MedChemComm

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Journal:	*Medicinal Chemistry Communications*
Manuscript ID:	MD-REV-06-2014-000255.R1
Article Type:	Review Article
Date Submitted by the Author:	06-Aug-2014
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Synthesis of α-brominated phosphonates and their application as phosphate bioisosteres

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(C. Cairo)

Abstract

Substrate phosphorylation is a key modulator of signal transduction. Abnormal phosphorylation *in vivo* is implicated in many diseases including cancer, diabetes, and Alzheimer's disease. Inhibitors of phosphate-recognizing proteins have potential as medicinal agents as well as tools to study phosphorylation pathways. A well-known and common inhibition strategy is to synthetically replace the labile phosphate moiety with a non-hydrolyzable phosphonate. Fully saturated, α-fluoro and α,α-difluorophosphonates are often effective phosphate bioisosteres and have been well studied. More recently α-brominated phosphonates have begun to emerge as inhibitors of phosphate recognizing enzymes, some of which operate by irreversible mechanisms. Herein we discuss the synthetic approaches to aliphatic and benzylic α-bromophosphonates and their biological activities.

Introduction: Phosphonates and α-halophosphonates as phosphate bioisosteres

Substrate phosphorylation is a pivotal modulator of cellular function through its regulation of signal transduction pathways. It is estimated that one-third of all cellular proteins are modified through phosphorylation, making this the second most prevalent post-translational modification after glycosylation.[1] Phosphorylation of target molecules is carried out by kinases, acting in opposition to phosphatases, which dephosphorylate these same targets.[2] A large number of kinases and phosphatases are found in humans which generate or modify phospho-serine, threonine, tyrosine, or histidine residues.[2,3] Additionally, phosphorylation pathways on lipid- and carbohydrate-based substrates play fundamental biochemical roles in metabolic processing and

protein–carbohydrate or lipid recognition.[4]

Due to the ubiquitous nature of substrate phosphorylation in cell signaling networks, these pathways are important to physiological processes including cellular differentiation, migration, metabolism, and apoptosis.[2,5,6] Aberrant phosphorylation pathways can have devastating physiological consequences and may play a role in pathologies including cancer,[6] diabetes,[7] and Alzheimer's disease.[8] As a result of these wide ranging effects, general strategies for the design of improved inhibitors and probes of this class of enzymes, or the receptors that recognize them, is of continued interest to medicinal chemists. Novel functional groups that can be exploited for inhibitor design may be valuable components of research tools or therapeutic strategies.

It has been known for many years that a general strategy for producing competitive inhibitors of phosphate recognizing proteins is to create substrate mimics that replace the hydrolyzable C–**O**–**P** bond of a phosphate with a non-hydrolyzable C–**C**–**P** bond to yield a phosphonate moiety. Phosphonates are not only resistant to enzymatic cleavage, hydrolysis of the functional group is also dramatically decreased allowing for synthetic manipulation of the molecule after installation of the phosphonate.[9] However, there are obvious shortcomings to this strategy. Firstly, replacement of an electronegative oxygen atom with a non-polar methylene group may reduce affinity for the phosphate-recognizing-protein active site. Solubility of the compounds in water may also be decreased. The phosphonate group may also exhibit changes in charge state, for example if the pKa of the phosphonic acid is increased above physiological pH;[10] this can make salt bridge or hydrogen bond contacts less favorable in an enzyme active site.[9,11]

An early approach to address this issue was the installation of fluorine atoms on the phosphonate

α-carbon, thus making the phosphonate a more effective electronic isostere of the phosphate group.[12,13] α-Fluorophosphonates also reduce the pKa of the phosphonic acid protons below physiological pH (pKa_2 ~5.6), restoring the group to the same charge state as a native phosphate (pKa_2 ~6.4) at physiological pH.[11,12]

Medicinal chemists have more recently exchanged fluorine for bromine to determine the activity of α-bromophosphonates as inhibitors. Typical C–X bond lengths within α-halophosphonate compounds are 1.41 Å (C–F),[14] 1.79 Å (C–Cl),[15] and 1.93 Å (C–Br).[16] Thus, α-bromophosphonates may have different steric requirements than their fluoro-derived counterparts. Additionally, while the bromine atom does not offer the same electronegativity as fluorine, it may act as a good leaving group offering the potential to create covalent labels of enzyme active sites that contain potent nucleophiles.[17,18] This unique combination of features makes α-bromophosphonates a useful tool for the medicinal chemist. Herein we will discuss the synthesis and examples of applications which have exploited α-bromophosphonate derivatives in biological studies.

1. Design, synthesis and evaluation of α-bromophosphonates as bioactive compounds

α-Bromophosphonates were first described in the literature over 90 years ago. The earliest example of an α-bromophosphonate was isolated as an α,β-dibromo phosphonic acid intermediate (**1**, Fig. 1) which was decomposed in the presence of base to provide a β-bromo-α,β-unsaturated phosphonic acid.[19] However, as the use of phosphonates expanded in medicinal

chemistry, additional reports of the synthesis of α-bromophosphonates as synthetic targets began to emerge. Early examples of α-bromophosphonates appeared in a 1953 study of pKa values of α-substituted phosphonic acids (**2**).[10] The pKa values of compound **2** were found to be 1.14 and 6.52, respectively,[20] indicating that the α-bromophosphonates provide a pKa lowering effect over CH_2-phosphonates (pKa_2 ~7.6) which more closely matches the acidity of the phosphonate to that of naturally occurring phosphates. This also indicates that the α-bromophosphonate group is 10-fold more basic than the α-fluorophosphonate moiety.[12]

The first biological studies of alkyl α-bromophosphonates (**3**) were conducted with α-bromophosphonates tested as agricultural chemicals. These compounds were found to be active in killing worms, insects, and bacteria.[21]

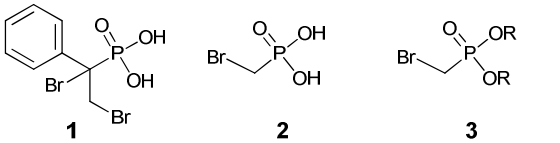


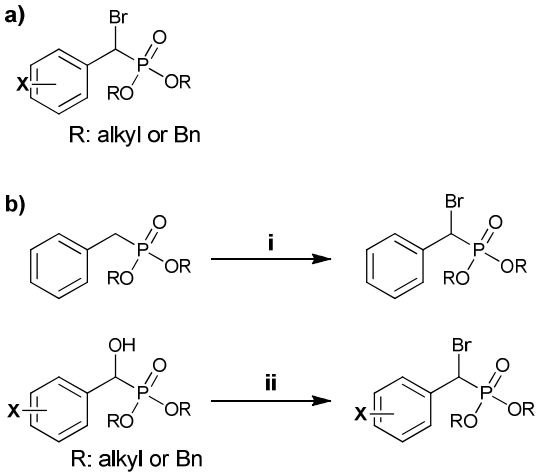
Fig. 1 Early examples of α-bromophosphonates.

1.1. Benzylic α-bromophosphonates

Synthetic access to benzylic α-bromophosphonates (BBP) has been well studied, and these are generally easier to access than their aliphatic counterparts (Scheme 1). This functional group has been exploited as a bioisostere of phosphotyrosine residues in medicinal chemistry. We highlight examples of BBP in the literature, and discuss general strategies for their synthesis below.

1.1.1. Synthetic approaches to BBP

Installation of bromine at the α-position of benzylic phosphonates is usually conducted through one of two general approaches, either (i) direct bromination or (ii) bromination of an α-hydroxy benzylphosphonate (Scheme 1b).

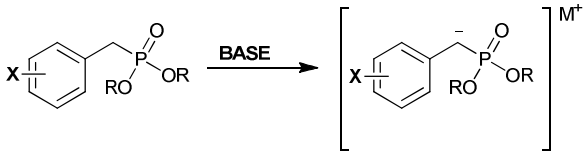


Scheme 1 a) A generic benzylic α-bromophosphonate (BBP). **b)** Synthetic strategies for obtaining BBPs.

A summary of reported conditions used through pathway i (Scheme 1b) is given in Table 1. The fully saturated phosphonate precursor is typically converted to the α-bromophosphonate through either radical bromination using *N*-bromosuccinimide (entries 1–4, Table 1) or through deprotonation of the α-proton to form a phosphorus-stabilized carbanion that is subsequently quenched with an electrophilic bromine source (entry 5, Table 1). Benzyl diethylphosphonate was subjected to three equivalents of lithium hexamethyldisilazide (LiHMDS) at −78 °C and the phosphorus-stablized anion was quenched with trimethylsilyl chloride (TMSCl) to temporarily

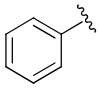
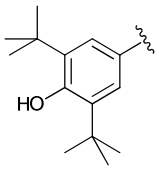
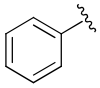
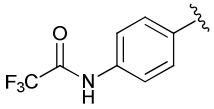
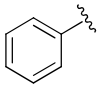
install a TMS group at the α position *in situ* (entry 5). Under these conditions, a second deprotonation occurred simultaneously which was subsequently quenched with an electrophilic bromine source, 1,2-dibromotetrachloroethane (TCDBE). The reaction was then warmed to 0 °C and lithium ethoxide was added to cleave the TMS group, furnishing the BBP adduct in good yield. Importantly, for all entries in Table 1, a 1:1 mixture of diastereomers at the α position is expected in the absence of any directing groups.

