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A single-step preparation of carbohydrate functionalized monoliths for separation and trapping of polar compounds

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ABSTRACT

A single-step copolymerization strategy was developed for the preparation of carbohydrate (glucose and maltose) functionalized monoliths using click reaction. Firstly, novel carbohydrate-functionalized methacrylate monomers were synthesized through Cu(I)-catalyzed 1,3-dipolar cycloaddition (alkyne-azide reaction) of terminal alkyne with azide of carbohydrate derivatives. The corresponding carbohydrate functionalized monolithic columns were then prepared through a single-step *in-situ* copolymerization. The physicochemical properties and performance of the fabricated monolithic columns were evaluated using scanning electron microscopy, Fourier-transform infrared spectroscopy, and nano-liquid chromatography. For the optimized monolithic column, satisfactory column permeability and good separation performance were demonstrated for polar compounds including nucleoside, phenolic compounds and benzoic acid derivatives. The monolithic column is also highly useful for selective and efficient enrichment of glycopeptides from human IgG tryptic digests. This study not only provided a novel hydrophilic column for separation and selective trapping of polar compounds, but also proposed a facile and efficient approach for preparing carbohydrate functionalized monoliths.

1. Introduction

Serving as energy sources and structural units in organisms, carbohydrates are widely distributed in nature [1]. Due to their important biophysiological functions, the specific interactions between carbohydrates and other biomolecules have attracted increasing interests in recent years [2,3]. To further study and utilize carbohydrate and its derivatives, a useful approach is to immobilize carbohydrates onto solid surfaces [4–7]. For example, carbohydrates have been successfully employed as chromatographic stationary phases for both separation (e.g. polar [5,8–11] and chiral compounds [12–14], and glycoproteins [15–17]) and enrichment (e.g. glycopeptides [13,18,19]). To date, most carbohydrate functionalized columns are based on silica particles, which have some inherent drawbacks such as low immobilization efficiency, complicated preparation, as well as limited pH working range [5,9]. So, it is of great interest to develop a simple and efficient approach for the preparation of the carbohydrate functionalized stationary phases.

Monolithic materials, especially polymeric monoliths, have been extensively used in the field of separation and enrichment because of their advantages, i.e. fast and simple preparation, favorable bio-compatibility, high permeability and good control of porosity [20,21]. However, only few reports were focused on the preparation of carbohydrate functionalized monolithic columns [15,22]. Tetala et al [22] reported a three-step modification method for preparing α-mannose functionalized monolithic columns in fused-silica capillaries. A poly(2-hydroxyethyl methacrylate (HEMA)-*co*-(+)-*N,N'*-diallyltartardiamide (DATD)) monolith was first prepared, then the 1,2-diol groups of DATD were converted to aldehyde groups via periodate treatment, followed by coupling with α-mannose, and finally reductive amination. Although this post-modification allowed to independently control the column morphology and easily obtained satisfied morphology and permeability, it still suffered from some disadvantages like laborious, time-consuming, and uncontrollable leading to poor reproducibility. In order to simplify the preparation process, the same group [15] later

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developed a single-step approach. Polymerizable carbohydrates (based on galactose, glucose, and mannose) were pre-synthesized with an alkene terminated tetraethylene glycol spacer, and the resultant functional monomers were then *in-situ* copolymerized with HEMA, DATD and 1,4-bisacryloyl-piperazine (PDA) to prepare the corresponding monoliths. Unfortunately, no further applications were reported, which are largely due to the lack of commercially available or easy-to-synthesize polymerizable carbohydrate-functionalized monomers (e.g. methacrylate).

