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Novel Phosphate Ester-Linked Resins: The Solid-Phase Generation of Phenyl Phosphate-Containing Compounds for SH2 Inhibition

Chester A. Metcalf III,* Chi B. Vu, Raji Sundaramoorthi, Virginia A. Jacobsen, Edgardo A. Laborde,† Jeremy Green,‡ Yinka Green,§ Karina J. Macek, Taylor J. Merry, Selvi G. Pradeepan, Mayumi Uesugi, Vaibhav M. Varkhedkar, and Dennis A. Holt

ARIAD Pharmaceuticals, Inc.
26 Landsdowne Street, Cambridge MA 02139-4234

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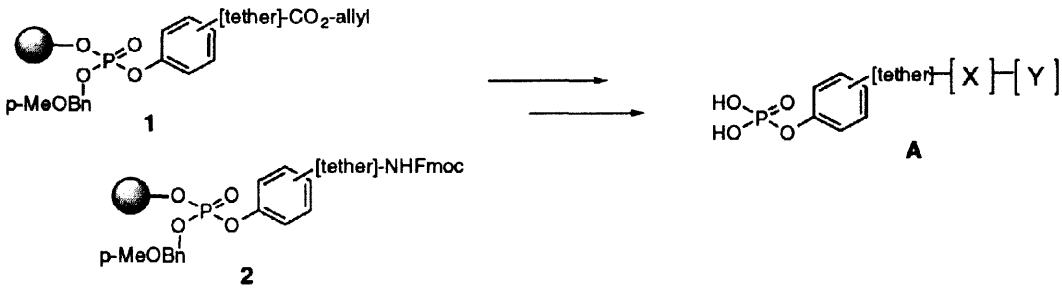
Abstract: An efficient method for the preparation of protected phosphate ester-linked resins in high yield and purity is presented. Variation in tether length/functionality and substitution pattern of the phenol precursors, as well as mild deprotection conditions make this system ideal for the combinatorial generation of phenyl phosphate SH2 inhibitor libraries.

Solid-phase combinatorial chemistry has provided the pharmaceutical industry with a valuable tool for drug discovery, as demonstrated by the generation of numerous interesting and significant lead compounds.[1] Given the enormous growth and interest in signal transduction over the past several years, it would seem logical to utilize this powerful technology to discover and develop small molecule leads in this area; signaling pathways involving Src homology 2 (SH2) mediated protein-protein interactions appear particularly attractive to therapeutic intervention.[2] Thus, the integration of solid-phase chemistry into our current SH2 drug discovery program[2b-f] has been accomplished through a series of hitherto unreported phenyl phosphate-linked resins, **1** and **2**, that easily lend themselves to a library format. A "pharmacophore" linking strategy[3] was utilized to enable our phenyl phosphate linkers to adopt the dual role of substrate attachment site and crucial SH2-binding element. Sequential monomer attachment and deprotection provide phenyl phosphate-containing compounds of type **A** as potential SH2 inhibitors.



Phosphoramidite **3** was generated by initial treatment of commercially available (Aldrich) bis(diisopropylamino)chlorophosphine with p-methoxybenzyl alcohol/triethylamine (Scheme 1).[4] This was followed by careful concentration under N_2 (moisture/air sensitive) and immediate transfer to a suspension of the protected amino/carboxy phenol and diisopropylamino tetrazole (DIAT) in CH_2Cl_2.[5] Intermediate **3** could be purified by flash chromatography and was usually carried through the remainder of the synthesis the

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following day, although storage at -20 °C for up to 1 week resulted in little if any loss in loading and purity of the generated resins. Attachment to Wang resin (1.15 mmol/g) was accomplished through sonication in a tetrazole/THF solution. A novel oxidation using 4-methylmorpholine N-oxide (NMO) provided the protected phosphate resin **4**. All phosphate resins provided a characteristic peak in the ^{31}P NMR (gel phase) spectrum at ~δ -5.5. The resins were routinely prepared in batch quantities of 20 g or more in excellent yield and purity as shown in Table 1.

Scheme 1.

