Synthesis of (Glycopyranosyl-triazolyl)-purines and Their Inhibitory Activities against Protein Tyrosine Phosphatase 1B (PTP1B)

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Development of novel purine derivatives has attracted considerable interest, since both purine and purine-based nucleosides display a wide range of crucial biological activities in nature. We report here a novel expansion of these studies by introducing gluco- or galactopyranosyl scaffold to the N- or 9-position (or both) of 6-Cl purine moiety *via* Cu^{I}-catalyzed *Huisgen* 1,3-dipolar cycloaddition. By such an efficient reaction, a series of glycosyl-triazolyl-purines were successfully synthesized in good yields. Biological evaluation showed that the majority of these glycoconjugates were good PTP1B inhibitors with IC_{50} values in low micromolar range (1.5–11.1 μM). The benzylated sugar derivatives displayed better inhibitory potency than that of the acetylated ones. Replacement of Cl by MeO at C(6) of the purine moiety decreased the inhibition in the case of benzylated (glycosyl-mono-triazolyl)-purines **11** and **12** ($IC_{50} > 80$ μM), whereas MeO-substituted benzylated bis[galactosyl-triazolyl]-purine **16** possessed the best inhibitory activity with an IC_{50} value of 1.5 μM. Additionally, these compounds exhibited 2- to 57-fold selectivity over other PTPs (TCPTP, SHP1, SHP2, and LAR).

Introduction. – Ever since purine, *i.e.*, imidazo[4,5-*d*]pyrimidine, was introduced by *Nobel* laureate *Emil Fischer* over 100 years ago [1], successive efforts have been devoted to its chemical preparation and diversification [2]. Indeed, being the central structure of adenine and guanine that are essential accessories for constituting RNA and DNA, the purine ring can be ubiquitously detected in Nature [3]. Owing to its N-containing heterocycle nature, it could be specifically recognized by numerous enzymes or receptors, thus being involved in a myriad of biological processes such as metabolic intermediation, cell signalling, and cell-cycle arrest [3–5].

Besides the versatile roles that purine-based compounds perform through a great variety of biological purposes [4], purine derivatives also possess significant therapeutic potentials serving as inhibitors of Hsp90 [6], Src kinase [7], p38α MAP kinase [8], *etc.* Moreover, purine-based nucleoside mimics have been identified as adenosine receptor antagonists [9][10], cholinesterase inhibitors [11] as well as anti-HIV-1 agents [12].

Among the different methodologies for forming new nucleoside derivatives, the Cu^{I}-catalyzed *Huisgen* alkyne-azide cycloaddition (CuAAC, also known as 'click reaction') [13] has apparently been one of the most frequently used reactions. As a consequence, various 1,2,3-triazole-linked nucleoside, nucleotide, and oligonucleotide mimics that are of exciting biological values have been successfully synthesized in high yields [14]. Hence, further development of structurally novel and biologically valuable purine-based nucleoside derivatives *via* CuAAC would be of a lasting interest.

Protein tyrosine phosphatase 1B (PTP1B), the most clearly characterized enzymatic member of the PTP superfamily, is considered as a negative factor to both Type 2 diabetes and obesity [15][16]. Very recent studies reveal that PTP1B may also function as an oncogene in the context of breast cancer [17]. These compelling results have led to considerable efforts to target PTP1B for therapeutic development [18–23].

We report here the synthesis of glycopyranosyl-triazolyl-purines *via* 'click chemistry' as a series of novel PTP1B inhibitors. This work was initiated by our preliminary biological assay which identified 2-amino-6-chloropurine (**1**; *Fig. 1.*) as a new heterocyclic PTP1B inhibitor with an IC_{50} value of 60 μM. Such a compound is similar to the previously described non-negatively charged PTP1B inhibitors pyridazine (*cf.* **A**; *Fig. 1.*) [24] and pyrimidine (*cf.* **B**; *Fig. 1.*) [25] analogs. With the aim of developing novel PTP1B inhibitors [26][27], we prepared a series of mono-glycosyl-triazolyl- or bis[glycosyl-triazolyl]-purines by using 6-Cl purine as the starting material. Most of the glycosylated purines showed moderate-to-good PTP1B inhibitory activity. Furthermore, these compounds displayed at least several fold selectivity over other homogenous PTPs including TCPTP, SHP1, SHP2, and LAR.

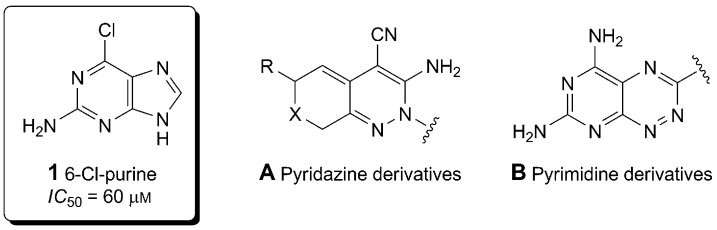


Fig. 1. *Non-negatively charged PTP1B inhibitors*

Result and Discussion. – The Cu^{I}-catalysed *Huisgen* 1,3-dipolar cycloaddition of alkynes with azides was employed for the synthesis of various glycosyl purines. Three azido sugars **4** [28], **5** [29], and **6** [30] (*Fig. 2*) were prepared according to literature procedures. Two propargyl purines were readily synthesized from the 6-Cl-purine. In

