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Note

Preparation of triazole-linked glycosylated α-ketocarboxylic acid derivatives as new PTP1B inhibitors

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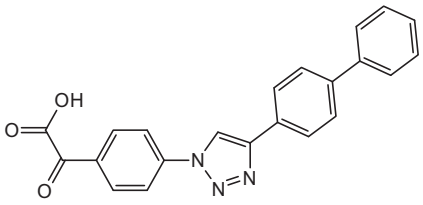
ABSTRACT

The synthesis of triazole-linked glycosyl acetophenone, benzoic acid, and α-ketocarboxylic acid derivatives was readily achieved via Cu(I)-catalyzed azide–alkyne cycloaddition ('click' reaction) in excellent yields of 93–97%. Among the synthesized glycoconjugates, the triazolyl α-ketocarboxylic acids were identified as the most potent protein tyrosine phosphatase 1B (PTP1B) inhibitors with micromole-ranged IC_{50} values and moderate-to-good selectivity over a panel of homologous PTPs including TCPTP (4.6-fold), LAR (>30-fold), SHP-1 (>30-fold) and SHP-2 (>30-fold). Moreover, a docking simulation was conducted to propose a plausible binding mode of the glucosyl α-ketocarboxylic acid triazole with the enzymatic target.

Protein tyrosine phosphatases (PTPs) are regulators of tyrosine phosphorylation-dependent cellular events that govern numerous critical physiological processes.[1] Among this large superfamily, protein tyrosine phosphatase 1B (PTP1B) represents one of the best therapeutically characterized enzymatic members and is related to several major human diseases. For example, PTP1B-knockout mice could lead to the increase of insulin sensitivity and resistance to diet-induced obesity while treatment with PTP1B antisense oligonucleotides resulted in the improvement of hyperglycemia in diabetes mice models.[2,3a] In addition, more recent reports indicated that PTP1B also performs as an oncogene in the context of breast cancer.[3b] These data suggest that inhibition of PTP1B may represent a practical strategy for the treatment of type 2 diabetes, obesity, as well as breast cancer.

Consequently, increasing efforts have been made toward the development of PTP1B inhibitors by both academia and the pharmaceutical industry.[4] Most of the reported bioactive compounds fall into isosteric phosphotyrosine (pTyr) surrogates that competitively target the PTP1B active site.[5] These nonhydrolyzable phosphonate and carboxylic acid mimetics including difluoromethylene phophonates (DFMP),[6] *N*-aryl oxamic acid,[7] isoxazole acid,[8] 2-oxalylamino benzoic acid,[9] and tyrosine acid derivatives[10] have provided considerable leading compounds for the structure or fragment based approaches to derive other novel and potent PTP1B inhibitors. The α-ketocarboxylic acid, bearing a fundamental carboxylic acid structure and one additional ketone group, was recognized as a promising pTyr mimetic and its derivatives have recently been identified as favorable PTP inhibitors.[11]

Carbohydrate-based drug discovery has lately attracted considerable interest due to its structural diversity, natural abundance, and high biocompatibility.[12] In addition, numerous glycosylated bioactive triazolyl compounds formed via click chemistry have been reported in the past decade.[13] With a continuing interest in preparing sugar-based inhibitors of negative enzymatic regulators associated with major human diseases,[14] we sought to synthesize triazole-linked glycosyl α-ketocarboxylic acid derivatives as new



A : IC_{50} = 84 μM (PTP1B)

Figure 1. Aryl α-ketocarboxylic acid-based PTP1B inhibitor **A**.[11]

