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SOLID-PHASE SYNTHESIS OF POTENTIAL PROTEIN TYROSINE PHOSPHATASE INHIBITORS VIA THE UGI FOUR-COMPONENT CONDENSATION

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Abstract: A library of 108 α,α-difluoromethylenephosphonic acids was prepared by Ugi four-component condensation using Rink-NH_2 resin, 4-[(diethoxyphosphinyl)difluoromethyl]benzoic acid, and a set of 18 aldehydes and 6 isonitriles. Following resin cleavage, the diethylphosphonate esters were hydrolyzed with trimethylsilyl bromide to yield the free acids which were assayed for inhibition of PTPα, PTPβ and PTPε.

Keywords: Solid phase synthesis; phosphonic acids and derivs; enzyme inhibitors

Tyrosine phosphorylation levels, controlled by the relative activity of protein tyrosine kinases (PTKs) and phosphatases[1] (PTPases), play an important role in various cellular events. The regulation of PTPase activity is believed to be of importance in conditions such as cancer, diabetes and microbial pathogenesis. One approach to PTPase inhibition is based on competitive substrate analogs in which phosphotyrosine (pTyr) is replaced by non-hydrolyzable mimetics such as phosphonates,[2] sulfonates,[3] and malonates.[4]

Following a report by Cao *et al.*[5] on a series of cinnamates (**1**, **Figure 1**) which were micromolar inhibitors of HePTP, we wished to examine a library of analogous phosphonates (**2**) against receptor-like PTPs. In **2**, α,α-difluorination should improve potency over simple benzylphosphonates, as the fluorine atoms lower the phosphonate pK_{a2} as well as enable hydrogen bonding interactions.[6] Besides potential PTPase inhibition, phosphonates **2** can also be screened for binding to pTyr recognition elements[7] such as SH2 domains.[8]

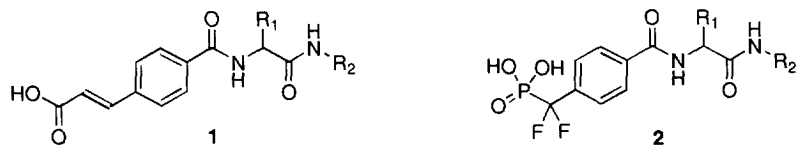

Figure 1

Our library preparation utilized the same strategy as Cao *et al.*: solid-phase synthesis[9] via an Ugi four component condensation.[10] Rink-NH_2 resin served as an ammonia equivalent, while both aldehyde and isonitrile components were varied. The desired 4-[(diethoxyphosphinyl)difluoromethyl]benzoic acid (**3**)[11] was obtained in three steps from 4-iodobenzoic acid (**Scheme 1**). The key step in this sequence, coupling of the iodide with an organozinc reagent, closely followed a recent report.[12]

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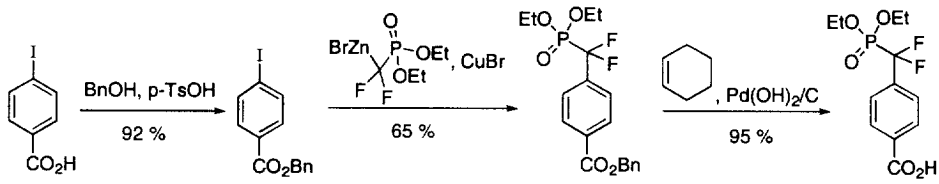
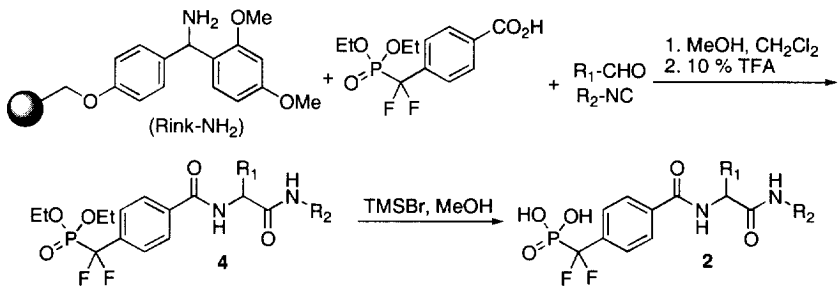


Scheme 1

Solid-phase Ugi reactions[13] (**Scheme 2**) with **3** were successful with a variety of aliphatic and aromatic aldehydes, while three commercially available isonitriles did not give good yields: 1*H*-benzotriazol-1-ylmethyl isocyanide, 2-morpholinoethyl isocyanide, and toluene-4-sulfonylmethyl isocyanide. Attempted resin-bound hydrolysis of the phosphonate esters with trimethylsilyl bromide (TMSBr) was problematic, presumably due to the sensitivity of the Rink resin to acidic conditions. Instead, post-cleavage hydrolysis was performed in solution to give the free phosphonic acids (**2**). As the byproducts of the TMSBr hydrolysis are volatile, purification was effected by simple evaporation.



Scheme 2

A library of 108 discrete diamides was prepared[14] using a set (**Table 1**) of six isonitriles and eighteen aldehydes. The identity of all intermediate phosphonate esters **4** was checked by ^{1}H NMR. After TMSBr hydrolysis, 20 % of the library was sampled and characterized by ^{1}H NMR and MS, while product purity was determined by HPLC analysis. Although product yields are variable, purities are uniformly high, reflecting the advantage of multicomponent condensations as unreacted synthetic intermediates do not build up on the resin.

