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Original article

Design, synthesis and biological activity of cyclohexane-bearing *C*-glucoside derivatives as SGLT2 inhibitors

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ABSTRACT

Seven cyclohexane-bearing *C*-glucoside derivatives (**7**, **9**, **12**, **13** and **17–19**) were designed and synthesized as SGLT2 inhibitors starting from a potent SGLT2 inhibitor we discovered in earlier work, (1*S*)-1-deoxy-1-[4-methoxy-3-(*trans*-*n*-propylcyclohexyl)methylphenyl]-D-glucose (**1**). The *in vitro* and *in vivo* biological activities were evaluated by hSGLT2/hSGLT1 inhibition and urinary glucose excretion (UGE), respectively. Among the synthesized compounds **12**, the 6-deoxy derivative of **1** was the most active and selective SGLT2 inhibitor (IC_{50} = 1.4 nmol/L against hSGLT2; selectivity = 1576). Compound **12** was a potent SGLT2 inhibitor, which could induce more urinary glucose than **1** and dapagliflozin in UGE.

1. Introduction

Diabetes is a kind of chronic metabolic disease that is characterized by hyperglycemia, either because the pancreas does not produce enough insulin and/or because the tissue cells poorly respond to the insulin that is produced. Diabetes without adequate treatment can cause a number of severe complications. Although there are many anti-diabetic drugs currently available, the hyperglycemia still can not be controlled in many cases, indicating that new anti-diabetic drugs with novel action mechanisms are urgently needed.

Most of the plasma glucose that is filtered in the renal glomerulus is reabsorbed into the blood mainly by sodium-glucose co-transporter 2 (SGLT2) in the renal proximal tubule [1,2]. Therefore, inhibition of SGLT2 is able to suppress the reabsorption of glucose from the glomerular filtrate into blood, which will lower the blood glucose levels. SGLT2 inhibitors have become a promising class of hypoglycemic agents for the treatment of type-2 diabetes, and many SGLT2 inhibitors are now in clinical trials. One of the SGLT2 inhibitors, dapagliflozin, has been approved recently in EU (Fig. 1).

In an earlier work, we discovered a class of novel SGLT2 inhibitors which incorporated a cyclohexane moiety in the molecules, among which a potent SGLT2 inhibitor, (1*S*)-1-deoxy-1-[4-methoxy-3-(*trans*-*n*-propylcyclohexyl)methylphenyl]-D-glucose (**1**), was discovered (Fig. 2) [3,4]. Encouraged by these promising results, we moved on to further study this class of novel SGLT2 inhibitors in anticipation of discovering more potent SGLT2 inhibitors, and herein would like to report the study on the compounds resulting from the derivatizations of the 6-OH of the sugar ring in **1** (Schemes 1–3).

2. Experimental

^{1}H NMR spectra were recorded on a Bruker AV400 spectrometer with DMSO-d_6 as solvent and TMS as internal standard. The HR-MS data were obtained on an Agilent Q-TOF 6510 mass spectrometer using electrospray ionization (ESI) technique. The synthetic routes of target compounds, **7**, **9**, **12**, **13**, **17–19**, are depicted in Schemes 1–3 and the steps involved are described in "Results and discussion".

A 50-mL dried flask was charged with 5 mL of dried MeOH and 0.10 g (4 mmol) of sodium, and the mixture was stirred at room temperature until all the sodium disappeared. Compounds **6**, **10**, **11** and **14–16** (1 mmol) were added to the solution, individually. The stirring was continued at room temperature until all the starting compounds were consumed completely (typical within

