Synthesis of an analogue of the bisphosphonate drug Ibandronate for targeted drug-delivery therapeutic strategies†‡

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An analogue of the bisphosphonate drug Ibandronate was prepared and coupled *via* a cleavable ester function to a bromoacetyl linker with specific reactivity for thiol groups. This compound should find useful applications in therapeutic strategies aiming to deliver bisphosphonate drugs specifically to cancer cells making use of proteins as vectors. The specific delivery of bisphosphonates to cancer cells instead of bone, the usual site of accumulation of these cytotoxic drugs, could greatly widen their therapeutic applications.

Introduction

Bisphosphonates are the most commonly used drugs for the treatment of bone diseases.[1,2] This specific therapeutic application results from two particular properties. First, bisphosphonates have a cytotoxic activity.[3–6] They are potent inhibitors of farnesyl pyrophosphate synthase, an enzyme involved in the biosynthesis of isoprenoids.[7] Inhibition of this enzyme prevents the formation of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, two isoprenoids used for protein prenylation. In absence of prenylation, many proteins, in particular proteins involved in intracellular signalling pathways, become mislocalised and non-functional, which leads to apoptosis of the cells.[4,8] Secondly, bisphosphonates have a biodistribution specificity for bones.[9] The characteristic bisphosphonate moiety present in all of these drugs gives them affinity for hydroxyapatite, the main component of bone mineral.[10]

Combined together, these two particular properties make bisphosphonates ideal drugs for the treatment of bone diseases as their cytotoxicity is restricted to bone cells. Osteoclasts, cells involved in bone resorption, are the cells most exposed to bisphosphonates and bisphosphonates are thus extensively used to prevent osteoporosis.[11,12] The bone binding property of the bisphosphonate moiety has also been used on its own to target various molecules to bones. Drugs, hormones and growth factors promoting bone formation have been attached to bisphosphonates in order to prevent osteoporosis.[13–15] Bisphosphonate-conjugated fluorescent and radioactive labels are also common clinical tools for bone imagery.[9,16] On the other hand, the use of bisphosphonates for their cytotoxic properties only has not been investigated to the same extent. Bisphosphonates have, however, an interesting anticancer activity.[17–20] Some of them are even approved as drugs for the treatment of bone metastases.[21] The applications of bisphosphonates in anticancer therapy could be greatly widened if a way to redirect bisphosphonates to cancer cells localised to soft tissue instead of bone could be found.

Conjugating bisphosphonates to vectors specific to cancer biomarkers could be a way to achieve this goal. Vectorisation strategies have proved very useful for rendering drug biodistribution more specific to the site of disease, especially for cytotoxic anticancer drugs.[22,23] Many of these drugs have a limited therapeutic usefulness because of their lipophilic character. This lipophilicity leads to high levels of side-effects due to the accumulation of the drug in non-targeted tissues. Conjugated to vectors specific for cancer cells, these drugs have, however, limited non-specific biodistribution and reduced side-effects.[24–26]

Among the different types of vectors investigated for targeted drug-delivery strategies to cancer cells, proteins and peptides are some of the most common. Many of the natural ligands of receptors overexpressed by cancer cells are proteins or peptides, which readily provides vectors with specificity for these cells. Monoclonal antibodies specific to cancer biomarkers have also proved to be valuable vectors for the targeting of cancer cells.[27,28] Their usefulness as vectors for targeted-drug delivery strategies has been demonstrated by the approval of Mylotarg®, an immunoconjugate composed of the cytotoxic drug calicheamicin attached to a monoclonal antibody specific to the CD33 receptor, for the treatment of acute myeloid leukemia.[29] In order to study the possibilities of targeted delivery of bisphosphonates to cancer cells, we have thus investigated the synthesis of bisphosphonates suitable for conjugation to proteins.

For a targeted drug delivery strategy to be successful, drug and vector must meet some requirements: the drug must be coupled to the protein without interfering with its target recognition properties; a mechanism allowing the release of the drug at the target site must be designed; the drug must finally be released in an active form. We tried to address all these issues in the design of conjugatable bisphosphonates for targeted drug delivery strategies. Cysteine residues were selected as sites for the coupling of the bisphosphonate. These amino acids are relatively rare in proteins, providing specific

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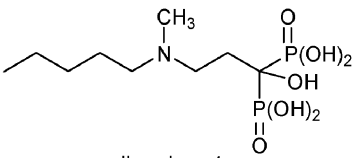
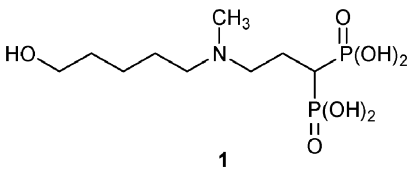
anchor points for the bisphosphonates. Using recombinant DNA techniques, they can also be engineered at specific positions in the protein to avoid interference with the target binding properties of the protein.[30] Finally, several thiol reactive groups such as maleimides or haloesters allow the specific conjugation of molecules to proteins.[26,31] We also anticipated an ester linkage to provide an appropriate stability *in vivo* as well as a mechanism to release the bisphosphonates inside cancer cells.[13] Esters should be cleaved under the acidic conditions of the endosomes after internalisation of the vectorised drug. Finally, an analogue of the commercial drug Ibandronate[12] with an additional alcohol located at the end of its side-chain was chosen for the vectorisation (Scheme 1). The structure of this analogue presents many of the characteristic elements of the pharmacophore required for FPPSase inhibition. As such, it is anticipated that bisphosphonate **3** will inhibit FPPSase while its alcohol group will provide the necessary functional group for the formation of an ester linkage with the carrier protein.[32,33]

Results and discussion

Compound **10**, a key intermediate in the synthesis of an Ibandronate analogue attached to a linker with thiol specific reactivity, was our first synthetic target.



