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Synthesis of a novel peptidic photoaffinity probe for the PTP-1B enzyme

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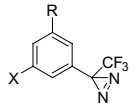
Abstract—The synthesis of a novel radioactive peptidic photoaffinity probe for the PTP-1B enzyme as well as some SAR leading to the choice of this compound as a photoaffinity probe are presented.

Upon binding of insulin to its receptor (IR), the latter is activated and its regulatory domain tyrosine residues are phosphorylated, resulting in the insulin signaling pathway.[1] The protein tyrosine phosphatase (PTPase) enzymes are known to be responsible for the dephosphorylation of tyrosine residues.[1] In particular, and of significant interest to us, the PTP-1B enzyme has been implicated in negative regulation of the insulin signaling pathway by dephosphorylating the IR.[2–4] It is believed that this negative regulation of insulin signaling could be a contributing factor to the insulin resistance observed in type 2 diabetes, noninsulin dependent diabetes mellitus.[5] Furthermore, studies with PTP-1B knock-out mice have demonstrated that they are resistant to diet-induced obesity and possess increased insulin sensitivity.[6] These results prompted us to pursue the preparation of a specific PTP-1B inhibitor as potential treatment for type 2 diabetes.

We felt that in order to better characterize the action of our specific PTP-1B inhibitors, such as their ability to penetrate the cell, the preparation of a radiolabeled photoaffinity probe would be quite useful. We had already prepared some very potent peptidic inhibitors of PTP-1B[7] and decided to prepare analogs of those peptides, which would contain a phenyl group substituted with a trifluoromethyldiazirine[8] unit as the photoactive group and an iodo as a potential radioactive group,[9] as shown in Figure 1.

The phenyl trifluoromethyldiazirine unit was prepared as described in the literature.[8,9] Where methyl 3,5-diiodobenzoate or methyl 3-bromo-5-iodobenzoate is used as starting material, at the benzyl alcohol stage either a bromination or an oxidation is performed to obtain the desired benzyl bromide or benzoic acid. For considerations of practicality, some compounds were originally prepared as the bromo substituted trifluoromethyldiazirine instead of the iodo, assuming that the SAR would be similar between the two compounds and that any potent compound could be synthesized as the iodo analog in order to prepare a radioactive version.

The chiral *o*-bromo difluoromethylphosphonophenylalanine (*o*-BrF_2PMP) was prepared as illustrated in



X = Br, I
R = CH_2Br,COOH

Figure 1.

Keywords: Photoaffinity probe; Protein tyrosine phosphatase PTP-1B.
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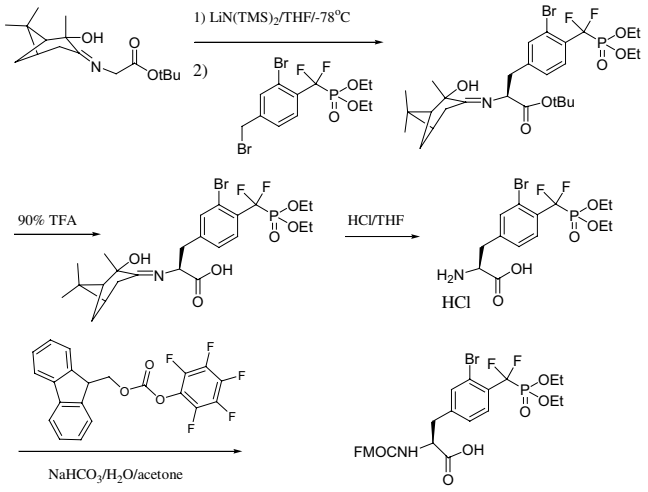
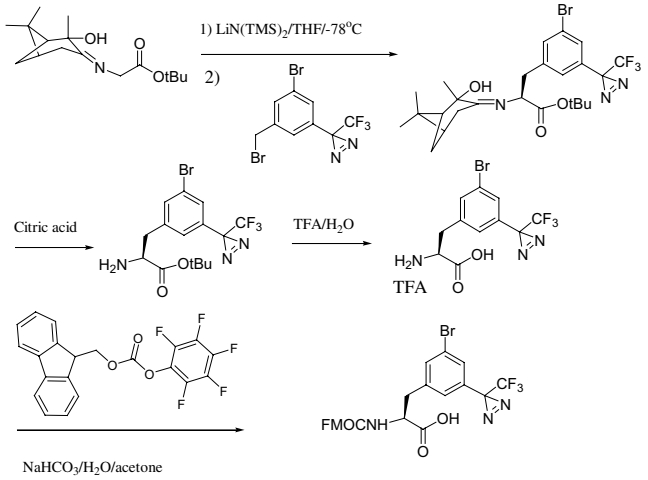


