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Synthesis of [Difluoro-(3-alkenylphenyl)-methyl]-phosphonic Acids on Non-crosslinked Polystyrene and Their Evaluation as Inhibitors of PTP1B

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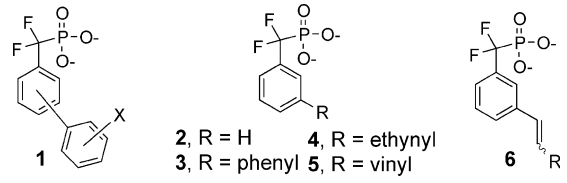
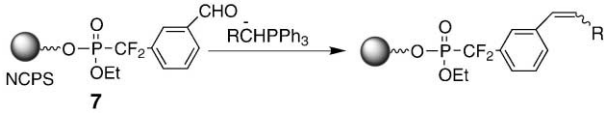
Abstract—A series of [difluoro-(3-alkenylphenyl)-methyl]-phosphonates were prepared on non-crosslinked polystyrene, a soluble polymer support. After cleavage from the support, the resulting phosphonic acids were examined for inhibition with protein tyrosine phosphatase 1B. Compound **20**, bearing an α,β-unsaturated allyl ester moiety, was the most potent of this series of compounds, being a reversible, competitive inhibitor with a K_i of 8.0 ± 1.4 μM.

Recently, there has been considerable interest in the development of inhibitors of protein tyrosine phosphatase 1B (PTP1B).[1] This stems for the relatively recent discovery that PTP1B knock-out mice are insulin sensitive and are resistant to obesity.[2] These studies suggest that PTP1B is involved in the down regulation of insulin signaling and that inhibitors of PTP1B may be useful for the treatment of type II diabetes and obesity.[2] As part of our program on the development of non-peptidyl PTP1B inhibitors,[3] we became interested in devising methods that would allow for the rapid synthesis of potential inhibitors bearing the difluoromethylenephosphonic acid group (DFMP), a highly effective non-hydrolyzable phosphate mimetic for obtaining PTP1B inhibtors.[4] Towards this end we developed an approach to the synthesis of biaryl derivatives of type **1** on non-crosslinked polystyrene (NCPS), a soluble polymer support.[5a,b] Biaryl derivatives were specifically targeted since we had previously demonstrated that substitution of **2** with a phenyl group at the *meta* position, **3** ($IC_{50} = 15$ μM), significantly increased inhibitory potency by 17-fold compared to **2**.[3a] Quite recently, Shibuya et al. reported that the ethynyl compound **4** ($IC_{50} = 19$ μM) was also a good inhibitor. These results indicate that certain unsaturated moieties at the *meta* position of **2** can significantly increase inhibitory potency which suggests that the vinyl derivative **5** and compounds of type **6** might also be effective inhibitors. However, Shibuya reported that they were unable to construct compound **5** in pure form.[6] Nevertheless, since the styryl derivative (**6**, R = Ph) was reported,[6] we reasoned that other substituted vinyl derivatives of type **6** should be readily obtainable. Here we report the polymer-supported synthesis of alkenes of type **6** and their evaluation as inhibitors of PTP1B.



Our approach to compounds of type **6** involved a Wittig reaction between NCPS-bound aldehyde **7** and a series of phosphorus ylids (Scheme 1). Although compounds of type **6** could potentially be prepared using Stille couplings,[6] we chose to use a Wittig reaction[7] since a wide



Scheme 1. Proposed route to alkenes from polymer-bound **7**.

