

Monosaccharide as a Central Scaffold Toward the Construction of Salicylate-Based Bidentate PTP1B Inhibitors *via* Click Chemistry

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The discovery of carbohydrate-based bioactive compounds has recently received considerable interest in the drug development. This paper stresses on the application of 1-methoxy-*O*-glucoside as the central scaffold, whereas salicylic pharmacophores were introduced with diverse spatial orientations probing into the structural preference of an enzymatic target, i.e. protein tyrosine phosphatase 1B (PTP1B). By employing regioselective protection and deprotection strategy, 2,6-, 3,4-, 4,6- and 2,3-di-*O*-propynyl 1-methoxy-*O*-glucosides were previously synthesized and then coupled with azido salicylate *via* click chemistry in forming the desired bidentate salicylic glucosides with high yields. The inhibitory assay of the obtained triazolyl derivatives leads to the identification of the 2,3-disubstituted salicylic 1-methoxy-*O*-glucoside as the structurally privileged PTP1B inhibitor among this bidentate compound series with micromole-ranged IC₅₀ value and reasonable selectivity over other homologous PTPs tested. In addition, docking simulation was conducted to propose a plausible binding mode of this authorized inhibitor with PTP1B. This research might furnish new insight toward the construction of structurally different bioactive compounds based on the monosaccharide scaffold.

Key Words : Monosaccharide, Structural preference, Click chemistry, Protein tyrosine phosphatase 1B (PTP1B) inhibitor

Introduction

Carbohydrates universally exist in nature governing a multitude of crucial biological and pathological events on cellular level. The synthesis of carbohydrate-based bioactive compounds has currently absorbed considerably many efforts as regards its natural abundance, structural diversity as well as high biocompatibility.¹ For example, the two drugs namely, zanamivir and oseltamivir, approved to date in combating both influenza A and B were derived from a pyranoglycosyl scaffold *via* computational study.²

Indeed, the carbohydrate structure that contains multiple stereo-centers and reactive hydroxyl groups may favorably allow modification toward the construction of numerous structurally diverse glycomimetics. For instance, a 1-methoxy-*O*-glucoside has a primary hydroxyl group on C6 and three secondary hydroxyl groups respectively on C2, C3 and C4 with different reactivities and spatial orientations on its side chain, shown in Figure 1. Such a molecule would be desirable as a central scaffold with the introduced pharmacophores onto its readily modifiable side-chain positions with diverse spatial orientations for investigating the structural preference of drug targets. Moreover, the high biocompatibility of carbohydrate itself might also be considered as an essential merit for enhancing the bioavailability of the fabricated sugar-contained bioactive compounds.

Recent studies indicate that enhanced insulin sensitivity and ameliorated glycemic control, and resistance to diet-induced obesity were observed on protein tyrosine phosphatase 1B (PTP1B)-null mice, suggesting that the inhibition of PTP1B may represent a promising strategy to combat both type 2 diabetes and obesity.^{3,4} As a consequence, a large number of PTP1B inhibitors have been disclosed in the past decade, which mainly fell into competitive nonhydrolyzable phosphotyrosine (pTyr) surrogates.⁵ Meanwhile, noncompetitive inhibitors identified such as the aryl diketoacid derivatives that target the open conformation of WPD loop also enabled an alternative way for PTP1B inhibition.⁶

As shown in Figure 1, we have recently synthesized series of triazolyl salicylate-hydroxyl *O*-glycoside conjugates *via* Cu(I)-catalyzed azide-alkyne 1,3-cycloaddition (click reaction).⁷ The sequential inhibitory assay identified these compounds as weak PTP1B inhibitors, (IC₅₀ > 50 μM) possibly due to the lack of hydrophobic moieties for generating nonpolar interactions with the proper enzymatic surface.^{6,7} As a result, we seek to continuously progress our study toward the preparation of new PTP1B inhibitors based on triazole-linked salicylic *O*-glycosides which simultaneously comprise aryl groups *via* the versatile click chemistry.⁸⁻¹⁰

The strategy for preparing 'bidentate' inhibitors simultaneously access the catalytic site and a neighboring pocket such as the second phosphotyrosine site¹¹ or the YRD motif has

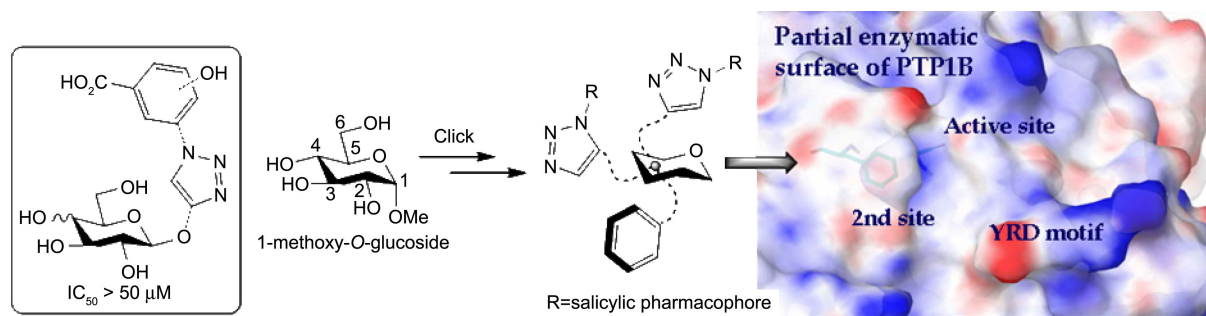


Figure 1. Depict the construction of bidentate PTP1B inhibitors *via* click reaction based on a glucosyl scaffold.

proven efficacious to achieve potent and selective PTP1B inhibition.⁵ We were consequently prompted to prepare bidentate inhibitors by introducing two salicylic pharmacophores^{12,13} onto a 1-methoxy-*O*-glucosyl scaffold. The benzyl 2,6-, 3,4-, 4,6- and 2,3-disubstituted di-2-*O*-propynyl glucosides concomitantly containing two benzyl groups and two activated alkynyl functionalities were prepared to couple with the azido salicylate, forming the desired bidentate salicylic glucosides. The inhibitory activity of the synthesized products on PTP1B and other homologous PTPs was assessed and discussed. Furthermore, the plausible binding mode of the most bioactive product with PTP1B was proposed *via* docking simulation.