The phosphonic acids were screened at 100 μM for inhibition of *p*-nitrophenyl phosphate hydrolysis by the intracellular catalytic domains of three receptor-like PTPs: PTPα, β, and ε. Three compounds, **2d**, **2w**, and **2x**, inhibited PTPε by more than 50 %. The low hit rate and the selectivity of inhibition suggests that subtle differences may exist between the active sites of closely related PTPs. In general, the activity observed is lower than that of the cinnamates against non-receptor HePTP. This may be due to differences between receptor and non-receptor PTPs, or a more complex mechanism than phosphotyrosine mimicry by the cinnamates.

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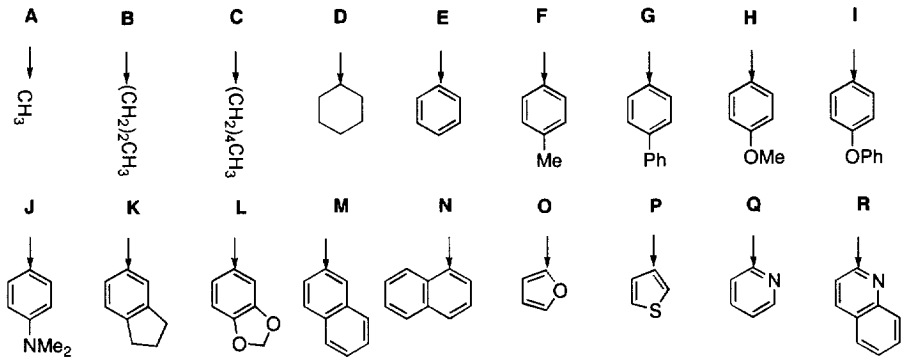
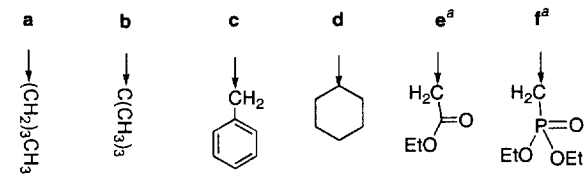
Table 1. Library Building Blocks, Yield and Purity of Representative Examples.

Aldehydes, R_1-CHO



Isonitriles, R_2-NC



Yield (%) and Purity[b] (%) of Phosphonic acids 2

Comp'd	R_1	R_2	Yield	Purity	Comp'd	R_1	R_2	Yield	Purity
2a	**A**	**a**	95	90	**2n**	**K**	**a**	50	84
2b	**A**	**e**	29	89	**2o**	**L**	**f**	77	48
2c	**B**	**c**	59	97	**2p**	**M**	**e**	23	94
2d	**C**	**c**	23	96	**2q**	**N**	**d**	46	97
2e	**D**	**d**	17	74	**2r**	**O**	**b**	13	79
2f	**E**	**b**	27	85	**2s**	**P**	**a**	49	95
2g	**F**	**b**	17	74	**2t**	**Q**	**b**	59	83
2h	**F**	**f**	10	88	**2u**	**R**	**c**	65	74
2i	**G**	**d**	42	97	**2v**	**J**	**a**	55	87
2j	**H**	**c**	68	97	**2w**	**I**	**c**	19	69
2k	**I**	**a**	34	97	**2x**	**J**	**c**	29	73
2l	**J**	**e**	15	78	**2y**	**Q**	**c**	67	75
2m	**J**	**f**	11	85					

[a]The esters were also hydrolyzed by TMSBr in the final products; [b]Based on HPLC analysis with UV detection at 210 nm.

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11. ^{1}H NMR ($CDCl_3$, 300 MHz) for **3**: δ 8.16 (d, 2H, *J* = 8.1 Hz), 7.72 (d, 2H, *J* = 8.1 Hz), 4.34-4.15 (m, 4H), 3.83 (br s, 1H, D_2O exchangeable), 1.34 (t, 6H, *J* = 7.0 Hz).
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14. Typical procedure: Aldehyde (0.4 mmol) and Rink-NH_2 resin (0.05 mmol) were agitated in CH_2Cl_2 (2 h, rt). Acid **3** (0.2 mmol) and isonitrile (0.2 mmol, both 0.5 M in MeOH) were added, and the resulting mixture was agitated (3 days, rt). The resin was filtered and washed (DMF, MeCN, CH_2Cl_2). A mixture of TFA/Et_3SiH/CH_2Cl_2 (10:5:85; 1 mL) was added and the resin agitated (30 min, rt), filtered, and rinsed with CH_2Cl_2. The filtrate was dried in vacuo, re-dissolved in anhydrous CH_2Cl_2 (1.0 mL), followed by addition of anisole (50 μL) and TMSBr (46 μL, 0.35 mmol). After 3 h, MeOH (1 mL) was added, and the mixture evaporated in vacuo to afford the final product. **2g:** ^{1}H NMR (CD_3OD, 300 MHz) δ 7.93 (d, 2H, *J* = 8.0 Hz), 7.69 (d, 2H, *J* = 8.0 Hz), 7.39 (d, 2H, *J* = 7.2 Hz), 7.20 (d, 2H, *J* = 7.2 Hz), 5.60 (s, 1H), 2.33 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.6, 168.4, 139.1, 138.0, 136.2, 130.3, 128.9, 128.6, 127.6, 127.5, 59.0, 52.3, 28.8, 21.1; MS (API) m/z, 453 (M^+-1); **2y**: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.74-8.52 (m, 1H), 8.11-7.98 (m, 1H), 7.92 (d, 2H, *J* = 8.0 Hz), 7.68 (d, 2H, *J* = 8.0 Hz), 7.64-7.48 (m, 1H), 7.32-7.03 (m, 6H), 5.67 (s, 1H), 4.52 (s, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 170.9, 168.6, 163.4, 149.5 139.0, 138.6, 138.5, 136.1 135.4, 131.6, 128.9, 128.2, 128..0, 127.5, 122.4, 120.7, 43.8; MS (API) *m/z*: 474 (M^+-1).