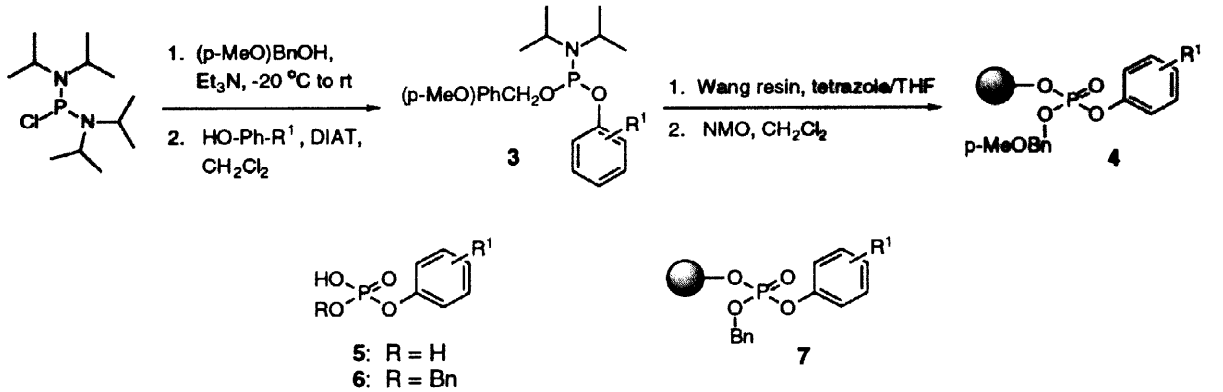


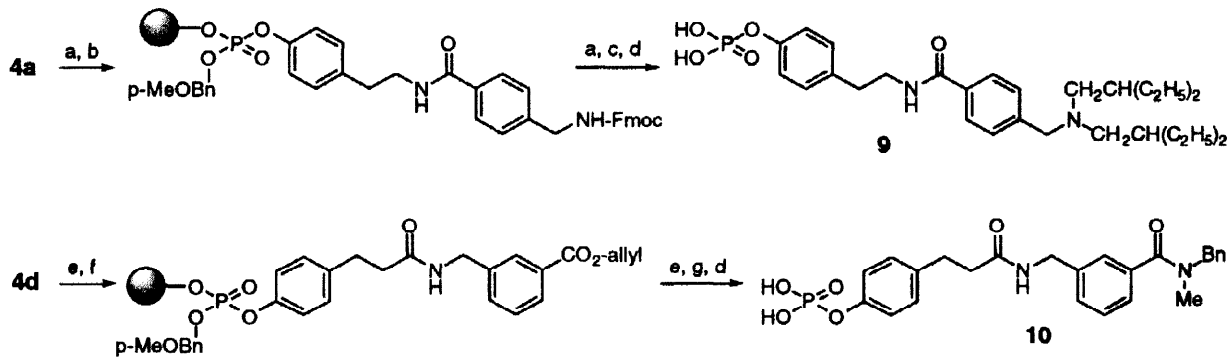
Table 1. Analysis of Resins **4** and **7**

Resin	R1	Loading Capacity[6,7] (mmol/g)	Yield[8] (%)	Qualitative Purity: ^{31}P NMR[a] (gel phase)	Qualitative Purity: MS (% TIC)[b]
4a	p-$CH_2CH_2NHFmoc$	0.659	93	-5.308 (sp)	-
4b	p-$CH_2NHFmoc$[10]	0.637	89	-5.344 (sp)	-
4c	m-$CH_2NHFmoc$[10]	0.627	88	-5.478 (sp)	-
4d	p-$CH_2CH_2CO_2$-allyl	0.730	92	-5.516 (sp)	100
4e	p-CH_2CO_2-allyl	0.672	84	-5.549 (sp)	100
4f	p-$CH_2CH(NHAc)CO_2Me$[11]	0.547	70	-5.801 (smp)	59
7g	p-OPh(p-$CH_2NHFmoc$)[12]	0.552	81	-5.390 (sp)	-
7h	p-OPh(m-$CH_2NHFmoc$)[12]	0.620	90	-5.346 (smp)	-
7i	p-OPh(o-$CH_2NHFmoc$)[12]	0.380	55	-5.227 (smp)	-
7j	p-CH=$CHCO_2$-allyl	0.535	66	-5.891 (smp)	85
7k	m-CH=$CHCO_2$-allyl	0.796	98	-5.578 (smp)	90
7l	m-CH_2CO_2-allyl	0.807	98	-5.740 (sp)	100

sp - single peak.
smp - single major peak [minor amount(s), <10%, of additional peak(s)].
[a] Gel phase ^{31}P NMR in $CDCl_3$, samples referenced against an external phosphoric acid standard (0.0 ppm) [b] Mass Spectrum (ES^-) of cleaved linker **5/6** expressed in percentage of total ion current (TIC), with a 10% BPI peak cutoff.

