Click chemistry: a new facile and efficient strategy for preparation of functionalized HPLC packings

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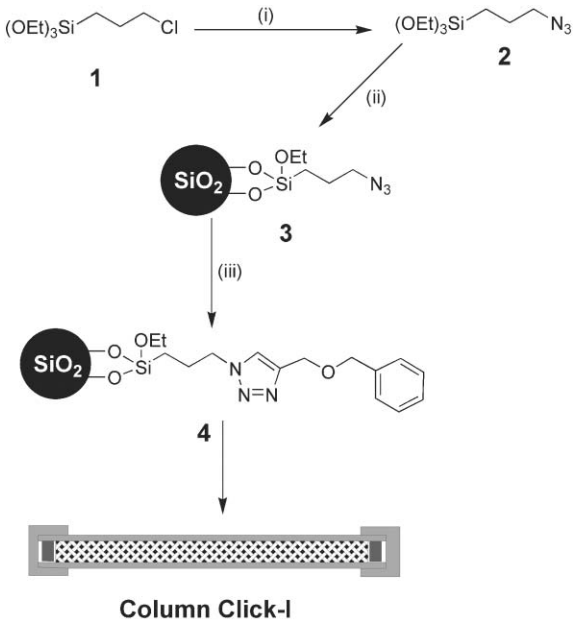
Click chemistry has been successfully extended into the field of preparation of functionalized HPLC packings, proving a novel facile and efficient strategy for covalently bonding stationary phases onto HPLC grade silica beads; the potential has been demonstrated by the preparation of "Click I–IV" columns and preliminary results in the separation of sugars.

Covalently bonded stationary phase on silica beads is the most popular separation medium for high performance liquid chromatography (HPLC). Although a long-chain alkyl is mostly used for reversed stationary phases, other functionalized molecules immobilized on silica beads acting as highly selective stationary phases are required in some cases, such as for separation of enantiomers,[1] positional isomers,[2] and some polar compounds,[3] *etc*. Therefore, exploring for highly selective stationary phases[1c,2,4] and the conjugation strategies[1b,5] between the functionalized molecules and the silica support have been receiving more and more attention. The traditional nucleophilic and electrophilic reactions are currently widely used as the conjugation methods.[1b,1c,5a,5b,5d] These classic conjugations involve reactive groups (such as $-NH_2$, –CNO, –CHO, –COCl, –COOH) on the supports and the functionalized molecules,[1b,1c,5a,5b,5d,6] which sometimes have low selectivity and efficiency. The poor selectivity of the immobilization reaction may cause some side reactions between the silica supports and the other functional groups of the stationary phases, which may result in reducing or even losing the function of the stationary phase.[5f] In addition, the reactive groups on the silica supports may not be fully converted due to the inefficient immobilization process, which will reduce the surface concentration of the stationary phases. There is no doubt that the remnant reactive groups on the support will exhibit different retention mechanisms from the stationary phases, which affects the separation selectivity.[7] Thus, developing a robust, reliable immobilization method with high selectivity under mild conditions for preparation of functionalized HPLC packings remains a challenge in separation chemistry.

The term "click chemistry" was first coined by K. B. Sharpless in 2001.[8] The Huisgen [3 + 2] dipolar cycloaddition between organic azides and terminal alkynes is the primary one of click chemistry,[8,9] which has received more and more attention in many chemistry research fields,[10] including combinatorial chemistry,[11] bioconjugation,[6,12] materials science,[13] solid state phase reaction,[14] surface modification,[15] and others.[16] This reaction has high selectivity and can be carried out in a variety of solvents, including aqueous solution, under mild conditions in the presence of Cu-catalyst. Recently, Finn *et al.* reported an example of the application of click chemistry in affinity chromatography with agarose supports.[6] Frechet and coworkers reported the application of click chemistry in the preparation of HPLC packings, in which they used acrylate polymer beads as the support.[16g] Hoffmann and coworkers investigated the [3 + 2] reaction of azide immobilized on silica with acetylenes and found that the azide groups on silica can be 100% converted to the 1,2,3-triazoles.[15b] Herein, we report that different functionalized molecules can be immobilized on HPLC grade silica beads *via* the "click strategy", and they can act as HPLC packings, which show potential in terms of separation performance and selectivity.

The synthetic route of this new strategy for the preparation of functionalized HPLC packings is shown in Scheme 1. The substitution of chloride from the commercially available reagent 3-chloropropyl triethoxysilane **1** with sodium azide gave the 3-azidopropyl triethoxysilane **2**. Compound **2** was then directly polymerized with silica beads, which yielded azide-silica beads. The "clicking" between the azide-silica **3** and functionalized terminal



Scheme 1 *Reagents and conditions*: (i) NaN_3, toluene, NaI, 70–80 °C; (ii) silica beads, toluene, 70–80 °C; (iii) benzyl propargyl ether, (C_2H_5OH : H_2O 2 : 1), $CuSO_4$, 5 mol%, sodium ascorbate, 15 mol%, RT.

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alkynes in the presence of Cu-catalyst produced the functionalized silica **4**, which was packed as the "Click I" column.

The "click" process can be monitored by FT-IR, in which the peak 2110 cm^{-1} (azide groups) disappeared on the completion of the "clicking" process. It indicates that the "click" strategy can efficiently immobilize the stationary phase on the silica support.[15b] The surface coverage of the stationary phases was detected by elemental analysis (Table 1).

With a similar procedure, two more "click" HPLC columns (Click II, Click III) were prepared, in which the stationary phases include long-chain alkyl groups and an ester group or urea group (entry 2 and entry 3 in Table 1).