Ibandronate



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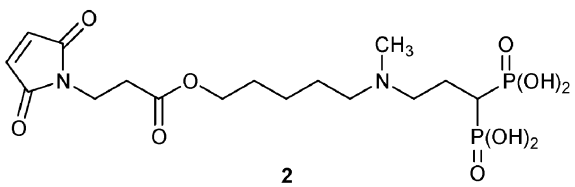
Ibandronate analogue functionalised for vectorisation by proteins



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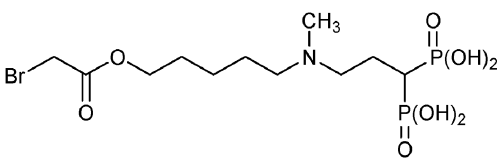

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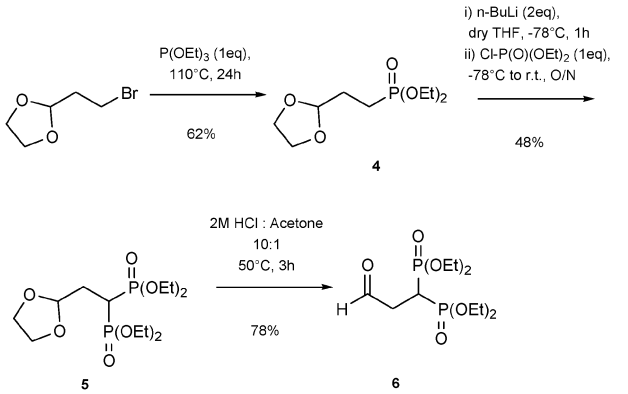
Examples of the Ibandronate analogue attached by a cleavable ester group to linkers with thiol specific reactivity

Scheme 1 Structure of Ibandronate and of its analogue functionalised for targeted drug-delivery strategies.

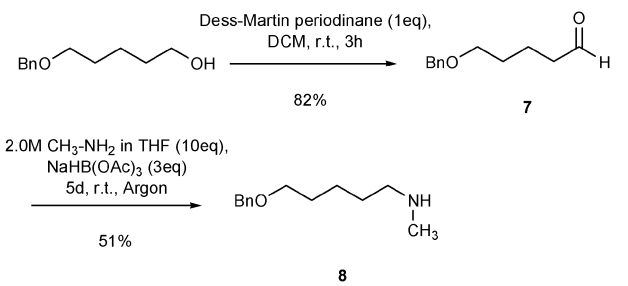


Scheme 2 Formation of the bisphosphonate moiety **6**.

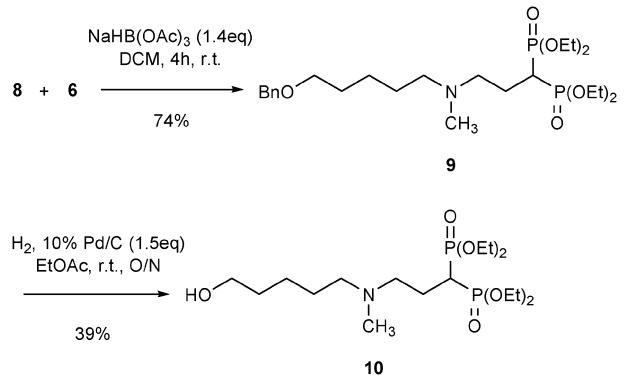
Its synthesis involved the formation of the bisphosphonate moiety **6**. Protection of the bisphosphonic acid moiety as a bisphosphonate tetraester was required due to the difficulty of purification of highly polar bisphosphonic acids. The aldehyde **6** was obtained in three steps from 2-bromoethyl-1,3-dioxolane (Scheme 2). The Michaelis-Arbusov reaction of this product with triethyl phosphite afforded the phosphonate **4** in 62% yield after 24 h heating at 110 °C.[34,35] To complete the formation of the *gem*-bisphosphonate moiety, phosphonate **4** was treated with *n*-BuLi and the resulting carbanion reacted with diethyl chlorophosphate to give the bisphosphonate product **5** in 48% yield.[35] As this phosphonoalkylation reaction was gradually inactivated by the acido-basic reaction between the phosphonate carbanion and the bisphosphonate product **5**, two equivalents of *n*-BuLi were required in total to achieve good conversion rates. Deprotection of the cyclic acetal **5** under acidic conditions (2 M HCl : acetone 10 : 1/ 50 °C/3 h) gave the aldehyde **6** in 78% yield.

The second part of the synthesis of compound **10** consisted of the formation of the side-chain of the Ibandronate analogue and its coupling to the bisphosphonate **6**. *N*-Methyl-5-benzyloxypentamine **8** was synthesized in two steps by Dess–Martin oxidation[36] of 5-benzyloxypentanol into 5-benzyloxypentanal **7** (82%) followed by reductive amination of this product with methylamine using sodium triacetoxyborohydride as a reducing agent (Scheme 3).[37] *N*-Methyl-5-benzyloxypentamine **8** was isolated in 51% yield after alumina gel chromatography.