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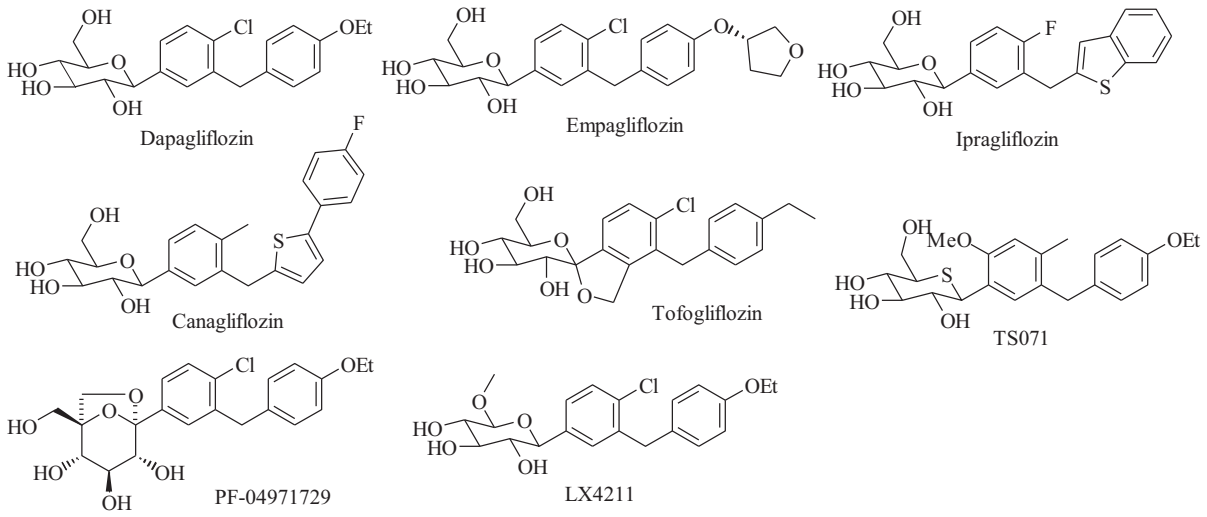


Fig. 1. Molecular structures of some SGLT2 inhibitors that are now in clinical trials or launched.

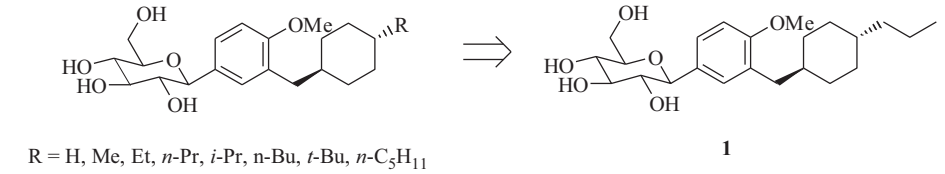
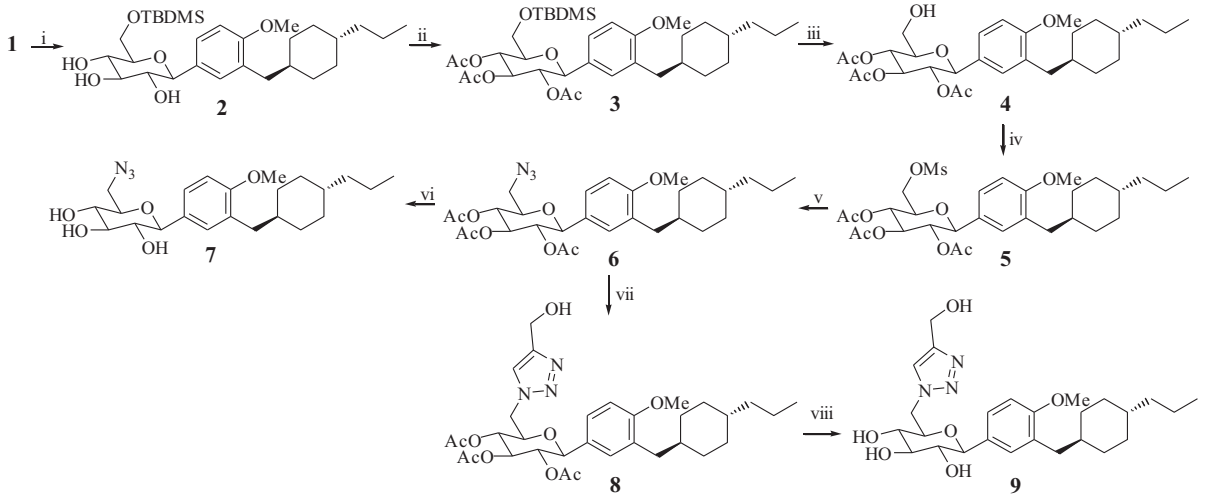


Fig. 2. The cyclohexane-bearing SGLT2 inhibitors discovered previously in our laboratories.

