doi:10.1246/cl.2010.1261
Published on the web November 6, 2010

Triazole-linked Benzylated Glucosyl, Galactosyl, and Mannosyl Monomers and Dimers as Novel Sugar Scaffold-based PTP1B Inhibitors

Yin-Jie Zhang,[1] Xiao-Peng He,[3,4] Cui Li,[3] Zhen Li,[1] De-Tai Shi,[1] Li-Xin Gao,[2] Bei-Ying Qiu,[2] Xiao-Xin Shi,[3] Yun Tang,*[3] Jia Li,*[2] and Guo-Rong Chen*[1]

[1]*Key Laboratory for Advanced Materials and Institute of Fine Chemicals, School of Chemistry and Molecular Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, P. R. China*

[2]*National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, P. R. China*

[3]*School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, P. R. China*

[4]*PPSM, ENS Cachan, CNRS, 61 av. President Wilson, F-94230 CACHAN, France*

(Received August 16, 2010; CL-100704; E-mail: ytang234@ecust.edu.cn, jli@mail.shcnc.ac.cn, mrs_guorongchen@ecust.edu.cn)

Monomeric and dimeric benzylated glycosyl benzenes were synthesized via copper-catalyzed [3 + 2] azide–alkyne cycloaddition. These compounds were then identified as protein tyrosine phosphatase (PTP) 1B inhibitors which displayed at least several fold selectivity over other homologous PTPases. The glucosyl, galactosyl, and mannosyl inhibitors exhibited different biological profiles, suggesting the monosaccharides may qualify as chiral scaffolds for probing the spatial preference of PTP1B. Furthermore, docking study suggested a plausible binding mode of this glycoside series with the enzymatic target.

Protein tyrosine phosphatase (PTP) 1B has been well characterized as a drug target for type 2 diabetes, obesity, and cancer.[1,2] However, most of the reported inhibitors[3] contain negatively charged nonhydrolyzable phosphotyrosine (pTyr) mimetics[4] due to the positively charged nature of the PTP1B catalysis site. The poor cell permeability has thus impeded the further advancement of these inhibitors as drug candidates. Therefore, novel chemical entities that may serve as potential PTP1B inhibitors are highly desirable.

Carbohydrates ubiquitously distribute in nature and govern various biological and pathological events.[5] This abundantly accessible natural source has aroused considerable interest in drug discovery due to their conformational flexibility, high biocompatibility, and intrinsic low toxicity.[6] However, sugar-based PTP1B inhibitors have scarcely been shown to date.[7] Recent efforts have been devoted to develop extremely potent and selective bidentate PTP1B inhibitors,[8] which contain two covalently conjugated pharmacophores via appropriate linkage, synergistically targeting the active site and one periphery pocket of the intended enzyme.

We report here the formation of several new monomeric glycosides and their corresponding dimers as PTP1B inhibitors. Indeed, the dimeric glycosides designed are envisioned to simultaneously target two different enzymatic pockets which would presumably enhance the resulting inhibitory potency compared to monomers. As shown in Figure 1, benzylated 6-azido-6-deoxy-glucoside **1**, 6-azido-6-deoxy-galactoside **2**, 6-azido-6-deoxy-mannoside **3**, and glucosyl azide **4** were employed as different glycodonors for achieving the copper-catalyzed [3 + 2] azide–alkyne cycloaddition[9] with mono- and di-2-propynyl amide and ester benzene bridges **5**–**8**. All triazolyl glycosyl monomers and dimers **9**–**24** were prepared efficiently under the promotion of sodium ascorbate (VcNa) and $CuSO_4 \cdot 5H_2O$ in moderate-to-excellent yields (Schemes 1 and 2). Compared with monomers, increased catalyst loading was