The "click" HPLC columns (Click I, Click II, Click III), which are similar to commercial reversed phase columns, exhibit excellent chromatographic properties. The result for the chromatographic characterization of the column Click I with uracil, benzene, toluene and naphthalene as the probes is shown in Fig. 1. The peaks' symmetry is 1.08 and column performance is 52000 plates m^{-1} calculated from naphthalene. The results indicate that "click chemistry" can be applied in the preparation of HPLC packings.

To further demonstrate the potential of "click chemistry" in separation chemistry, we prepared another HPLC column, "Click IV" (Table 1, entry 4), with a polar stationary phase. It is well known that the separation of highly polar compounds remains one of the problems on reversed stationary phases.[17] To address this problem, the hydroxyl group was immobilized on the silica support through the click reaction of the propargyl alcohol with the azide-silica beads.

As shown in Fig. 2, uracil, a standard polar compound which is used to test the dead time in RP-HPLC mode, has good retention and elutes after naphthalene on the column Click IV with acetonitrile–water as the mobile phase. This is the unique character of so-called hydrophilic interaction liquid chromatography (HILIC). It proves that the mono-hydroxyl group immobilized on silica beads *via* click chemistry is a successful polar stationary phase which can demonstrate the function of HILIC.

The evaluation of this "click" HPLC column (column Click IV) in the separation of polar compounds has been extended to the separation of sugars. It is pleasing to find that four kinds of monosaccharide and disaccharide can be successfully separated with the column Click IV (Fig. 3). Compared with the commercial amino columns for the separation of highly polar compounds, the hydroxyl group is a simple and efficient functional group for the separation of polar compounds in HILIC mode. Furthermore, the "click" hydroxyl column is more stable than the amino group in a wide range of pH.

In summary, click chemistry is a sustainable strategy for the preparation of functionalized HPLC packings. The immobilization of long-chain alkyl and hydroxyl groups on HPLC-grade silica

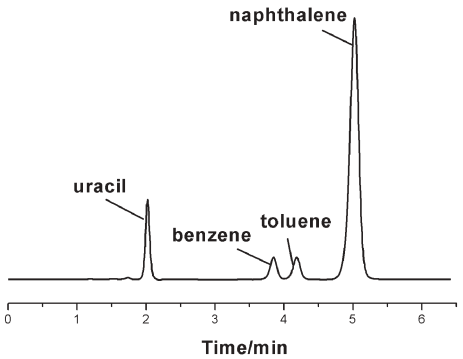


Fig. 1 Chromatogram obtained with the column Click I (4.6 × 150 mm). Conditions: flow rate 0.8 ml min^{-1}; 30 °C; mobile phase: A: water 30%, B: acetonitrile 70%; UV: 254 nm.

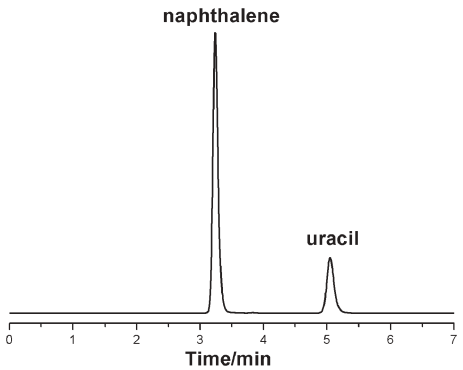
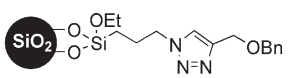
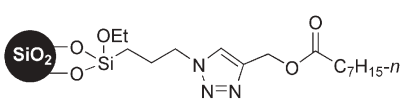
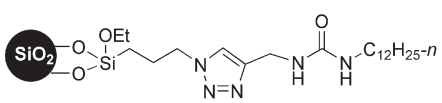
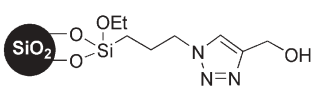


Fig. 2 Chromatogram obtained with the column Click IV (4.6 × 150 mm). Conditions: flow rate 0.6 ml min^{-1}; 30 °C; mobile phase: A: water 5%, B: acetonitrile 95%; UV: 254 nm.

Table 1 Illustrations of the functionalized HPLC packings and the surface coverage of the functional molecules

Entry	HPLC columns	Functionalized HPLC packings	Surface coverage[a] [μmol m^{-2}]
1	Click I		1.76
2	Click II		1.77
3	Click III		1.67
4	Click IV		1.20

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

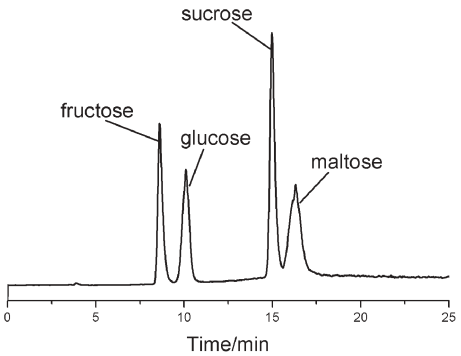


Fig. 3 Separation of monosaccharide and disaccharide on column Click IV. Column: 4.6 × 150 mm. Conditions: flow rate 0.6 ml min^{-1}; 30 °C; mobile phase: A: water, B: acetonitrile; gradient: 0–10 min: 5 → 10% A, 10–25 min: 10% A; ELSD: nitrogen nebulizer gas: 15 psi; tube temperature: 40 °C; gain: 10.

beads has been examined, and the results firmly illustrate the potential of click chemistry in separation chemistry. The scope of this protocol in the preparation of functionalized HPLC packings, and the applications in the separation of polar compounds are undergoing investigation in our laboratory, and will be reported in due course.

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