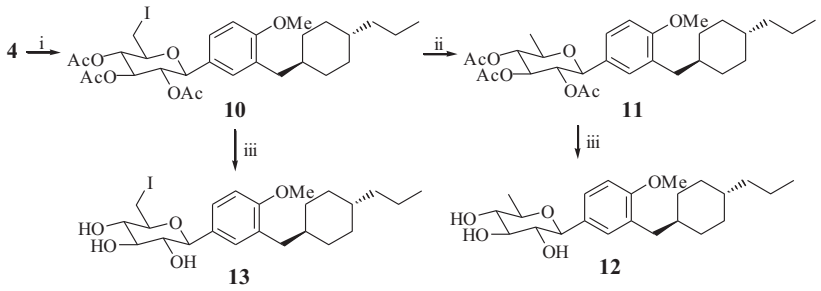
1 h) as demonstrated by TLC. On completion, 2 g of dried acidic resin was added to the reaction mixture and the stirring was continued at room temperature until the pH 7. The reaction mixture was filtered, and the filtrate was evaporated on a rotary evaporator to afford a white residue as crude products, which were further dried by vacuum oil pump to yield the pure products **7**, **13**, **12** and **17**–**19**, respectively.

A 50-mL flask was charged with **8** (1 mmol) and 10 mL of EtOH. The mixture was stirred at room temperature, followed by addition of 2 mL of 30% aqueous NaOH. The mixture thus obtained was refluxed for 10 min, cooled to room temperature and poured to 100 mL of water. The aqueous mixture was adjusted to pH 7 with concentrated hydrochloric acid and extracted with CH_2Cl_2 (15 mL × 3). The combined extracts were washed with saturated brine, dried over Na_2SO_4 and evaporated on a rotary evaporator to afford a residue, which was further dried *in vacuo* to yield the pure product **9**.

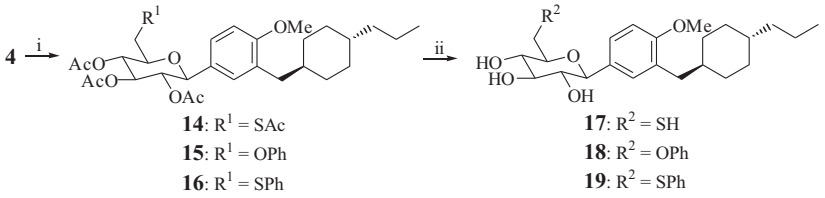
7: White foam, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.10 (dd, 1 H, J = 2.0 Hz and 8.4 Hz), 7.02 (d, 1H, J = 2.0 Hz), 6.85 (d, 1H, J = 8.4 Hz), 5.15 (d, 1H, J = 4.8 Hz), 4.97 (d, 1H, J = 4.8 Hz), 4.76 (d, 1H, J = 5.6 Hz), 4.01 (d, 1H, J = 9.6 Hz), 3.73 (s, 3H), 3.52 (dd, 1H, J = 2.0 Hz and 13.2 Hz), 3.42–3.44 (m, 1H), 3.21–3.37



Scheme 1. Synthetic route of target molecule **7** and **9**. Reaction conditions: i) TBDMSC l (1.1 eq), imidazole, dried DMF, 0-r.t.; ii) Ac_2O, DMAP, pyridine, 0-r.t.; iii) 90% AcOH, 45 °C; iv) MsCl, EtN, CH_2Cl_2, 0-r.t.; v) NaN_3, DMF, 100 °C; vi) MeONa, MeOH, r.t., then strongly acidic ion exchange resin (H^+ form); vii) propargyl alcohol, Cu(I), DMF, r.t.; viii) 30% NaOH, EtOH, reflux.



Scheme 2. Synthetic route of target molecule **12** and **13**. Reaction conditions: i) I_2, PPh_3, imidazole, CH_2Cl_2, r.t.; ii) *n*-Bu_3SnH, AIBN, PhMe, r.t.; iii) MeONa, MeOH, r.t., then strongly acidic ion exchange resin (H^+ form).



Scheme 3. Synthetic route of target molecule **17**–**19**. Reaction conditions: i) R^1H, PPh_3, DEAD, THF, r.t.; ii) MeONa, MeOH, then strongly acidic ion exchange resin (H^+ form).