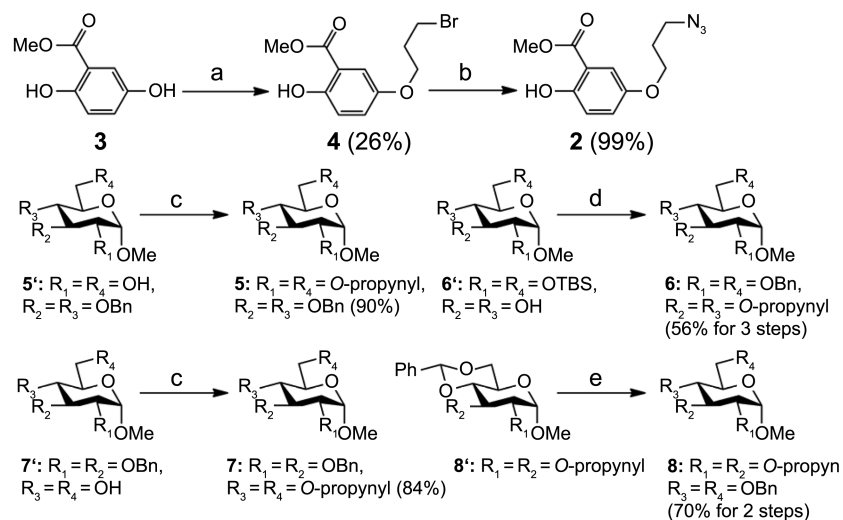
Result and Discussion

Synthesis. For the synthesis of the desired products, methyl 5-(3-azidopropoxy)-2-hydroxybenzoate **2** and di-*O*-propynyl 1-methoxy-*O*-glucosides **5-8** were first prepared. As shown in Scheme 1, the salicylic azide **2** was readily synthesized by the etherification of commercially available methyl 2,5-dihydroxybenzoate **3** with 1,3-dibromopropane in the presence of K_2CO_3 followed by azide substitution. A 3C-alkyl linkage was employed to build this salicylic azide

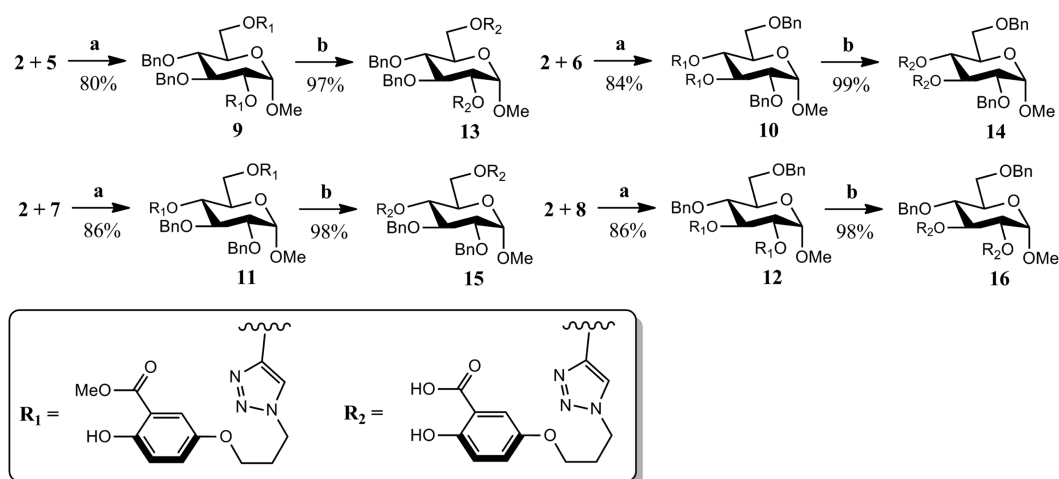
for allowing adequate structural flexibilities toward the extension of the pharmacophores to appropriate docking sites.

The 2,6-di-*O*-propynyl glucoside **5** was synthesized by introducing propargyl bromide onto the 2,6-positions of the known 3,4-di-*O*-benzyl glucoside **5'**¹³ in the presence of NaH. Similarly, the synthesis of 4,6-di-*O*-propynyl glucoside **7** was realized by the *O*-propargylation of methyl 2,3-di-*O*-benzyl- α -D-glucopyranoside **7'**.¹⁴ The benzylated 3,4-di-*O*-propynyl glucoside **6** was then prepared from the selectively silylated **6'**¹⁵ *via* a 3-steps sequence which includes *O*-propargylation, AcCl catalyzed desilylation and *O*-benzylation. The preparation of 2,3-di-*O*-propynyl glucoside **8** was eventually achieved by *O*-propargylation of the known benzylidene derivative **8'**¹⁶ which was then converted to the benzyl derivative **8** by the removal of benzylidene group with TFA and the following *O*-benzylation as shown in Scheme 1.