The chemical stability of our phenyl phosphate linkers under a variety of solid-phase reaction conditions has been demonstrated through the outlined synthetic routes utilizing resins **4a** and **4d** (Scheme 2). Cleavage from the solid supports with 30% TFA/CH_2Cl_2 (5% H_2O) and concentration afforded phenyl phosphates **9** and **10** with HPLC purities of 86% [MS (ES^+): *m/z* 519, M+H, 95% TIC] and 66%[13] [MS (ES^+): *m/z* 483, M+H, 80% TIC], respectively.

Scheme 2.



(a) 1% DBU/DMA. (b) p-(HO_2C)$PhCH_2NH$-Fmoc, TBTU, DIEA, DMA. (c) 2-ethylbutyraldehyde (4.0 eq), $Na(OAc)_3BH$, DMA. (d) 30% TFA/CH_2Cl_2 (5% H_2O). (e) $Pd(Ph_3P)_4$, HOBt, Ph_3P, CH_2Cl_2, DMF. (f) m-(NH_2CH_2)$PhCO_2$allyl, TBTU, DIEA, DMA. (g) NH(Me)Bn, TBTU, DIEA, DMA.

In summary, we have provided a simple and efficient method to generate phosphate ester-linked resins on large scale with varying substitution pattern, with the capacity to generate potential SH2-binding phenyl phosphate derivatives. A recent publication[14] on a related series of *de novo* designed phenyl phosphate ligands targeting Src SH2 domain exemplifies the significance of such compounds. Phenyl phosphate combinatorial libraries have been successfully generated using the described solid phase methodology. The solid-phase library synthesis and structure-activity results of this work will be disclosed elsewhere.

Representative Procedure for the Preparation of Resin 4

Tyramine Resin 4a: To a cooled (-20 °C) suspension of bis(diisopropylamino)chlorophosphine (20.0 g, 0.075 mol) in 300 mL of anhydrous Et_2O, under an atmosphere of N_2, was added 11.1 mL of triethylamine (0.080 mol) followed by 9.3 mL of 4-methoxybenzyl alcohol (0.075 mol). The resulting reaction mixture was warmed to ambient temperature and stirred for an additional 40 min. To this was added hexanes (~100 mL) and the supernatant filtered through a fritted funnel via cannulation (16G needle used). *It is imperative to rigorously maintain an atmosphere of N_2 throughout this process.*[5] The solvent was removed under reduced pressure (rotary evaporator equipped with an in-line drying tube and vented with N_2) and the resulting residue further concentrated *in vacuo* (~90 min) to remove the remaining Et_3N. A solution of the cloudy oil in 200 mL of CH_2Cl_2 was then cannulated into a suspension containing 18.9 g of N-Fmoc tyramine (0.052 mol) and 4.62 g of DIAT (0.027 mol) in 240 mL of CH_2Cl_2. The resulting solution was stirred at ambient temperature for 1.5 h, upon which it was poured into 100 mL of saturated aqueous $NaHCO_3$. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated. Purification by flash chromatography (4:1, hexanes/EtOAc with 1% Et_3N) provided 29.8 g of phosphoramidite **3a** as a clear oil.

^1H NMR (DMSO) δ 7.88 (d, 2H), 7.66 (d, 2H), 7.43-7.25 (m, 7H), 7.09 (d, 2H), 6.92-6.87 (m, 4H), 4.69-4.65 (m, 2H), 4.28 (brd, 2H), 4.21-4.16 (m, 1H), 3.74 (s, 3H), 3.71-3.63 (m, 2H), 3.19-3.15 (m, 2H), 2.68-2.63 (m, 2H), 1.17-1.11 (m, 12H); ^{31}P NMR (DMSO) δ 151.26.

