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Design and Synthesis of Phosphotyrosine Mimetics

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Abstract—Selective inhibitors of protein tyrosine phosphatases (PTPases) are of great interest as therapeutic agents and research tools. Several phenylalanine derivatives (**1**, **2**) designed as phosphotyrosine mimetics or irreversible active site inhibitors were successfully synthesized, then incorporated into a combinatorial library based on a peptidomimetic β-strand template.
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The protein tyrosine phosphatases (PTPases) play an important role in controlling the status of tyrosine phosphorylation and the regulation of cellular function.[1] The ability to selectively inhibit tyrosine phosphatases holds enormous therapeutic potential for the treatment of diseases such as diabetes, cancer and osteoporosis.[2] As a part of our ongoing research program,[3] we have designed and synthesized several phenylalanine derivatives **1** and **2** (Fig. 1).[4] The type-1 derivatives were designed to mimic a phosphorylated tyrosine,[5] while the type-2 derivatives include aryl substituents similar to a 1-benzothiopyran-1,1,4-trione moiety, previously reported to be a selective irreversible inhibitor of PTP1B.[6]

The synthesis of the unusual amino acids **1** began with commercially available Fmoc-L-Tyr-(3-NO_2)-OH (**3**) as depicted in Scheme 1. The amino acid was esterified with CDI and MeOH, followed by reduction of the nitro group with $SnCl_2$ and cyclization of the resulting aminophenol **4** with phosgene to furnish **5**. Compound **4** was also treated with TsCl in the presence of pyridine, then cyclized with SO_2Cl_2 to afford **7**. Both compounds **5** and **7** were then carefully hydrolyzed with LiOH to the corresponding acids **1a** and **1b**.

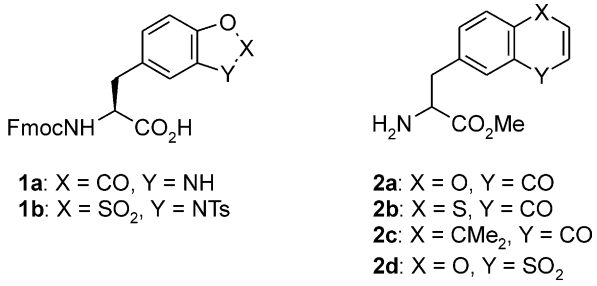
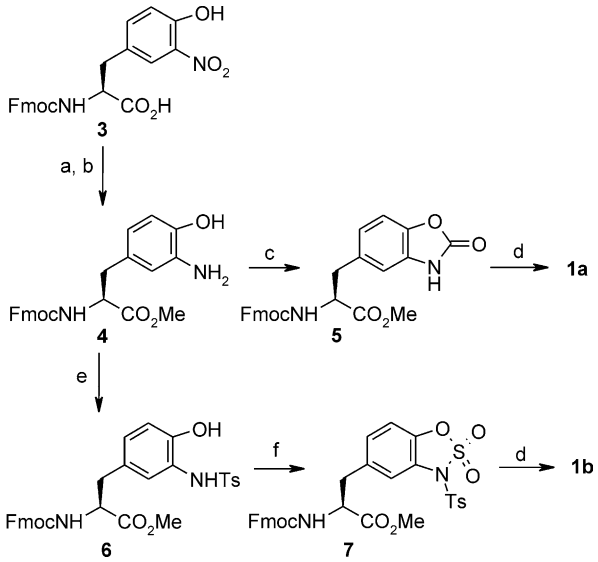


Figure 1.



Scheme 1. (a) CDI in MeOH, rt, 85%; (b) $SnCl_2$, EtOH, reflux, 88%; (c) phosgene, THF, rt, 65%; (d) 0.2 M LiOH (aq), THF, 0 °C, 61–62%; (e) TsCl, Py, CH_2Cl_2, rt, 40%; (f) SO_2Cl_2, TEA, CH_2Cl_2, rt, 68%.

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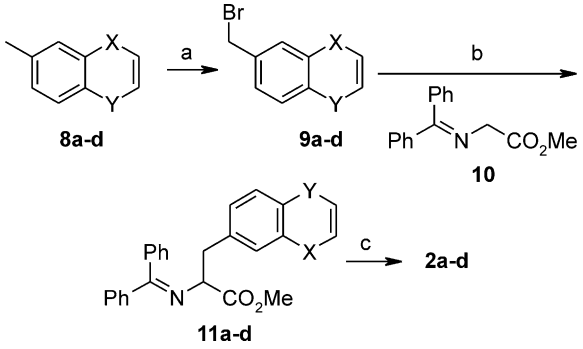
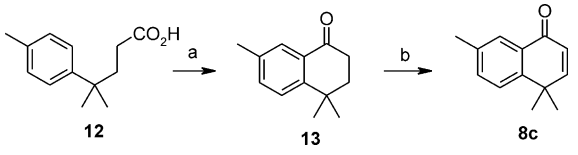
The synthesis of the title compounds **2** is described in Scheme 2. Starting material **8a** was commercially available, while the synthesis of **8b** has been previously reported.[7] The 4,4,7-trimethyl-4H-naphthalen-1-one **8c** was prepared from *gem*-dimethyl carboxylic acid **12**[8] via treatment with TFAA in TFA (Scheme 3). Resulting ketone **13** was deprotonated with LHMDS and selenated with PhSeBr, followed by oxidative elimination to afford **8c**. The synthesis of **8d** required the intermediate **15**, prepared from **14**[9] by reaction with SO_2Cl_2, then quinoline (Scheme 4). The thioenol **15** was then treated with H_2O_2 to give **8d** in 76% yield.

