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Discovering the distinct inhibitory effects between C4-epimeric glycosyl amino acids: new insight into the development of protein tyrosine phosphatase inhibitors

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Abstract There has been increasing interest in the development of drug candidates based on sugar templates that possess rich structural and, especially, configurational diversities. We disclose herein that the epimeric identity between methyl 3,4-bis-phenylalanyl/tyrosinyl triazolyl-alpha-D-galactopyranoside and glucopyranoside may lead to their distinct inhibitory effects on specific protein tyrosine phosphatases (PTPs). Subsequently performed molecular docking study elucidated the plausible binding behaviors of the more potent galactosyl inhibitors with their primary PTP target, i.e. Cell Division Cycle 25B (CDC25B) phosphatase.

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Nature creates sugars for the signal transductions involved in numerous crucial biological and pathological processes between cells through specific sugar-protein recognitions. Possessing such unique function, certain glycan entities have been fabricated into vaccines or drugs toward disease protection or therapeutics [1–5]. In addition, the glycosylation is noted to enhance the pharmacodynamics and/or pharmacokinetics of some known bioactive non-sugar compounds [6]. However, during the advancement of *de novo* drug design, simple sugar frameworks such as a monosaccharide are relatively less-regarded starting materials.

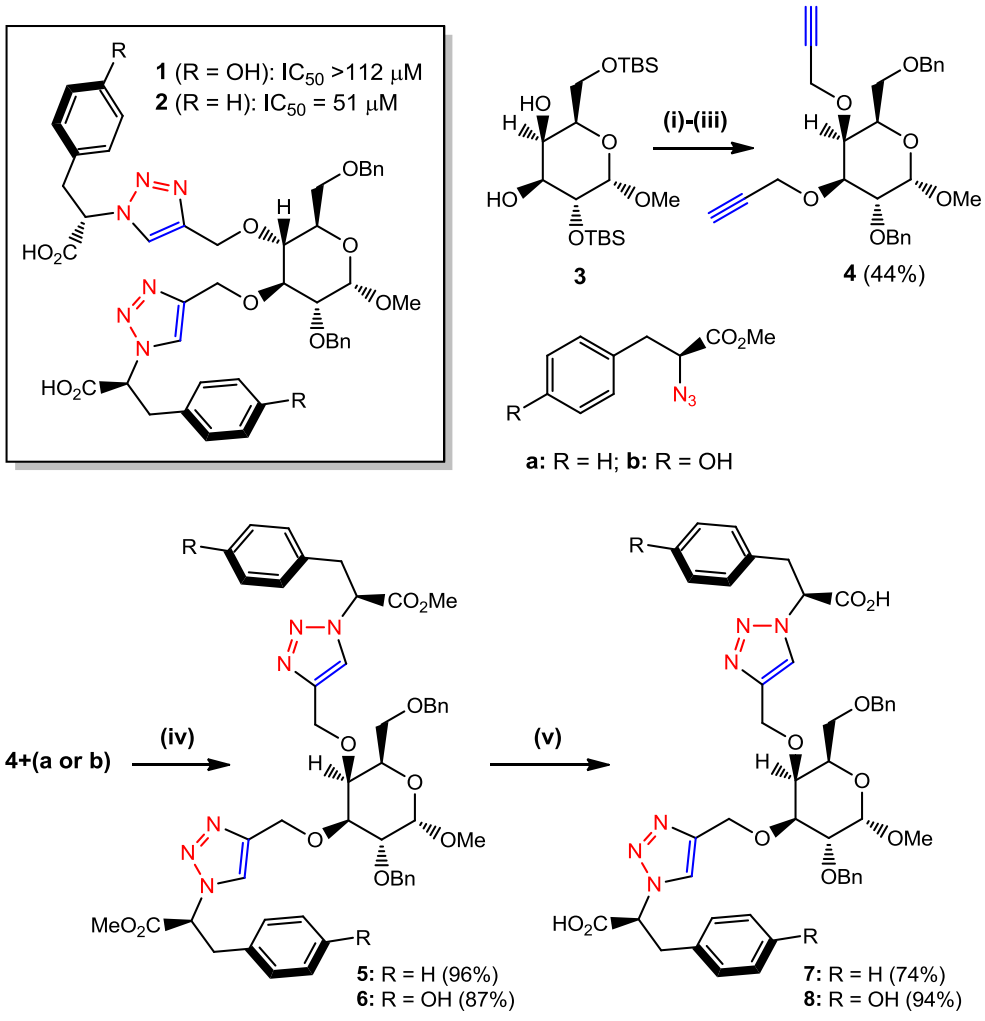
Becker and co-workers have noticed in a recent review that the presentation of pharmacophores on a rigid sugar template owing well-defined stereoinformation represents a compelling strategy for drug discovery [7]. Indeed, to complement the conformationally folded docking cavities of proteins, small molecules containing "three-dimensional" structures would be advantageous. Sugars are among arguably the most readily accessible raw materials provided by nature that may answer for such exceptional purpose. For example, glucose (C4-equatorial bond) and galactose (C4-axial bond) represent a pair of simplest monosaccharide C4-epimers, whereas their only difference in the C4-sterecontrol may result in their distinct binding preference by lectins (carbohydrate-binding proteins) [1, 8].

