Hydroxy-1,1-bisphosphonates with protected hydroxyl functionality represent extremely valuable synthetic intermediates because in the absence of a free hydroxyl group, undesired rearrangement depicted in Figure 3 cannot take place. In this context, Niemi and Turhanen investigated the use *O*-acetyletidronate derivatives.[4c,d,l] However, we found that the O-acylation strategy was not applicable to alendronate derivatives mainly because of an intramolecular O → N acyl transfer of tetraalkyl *O*-acylalendronate **8** to tetraalkyl *N*-acylalendronate **9** via an unusually kinetically favorable 7-exo-trig mechanism (Scheme 2).[16] Despite the fact that O → N acyl transfer may be utilized for the synthesis of *N*-acylalendronates, a more general protective group strategy was needed for the preparation of other alendronate derivatives. In addition, the formation of **6** and **7** was low-yielding and not practical for the preparation of large quantities of **9** (Scheme 2).

The use of silicon-based protective groups proved to be the most synthetically useful strategy for the preparation of alendronate derivatives. We based our approach on the previously optimized conditions outlined in Scheme 1. Dichloromethane with DMAP was found to be the optimal condition for all of the following elements of the overall synthetic sequence: the Michaelis−Arbuzov reaction, suppression of the rate of the undesired rearrangement, suppression of the rate of silylenolether formation resulting from the base-promoted enolization of α-ketophosphonate prior to the Pudovik reaction, the Pudovik reaction, and the *O*-TBS protection of the Pudovik reaction product.[17] The optimized conditions enabled us to prepare 1-trialkylsilyloxy-1,1-bisphosphonate **10** in 45% overall yield (three steps) via a practical one-pot sequence, overcoming all caveats of the preexisting alternatives (Scheme 3).[18,19] Silylated chloride **10** can be subsequently converted to azide **11** in

Scheme 3. Practical One-Pot, Three-Step Synthetic Route to Protected *N*-Acyl-1-trialkylsilyloxy-1,1-bisphosphonate **10** and Its Conversion to *N*-Acylalendronic Acid **15**[a]



[a] Conditions: (a) $P(OEt)_3$, 0 °C; (b) $HOP(OEt)_2$, DMAP, CH_2Cl_2, room temp, 1 h; (c) TBSCl, 15 h, 45% (three steps, one-pot); (d) NaN_3, DMF, 75 °C, 2 h, 82%; (e) Pd−C, 50 psi of H_2, AcOEt, 15 h, >99%; (f) RCOCl, Et_3N, CH_2Cl_2 or RCO_2H, EDC, DIPEA, MeCN; (g) TMSI, MeCN and then MeOH, 90%; (h) TBAF, THF, 93%.

significantly improved yield compared to its acetylated counterpart **6** and further quantitatively reduced to amine **12**. Because of the practical protection of the hydroxyl functionality, **12** may now be derivatized under a variety of conditions to afford *N*-acylalendronates **13a**−**d**. Standard nucleophilic dealkylation[20] of both phosphonate groups afforded *O*-TBS-*N*-acylalendronic acid **14**; deprotection of **14** was accomplished using aqueous hydrofluoric acid or tetrabutylamonium fluoride (TBAF) to afford *N*-acylalendronic acid **15**.

Acids **1**, **14**, and **15** are stable in solutions at wide ranges of temperature and pH regardless of the solvent and do not undergo the rearrangement outlined in Figure 3. Having established their general stability, we then tested the potential for the amidic functionality to undergo a hydrolytic cleavage. In in vitro experiments, incubation of **15a** with human intestinal mucosa cell homogenate and with human and dog plasma did not provide detectable amounts of released parent drug. A study of uptake and conversion of **15a** across rat intestinal tissue also failed to provide any evidence of the conversion of **15a** to the parent prodrug.