Similar reductive amination conditions were used to couple *N*-methyl-5-benzyloxypentamine **8** to the aldehyde **6** (Scheme 4). Compound **9** was isolated in 74% yield after purification by column chromatography. The alcohol **10** was



Scheme 3 Synthesis of the side-chain of the Ibandronate analogue **1**.



Scheme 4 Synthesis of compound **10**, a key intermediate in the synthesis of Ibandronate analogues functionalised for targeted drug delivery.

finally obtained in 39% yield after hydrogenation with a palladium catalyst.

Different approaches then had to be investigated in order to couple this Ibandronate analogue to a thiol reactive linker. The type of linker, the conditions used for the deprotection of the bisphosphonate moiety and the order of the linker coupling and bisphosphonate deprotection reactions were varied in order to find conditions allowing the formation of an Ibandronate analogue coupled to a thiol reactive linker.

In a first attempt, a linker with a maleimide group, a frequently used thiol reactive moiety,[24] was used. *N*-Maleimidopropionic acid, a commercial product, was turned into its acyl chloride derivative using thionyl chloride and reacted directly with the alcohol **10** in the presence of triethylamine to form the ester **11** in 70% yield (Scheme 5). The bisphosphonate group of compound **11** could be deprotected by silyl-dealkylation using a large excess of TMSBr (20 equiv.) in dry DCM for 72 h.[38] However, using these deprotection conditions, an addition of bromide also occurred on the maleimide double bond.

A different approach to the bisphosphonate deprotection and the linker coupling was also investigated using the same *N*-maleimidopropionic acid linker. In this approach, the bisphosphonate group of the alcohol **10** was deprotected under strong acidic conditions (6 M HCl /reflux /12 h) (Scheme 5).[39] If the bisphosphonate group of compound **10** was cleanly deprotected into a bisphosphonic acid, an additional modification occurred again: about 60% of the alcohol group was converted into a chloride. However, this chloride could be completely converted back into an alcohol by simply heating to reflux the mixture of alcohol and chloride in distilled water for 24 h. The Ibandronate analogue **1** could be obtained in near quantitative yields with this method. However, attempts to esterify the deprotected Ibandronate analogue **1** using conditions similar to the ones used for the formation of amide bonds in water (HBTU (1.1 equiv.)/ triethylamine (2.2 equiv.)/dry DMF/r.t./O/N) remained unsuccessful.[40]

The use of α-haloacyl linkers, alternative linkers with thiol specific reactivity,[26] was considered as a possible solution to attach an Ibandronate analogue to a thiol reactive linker. The alcohol **10** was thus esterified with bromoacetyl bromide[13] in the presence of pyridine and gave the ester **12** in 60% yield after purification by silica gel chromatography (Scheme 6). The bisphosphonate group of compound **12** was deprotected by silyl-dealkylation (TMSBr (20 equiv.)/dry DCM/25 °C/72 h, followed by methanolysis) to give almost quantitatively the linker attached bisphosphonate **3**.

No other side reaction was observed under these conditions. Bisphosphonate **3** was, however, prone to degradation when stored under non-buffered conditions, the acidity of the bisphosphonic acid group causing the hydrolysis of the ester group linking the drug to the bromoacetyl linker.

Conclusions

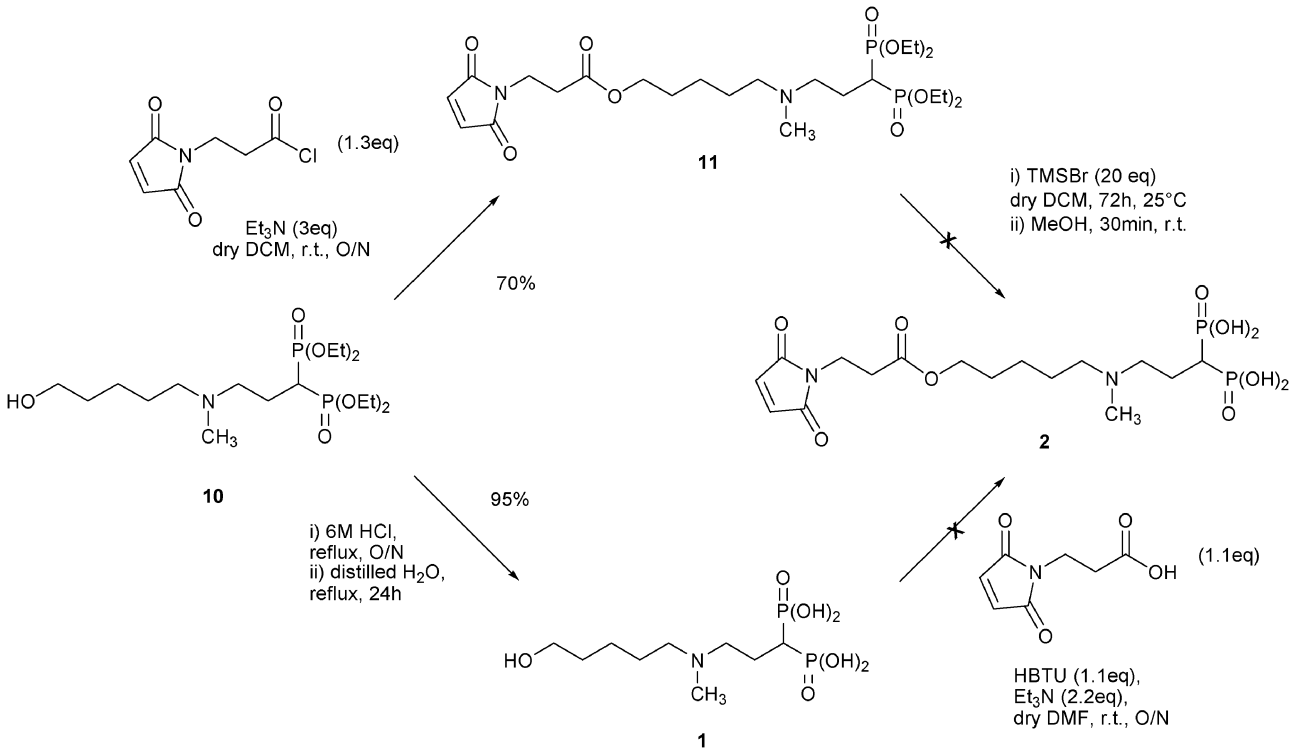
We have developed an effective synthetic sequence that gives access to conjugatable bisphosphonates and successfully synthesised an activated bisphosphonate analogue of Ibandronate. The conjugation of the bromoacetyl linker coupled Ibandronate analogue **12** to a protein carrier and the biological activity of both the free Ibandronate analogue **1** and its vectorised form will be reported in due course to demonstrate the broad potential of vectorised bisphosphonates in anticancer therapy.