The click reaction between the azide **2** and sugar alkynes **5-8** was then performed. As shown in Scheme 2, in the presence of 4 equiv. Na ascorbate and 2 equiv. $CuSO_4 \cdot 5H_2O$, the *Huisgen* [3 + 2] cycloaddition proceeded smoothly in a solvent mixture of water/ CH_2Cl_2 (1:1, V/V) within 12 h, affording the desired bis-triazolyl glucoside **9, 10, 11** and **12**



Scheme 1. Synthesis of the azide and sugar alkynes: Reagents and conditions: (a) 1,3-dibromopropane, K_2CO_3 , CH_3CN , reflux. (b) NaN_3 , CH_3CN , reflux. (c) propargyl bromide, NaH, DMF, 0 °C-r.t. (d) (i) propargyl bromide, NaH, DMF, 0 °C-r.t.; (ii) AcCl, MeOH, r.t.; (iii) BnBr, NaH, DMF, 0 °C-r.t. (e) (i) TFA, MeOH, r.t.; (ii) BnBr, NaH, DMF, 0 °C-r.t.



Scheme 2. Synthesis of the desired triazole-linked glucosyl salicylates: Reagents and conditions: (a) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Na ascorbate, CH_2Cl_2 , water, r.t. (b) $\text{LiOH} \cdot \text{H}_2\text{O}$, water, THF, r.t.

in good yields of 80, 84, 86 and 86%, respectively. The following saponification with $\text{LiOH} \cdot \text{H}_2\text{O}$ gave the free acids **13–16** in almost quantitative yields (97–99%).

Inhibitory Assay. The inhibitory activity of the prepared triazole-linked glucosyl salicylic derivatives **13**, **14**, **15** and **16** on a panel of PTPs including PTP1B, TCPTP, LAR, SHP-1 and SHP-2 was tested at the concentration of $100 \mu\text{g}/\text{mL}$ via our previously established methods.¹⁷ As listed in Table 1, the 3,4-disubstituted glucoside **14** was first found to be inactive on all PTPs tested. The 2,6- and 4,6-disubstituted glucosides **13** and **15** then exhibited similar inhibitory activities on PTP1B with IC_{50} values equal to 30 and $25 \mu\text{M}$, respectively. Additionally, the 2,6-disubstituted compound **13** simultaneously displayed moderate inhibitory activity on SHP-1 ($\text{IC}_{50} = 59 \mu\text{M}$) and SHP-2 ($\text{IC}_{50} = 39 \mu\text{M}$) whereas both compounds **13** and **15** were not inhibitors of TCPTP, LAR and SHP-1 ($\text{IC}_{50} > 100 \mu\text{M}$). Eventually, to our delight, the 2,3-disubstituted glucoside **16** displayed further enhanced inhibitory activity on PTP1B compared to other bidentate salicylic glucosides assessed with an IC_{50} value = $7.7 \mu\text{M}$. This inhibitor also exhibited good selectivity over TCPTP (7-fold) and excellent selectivity over LAR (> 13-fold), SHP-1 (> 13-fold) and SHP-2 (> 13-fold).

Obviously, the four structurally diverse bidentate salicylic glucosides synthesized exhibited different PTP1B inhibitory potency as depicted above. The 3,4-disubstitution on glucosyl

scaffold was proved unsuitable for achieving PTP1B inhibition whereas, both 2,6- and 4,6-disubstituted glucosides exhibited moderate inhibitory activity toward PTP1B. Notably, the 2,3-disubstitution pattern finally leads to an at least 3-fold increased inhibitory activity of compound **16** compared to that of the rest (Table 1). These results unambiguously supported the notion of proposing the monosaccharide as a central scaffold for probing the structural preference of PTP1B.

Furthermore, the inhibitory activity of compound **16** ($\text{IC}_{50} = 7.7 \mu\text{M}$) is almost 7-fold improved compared to that of our previously synthesized salicylic glycosides ($\text{IC}_{50} > 50 \mu\text{M}$, Fig. 1) with exposed hydroxyl groups, demonstrating the existence of the benzyl groups on the glucosyl scaffold necessary for enhancing PTP1B binding affinity.

Docking Simulation. We then tended to propose a plausible binding mode of the inhibitor **16** with PTP1B by starting with a crystal structure in complex with a reference ligand (PDB code: 3EB1, resolution: 2.40 \AA). Water was removed from the original structure, and the remaining protein was prepared using the Protein preparation wizard

Table 1. Inhibitory activity of compounds **13**, **14**, **15** and **16** on PTPs

Compd.	$\text{IC}_{50} - \mu\text{M}^a$				
	PTP1B	TCPTP	LAR	SHP-1	SHP-2
13	30.0	> 100	> 100	58.8	38.8
14	> 100	> 100	> 100	> 100	> 100
15	25.0	> 100	> 100	> 100	> 100
16	7.7	54.3	> 100	> 100	> 100

^aValues are means of three experiments.