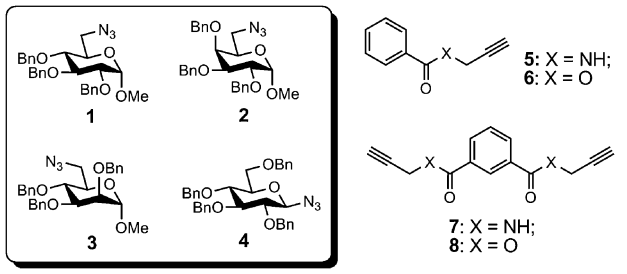
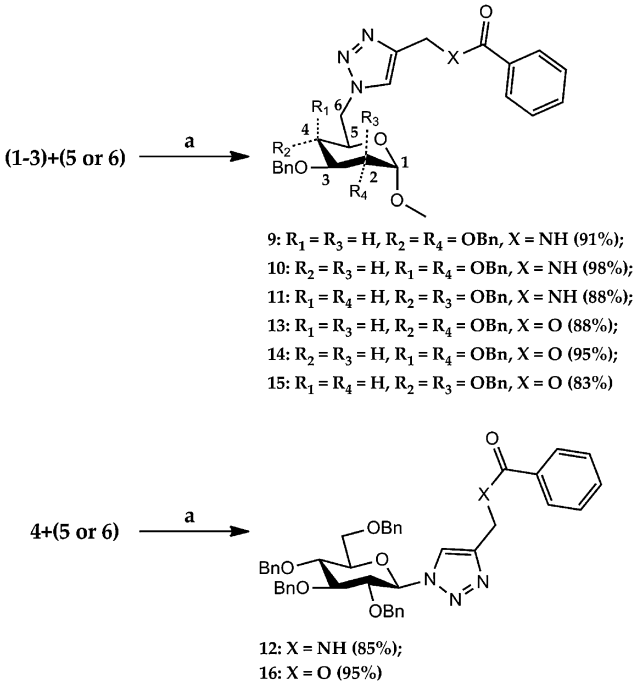
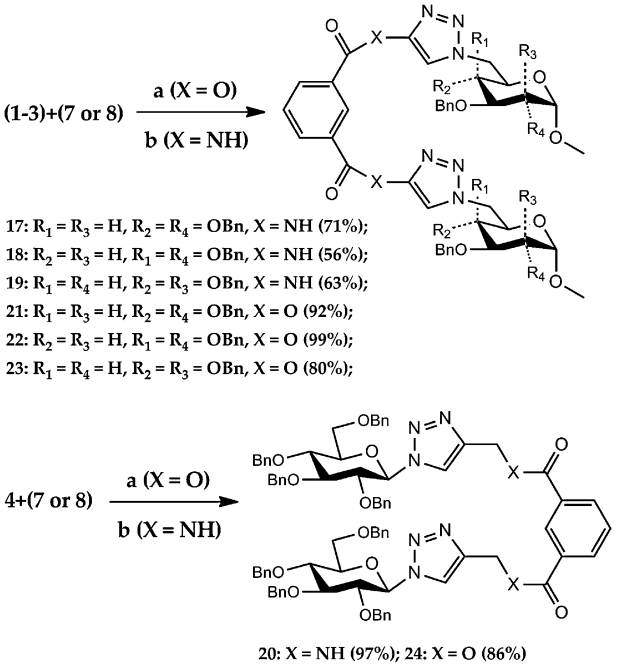


Figure 1. Azido glycodonors and 2-propynyl bridges used in this study.



Scheme 1. Synthesis of monomeric glycosides via click chemistry, reagents and conditions: (a) VcNa (3 equiv), $CuSO_4 \cdot 5H_2O$ (2 equiv) in CH_2Cl_2/H_2O, 6 h.



Scheme 2. Synthesis of monomeric glycosides via click chemistry, reagents and conditions: (a) VcNa (8 equiv), $CuSO_4 \cdot 5H_2O$ (6 equiv) in CH_2Cl_2/H_2O, 6 h; (b) VcNa (8 equiv), $CuSO_4 \cdot 5H_2O$ (6 equiv) in CH_2Cl_2/H_2O/DMF, 24 h.

required for dimers (VcNa from 3 equiv to 8 equiv and $CuSO_4 \cdot 5H_2O$ from 2 equiv to 6 equiv).

The mixed solvent system CH_2Cl_2/H_2O for the cycloaddition of di-2-propynylamide benzene **7** with the azidoglycosides **1**–**4** rendered unsatisfactory result due probably to the poor solubility of this propargyl material. We then tended to add DMF (CH_2Cl_2/H_2O/DMF = 5:2:1, V/V/V) into the system for simultaneously enhancing the solubility of the starting material and the formed products (Scheme 2). To our delight, under such improved condition with vigorous stirring for 24 h, amide triazolyl glycosyl dimers were afforded smoothly in moderate yields (71% for **17**, 56% for **18**, and 63% for **19**) with the 1-modified triazole **20** being an exception that was yielded almost quantitatively (97%).