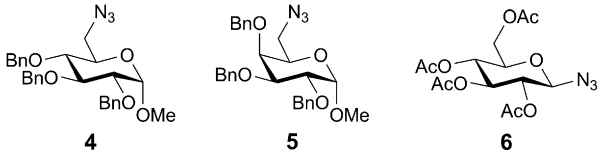
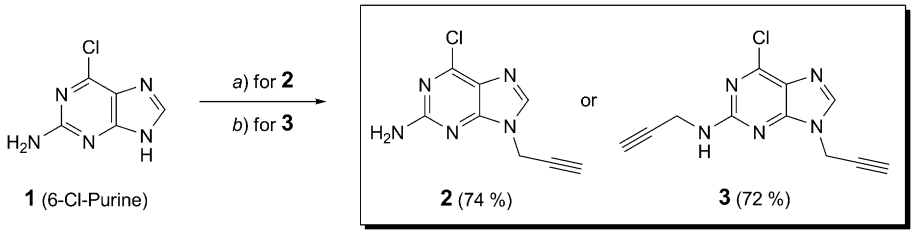


Fig. 2. *Azido-Sugars used in this study*

the presence of K_2CO_3 and propargyl (= prop-2-yn-1-yl) bromide, the known 9-substituted mono propargyl-purine **2** [31] was obtained in 74% yield. Treatment with excessive KOH and propargyl bromide led to 6-chloro-2,9-dipropargyl-purine **3** (72%; *Scheme 1*).

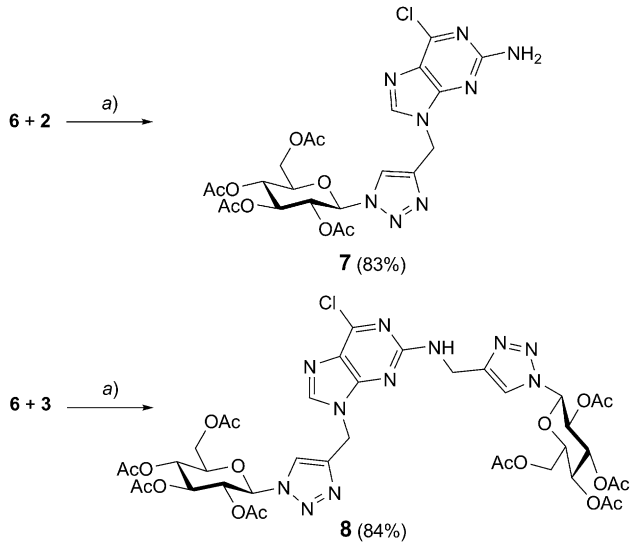
Scheme 1



a) Propargyl bromide, K_2CO_3, DMF. *b*) Propargyl bromide, KOH, DMF.

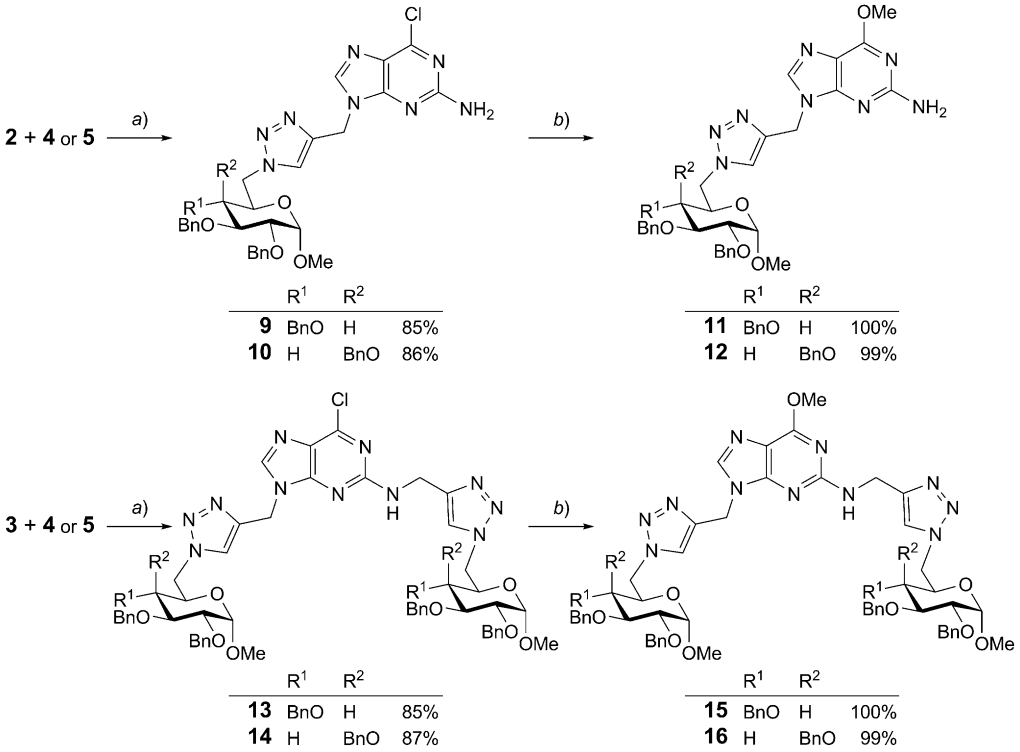
With azido sugars and propargyl-purines in hand, we then performed the 'click reaction'. As illustrated in *Scheme 2*, the acetylated azido sugar **6** was first ligated to either the mono- **2** or the dipropargyl purine **3**. In the presence of sodium ascorbate and $CuSO_4$ in a multi-solvent system (THF/H_2O/*t*-BuOH 3:1:1), the desired mono-triazolyl-purine **7** or bis[triazolyl]-purine **8** were obtained in 83 and 84% yield, respectively. Similarly, benzylated 6-azido-glucoside **4** and -galactoside **5** led to the corresponding mono-triazolyl-purines **9** and **10**, and bis[triazolyl]purines **13** and **14** in good yields (> 80%; *Scheme 3*).