Finally, the desired benzyl bromides **9** were obtained in moderate yield by benzylic bromination of **8** with NBS in the presence of AIBN. With the starting materials **9** in hand, we adopted O'Donnell's protocol[10] to synthesize **2** (Scheme 2). The glycine Schiff base **10**, derived from benzophone imine and glycine methyl ester, was benzylated with **9** to give **11** in 42–60% yield. For the final step, imino methyl ester **11** was treated with 1 N HCl to provide the title compounds **2** in good to excellent yields (Table 1).

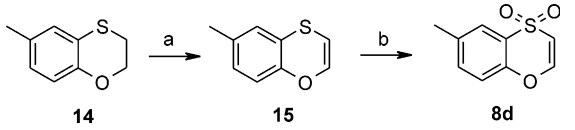
The phosphotyrosine mimetics were successfully incorporated into a library containing triazolopyridazine β-strand templates[11] via solid-phase synthesis. Preliminary screening for inhibition of four phosphatases (CD45, LAR, TCPP, and PTP1B) was accomplished by flourometric assays, using 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, Molecular Probes) as substrate.[12] Some derivatives exhibited moderate potency (up to 85% inhibition at 10 μM sample concentration) and selectivity against the tyrosine phosphatases assayed (see Table 2), with the type **2d** phosphotyrosine mimetic providing the best activity.[13]



Scheme 2. (a) NBS/AIBN, CCl_4; (b) 10% NaOH(aq), CH_2Cl_2, $nBu_4N^+HSO_4^-$; (c) 1 N HCl.



Scheme 3. (a) TFAA, TFA, 0 °C to rt, 63%; (b) (i) LHMDS, PhSeBr, −78 °C, THF, 86%; (ii) 30% H_2O_2, AcOH, THF, 80%.

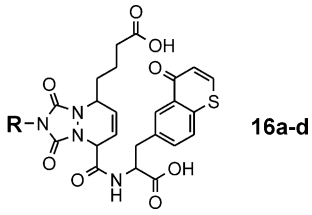


Scheme 4. (a) SO_2Cl_2, CH_2Cl_2, reflux, then quinoline, 150 °C, 50%; (b) 30% H_2O_2, AcOH, reflux, 76%.

Table 1. Yields of **9**, **11** and **2** (%)

8	9	11	2
a: X = O, Y = CO	61	42	100
b: X = S, Y = CO	53	54	90
c: X = CMe_2, Y = CO	66	59	86
d: X = O, Y = SO_2	50	60	68

Table 2. Screening results for inhibition of tyrosine phosphatases



Compd	R	PTP1B % inhibition (100 μM, 10 μM)	TC-PTP % inhibition (100 μM, 10 μM)	LAR % inhibition (100 μM, 10 μM)	CD45 % inhibition (100 μM, 10 μM)
16a	2,5-F_2-Bn	95, 0	100, 85	50, 25	50, 5
16b	4-PhO-Ph	100, 75	100, 85	70, 50	70, 20
16c	PhCH(Me)CH_2	95, 55	90, 20	50, 10	55, 15
16d	*c*HexCH_2	70, 40	20, 10	10, 0	70, 20

Acknowledgements

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12. Samples were preincubated with 0.2 units/mL enzyme in buffer (100 mM HEPES, 1 mM EDTA, 5 mM DTT, 0.05% Tween-20, 0.01% BSA) for 20 min at rt in black 96-well plates. DiFMUP was then added (final concentration 3.6 μM for PTP1B, 2.1 μM for TCPTP, 15 μM for LAR and 200 μM for CD45) and the plates read on a Fluoroskan (abs. 358, em. 452).
13. Regretfully, further analysis of the enzymatic inhibition was not possible due to the termination of the operations of Molecumetics. The mode of inhibition of compounds containing the purported irreversible inhibitor **2d** is of particular interest, as are IC_{50} or K_i values for the most active compounds against the various enzymes in order to determine selectivity.