The phosphorous-stabilized α-carbanion (Scheme 2) has been examined by several groups. The pKa of the alkyl phosphonate α proton is relatively high (~29)[22] and deprotonation requires the use of a strong and non-nucleophilic base to produce the anion.[12] Cantat et al. confirmed that anionic C–P bonds are shorter than C–P bonds of the corresponding saturated compounds using crystallography. Computational analysis suggested stabilization of the anion through hyperconjugation resulting from $p(C) \rightarrow \sigma^*(P–O)$ donation. It was concluded that contact with the metal cation is shared between the O=P–C atoms.[23]



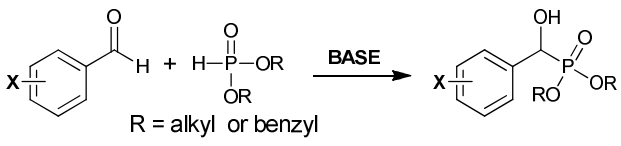
Scheme 2 Deprotonation of a benzylic phosphonate to form a phosphorus-stabilized carbanion.

Table 1 Conditions for generation of benzylic α-bromophosphonates by bromination of a phosphonate precursor.

Entry	Ar	R	Conditions	Yield[a]	Reference
1		Me	$(PhCO)_2$, NBS, 76 °C	41%	Collins et al., 1984[24]
2		Et Me	NBS	55% 71%	Gross et al., 1990[25] Andriyashin et al., 2012[26]
3		^{i}Pr	$(PhCO)_2$, NBS, 76 °C	82%	Chakraborty et al., 1991[27]
4		Et	NBS, $h\lambda$	N/A	Pieper et al., 2000[28]
5		Et	1. LiHMDS, TMSCl, −78 °C 2. TCDBE,[b] −78 °C 3.LiOEt, 0 °C	87%	Iorga et al., 1999[29]

(a) The R group shown is for the highest yielding analog. (b) 1,2-dibromotetrachloroethane.

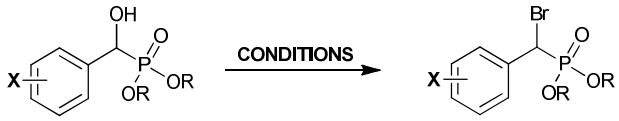
The more common synthetic approach to form BBP is *via* an α-hydroxyphosphonate intermediate (pathway ii, Scheme 1b). This strategy typically involves milder conditions than the strong basic conditions required for formation of the α-carbanion. Shown in Scheme 3 is a general approach to the synthesis of the parent α-hydroxy adduct *via* Pudovik reaction.[30] In this transformation, a benzyaldehyde derivative is subjected to treatment with a deprotonated dialkyl- or diarylphosphite to furnish a diastereomeric mixture of the α-hydroxy analog.

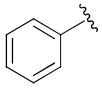
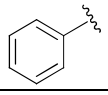
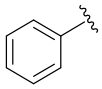
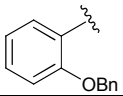
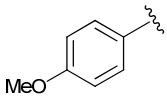
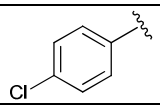
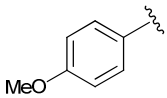
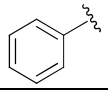
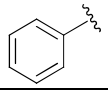
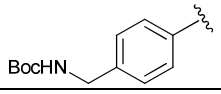
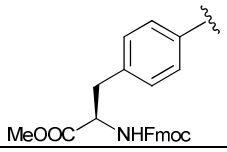
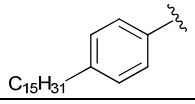
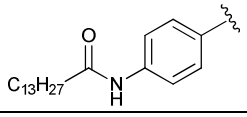


Scheme 3 General form of the Pudovik reaction to generate a benzylic α-hydroxyphosphonate.

After obtaining the α-hydroxy precursor, the subsequent bromination reaction can be performed using a variety of conditions. Several methods used to achieve this transformation are summarized in Table 2. Entries 1–8 are examples that achieved benzylic α-bromination with an electrophilic bromide source, with yields ranged from 42% to 100%. Entries 9–13 summarize conditions employed to obtain α-brominated phosphonates which were ultimately screened for activity against phosphate recognizing proteins. In all instances, the syntheses required no more than three chemical steps to obtain the target compound. Several analogs tested for activity from the references included in Table 2 are illustrated in Fig. 2. We discuss the biological activity of these species below.

Table 2 Benzylic α-bromination strategies.



Entry	Ar	R	Conditions	Yield[a]	Reference
1		Et	CBr_4, PPh_3, 80 °C	42%	Gajda, 1990[31]
2		Et	PPh_3/Br_2, C_5H_5N	42%	Gajda, 1990[31]
3			$SOBr_2$ or PBr_3	79%	Kumaraswamy et al., 1997[32]
4		Et	$SOBr_2$, C_5H_5N	88%	Gross et al., 1993[33]
5		Et	CDI,[b] allyl bromide	100%	Green et al., 1996[34]
6		Et	*n*-BuNBr, PPh_3, DDQ	98%	Firouzabadi et al., 2004[35]
7		Et	APDPP,[c] Br_2	100%	Iranpoor et al., 2006[36]
8		Et	$SOBr_2$	80%	Balczewski et al., 2006[37]
9		Et	NBS, C_5H_5N	N/A	Taylor et al., 1996[38]
10		Et	PPh_3/Br_2, C_5H_5N	51%	Kumar et al., 2004[39]
11		Me	$SOBr_2$, C_5H_5N	88%	Tulsi et al., 2010[40]
12		Me	PBr_3	N/A	Gupte et al., 2011[41]
13		Et	CBr_4, PPh_3	81%	Jiang et al., 2011[42]

(a) The R group shown is for the highest yielding analog. (b) *N,N*-carbonyldiimidazole; (c) 4-aminophenyldiphenylphosphinite (APDPP).

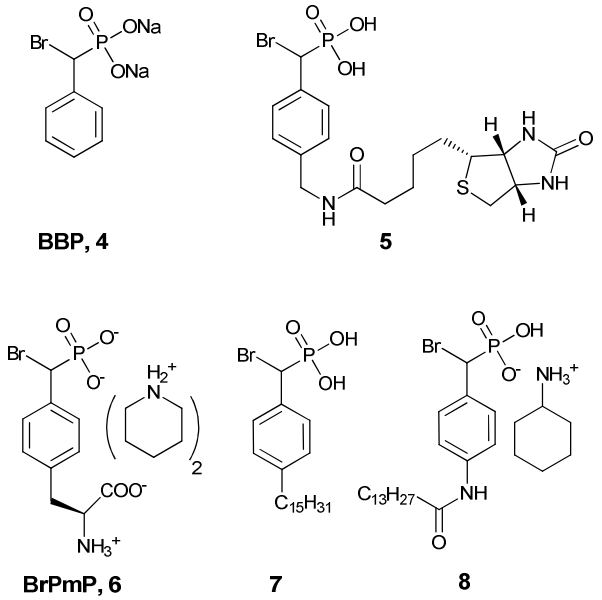
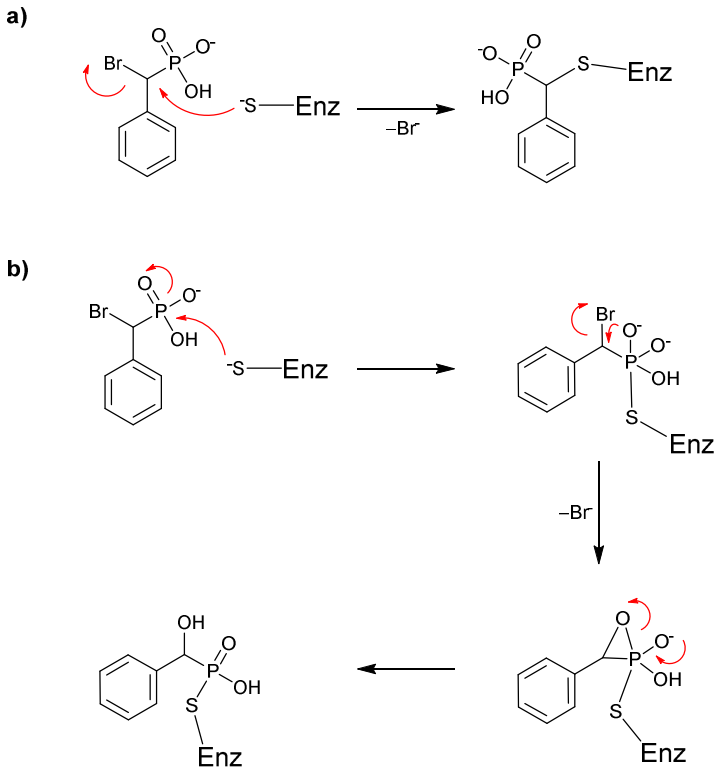