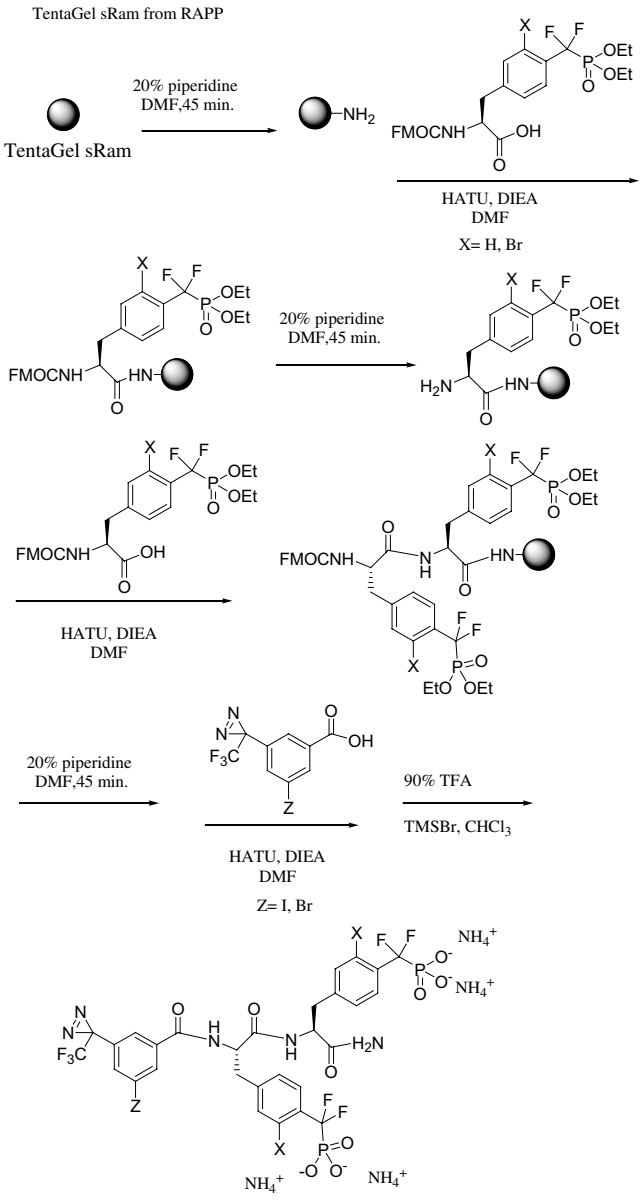
Scheme 1. Synthesis of chiral *o*-bromo difluoromethylphosphonophenylalanine.

Scheme 1, utilizing a bicyclic Schiff base chiral auxiliary.[10] Using the same chiral auxiliary an analog of tyrosine with a trifluoromethyldiazirine unit was prepared as in Scheme 2.

Scheme 3 shows the synthesis of a representative peptide substituted with the required trifluoromethyldiazirine-benzoic acid capping the N-terminus using standard procedures.[11–13] Typically, TentaGel sRam from RAPP was deprotected using piperidine in DMF for 30 min, then washed with DMF (5×), and CH_2Cl_2 (5×). A Fmoc-protected amino acid was then coupled to the resin using HATU and diisopropylethylamine in DMF, deprotection with piperidine then affords the free amine ready for coupling with another carboxylic acid using the same procedure. The peptide was cleaved from the resin by treatment with trifluoroacetic acid and water, subsequent treatment with TMSBr in $CHCl_3$ afforded the phosphonic acid directly or the ammonium salt after reverse-phase HPLC purification using an ammonium acetate buffer.



Scheme 2. Synthesis of chiral trifluoromethyldiazirine phenylalanine.



Scheme 3. Representative synthesis of peptides.