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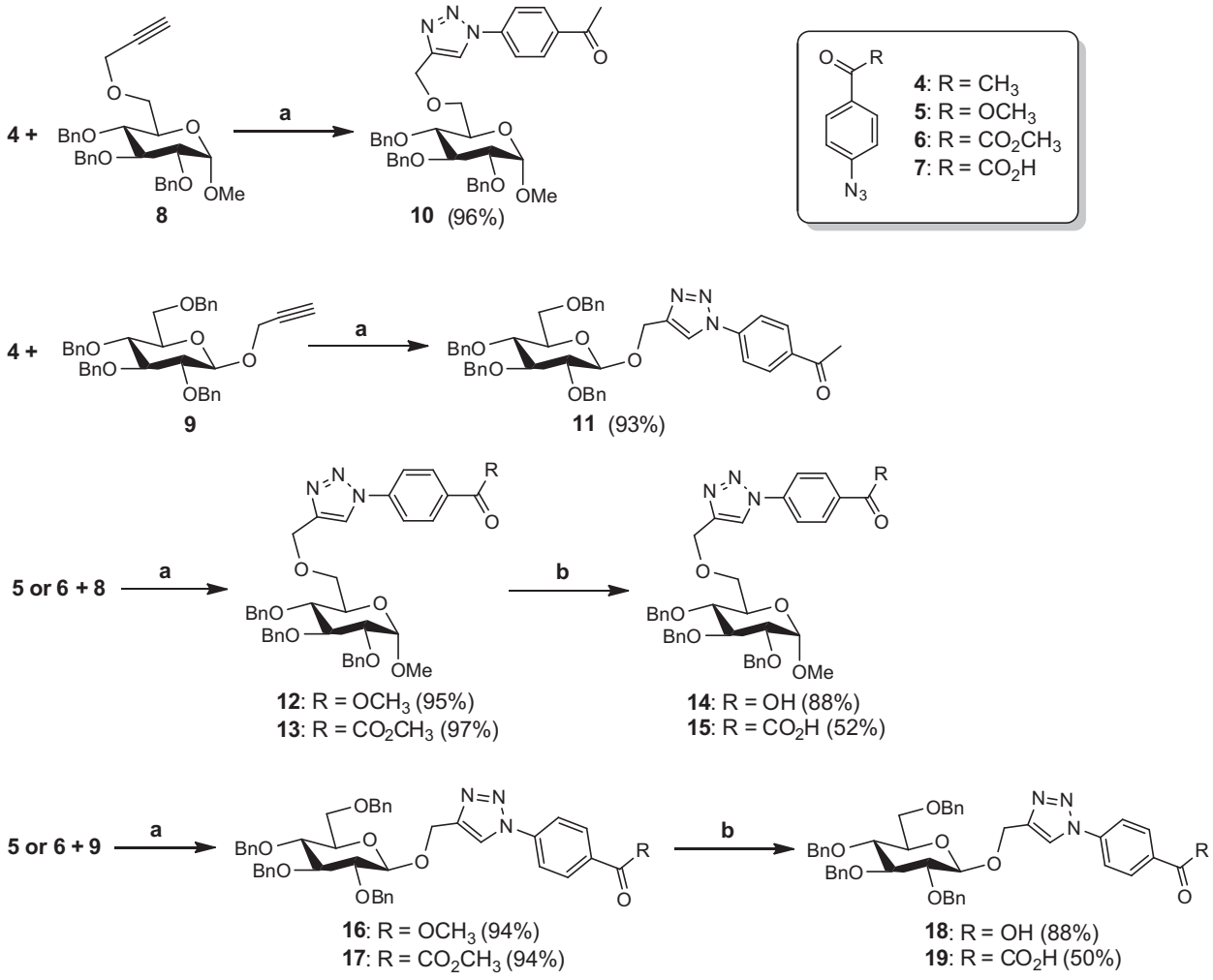
PTP1B inhibitors. Meanwhile, glycosyl acetophenone and benzoic acid derivatives were also prepared for SAR (structure–activity relationship) study. Recently, an α-ketocarboxylic acid triazole with a distal biphenyl moiety (Compound **A**, Fig. 1) has been reported to possess moderate PTP1B inhibitory activity (84 μM).[11] Meanwhile, it is also well-noted that aryl moieties covalently coupled with the active site-oriented precursor may simultaneously generate hydrophobic interactions with periphery enzyme surface (such as the aryl second phosphotyrosine site or YRD motif) of PTP1B.[14a,15] We consequently chose to use benzylated 1-*O*-propargyl or 6-*O*-propargyl glucosides as glycodonors to comparatively investigate whether such an aryl sugar scaffold would lead to enhanced affinity.

As shown in Scheme 1, 1-(4-azidophenyl)ethanone **4**,[16] methyl 4-azidobenzoate **5**[16] and methyl 2-(4-azidophenyl)-2-oxoacetate **6**[11] were used as the azido reactants while the *O*-propargyl glycosyl donors **8** and **9** were prepared according to the known literature reports.[17] Furthermore, the 2-(4-azidophenyl)-2-oxoacetic acid **7**[11] was also synthesized for inhibitory assay.

The Cu-catalyzed azide–alkyne cycloaddition (click reaction)[18] was achieved in the presence of sodium ascorbate and $CuSO_4{\cdot}5H_2O$ in CH_2Cl_2/H_2O (1:1, v/v) solvent mixture. As illustrated in Scheme 1, azide **4** was first coupled with propargyl **8** and **9**, which favorably yielded the desired triazole-linked glycoconjugates **10** and **11** in yields of 96% and 93%, respectively. The click reaction of azido esters **5** and **6** with alkynes **8** and **9** was then realized to afford the glycosyl esters in excellent yields of 95% (**12**), 97% (**13**), 94% (**16**) and 94% (**17**), respectively. Saponification with LiOH finally gave the free acids **14**, **15**, **18,** and **19**. Notably, the relatively low yields of the obtained glycosyl α-ketocarboxylic acids (52% for **15** and 50% for **19**) was most likely rendered by partial conversion of the ketoacids into carboxylic acids in basic condition.[19]