The phosphoramidite intermediate **3a** (29.8 g, 0.047 mol) was dissolved in 500 mL of anhydrous THF and added 13.8 g of Wang resin (0.016 mol, loading = 1.15 mmol/g) followed by 5.54 g of tetrazole (0.079 mol). The reaction mixture was swirled and periodically sonicated for brief periods until all the tetrazole had dissolved, upon which sonication was maintained for 1.5 h. The mixture was left to stand for 30 min and then diluted with 100 mL of DMA. After a short sonication period (~10 min) the resin was filtered and washed successively with DMA (5x100 mL), Et_2O (2x100 mL), and CH_2Cl_2 (3x100 mL). The resin was then transferred to a r.b. flask along with 350 mL of CH_2Cl_2 and added 3.71 g of NMO (0.032 mol). The mixture was sonicated for 2 h, after which the resin was filtered and washed successively with DMA (2x100 mL),

CH_2Cl_2 (4x100 mL), Et_2O (2x100 mL), CH_2Cl_2 (1x100 mL), Et_2O (1x100 mL), and CH_2Cl_2 (2x100 mL). Excess solvent was removed *in vacuo* overnight to provide 22 g of resin **4a**. The loading capacity was determined to be 0.66 mmol/g.[5]

^{31}P NMR (gel phase, $CDCl_3$) δ -5.308 (br).

Phenyl Propionate Resin 4d: Resin **4d** was prepared in an analogous fashion to that described for **4a** to provide 19 g of resin. The loading capacity was determined to be 0.77 mmol/g.[6]

^{31}P NMR (gel phase, $CDCl_3$) δ -5.549 (br).

A small sample of resin was cleaved using 20% TFA/CH_2Cl_2 to provide the following analytical data (**5d**): ^1H NMR (DMSO) δ 7.19 (d, 2H), 7.06 (d, 2H), 5.95-5.82 (m, 1H), 5.29-5.17 (m, 2H), 4.53 (d, 2H), 3.80-3.58 (m, 2H), 2.83 (t, 2H), 2.64 (t, 2H); ^{31}P NMR (DMSO) δ -0.924; MS (ES^-) *m/z* 285 (M-H).

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E-mail: * metcalfc@ariad.com. Present Addresses: † Terrapin Technologies, Inc., So. San Francisco, CA. ‡ Vertex Pharmaceuticals, Cambridge, MA. § Astra Hassle, Cambridge, MA.

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4. Substituting benzyl alcohol for p-methoxybenzyl alcohol in the synthesis affords resin **7** with the same relative high purity and loading capacity as resin **4**. However, removal of the benzyl protecting group following cleavage to generate **5** requires prolonged exposure to 95% TFA (5% H_2O).
5. The electron-withdrawing aryloxy groups render these intermediates susceptible to nucleophilic degradation (by H_2O, etc.) and therefore require greater care when handled as compared to analogous intermediates used in oligonucleotide synthesis.
6. The loading capacities of resins **4a-c/7g-i** were determined by spectrophotometric measurement of the cleaved Fmoc group from a pre-dried/weighed resin sample.
7. The loading capacities of resins **4d-f/7j-l** were determined using the total mass of the cleaved (20% TFA/CH_2Cl_2) phosphate products **5d-f/6j-l** generated from a pre-dried/weighed resin sample. Cleaved products were stripped down several times with CH_2Cl_2, concentrated *in vacuo* to constant weight, and analyzed for product verification using ^1H NMR, ^{31}P NMR, and MS (ES^-) prior to weighing.
8. % Yield = actual loading capacity/theoretical loading capacity.[9]
9. Theoretical loading capacity = 1.15 mmol/[1g + ($1.15x10^{-3}$ mol x MW added to resin)] for Wang resin 1.15 mmol/g loading.
10. 3- and 4-Hydroxybenzyl amines were prepared from their respective 3- and 4-cyanophenols by repeated hydrogenation (H_2, Pd/C, aq HCl/EtOH, 60 psi) followed by recrystallization from Et_2O/MeOH.
11. The methyl ester protecting group was easily removed using LiOH (2.2 eq)/THF:H_2O (2:1) conditions (2h) without any measurable loss in loading capacity.
12. The biphenyl ether linkage was established via solution phase Ullmann coupling (K_2CO_3, CuO, pyr, 140 °C) between 4-benzyloxyphenol and the appropriately substituted cyanophenyl bromide.
13. Compound **10** contains 21% of the coupled N-methylbenzamide of deprotected linker **4d** [MS (ES^+): *m/z* 350, M+H] as determined by LCMS of the crude cleaved product.
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