Because it is difficult for in vitro models to accurately reflect the in vivo behavior of prodrug conversions, in particular, the lack of conversion of **1** and **15** to the parent drug, we resorted to in vivo pharmacokinetic experiments in the rat. Four groups of rats (three rats per group) were administered iv[21] with alendronic acid, **1**, **15a**, and **15b**, respectively, at a dose equivalent to 0.1 mpk of free alendronic acid. In the group of rats dosed with parent alendronic acid and alendronate prodrugs, 30% of alendronic acid, 8% of **15a**, 4% of **1**, and <1% of **15b** were eliminated in urine as free alendronic acid within 24 h after dosing. The rapid excretion of alendronic acid for animals dosed with parent drug is fully consistent with previously published work that demonstrated that alendronic acid not absorbed by bone tissue was rapidly excreted and served as biomarker readout for the quantitation of the amounts actually absorbed by the bone tissue.[22] Although the amounts of alendronic acid excreted after dosing with prodrugs were generally lower compared to amounts from dosing with the parent drug, the data indicate that at least two of the prodrugs, **1** and **15a**, were converted to the parent alendronic acid in vivo. Acid **15a** represents a particularly promising lead because 25% of the total amount of **15a** dosed underwent an in vivo conversion to alendronic acid. Although the mechanism of the in vivo cleavage of the amidic functionality of **15a** is presently unknown, we speculate that it is unlikely to be a simple unactivated hydrolysis because of the absence of such cleavage in the case of **15b**. To our knowledge, activated conversion of **15a** to its parent alendronic acid is the first in vivo pharmacokinetic evidence that a prodrug strategy may be a viable option for alendronate derivatives.

To establish the oral bioavailability of potential prodrugs, each of the four groups of rats ($n = 3$ per group) was orally dosed with alendronic acid, **1**, **15a**, and **15b** at 1, 2, and 5 mpk equivalents to free alendronic acid. In the animals orally administrated with alendronic acid and alendronate prodrugs, 0.23% of alendronic acid, 0.003% of **1**, 0.02% of **15a**, and <0.001% of **15b** were excreted in urine as free alendronic acid within 48 h after dosing. None of the investigated prodrugs provided an enhanced level of oral bioavailability. Improving the overall pharmacokinetic profile of these derivatives including modifications of their formulation for oral administration is ongoing and will be a subject of future disclosures.

In conclusion, we have investigated the alendronic acid prodrugs. We have devised general synthetic strategies for the preparation of several classes of alendronate derivatives, producing intermediates in practical and reproducible yields. The key steps of the syntheses include optimized Michaelis−Arbuzov and Pudovik reactions performed in a one-pot sequence. The detailed knowledge of the chemical and physical properties of alendronate derivatives enabled us to predict each class's potential to serve as prodrugs. We have identified *N*-acylalendronates as the most promising class of alendronic acid prodrugs where 25% of the leading example, *N*-myristoylalendronic acid (**15a**), is converted to the parent produg in vivo after iv dosing in the rat. This pharmacodynamic evidence represents the first proof-of-concept of the prodrug strategy for any alendronate derivative reported to this date.

Acknowledgment. The authors express their gratitude to Falguni Patel and Dr. Bernard Choi for performing HRMS analyses.

Supporting Information Available: Experimental procedures and characterization of **1**, **4**−**7**, and **9**−**15** by ^1H, ^{13}C, and ^{31}P NMR and HRMS analyses and HPLC analysis traces under two diverse conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Examples of our efforts to directly convert alendronic acid and *N*-(carbobenzyloxy)alendronic acid (**1**) into their respective tetraalkyl alendronates include alkylation under basic conditions with methyl iodide and under acidic conditions with methyl sulfate, esterification using coupling reagents, protic and Lewis acids, and methanol or methanol equivalents, diazomethane and trimethylsilyldiazomethane, and various orthoformates at elevated temperatures. In most cases, traces of the desired product were observed by ^{31}P NMR analysis of the reaction mixture along with the rearranged 1-phosphonate-1-phosphate byproduct. The desired product was never isolated in substantial yield.

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(12) We were unable to reproduce several published procedures that relied on the use of catalytic amounts of secondary and tertiary amines as sufficient to promote the reaction. When 0.1 and 0.2 equiv of various bases were used, an average conversion of only 9.3 ± 1.2% and 21.1 ± 2.3% was observed, respectively.