Next, the inhibitory activity of the prepared glycosides (**10**–**19**) as well as the azido α-ketocarboxylic acid **7** was assayed toward PTP1B. The recombinant human PTP1B was first expressed and purified. In a typical 100 μL assay, a mixture containing 50 mM MOPS, pH 6.5, 2 mM *p*NPP and recombinant enzymes, PTP1B activities were continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. IC_{50} was calculated from the nonlinear curve fitting of percent inhibition (inhibition (%)) versus inhibitor concentration [*I*] by using the following equation: inhibition (%) = $100/\{1 + (IC_{50}/[I])k\}$, where *k* is the Hill coefficient.[20a]

As listed in Table 1, the azido ketoacid **7** (entry 1) was first observed to have moderate inhibitory activity with IC_{50} value = 30.2 μM. The glycosylated acetophenones **10** (entry 2) and **11** (entry 3), lacking the carboxylic acid functionality, weakly inhibited PTP1B (about 50% inhibition at 100 μg/mL). In addition, both glucosides substituted on 6 position by triazolyl benzoic ester **12** (entry 4) and benzoic acid **14** (entry 6) showed weak inhibition whereas the glucoside 6-substituted by α-keto ester **13** (entry 5)



Scheme 1. Reagents and conditions: (a) Na ascorbate, $CuSO_4{\cdot}5H_2O$ in CH_2Cl_2/H_2O; (b) LiOH in H_2O/MeOH.

Table 1
Inhibitory activity of **7** and **10**–**19** on PTP1B[a]

Entry	Compd	PTP1B	
		Inhibition rate (%)	IC_{50} (μM)
1	**7**	96.9	30.2
2	**10**	44.1	>100
3	**11**	48.2	>100
4	**12**	51.5	>100
5	**13**	99.5	12.7
6	**14**	18.5	>100
7	**15**	**97.9**	**3.2**
8	**16**	25.6	>100
9	**17**	99.5	12.3
10	**18**	100	11.1
11	**19**	**99.3**	**5.6**
12	**A**	–	84[b]

[a] Data are means of three experiments at a concentration of 100 μg/mL.
[b] This IC_{50} value is cited in the Ref. 11.

displayed excellent inhibition (99.5%) and reasonable IC_{50} value (12.7 μM) on PTP1B. Its acid-free derivative **15** (entry 7) was then determined to possess the best inhibitory potency among all assayed compounds and owns an almost 10-fold enhanced IC_{50} value (3.2 μM) comparing to the model compound **7**.

Comparing to the glucosides 6-substituted by benzoic moieties (**12** and **14**), the 1-substituted ester **16** (entry 8) similarly showed no inhibitory activity on PTP1B, while the corresponding acid **18** (entry 10) displayed favorable inhibition (100%) with an IC_{50} value of 11.1 μM. On the other hand, despite that the 1-substituted α-ketoester **17** (entry 9) exhibited nearly equal IC_{50} value to that of its 6-substituted counterpart (**13**), the inhibitory activity of the corresponding 1-substituted acid **19** (entry 11) was slightly lower (IC_{50} = 5.6 μM) than that of the 6-substituted acid **15** (IC_{50} = 3.2 μM). Furthermore, ketoacid **19** displayed an approximately twofold increased inhibitory activity on PTP1B comparing to the carboxylic acid **18**, unambiguously demonstrating that the α-ketocarboxylic acids are more effective as a PTP1B inhibitor than the corresponding benzoic acid derivatives.

In addition, by comparing the inhibitory activity of the reported biphenyl ketoacid triazole (compound **A**, Table 1, entry 12) with that of the identified glucosyl ketoacid triazoles **15** and **19**, the glucosyl compounds exhibited remarkably 26- and 15-fold increased inhibitions. This clearly demonstrated that the natural glucosyl scaffold employed in this study was advantageous as a chiral scaffold for the enhancement of binding affinity toward PTP1B.

The specificity of the triazole-linked glucosyl α-ketocarboxylic acid derivatives **15** and **19** over a panel of homologous PTPs including TCPTP (74% identity with PTP1B), LAR, SHP-1 and SHP-2 was sequentially assessed using the method similar to that employed for PTP1B.[20b] As shown in Table 2, whereas the 1-substituted glycoside **19** (entry 2) displayed almost identical IC_{50} value (IC_{50} = 6.4 μM on TCPTP and 5.6 μM on PTP1B), the 6-substituted glycoside **15** showed 4.6-fold selectivity over TCPTP (IC_{50} = 14.5 μM on TCPTP and 3.2 μM on PTP1B). In addition, glycoside **15** exhibited excellent selectivity over LAR, SHP-1, and SHP-2 (IC_{50} >100 μM) while the glycoside **19** simultaneously displayed low inhibitory activity on SHP-2 (IC_{50} = 55.4 μM) with no inhibitory potency on LAR and SHP-1 (IC_{50} >100 μM).