Figure 2 lists all the analogs that were prepared. We considered two factors for what would constitute a desirable photoaffinity probe. The most important criterion was the compounds' ability to inhibit the PTP-1B enzyme in the fluorescein diphosphate (FDP) assay.[14] Secondly, the probe had to be irreversibly bound to the enzyme. The co-valent binding of the photoprobe to the enzyme was assessed by irradiating a solution of the photoprobe and the PTP1B enzyme with a 40 W lamp and the amount of adduct formed was measured by mass spectrometry. The results are summarized in Table 1. First, we noted that there was a significant increase in potency in going from a monopeptide/monophosphonic acid, **8**, to a dipeptide/diphosphonic acid **1**, as indicated in Table 1. As expected, having two trifluoromethyldiazirine units, **9**, did not improve the potency. The introduction of a glutamic acid to form a tripeptide, **7**, did not improve the potency over the dipeptide where

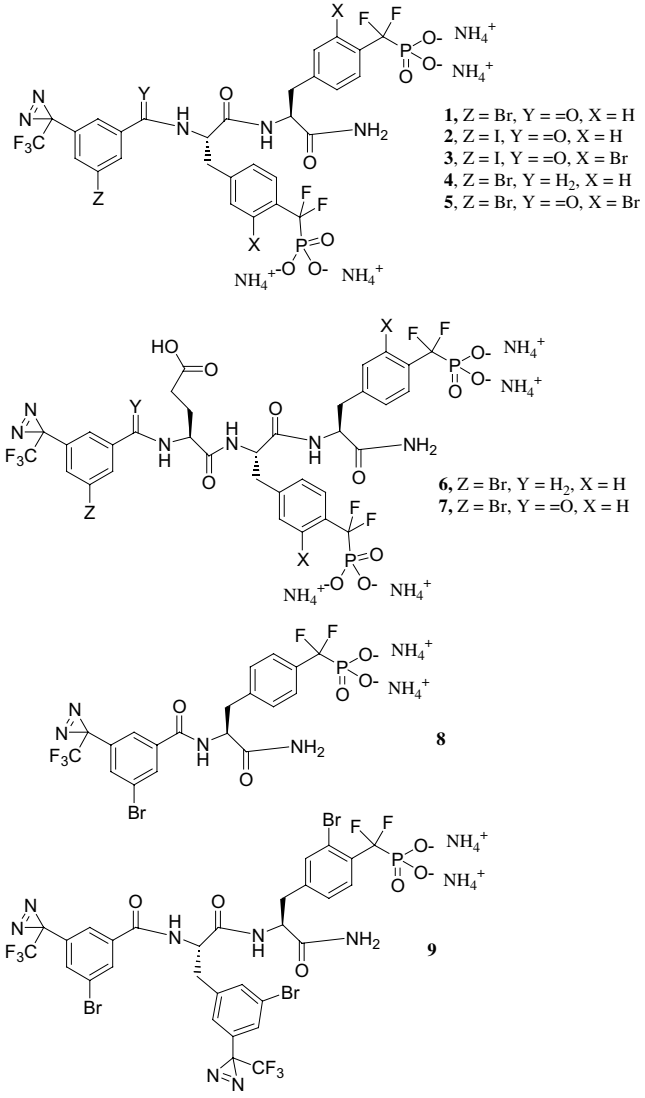


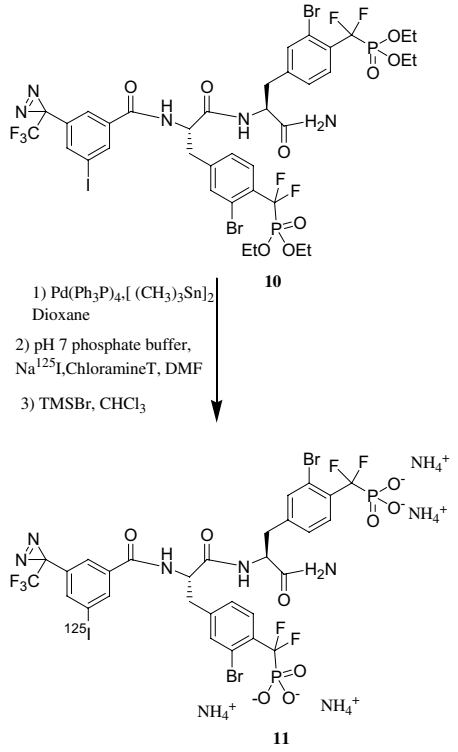
Figure 2. Peptidic analogs synthesized.

