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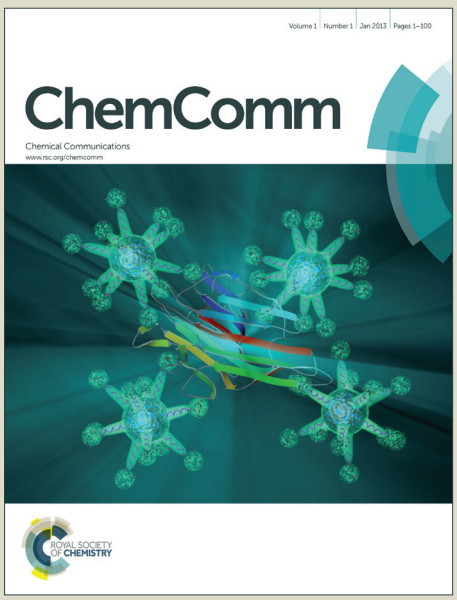

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Solventless synthesis of acyl phosphonamidates, precursors to masked bisphosphonates

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A series of acyl phosphonamidates, the synthetic precursors to bisphosphonates, have been readily prepared from phosphoramidite type reagents and a range of acid chlorides. These reactions were performed using solventless conditions, where purification was easily achieved using column chromatography with yields ranging from 71-90%. Furthermore, we have demonstrated that these acyl phosphonamidates could be used for the preparation of unsymmetrical bisphosphonates, which do date are scarcely reported in the literature.

Introduction

Acyl phosphorous compounds are a unique class of phosphorous reagents with unique reactivity patterns, where the C-P bond is susceptible to facile cleavage under nucleophilic attack, including acid, base and oxidative hydrolysis. For this reason, they are often considered as synthetic equivalents to acyl chlorides.[1] Because the reactivity of the carbonyl towards nucleophilic addition can be modulated by controlling reaction conditions such as temperature and the nature of the nucleophile,[2] acylphosphonates have been widely employed in the generation of phosphorous-containing analogues of biologically active compounds. These include covalent protein probes that target the active sites of phosphotyrosine binding proteins,[3] hydrogels derived from bisphosphonate-based gelator molecules[4] and triethylene glycol-bisphosphonate conjugates, which are potential candidates for nanoparticle targeting.[5] They have also been employed as competitive inhibitors of the MenD enzyme which is required for menaquinone (vitamin K_2) biosynthesis in many bacteria.[6] Of note in this class of phosphorus containing compounds are the bisphosphonates (BP's) which are structural analogues of pyrophosphates and are characterized by a stable P-C-P linkage (Figure 1).

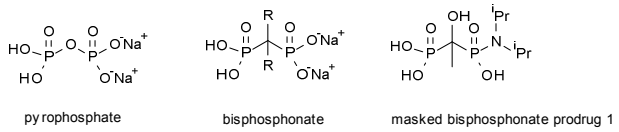

Figure 1 Structure of pyrophosphate, bisphosphonate and masked bisphosphonate

The BP's particular high binding affinity of hydroxyapathite, and therefore the bone matrix, has led to their use in the treatment of bone disorders such as osteoporosis and Paget's disease.[7] In this context, compounds like alendronate, pamidronate, zoledronate, risedronate and etidronate (Figure 2, **2-6**) are currently widely prescribed for the treatment of osteoporosis.[8] Furthermore, another bisphosphonate, clodronate (Figure 2, **7**), is used as a palliative agent during cancer treatment whereby it displays dual pharmacological properties, one as a Ca^{2+} chelator and the other as an anti-resorptive agent where it is metabolised into adenosine-β-γ-dichloromethylene triphosphate (Figure 2, **8**). [9] BP's polyanionic nature at physiological pH precludes their uptake into extraskeletal tissues and induces poor bioavailability.[10]

To overcome this, many masking prodrug strategies have been put in place in order to achieve higher circulation concentrations of these drugs.[11] Wiemer and co- workers have adopted such approaches by developing a series of pivaloyloxymethyl (POM) protected phosphonates.[12]