Table 2
Inhibitory activity of **15** and **19** on PTPs[a]

Entry	Compd	IC_{50} (μM)				
		PTP1B	TCPTP	LAR	SHP-1	SHP-2
1	**15**	3.2	14.5	>100	>100	>100
2	**19**	5.6	6.4	>100	>100	55.4

[a] Data are means of three experiments at a concentration of 100 μg/mL.

A plausible binding mode of the triazole-linked glucosyl α-ketocarboxylic acid **15** with PTP1B was then proposed via docking simulation by starting with a crystal structure in complex with a reference ligand (PDB code: 3EB1, resolution: 2.40 Å).[15] Water was removed from the original structure, and the rest of the protein was prepared using the 'Protein preparation wizard' workflow in Maestro (Schrödinger, LLC, New York, NY, 2005). Hydrogens were added, bond orders assigned, and overlaps treated. The impref utility was run to perform restrained optimizations of the protein and the compound **15** was prepared using Ligprep 2.1 (Schrödinger, LLC, New York, NY, 2005). OPLS2005 Force field were employed, and Epik used to perform the ionization followed by chiralities correction. Then the compound **15** were docked to the active site of the protein using the Induced Fit Docking workflow (Schrödinger, LLC, New York, NY, 2005). The center atom was set to be a virtual center of referenced key residues: Phe182, Cys215 and Gly259. The inhibitor was initially docked by Glide, then the protein–inhibitor complex was minimized by Prime, and the complex structure redocked by Glide within a specified energy of the lowest-energy structure.

As illustrated in Figure 2, the triazolyl α-ketocarboxylic acid precursor coupled on the C6-position of the glucopyranoside located well in the active site cleft of PTP1B, adopting a competitive inhibition manner. Densely functionalized hydrogen bonds were mainly made between the extended carboxylic acid group and residues including Cys215, Ser216, Ala217, Gly218, Ile219, and Gly220 of the catalysis pocket which stably fixed the small molecule. Thus, the limited inhibition of the glycosyl benzoic acid derivative **14** could be presumably ascribed to the lack of one additional ketone group toward the extension of carboxylic group to the catalysis groove. Interestingly, one hydrogen bond was also made between the nitrogen atom of the triazole and Gly183 while the benzene moiety of Phe182 of the WPD loop covers the triazole ring like a lid. This is quite different from the positioning manner of pTyr substrate where the benzene moiety of Phe182 residue chooses to cover the phosphorylated phenol moiety of the tyrosine residue.[15] Moreover, hydrophobic interaction of the C2-benzyl

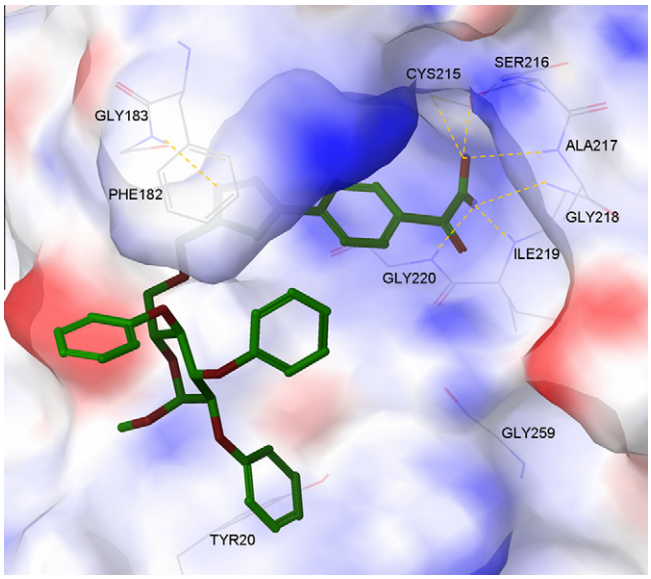


Figure 2. PTP1B in complex with compound **15**. The surface of the protein was shown as colored in property of electrostatic potential. The compound was shown as green stick, and residues of the active site in PTP1B were shown as light grey line. Nitrogen atoms are in blue and oxygen atoms in red. Hydrogen bonds were shown as yellow dash line and all nonpolar hydrogens were hidden. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group on sugar scaffold with Tyr20 of the second phosphotyrosine site was also possibly generated for enhancing the binding affinity.

In summary, we have efficiently synthesized glycosylated acetophenone, benzoic acid, and α-ketocarboxylic acid derivatives via Cu(I)-catalyzed 1,3-dipolar cycloaddition in excellent yields. The glycosyl α-ketocarboxylic acids were identified as promising sugar-based PTP1B inhibitors for the first time with at least several fold selectivities over a panel of homologous PTPs. In addition, docking study plausibly proposed a typical competitive inhibitory manner of ketoacid **15** with the enzymatic target, suggesting that both the triazolyl α-ketocarboxylic acid precursor and the benzylated glucosyl scaffold being the contributors. Our work may provide new insight toward the preparation of sugar scaffold-based PTP1B inhibitors.