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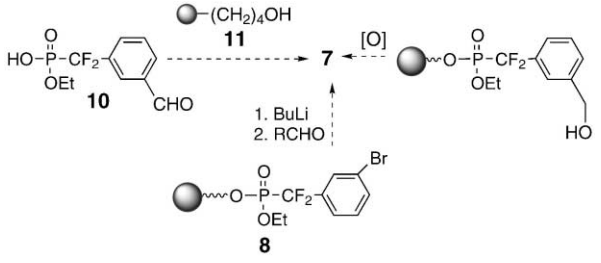
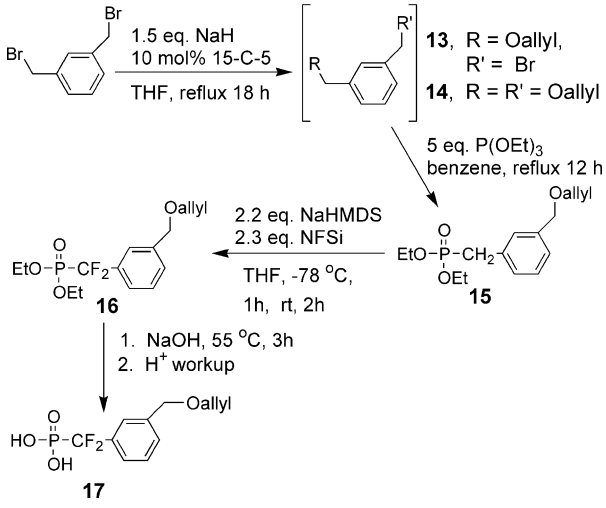
variety of phosphonium salts are commercially available and these reactions could be performed at room temperature. Polymer-supported chemistry would facilitate the removal of triphenylphosphine oxide from the reaction mixtures. NCPS is soluble in many aprotic organic solvents yet is insoluble in polar protic solvents such as MeOH or water. This approach has certain advantages over the more conventional solid phase methodologies since reactions are carried out under homogeneous conditions, which results in faster reaction rates[8] and facile monitoring of the reaction using conventional solution NMR. Pure polymer-bound product is obtained by precipitation followed by filtration.

Initially, we wished to prepare aldehyde **7** by lithiation of polymer bound aryl bromide **8**[5a] followed by formylation (Scheme 2). However, such reactions with model compound **9** in solution indicated that this would not be a suitable approach due to the formation of numerous impurities. An alternative approach was to prepare the aldehyde **10** (or its acetal equivalent), followed by attachment to functionalized polymer **11**[5a] by a Mitsonobu reaction (Scheme 2).[5a] We initially examined this approach using the known aldehyde **12**[9] as a model substrate. However, we were unable to attach this compound to polymer **11** cleanly and in good yield under a wide variety of conditions. Attempts to convert acetal derivatives of **12** to the acid chloride and attach them to **11** were also unsuccessful. Our third approach was by oxidizing a polymer-bound alcohol to the aldehyde. This involved first preparing the protected alcohol

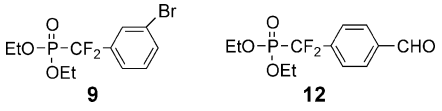


Scheme 2. Potential routes to **7**.



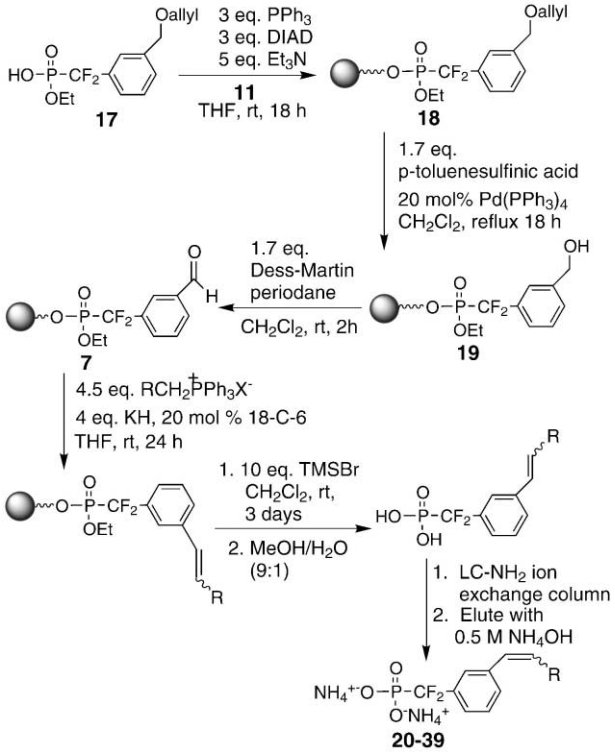
Scheme 3. Synthesis of **17**.