(m, 3H), 3.11–3.13 (m, 1H), 2.38–2.39 (m, 2H), 1.59–1.66 (m, 4H), 1.40 (bs, 1H), 1.23–1.28 (m, 2H), 1.08–1.11 (m, 3H), 0.74–0.92 (m, 7H); HR-ESI-MS, calcd. for $C_{23}H_{39}N_4O_5$ ($[M+NH_4]^+$) 451.2920, found 451.2915.

9: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.71 (s, 1H), 7.04–7.07 (m, 1H), 6.95 (s, 1H), 6.84 (d, 1H, J = 8.4 Hz), 5.34 (d, 1H, J = 5.2 Hz), 5.09 (t, 1H, J = 5.6 Hz), 5.02 (d, 1H, J = 4.8 Hz), 4.77 (d, 1H, J = 6.0 Hz), 4.69 (d, 1H, J = 12.0 Hz), 4.45–4.46 (m, 2H), 4.46 (d, 2H, J = 5.6 Hz), 3.95 (d, 1H, J = 9.6 Hz), 3.73 (s, 3H), 3.59–3.62 (m, 1H), 3.03–3.09 (m, 2H), 1.58–1.67 (m, 4H), 1.39 (s, 1H), 1,23–1,29 (m, 2H), 1.09–1.12 (m, 3H), 0.76–0.93 (m, 7H); HR-ESI-MS, calcd. for $C_{26}H_{40}N_3O_6$ ($[M+H]^+$) 490.2917, found 490.2910.

12: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.07 (dd, 1H, J = 2.0 Hz and 8.4 Hz), 6.97 (d, 1H, J = 2.0 Hz), 6.84 (d, 1H, J = 8.4 Hz), 4.91 (d, 1H, J = 5.6 Hz), 4.84 (d, 1H, J = 4.4 Hz), 4.62 (d, 1H, J = 5.2 Hz), 3.91 (d, 1H, J = 9.2 Hz), 3.73 (s, 3H), 3.12–3.28 (m, 3H), 2.89–2.94 (m, 1H), 2.44 (dd, 1H, J = 6.8 Hz and 12.8 Hz), 2.35 (dd, 1H, J = 7.2 Hz and 12.8 Hz), 1.59–1.67 (m, 4H), 1.39 (s, 1H), 1.23–1.29 (m, 3H), 1.07–1.15 (m, 5H), 0.88–0.96 (m, 7H); HR-ESI-MS, calcd. for $C_{23}H_{40}NO_5$ ($[M+NH_4]^+$) 410.2906, found 410.2916.

13: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.11 (dd, 1H, J = 2.0 Hz and 8.4 Hz), 7.02 (d, 1H, J = 2.0 Hz), 6.87 (d, 1H, J = 8.4 Hz), 5.16 (bs, 1H), 4.78 (bs, 2H), 4.04 (d, 1H, J = 9.2 Hz), 3.74 (s, 3H), 3.52 (dd, 1H, J = 2.6 Hz and 10.6 Hz), 3.39 (dd, 1H, J = 5.2 Hz and 10.4 Hz), 3.31 (t, 1H, J = 8.8 Hz), 3.08–3.15 (m, 2H), 2.95–2.99 (m, 1H), 2.34–2.46 (m, 2H), 1.64–1.67 (m, 4H), 1.41 (s, 1H), 1.21–1.30 (m, 2H), 1.07–1.12 (m, 3H), 0.83–0.97 (m, 7H); HR-ESI-MS, calcd. for $C_{23}H_{35}INaO_5$ ($[M+Na]^+$) 541.1427, found 541.1431.

17: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.10 (dd, 1H, J = 1.8 Hz and 8.2 Hz), 7.00 (d, 1H, J = 1.6 Hz), 6.86 (d, 1H, J = 7.6 Hz), 5.06 (d, 1H, J = 5.2 Hz), 4.92 (d, 1H, J = 4.0 Hz), 4.70 (d, 1H, J = 6.0 Hz), 3.96 (d, 1H, J = 9.6 Hz), 3.11–3.29 (m, 4H), 2.85–2.91 (m, 1H), 2.56–2.63 (m, 1H), 2.34–2.47 (m, 2H), 2.00 (t, 1H, J = 7.6 Hz), 1.61–1.67 (m, 4H), 1.38–1.42 (m, 1H), 1.21–1.30 (m, 2H), 1.07–1.18 (m, 3H), 0.76–0.98 (m, 7H); HR-ESI-MS, calcd. for $C_{23}H_{40}NO_5S$ ($[M + NH_4]^+$) 442.2627, found 442.2621.