Scheme 2



a) $CuSO_4$, sodium ascorbate, THF/H_2O/*t*-BuOH 3:1:1, r.t.

Scheme 3



	R^1	R^2	
9	BnO	H	85%
10	H	BnO	86%
11	BnO	H	100%
12	H	BnO	99%
13	BnO	H	85%
14	H	BnO	87%
15	BnO	H	100%
16	H	BnO	99%

a) $CuSO_4$, sodium ascorbate, THF/H_2O/*t*-BuOH (3:1:1), r.t. *b*) 0.5M MeONa/MeOH, r.t.

Finally, the replacement of Cl by the MeO group was accomplished under conventional condition (0.5M MeONa/MeOH) to afford the desired products, **11**, **12**, **15**, and **16**, in almost quantitative yield (> 99%).

The inhibitory activities of the newly synthesized compounds toward PTP1B were consecutively measured using *p*NPP as the substrate (*Table*) [32]. The 2-amino-6-chloropurine (**1**) was used as a positive control (IC_{50} 60 μM). Propargylated purines **2** and **3** possessed lower inhibitory potencies, compared to **1**, with IC_{50} values of 232 and 76 μM, respectively. The benzylated monoglucosyl and -galactosyl purines **9** and **10** possessed enhanced PTP1B inhibitory potencies with IC_{50} values of 7.2 and 11.1 μM, respectively, compared to **1**. In contrary, the acetylated monoglycosyl and diglycosyl purines **7** and **8**, respectively, were not PTP1B inhibitors ($IC_{50} > 80$ μM), possibly due to the lack of benzene groups for generating hydrophobic interactions with enzyme. In addition, when the Cl group at C(6) of purine precursor was replaced by a MeO group in the case of benzylated monoglycosyl purines **11** and **12**, no inhibition was observed.

Fortunately, all benzylated diglycosyl purines were shown to be good PTP1B inhibitors. Compared to the corresponding mono-triazoles, the inhibitory activities of 6-Cl derivatives **13** (IC_{50} 2.2 μM) and **14** (IC_{50} 3.2 μM) were enhanced by more than

Table. In vitro *Activities of Purine Analogs* **1**–**3** *and Purine-Based Triazoyl Glycomimics* **7**–**16**

	IC_{50} [μM][a])		Fold (TC-PTP/PTP1B)	IC_{50} [μM]		
	PTP1B	TC-PTP		LAR-PTP	SHP1	SHP2
1	59.7 ± 30.9	> 80	–	> 80	> 80	> 80
2	232.4 ± 16.7	> 80	–	> 80	> 80	> 80
3	75.7 ± 15.9	> 80	–	> 80	> 80	> 80
7	> 80	> 80	–	> 80	> 80	> 80
8	> 80	> 80	–	> 80	> 80	> 80
9	7.2 ± 0.1	29.9 ± 2.6	4.2	> 80	> 80	> 80
10	11.1 ± 2.1	53.6 ± 5.1	4.8	> 80	> 80	> 80
11	> 80	> 80	–	> 80	> 80	> 80
12	> 80	> 80	–	> 80	> 80	> 80
13	2.2 ± 0.2	11.0 ± 2.2	5	> 80	> 80	> 80
14	3.2 ± 0.03	18.0 ± 1.1	**5.6**	> 80	> 80	> 80
15	7.9 ± 0.5	16.2 ± 1.0	2.1	> 80	> 80	> 80
16	**1.5 ± 0.2**	2.9 ± 0.1	1.9	> 80	24.9 ± 1.2	35.7 ± 7.7

[a]) The half maximal inhibitory concentration (IC_{50}) values on Protein Tyrosine Phosphatase 1B (PTP1B), T-Cell Protein Tyrosine Phosphatase (TC-PTP), Leukocyte Antigen-Related Protein Tyrosine Phosphatase (LAR-PTP), SH2-Containing Protein Tyrosine Phosphatase1 (SHP1), and SH2-Containing Protein Tyrosine Phosphatase2 (SHP2) given for tested compounds are means of three experiments.

threefold, evidencing that the bidentate glycosyl-purines are more effecting for increasing the inhibitory potency. More interestingly, contrary to the glycosyl-triazolyl-purines, **11** and **12**, the replacement of Cl by the MeO group on purine moiety of the bis[glycosyl-triazolyl]-purines, **15** and **16**, was found not to be deleterious. Although the MeO substitution of **15** decreased the inhibitory activity 3.6-fold (**15** *vs.* **13**, IC_{50} 7.9 μM for **15**), the MeO-substituted bis[galactosyl-triazolyl]-purine **16** beneficially displayed better inhibitory activity with an IC_{50} value of 1.5 μM towards PTP1B. This result suggests that the bis[triazolyl] derivatives may bind with PTP1B in a distinct manner compared to the corresponding monotriazolyl compounds.