17 as outlined in Scheme 3. Thus, α,α′-dibromo-*meta*-xylene was reacted with allyl alcohol in the presence of NaH and 15-C-5 to give a mixture of the mono and diallylated compounds **13** and **14**. These compounds were not separated but instead were subjected to excess triethytlphosphite in refluxing benzene to give the phosphonate **15** in 54% yield (two steps). Electrophilic fluorination[10] using *N*-fluorobenzenesulfonimide (NFSi) gave compound **16** in 80% yield. Basic hydrolysis followed by acidification gave acid **17** in 97% yield.



Compound **17** was attached to polymer **11** (0.4 mmol alcohol moiety/gram of polymer)[5a] in quantitative yields (as determined by NMR) by a Mitsonobu reaction (Scheme 4).[5a] Pure polymer-bound **18** was obtained by addition of the reaction mixture to MeOH–water (9:1) followed by filtration. Polymer recovery was approximately 97%. Using the conditions developed by Honda et al.[11] for removal of allyl groups, polymer bound alcohol **19** was obtained in near quantitative yields as determined by ^{19}F NMR. By subjecting **19** to the Dess–Martin periodane in methylene chloride at room temperature for 2 h, aldehyde **7** was obtained in quantitative yields as determined by ^{19}F NMR with the only loss of material being due to the precipitation/filtration step (3% for the deprotection and oxidation).

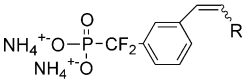
Wittig reactions using 400 mg of **7** were performed at room temperature using an excess of phosphorus ylid



Scheme 4. Synthesis of alkenes **20**–**39**.

(generated from commercially available phosphonium salts and KH) in the presence of 20 mol% 18-C-6 in THF (Scheme 4). The reactions were quenched by adding the reaction mixture to a stirred 9:1 MeOH–water solution followed by filtration. With these smaller scale reactions, recovery of the polymer ranged from 80 to 95%. Simultaneous cleavage from the support and phosphonate deprotection was achieved using TMSBr.[5a] The reactions were concentrated by rotary evaporation and the residue redissolved in methylene chloride which was then added to a 9:1 solution of MeOH and water and stirred for 12 h. The polymer was removed by filtration and the filtrate concentrated to give the phosphonic acids. The acids were applied to an LC-NH_2 weak anion exchange resin[12] and washed with THF, MeOH and water. The compounds were removed from the column as their diammonium salts by elution with 0.5 M NH_4OH followed by repeated lyophilization. 20 different alkenes were constructed (**20**–**39**, Table 1). Compounds were characterized by ^1H, ^{31}P, ^{19}F NMR and negative FABMS.[13] Yields ranged from 28 to 83%. The purities for the majority of the compounds were

Table 1. Yields and purities of alkenes **20**–**39**



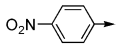
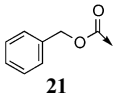
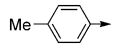
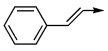
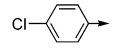
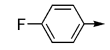
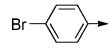
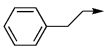
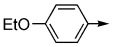
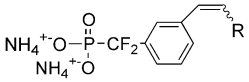
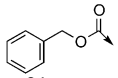
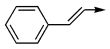
R	Crude yield	Purity		R	Crude yield	Purity	
		NMR (^{19}F, ^{31}P)	HPLC			NMR (^{19}F, ^{31}P)	HPLC
 **20**	83	96, 100	100	 **30**	68	98, 100	95
 **21**	64	88, 100	92	 **31**	56	98, 98	90
 **22**	44	90, 97	95	 **32**	62	99, 98	99
 **23**	70	88, 97	93	 **33**	62	96, 100	97
 **24**	41	97, 98	97	 **34**	61	98, 97	99
25	82	95, 99	91	 **35**	65	92, 98	95
 **26**	82	96, 100	80	CI **36**	83	98, 95	65
 **27**	60	99, 93	100	CH_3CH_2 **37**	28	100, 98	100
 **28**	82	100, 100	100	 **38**	38	100, 98	100
 **29**	71	98, 85	88	 **39**	31	97, 85	72