Fig. 2 Biologically active BBPs.[43]

1.1.2. Benzylic α-bromophosphonates as irreversible inhibitors

The Widlanski group was the first to examine α-bromophosphonates as inhibitors of phosphate recognizing proteins.[38] BBP analog **4** was screened against a truncated recombinant form of the protein tyrosine phosphatase (PTP), Yop51 (Yop51*Δ162), from *Yersinia enterocolitica.* The PTP superfamily, which includes Yop51, consists of a key conserved Cys-(X_5)-Arg active site motif, with the cysteine being responsible for catalysis.[39] Subsequent work has investigated the role of the nucleophilic cysteine residue in displacement of the α-bromide to produce a covalent adduct. Two mechanisms for covalent inhibition have been proposed (Scheme 4); in the first, the active site thiolate undergoes a direct S_N2 displacement of the α-bromide, creating a covalent

C–S bond that inactivates the enzyme. In the second proposed mechanism the nucleophilic cysteine attacks phosphorus to provide a pentavalent intermediate. This intermediate then collapses to concomitantly displace the α-bromide, resulting in a three-membered oxaphosphetane-like intermediate that may decompose to form the *S*-linked α-hydroxyphosphonate.[38] Mutagenesis experiments implicate a central role for the active site Cys; however, it is not clear which of these pathways is specifically responsible for enzyme inactivation.



Scheme 4 Proposed mechanisms for covalent inhibition of a PTP by BBP: **a)** S_N2 displacement of bromide or **b)** intramolecular formation of a three-membered oxaphosphetane-like intermediate.

Taylor et al. concluded that BBP **4** was an active site-directed covalent inhibitor of Yop51 at low mM concentration.[38] The absence of activity for probe **4** with alkaline phosphatase confirmed its specificity for PTPs. Zhang and coworkers expanded on this finding by constructing BBP analog **5**, which was proposed as an activity-based proteomic probe (ABPP) to covalently tag phosphatase enzymes.[44,45] Analog **5** built on the original BBP design by including an affinity tag, biotin, linked through the *para*-position of the aromatic ring. This modification enabled streptavidin to be used for detection of bioconjugates formed in the reaction of **5** with its targets. Testing of analog **5** as an irreversible inhibitor of YopH, another PTP from *Yersinia,* showed that its activity was comparable to **4** (Table 3). Compound **5** followed a similar inactivation time course to **4**; however, it displayed 5-fold improved specificity for the enzyme.[39]

Table 3 Equilibrium binding constant (K_I) and inactivation rate constant (k_i) for irreversible inhibitors **4**, **5**, and **9** & **10** against YopH.

Analog	K_I **(μM)**	k_i **(min^{-1})**
4	4100	0.11
5	740	0.17
9 & **10**	690	0.52

Importantly, when probe **5** was introduced into a mixture PTPs (YopH, PTP1b, HePTP, SHP2, FAP-1, PTPα, DEP-1, VHR, Cdc14 and PRL-3) at 1 mM concentration, covalent adducts were formed with all enzymes. Specificity of the reagent toward PTPs was confirmed by comparison to other phosphatase enzymes (alkaline, potato, prostatic acid, DSP and λ phosphatases) *in vitro* as well and among the melieu of cellular proteins found in the cell lysate of *E. coli*.[39] These studies were the first to demonstrate that α-bromobenzylphosphonates could be used

successfully for selective, irreversible inhibition of PTPs as an enzyme class. More recently α-BBP analog **5** was used by Boivin et al. to identify PTPs and monitor platelet-derived growth factor (PDGF) receptor signaling in an angiomyolipoma cancer cell model.[46] It is worth noting that these studies also raise the concern that, while extremely active, the α-BBP functional group alone does not provide specificity among different PTPs.[39]

Fluorescent tags (**9** and **10**, Fig. 3) have been incorporated into α-BBP derivatives to detect PTPs in breast, lung, liver, ovarian and cervical cancer cell lines. The cell lines could be specifically labeled using these fluorescent probes at a concentration of 1 mM. These rhodamine-based regioisomers of α-BBP were isolated as a mixture and were not separated (Fig. 3). These analogs offered a slight improvement in K_I over analog **5** (Table 3), but provided 1000-fold more sensitivity for detection of labeled enzyme.[47]

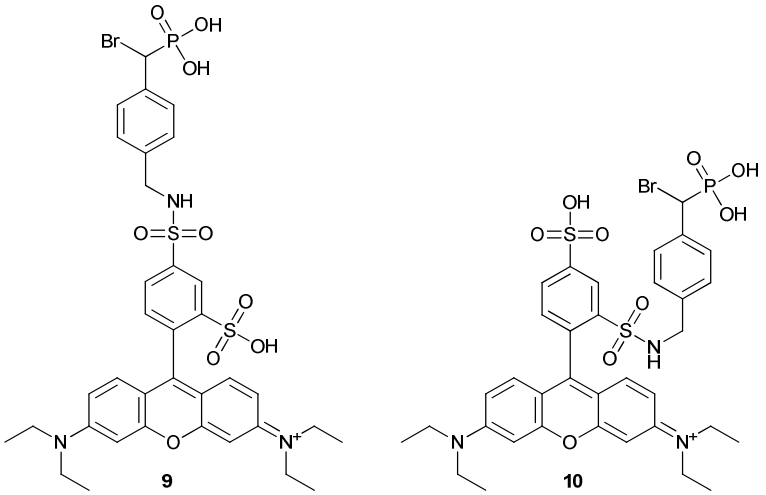
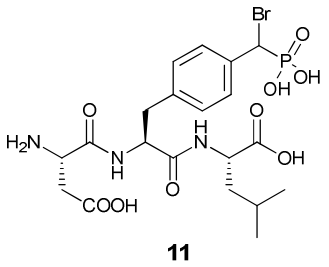


Fig. 3 The structure of fluorescent rhodamine-based α-bromophosphonate regioisomers **9** and **10**.[47]

More recently Tulsi et al. developed an improved strategy for the design of BBP-based inhibitors to address the problem of selectivity among PTP enzymes. They approached this issue by developing a method to incorporate α-BBP-based analogs into peptides, offering the possibility of increased specificity for individual PTP enzymes and modular synthesis of new inhibitors. The analog was synthesized as a protected phosphotyrosine analog incorporating the α-BBP functional group, L-bromophosphonomethylphenylalanine (BrPmp, **6**). BrPmp was the first attempt to incorporate BBP into an amino acid derivative. BrPmp was synthesized from L-tyrosine in 14 steps and 22% overall yield. It was then incorporated into the tripeptide sequence, Asp-BrPmp-Leu (**11**, Fig. 4) using solid phase peptide synthesis.[40] This sequence was selected as an example of a short sequence that contained an acidic residue *N*-terminal to the phosphotyrosine site, and a hydrophobic residue at the *C*-terminal side—both of which are features commonly found in phosphopeptide substrates of the human PTP enzyme, CD45.[23] While BBP **4** was only a weak inhibitor, BrPmp inhibited CD45 both as an individual amino acid and as part of tripeptide analog **11**.[40]



11

Fig. 4 Synthetic tripeptide incorporating BrPmp shown to have specificity for immune PTP, CD45.[40]

Tulsi et al. confirmed that **6** was indeed a covalent inhibitor of CD45 using a Kitz-Wilson

analysis with similar rate of inactivation to that of tripeptide **11**. Importantly, they found that BrPmp in the context of the tripeptide had 4-fold increased specificity for the enzyme over BrPmp alone (Table 4).[40] These results strongly suggest that an appropriate BrPmp peptide sequence could be used to target an individual PTP enzyme with high potency. The increased specificity of BrPmp in a peptide sequence provides support for this strategy as a means to design new inhibitors or probes that target individual PTPs.

Table 4 K_I and k_i for inhibitors **6** and **11** against CD45.