We are currently interested in the construction of functional triazologlycomimetics [9–12] via the modular and regioselective Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition [13, 14] (the best example of click chemistry) [15], which has proven to be very efficacious for the construction

of bioactive compounds [16, 17]. Protein tyrosine phosphatases (PTPs) are regulators of myriads of pathological events, thus the development of PTPs inhibitors have absorbed considerable attention from the academia and the pharmaceutical industry [18, 19]. As shown in Scheme 1, we have recently synthesized C3,4-disubstituted phenylalaninyl (**1**) and tyrosinyl (**2**) glucosides, which were determined as weak PTP1B inhibitors [20]. We sequentially envision whether the galactosyl epimers of these compounds would exhibit markedly varied inhibitory profile toward the PTPs.

In order to prove such hypothesis, we first prepared C3,4-bis-triazolyl phenylalaninyl and tyrosyninyl galactosides by starting with the known 2,6-disilylated 1-*O*-methyl-α-D-galactoside **3** [21], shown in Scheme 1. The benzylated 3,4-propargyl galactoside was readily synthesized by a 3-step sequence (yield: 44%) involving *O*-propargylation, desilylation and *O*-benzylation [20]. Then, the previously prepared azido phenylalanine (**a**) and tyrosine (**b**) [20, 22] were used for introducing amino acid residues onto this sugar alkyne via a microwave-assisted [23] dual click reaction. In a solvent mixture of $CH_2Cl_2/H_2O/t$BuOH (1:1:1, V/V/V) [24], the sugar alkyne (**4**) and amino acid azides (**a** or **b**) were mixed, followed by a catalytic amount of Na ascorbate (0.4 equiv) and $CuSO_4{\cdot}5H_2O$ (0.2 equiv). This mixture was then transferred to a Yalian (YL8023B1) microwave oven with a ramp time of 8 min until the temperature reached 60°C. To our delight, after 30-min stirring, the methyl 2,6-di-O-benzyl-3,4-di-O-{1-[(1(S)-methoxycarbonyl-2-phenylethyl)]-4-methyl-1H-1,2,3-triazole-4-yl -alpha-D-galactopyranoside **5** was afforded in an excellent yield of 96%.

However, the reaction of compound **4** with the tyrosinyl azide **b** was incomplete under such condition, probably due to the insufficient catalyst loading [25, 26]. Therefore, after the loading of Na ascorbate and $CuSO_4{\cdot}5H_2O$ was increased up to 4 equiv and 2 equiv, respectively, the desired methyl 2,6-di-O-benzyl-3,4-di-O-{1-[(1(S)-methoxycarbonyl-2-p-hydroxyphenylethyl)]-4-methyl-1H-1,2,3-triazole-4-yl -alpha-D-galact opyranoside **6** was afforded in a good yield of 87% under microwave heating (60°C) in 30 min. The saponification of esters **5** and **6** with LiOH (1.5 equiv/ester) sequentially led to the bis-triazoloacids **7** and **8** for biological assays.