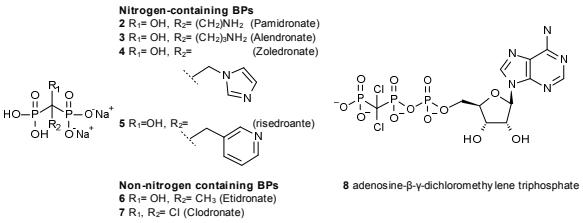

Figure 2 Structure of clinically prescribed BP's and clodronate metabolite, adenosine-β-γ-dichloromethylene triphosphate

Hydroxymethylene-1,1-bisphosphonate (HMBPs) derivatives (Figure 3, **9a-g**) have also been utilized in this regard, where Lecouvey *et al* synthesised a series of HMBP partial esters *via* a range of bis(silylated) α- ketophosphonates. These molecules have been shown to be useful against metastic cancer and have been used in prodrug strategies to bisphosphonates.[13] Moreover, phosphoramidate masking approaches have been applied to a variety of drug molecules and have been evaluated for the treatment for a variety of disease states, including HIV, type 2 diabetes, hepatitis C and cancer.[14] More recently, Meyers *et al* developed a bisphosphonamidate prodrug strategy which enhanced the membrane permeability of the BP clodronate (Figure 3, **10**) *via*

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the incorporation of biodegradable nitroaryl delivery groups and two chlorobutylamine masking groups.[15]

The synthesis of acylphosphonates is well documented in the literature being generally prepared *via* well-known Arbuzov-type reactions,[16] or from hydroxyphosphonates using oxidants such as alumina-supported CrO_3[17] and zinc dichromate trihydrate.[18] Yet, the synthesis of non-symmetrically substituted acylphosphonates still remains problematic, where the reaction often results in a mixture of the both symmetric and non-symmetric acylphosphonates leading to low yields.[19] Furthermore, access to acyl phosphonamidates also remains synthetically challenging. For instance, *Breuer et al* described the synthesis of a series of acyl phosphonamidates using methyl benzoylphosphonochloridate and a series of amines in DCM in the presence of pyridine or excess amine.[20] Unfortunately, the products could not be isolated in pure form. Facing similar challenges and despite their prevalence as synthetic precursors to oligonucleotides, phosphoramidites have been rarely used as nucleophilic starting materials to access phosphonamidates.[21] These reactions are often hampered by low yields and require strenuous conditions. On many occasions, the stability of the acyl phosphonamidate intermediate is viewed as a limiting factor.[22]

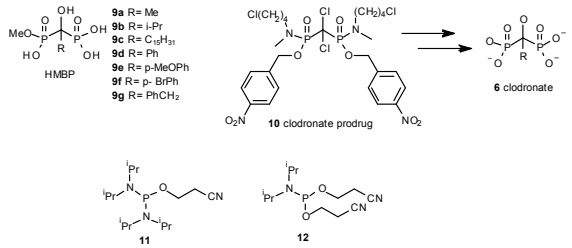

Figure 3 Structure of bisphosphonate prodrug analogues and their acyl phosphonamidate synthetic precursors

To date, there is no straightforward protocol towards the synthesis of acyl phosphonamidates, in particular unsymmetrical acyl phosphonamidates, despite their synthetic importance. The phosphoramidite reagents required for the synthesis of these reagents are themselves highly reactive and hydrolytically unstable meaning their preparation and handling is often problematic. Within our laboratory, we have demonstrated that the use of ionic liquids (IL's) can be used to enhance the chemoselectivity, as well as, the hydrolytic stability for the preparation of a series of phosphoramidite reagents.[23] As such, we have implemented the use of this ionic liquid protocol for the preparation of the synthetic precursors **11** and **12** to acyl phosphonamidates, Figure 3.

Our initial attempts towards the synthesis of acyl phosphonamidates involved the reaction of phosphorodiamidite **11** with benzoyl chloride. Gratifyingly, the reaction proceeded cleanly giving the desired phosphonamidate **14** in high yield (92 %), Figure 4. We envisaged the use of a bulky amino substituent in order to enhance the stability of the phosphonamidate products. It has been previously demonstrated that smaller amino substituents result in molecules that are highly unstable. In fact, in our hands, if the diisopropylamino substituents were replaced with morpholino substituents and reacted with **13a**, the reactions were highly exothermic and extensive fuming was observed, even when performed at low temperature. Analysis of the crude ^{31}P NMR indicated a significant amount of degradation as well as the formation of trivalent phosphorus species as opposed to the desired phosphonamidate, as has been previously observed by Antokhina and Alimov and later by Gazizov *et al*.[24] The formation of a trivalent "P" species is possible *via* acetylation of the nitrogen centre, which is less likely when bulky amino substituents are used due to the steric shielding of the nitrogen lone pair. As such, diisopropyl substituents were favoured for subsequent reactions. In comparison, the synthesis of phosphonamidate **14** was successfully carried out in the absence of solvent at room temperature.