Analog	K_I (μM)	k_i (min^{-1})
6	40 ± 8	0.041 ± 0.001
11	16 ± 4	0.048 ± 0.003

1.1.3. Benzylic α-bromophosphonates as inhibitors of invasive and metastatic pathways.

Benzylic α-bromophosphonates have been investigated as inhibitors of invasion and metastasis in cancer cell lines. α-Bromobenzylphosphocholine derivatives have been synthesized as potential inhibitors of autotaxin (ATX), which catalyzes the hydrolysis of lysophosphatidylcholine to form the bioactive lipid lysophosphatidic acid (LPA, Fig. 5).[48] ATX expression is upregulated in numerous cancers and has been shown to aid in tumor cell motility, resulting in implications for metastasis and invasion, as well as chemotherapy resistance.[41,42,49] LPA itself has demonstrated mitogenic, motogenic and anti-apoptotic properties that increase survival of carcinomas that use LPA in an autocrine and paracrine fashion.[41] Therefore, inhibitors of ATX provide an opportunity to reduce both ATX-induced and LPA-induced effects in cancer.[50]

In 2008, ATX inhibitor S32826 (Fig. 5) was identified as the first nanomolar inhibitor of ATX (K_i = 9.0 nM)[42] by high-throughput screening; however, it showed no inhibitory activity *in vitro* or *in vivo*.[41] Gupte et al.[41] postulated that the reason for this lack of activity was due to cleavage of the amide bond by other enzymes. The authors synthesized a library of S32826-based analogs, including α-bromophosphocholine **7**, which offered the advantage of a non-hydrolyzable aliphatic chain *para* to the alkyl chain. Compound **7** demonstrated a mixed mode of inhibition for ATX (IC_{50} 10.1 μM) with a K_i value of 6.10 μM and K_i' value of 2.97 μM.[51] Compound **7** was a much better inhibitor of ATX than the α-chloro derivative and was similar to the α-fluoro analog of the same scaffold. Interestingly, the analogous fully saturated phosphonate **12** (Fig. 5) was a much more potent inhibitor of ATX (IC_{50} = 0.17 μm, K_i = 0.27 μm, and K_i' = 0.28 μm) and was therefore tested more rigorously. Analog **12** showed significantly ($p < 0.05$) decreased LPA-dependent MM1 heptocarcinoma cell invasion of human umbilical vein endothelial cells (HUVEC), at a concentration of 3 μM. The authors observed that compound **12** exhibited anti-metastatic properties in B16-F10 melanoma cells, with a reduction in metastasis ($p < 0.05$) at concentrations of 0.5 mg·kg^{-1}day^{-1} for 21 days.[41]

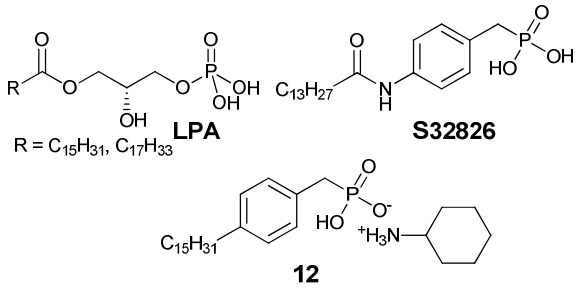


Fig. 5 LPA and related synthetic analogs.

The Prestwich group tested an α-BBP derivative of S32826, compound **8**. They argued that the lack of *in vitro* and *in vivo* activity was due to the low solubility of S32826. As a result, they opted to preserve the amide functionality and reduce the aliphatic chain length found in S32826. One resulting compound, α-bromophosphonate **8**, was found to inhibit ATX with nearly a 1000-fold improvement in potency (K_i = 8.1 nM) over compound **7**. This activity was also a 20-fold improvement over compound **12** and offered slight improvement over S32826.[42] These data suggest that the amide bond is crucial to potency toward ATX. We speculate that the limited activity of **12** and S32826 *in vitro* or *in vivo* could also be due to the reduced stability of these compounds, as hypothesized by Gupte et al.[41]

Although there are an appreciable number of early synthetic examples that incorporate the α-BBP functionality, interest in their application to inhibit phosphate recognizing proteins was not explored until the mid-nineties. We believe that the examples discussed above highlight the untapped potential of benzylic α-bromophosphonates as irreversible inhibitors and probes of enzyme activity.

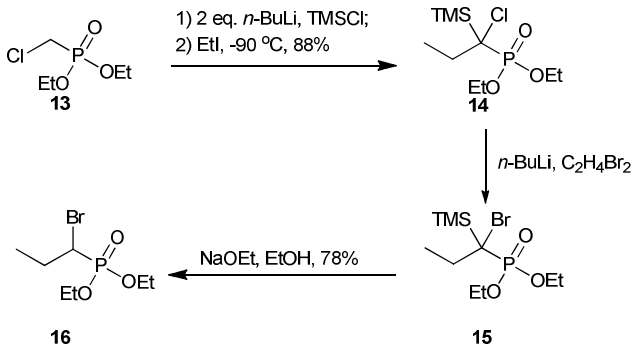
1.2. Aliphatic α-bromophosphonates

There are far fewer synthetic examples of aliphatic α-bromophosphonates relative to α-benzylbromophosphonates; however, several biologically active analogs have been identified and tested. We summarize the synthesis and biological properties of these below.

1.2.1. Synthetic approaches

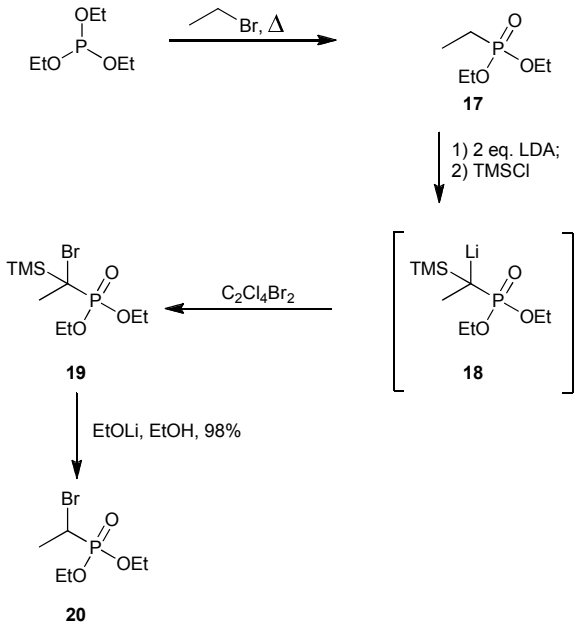
Synthesis of aliphatic α-bromophosphonates and α-halophosphonates is generally more difficult than that of α-BBPs. Interestingly, only a few synthetic reports have utilized an α-hydroxy precursor, which is the most commonly used parent compound for creating benzylic α-bromophosphonates.

An early example of an aliphatic α-bromophosphonate synthesis was reported by Teulade and Savignac, where they synthesized a series of five aliphatic α-bromophosphonates. The synthesis of propyl α-bromophosphonate derivative **16** is shown in Scheme 5. Compound **14** was generated *via* silylation of a phosphorus-stabilized carbanion intermediate and subsequent alkylation in one pot using two equivalents of *n*-BuLi, TMSCl, and ethyl iodide. Subsequent treatment of analog **14** with *n*-BuLi in the presence of 1,2-dibromoethane resulted in halogen–lithium exchange of the chlorine atom (**15**). In a second one-pot procedure, the α-bromo-α-silylphosphonate was converted to the final α-bromo product by removal of the TMS group with sodium ethoxide in ethanol to furnish **16** in 78% yield as a diastereomeric mixture.[52]



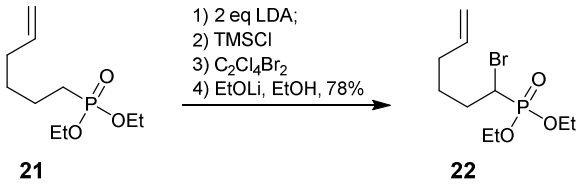
Scheme 5 The synthesis of an aliphatic α-bromophosphonate derivative.[52]

Savignac and coworkers later reported an improvement to this aliphatic bromination strategy that avoided the need for α-chlorophosphonate **13** (Scheme 6).[53] Instead, they utilized the Michaelis–Arbuzov reaction by treatment of triethylphosphite with an alkyl halide under reflux to obtain the starting alkyl phosphonate.[54] A number of alkyl phosphonates were synthesized using this method; however, Scheme 6 only depicts the synthesis of a single representative ethyl adduct, **20**. Treatment of compound **17** with 2 equivalents of lithium diisopropyl amide (LDA) to generate a phosphorus-stabilized carbanion, followed by dropwise addition of TMSCl, produced intermediate **18** which was confirmed through ^{31}P NMR studies. Lithium–bromine exchange was performed using tetrachlorodibromoethane. Finally, intermediate **19** was desilylated using lithium ethoxide in ethanol to furnish α-bromophosphonate **20** as a diastereomeric mixture in nearly quantitative yield from **17**.[53] In 2007, the Fu group also synthesized **20** using this procedure, but reported a more modest yield of 70%.[55]



Scheme 6 An improved synthetic strategy to alkyl α-bromophosphonates.[53]

To the best of our knowledge, the only other use of this methodology has been reported by Wnuk et al. Lithium–bromine exchange was used to convert saturated phosphonate **21** into α-bromo analog **22** as a precursor to radical cyclization (Scheme 7).[56]

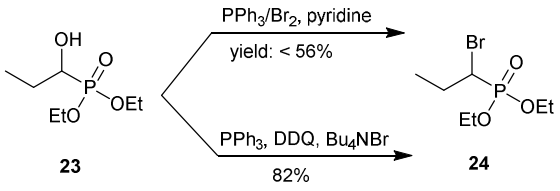


Scheme 7 An α-bromophosphonate as a precursor for radical cyclization.[56]

Although methodology for obtaining aliphatic α-bromophosphonates *via* an α-hydroxy intermediate has not been explored as thoroughly as for the α-BBP moiety, it has been used with moderate to good yields. This conversion generally utilizes far milder conditions and provides the opportunity to obtain enantiopure products, which has not yet been achieved *via* lithium–bromine exchange.

