“Click saccharides”: novel separation materials for hydrophilic interaction liquid chromatography

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Received (in Cambridge, UK) 6th February 2007, Accepted 9th March 2007
First published as an Advance Article on the web 27th March 2007
DOI: 10.1039/b701831b

Bonded mono-, di- and oligosaccharides were developed as novel separation materials for HILIC *via* click chemistry and proven to have excellent chromatographic properties for separation of polar compounds.

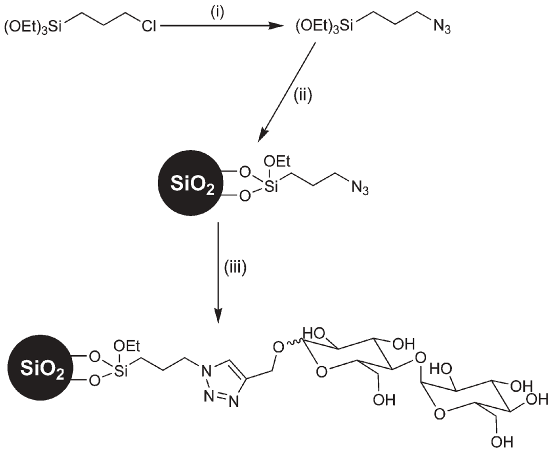
Separation of highly polar compounds by HPLC is a great challenge in separation science and has become a universal problem in many research fields, such as the study of natural products, medicine, metabonomics, proteomics, *etc.* Hydrophilic interaction liquid chromatography (HILIC) which was first put forward by Alpert in 1990,[1] is an alternative HPLC mode for separation of polar compounds with polar stationary phases as that in NP-HPLC but aqueous mobile phase similar to RP-HPLC mode.[1,2] Developing HPLC separation methods with HILIC mode and novel separation materials for HILIC has attracted increasing attention in recent years.[2,3] More and more special separation materials designed for HILIC have appeared in recent years, such as underivatized silica, amido silica, poly(succinimide)-bonded silica, polyhydroxyl silica, *etc.*[2,3a,3h] These special separation materials for HILIC demonstrate well selectivity and reproducibility for separation of polar compounds. However, different types of separation materials for HILIC have different retention characteristics and separation selectivity.[3h] Besides, the preparation procedure is usually complicated and difficult due to the complex structures and active groups on the bonded stationary phases.[2] Mono-, di- and oligosaccharides are typical polar compounds with several polar groups and unique structures, which are ideal bonded stationary phases for HILIC. The CDs, a series of cyclo-oligosaccharide with 1,4-α-bonds, have been developed as HILIC separation materials and used in the separation of sugars, amino acids, peptides, *etc.*[2,3a] Noncyclo-oligosaccharides also have polar groups and have more flexible structures than CDs. Bonded noncyclo-saccharides on silica support may demonstrate more interesting chromatographic properties in HILIC mode. However, the immobilization of saccharides on solid supports is a difficult task with the traditional conjugation strategies. To the best of our knowledge, there are no reports about bonded noncyclo-oligosaccharides as separation materials for HPLC. In recent years, immobilization of saccharides on solid supports has been developed *via* click chemistry[4] due to the power of click chemistry and its application to surface modification.[5]

In our previous report,[5h] we demonstrated the suitability of click chemistry in developing silica based HPLC separation materials. Frechet and co-workers studied click chemistry in the preparation of HPLC separation materials on polymer support.[5i] More recently, Lindner and co-workers studied the preparation of chiral stationary phases *via* click chemistry.[5j] However, these reports mainly focused on demonstrating the suitability of click chemistry in the preparation of separation materials for HPLC. The functionalized molecules immobilized on the silica or polymer supports *via* click chemistry were mainly traditional stationary phases, which were not helpful to resolve the problems in separation chemistry.

The objective of this communication is to introduce bonded saccharides as separation materials for HILIC and the preparation strategy *via* click chemistry for the first time and to further demonstrate the power of click chemistry in the preparation of separation materials with complex bonded stationary phases.