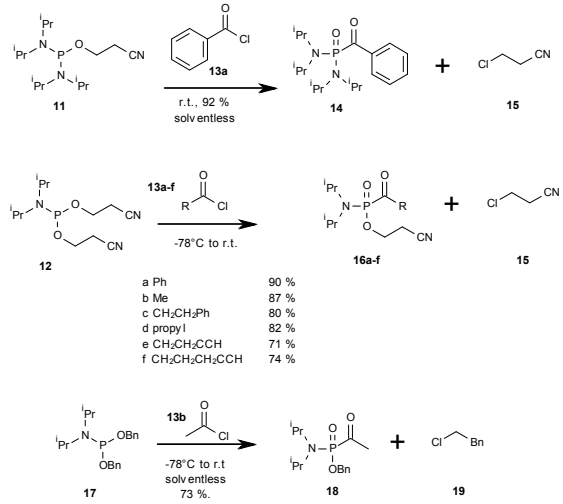

Figure 4 Solventless synthesis of phosphonamidate **14** and acyl phosphonamidates **16a-f** and **18**

^{31}P NMR analysis of the reaction mixture indicated that **14** had been obtained quantitatively as evidenced by the sole peak at δ 14 ppm in the ^{31}P NMR spectrum. ^{1}H NMR of the crude mixture also confirmed the presence of **14** as well as the by-product, **15**, which confirmed the reaction had proceeded *via* a Michaelis-Arbuzov type rearrangement. **15** was observed as two triplets in the crude ^{1}H NMR spectra at 2.85 ppm and 3.71 ppm with *J* values of 6.7 Hz. Moreover, no hydrolysis of **11** was observed, highlighting the significant advantage of solventless protocols when applied to reactive phosphorus reagents as well as the incorporation of bulky amino substituents. Acyl phosphonamidate **14** was readily isolated, proved to be highly stable and could be stored at room temperature without the need for an inert atmosphere. ^{1}H NMR of the isolated material revealed there was no free rotation about the P-N bond as evidenced by the presence of two doublets at 1.17 and 1.31 ppm with *J* values of 7.2 Hz, which were indicative of the CH_3 protons of isopropyl groups. Unfortunately, when **14** was subsequently reacted with triethylphosphite $P(OEt)_3$ to assess its suitability as a precursor to its corresponding masked BP, no reaction was observed as indicated by ^{31}P NMR. It has been well reported that the use of Lewis acid catalysts can accelerate the reaction between a phosphite and a reactive carbonyl centre.[25] As a more user friendly alternative, Kolodyazhnyi and co-workers demonstrated that pyridinium salts, in particular pyridinium perchlorate ($Py.HClO_4$), could be used as an alternative easily recyclable catalyst for this transformation.[25] However, even under these conditions, no BP formation was observed and **14** was recovered without any degradation. We concluded that the steric bulk imparted by two *N*-substituents is likely to play a role in a sterically crowded transition state leading to the masked