Experimental

Chemicals were purchased from Sigma Aldrich. Solvents were, when necessary, dried and stored for up to three weeks on molecular sieves. DCM and Et_3N were dried over $CaCl_2$, and THF over Na/benzophenone. DMF was dried by storing a fresh bottle of solvent over activated molecular sieves under argon. All reactions were done under argon atmosphere and monitored on commercially available pre-coated TLC plates (layer thickness 0.25 mm) of Kieselgel 60 F254. Compounds were visualised by use of a UV lamp and/or a suitable dipping solution and heating. Column chromatography was performed either manually or using a Biotage System with Merck 60 (40–60 mm) silica gel or aluminium oxide (activated, neutral, Brockmann grade I) as the solid phase. Mass spectrometric data (MS) were obtained by electrospray (ES) on a Waters LCT Premier Mass Spectrometer. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$ and D_2O on Bruker Avance DRX 500 or DRX 300 spectrometers. TMS (0 ppm, ^{1}H NMR), $CHCl_3$ (77 ppm, ^{13}C) and triethyl phosphate (0.2 ppm, ^{31}P NMR) were used as internal references. The chemical shifts (δ) are reported in ppm (parts per million) and the coupling constants (J values) in Hz.

Diethyl 2-(1,3-dioxolan-2-yl)ethylphosphonate 4

Triethyl phosphite (3.43 mL, 20 mmol, 1 equiv.) was added dropwise to neat 2-(2-bromoethyl)-1,3-dioxolane (4.70 mL, 40 mmol, 2 equiv.) at r.t. under argon. The reaction mixture was heated to 110 °C until the complete disappearance of triethyl phosphite was confirmed by ^{31}P NMR analysis of the reaction mixture. Purification of the crude product by silica gel column chromatography (MeOH–DCM, 0 : 100–5 : 95) afforded an inseparable mixture of **4**, diethyl ethylphosphonate and diethyl *H*-phosphonate, which was distilled under reduced pressure. Pure product **4** (2.93 g, 62%) was obtained as a pale



Scheme 5 Strategies investigated for the coupling of a *N*-maleimidopropionic acid linker to the Ibandronate analogue **1**.

oil after vacuum distillation of diethyl *H*-phosphonate (46 °C at 1 mmHg) and diethyl ethylphosphonate (60 °C at 1 mmHg). δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +33.2; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.32 (6 H, t, *J* 7, OCH_2CH_3), 1.79-2.00 (4 H, m, PCH_2CH_2), 3.84-3.99 (4 H, m, OCH_2CH_2O), 4.06-4.15 (4 H, m, OCH_2CH_3), 4.95 (1 H, t, *J* 4, $PCH_2CH_2CHO_2$); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.8 (2 C, d, $^3J_{P-O-C-C}$ 6), 19.9 (d, $^1J_{P-C}$ 144), 27.3 (d, $^2J_{P-C-C}$ 4), 61.9 (2 C, d, $^2J_{P-O-C}$ 6), 65.5 (2 C, s), 103.7 (d, $^3J_{P-C-C-C}$ 19); MS *m*/*z* (positive, ES) calcd for $C_9H_{19}O_5NaP$ $[M + Na]^+$ 261.0875 found 261.0868.