β-CD, glucose and maltose were chosen as model molecules of cyclo-oligosaccharide and nocyclo-saccharide, respectively. The “Click Maltose” was prepared (shown in Scheme 1) according to the “click strategy” as described in our previous reports.[5h]

The other two “click saccharides” were prepared with the same route and the structures are shown in Table 1 (entries 1 and 2). The key intermediate alkyne-saccharides were synthesized according to the literature.[6] In the immobilization process of these alkyne-saccharides, only the alkyne groups on the saccharides could react



Scheme 1 *Reagents and conditions*: (i) NaN_3, DMF, KI, 90–100 °C; (ii) silica beads, DMF, 100–110 °C; (iii) 1-*O*-propargylmaltose ($CH_3OH–H_2O$, 1 : 1), $CuSO_4$, 5 mol%, NaAsc, 15 mol%, RT.

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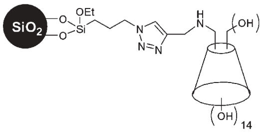
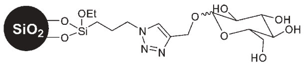
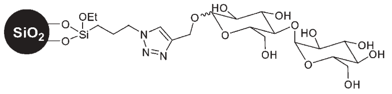
Table 1 The illustrations of separation materials and the surface coverage of the saccharides

Entry	Separation material	Functionalized HPLC packing	Surface coverage[a]/μmol m^{-2}
1	**Click CD**		0.310
2	**Click Glucose**		1.366
3	**Click Maltose**		0.801

[a] The surface coverage was obtained *via* calculation based on the increase of carbon content from the elemental analysis.

with the azide group on the silica. Therefore, the side-reaction aroused by the hydroxyl groups can be avoided in this click strategy and the "click saccharides" bonded on the silica surface could retain their inherent structures and properties. The processes can be monitored by FT-IR, in which the peak at 2110 cm^{-1} (azide groups) disappeared on the completion of the "clicking" process.[5d] The azide-silica and the "click saccharide"-silica were characterized by FT-IR and EA (Table 1).

To investigate the separation applicability of these "click saccharide" silica based separation materials, chromatographic studies were performed with polar compounds as probes. All these three separation materials demonstrate good performance in HILIC mode. Taking Click Maltose as an example, the peaks' symmetry is 0.95 and column performance is 43 000 plates m^{-1} calculated from adenosine (Fig. 1). This proved that the "click saccharides" silica are suitable as separation materials for HILIC.

To further demonstrate the potential of "click saccharides" in the separation of polar compounds, they were used for separation of a mixture of typical polar compounds, such as base, nucleoside and sugar. The mixture of polar compounds could be well separated on these "click saccharides" in HILIC mode. The

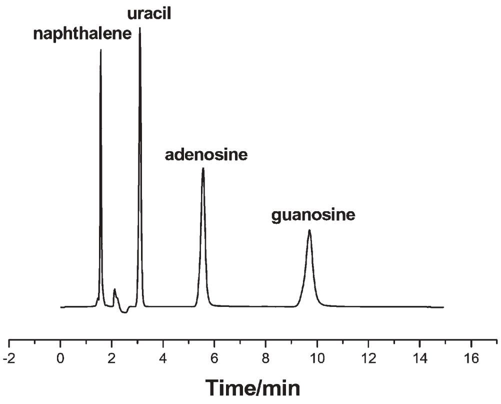


Fig. 1 The chromatogram obtained with the column Click Maltose (4.6 × 150 mm). *Conditions*: flow rate: 1.0 ml min^{-1}; 30 °C; mobile phase: A: 10 mM ammonium acetate aqueous solution, B: acetonitrile; isocratic: 15% A; UV: 254 nm.