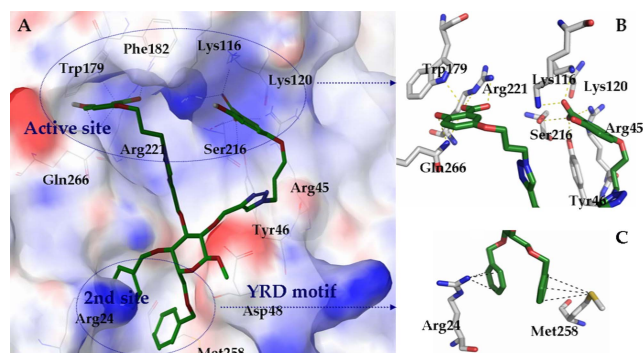


Figure 2. PTP1B in complex with compound **16**. The compound is shown as green stick, and residues of the active site in PTP1B shown as light grey line. Nitrogen atoms are in blue and oxygen atoms in red. Hydrogen bonds are shown as yellow dash line and nonpolar interactions shown in black.

(Schrödinger, LLC, New York, NY, 2005). Then compound **16** was docked into 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.

As illustrated in Figure 2A, densely functionalized hydrogen-bonding network was generated between the two salicylic precursors of the molecule and the active site region while additional nonpolar interactions were made between the benzyl groups on the sugar ring with the second site of PTP1B, synergistically fixing compound **16** in this plausible location. As shown more lucidly in Figure 2B, the carboxylic acid and hydroxyl groups of the glucosyl C3-attached salicylic precursor made six hydrogen bonds with Arg221 and Trp179 in the WPD loop and Gln266. Interestingly, the other salicylic precursor attached on C4 position of the glucosyl scaffold also generated several hydrogen-bonding interactions with the amino acid residues on peripheric surface of the first site including Ser216, Lys116, Lys120, Arg45, and Tyr46. In addition, as shown in Figure 2C, the distal benzene groups on C4 and C6 position of the glucoside made cation- π interaction with the guanidinium group of Arg24 and hydrophobic interaction with Met258, which may account for the enhancement of the inhibitory activity of this compound compared to our previously prepared glycosides with exposed side-chain hydroxyl groups.

Conclusion

In summary, we have efficiently prepared in this study a series of bidentate triazolyl glucosides bearing two salicylic precursors on 2,6-, 3,4-, 4,6- and 2,3-position of the central glucosyl scaffold *via* click chemistry. The following inhibitory assay identified the 2,3-disubstituted glucoside **16** as the most potent inhibitor with at least 3-fold enhanced inhibitory activity compared to the rest, which also possesses favorable selectivity over a panel of homologous PTPs. Docking simulation was then conducted to propose a plausible binding mode of this inhibitor with PTP1B and the crucial role of the benzyl groups on the glycosyl moiety for enhancing the binding affinity. Our work presented here would thus provide new insight toward the utilization of monosaccharide as the central scaffold whereas diverse pharmacophores could be flexibly attached for the probing of the structural preference of drug targets.

Experimental Section

General. Chemicals and solvents are of analytical grade and are used without further purifications. All reactions and chromatographic fractions were monitored by thin-layer chromatography (TLC) using precoated silica gel plates (Yantai Marine Chemical Co. Ltd., China), and spots were visualized either under UV light (254 nm) or by sulfuric acid spray. Silica gel (200-300 mesh, Yantai Chemical Industry Research Institute, China) was used for column chromatography.

¹H and ¹³C NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethylsilane (TMS) as the internal standard chemical shifts in ppm. Coupling constants (*J*) are in hertz. Optical rotations were measured using a Perkin-Elmer 241 polarimeter at room temperature and a 10-cm 1-mL cell. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV).

Synthesis of Methyl 5-(3-bromopropoxy)-2-hydroxybenzoate (4). **3** (1800 mg, 10.7 mmol) and 1,3-dibromopropane (1.3 mL, 12.9 mmol) were dissolved in anhydrous acetonitrile (30 mL) and heated to 100 °C. Potassium carbonate was added in 3 portions and the resulting mixture was refluxed for 3 hours. The solvent was then removed in vacuo and the residue was taken into ethyl acetate (60 mL) followed by extraction with 1 N HCl and brine and dried with anhydrous MgSO₄. Upon concentration in vacuo, the crude product was further purified by column chromatography (Pet/EtOAc, 11:1) to furnish **4** (795 mg, 25.7%).

Synthesis of Methyl 5-(3-azidopropoxy)-2-hydroxybenzoate (2). To a solution of compound **4** (795 mg, 2.75 mmol) in anhydrous acetonitrile (30 mL) was added sodium azide (1.07 g, 16.50 mmol) and this mixture was stirred at reflux for 3 hours. The solvent was then removed under reduced pressure and the residue was taken into dichloromethane and washed with water and brine. The combined organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo to afford a pale yellow product **2** (683 mg, 98.9%). ¹H NMR (400 MHz, CDCl₃): δ = 10.36 (s, 1H), 7.29 (d, *J* = 3.1 Hz, 1H), 7.07 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 4.00 (t, *J* = 5.9 Hz, 2H), 3.94 (s, 3H), 3.51 (t, *J* = 6.6 Hz, 2H), 2.08-1.98 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ = 170.4, 156.4, 151.2, 124.6, 118.7, 113.0, 112.0, 65.4, 52.4, 48.3, 28.9.

General Procedure for the *O*-Propargylation. The alcohol was dissolved in anhydrous DMF and NaH (3-5 equiv) was added to this mixture at 0 °C, stirring for 20 min. Then propargyl bromide (3-5 equiv) was slowly added at 0 °C and the mixture was warmed to r.t., stirring for another 12 h. The solvent was then removed and the resulting residue was diluted with EtOAc, washed successively with water and brine. The combined organic layers were dried over MgSO₄, filtered and concentrated to give a crude product which was purified by column chromatography, yielding product **5** and **7**.