Tetraethyl 2-(1,3-dioxolan-2-yl)ethane-1,1-diyldiphosphonate 5

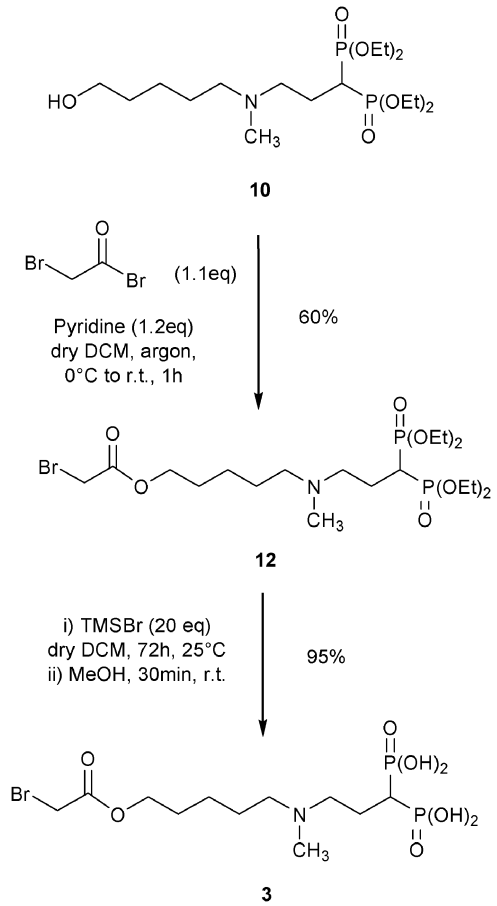
n-BuLi (3.44 mL of a 1.6 M solution in hexane, 5.5 mmol, 1.1 equiv.) was added dropwise to a solution of phosphonate **4** (1.19 g, 5.0 mmol, 1 equiv.) in dry THF (30 mL) under argon, cooled down to −78 °C. After stirring for 1 h at −78 °C, diethyl chlorophosphate (759 µL, 5.25 mmol, 1.05 equiv.) was added to the reaction mixture. After warming to r.t. overnight, the reaction mixture was cooled down again to −78 °C. *n*-BuLi (3.0 mL of a 1.6 M solution in hexane, 4.8 mmol, 0.95 equiv.) was added dropwise to the reaction mixture followed, after 1 h stirring at –78 °C, by diethyl chlorophosphate (380 µL, 2.63 mmol, 0.53 equiv.). The reaction mixture was allowed to warm to r.t. overnight. A saturated aqueous NH_4Cl solution (30 mL) was used to quench the reaction mixture. The aqueous layer was extracted with diethyl ether (3 × 40 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to give 1.59 g of crude product. Purification by silica gel column chromatography (MeOH–DCM, 1 : 99–5 : 95) afforded the pure bisphosphonate **5** (907 mg, 48%) as a yellow oil. δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +24.4; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.34 (12 H, t, *J* 7, OCH_2CH_3), 2.18 (2 H, tt, $^3J_{P-C-C-H}$ 17, *J* 6, P_2CHCH_2), 2.63 (1 H, tt, $^2J_{P-C-H}$ 24, *J* 6, P_2CHCH_2), 3.85-3.99 (4 H, m, OCH_2CH_2O), 4.13-4.24 (8 H, m, OCH_2CH_3), 5.25 (1 H, t, *J* 6, $P_2CHCH_2CHO_2$); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.8 (4 C, d, $^3J_{P-O-C-C}$ 7), 30.5 (t, $^2J_{P-C-C}$ 5), 32.8 (t, $^1J_{P-C}$ 134), 63.0 (2 C, d, $^2J_{P-O-C}$ 7), 63.1 (2 C, d, $^2J_{P-O-C}$ 7), 65.2 (2 C, s), 102.8 (t, $^3J_{P-C-C-C}$ 8); MS *m*/*z* (positive, ES) calcd for $C_{13}H_{28}O_8NaP_2$ $[M + Na]^+$ 397.1157 found 397.1171.

Tetraethyl 3-oxopropane-1,1-diyldiphosphonate 6

A solution of dioxolane **5** (2.68 g, 7.15 mmol) in a 10 : 1 2 M HCl_{aq} : acetone mixture (55 mL) was heated to 50 °C for 3 h. The reaction mixture was cooled down to r.t.. Volatiles were evaporated *in vacuo*. The reaction mixture was then extracted with DCM (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to give the pure aldehyde **6** (1.85 g, 78%) as a pale oil. δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +23.3; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.32 (6 H, t, *J* 7, OCH_2CH_3), 1.32 (6 H, t, *J* 7, OCH_2CH_3), 2.93-3.25 (3 H, m, P_2CHCH_2) 4.11-4.22 (8 H, m, OCH_2CH_3), 9.72 (1 H, t, *J* 1, C(O)*H*); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.6 (4 C, d, $^3J_{P-O-C-C}$ 6), 30.2 (t, $^1J_{P-C}$ 140), 39.5 (t, $^2J_{P-C-C}$ 7), 63.2 (2 C, d, $^2J_{P-O-C}$ 7), 63.4 (2 C, d, $^2J_{P-O-C}$ 7), 197.7 (t, $^3J_{P-C-C-C}$ 8); MS *m*/*z* (positive, ES) calcd for $C_{11}H_{25}O_7P_2$ $[M + H]^+$ 331.1076 found 331.1086; $[M + Na]^+$ 353.0895 found 353.0911.