All compounds were then tested for inhibition on other homogenous PTPs including TC-PTP (with 77% sequence identity with PTP1B), SHP1, SHP2, and LAR-PTP for specificity assessment. The results were compiled in the *Table*. Apparently, the 2-amino-6-chloropurine (**1**), mono-propargyl and dipropargyl purines, **2** and **3**, respectively, were established as PTP1B inhibitors, since no inhibitory activity could be monitored on other PTPs. All glycosyl-triazole-linked purines exhibiting PTP1B inhibitory activities were found to possess at least several-fold selectivity over other tested PTPs. Chlorine-containing compounds, **9**, **10**, **13**, and **14**, were more selective than MeO-substituted bis[triazolyl] derivatives **15** and **16** for TC-PTP, with bis[galactosyl-triazolyl] compound **14** being the most selective (5.6-fold). Moreover, these compounds showed no inhibition toward LAR-PTP, SHP1, and SHP2, with exception of **16**, which displayed moderate inhibitory activity toward SHP1 and SHP2 (IC_{50} 24.9 and 35.7 μM, resp.). To the best of our knowledge, compound **16** represented the first example of a purine nucleoside mimic as SHP inhibitor.

Conclusions. – In summary, we have efficiently synthesized a series of gluco- or galactopyranosyl-triazolyl and bis[gluco- or galactopyranosyl-triazolyl]-purines by Cu^{I}-catalyzed click chemistry. The consecutive biological assays identified most of the newly constructed glycoconjugates as PTP1B inhibitors with IC_{50} values in low μM range. Further specificity examinations revealed that these compounds possessed up to 5.6-fold selectivity over TC-PTP, the most homogenous enzyme of PTP1B, and at least more than tenfold selectivity over SHP1, SHP2, and LAR-PTP. Such encouraging result prompts us to prepare more potent and selective PTP1B inhibitors based on purine precursors.

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Experimental Part

General. Solvents were purified by standard procedures. All reactions were monitored by TLC (*Yantai Marine Chemical Co., Ltd.*, P. R. China). Optical rotations: *Perkin-Elmer 241* polarimeter at r.t. in a 10-cm, 1-ml cell. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer in $CDCl_3$ or (D_6)DMSO solns.; TMS as the internal standard (chemical shifts in ppm). HR-MS: *Waters LCT Premier XE* spectrometer using standard conditions (ESI, 70 eV).

General Procedure for the N-*Propargylation*. To a soln. of compound **1** (1 equiv.) in anh. DMF at r.t., K_2CO_3 (1.5 equiv.) or KOH (3 equiv.) was added. After 20-min stirring, propargyl bromide (1.5 or 3 equiv.) was slowly added. The mixture was stirred for *ca.* 12 h. Then, DMF was evaporated, and the resulting residue was diluted with AcOEt, washed successively with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated to give a crude product which was then purified by column chromatography (CC).

*6-Chloro-9-(prop-2-yn-1-yl)-9*H-*purin-2-amine* (**2**) [32]. From **1** (339 mg, 2.0 mmol), K_2CO_3, and propargyl bromide. CC (CH_2Cl_2/MeOH 15:1) afforded **2** (368 mg, 74%). Yellow powder. R_f (CH_2Cl_2/MeOH 15:1) 0.79. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.97 (*s*, 1 H); 5.13 (br. *s*, 2 H); 3.49 (*s*, 2 H); 2.52 (*s*, 1 H).

*6-Chloro-*N,*9-di(prop-2-yn-1-yl)-9*H-*purin-2-amine* (**3**). From compound **1** (509 mg, 3.0 mmol), KOH, and propargyl bromide. CC (AcOEt/petroleum ether (PE) 1:1) afforded **3** (530 mg, 72%). Yellow powder. R_f (AcOEt/PE 1:1) 0.68. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.90 (*s*, 1 H); 7.24 (*t*, J = 6.4, 1 H); 5.69 (*d*, J = 6.4, 2 H); 4.66 (*s*, 2 H); 2.23 (*s*, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 157.1; 152.2; 151.3; 139.8; 125.1; 92.7; 88.9; 79.0; 71.8; 36.0; 29.7. HR-MS: 268.0358 ($C_{11}H_8ClN_5Na^+$; calc. 268.0366).