1. Experimental

1.1. General

Solvents were purified by standard procedures. ^{1}H and ^{13}C NMR spectra were recorded on Bruker JEOL DX-400 spectrometers in $CDCl_3$ solution. Optical rotations were measured using a Perkin–Elmer 241 polarimeter at room temperature and a 10-cm 1-mL cell. Analytical thin-layer chromatography was performed on E. Merck aluminum percolated plates of Silica Gel 60F-254 with detection by UV and by spraying with 6 N H_2SO_4 and heating about 2 min at 300 °C. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV). Analytical HPLC was measured using Agilent 1100 Series equipment.

1.2. General procedure for Cu(I)-catalyzed 1,3-dipolar cycloaddition

To a biphasic solution of alkynyl sugar (1 equiv) and azide (1 equiv) in CH_2Cl_2 (5 mL) and H_2O (5 mL), Na ascorbate (4 equiv) and $CuSO_4{\cdot}5H_2O$ (2 equiv) were added. Then the mixture was stirred vigorously at rt. for 6 h. After completion of the reaction, the resulting mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$, filtered, and evaporated to give a crude residue which was purified by column chromatography.

1.2.1. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[1-(4-acetylphenyl)-1*H*-1,2,3-triazol-4-ylmethyl] α-D-glucopyranoside (10)

From compound **4** (58 mg, 0.36 mmol) and **8** (150 mg, 0.30 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **10** as a yellow powder (191 mg, 96%). R_f = 0.52 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ +12 (*c* 0.1 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, 2H, *J* = 8.8 Hz), 7.97 (s, 1H), 7.79 (d, 2H, *J* = 8.8 Hz), 7.36–7.20 (m, 15H), 4.98 (d, 1H, *J* = 10.8 Hz), 4.87 (d, 1H, *J* = 10.8 Hz), 4.82–4.77 (m, 3H), 4.70 (d, 1H, *J* = 14.4 Hz), 4.66 (d, 1H, *J* = 12.4 Hz), 4.61 (d, 1H, *J* = 3.6 Hz), 4.55 (d, 1H, *J* = 11.2 Hz), 4.00 (t, 1H, *J* = 9.2 Hz), 3.83 (dd, 1H, *J* = 3.6, 10.4 Hz), 3.79–3.74 (m, 2H), 3.62 (t, 1H, *J* = 9.2 Hz), 3.55 (dd, 1H, *J* = 3.2, 9.6 Hz), 3.38 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.3, 146.2, 140.0, 138.3, 138.2, 136.8, 130.1, 128.5, 128.4, 128.0, 127.9, 127.7, 127.6, 120.6, 120.0, 98.3, 82.1, 79.9, 75.8, 75.0, 73.4, 70.0, 69.2, 64.8, 55.3, 26.7; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{39}H_{41}N_3O_7$: 664.3023; found: 664.3038.

1.2.2. 1-(4-Acetylphenyl)-1*H*-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (11)

From compound **4** (24 mg, 0.15 mmol) and **9** (80 mg, 0.14 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **11** as a white powder (95 mg, 93%). R_f = 0.36 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ −1 (*c* 0.1 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 8.02 (d, 2H, *J* = 8.0 Hz), 7.63 (d, 2H, *J* = 8.8 Hz), 7.34–7.26 (m, 18H), 7.17–7.15 (m, 2H), 5.09 (d, 1H, *J* = 13.6 Hz), 5.03 (d, 1H, *J* = 13.2 Hz), 4.94 (d, 1H, 11.2 Hz), 4.92 (d, 1H, 11.2 Hz), 4.83–4.78 (m, 3H), 4.62 (d, 1H, *J* = 12.4 Hz), 4.56–4.52 (m, 3H), 3.77 (dd, 1H, *J* = 1.6, 10.8 Hz), 3.71 (dd, 1H, *J* = 4.8, 10.8 Hz), 3.66–3.63 (m, 2H), 3.53 (t, 1H, *J* = 8.0 Hz), 3.51–3.48 (m, 1H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.6, 146.3, 140.0, 138.5, 138.0, 136.7, 130.0, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 121.0, 119.9, 102.8, 84.7, 82.3, 77.8, 75.8, 75.1, 74.9, 74.8, 73.5, 69.0, 63.1, 26.7; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{45}H_{45}N_3O_7$: 740.3336; found: 740.3333.