5-Benzyloxypentan-1-al 7

Dess–Martin periodinane (2.67 g, 6.3 mmol, 1.05 equiv.) was added portionwise to a solution of 5-benzyloxypentan-1-ol (1.16 mL, 6 mmol, 1 equiv.) in DCM (60 mL) at 0 °C. The



Scheme 6 Coupling of a bromoacetic acid linker to the Ibandronate analogue **1**.

reaction mixture was allowed to warm to r.t. and stirred for 2 h. A 1 M NaOH aqueous solution (60 mL) was then added to the reaction mixture and stirred until complete dissolution of all precipitates from the organic layer. The aqueous layer was extracted with DCM (2 × 30 mL). The organic layers were combined, washed with 1 M $NaOH_{aq}$ (30 mL) and brine (2 × 30 mL), dried over $MgSO_4$ and concentrated under vacuum to afford the crude product (1.26 g) as a clear oil. Pure aldehyde **7** (942 mg, 82%) was obtained after purification by silica gel column chromatography (EtOAc : PE, 33 : 67–100 : 0). δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.60-1.80 (4 H, m, CH_2CH_2), 2.46 (2 H, td, J_1 7, J_2 2, $CH_2C(O)H$), 3.49 (2 H, t, J 6, OCH_2), 4.50 (2 H, s, $PhCH_2$), 7.27-7.38 (5 H, m, *Ph*), 9.76 (1H, t, J 2, C(O)*H*); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 19.4, 29.6, 44.0, 70.2, 73.4, 128.0, 128.0 (2 C, s), 128.8 (2 C, s), 138.9, 202.9.

5-(Benzyloxy)-*N*-methylpentan-1-amine 8

Sodium triacetoxyborohydride (2.42 g, 11.4 mmol, 1.4 equiv.) was added portionwise to a stirred solution of 5-benzyloxy-pentan-1-al **7** (1.57 g, 8.2 mmol, 1 equiv.) and methylamine (41 mL of a 2 M solution in THF, 82 mmol, 10 equiv.) under argon. Reaction progress was monitored by ^{1}H NMR. More sodium triacetoxyborohydride (2.42 g, 11.4 mmol, 1.4 equiv.) was added after 4 d stirring at r.t. After another day stirring at r.t., a 1 M NaOH aqueous solution (100 mL) was added to the reaction mixture and stirred until complete dissolution of all precipitates. The solution was extracted with diethyl ether (3 × 50 mL). Organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to afford the crude amine (1.68 g). Purification by alumina gel column chromatography (MeOH–DCM, 0 : 100–10 : 90) afforded the pure amine **8** (870 mg, 51%). δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.35-1.56 (4 H, m, CH_2CH_2), 1.64 (2 H, quintet, J 7, CH_2), 2.42 (3 H, s, CH_3), 2.57 (2 H, t, J 7, CH_2N), 3.47 (2 H, t, J 7, OCH_2), 4.50 (2 H, s, $PhCH_2$), 7.27-7.36 (5 H, m, *Ph*); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 22.9, 28.6, 35.4, 51.0, 69.3, 71.9, 126.5, 126.6 (2 C, s), 127.3 (2 C, s), 137.6; MS m/z (positive, ES) calcd for $C_{13}H_{22}NO$ $[M + H]^+$ 208.1701 found 208.1698.

Tetraethyl 3-((5-(benzyloxy)pentyl)(methyl)amino)propane-1,1-diyldiphosphonate 9

Sodium triacetoxyborohydride (1.34 g, 6.3 mmol, 1.5 equiv.) was added portionwise to a stirred solution of 5-(benzyloxy)-*N*-methylpentan-1-amine **8** (870 mg, 4.2 mmol, 1 equiv.) and aldehyde **6** (1.45 g, 4.4 mmol, 1.1 equiv.) in dry DCM (50 mL) under argon. After 4 h stirring at r.t., a 1 M NaOH aqueous solution (50 mL) was added to the reaction mixture and stirred until complete dissolution of all precipitates. The aqueous layer was extracted with DCM (3 × 50 mL). Organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to afford the crude amine (2.09 g). Purification by alumina gel column chromatography (MeOH–DCM, 0 : 100–10 : 90) afforded the pure amine **9** (1.54 g, 74%). δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +25.7; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.33 (12 H, t, J 7, OCH_2CH_3), 1.37-1.52 (4 H, m, CH_2CH_2), 1.63 (2 H, quintet, J 7, CH_2), 1.96-2.14 (2 H, m, P_2CHCH_2), 2.19 (3 H, s, CH_3), 2.34 (2 H, t, J 7, $P_2CHCH_2CH_2N$), 2.54 (2 H, t, J 7, CH_2N), 2.66 (1 H, tt, $^2J_{P-C-H}$ 24, J 6, P_2CH), 3.46 (2 H, t, J 7, OCH_2), 4.11-4.22 (8 H, m, OCH_2CH_3), 4.50 (2 H, s, $PhCH_2O$), 7.27-7.37 (5 H, m, *Ph*); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.8 (4 C, d, $^3J_{P-O-C-C}$ 6), 23.6 (t, $^2J_{P-C-C}$ 5), 24.5, 27.6, 30.2, 33.7 (t, $^1J_{P-C}$ 134), 42.1, 56.1 (t, $^3J_{P-C-C-C}$ 7), 58.1, 62.7 (2 C, d, $^2J_{P-O-C}$ 7), 62.9 (2 C, d, $^2J_{P-O-C}$ 7), 70.8, 73.3, 127.9, 128.0 (2 C, s), 128.7 (2 × s), 139.1; MS m/z (positive, ES) calcd for $C_{24}H_{46}NO_7P_2$ $[M + H]^+$ 522.2750 found 522.2742.