Next, all synthesized triazolyl-glycosides were assayed through PTP1B and a panel of homologous PTPases including TCPTP (77% sequence identity with PTP1B), SHP-1, SHP-2, and LAR by previously developed methods.[10,11] The data are listed in Table 1 (for PTP1B and TCPTP) and Table S1 (for SHP and LAR).[13] The monomers were first observed to display micromole-ranged PTP1B inhibitory activity (Table 1, compounds **9**–**16**). Obviously, the 1-distributed products **12** ($IC_{50} = 9.3\,\mu mol\,L^{-1}$) and **16** ($IC_{50} = 14.1\,\mu mol\,L^{-1}$) were more active toward PTP1B than the 6-distributed ones (compounds **9**–**11**, $IC_{50} = 13.4$–$31.2\,\mu mol\,L^{-1}$; compounds **13**–**15**, $IC_{50} = 16.8$–$25.8\,\mu mol\,L^{-1}$), indicating that the conjugation pattern of anomeric carbon-modified glycosides was structurally advantageous comparing to that of the side-chain (C-6) modified structures.

Generally, the dimers (Table 1, compounds **17**–**24**, except **22**) showed at least 2-fold enhanced activity compared to their corresponding monomers which demonstrated our design of bidentate inhibitors desirable. For the ester series **21**–**24**, the

Table 1. In vitro PTP1B and TCPTP inhibitory activities of **9**–**24**

Compound	$IC_{50}/\mu M$[a]		Fold[b]
	PTP1B	TCPTP	
9	13.4 ± 1.1	n.d.	—
10	31.2 ± 1.4	>100	**>3.2**
11	20.4 ± 3.0	35.7 ± 2.8	1.8
12	9.3 ± 0.8	33.6 ± 2.3	3.6
13	16.8 ± 2.9	37.8 ± 1.0	2.3
14	25.8 ± 1.5	>100	**>3.9**
15	19.4 ± 1.2	47.6 ± 0.8	2.5
16	14.1 ± 1.4	44.8 ± 0.8	3.2
17	**1.5 ± 0.1**	15.5 ± 0.1	**10.3**
18	7.7 ± 0.2	17.9 ± 0.06	2.3
19	6.6 ± 1.5	20.0 ± 1.2	3.0
20	4.6 ± 0.8	12.6 ± 0.5	2.7
21	4.5 ± 0.6	11.7 ± 2.1	2.6
22	19.4 ± 1.1	28.6 ± 1.0	1.5
23	4.1 ± 0.03	5.2 ± 0.3	1.3
24	7.0 ± 0.9	10.0 ± 1.3	1.4

[a]Values are mean of three experiments (n.d.: not detected).
[b]This is the inhibition ratio of TCPTP/PTP1B.

dimeric glucosyl **21** and mannosyl **23** showed best inhibitory activities ($IC_{50} = 4.5$ and $4.1\,\mu mol\,L^{-1}$, respectively) and the galactosyl dimer **22** was almost 5-fold less active ($IC_{50} = 19.4\,\mu mol\,L^{-1}$). This suggests the conformational change on the C-2 position of sugar moiety rendered little impact toward PTP1B activity whereas the C-4 epimerization was crucial for inhibition with the equatorial bond being privileged. However, the 1-distributed glucosyl dimer **24** ($IC_{50} = 7.0\,\mu mol\,L^{-1}$) was less potent compared to the side chain modified **21** and **23**, which is different from the structure–activity relationship (SAR) pattern of related monomers (**16** vs. **13** and **15**) described above.