1.2.3. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[1-(4-(methoxycarbonyl)-phenyl)-1*H*-1,2,3-triazol-4-ylmethyl] α-D-glucopyranoside (12)

From compound **5** (71 mg, 0.40 mmol) and **8** (201 mg, 0.40 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **12** as a yellow powder (260 mg, 95%). R_f = 0.35 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ +40 (*c* 0.1 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, 2H, *J* = 8.4 Hz), 7.96 (s, 1H), 7.75 (d, 2H, *J* = 8.4 Hz), 7.36–7.20 (m, 15H), 4.98 (d, 1H, *J* = 10.8 Hz), 4.86 (d, 1H, *J* = 11.2 Hz), 4.83–4.77 (m, 3H), 4.69 (d, 1H, *J* = 12.4 Hz), 4.66 (d, 1H, *J* = 12.4 Hz), 4.62 (d, 1H, *J* = 3.6 Hz), 4.56 (d, 1H, *J* = 10.8 Hz), 4.00 (t, 1H, *J* = 9.2 Hz), 3.95 (s, 3H), 3.83 (dd, 1H, *J* = 3.6, 10.4 Hz), 3.79–3.74 (m, 2H), 3.62 (t, 1H, *J* = 9.2 Hz), 3.55 (dd, 1H, *J* = 3.6, 9.6 Hz), 3.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 146.2, 140.0, 138.8, 138.3, 138.2, 131.3, 130.2, 128.5, 128.4, 128.0, 127.9, 127.7, 127.6, 120.5, 119.9, 98.3, 82.1, 79.9, 75.8, 75.0, 73.4, 70.0, 69.1, 64.8, 55.3, 52.5; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{39}H_{41}N_3O_8$: 680.2972; found: 680.2984.

1.2.4. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[1-(4-(2-methoxy-2-oxoacetyl)phenyl)-1*H*-1,2,3-triazol-4-yl methyl] α-D-glucopyranoside (13)

From compound **6** (82 mg, 0.40 mmol) and **8** (200 mg, 0.40 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **13** as a yellow powder (274 mg, 97%). R_f = 0.42 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ +55 (*c* 0.1 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, 2H, *J* = 8.4 Hz), 8.00 (s, 1H), 7.84 (d, 2H, *J* = 8.8 Hz), 7.35–7.22 (m, 15H), 4.99 (d, 1H, *J* = 10.8 Hz), 4.87 (d, 1H, *J* = 11.2 Hz), 4.82–4.77 (m, 3H), 4.70 (d, 1H, *J* = 12.0 Hz), 4.66 (d, 1H, *J* = 12.0 Hz), 4.62 (d, 1H, *J* = 3.6 Hz), 4.56 (d, 1H, *J* = 11.2 Hz), 4.00 (t, 1H, *J* = 9.2 Hz), 4.00 (s, 3H), 3.84 (dd, 1H, *J* = 4.0, 10.8 Hz), 3.80–3.74 (m, 2H), 3.62 (t, 1H, *J* = 9.2 Hz), 3.55 (dd, 1H, *J* = 3.6, 9.6 Hz), 3.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.1, 163.3, 146.5, 141.2, 138.8, 138.3, 138.2, 132.2, 132.0, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7 (1), 127.7 (2), 127.6, 120.6, 120.1, 98.3, 82.1, 79.9, 75.8, 74.9, 73.4, 70.0, 69.2, 64.8, 55.3, 53.1; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{40}H_{41}N_3O_9$: 708.2921, found: 708.2927.

1.2.5. 1-(4-(Methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (16)

From compound **5** (55 mg, 0.31 mmol) and **9** (150 mg, 0.26 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **16** as a white powder (196 mg, 94%). R_f = 0.33 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ −4 (*c* 0.3 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, 2H, *J* = 8.4 Hz), 8.01 (s, 1H), 7.60 (d, 2H, *J* = 8.4 Hz), 7.34–7.26 (m, 18H), 7.17–7.15 (m, 2H), 5.09 (d, 1H, *J* = 13.2 Hz), 5.03 (d, 1H, *J* = 13.2 Hz), 4.93 (d, 2H, *J* = 12.0 Hz), 4.83–4.80 (m, 3H), 4.62 (d, 1H, *J* = 12.0 Hz), 4.54 (m, 3H), 3.96 (s, 3H), 3.76–3.69 (m, 2H), 3.67–3.61 (m, 2H), 3.52 (t, 1H, *J* = 8.0 Hz), 3.50–3.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 146.2, 140.0, 138.4, 137.9, 131.2, 130.0, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 120.9, 119.7, 102.7, 84.7, 82.3, 77.8, 75.0, 74.8, 73.5,

68.9, 63.0, 52.4; ESIMS *m*/*z*: $[M+Na]^+$ calcd for $C_{45}H_{45}N_3O_8$: 778.3104; found: 778.3111.