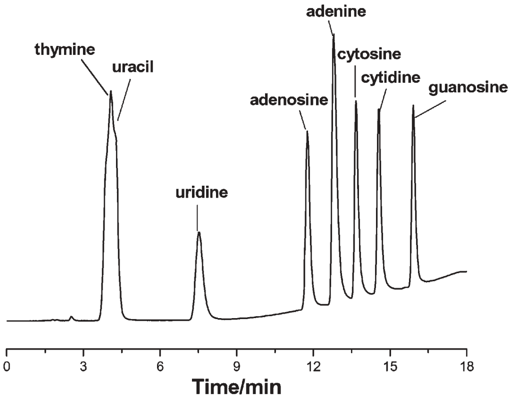


Fig. 2 Separation of base and nucleoside on Click Glucose (4.6 × 150 mm). *Conditions*: flow rate 1.0 ml min^{-1}; 30 °C; mobile phase: A: 10 mM ammonium acetate aqueous solution, B: acetonitrile; gradient: 0–5 min: 3% A; 5–20 min: 3 → 20% A; UV: 270 nm.

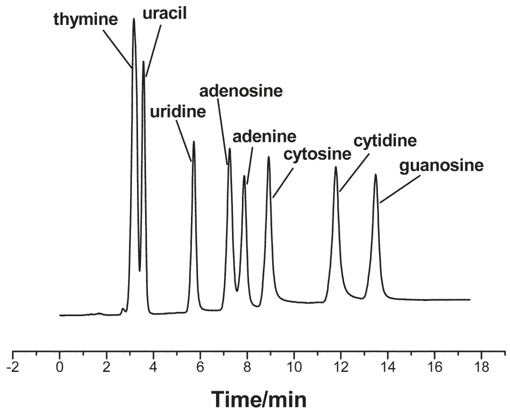


Fig. 3 Separation of base and nucleoside on Click Maltose (4.6 × 150 mm). *Conditions*: flow rate 1.0 ml min^{-1}; 30 °C; mobile phase: A: 10 mM ammonium acetate aqueous solution, B: acetonitrile; gradient: 0–20 min: 10 → 20% A; UV: 275 nm.

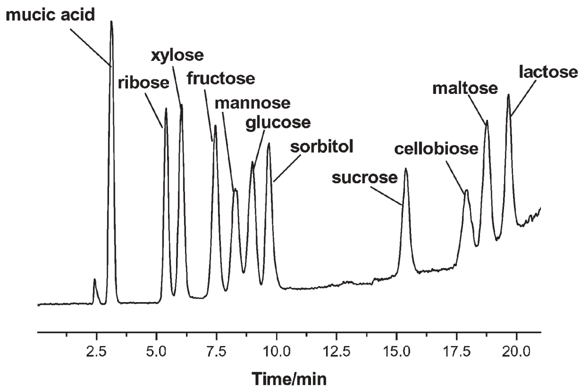


Fig. 4 Separation of sugars and sugar derivates on Click β-CD. Column: 4.6 × 150 mm. *Conditions*: flow rate 1.0 ml min^{-1}; 30 °C; mobile phase: A: 10 mM ammonium acetate aqueous solution, B: acetonitrile; gradient: 0–15 min: 10 → 15% A, 15–25 min: 15 → 25% A; ELSD: nitrogen nebulizer gas: 30 psi; tube temperature: 50 °C; gain: 10.

chromatograms obtained with Click Glucose and Click Maltose are shown in Fig. 2 and Fig. 3, respectively, in which the mixture of base and nucleoside are well separated. The mixture of sugars and sugar derivatives were well separated on Click CD (shown in Fig. 4). Compared with previous reports,[2,3h] these "click saccharides" silica based separation materials have equal or better separation selectivity for separation of polar compounds.

In summary, this work introduces bonded saccharides as novel separation materials for HILIC and shows an efficient strategy for immobilization of saccharides on silica supports. β-CD, glucose and maltose have been immobilized on silica support *via* click chemistry. Moreover, the chromatographic results firmly illustrate the potential of "click saccharides" as separation materials for HILIC and the power of click chemistry in the preparation of complex separation materials. The chromatographic properties and application of these "click saccharides" in the separation of polar compounds are underway in our laboratory. We believe that the "click saccharides" silica, together with the preparation strategy, will become more useful for separation of polar compounds in many research fields.

We acknowledge the grant of key project of the knowledge innovation program supported by the Chinese Academy of Sciences (Grant: KGCX2-SW-213) and thank the start-up fund of the Green Catalysis Institute from Wuhan University. We also thank the financial support of the Key Project of National Science Foundation of China (Grant: 20235020).