Fu and co-workers conducted a methodological study on this transformation by bromination of a library of α-hydroxyphosphonates using mild conditions. Scheme 8 shows an example using an alkyl phosphonate. α-Hydroxypropylphosphonate **23** was brominated to obtain **24** using either PPh_3/Br_2 and pyridine or PPh_3, DDQ and Bu_4NBr.[57] Mechanistic studies appear to rule out a radical pathway.[35] Analog **24** was used as an intermediate for subsequent fluorination *via* a phosphorus-stabilized carbanion using *N*-fluorobenzenesulfonimide (NFSI). Although the

authors discussed the potential importance of these α-bromo-α-fluorophosphonate analogs as inhibitors of PTPs, they did not report biological testing.[57]

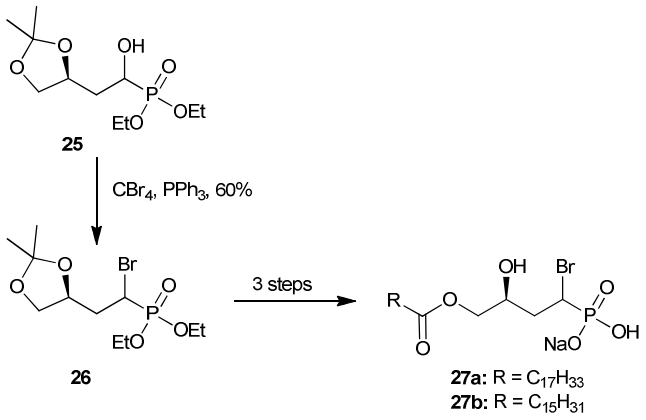


Scheme 8 Electrophilic bromination of aliphatic α-bromophosphonates.[57]

We focus now on examples of aliphatic α-bromophosphonates that have been assayed as inhibitors of phosphate recognizing proteins.

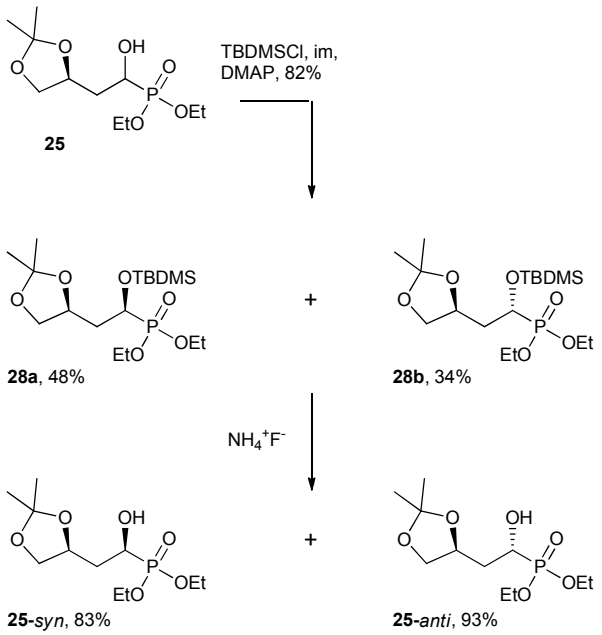
1.2.2. Aliphatic α-bromophosphonate analogs of LPA.

Aliphatic α-bromophosphonates have been screened against phosphate recognizing proteins involved in the ATX–LPA pathway as anti-invasive and anti-metastatic agents (*vide supra*). Prestwich and co-workers used an α-hydroxyphosphonate as the bromination precursor, which allowed for separation of the two epimers downstream (Scheme 10). Scheme 9 depicts the synthesis of α-bromophosphonates **27a** and **27b**. The parent α-hydroxy analog, **25**, was treated with CBr_4 and PPh_3 to obtain alkyl α-bromo analog **26** in moderate yield. Biologically active phosphonates **27a** and **27b** were then obtained through acylation and phosphonate ester hydrolysis.[50]



Scheme 9 The synthesis of biologically active α-bromo analogs **27a** and **27b** *via* α-hydroxy intermediate **25**.[50]

By treating analog **25** with *tert*-butyldimethylsilyl chloride (TBDMSCl) under basic conditions, the resulting bulky siloxane product could be separated using flash chromatography. The TBDMS group was then cleaved under mildly acidic conditions to furnish the isolated α-hydroxy diastereomers (**25**-*syn*, **25**-*anti*, Scheme 10).[50]



Scheme 10 The separation of α-hydroxy derivative **25** into its two diastereomers.[50]

1.2.3. α-Bromophosphonates as inhibitors of cell invasion and metastasis

Aliphatic derivatives **27a** and **27b** were designed to target the G-protein coupled receptors (GPCRs) of LPA, which regulate cancer cell proliferation, invasion, and angiogenesis. Compounds **27a** and **27b** were found to inhibit the four GPCRs screened, LPA_1, LPA_2, LPA_3 and LPA_4 with K_i values ranging from 0.17 μM to 2.5 μM. Interestingly, the analogous α-chloro and α-hydroxy derivatives of **27a** and **27b** were less active against $LPA_{1–4}$.[50] It is not clear if the mode of action involves covalent modification of the receptors.

In a follow up study, Prestwich and coworkers tested the activity of diastereomeric α-bromo derivatives **27b**-*syn* and **27b**-*anti* in LPA−ATX signal transduction (Fig. 6). They termed the collective *syn* and *anti* isomers of analog **27b** as Br-LPA. In initial tests of the *syn* and *anti*-isomers as separate compounds against LPA_{1-4} receptors, both were found to be inhibitors of the GPCRs on their own, as had been predicted using molecular modeling. When screened against ATX, the *anti*-isomer (IC_{50} = 22 nM) was determined to be a better inhibitor than the *syn*-isomer (IC_{50} = 165 nM); however, both isomers inhibited ATX in a dose-dependent manner. Furthermore, compounds **27b**-*syn*, **27b**-*anti*, and Br-LPA knocked out 98% of ATX activity at a concentration of 10 μM, but were only weakly active against platelet-activating LPA_5.[5]

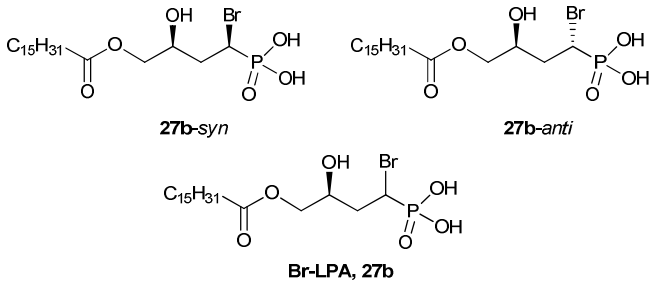


Fig. 6 The structures of two LPA-based α-bromophosphonate diastereomers, **27b**-*syn* and **27b**-*anti*. A 1:1 mixture of **27b**-*syn* and **27b**-*anti* was termed Br-LPA (**27b**).[5]

Further exploration of Br-LPA in breast and lung cancers *in vitro* and *in vivo* have suggested these compounds may have therapeutic potential.[58] Compounds **27b**-*syn* and **27b**-*anti* were tested as inhibitors of LPA-dependent cancer cell migration. Both analogs reduced migration of MDA-MB-231 breast cancer cells at a concentration of 40 μM, using a scratch wound assay. Both isomers also reduced MDA-MB-231 breast cancer cell invasion by ~50%; interestingly, the potency of the two diastereomers were not significantly different.[5] Follow-up studies have since found that Br-LPA can reduce tumor size in breast[5] and lung[59] tumor models in mice, and the compound had limited toxicity.