Methyl 3,4-di-*O*-benzyl-2,6-di-*O*-propargyl- α -D-glucopyranoside (5): Yellow-brown syrup (90.5%); $[\alpha]_D^{25} = +70.0$ (*c* 0.1, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.41-7.26 (m, 10H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.78 (d, *J* = 12.4 Hz, 1H), 4.66 (t, *J* = 12.4 Hz, 2H), 4.58-4.10 (m, 5H), 3.88 (t, *J* = 9.6 Hz, 2H), 3.83 (dd, *J* = 3.6, 10.4 Hz, 1H), 3.73-3.70 (m, 1H), 3.65 (dd, *J* = 2.0, 10.4 Hz, 1H), 3.56 (t, *J* = 9.6 Hz, 1H), 3.49 (dd, *J* = 3.6, 9.6 Hz, 1H), 3.34 (s, 3H), 2.45 (t, *J* = 2.4 Hz, 1H), 2.38 (t, *J* = 2.4 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 138.4, 138.1, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 98.1, 81.7, 80.4, 79.6, 79.5, 76.9, 75.2, 75.1, 74.3, 73.4, 69.7, 68.0, 60.5, 58.6, 55.2; HR(ESI)MS: calcd for

$C_{27}H_{30}O_6+Na$ 473.1940, found 473.2929.

Methyl 2,3-di-*O*-benzyl-4,6-di-*O*-propargyl- α -D-glucopyranoside (7): Yellow-brown syrup (84.0%); $[\alpha]_D = +96.8$ (*c* 1.2, CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.27 (m, 10H), 4.96 (d, 1H, *J* = 10.8 Hz), 4.82 (d, 1H, *J* = 10.8 Hz), 4.78 (d, 1H, *J* = 12.0 Hz), 4.64 (d, 1H, *J* = 12.0 Hz), 4.59 (d, 1H, *J* = 3.2 Hz), 4.46-4.13 (m, 2H), 4.26-3.96 (m, 2H), 3.94 (t, 1H, *J* = 9.2 Hz), 3.83 (dd, 1H, *J* = 4.0, 10.4 Hz), 3.75-3.71 (m, 2H), 3.51-3.47 (m, 2H), 3.38 (s, 3H), 2.45-2.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.0, 128.5, 128.4, 128.2, 128.1, 128.0, 127.0, 98.2, 81.8, 80.1, 79.6, 79.3, 77.0, 75.8, 75.0, 74.3, 73.4, 69.5, 68.1, 60.1, 58.6, 55.3; HR(ESI)MS: calcd for $C_{27}H_{30}O_6+K$ 489.1679, found 489.1682.

Synthesis of Methyl 2,6-di-*O*-benzyl-3,4-di-*O*-propargyl- α -D-glucopyranoside (6). To a solution of **6'** in anhydrous DMF at 0 °C, NaH (5 equiv.) was added, stirring for 20 min. Propargyl bromide (5 equiv.) was then slowly added at 0 °C, stirring for another 12 h at r.t. After completion of the reaction, the solvent was removed in vacuum and the resulting residue was diluted with EtOAc, washed successively with water and brine, dried over $MgSO_4$, filtered and concentrated to give crude products. The disilylated glycosyl alkyne was directly dissolved in MeOH, followed by the addition of AcCl (0.5 equiv.), stirring for 6 h. The mixture was then concentrated, extracted with CH_2Cl_2 , washed with sat. $NaHCO_3$ and brine. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude product which was dissolved directly in anhydrous DMF. NaH (5 equiv.) was added at 0 °C, stirring for 20 min. Then BnBr (5 equiv.) was added dropwise at 0 °C, stirring for another 12 h at r.t. Upon completion of the reaction, solvent was removed and the resulting residue was diluted with EtOAc, washed with water and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude product which was eventually purified by column chromatography. Yellow-brown syrup (56% for 3 steps); $[\alpha]_D = +76.4$ (*c* 0.1, CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.27 (m, 10H), 4.94 (d, *J* = 3.6 Hz, 1H), 4.88 (d, *J* = 11.2 Hz, 1H), 4.79 (d, *J* = 10.8 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.41-4.30 (m, 3H), 4.23 (dd, *J* = 2.0, 13.2 Hz, 1H), 3.92 (t, *J* = 9.2 Hz, 1H), 3.76-3.72 (m, 3H), 3.66 (dd, *J* = 3.6 Hz, 6.0 Hz, 1H), 3.56 (t, *J* = 9.2 Hz, 1H), 3.44 (s, 3H), 2.44 (brs, 1H), 2.40 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.0, 128.5, 128.4, 128.2, 128.2, 128.4, 128.0, 127.8, 127.7, 98.1, 81.8, 80.0, 79.9, 79.4, 77.4, 75.7, 75.0, 74.4, 73.5, 69.8, 68.7, 60.0, 58.8, 55.2; HR(ESI)MS calcd for $C_{27}H_{30}O_6+K$ 489.1679, found 489.1686.