18: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.23–7.27 (m, 2H), 7.09 (dd, 1H, J = 2.0 Hz and 8.4 Hz), 6.98 (d, 1H, J = 2.0 Hz), 6.84–6.93 (m, 4H), 5.18 (d, 1H, J = 4.8 Hz), 4.98 (d, 1H, J = 4.0 Hz), 4.72 (d, 1H, J = 5.6 Hz), 4.25 (d, 1H, J = 10.0 Hz), 3.98–4.04 (m, 2H), 3.72 (s, 3H), 3.55–3.59 (m, 1H), 3.27–3.32 (m, 3H), 3.15–3.21 (m, 1H), 2.44 (dd, 1H, J = 6.8 Hz and 12.8 Hz), 2.34 (dd, 1H, J = 7.0 Hz and 13.0 Hz), 1.59–1.66 (m, 4H), 1.39 (s, 1H), 1.21–1.28 (m, 2H), 1.06–1.11 (m, 3H), 0.78–0.96 (m, 7H); HR-ESI-MS, calcd. for $C_{29}H_{44}NO_6$ ($[M+NH_4]^+$) 502.3169, found 502.3170.

19: White foam, ^1H NMR (400 MHz, DMSO-d_6): δ 7.31 (d, 2H, J = 7.6 Hz), 7.25 (t, 2H, J = 7.6 Hz), 7.13 (t, 1H, J = 7.2 Hz), 7.05 (dd, 1H, J = 1.6 Hz and 8.4 Hz), 6.97 (d, 1H, J = 1.6 Hz), 6.84 (d, 1H, J = 8.4 Hz), 5.21 (d, 1H, J = 4.4 Hz), 4.96 (s, 1H), 4.72 (d, 1H, J = 5.6 Hz), 3.95 (d, 1H, J = 9.6 Hz), 3.73 (s, 3H), 3.42–3.45 (m, 2H), 3.22–3.24 (m, 2H), 3.11–3.17 (m, 1H), 3.02 (dd, 1H, J = 8.0 Hz and 14.0 Hz), 2.44 (dd, 1H, J = 6.8 Hz and 12.8 Hz), 2.33 (dd, 1H, J = 7.0 Hz and 13.0 Hz), 1.60–1.67 (m, 4H), 1.39 (s, 1H), 1.21–1.30 (m, 3H), 1.07–1.12 (m, 3H), 0.81–0.96 (6H); HR-ESI-MS, calcd. for $C_{29}H_{44}NO_5S$ ($[M+NH_4]^+$) 518.2940, found 518.2926.

3. Results and discussion

As shown in Scheme 1, the starting material **1** [3] was treated with 1.1 eq. of TBDMSCl (*t*-butyldimethylsilyl chloride) in the presence of imidazole in DMF to regioselectively protect its 6-OH with TBDMS to give **2**, which was further peracetylated to afford triacetate **3** by treatment with Ac_2O in pyridine in the presence of DMAP (4-dimethylaminopyridine) at room temperature [5]. The TBDMS in **3** was then cleaved by 90% aqueous AcOH at 45 °C to yield **4**. Conversion of **4** to its mesylate **5** was followed by S_N2 substitution of mesylate with azide to furnish the azide **6**. Deacetylation of **6** by treatment with MeONa afforded **7**. Click reaction of azide **7** and propargy alcohol in the presence of Cu(I) generated by *in situ* reduction of $CuSO_4$ with ascorbic acid produced the 1,2,3-triazole **8** [6], which afforded **9** on saponification with aqueous NaOH in refluxing EtOH. Compound **4** was converted to iodide **10** by treatment with I_2/PPh_3/imidazole. Reduction of iodide **10** with *n*-Bu_3SnH in the presence of AIBN (azobisisobutyronitrile) in toluene gave **11**, which was deacetylated with MeONa to yield **12**. Direct deacetylation of **10** with MeONa afforded **13**. Mitsunobu reactions of **4** and AcSH, PhOH and PhSH gave **14**–**16**, respectively. Compounds **14**–**16** were deacetylated with MeONa to yield **17**–**19**, respectively.

Table 1
hSGLT1 and hSGLT2 inhibitory potency and UGE results for the synthesized SGLT2 inhibitors.