bisphosphonate. Bis-(2-cyanoethyl)-*N*,*N*-diisopropylphospho-ramidite, **12**, was then considered as the synthetic precursor to acyl phosphonamidates. The inclusion of one diisopropylamino substituent was retained as it was thought it would provide the best balance between reagent stability and reactivity, by virtue of steric hindrance. The reaction between **12** and acid chlorides **13a-f** were performed under solventless conditions, according to Figure 4. These reactions were highly selective towards the formation of the desired acyl phosphonamidates, **16a-f**, and were readily isolated in high yields, Figure 4. The acid chlorides **13e** and **13f** were prepared from their corresponding acid and oxalyl chloride, where the completion of the reaction was determined by ^{1}H NMR, where a proton shift of the CH_2 protons adjacent to the carbonyl centre was observed in each instance. Notably, when the reactions described in Figure 4 were performed at room temperature they were extremely exothermic and thus required external cooling. This cooling also helped to limit a troublesome side reaction occurring, due to the ability of alkyl/ acylphosphonates to exist in equilibrium with their enol form. For example, upon the reaction of **13b** with **12** the crude ^{31}P NMR indicated two peaks at δ 7.88 ppm and 12.33 ppm. Further analysis of the ^{1}H NMR spectra of this reaction mixture suggested the presence of an alkene product (δ dd, 2H, 5.69 ppm and 6.01 ppm). The presence of these doublets suggested the existence of the keto/enol forms of **16b**, where this enol form reacts with excess acetyl chloride to produce the acetyoxyvinylphosphonic diamide (see ESI). As expected, this by-product was not observed for the reaction of **12** with benzoyl chloride. Gratifyingly, cooling of the reaction vessel helped to limit the amount of arkyl/arylvinyloxyphosphonic diamide formed in each reaction, where the desired acyl phosphonamidates were isolated in high yields *via* column chromatography (71-90 %). This protocol was readily extended to incorporate –OBn protecting groups on the phosphoramidite starting material, as shown in Figure 4 by the reaction of **17** with **13b**. This reaction once again proceeded cleanly to afford the desired phosphonamidate **18** in 73 % yield after isolation. Acyl phosphonamidates **16a-f** and **18,** represent viable tools in a plethora of organophosphorous based chemistry. For example, their comparable α-ketophosphonates have been shown to be instrumental in enantioselective organocatalysis, for the preparation of biologically interesting enantiomerically pure products *via* asymmetric reduction and as dionophiles in hetero Diels-Alder reactions for the preparation of glycosyl type phosphonates.[26] Moreover, the synthesis of BP's with the incorporation of a P-N substituent has not been well reported; however Barbosa *et al* have reported the synthesis of a series of bis-heterocyclic bisphosphonates and phosphonamidates utilizing the Pudovick reaction and the catalyst Nb_2O_5.[27]

To establish the applicability our newly synthesised acyl phosphonamidates for the preparation of BP prodrug candidates, **16b** and **18** were reacted with $P(OEt)_3$ in the presence of $Py.HClO_4$ (Figure 5). Two molar equivalents of $Py.HClO_4$ along with an equimolar amount of $P(OEt)_3$ reacted with **13b** to afford compounds **20** and **21**, the precursors to the masked BP, **1**. An excess of $P(OEt)_3$ was used due to the hydroscopic nature of $Py.HClO_4$, despite being dried prior to use. This resulted in the hydrolysis of $P(OEt)_3$ during the reaction. Nevertheless, this hydrolysis product could be easily removed under vacuum due to its low boiling point of ~50°C. Moreover, when the reaction was performed in the absence of a catalyst, no reaction occurred. Compounds **20** and **21** were isolated in 71 % and 70 % yield. It is also important to note that the $Py.HClO_4$

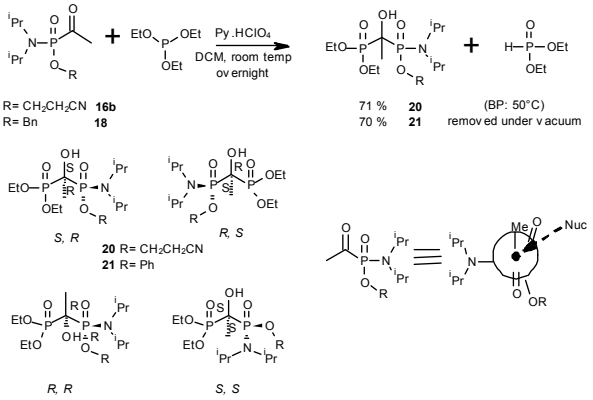

Figure 5 Preparation of compounds **20** and **21** from acyl phosphonamidates **16b** and **18** and the possible diastereoisomers