Scheme 1 Reagents and conditions: (i) propargyl bromide/NaH, DMF; (ii) AcCl, MeOH; (iii) BnBr/NaH, DMF; (iv) Na ascorbate/$CuSO_4{\cdot}5H_2O$, $CH_2Cl_2/H_2O/t$BuOH; (v) LiOH, MeOH/H_2O

In employing our previously developed methods [27, 28], the inhibitory activities of compounds **7** and **8** were assayed on a panel of homologous PTPs including PTP1B, CDC25B, T-Cell PTP (TCPTP), SH2-Containing PTP-1 (SHP-1), SHP-2 and Leukocyte Antigen-Related Tyrosine Phosphatase (LAR) while those of compounds **1** and **2** were also evaluated on CDC25B (their IC_{50} values on the rest of the tested PTPs have been previously acquired) [20]. As shown in Table 1, the methyl 2,6-di-O-benzyl-3,4-di-O-{1-[(1(S)-carboxy-2-phenyl-ethyl)]-4-methyl-1H-1,2,3-triazole-4-yl -alpha-D-glucopyranoside **1** is not a PTPs inhibitor with IC_{50} values >112 μM whereas its tyrosinyl counterpart **2** possesses weak inhibitory activity on PTP1B (IC_{50}=51 μM), slightly improved activity on CDC25B (IC_{50}=36 μM) and no inhibition on TCPTP, SHP and LAR (IC_{50} >112 μM). In sharp contrast, galactoside **7** that is the epimer of glucoside **1** displayed measurably much enhanced inhibitory potency on both CDC25B (IC_{50}=11 μM) and PTP1B (IC_{50}=25 μM) with retained selectivity over TCPTP (IC_{50}=112 μM), SHP and LAR (IC_{50} >112 μM). Moreover, the galactoside **8** exhibited twofold decreased inhibitory potency on both CDC25B (IC_{50}=75 μM) and PTP1B (IC_{50}=98 μM) comparing to its glucosyl epimer **2**. These data positively indicate that the substitution of pharmacophores on, directly, the C4-epimeric sites of glucose and galactose may lead to the remarkable inhibitory divergence toward PTPs. On the other hand, the tyrosinyl derivative **8** is several-fold less active than its phenylalaninyl counterpart **7**, demonstrating that the additional hydroxyl groups on its tyrosinyl residue may bring on deleterious impact toward PTP inhibition.

We then attempted to study the plausible binding modes of galactosides **7** and **8** with their primary target CDC25B via docking simulation in order to gain more understanding on the potential enzyme-inhibitor interactions. As shown in Fig. 1a, the carboxylic acid of the galactosyl C3-triazolophenylalaninyl residue of compound **7** could generate extensive hydrogen-bonding interactions with amino acid residues including Glu474, Phe475, Ser476, Ser477 and Glu478 the CDC25B catalytic site that has been suggested to accommodate polar moieties [29]. The other phenylalaninyl residue on C4-position of this molecule also made one hydrogen bond with a peripheral amino acid residue (Arg482) by its carboxylic acid group. In addition, non-polar contacts that are reported to largely enhance the binding affinity of the inhibitors with PTPs [18] were also observed in this complex. A π-π stacking was generated by the benzene moiety of galactosyl C3-phenylalaninyl residue with Phe475 while the benzene group of the C4-residue also made hydrophobic contacts with Arg482. Moreover, hydrophobic interactions were made between the two benzyl groups on C2,6-position of the sugar moiety and Met531 and Trp550, respectively.

Notably, from this proposed binding mode illustrated in Fig. 1a, the triazolophenylalaninyl residue on the C4-