1.2.4. Br-LPA acts a substrate for LPA-induced behavior in neuropathic pain

Recently, the Rosenbaum group determined that LPA acts as a direct substrate for the TRPV1 ion channel. This channel is expressed in primary afferent nociceptors, and is sensitive to stimuli such as high temperature and low pH, which result in hyperalgesia in neuropathic pain. Furthermore, Rosenbaum and coworkers showed that none of the LPA receptors discussed above are implicated in TRPV1 channel activation by LPA. In order to conclude that LPA receptors

were not implicated in TRPV1-induced hyperalgesia, the authors compared LPA-induced activation of TRPV-1 to Br-LPA-induced activation. They observed that both LPA and Br-LPA activated TRPV1 with similar dose responses (LPA: K_D = 754 nM; Br-LPA: K_D = 1.1 ± 0.25 μM). These data were very encouraging, as Br-LPA is a pan-selective antagonist of LPA receptors. Therefore, because Br-LPA has a similar dose response to LPA, this indicates direct TRPV1 channel activation by LPA and that the LPA receptor pathway is not involved in the response. They also demonstrated that Br-LPA injections in wild-type mice mimicked the effects of LPA at the same dose and little effect was observed in *Trpv1*$^{-/-}$ knock-out mice, comparable to LPA.[60]

1.3. α-Bromophosphonates on a carbohydrate scaffold

Phosphonate-based mimics of carbohydrates are also well known in the literature.[9] The first example of phosphonate synthesis in carbohydrates appeared over 50 years ago by Griffin and Burger when they synthesized glucose-6-phosphate analog, **29** (Fig. 7).[61] Since that time, a range of carbohydrate phosphonate analogs have been targeted with general access available at all positions of pyranosides and furanosides (eg. **30**).[9,62,63] Work to date towards glycosyl α-halophosphonate analogs has focused on the introduction of α-fluoro and α,α-difluorophosphonates at various positions of *O*- and *C*-glycosides (eg. **31**).[12,64] Important advances in the synthesis of both α-fluorinated and α,α-difluorinated glucosyl-6-phosphonates have been described by the Berkowitz group (Scheme 11). By treating α-hydroxyphosphonate **32** with diethylaminosulfur trifluoride (DAST) α-fluorophosphonate **33** was obtained in moderate yield, which could be resolved as a diastereomeric mixture by column chromatography.[11] To

obtain the related α,α-difluorophosphonate, Berkowitz and co-workers displaced triflate **34** using a phosphorus-stabilized carbanion, [diethylphosphono(difluoromethyl)]lithium, to provide α,α-difluorophosphonate **35** in good yield.[11,65–67] These examples illustrate the accessibility of α-fluorophosphonates; however, until recently there was no corresponding precedent to the α-brominated phosphonates on a carbohydrate scaffold.

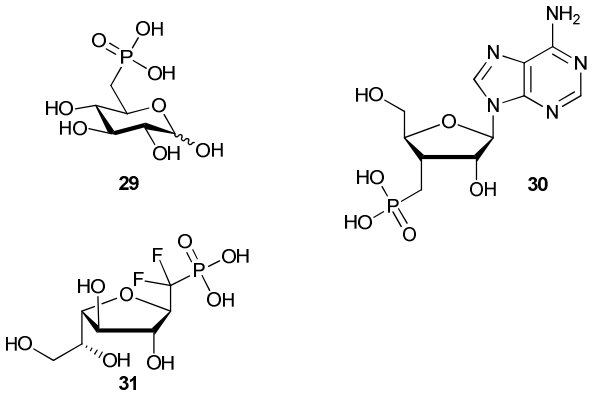
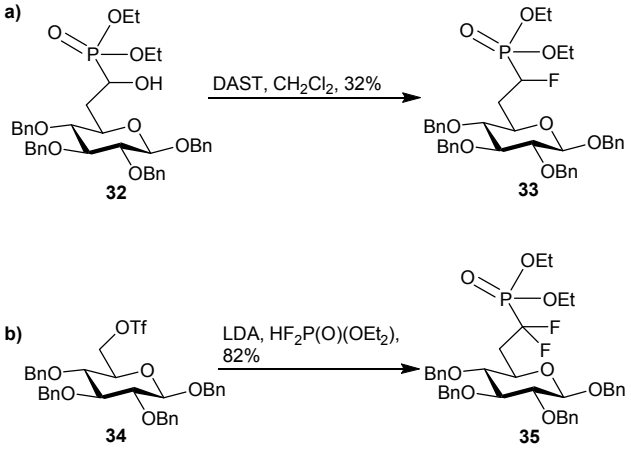


Fig. 7 Examples of known carbohydrate-based phosphonates.



Scheme 11 Known methods to accessing (a) α-fluoro and (b) α,α-difluorophosphonates on glucose scaffolds.[11,65–67]

We recently approached this problem by developing synthetic methods to access glucose-6-phosphate (G6P) derived α-bromophosphonates (**36**, Fig. 8) as inhibitors of glucose-6-phosphatase (G6Pase).[68] Specific inhibitors of G6Pase are of interest to both medicinal and biochemists[69] as G6Pase activity is upregulated in type II diabetes and no crystal structures of human G6Pase are available.[7,70] G6Pase is known to contain a nucleophilic histidine and G6Pase analog **37** was hypothesized to be a potent or even irreversible inhibitor of the enzyme.[68]

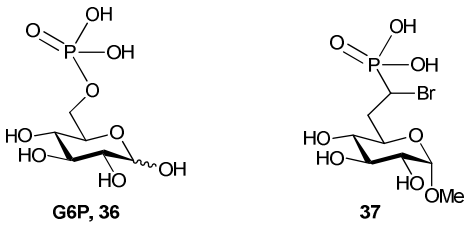
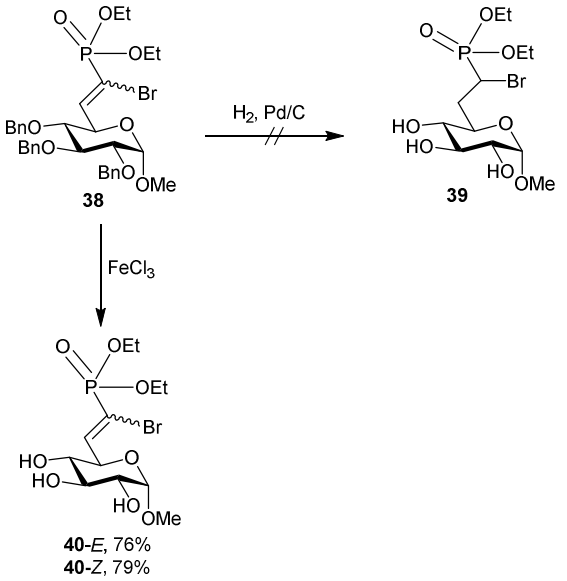


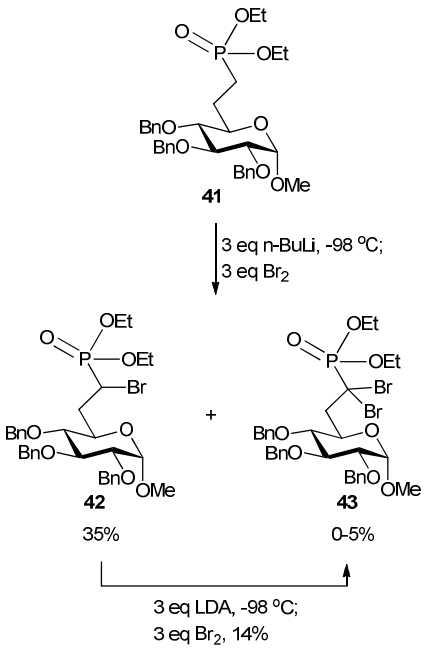
Fig. 8 Structures of G6P (**36**) and targeted G6P analog **37**.[68]

Initial access to compound **37** was attempted *via* reduction of an α-bromo-α,β-unsaturatedphosphonate intermediate (**38**, Scheme 12) using standard hydrogenation conditions. This failed due to concomitant hydrogenolysis of the C–Br bond;[68] however, the α-bromo-α,β-unsaturated phosphonate scaffold has been shown to be biologically active in other systems[71] and was unprecedented in carbohydrates.



Scheme 12 Attempted access to α-bromo G6P analog **37** *via* standard olefinic reduction. Biologically active *E* and *Z* isomers of α-bromo-α,β-unsaturated phosphonate **40** could be isolated *via* debenzylation using $FeCl_3$.[68]

Access to compound **37** was ultimately gained through a phosphorus-stabilized carbanion generated by *n*-BuLi that was quenched with Br_2 at –98 ^oC (Scheme 13). Deprotection of the 2-, 3-, and 4-OH groups and the phosphonate ester furnished target compound **37** in an additional two steps (not shown). Interestingly, the unique α,α-dibromophosphonate analog **43** could be isolated by subsequent treatment of the α-bromo analog **42** with LDA and quenching with Br_2 (Scheme 13). Similar methodology to provide an α,α-dibromophosphonate is known, but not on a carbohydrate scaffold.[72]



Scheme 13 Partial synthesis of α-brominated phosphonate **37** with access to the α,α-dibromophosphonate **43**.[68]

Screening of target coumpound **37** displayed greater potency for G6Pase than the native G6P substrate in Wister rat liver microsomes; however, a 60 h incubation was required to achieve inhibition. The phosphonate ester **44** (Fig. 9) also displayed potency that exceeded the K_m of G6P, but with an incubation time of only 1 h. The α,α-dibromo analog **45** also displayed similar potency under the same conditions, indicating that α,α-dibromophosphonates are worthy of exploration as inhibitors of phosphate recognizing enzymes. Of note, the stereoisomers of α-bromo-α,β-unsaturatedphosphonate **40** also had similar potency against G6Pase.