Tetraethyl 3-((5-hydroxypentyl)(methyl)amino)propane-1,1-diyldiphosphonate 10

10% Pd/C (6.12 g, 2.88 mmol, 1.5 equiv.) was added to a stirred solution of the bisphosphonate **9** (1.0 g, 1.92 mmol, 1 equiv.) in EtOAc under argon. After three vacuum/H_2 cycles to remove argon from the reaction flask, **9** was hydrogenated (balloon) at r.t. overnight under vigorous stirring. The reaction mixture was then filtered on a celite pad and the filtrate concentrated under vacuum to afford pure alcohol **10** (323 mg, 39%) as a pale oil. δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +25.7; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.34 (12 H, t, J 7, OCH_2CH_3), 1.37-1.52 (4 H, m, CH_2-CH_2), 1.58 (2 H, quintet, J 7, CH_2), 2.06 (2 H, tq, $^3J_{P-C-C-H}$ 24, J 6.5, P_2CHCH_2), 2.19 (3 H, s, CH_3), 2.35 (2 H, t, J 6.5, $P_2CHCH_2CH_2N$), 2.55

(2 H, t, *J* 7, CH_2N), 2.71 (tt, $^2J_{P–C–H}$ 24, *J* 6.5, P_2CH), 3.63 (2 H, t, *J* 7, CH_2OH), 4.11-4.22 (8 H, m, OCH_2CH_3); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.8 (4 C, d, $^3J_{P–O–C–C}$ 6), 22.6 (t, $^2J_{P–C–C}$ 4.5), 23.7, 26.0, 32.6, 33.9 (t, $^1J_{P–C}$ 134), 41.6, 55.3 (t, $^3J_{P–C–C–C}$ 7), 57.2, 62.7, 63.0 (2 C, d, $^2J_{P–O–C}$ 6.5Hz), 63.2 (2 C, d, $^2J_{P–O–C}$ 6.5); MS *m*/*z* (positive, ES) calcd for $C_{17}H_{40}NO_7P_2$ $[M + H]^+$ 432.2280 found 432.2269.

N-Maleimidopropionyl chloride

Thionyl chloride (140 μL, 1.92 mmol, 8 equiv.) was added dropwise to a solution of 3-maleimidopropionic acid (40 mg, 0.24 mmol, 1 equiv.) in dry DCM under argon. The reaction mixture was heated to reflux for 20 h. Concentration of the reaction mixture under vacuum afforded pure *N*-maleimidopropionylchloride (44 mg, 100%) as a pale oil crystallizing over time. δ_H(300 MHz; $CDCl_3$; Me_4Si) 3.25 (2 H, t, *J* 7, $CH_2C(O)Cl$), 3.86 (2 H, t, *J* 7, NCH_2), 6.73 (2 H, s, CH=CH); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$), 33.2, 45.0, 134.5 (2 C, s), 170.1 (2 C, s), 171.5.

5-((3,3-Bis(diethoxyphosphoryl)propyl)(methyl)amino)-pentyl 3-(2,5-dioxo-2*H*-pyrrol-1(5*H*)-yl) propanoate 11

N-Maleimidopropionyl chloride (24 mg, 0.129 mmol, 1.1 equiv.) was added to a solution of the alcohol **10** (50 mg, 0.116 mmol, 1 equiv.) and triethylamine (49 μL, 0.349 mmol, 3 equiv.) in dry DCM. The reaction mixture was stirred overnight at r.t. Concentration of the reaction mixture under vacuum afforded the crude ester **11** (113 mg). Purification by silica gel column chromatography (MeOH–DCM, 0 : 100–10 : 90) afforded the pure ester **11** (43 mg, 70%). δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +25.6; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.34 (14 H, t, *J* 7, $OCH_2CH_3 + CH_2$), 1.48 (2 H, br s, CH_2), 1.63 (2 H, quintet, *J* 7, CH_2), 2.08 (2 H, br s, P_2CHCH_2), 2.20 (3 H, br s, CH_3), 2.34 (2 H, br s, CH_2N), 2.55 (2 H, br s, $P_2CHCH_2CH_2N$), 2.64 (2 H, t, *J* 7, $NCH_2CH_2C(O)O$), 2.66 (1 H, tt, $^2J_{P–C–H}$ 24, *J* 6, P_2CH), 3.83 (2 H, t, *J* 7, $NCH_2CH_2C(O)O$), 4.06 (2 H, t, *J* 7, $C(O)OCH_2$), 4.12–4.23 (8 H, m, OCH_2CH_3), 6.72 (2 H, s, CH=CH); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.6 (4 C, d, $^3J_{P–O–C–C}$ 4), 23.3, 23.9, 27.1, 28.6, 33.1, 33.5 (t, $^1J_{P–C}$ 133), 33.8, 41.8, 55.9, 57.7, 62.5 (4 C, d, $^2J_{P–O–C}$ 24), 65.0, 134.4 (2 C, s), 170.5 (2 C, s), 170.9; MS *m*/*z* (positive, ES) calcd for $C_{24}H_{45}N_2O_{10}P_2$ $[M + H]^+$ 583.2549 found 583.2560.