(13) The role of solvent polarity on the rearrangement of monophosphonates to monophosphates follows a trend similar to that reported here for bisphosphonates. Compare this to a recent disclosure for monophosphonates: El Kaim, L.; Gaultier, L.; Grimaud, L.; Dos Santos, A. *Synlett* **2005**, 2335.

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(15) Half-life ($t_{1/2,4\rightarrow5}$) determined by ^{31}P NMR for a sample stored under an inert atmosphere of argon at 20 ± 3 °C.

(16) The intramolecular mechanism of the 7-exo-trig O → N acyl transfer was established by a crossover experiment in which a mixture of 3 equiv of **6b** and 1 equiv of **7a** was hydrogenated. No cross-acylation to produce *N*-acetyl **9b** was observed, and the sole product of this reaction was *N*-myristoyl **9a**.

(17) While the use of DMAP afforded a ratio of the desired *O*-TBS-alendronate to the rearranged 1-phosphonate-1-phosphate of 9:1, the use of other bases provided the following ratios: imidazole, 5:1; triethylamine, 6:1; Hunigs base, 8:1; DABCO, 6:1; DBU, 0.5:1. In addition, the use of imidazole did not affect completion of the silylation step and the use of the Huenigs base led to undesired formation of silylenolethers from the α-ketophosphonate, effectively reducing the overall yield. The use of less polar solvents such as cyclohexane or toluene with a potential of increasing the content of desired Pudovik adduct over undesired rearranged byproduct prior to silylation (see Table 1) led to heterogeneous reaction mixtures and irreproducible overall yields.

(18) *O*-TBS-1-hydroxy-1,1-bisphosphonates $Cl(CH_2)_3C(OTBS)(PO(OMe)_2)_2$, $BnO_2C(CH_2)_3C(OTBS)(PO(OEt)_2)_2$, and $BnO_2C(CH_2)_3C(OTBS)(PO(OMe)_2)_2$, were prepared in 57%, 89%, and 71% overall isolated yield, respectively, using the same general strategy.

(19) Experimental procedure for the preparation of **10**: A 1 L oven-dried round-bottomed flask under an inert atmosphere of nitrogen was charged with 4-chlorobutyryl chloride (14 g, 0.10 mol). Triethyl phosphite (16.6 g, 0.10 mol) was added dropwise at 0 °C via syringe over 5 min with stirring. The resulting mixture was allowed to reach room temperature and stirred an additional 15 min, after which anhydrous methylene chloride (300 mL) was added via canella. Diethyl phosphite (15.2 g, 0.11 mol) and 4-(dimethylamino)pyridine (12.2 g, 0.10 mol) were added sequentially. The mixture was stirred at room temperature for 1 h, and *tert*-butyldimethylsilyl chloride (16.5 g, 0.11 mol) was added. The reaction stirred at room temperature for an additional 15 h. The mixture was washed with 0.1 M aqueous hydrochloric acid (2 × 300 mL), dried over sodium sulfate, and concentrated. Analytically pure **10** (22.3 g, 45%) was obtained using Biotage 75L liquid chromatography on silica gel (eluent: 50% ethyl acetate in *n*-heptane). ^{1}H NMR (400 MHz, CDCl3) δ 0.13 (s, 6H), 0.83 (s, 9H), 1.30 (t, J = 7.0 Hz, 12H), 2.07 (m, 2H), 2.16 (m, 2H), 3.49 (t, J = 6.4 Hz, 2H), 4.07 (m, 8H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 16.4, 19.0, 25.1, 27.1, 33.4, 45.4, 53.7, 64.2 (m); ^{31}P NMR (400 MHz, $CDCl_3$) δ 19.21; HRMS calcd m/z 494.1785, obsd m/z 494.1789.

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(21) Intravenous dosing was chosen over oral administration to circumvent potential errors in the overall biomarker readout due to any phase I metabolism of *N*-acylalendronates or substantial variations in their oral bioavailability compared to the parent drug.

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