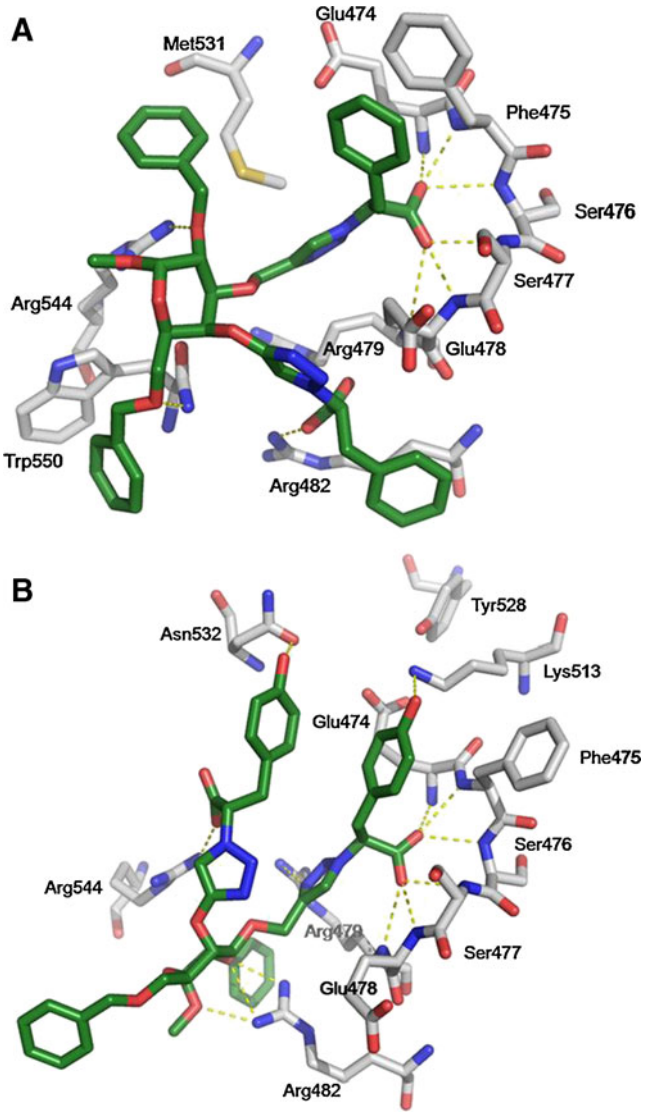


Fig. 1 Plausible binding modes of (**a**) compound **7** and (**b**) compound **8** with CDC25B: the carbon atoms of the inhibitors are shown in green sticks and those for the enzyme are shown in gray sticks. All oxygen atoms are shown in red, nitrogen atoms in blue and the sulphur atom in yellow. Hydrogen bonds are shown in yellow dashed lines

Table 1 Inhibitory activities of compounds **1**, **2**, **7** and **8** on PTPs

Compd.	Structure[a]	IC_{50} (μM)[b,c]		
		CDC25B	PTP1B	TCPTP
1	Glc-Phe	>112	>112	>112
7	Gal-Phe	11.1±1.4	25.3±3.3	111.9±2.7
2	Glc-Tyr	35.6±2.2	51.0±5.3	>112
8	Gal-Tyr	75.2±10.3	98.4±17.0	>112

[a] Abbreviations: Glc: glucosyl; Gal: galactosyl; Phe: phenylalanine; Tyr: tyrosine

[b] Values are means of three experiments

[c] The IC_{50} values of compounds **1**, **2**, **7** and **8** on SHP-1, SHP-2 and LAR are >112 μM

position of galactoside is observed to be fixed right above residue Arg482 with which molecular contacts were generated. While this C4-axial bond is inversed, steric clash would be obviously caused, which might sequentially hamper the insertion of the C3-triazolophenylalaninyl residue into the adjacent catalytic site. Hence, we speculate that the inhibitory deficiency of glucoside **1** toward CDC25B is ascribable to its equatorially positioned C4-triazolophenylalaninyl fragment on the sugar scaffold.

As shown in Fig. 1b, the C3-triazolotyrosinyl residue of compound **8** similarly occupies the catalytic site of CDC25B by making hydrogen bonding interactions with Phe475, Ser476, Ser477, Glu478 and Arg479 while its additional OH-group simultaneously generated one hydrogen bond with Lys513. In stark contrast, instead of approaching Arg482, the other triazolotyrosinyl residue on C4 position of the galactoside is prone to make hydrogen bonds with Asn532 and Arg544 using, its hydroxyl and carboxylic acid groups, respectively. As a consequence, the spatial orientation of C2,6-benzyl groups on the sugar moiety might be largely altered, which may result in their binding-free fashion without generating any non-polar contacts with the peripheral amino acid residues adjacent to the catalytic site. Such decrease in hydrophobic interactions of compound **8** (75 μM) with CDC25B could probably account for its several-fold weakened IC_{50} value compared to its phenylalaninyl counterpart **7** (11 μM).

In conclusion, we have discovered herein that the epimeric identity of C3,4-bis-triazolyl amino acid-glucoside conjugates is determinant toward their binding preference with CDC25B and PTP1B, two validated therapeutic targets that belong to the superfamily of pathologically crucial PTPs. This unique feature would make "clickable" sugar templates promising for the efficient construction of triazologlycomimetic libraries by simply introducing the abundant and biocompatible amino acids onto their epimeric sites via the modular click chemistry. The fabricated sugar epimers with identical molecular weights but diversified configurations would constitute new "three-dimensional" chemical entities with different PTPs binding affinities, thereby leading to a new way for screening potent and potentially biocompatible PTPs inhibitors.

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Supporting Information Additional Supporting Information may be found in the online version: Full characterization of all new compounds and ^{1}H NMR and ^{13}C NMR spectra of title compounds.

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