1.2.6. 1-(4-(2-Methoxy-2-oxoacetyl)phenyl)-1*H*-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (17)

From compound **6** (80 mg, 0.40 mmol) and **9** (208 mg, 0.36 mmol), column chromatography (EtOAc/petroleum ether, 1:2) afforded **17** as a yellow powder (264 mg, 94%). R_f = 0.27 (EtOAc/petroleum ether, 1:1); $[\alpha]_D$ −3 (*c* 0.1 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, 2H, *J* = 8.8 Hz), 8.07 (s, 1H), 7.65 (d, 2H, *J* = 8.8 Hz), 7.36–7.23 (m, 18H), 7.18–7.15 (m, 2H), 5.09 (d, 1H, *J* = 13.6 Hz), 5.04 (d, 1H, *J* = 13.6 Hz), 4.94 (d, 2H, *J* = 10.8 Hz), 4.84–4.79 (m, 3H), 4.62 (d, 1H, *J* = 12.0 Hz), 4.56–4.53 (m, 3H), 4.00 (s, 3H), 3.78 (dd, 1H, *J* = 2.8, 10.8 Hz), 3.72 (dd, 1H, *J* = 4.8, 10.8 Hz), 3.67 (d, 1H, *J* = 8.8 Hz), 3.63 (d, 1H, *J* = 8.8 Hz), 3.54 (t, 1H, *J* = 8.0 Hz), 3.53–3.49 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.0, 163.3, 146.5, 138.0, 127.8, 121.0, 119.9, 102.8, 77.8, 74.9, 74.8, 73.5, 69.0, 63.1, 53.1; ESIMS *m*/*z*: $[M+Na]^+$ calcd for $C_{46}H_{45}N_3O_9$: 806.3054, found: 806.3051.

1.3. General procedure for saponification

To a solution of methyl ester in MeOH (5 mL) and water (5 mL) were added LiOH (1.5 equiv/ester). The mixture was stirred at rt for 6 h, then acidified with resin H^+, filtered, and evaporated to give the free acid and purified by column chromatography.

1.3.1. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[1-(4-carboxyphenyl)-1*H*-1,2,3-triazol-4-ylmethyl] α-D-glucopyranoside (14)

From compound **12** (282 mg, 0.42 mmol) and LiOH (35 mg, 0.84 mmol), column chromatography (CH_2Cl_2/MeOH, 10:1) afforded **14** as a yellow powder (242 mg, 88%). R_f = 0.62 (CH_2Cl_2/MeOH, 7:1); $[\alpha]_D$ +19 (*c* 0.2 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.23 (br s, 2H), 7.97 (s, 1H), 7.76 (br s, 2H), 7.34–7.22 (m, 15H), 4.98 (d, 1H, *J* = 10.8 Hz), 4.87 (d, 1H, *J* = 10.8 Hz), 4.81 (d, 1H, *J* = 10.8 Hz), 4.79 (d, 2H, *J* = 12.0 Hz), 4.68–4.62 (m, 3H), 4.56 (d, 1H, *J* = 10.8 Hz), 4.00 (t, 1H, *J* = 9.2 Hz), 3.84 (d, 1H, *J* = 10.0 Hz), 3.80–3.75 (m, 2H), 3.63 (t, 1H, *J* = 9.2 Hz), 3.56 (dd, 1H, *J* = 2.4, 9.2 Hz), 3.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9, 146.2, 140.4, 138.7, 138.2, 138.1, 131.9, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 120.5, 119.9, 98.3, 82.1, 79.7, 75.8, 75.0, 73.4, 70.0, 69.1 64.8, 63.7, 55.3; ESIMS *m*/*z*: $[M-H]^-$ calcd for $C_{38}H_{39}N_3O_8$: 664.2659, found: 664.2651.

1.3.2. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[1-(4-(carboxycarbonyl)phenyl)-1*H*-1,2,3-triazol-4-ylmethyl] α-D-glucopyranoside (15)

From compound **13** (170 mg, 0.24 mmol) and LiOH (10 mg, 0.24 mmol), column chromatography (CH_2Cl_2/MeOH, 10:1) afforded **15** as a white powder (87 mg, 52%). R_f = 0.32 (CH_2Cl_2/MeOH, 7:1); $[\alpha]_D$ +27 (*c* 0.1 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (d, 2H, *J* = 7.2 Hz), 7.97 (s, 1H), 7.80 (d, 2H, *J* = 7.6 Hz), 7.32–7.21 (m, 15H), 4.97 (d, 1H, *J* = 10.8 Hz), 4.86 (d, 1H, *J* = 11.2 Hz), 4.82–4.76 (m, 4H), 4.63–4.60 (m, 2H), 4.55 (d, 2H, *J* = 10.8 Hz), 3.99 (t, 1H, *J* = 9.2 Hz), 3.75 (t, 2H, *J* = 10.8 Hz), 3.60 (t, 1H, *J* = 9.2 Hz), 3.53 (dd, 1H, *J* = 3.2, 9.6 Hz), 3.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.6, 171.2, 138.8, 138.2, 138.1, 131.3, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 120.5, 98.3, 82.0, 79.9, 75.7, 75.0, 73.3, 70.0, 69.2, 60.4, 55.3; ESIMS *m*/*z*: $[M-H]^-$ calcd for $C_{39}H_{39}N_3O_9$: 692.2608; found: 692.2605. Analytical HPLC: t_R = 5.7 min (solvent: MeOH, 0.6 mL/min over 32 min, purity 97%).