Furthermore, the IC_{50} value of dimeric galactoside containing amide bonds (compound **18**, $7.7\,\mu mol\,L^{-1}$) lowered remarkably in comparison with its ester counterpart (compound **22**, $19.4\,\mu mol\,L^{-1}$) while the same tendency was also observed for the glucosyl derivatives (**17** vs. **21**) but not for mannosyl derivatives (**19** vs. **23**). Finally, the best hit (amide 6-triazolyl dimeric glucoside **17**, $IC_{50} = 1.5\,\mu mol\,L^{-1}$) was discovered which exhibited almost 10-fold and 3–13-fold enhanced inhibitory potency, respectively, compared to its corresponding monomer **9** ($IC_{50} = 13.4\,\mu mol\,L^{-1}$) and other dimeric glycosides.

Our next attempt was to assess the specificity of the acquired glycoside inhibitors. As noted, achievement of selective PTP1B inhibitors over other homologous PTPases (especially TCPTP with 77% sequence identity) is a challenging task.[3] Evidently, all assayed products **9**–**24** exhibited at least several fold selectivity for PTP1B over TCPTP, SHP-1 (>3.3-fold), SHP-2 (>4.9-fold), and LAR (>4.9-fold).

Monomeric glucosides **12**, **13**, and **16** and mannosides **11** and **15** exhibited satisfactory specificity over SHP-1, SHP-2, and LAR (Table S1)[13] though moderate selectivity over TCPTP (1.8–3.6-fold, Table 1) was gained. Interestingly, the monomeric galactosides **10** and **14** displayed culminant selectivity over TCPTP as well as SHP-1, SHP-2, and LAR among the monomer

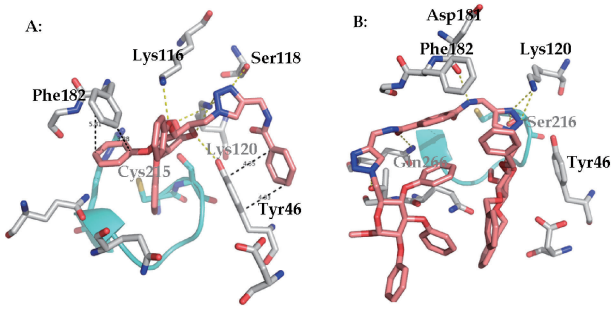


Figure 2. Binding mode of ligands (A) **9** and (B) **17** to PTP1B by docking simulation. Carbon atoms are in gray for PTP1B and pink for ligands, nitrogen atoms are in blue, and oxygen atoms are in red.

series. Such clear evidence may qualify the triazole-linked galactosyl monomer as a promising chiral scaffold for the further development of monosaccharide-based selective PTP1B inhibitors. The amide 6-triazolyl-glucoside **17** that showed the best IC_{50} value (1.5 μmol L^{-1}, Table 1) concomitantly displayed considerable specificity over TCPTP (>10-fold), SHP-1, SHP-2, and LAR (>67-fold).

We then sought to propose a plausible binding mode for this compound series with PTP1B via molecular docking study. As shown in Figure 2, the binding modes of glucosyl monomer **9** (A) and its corresponding dimer **17** (B) were simulated.

Obviously, unlike traditional competitive inhibitors, they did not deeply insert into the active site of PTP1B (Cys215, Ser216, etc.). For the monomer **9** (Figure 2A), the oxygen atom on pyranoglucosyl skeleton generated one hydrogen bond with Tyr46 in the YRD motif and two other hydrogen bonds were also possibly made between the 1-methoxy group of glycoside and the periphery amino acid residues such as Lys116 and Lys120. Additionally, multiple hydrogen bonds were made by the triazole moiety with Ser118 and Lys120 which should be crucial for immobilizing the ligand at this position being distant from the active site. Hydrophobic interactions of the distal benzene rings on triazole and the benzyl group on C-3 of glycoside with the residual phenol group of Tyr46 and benzene group of Phe182 (in WPD loop) also contributed to the inhibitory activity. This possible docking mode dominated by benzene-mediated hydrophobic interactions is similar to that of the aryl diketoacid derivatives reported by Zhang et al.[12]

The plausible location of the dimer **17** (Figure 2B) was slightly different from that of the monomer **9**. Half of the triazole ring performed as hydrogen donor by interacting with Lys120 and Ser216 while the NH group adjacent to this triazole group generated one hydrogen bond with the WPD loop for co-immobilization. Although only limited hydrogen bonds were made between the other half with Gln266, the benzyl glycoside moiety appended on this half successfully extended to the second phosphotyrosine site of PTP1B (Gln266, Gln262, etc.) and might presumably make nonpolar and hydrophobic interactions with the residues inside. Such unique dual binding of this dimeric glycoside may account for its enhanced inhibitory activity and selectivity.[3,8]