General Procedure for the Cu^I-Catalyzed Huisgen *Cycloaddition*. To a soln. of propargyl purine (1 equiv.) and azido sugar (1.1 or 2.2 equiv.) in a mixture of THF/H_2O/*t*-BuOH 3:1:1, sodium ascorbate soln. (1M in H_2O, 0.6 equiv., freshly prepared) and $CuSO_4$ soln. (7.5% in H_2O, 0.15 equiv., freshly prepared) were added. After stirring for 8–16 h at r.t. (until TLC indicated the disappearance of the starting material), the mixture was diluted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), filtered, and evaporated to give a crude residue, which was then purified by CC.

*2-Amino-6-chloro-9-{[1-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methyl}-9*H*-purine* (**7**). From **2** (207 mg, 1.0 mmol) and **6** (410 mg, 1.1 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **7** (479 mg, 83%). Yellow powder. R_f (AcOEt/PE 1:2) 0.58. $[\alpha]_D = -27.9$ (c = 4.8, CH_2Cl_2). ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.36 (*s*, 1 H); 8.14 (*s*, 1 H); 6.94 (*s*, 2 H); 6.31 (*d*, J = 9.2, 1 H); 5.61 (*t*, J = 9.2, 1 H); 5.53 (*t*, J = 9.2, 1 H); 5.42–5.34 (*m*, 2 H); 5.15 (*t*, J = 9.6, 1 H); 4.35–4.32 (*m*, 1 H); 4.11 (*dd*, J = 7.2, 12.8, 1 H); 4.07–4.04 (*m*, 1 H); 2.01 (*s*, 3 H); 1.98 (*s*, 3 H); 1.95 (*s*, 3 H); 1.76 (*s*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.5; 170.0; 169.8; 168.9; 160.4; 154.3; 149.9; 143.6; 143.3; 123.6; 123.0; 84.3; 73.7; 72.5; 70.6; 67.9; 62.2; 38.6; 20.9; 20.8; 20.7; 20.3. HR-MS: 603.1331 ($C_{22}H_{25}ClN_8NaO_9^+$; calc. 603.1331).

6-Chloro-9-{[1-(2,3,4,6-tetra-O-*acetyl-β*-D-*glucopyranosyl)-1*H-*1,2,3-triazol-4-yl]methyl}-2-({[1-(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-1*H-*1,2,3-triazol-4-yl]methyl}amino)-9*H-*purine* (**8**). From **3** (207 mg, 1.0 mmol) and **6** (834 mg, 2.2 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **8** (1.058 g, 84%). Yellow powder. R_f (AcOEt/PE, 1:2) 0.65. $[\alpha]_D = -35.9$ ($c = 2.0$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.88 (*s*, 1 H); 7.83 (*s*, 2 H); 7.22 (*t*, *J* = 6.4, 1 H); 5.86 (*d*, *J* = 8.8, 2 H); 5.67 (*d*, *J* = 6.4, 2 H); 5.46–5.38 (*m*, 2 H); 5.23 (*t*, *J* = 9.6, 2 H); 5.09 (*d*, *J* = 15.6, 2 H); 4.92 (*d*, *J* = 15.6, 2 H); 4.29 (*dd*, *J* = 7.6, 12.8, 2 H); 4.15 (*d*, *J* = 12.4, 2 H); 4.00 (*dd*, *J* = 2.8, 10.0, 2 H); 2.07 (*s*, 6 H); 2.06 (*s*, 6 H); 2.03 (*s*, 6 H); 1.85 (*s*, 6 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 202.4; 170.5; 169.9; 169.4; 168.9; 157.9; 152.5; 151.2; 145.2; 139.6; 124.8; 121.2; 92.8; 88.9; 85.7; 75.1; 72.6; 70.2; 67.7; 61.6; 42.3; 20.7; 20.6; 20.5; 20.2. HR-MS: 1014.2606 ($C_{39}H_{46}ClN_{11}NaO_{18}^+$; calc. 1014.2609).

*Methyl 6-{4-[(2-Amino-6-chloro-9*H-*purin-9-yl)methyl]-1*H-*1,2,3-triazol-1-yl}-2,3,4-tri*-O-*benzyl-6-deoxy-α*-D-*glucopyranoside* (**9**). From **2** (207 mg, 1.0 mmol) and **4** (538 mg, 1.1 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **9** (593 mg, 85%). Yellow powder. R_f (AcOEt/PE 1:2) 0.48. $[\alpha]_D = +11.5$ ($c = 1.2$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.93 (*s*, 1 H); 7.61 (*s*, 1 H); 7.35–7.30 (*m*, 15 H); 5.37 (br. *s*, 2 H); 5.11 (br. *s*, 2 H); 4.99 (*d*, *J* = 10.4, 1 H); 4.92 (*d*, *J* = 10.8, 1 H); 4.81 (*d*, *J* = 10.8, 1 H); 4.78 (*d*, *J* = 10.8, 1 H); 4.71 (*d*, *J* = 10.8, 1 H); 4.63 (*d*, *J* = 12.0, 1 H); 4.55–4.49 (*m*, 3 H); 4.00 (*t*, *J* = 9.2, 1 H); 3.95–3.91 (*m*, 1 H); 3.40 (*dd*, *J* = 2.8, 9.6, 1 H); 3.14 (*t*, *J* = 9.6, 1 H); 3.11 (*s*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 159.3; 153.5; 151.3; 142.2; 141.9; 138.3; 137.9; 137.8; 128.6; 128.5; 128.4; 128.2; 128.0; 127.8; 125.0; 124.3; 98.0; 81.7; 79.9; 77.9; 75.8; 74.9; 73.4; 69.0; 55.2; 50.9; 38.6. HR-MS: 719.2478 ($C_{36}H_{37}ClN_8NaO_5^+$; calc. 719.2473).