To explore the activity of compound **44**, ^{31}P NMR was used to confirm that the phosphonate ester could be hydrolyzed to compound **37** when incubated for 12 hours in rat liver microsomes.

This finding suggested that inhibition may be a result of phosphonic acid analog **37** after cleavage by a native esterase. There was no evidence of irreversible inhibition of G6Pase by any of the α-bromophosphonates screened, and future work will be required to confirm the mode of inhibition.[68]

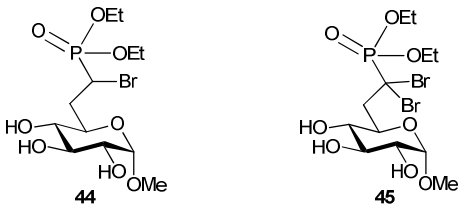
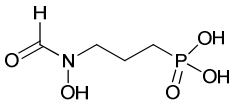


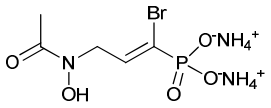
Fig. 9 Phosphonate ester-based inhibitors of G6Pase.[68]

1.4. α-Bromo-α,β-unsaturated phosphonates

The α-bromo-α,β-unsaturated phosphonate functional group of compound **40** generated active inhibitors of G6Pase.[68] This novel functional group has been tested for biological activity in only one other instance. Fosmidomycin (**46**, Fig. 10) has shown promise in clinical trials for malaria by targeting the mevalonate-independent isoprenoid biosynthesis pathway in this bacterium.[71,73,74] Fosmidomycin inactivates the essential 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) enzyme in this pathway (IC_{50} = 35 nM in recombinant *E. coli* DXR). Devreux et al. synthesized a small library of α-arylphosphonates (not shown) using either Stille or Suzuki coupling *via* fully protected α-bromophosphonate **47**. The results of assays with the α-functionalized phosphonates against recombinant *E. coli* DXR, found that only α-bromophosphonate **47** had a sub-micromolar IC_{50} (450 nM).[71]



Fosmidomycin, 46



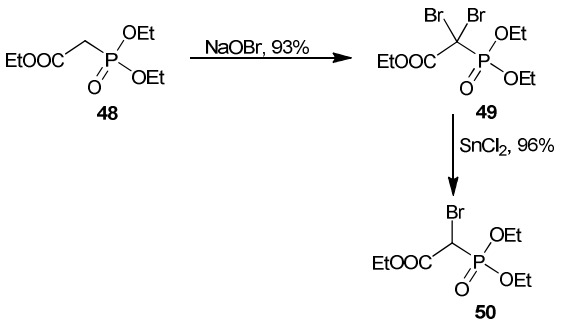
47

Fig. 10 Structures of fosmidomycin (**46**) and α-bromo-α,β-unsaturatedphosphonate **47** that was screened against recombinant *E. coli* DXR.[71]

1.5. α-Bromophosphonocarboxylates and α-bromobisphosphonates

Phosphonocarboxylates and bisphosphonates as bioisosteres of pyrophosphate have a host of medicinally important effects ranging from the prevention of bone resorption to cancer chemotherapy.[75,76] Bisphosphonates are well known to have affinity for bone.[76] The reactivity of both bisphosphonate and phosphonocarboxylate analogs is dramatically increased over their monophosphonate counterparts. Additionally, both mono- and dibrominated bisphosphonates have been reported. We briefly highlight synthetic access to α-bromophosphonocarboxylates and α-bromobisphosphonates and offer a discussion of their applications in medicinal chemistry.

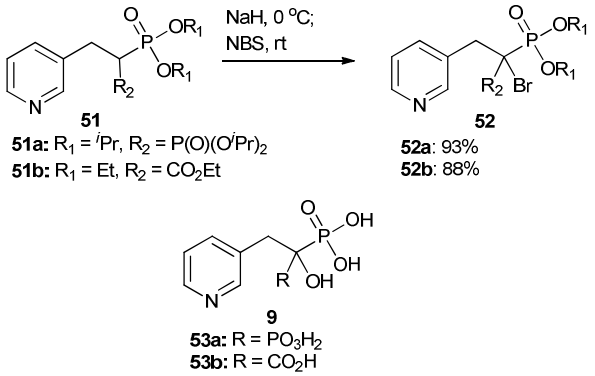
Synthetic efforts by McKenna and coworkers have demonstrated di- or mono-bromination of triethyl phosphonoacetate (**48**), in one or two steps, respectively (Scheme 14). Treatment of **48** with NaOBr provided triethyl dibromophosphonacetate (**49**) in good yield, and the product could be reduced to the mono-brominated analog **50** in the presence of 0.96 equiv of $SnCl_2$ if desired.[77] Several researchers have utilized this methodology to obtain α-bromo and α,α-dibromophosphonoacetates on similar scaffolds with moderate to excellent yields.[78–86] Examples of access to brominated bisphosphonates using similar conditions are available.[87–89]



Scheme 14 Access to α-bromo and α,α-dibromophosphonoacetates.

We note that the pKa of the α-proton in phosphonocarboxylates and bisphosphonates is significantly lowered, allowing for the formation of either a bisphosphonate-stablized carbanion or phosphorus–carboxylate-stablized carbanion to occur under milder conditions than in monophosphonates. Quenching the anion with an electrophilic bromine source subsequently provides the expected α-brominated phosphonocarboxylate and bisphosphonate analog. Suitable bases include *n*-BuLi,[90] LDA,[91] LiHMDS,[92] and NaH.[68,81,93–95]

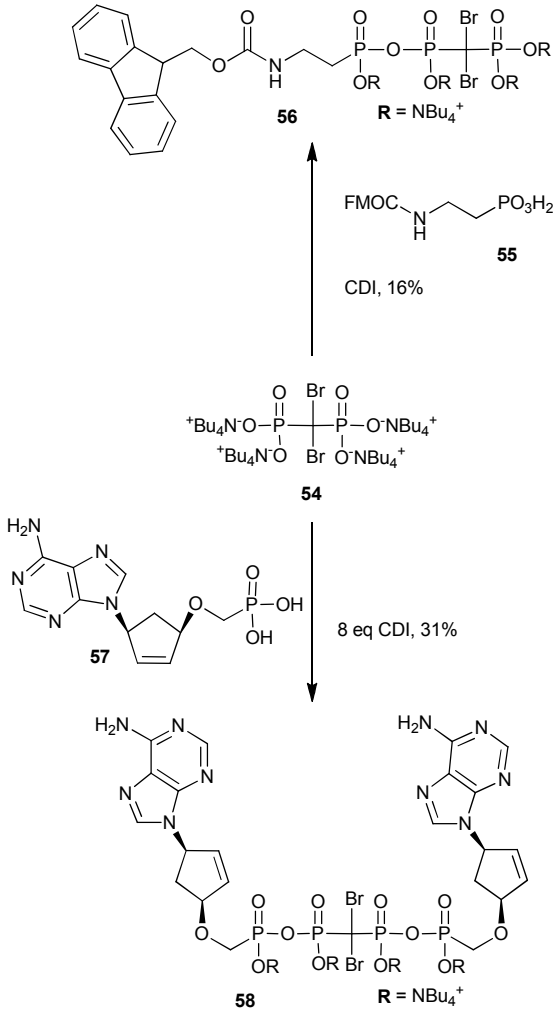
The McKenna group was able to develop α-halo derivatives of α-hydroxy compounds **53a** and **53b** (Scheme 15), which are known anti-osteoporotic agents that act through blockage of prenylation. These compounds are also known to bind bone minerals.[75,76,96] Protected benzylic bisphosphonates and phosphonocarboxylates were brominated *via* a stabilized carbanion generated by treatment with NaH and quenched with NBS. This strategy provided analogs **52a** and **52b** in good yields (Scheme 15).[75,96] Unfortunately, testing of the biologically active, free acid form of α-bromo analogs **52a** and **52b** indicated they were generally less active than the previously studied α-OH analogs **53a** and **53b**.[75,96]



Scheme 15 α-Bromination of benzyl bisphosphonates and phosphonocarboxylates *via* a bisphosphonate-stabilized (**51a** to **52a**) or phosphorus–carboxylate-stabilized (**51b** to **52b**) carbanion.[75,96]

1.6. Mono- and dibrominated polyphosphonates

Both nucleoside and non-nucleoside polyphosphonate analogues have been proposed for use in medicinal applications. We highlight the syntheses that incorporate an α,α-dibromophosphonate moiety representative of this class of molecules (Scheme 16). General access to these seemingly intricate α,α-dibromo polyphosphonate analogs begins from α,α-dibromo-diphosphonic acid (**54**) and a carbodiimide (CDI)-activated phosphonate (**55** or **57**). Yields are generally moderate, and both the adenine and guanine analogs of carbocyclic dinucleoside **57** were synthesized (e.g. **58**). In all instances the NBu_4^+ phosphonate salts were isolated.