Table 2. Percent Inhibition of PTP1B with 50 μM compounds **20–26**



R	% Inhibition	R	% Inhibition
 **20**	84	 **24**	53
 **21**	71	**25**	45
 **22**	70	 **26**	23
 **23**	60		

greater than 85% as judged by ^{19}F and ^{31}P NMR and HPLC (Table 1). The exceptions to this were compounds **26**, **36** and **39** which were obtained in purities of 80%, 65 and 72% as judged by HPLC. ^{19}F and ^{31}P NMR of these three compounds suggested that their purity was considerably greater than that determined by HPLC.

A rapid screen of **20–39** for PTP1B inhibition was performed by determining the percent inhibition in the presence of 50 μM **20–39** and using fluorescein diphosphate as substrate at K_m concentration (20 μM) at pH 6.5.[14] Compounds **20–26** were the most potent inhibitors (Table 2). Compounds **35–39** showed no inhibition. Compounds **27–34** showed little or no inhibition and these results are consistent with the studies of Shibuya et al. who reported that the styryl derivative (**6**, R = -Ph) was a weak inhibitor ($IC_{50} = 386$ μM).[6] Interestingly, compound **22**, which has an additional double bond exhibited 70% inhibition (Table 2) while its more saturated analogue, **38**, showed no inhibition. The data in Table 2 reveals that an α,β-unsaturated ester moiety aids inhibition and that an unsaturated group in the alcohol portion of the ester further enhance inhibition. The IC_{50} of the most potent inhibitor, **20**, was determined to be 12.2 ± 1.0 μM indicating that it is slightly more potent than compounds **3** and **4**. Further kinetic studies revealed that **20** was a reversible competitive inhibitor with a K_i of 8.0 ± 1.4 μM. We also prepared **20** in solution from difluoro-(3-formylphenyl)-methyl]-phosphonic acid diethyl ester[15] using the room temperature Wittig coupling conditions of Belluci et al.[16] which are known to give exclusively the E isomer. This compound exhibited identical ^{19}F, ^{31}P and ^{1}H NMR spectra to that prepared on the polymer which is consistent with the product being formed from a stabilized ylid. The compound prepared in solution also exhibited identical inhibition properties. Recent theoretical studies on compound **3** and PTP1B suggest that the *meta*-phenyl ring increases potency by pi-cation interaction with Lys 116 and Lys 120.[17] It is possible that similar interactions are occurring with some of the compounds reported here.

In summary, polymer-supported organic synthesis was used to prepare a series of alkenes of type **6**. Inhibition studies reveal that an α,β-unsaturated allyl ester at the *meta*-position on the aryl ring significantly increases potency in that compound **20** is a 76-fold more potent inhibitor than the parent compound **2**. Further studies to increase compound diversity by utilizing other reactions on polymer bound aldehyde **7**, as well as alcohol **19**, and their *para* analogues, are in progress.

Acknowledgements

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12. Available from Supelco.
13. No attempt was made to identify the proportions of geometric isomers formed.
14. Specific assay conditions were: 0.2 μg/mL PTP1B in 50 mM Bis–Tris (pH 6.5), 2 mM EDTA, 5 mM DTT, 0.01% triton X-100 and 5% DMSO. Assays were carried out in 96-well plates using a SPECTRAmax (Molecular Devices) microplate spectrofluorometer, (excitation at 485 nm, emission at 538 nm).
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