Synthesis of Methyl 4,6-di-*O*-benzyl-2,3-di-*O*-propargyl- α -D-glucopyranoside (8). To a solution of **8'** (306 mg, 0.82 mmol) in CH_2Cl_2 , TFA (0.45 mL, 5.74 mmol) was added. After stirring for 5 h at r.t., the mixture was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 . The combined organic layers were then washed with brine, dried over $MgSO_4$ and concentrated in vacuum to give the crude product which was directly dissolved in anhydrous DMF at

0 °C. NaH (5 equiv) was then added, stirring for 20 min, followed by careful addition of BnBr (5 equiv) at 0 °C. The reaction mixture was warmed to r.t. and stirred for another 12 h. Solvent was then removed and the resulting residue was diluted with EtOAc, washed successively with water and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude product which was purified by column chromatography. Yellow-brown syrup (70.4% for 2 steps); $[\alpha]_D = +96.8$ (*c* 1.2, CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 7.36-7.23 (m, 10H), 4.94 (d, *J* = 3.2 Hz, 1H), 4.91 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.54-4.38 (m, 6H), 3.87 (t, *J* = 9.2 Hz, 1H), 3.77-3.41 (m, 5H), 3.42 (s, 3H), 2.47 (t, *J* = 2.0 Hz, 1H), 2.44 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 137.9, 128.4, 128.3, 128.2, 128.0, 127.8, 98.0, 81.6, 80.2, 79.8, 79.3, 77.3, 75.2, 75.0, 74.3, 73.5, 70.0, 68.3, 60.4, 58.7, 55.1; HR(ESI)MS calcd for $C_{27}H_{30}O_6+Na$ 473.1940, found 473.1941.

General Procedure for CuAAC. To a solution of the azide **2** (2.4 equiv.) and di-*O*-propynyl glucoside (1 equiv.) in a solvent mixture of water/ CH_2Cl_2 (1:1, V/V), Na ascorbate (4 equiv.) and $CuSO_4 \cdot 5H_2O$ (2 equiv.) were added at r.t. The resulting mixture was stirred for 8-12 h and was then diluted with CH_2Cl_2 , washed with water and saturated EDTA, respectively. The combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by column chromatography to provide compounds **9**, **10**, **11** and **12**.

3,4-Di-*O*-benzyl-2,6-di-*O*-{1-[3-(4-hydroxy-3-(methoxycarbonyl)phenoxy)propyl]-1*H*-1,2,3-triazole-4-yl}- α -D-glucopyranoside methyl ether (9): Colorless syrup (80.0%). $[\alpha]_D -9.2$ (*c* 1.0, CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, 1H), 10.36 (s, 1H), 7.47 (s, 1H), 7.36-7.30 (m, 6H), 7.26-7.21 (m, 5H), 7.05-7.01 (m, 2H), 6.91 (d, *J* = 6.1 Hz, 1H), 6.89 (d, *J* = 6.1 Hz, 1H), 5.06 (d, *J* = 12.1 Hz, 1H), 4.96 (d, *J* = 12.1 Hz, 1H), 4.78 (d, *J* = 11.0 Hz, 1H), 4.74 (d, *J* = 8.4 Hz, 1H), 4.70 (d, *J* = 8.8 Hz, 1H), 4.65-4.59 (m, 3H), 4.48-4.44 (m, 3H), 4.40 (t, *J* = 6.7 Hz, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.89-3.81 (m, 4H), 3.77 (dd, *J* = 3.4 Hz, 7.0 Hz, 1H), 3.69-3.67 (m, 2H), 3.55-3.47 (m, 3H), 3.34 (s, 3H), 2.32-2.21 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.1, 156.4, 156.3, 150.8, 150.7, 145.5, 144.8, 138.3, 138.1, 128.5, 128.4, 128.1, 127.9, 127.7, 127.6, 124.4, 124.3, 122.9, 122.8, 118.7, 118.6, 112.9, 111.9, 98.1, 81.8, 79.7, 77.3, 74.8, 73.2, 69.9, 68.7, 66.6, 64.9, 64.8, 64.7, 55.2, 52.3, 47.0, 46.8, 29.9, 29.8; HR(ESI)MS calcd. for $C_{49}H_{56}N_6O_{14}+H$ 953.3933, found 953.3928.

2,6-Di-*O*-benzyl-3,4-di-*O*-{1-[3-(4-hydroxy-3-(methoxycarbonyl)phenoxy)propyl]-1*H*-1,2,3-triazole-4-yl}- α -D-glucopyranoside methyl ether (10): Colorless syrup (83.6%). $[\alpha]_D -44.4$ (*c* 1.0, CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, 2H), 7.39 (s, 1H), 7.35-7.30 (m, 7H), 7.28 (d, *J* = 1.8 Hz, 1H), 7.25-7.23 (m, 2H), 7.15 (s, 1H), 7.06 (dd, *J* = 1.0 Hz, 3.1 Hz, 1H), 7.03 (dd, *J* = 1.0 Hz, 3.1 Hz, 1H), 6.92 (d, *J* = 0.9 Hz, 1H), 6.90 (d, *J* = 0.9 Hz, 1H), 4.91 (d, *J* = 11.7 Hz, 1H), 4.89-4.78 (m, 5H), 4.66 (d, *J* = 11.7 Hz, 1H), 4.62 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 4.47-4.40 (m,

4H), 3.93 (s, 3H), 3.92 (s, 3H), 3.90-3.86 (m, 4H), 3.75-3.59 (m, 5H), 3.38 (s, 3H), 2.32-2.26 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 170.1, 156.4, 150.8, 145.0, 138.1, 128.4, 128.3, 127.8, 127.7, 127.6, 127.5, 124.4, 123.0, 122.8, 118.7, 112.9, 112.8, 111.9, 97.7, 81.8, 79.8, 77.7, 75.4, 73.5, 70.1, 68.5, 66.1, 64.9, 64.8, 64.6, 55.1, 52.4, 47.0, 46.8, 29.9; HR(ESI)MS calcd. for $\text{C}_{49}\text{H}_{56}\text{N}_6\text{O}_{14}+\text{H}$ 953.3933, found 953.3938.