Notes and references

1 A. J. Alpert, *J. Chromatogr.*, 1990, **499**, 177–196.
2 P. Hemstrom and K. Irgum, *J. Sep. Sci.*, 2006, **29**, 1784–1821.
3 (*a*) M. A. Strege, *Anal. Chem.*, 1998, **70**, 2439–2445; (*b*) D. S. Risley and M. A. Strege, *Anal. Chem.*, 2000, **72**, 1736–1739; (*c*) M. A. Strege, S. Stevenson and S. M. Lawrence, *Anal. Chem.*, 2000, **72**, 4629–4633; (*d*) B. A. Olsen, *J. Chromatogr., A*, 2001, **913**, 113–122; (*e*) V. V. Tolstikov and O. Fiehn, *Anal. Biochem.*, 2002, **301**, 298–307; (*f*) H. Schlichtherle-Cerny, M. Affolter and C. Cerny, *Anal. Chem.*, 2003, **75**, 2349–2354; (*g*) R. Oertel, V. Neumeister and W. Kirch, *J. Chromatogr., A*, 2004, **1058**, 197–201; (*h*) Z. Guo and S. Gaiki, *J. Chromatogr., A*, 2005, **1074**, 71–80; (*i*) G. Karlsson, S. Winge and H. Sandberg, *J. Chromatogr., A*, 2005, **1092**, 246–249; (*j*) H. L. Koh, A. J. Lau and E. C. Y. Chan, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 1237–1244; (*k*) Y. Iwasaki, M. Hoshi, R. Ito, K. Saito and H. Nakazawa, *J. Chromatogr., B*, 2006, **839**, 74–79.
4 (*a*) F. Fazio, M. C. Bryan, O. Blixt, J. C. Paulson and C. H. Wong, *J. Am. Chem. Soc.*, 2002, **124**, 14397–14402; (*b*) M. C. Bryan, F. Fazio, H. K. Lee, C. Y. Huang, A. Chang, M. D. Best, D. A. Calarese, C. Blixt, J. C. Paulson, D. Burton, I. A. Wilson and C. H. Wong, *J. Am. Chem. Soc.*, 2004, **126**, 8640–8641.
5 (*a*) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021; (*b*) J. P. Collman, N. K. Devaraj and C. E. D. Chidsey, *Langmuir*, 2004, **20**, 1051–1053; (*c*) S. Lober and P. Gmeiner, *Tetrahedron*, 2004, **60**, 8699–8702; (*d*) T. Lummerstorfer and H. Hoffmann, *J. Phys. Chem. B*, 2004, **108**, 3963–3966; (*e*) S. Punna, E. Kaltgrad and M. G. Finn, *Bioconjugate Chem.*, 2005, **16**, 1536–1541; (*f*) J. P. Collman, N. K. Devaraj, T. P. A. Eberspacher and C. E. D. Chidsey, *Langmuir*, 2006, **22**, 2457–2464; (*g*) N. K. Devaraj, P. H. Dinolfo, C. E. D. Chidsey and J. P. Collman, *J. Am. Chem. Soc.*, 2006, **128**, 1794–1795; (*h*) Z. M. Guo, A. W. Lei, X. M. Liang and Q. Xu, *Chem. Commun.*, 2006, 4512–4514; (*i*) M. Slater, M. Snauko, F. Svec and J. M. J. Frechet, *Anal. Chem.*, 2006, **78**, 4969–4975; (*j*) K. M. Kacprzak, N. M. Maier and W. Lindner, *Tetrahedron Lett.*, 2006, **47**, 8721–8726.
6 (*a*) H. B. Mereyala and S. R. Gurrala, *Carbohydr. Res.*, 1998, **307**, 351–354; (*b*) A. K. Pathak, V. Pathak, J. M. Riordan, S. S. Gurcha, G. S. Besra and R. C. Reynolds, *Carbohydr. Res.*, 2004, **339**, 683–691; (*c*) X. H. Lai and S. C. Ng, *Tetrahedron Lett.*, 2003, **44**, 2657–2660.