*Methyl 6-{4-[(2-Amino-6-chloro-9*H-*purin-9-yl)methyl]-1*H-*1,2,3-triazol-1-yl}-2,3,4-tri*-O-*benzyl-6-deoxy-α*-D-*galactopyranoside* (**10**). From **2** (207 mg, 1.0 mmol) and **5** (538 mg, 1.1 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **10** (599 mg, 86%). Yellow powder. R_f (AcOEt/PE 1:2) 0.52. $[\alpha]_D = +40.4$ ($c = 2.8$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.85 (*s*, 1 H); 7.54 (*s*, 1 H); 7.40–7.26 (*m*, 15 H); 5.30 (br. *s*, 4 H); 5.02 (*d*, *J* = 11.6, 1 H); 4.89 (*d*, *J* = 11.6, 1 H); 4.82 (*d*, *J* = 12.0, 1 H); 4.73 (*d*, *J* = 11.6, 1 H); 4.65 (*d*, *J* = 12.4, 1 H); 4.60 (*d*, *J* = 11.6, 1 H); 4.51 (*d*, *J* = 3.6, 1 H); 4.31 (*dd*, *J* = 9.6, 14.0, 1 H); 4.16 (*dd*, *J* = 2.4, 14.0, 1 H); 4.00 (*dd*, *J* = 3.6, 10.0, 1 H); 3.95–3.91 (*m*, 1 H); 3.90 (*dd*, *J* = 2.8, 12.8, 1 H); 3.82 (br. *s*, 1 H); 2.79 (*s*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 159.1; 153.3; 151.4; 142.0; 141.6; 138.4; 138.2; 137.8; 128.6; 128.5; 128.5; 128.4; 128.2; 128.1; 127.8; 127.9; 127.6; 125.0; 124.3; 98.7; 78.6; 76.1; 75.0; 74.6; 73.9; 73.6; 69.3; 54.9; 51.4; 38.5. HR-MS: 697.2666 ($C_{36}H_{38}ClN_8O_5^+$; calc. 697.2654).

*6-Chloro-*N*,9-bis[(1-{[(2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-tetrahydro-6-methoxy-2*H-*pyran-2-yl]methyl}-1*H-*1,2,3-triazol-4-yl)methyl]-9*H-*purin-2-amine* (**13**). From **3** (207 mg, 1.0 mmol) and **4** (1.076 g, 2.2 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **13** (1.044 g, 85%). Yellow powder. R_f (AcOEt/PE 1:2) 0.42. $[\alpha]_D = -45.1$ ($c = 1.0$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.81 (*s*, 1 H); 7.68 (br. *s*, 2 H); 7.35–7.27 (*m*, 30 H); 7.16 (*t*, *J* = 6.4, 1 H); 5.63 (*d*, *J* = 6.4, 2 H); 4.97 (*d*, *J* = 10.8, 4 H); 4.96 (*d*, *J* = 8.0, 2 H); 4.90 (*d*, *J* = 10.8, 2 H); 4.80 (*d*, *J* = 10.4, 2 H); 4.72 (*d*, *J* = 10.0, 4 H); 4.61 (*d*, *J* = 12.0, 2 H); 4.53 (*dd*, *J* = 2.8, 14.0, 4 H); 4.48 (*dd*, *J* = 6.0, 14.4, 2 H); 3.99 (*t*, *J* = 9.6, 2 H); 3.95–3.91 (*m*, 2 H); 3.39 (*dd*, *J* = 3.6, 9.6, 2 H); 3.15 (*t*, *J* = 9.2, 2 H); 3.13 (*s*, 6 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 202.3; 160.9; 158.1; 152.8; 145.1; 138.4; 137.9; 137.8; 136.7; 128.5; 128.2; 128.1; 128.0; 127.8; 123.8; 98.0; 92.9; 88.5; 81.8; 80.0; 78.0; 75.8; 75.0; 73.4; 69.2; 55.2; 50.7; 42.5. HR-MS: 1246.4877 ($C_{67}H_{70}ClN_{11}NaO_{10}^+$; calc. 1246.4893).