2,3-Di-*O*-benzyl-4,6-di-*O*-{1-[3-(4-hydroxy-3-(methoxycarbonyl)phenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (11): Colorless syrup (yield 85.5%). $[\alpha]_{\text{D}} -5.8$ (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 10.37 (s, 2H), 7.65 (s, 1H), 7.38 (s, 1H), 7.37-7.28 (m, 8H), 7.25 (t, *J* = 3.1 Hz, 2H), 7.07-7.03 (m, 2H), 6.91 (d, *J* = 1.9 Hz, 1H), 6.91 (d, *J* = 1.9 Hz, 1H), 4.96 (d, *J* = 11.0 Hz, 1H), 4.90 (d, *J* = 11.8 Hz, 1H), 4.79 (d, *J* = 8.9 Hz, 1H), 4.74 (d, *J* = 3.9 Hz, 1H), 4.71 (t, *J* = 4.5 Hz, 1H), 4.67-4.57 (m, 4H), 4.54 (t, *J* = 6.8 Hz, 6.6 Hz, 2H), 4.47 (t, *J* = 6.8 Hz, 2H), 3.93 (s, 6H), 3.91-3.89 (m, 4H), 3.78-3.75 (m, 1H), 3.70-3.64 (m, 2H), 3.57-3.54 (m, 1H), 3.50 (dd, *J* = 3.6, 6.1 Hz, 1H), 3.35 (s, 3H), 2.37-2.28 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 170.1, 156.3, 150.8, 145.1, 144.7, 138.9, 138.1, 128.5, 128.4, 128.1, 127.9, 127.6, 124.4, 123.2, 123.0, 118.7, 112.9, 112.8, 111.9, 98.2, 81.8, 79.8, 77.5, 75.6, 73.3, 69.8, 68.6, 66.1, 64.9, 64.8, 55.2, 53.5, 52.4, 47.1, 46.9, 29.9; HR(ESI)MS calcd. for $\text{C}_{49}\text{H}_{56}\text{N}_6\text{O}_{14}+\text{H}$ 953.3933, found 953.3932.

4,6-Di-*O*-benzyl-2,3-di-*O*-{1-[3-(4-hydroxy-3-(methoxycarbonyl)phenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (12): Colorless syrup (86.4%). $[\alpha]_{\text{D}} -52.2$ (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 10.37 (s, 1H), 10.36 (s, 1H), 7.33-7.24 (m, 10H), 7.18 (d, *J* = 6.9 Hz, 2H), 7.05 (t, *J* = 8.6 Hz, 8.8 Hz, 2H), 6.90 (d, *J* = 2.5 Hz, 2H), 6.87 (d, *J* = 2.6 Hz, 2H), 5.01-4.76 (m, 6H), 4.63 (d, *J* = 12.1 Hz, 2H), 4.51-4.46 (m, 4H), 4.12 (d, *J* = 7.2 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.89-3.85 (m, 3H), 3.76-3.41 (m, 7H), 3.39 (s, 3H), 2.38-2.25 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 170.2, 170.1, 156.3, 150.9, 150.8, 138.3, 137.9, 128.4, 127.9, 127.7, 127.6, 124.5, 124.4, 118.6, 112.9, 112.8, 111.9, 97.7, 81.7, 79.8, 77.6, 74.9, 73.5, 70.1, 68.4, 65.0, 64.8, 55.1, 52.4, 47.1, 47.0, 29.7; HR(ESI)MS calcd. for $\text{C}_{49}\text{H}_{56}\text{N}_6\text{O}_{14}+\text{H}$ 953.3933, found 953.3930.

General Procedure for Saponification. To a solution of methyl ester in THF/water (4:1, v/v) was added LiOH·H₂O (4.0 equiv./ester), stirring at r.t. overnight. Then the mixture was acidified to pH < 4 with 1N HCl. The solvent was removed in vacuum and the crude product was purified by column chromatography to furnish compounds **13**, **14**, **15**, **16**.

3,4-Di-*O*-benzyl-2,6-di-*O*-{1-[3-(4-hydroxy-3-carboxylphenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (13): Colorless syrup (96.6%). $[\alpha]_{\text{D}} +2.2$ (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CDCl_3) δ 8.10 (s, 1H), 7.97 (s, 1H), 7.37-7.25 (m, 10H), 7.20 (d, *J* = 6.8 Hz, 2H), 6.99 (dd, *J* = 2.8, 6.0 Hz, 2H), 6.76 (dd, *J* = 2.7, 6.2 Hz, 2H), 4.88 (d, *J* = 11.8 Hz, 1H), 4.79-4.77 (m, 2H), 4.71 (d, *J* = 10.9 Hz, 1H), 4.64 (s, 2H), 4.56 (d, *J* = 12.3 Hz, 1H), 4.50-

4.39 (m, 7H), 3.74 (t, *J* = 9.2 Hz, 3H), 3.60 (s br, 3H), 3.55-3.51 (m, 2H), 3.35 (d, *J* = 9.5 Hz, 1H), 3.27 (s, 3H), 2.19-2.15 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 171.7, 155.8, 150.0, 144.4, 143.7, 138.5, 138.4, 128.2, 128.1, 127.7, 127.6, 127.4, 127.4, 124.0, 123.6, 121.9, 117.3, 114.1, 96.8, 80.8, 79.4, 77.1, 73.9, 71.5, 69.6, 68.4, 65.6, 65.1, 65.0, 63.7, 54.5, 46.4, 46.3, 29.5; HR(ESI)MS calcd. for $\text{C}_{47}\text{H}_{52}\text{N}_6\text{O}_{14}+\text{H}$ 925.3620, found 925.3618.