Table 1. Photoprobe results summary

Compound	PTP-1B inhibition IC_{50}, (μM)	% Photolysis after 5 min[a]	% Adduct formation
1	0.012	52	
2	0.009	45	8
3	0.004	33	8
4	1.7	44	
5	0.0003	58	6
6	0.003	50	5
7	0.014	40	8
8	9.5	50	
9	0.015	64[b]	<5

[a] 50 μL of 25 μM photoprobe in water, irradiated with 40 W lamp for 5 min, product formation measured by MS, loss of N_2, gain of H_2O.
[b] 64% mono, 24% di.

the trifluoromethyldiazirine is attached as an amide, **1**. However, tripeptide **6** did show an increase in potency from the dipeptide **4** where the trifluoromethyldiazirine is attached as an amine. However, because of the low (5%) amount, of adduct formation (which is a measure of irreversibility) of **6**, it was not considered a suitable



Scheme 4.

compound. The introduction of an *ortho* bromo substituent on the phenyl ring, **3** and **5** versus **1** and **2** had a marked improvement on the potency against the PTP-1B enzyme. We therefore opted to prepare compound **3** as a radioactive photoaffinity probe for the PTP-1B enzyme.

As illustrated in Scheme 4, treatment of compound **10**, with 10 equiv of hexamethylditin and 0.1 equiv of tetrakistriphenylphosphine palladium in dioxane at 50 °C for 90 min afforded the tin intermediate, which was treated (1 mg) with a 50 μL (2.5 mCi) solution of $Na^{125}I$ in water, 0.2 M pH 7 phosphate buffer (38 μL), and Chloramine T (27 μL of a 10 mg/mL aqueous solution) in DMF for 1 h, and quenched with 2 M $NaHSO_3$ (100 μL) to afford **11** after reverse-phase HPLC purification followed by cleavage of the ester in a 1:2 mixture of TMSBr–$CHCl_3$.

In summary, we have prepared a novel photoaffinity probe for the PTP-1B enzyme utilizing a dipeptide substituted with a trifluoromethyldiazirine unit and an iodo substituent giving us the ability to make the radioactive analog. By adding an *ortho* bromo substituent on the phenyl difluoromethylphosphonate unit we were able to increase the intrinsic potency of the compound. This probe binds irreversibly to the PTP-1B enzyme upon irradiation by light, further studies with this probe will be published at a later date.

References and notes

1. Evans, J. L.; Jallal, B. *Exp. Opin. Invest. Drugs* **1999**, *8*, 139.

2. Bandyopadhyay, D.; Kusari, A.; Kenner, K. A.; Liu, F.; Chernoff, J.; Gustafson, T. A.; Kusari, J. *J. Biol. Chem.* **1997**, *272*, 1639.
3. Kenner, K. A.; Anyanwu, E.; Olefsky, J. M.; Kusari, J. *J. Biol. Chem.* **1996**, *271*, 19810.
4. Ahmad, F.; Li, P. M.; Meyerovitch, J.; Goldstein, B. J. *J. Biol. Chem.* **1995**, *270*, 20503.
5. Kahn, C. R. *Diabetes* **1994**, *43*, 1066.
6. Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science (Washington, DC)* **1999**, *283*, 1544.
7. Desmarais, S.; Wang, Q.; Ly, H.; Scheigetz, J.; Govindarajan, A.; Ramachandran, C.; Zamboni, R.; Huang, Z. *J. Biomol. Screening* **1999**, *4*, 327.
8. Brunner, J.; Senn, H.; Richards, F. M. *J. Biol. Chem.* **1980**, *255*, 3313.
9. Gallant, M.; Sawyer, N.; Metters, K. M.; Zamboni, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 63.
10. Jacob, M.; Roumcstant, P.; Martinez, J. *Synlett* **1997**, 691.
11. Dufresne, C.; Roy, P.; Wang, Z.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Xu, L.; Gordon, R.; Chan, C. C.; Waddleton, D.; Ramachandran, C.; Kennedy, B. P.; Leblanc, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1039.
12. Burke, T. R. J.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129.
13. Solas, D.; Hale, R. H.; Patel, D. V. *J. Org. Chem.* **1996**, *61*, 1537.
14. Huang, Z.; Wang, Q.; Ly, H. D.; Govindarajan, A.; Scheigetz, J.; Zamboni, R.; Desmarais, S.; Ramachandran, C. *J. Biomol. Screening* **1999**, *4*, 327.