1.3.3. 1-(4-Carboxyphenyl)-1*H*-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (18)

From compound **16** (55 mg, 0.07 mmol) and LiOH (6 mg, 0.14 mmol), column chromatography (CH_2Cl_2/MeOH, 10:1) afforded **18** as a white powder (48 mg, 88%). R_f = 0.73 (CH_2Cl_2/MeOH, 7:1); $[\alpha]_D$ −16 (*c* 0.1 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 2H, *J* = 8.8 Hz), 7.96 (s, 1H), 7.55 (d, 2H, *J* = 8.8 Hz), 7.26–7.17 (m, 18H), 7.10–7.07 (m, 2H), 5.04 (d, 1H, *J* = 13.6 Hz), 4.98 (d, 1H, *J* = 13.6 Hz), 4.88 (d, 1H, *J* = 11.2 Hz), 4.86 (d, 1H, *J* = 11.2 Hz), 4.76–4.72 (m, 3H), 4.55 (d, 1H, *J* = 12.0 Hz), 4.47 (d, 1H, *J* = 12.0 Hz), 4.50–4.44 (m, 2H), 3.70 (d, 1H, *J* = 9.6 Hz), 3.66 (d, 1H, *J* = 8.8 Hz), 3.63–3.56 (m, 2H), 3.45 (t, 1H, *J* = 8.0 Hz), 3.45–3.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 145.2, 139.4, 137.4, 137.3, 136.9, 136.8, 130.8, 128.3, 127.5, 127.4, 127.3, 126.9, 126.8, 126.7, 126.6, 120.0, 118.7, 101.6, 83.7, 81.3, 76.8, 74.7, 74.0, 73.9, 73.7, 72.5, 67.9, 61.9; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{44}H_{43}N_3O_8$: 742.3128, found: 742.3126.

1.3.4. 1-(4-(Carboxycarbonyl)phenyl)-1*H*-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (19)

From compound **17** (96 mg, 0.12 mmol) and LiOH (5 mg, 0.12 mmol), column chromatography (CH_2Cl_2/MeOH, 10:1) afforded **19** as a white powder (47 mg, 50%). R_f = 0.21 (CH_2Cl_2/MeOH, 7:1); $[\alpha]_D$ −16 (*c* 0.1 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, 2H, *J* = 8.4 Hz), 8.03 (s, 1H), 7.62 (d, 2H, *J* = 8.4 Hz), 7.34–7.26 (m, 18H), 7.17–7.15 (m, 2H), 5.10 (d, 1H, *J* = 13.2 Hz), 5.04 (d, 1H, *J* = 13.6 Hz), 4.94 (dd, 2H, *J* = 2.4, 11.2 Hz), 4.84–4.79 (m, 3H), 4.62 (d, 1H, *J* = 12.0 Hz), 4.56–4.52 (m, 3H), 3.77 (dd, 1H, *J* = 1.2, 10.8 Hz), 3.71 (dd, 1H, *J* = 4.8, 10.8 Hz), 3.67–3.62 (m, 3H), 3.53 (t, 1H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3, 164.7, 146.3, 142.8, 138.5, 138.4, 138.1, 138.0, 128.4, 127.9, 114.1, 102.8, 84.4, 81.6, 77.8, 75.6, 75.0, 73.5, 68.9, 62.7; ESIMS *m*/*z*: $[M+H]^+$ calcd for $C_{45}H_{43}N_3O_9$: 770.3078, found: 770.3077. Analytical HPLC: t_R = 4.1 min (solvent: MeOH, 0.6 mL/min over 14 min, purity 99%).

1.4. Inhibitory assay

Recombinant human PTP1B catalytic domain was expressed and purified according to procedures described previously.[20a] Enzymatic activity of PTP1B was determined at 30 °C by monitoring the hydrolysis of *p*NPP. Dephosphorylation of *p*NPP generates product *p*NP, which can be monitored at 405 nm. In a typical 100 μL assay, mixtures containing 50 mM MOPS, pH 6.5, 2 mM *p*NPP, and recombinant enzymes, PTP1B activities were continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. For calculating IC_{50}, inhibition assays were performed with 30 nM recombinant enzyme, 2 mM *p*NPP in 50 mM MOPS at pH 6.5, and the inhibitors diluted around the estimated IC_{50} values. IC_{50} was calculated from the nonlinear curve fitting of percent inhibition (inhibition (%)) versus inhibitor concentration [*I*] by using the following equation: inhibition (%) = $100/\{1 + (IC_{50}/[I])k\}$, where *k* is the Hill coefficient.

To study the inhibition selectivity on other PTP family members, human TCPTP, SHP-1, SHP-2, and LARD1 were prepared and assays were performed according to procedures described previously.[20b]

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