Compounds[a]	IC_{50} (nmol/L)		Selectivity [b]	Urinary glucose (mg/200 g)[c]
	hSGLT2	hSGLT1		
Vehicle	–	–	–	13 ± 2
Dapagliflozin	1.3	1289	992	1612 ± 102
1	6.3	3834	609	1633 ± 87
7	155	4766	31	620 ± 76
9	456	7812	140	541 ± 69
12	1.4	2206	1576	1844 ± 71
13	223	8331	37	673 ± 107
17	79	2987	38	994 ± 97
18	207	9947	48	319 ± 45
19	358	9701	27	336 ± 42

[a] Dose: 10 mg/kg (po).
[b] IC_{50} (hSGLT1)/IC_{50} (hSGLT2).
[c] The urinary glucose excreted 24 h post-challenge (mean ± SD) is expressed as mg/200 g of rat weight (n = 10).

The *in vitro* (hSGLT2 and hSGLT1 inhibitory activity) and *in vivo* hypoglycemic activity (UGE, urinary glucose excretion) of the synthesized compounds were evaluated according to known methods [1] and summarized in Table 1. As shown in Table 1, all the synthesized compounds were selective SGLT2 inhibitors (all selectivities >30), with **12** being the most selective one (1576), which was even more selective than **1** (609) and dapagliflozin (992). **12** was most active against hSGLT2 (1.4 nmol/L), which was more active than **1** (1.4 nmol/L *vs* 6.3 nmol/L) and comparable with dapagliflozin (1.4 nmol/L *vs* 1.3 nmol/L), indicating that the 6-deoxylation could be well tolerated in this case. **12** was also the most potent one in *in vivo* evaluation, which was significantly more potent than **1** (1844 *vs* 1633, $P < 0.05$) and dapagliflozin (1844 *vs* 1612, $P < 0.05$). The other synthesized compounds were much less potent, indicating that except for 6-deoxylation, other modifications at 6-OH, including introduction of small functional groups, such as N_3, SH and I and bulky groups, such as 1,2,3-triazole, OPh and SPh, were not well tolerated. Noteworthy is that compound **12** could induce more urinary glucose excretion than dapagliflozin, although their IC_{50}s against hSGLT2 were comparable (1.4 nmol/L *vs* 1.3 nmol/L), presumably because **12** possessed better pharmacokinetic properties than dapagliflozin as determined by *in vivo* evaluation.

4. Conclusion

In conclusion, modifications at the 6-OH position in the sugar ring of (1*S*)-1-deoxy-1-[4-methoxy-3-(*trans*-*n*-propylcyclohexyl)-methylphenyl]-D-glucose (**1**), a potent SGLT2 inhibitor discovered earlier in our laboratories, resulted in the discovery of a much more potent SGLT2 inhibitor **12**, which was the 6-deoxy derivative of **1**.

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References

[1] M. Meng, B.A. Ellsworth, A.A. Nirschl, et al., Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes, J. Med. Chem. 51 (2008) 1145–1149.
[2] W.N. Washburn, Development of the renal glucose reabsorption inhibitors: a new mechanism for the pharmacotherapy of diabetes mellitus type 2, J. Med. Chem. 52 (2009) 1785–1794.
[3] H. Shao, Y.L. Gao, Y.Y. Lou, et al., Design, synthesis and antihyperglycemic activity of trans-cyclohexane-bearing C-glucosides as SGLT2 inhibitors, Chin. J. Org. Chem. 31 (2011) 836–842.
[4] W.J. Zhao, X.L. Yu, H. Shao, et al., Synthesis and anti-hyperglycemic activities of trans-cyclohexane-bearing C-glucosides as SGLT2 inhibitors II, Chin. J. Synth. Chem. 20 (2012) 527–536.
[5] K.J. Liao, X.F. Jin, X.B. Meng, et al., Synthesis of an antimetastatic tetrasaccharide β-D-Gal-(1 → 4)-β-D-GlcpNAc-(1 → 6)-α-D-Manp-(1 → 6)-β-D-Manp-OMe, Chin. Chem. Lett. 23 (2012) 1371–1374.
[6] V.V. Rostovtsev, L.G. Green, V.V. Fokin, et al., A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes, Angew. Chem. Int. Ed. Engl. 41 (2002) 2596–2599.