Scheme 16 Synthesis of dibromo polyphosphonate analogs **56** and **58**.[97–99]

Non-nucleoside triphosphate analogs are inhibitors of terminal deoxynucleotidyl transferase (TDT), which has upregulated expression in leukemia.[98,100] Arzmanov et al. screened dibromo species **56** against TDT but it was found to have lower affinity for TDT than the analogous P–CF_2–P phosphonate or the P–O–P triphosphonate analog.[98]

Carbocyclic dinucleoside polyphosphonates have shown activity against HIV reverse transcriptase-catalyzed DNA synthesis and hence demonstrate antiviral activity.[97,99]

Polyphosphonate **58** displayed weaker anti-HIV activity than the equivalent CF_2 and P–O–P tetraphosphonate analogs.[99]

It is important to note here that synthetic access to biologically interesting cyclic and acyclic α-fluorophosphonate and α,α-difluorophosphonate nucleoside(-tide) analogs has been well studied and reviewed (e.g. **59**,[101] **60**,[102] **61**,[103] **62**,[104] and **63**,[105] Fig. 11).[12] Analog **63** was tested as an inhibitor of purine nucleoside phosphorylase (PNP) which catalyzes the phosphorolysis of (deoxy)guanosine and (deoxy)inosine into guanidine and hypoxanthine. This class of inhibitors for PNP are of interest as potential immunosuppressive agents.[12] The corresponding fully-saturated phosphonate of compound **63** was found to inhibit human erythrocyte PNP with a K_i of 174 nM, while **63** had substantially increased activity.[105] These examples demonstrate the potential of α-halogenated phosphonates as immunosuppressive agents, and the corresponding α-bromo phosphonate compounds remain to be explored.

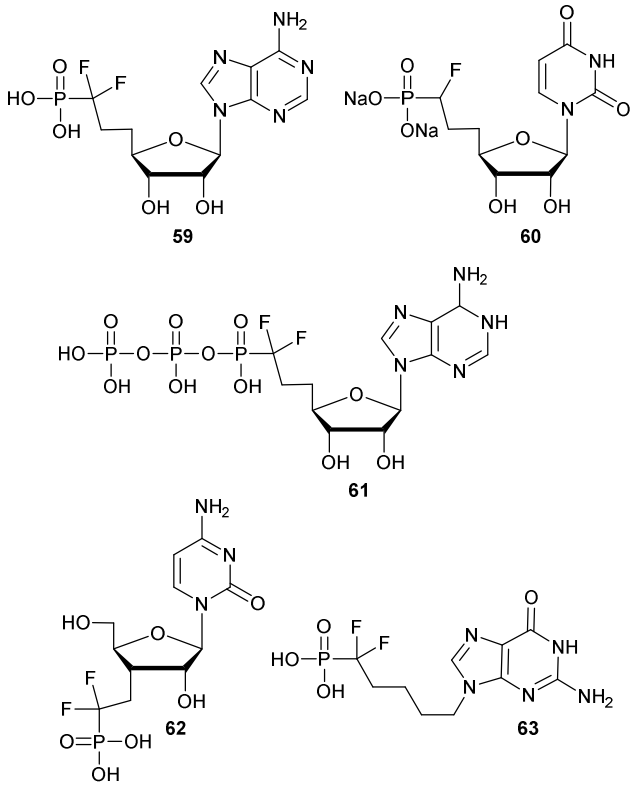
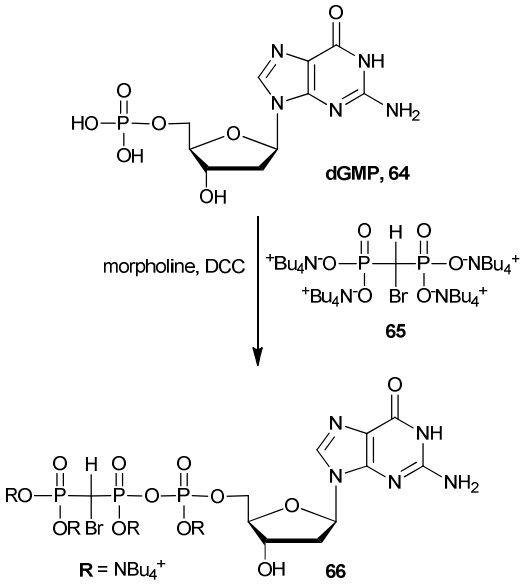


Fig. 11 Examples of α-fluorinated methylenephosphonate-based nucleosides(tides).

More recently it has been demonstrated that β,γ-methylene-dGTP analogs can act as substrate mimics of dNTPs for DNA polymerase β.[106–109] Monobromo dGTP analog **66** was synthesized in one step by coupling *N*,*N*′-dicyclohexylcarbodiimide (DCC)-activated dGMP with α-bromodiphosphonic acid (**65**) in 40% yield (Scheme 17). This synthetic strategy mirrors that of the nucleosides presented above (Scheme 16). The dibromo dGMP analog has also been reported, which was accessed *via* phosphonic acid **54** in 40% yield (not shown).[107] To demonstrate the ability of β,γ-methylene-dGTP analogs to function as substrate mimics of DNA polymerase β, the authors obtained crystal structures of numerous halogenated β,γ-methylene-dGTP–DNA polymerase β ternary complexes, including analog **66**.[109] They used this data to

study the fidelity of DNA polymerase β by looking at specific alterations in the active site due to binding of foreign nucleoside analogs.[107,109] Recently, these authors expanded the library of β,γ-methylene-dNTP to include halogenated thymidine and cytidine analogs. They showed that binding of analog **66** and its dibrominated counterpart, as well as and other halogenated β,γ-methylene dNTPs have tremendous effects on the transition state energies of DNA polymerase β-mediated catalysis.[110] These results certainly show promise for a broadened utility of β,γ-bromomethylene dNTP analogs to function as substrate mimics for other polymerases and other enzyme systems.



Scheme 17 Synthesis bromo-GTP analog **66** as a substrate for DNA polymerase β.[107]

2. Conclusions and outlook

There are a range of available methods to synthesize α-bromophosphonates, and these allow

versatile access to this functional group from alkyl, α-hydroxy, or benzyl substituted phosphonate starting materials. The reports summarized here provide a survey of the known biological activities observed for α-bromophosphonates and α,α-dibromophosphonates. This functional group has become an important bioisostere of the phosphate moiety, which can be used in the development of inhibitors and probes of phosphate-recognizing proteins. The ability of α-bromophosphonates to act as specific covalent labels in PTP systems has enormous potential in the identification of new enzymes, and in the development of new specific inhibitors. The scope of the inhibitor activity of α-bromophosphonates has recently been broadened to include GPCRs, ion channels, and glucose-6-phosphatase. The application of these compounds in mechanistic studies of enzymes, e.g. as dNTP mimics with polymerases, remains an area primed for expansion. The α-brominated phosphonates are an important functional group in the toolbox of medicinal chemists and chemical biologists.

Acknowledgments

CWC would like to acknowledge support from the Natural Sciences and Engineering Research Council of Canada. AMD would like to thank the University of Alberta for a Queen Elizabeth II Graduate Scholarship.

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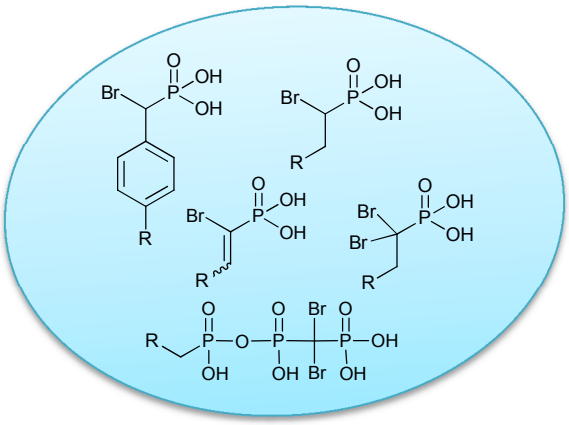
Graphical Abstract



Description: A review of the synthesis and biological activity of α-bromo-phosphonate groups as phosphate bioisosteres.