To summarize, we have efficiently prepared a novel series of triazole-linked benzylated glycosyl monomers and dimers through copper-catalyzed [3 + 2] azide–alkyne cycloaddition in good yields. Subsequent biological assays identified these aryl-triazolyl-glycosides as PTP1B inhibitors with micromole-ranged inhibitory activities and moderate-to-good selectivity over TCPTP, SHP-1, SHP-2, and LAR. Molecular docking study was also conducted to propose a plausible binding mode for this glycoside series. More importantly, we have discovered that carbohydrates might qualify as promising chiral scaffolds (such as compounds **10**, **14**, and **17**) for the probing of structural and conformational preference of PTP1B. Such interestingly featured triazolyl-glycosides should provide new and alternative insight for the development of sugar scaffold-based PTP1B inhibitors.

This work was supported by National Natural Science Foundation of China (Grant No. 20876045), National Science & Technology Major Project of China "Key New Drug Creation and Manufacturing Program" (No. 2009ZX09302-001), Shanghai Science and Technology Community (Nos. 10410702700 and 08DZ2291300), and Chinese Academy of Sciences (No. KSCX2-YW-R-168). X.-P. H. also thanks the French Embassy in China for a co-tutor doctorate fellowship and his French advisor Prof. J. Xie (ENS Cachan).

References and Notes

1 M. Elchebly, P. Payette, E. Michaliszyn, W. Cromlish, S. Collins, A. L. Loy, D. Normandin, A. Cheng, J. Himms-Hagen, C.-C. Chan, C. Ramachandran, M. J. Gresser, M. L. Tremblay, B. P. Kennedy, *Science* **1999**, *283*, 1544.
2 N. Dubé, A. Cheng, M. L. Tremblay, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1834.
3 a) S. Zhang, Z.-Y. Zhang, *Drug Discovery Today* **2007**, *12*, 373. b) V. V. Vintonyak, A. P. Antonchick, D. Rauh, H. Waldmann, *Curr. Opin. Chem. Biol.* **2009**, *13*, 272.
4 A. P. Combs, *J. Med. Chem.* **2010**, *53*, 2333.
5 C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357.
6 B. Ernst, J. L. Magnani, *Nat. Rev. Drug Discovery* **2009**, *8*, 661.
7 a) L. Lin, Q. Shen, G.-R. Chen, J. Xie, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6348. b) L. Lin, Q. Shen, G.-R. Chen, J. Xie, *Bioorg. Med. Chem.* **2008**, *16*, 9757.
8 a) Y. A. Puius, Y. Zhao, M. Sullivan, D. S. Lawrence, S. C. Almo, Z.-Y. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13420. b) J. Xie, C. T. Seto, *Bioorg. Med. Chem.* **2007**, *15*, 458. c) R. Srinivasan, M. Uttamchandani, S. Q. Yao, *Org. Lett.* **2006**, *8*, 713.
9 a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004. b) T. Michinobu, Y. Inazawa, K. Hiraki, Y. Katayama, E. Masai, M. Nakamura, S. Ohara, K. Shigehara, *Chem. Lett.* **2008**, *37*, 154. c) I. Kumar, C. V. Rode, *Chem. Lett.* **2007**, *36*, 592.
10 W. Zhang, D. Hong, Y.-Y. Zhou, Y.-N. Zhang, Q. Shen, J.-Y. Li, L.-H. Hu, J. Li, *Biochim. Biophys. Acta* **2006**, *1760*, 1505.
11 L. Shi, H.-P. Yu, Y.-Y. Zhou, J.-Q. Du, Q. Shen, J.-Y. Li, J. Li, *Acta Pharmacol. Sin.* **2008**, *29*, 278.
12 S.-J. Liu, L.-F. Zeng, L. Wu, X. Yu, T. Xue, A. M. Gunawan, Y.-Q. Long, Z.-Y. Zhang, *J. Am. Chem. Soc.* **2008**, *130*, 17075.
13 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