*6-Chloro-*N*,9-bis[(1-{[(2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-tetrahydro-6-methoxy-2*H-*pyran-2-yl]methyl}-1*H-*1,2,3-triazol-4-yl)methyl]-9*H-*purin-2-amine* (**14**). From **3** (207 mg, 1.0 mmol) and **5** (1.076 g, 2.2 mmol). Click reaction and CC (AcOEt/PE 1:6 to 1:4) afforded **14** (1.058 g, 87%). Yellow powder. R_f (AcOEt/PE 1:2) 0.43. $[\alpha]_D = +32.4$ ($c = 1.0$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.83 (*s*, 1 H); 7.52 (br. *s*, 2 H); 7.32–7.24 (*m*, 30 H); 7.09 (*t*, *J* = 6.8, 1 H); 5.60 (*d*, *J* = 6.8, 2 H); 5.01 (*d*, *J* = 11.6, 2 H); 4.90 (br. *s*, 2 H); 4.87 (*d*, *J* = 11.6, 2 H); 4.80 (*d*, *J* = 12.0, 2 H); 4.72 (*d*, *J* = 11.6, 2 H); 4.64 (*d*, *J* = 12.0, 2 H); 4.59 (*d*, *J* = 11.6, 2 H); 4.53 (br. *s*, 2 H); 4.29 (*dd*, *J* = 9.2, 14.0, 2 H); 4.18 (*dd*, *J* = 3.2, 14.0, 2 H); 3.99 (*dd*, *J* = 3.2, 10.0, 2 H); 3.96 (*dd*, *J* = 2.8, 9.2, 2 H); 3.89 (*dd*, *J* = 2.8, 10.0, 2 H); 3.82 (br. *s*, 2 H); 2.85 (*s*, 6 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 202.2; 157.7; 152.3; 151.0; 144.1; 139.4; 138.4; 138.2; 137.9; 128.5; 128.4; 128.1; 127.8; 127.7; 127.6; 124.3; 98.6; 92.5; 88.9; 78.7; 76.1; 75.0; 74.6; 73.8; 73.6; 69.4; 54.9; 51.2; 42.5. HR-MS: 1246.4893 ($C_{67}H_{70}ClN_{11}NaO_{10}^+$; calc. 1246.4893).

General Procedure for the Methoxylation. Benzylated-glycosyl 2-amino-6-chloro purine (1.0 equiv.) was suspended in a soln. of 0.5M MeONa in MeOH (8.0 equiv.), and stirred at r.t. for *ca*. 6 h. Then, the mixture was filtered, and the filter cake was washed 2× with CH_2Cl_2/MeOH 9:1. The resulting filtrate was directly evaporated to give the crude product, which was then purified by CC.

*Methyl 6-{4-[(2-Amino-6-methoxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-2,3,4-tri*-O*-benzyl-6-deoxy-α-*D*-glucopyranoside* (**11**). From **9** (696 mg, 1.0 mmol), CC (AcOEt/PE 1:2) afforded **11** (692 mg, 100%). White powder. R_f (AcOEt/PE 1:2) 0.53. $[\alpha]_D = +19.4$ ($c = 1.2$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.93 (*s*, 1 H); 7.80 (*s*, 1 H); 7.32 (br. *s*, 15 H); 5.41–5.27 (*m*, 2 H); 4.97 (*d*, $J = 10.8$, 2 H); 4.89 (*d*, $J = 10.8$, 1 H); 4.79 (*d*, $J = 11.2$, 1 H); 4.74 (*d*, $J = 14.4$, 1 H); 4.69 (*d*, $J = 11.2$, 1 H); 4.61 (*d*, $J = 12.0$, 1 H); 4.51–4.42 (*m*, 3 H); 4.10–4.05 (*m*, 3 H); 3.97 (*t*, $J = 8.4$, 1 H); 3.96–3.91 (*m*, 1 H); 3.45–3.39 (*m*, 1 H); 3.14 (*t*, $J = 7.6$, 1 H); 3.06 (*s*, 3 H); 2.54 (br. *s*, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 161.3; 152.6; 152.1; 141.9; 141.4; 139.1; 138.3; 137.9; 137.8; 128.5; 128.4; 128.2; 128.0; 127.8; 124.8; 117.9; 98.0; 81.8; 79.9; 77.9; 75.8; 74.9; 73.4; 69.0; 55.2; 54.5; 50.9; 38.9. HR-MS: 715.2974 ($C_{37}H_{40}N_8NaO_6^+$; calc. 715.2969).

*Methyl 6-{4-[(2-Amino-6-methoxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-2,3,4-tri*-O*-benzyl-6-deoxy-α-*D*-galactopyranoside* (**12**). From **10** (696 mg, 1.0 mmol), CC (AcOEt/PE 1:2) afforded **12** (685 mg, 99%). White powder. R_f (AcOEt/PE 1:2) 0.52. $[\alpha]_D = +35.5$ ($c = 1.7$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.65 (*s*, 1 H); 7.52 (*s*, 1 H); 7.41–7.26 (*m*, 15 H); 5.29 (*s*, 2 H); 5.02 (*d*, $J = 11.6$, 1 H); 4.93 (br. *s*, 2 H); 4.89 (*d*, $J = 12.0$, 1 H); 4.82 (*d*, $J = 12.0$, 1 H); 4.73 (*d*, $J = 11.6$, 1 H); 4.66 (*d*, $J = 12.0$, 1 H); 4.60 (*d*, $J = 12.0$, 1 H); 4.53 (*d*, $J = 3.6$, 1 H); 4.29 (*dd*, $J = 9.6, 14.0$, 1 H); 4.15 (*dd*, $J = 2.8, 14.0$, 1 H); 4.06 (*s*, 3 H); 3.99 (*dd*, $J = 6.4, 10.0$, 1 H); 3.94–3.88 (*m*, 2 H); 3.81 (*m*, 1 H); 2.79 (*s*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 161.6; 159.4; 153.4; 142.4; 138.9; 138.4; 138.2; 137.8; 128.6; 128.5; 128.4; 128.4; 128.2; 128.1; 127.9; 127.8; 127.7; 124.2; 115.4; 98.7; 78.7; 76.1; 75.0; 74.6; 73.9; 73.6; 69.3; 55.0; 53.9; 51.3; 38.4. HR-MS: 715.2974 ($C_{37}H_{40}N_8NaO_6^+$; calc. 715.2969).