Figure 6 Preparation of masked BP **1**

Furthermore, it has been well documented that isomerization of the P–C–P bridge to give a P–C–O–P bridge can readily occur, particularly when all four phosphonic –OH groups are masked.[28] As such, esterification of the bridging carbons alcohol moiety (C-OH) has been implemented to prevent rapid isomerisation of tetraester BP's. The acyl phosphonamidates synthesised herein have been demonstrated to be stable to this isomerization, thus negating the need for hydroxyl manipulation prior to BP synthesis. Finally, four distinct ^{31}P chemical shifts were observed for compounds **20** and **21**, confirming that each product exists as diastereoisomers invoked by the two chiral centres inherent in both molecules, Figure 5. The stereoisomers exist in a 5:1 ratio as indicated by the ratio of the peaks in the ^{31}P NMR spectrum. We suggest that this ratio is governed by the chiral nature of the starting phosphonamidates, where the Felkin-Ahn model predicted in Figure 5 seems to offer the best rationalisation for nucleophilic attack from the incoming phosphite, and therefore the resultant diastereoisomeric ratios observed. Compounds **20** and **21** were then subjected to treatment with TMSBr followed by subsequent hydrolysis with $MeOH/H_2O$ in an attempt to reveal the fully deprotected masked BP, **1**. Under these conditions, **21** was fully deprotected to afford **1** without any degradation of the amino substituent. Deprotection of **20** under the same conditions, however, showed that although complete deprotection of the ethyl moieties had taken place, complete removal to the cyanoethyl protecting group had not occurred as evident by ^{1}H NMR. In fact, even when the deprotection of **20** was attempted using 6M HCl, complete removal of the cyanoethyl protecting groups was still unsuccessful. We therefore concluded that -OBn protecting groups were preferred to that of cyanoethyl's in our novel synthesis towards the BP prodrug analogue **1**, Figure 6. To further demonstrate the versatility of our synthetic route towards masked BP's, we explored the versatility of the amino substituent. Hence, phosphoramidite **22** was reacted with $P(OEt)_3$ in the presence of $Py.HClO_4$. However, under these conditions, chlorophosphite **23** was formed in favour of the expected BP **26**, Figure 7. Under these conditions, BP formation was not favoured

due to the enhanced electrophilic nature of the phosphorus centre when small amino substituents are present, resulting in the formation of the corresponding bis-alkyoxychlorophosphine **23** (observed at δ 165 ppm in the ^{31}P NMR). This is possible *via* acetylation of the amino group in favour of the phosphorus centre. We therefore explored the possibility of synthesising **26** *via* the ketophosphonate intermediate **25**. We postulated that **25** would behave as a softer electrophile in relation to acetyl chloride therefore favouring our desired reaction pathway towards the unsymmetrical BP **26**. Gratifyingly, when phosphoramidite **22** was reacted with ketophosphonate **25** in the presence of Py.$HClO_4$, **26** was obtained as a diastereoisomeric mixture.

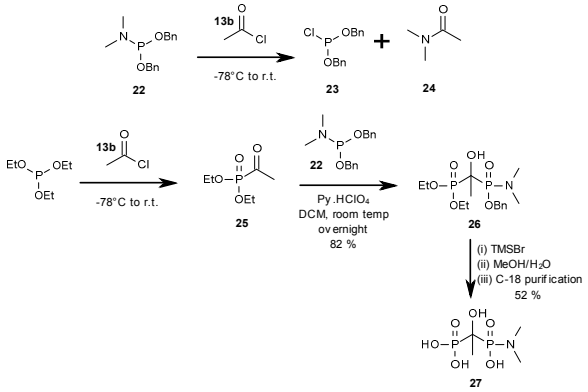

Figure 7 Synthesis of masked BP with small amino substituent

However, ^{31}P NMR also showed the presence of two unidentified trivalent 'P' species which were inseparable from the product by standard silica column chromatography. Nevertheless, the crude mixture was subjected to deprotection conditions with TMSBr and purified by careful C-18 reverse phase column chromatography to afford the fully deprotected masked BP **27**.

Conclusions

Herein, it has been demonstrated that phosphorus reagents of the type $P(OR)(NR'_2)_2$ and in particular $P(OR)_2(NR'_2)$, are extremely useful reagents for the rapid production of acyl phosphonamidates from a range of acid chlorides. We have demonstrated that the phosphonamidates are highly stable and valuable intermediates for the preparation of the BP prodrug analogues **1** and **27**. They display significant advantages over their phosphonate counterparts in that the incorporation of bulky amino moieties into these structures limits the possibility for BP rearrangement whilst imparting reagent stability. Finally, the acyl phosphonamidates synthesised herein would allow for access to functionalisable bisphosphonate analogues *via* P-N bond activation.

Acknowledgements

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