3-((5-Hydroxypentyl)(methyl)amino)propane-1,1-diyldi-phosphonic acid 1

Tetraethyl bisphosphonate **10** (15 mg, 0.035 mmol, 1 equiv.) was dissolved in a 6 M HCl aqueous solution (5 mL) and heated to reflux for 20 h. The reaction mixture was concentrated under vacuum. The concentrate was dissolved in distilled water (5 mL) and heated to reflux for 20 h. Concentration of the reaction mixture under vacuum afforded pure bisphosphonic acid **1** (11 mg, 95%). δ_P(121 MHz; D_2O; $(EtO)_3P(O)$) +21.2; δ_H(300 MHz; D_2O) 1.45 (2 H, q, *J* 8, CH_2), 1.62 (quintet, *J* 7, CH_2), 1.73-1.85 (2 H, br m, CH_2), 2.36 (3 H, br s, $P_2CH + P_2CHCH_2$), 2.91 (3 H, s, CH_3), 3.13 (1 H, m, CH_AH_BN), 3.24 (1 H, m, CH_AH_BN), 3.42 (1 H, m, $P_2CHCH_2CH_AH_BN$), 3.52 (1 H, m, $P_2CHCH_2CH_AH_BN$), 3.63 (2 H, t, *J* 7, CH_2OH); δ_C(75.5 MHz; D_2O) 21.0 (br), 22.5, 23.7, 31.1, 36.5 (t, $^1J_{P–C}$ 124), 40.0, 55.9 (t, $^2J_{P–C–C}$ 7), 56.6, 61.7; MS *m*/*z* (positive, ES) calcd for $C_9H_{23}NO_7P_2Na$ $[M + Na]^+$ 342.0847 found 342.0840; calcd for $CH_{22}NO_7P_2Na_2$ $[M - H + 2Na]^+$ 364.0667 found 364.0665.

5-((3,3-Bis(diethoxyphosphoryl)propyl)(methyl)amino)-pentyl 2-bromoacetate 12

Bromoacetyl bromide (6.4 μL, 74 μmol, 1.1 equiv.) was added dropwise to a stirred solution of the alcohol **10** (29 mg, 67 μmol, 1 equiv.) and pyridine (6.5 μL, 81 μmol, 1.2 equiv.) in dry DCM (4 mL) cooled down to 0 °C under argon. The reaction mixture was allowed to warm to r.t. and stirred for 1 h. The reaction mixture was concentrated under vacuum to give the crude ester **12** (58 mg). Purification by silica gel column chromatography (MeOH–DCM, 0 : 100–10 : 90) afforded the pure ester **12** (22 mg, 60%). δ_P(121 MHz; $CDCl_3$; $(EtO)_3P(O)$) +22.6; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.36 (12 H, t, *J* 7, OCH_2CH_3), 1.46 (2 H, quintet, *J* 7, CH_2), 1.73 (2 H, quintet, *J* 7, CH_2), 1.94 (2 H, br s, CH_2), 2.46 (2 H, br s, P_2CHCH_2), 2.62 (1 H, tt, $^2J_{P–C–H}$ 24, *J* 6, P_2CH), 2.78 (3 H, s, CH_3), 2.93-3.15 (2 H, br m, $P_2CHCH_2CH_2N$), 3.27-3.58 (2 H, br m, CH_2N), 3.84 (2 H, s, $BrCH_2$), 4.18 (2 H, t, *J* 6, $C(O)OCH_2$), 4.21 (8 H, quintet, *J* 7, OCH_2CH_3); δ_C(75.5 MHz; $CDCl_3$; $CHCl_3$) 16.6 (4 C, d, $^3J_{P–O–C–C}$ 6), 20.7 (t, $^2J_{P–C–C}$ 5), 23.4, 26.0, 27.0, 28.0, 34.3 (t, $^1J_{P–C}$ 134), 40.1, 54.4 (t, $^3J_{P–C–C–C}$ 6.5), 56.2, 63.5 (4 C, br s), 65.6, 167.4; MS *m*/*z* (positive, ES) calcd for $C_{19}H_{41}NO_8P_2Br$ $[M + H]^+$ 552.1491 found 552.1533.

3-((5-(2-Bromoacetoxy)pentyl)(methyl)amino)propane-1,1-diyldiphosphonic acid 3

Bromotrimethylsilane (120 μL, 0.91 mmol, 24 equiv.) was added dropwise to a stirred solution of tetraethyl bisphosphonate **12** (21 mg, 38 μmol, 1 equiv.) in dry DCM (5 mL) under argon. The reaction mixture was stirred at 25 °C in the dark under argon for 3 d. The reaction mixture was then concentrated under vacuum. The concentrate was solvolysed with methanol (2 mL) at r.t. for 30 min and concentrated again under vacuum. The concentrate was dissolved in distilled water (2 mL). The aqueous layer was washed with DCM (4 × 2 mL) and freeze-dried to afford the bisphosphonic acid **3** (17 mg, 95%) as a sticky gum. δ_P(121 MHz; D_2O; $(EtO)_3P(O)$) +20.7; δ_H(300 MHz; D_2O) 1.41 (2 H, quintet, *J* 7, CH_2), 1.64-1.77 (4 H, m, $CH_2 + CH_2$), 2.13-2.33 (3 H, br m, $P_2CH + P_2CHCH_2$), 2.83 (3 H, s, CH_3), 3.11 (1 H, m, CH_AH_BN), 3.19 (1 H, m, CH_AH_BN), 3.34 (1 H, m, $P_2CHCH_2CH_ACH_BN$), 3.44 (1 H, m, $P_2CHCH_2CH_ACH_BN$), 3.99 (2 H, s, CH_2Br), 4.24 (2 H, t, *J* 6, $C(O)OCH_2$); δ_C(75.5 MHz; D_2O) 21.0 (br s), 22.6, 23.6, 26.9, 27.5, 36.4 (t, $^1J_{P–C}$ 124), 40.0, 55.9 (br s), 56.5, 67.1, 170.6.

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