*6-Methoxy-*N*,9-bis[(1-{[(2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-tetrahydro-6-methoxy-2*H*-pyran-2-yl]methyl}-1*H*-1,2,3-triazol-4-yl)methyl]-9*H*-purin-2-amine* (**15**). From **13** (612 mg, 0.5 mmol), CC (AcOEt/PE 1:2) afforded **15** (610 mg, 100%). White powder. R_f (AcOEt/PE 1:2) 0.56. $[\alpha]_D = +43.9$ ($c = 2.8$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.72 (*s*, 1 H); 7.56 (br. *s*, 2 H); 7.31–7.28 (*m*, 30 H); 7.18 (*t*, $J = 6.4$, 1 H); 5.59 (*d*, $J = 6.4$, 2 H); 5.01–4.93 (*m*, 6 H); 4.89 (*d*, $J = 10.8$, 2 H); 4.79 (*d*, $J = 10.8$, 2 H); 4.72 (*dd*, $J = 8.8, 11.6$, 4 H); 4.59 (*d*, $J = 12.0$, 2 H); 4.53–4.48 (*m*, 4 H); 4.44 (*dd*, $J = 6.4, 14.4$, 2 H); 4.01 (*s*, 3 H); 3.98 (*t*, $J = 9.2$, 2 H); 3.92 (*td*, $J = 2.8, 6.4$, 2 H); 3.38 (*dd*, $J = 3.2, 9.6$, 2 H); 3.13 (*t*, $J = 9.2$, 2 H); 3.08 (*s*, 6 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 202.3; 160.9; 158.1; 152.8; 145.1; 138.4; 137.92; 137.88; 136.7; 128.5; 128.5; 128.2; 128.0; 127.8; 123.8; 98.0; 92.9; 88.5; 81.8; 79.9; 78.0; 75.8; 75.0; 73.4; 69.2; 55.2; 53.8; 50.6; 42.5. HR-MS: 1242.5393 ($C_{68}H_{73}N_{11}NaO_{11}^+$; calc. 1242.5389).

*6-Methoxy-*N*,9-bis[(1-{[(2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-tetrahydro-6-methoxy-2*H*-pyran-2-yl]methyl}-1*H*-1,2,3-triazol-4-yl)methyl]-9*H*-purin-2-amine* (**16**). From **14** (612 mg, 0.5 mmol), CC (AcOEt/PE 1:2) afforded **16** (606 mg, 99%). White powder. R_f (AcOEt/PE 1:2) 0.53. $[\alpha]_D = +41.8$ ($c = 1.1$, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.71 (*s*, 1 H); 7.46 (br. *s*, 2 H); 7.37–7.26 (*m*, 30 H); 7.13 (*t*, $J = 6.8$, 1 H); 5.59 (*d*, $J = 6.8$, 2 H); 5.02 (*d*, $J = 11.6$, 2 H); 4.96–4.86 (*m*, 4 H); 4.81 (*d*, $J = 12.0$, 2 H); 4.73 (*d*, $J = 12.0$, 2 H); 4.67 (*d*, $J = 12.0$, 2 H); 4.60 (*d*, $J = 11.6$, 2 H); 4.51 (*d*, $J = 3.6$, 2 H); 4.29 (*dd*, $J = 9.2, 14.4$, 2 H); 4.18 (*dd*, $J = 3.2, 14.0$, 2 H); 4.01–3.98 (*m*, 2 H); 4.00 (*s*, 3 H); 3.96 (*dd*, $J = 2.8, 9.2$, 2 H); 3.90 (*dd*, $J = 2.8, 10.0$, 2 H); 3.83 (br. *s*, 2 H); 2.82 (*s*, 6 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 202.3; 160.9; 158.0; 152.7; 145.0; 138.4; 138.2; 137.9; 136.6; 128.1; 127.8; 127.7; 127.6; 123.6; 114.0; 98.7; 92.8; 88.4; 78.8; 76.1; 75.1; 74.6; 73.8; 73.6; 69.5; 54.9; 53.7; 51.2; 42.4. HR-MS: 1242.5396 ($C_{68}H_{73}N_{11}NaO_{11}^+$; calc. 1242.5389).

Inhibitory Assays. Recombinant human PTP1B catalytic domain was expressed and purified according to procedures described in [31]. Enzymatic activity of PTP1B was determined at 30° 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, 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°, and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. To calculate the IC_{50}, values 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. The IC_{50} values were calculated from the nonlinear

curves fitting percent inhibition (inhibition [%]) *vs.* 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 TC-PTP, SHP1, SHP2, and LAR-PTPD1 were prepared, and assays were performed according to procedures described in [33].

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