2,6-Di-*O*-benzyl-3,4-di-*O*-{1-[3-(4-hydroxy-3-carboxylphenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (14): Colorless syrup (98.5%). $[\alpha]_{\text{D}} -15.4$ (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CDCl_3) δ 8.08 (s, 1H), 7.96 (s, 1H), 7.38-7.24 (m, 12H), 6.97 (d, *J* = 2.9 Hz, 1H), 6.95 (d, *J* = 2.9 Hz, 1H), 6.74 (d, *J* = 8.8 Hz, 2H), 4.81-4.76 (m, 3H), 4.70 (s, 2H), 4.64 (t, *J* = 11.0 Hz, 2H), 4.51-4.30 (m, 7H), 3.68 (t, *J* = 9.0 Hz, 2H), 3.56-3.44 (m, 7H), 3.26 (s, 3H), 2.25-2.18 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 171.7, 155.9, 149.9, 144.1, 144.0, 138.7, 138.3, 128.2, 128.0, 127.8, 127.6, 127.4, 127.3, 123.9, 123.8, 121.5, 117.1, 114.3, 114.2, 96.9, 81.1, 79.1, 77.1, 74.3, 72.3, 69.6, 68.6, 65.2, 65.0, 63.2, 54.4, 46.5, 46.4, 29.6; HR(ESI)MS calcd. for $\text{C}_{47}\text{H}_{52}\text{N}_6\text{O}_{14}+\text{H}$ 925.3620, found 925.3618.

2,3-Di-*O*-benzyl-4,6-di-*O*-{1-[3-(4-hydroxy-3-carboxylphenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (15): Colorless syrup (98.2%). $[\alpha]_{\text{D}} -18.0$ (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CDCl_3) δ 8.13 (d, *J* = 5.8 Hz, 1H), 8.04 (s, 1H), 7.34-7.29 (m, 10H), 7.24 (s, 2H), 7.10 (dd, *J* = 2.3 Hz, 8.9 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.80-4.72 (m, 4H), 4.64-4.46 (m, 9H), 3.91 (s, 2H), 3.90 (s, 2H), 3.72-3.63 (m, 2H), 3.57-3.45 (m, 4H), 3.26 (s, 3H), 2.13-1.93 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 171.5, 155.5, 150.5, 143.9, 143.8, 138.7, 138.6, 138.4, 128.2, 128.1, 127.7, 127.6, 127.5, 127.3, 124.0, 123.6, 118.0, 113.6, 96.8, 81.5, 81.0, 79.4, 77.3, 77.1, 74.4, 74.3, 73.5, 71.4, 69.6, 68.6, 68.5, 68.4, 65.2, 65.1, 56.0, 54.5, 46.5, 46.4, 29.5; HR(ESI)MS calcd. for $\text{C}_{47}\text{H}_{52}\text{N}_6\text{O}_{14}+\text{H}$ 925.3620, found 925.3619.

4,6-Di-*O*-benzyl-2,3-di-*O*-{1-[3-(4-hydroxy-3-carboxylphenoxy)propyl]-1*H*-1,2,3-triazole-4-yl]- α -D-glucopyranoside methyl ether (16): Colorless syrup (97.6%). $[\alpha]_{\text{D}} -10.6$ (*c* 5.1, CH_3OH); ^1H NMR (400 MHz, CDCl_3) δ 8.16 (s, 1H), 8.01 (s, 1H), 7.33-7.26 (m, 10H), 7.16 (d, *J* = 7.6 Hz, 2H), 6.86-6.83 (m, 2H), 6.64 (d, *J* = 7.9 Hz, 2H), 4.83 (d, *J* = 11.7 Hz, 1H), 4.77 (d, *J* = 3.2 Hz, 1H), 4.74-4.69 (m, 4H), 4.51-4.38 (m, 9H), 3.86-3.36 (m, 8H), 3.25 (s, 3H), 2.23-2.12 (m, 4H); ^{13}C NMR (400 MHz, CDCl_3) δ 171.9, 155.7, 149.6, 144.3, 144.2, 138.3, 138.2, 128.2, 128.1, 127.7, 127.6, 127.4, 124.0, 123.8, 120.4, 116.6, 114.8, 96.9, 96.4, 80.8, 79.2, 79.1, 77.2, 74.0, 72.3, 72.2, 69.6, 68.6, 68.5, 65.5, 65.0, 64.9, 63.2, 54.4, 46.5, 29.6, 29.0; HR(ESI)MS calcd. for $\text{C}_{47}\text{H}_{52}\text{N}_6\text{O}_{14}+\text{H}$ 925.3620, found 925.3620.

Inhibitory Assay. Recombinant human PTP1B catalytic domain was expressed and purified according to procedures described previously.^{17a} 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, 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. For calculating IC₅₀, inhibition assays were performed with 30 nM recombinant enzyme, 2 mM pNPP in 50 mM MOPS at pH 6.5, and the inhibitors diluted around the estimated IC₅₀ values. IC₅₀ was calculated from the nonlinear curve fitting of percent inhibition (inhibition (%)) vs. inhibitor concentration [I] by using the following equation: inhibition (%) = 100/{1 + (IC₅₀/[I])^k}, where *k* is the Hill coefficient. To study the inhibition selectivity on other PTP family members, human CDC25B, TCPTP, SHP-1, SHP-2 and LARD1 were prepared and assays were performed according to procedures described previously.^{17b}

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