Recently, Click chemistry (including alkyne-azide, thiol-ene, and thiol-yne reactions) has been introduced into the preparation of carbohydrate functionalized columns [23]. Among them, the alkyne-azide reaction of Cu(I)-catalyzed 1,3-dipolar cycloaddition, is considered as a common-used click reaction [24], because of its high efficiency, high selectivity, high stereoselectivity, less side reaction and mild reaction condition. In our previous study, the alkyne-azide reaction was applied to synthesize a series of polymerizable β-cyclodextrin functionalized monomers, such as mono-(1H-1,2,3-triazol-4-ylmethyl)2-methylacryl-β-cyclodextrin (PMA-β-CD) and its derivatives [25]. These remind us a new routine to synthesize the polymerizable carbohydrates.

In order to avoid time-consuming and uncontrollable post-modifications mentioned above, facile and rapid approaches are preferred for the preparation of the carbohydrate functionalized monolithic columns. In this work, glucose and maltose, two common sugar units in nature were chosen as the model ligands. Combining with click reaction, a simple *in-situ* single-step strategy were employed to obtain two novel carbohydrate-functionalized monolithic columns, i.e. poly(mono-(1H-1,2,3-triazol-4-ylmethyl) 2-methylacryl-β-D-glucose-*co*-*N,N*-methylenebisacrylamide) (poly (PMA-glucose-*co*-MBA) and poly(mono-(1H-1,2,3-triazol-4-ylm ethyl)2-β-D-methylacryl-maltose-*co*-*N,N*-methylenebisacrylamide) poly(PMA-maltose-*co*-MBA)). The separation and enrichment performance of the two optimized carbohydrate functionalized monolithic columns were systematically study.

2. Experimental

2.1. Chemicals and materials

β-D-glucose (glucose), hydrobromic acid, *N,N*-methylenebisa crylamide (MBA), 2,2′-azobisisobutyronitrile (AIBN), acetonitrile (ACN), formic acid (FA), ammonium formate (AF) and test compounds (including toluene, thiourea, uracil, uridine, adenosine, inosine, cytidine, guanosine, 2,6-dihydroxybenzoic acid (2,6-DHB), 2-hydroxybenzoic acid (2-HB), benzoic acid (B), 4-hydroxybenzoic acid (4-HB) and 2,4-dihydroxybenzoic acid (2,4-DH)) were all purchased from Aladdin Chemical (Shanghai, China). Octa-O-acetyl-β-D-lactopyranose, sodium bicarbonate, tetrabutylammonium bromide, acetic anhydride, sodium ascorbate, cupric sulfate pentahydrate, MeOD-d_4, and DMSO-d_6 were obtained from Macklin Biochemical (Shanghai, China) or Energy Chemical (Shanghai, China). Sodium azide was bought from Beilian Chemical (Tianjin, China). 3-(Trimethoxysilyl)propyl methacrylate (γ-MAPS), propargyl methacrylate (PMA), human serum immunoglobulin G (human IgG), and trypsin (bovine pancreas, tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated) were obtained from Sigma-Aldrich (Shanghai, China). HPLC grade ACN and MeOH were supplied by Merck (Shanghai, China). The fused-silica capillaries (375 μm O.D. $\times$ 100 μm I.D.) were purchased from Ruifeng Chromatography (Hebei, China), and distilled H_2O was purified by Milli-Q system (Massachusetts, MA, USA). All the other reagents are of analytical grade and were purchased from Aladdin Chemical (Shanghai, China).

Tryptic digestion of IgG was prepared as follow: a solution of IgG (1 mg·mL^{-1}) in 50 mM NH_4HCO_3 buffer was denatured at 95°C for 5 min. Then, 40 μL trypsin solution (1 mg·mL^{-1}) was added into the IgG solution, and the resulting solution was incubated at 37°C for 12 h. The tryptic digestion was lyophilized and stored at -20°C until use.

2.2. Instrumentation

^{1}H NMR (300 or 500 MHz) and ^{13}C NMR (75 or 126 MHz) spectra were recorded on a Bruker-AVANCE III Digital NMR Spectrometer (Karlsruhe, Germany). Molecular mass was determined on an Agilent 6210 LC/MS-TOF system. The monolithic columns were prepared using a Jinghong DK-S22 water bath (Shanghai, China). The morphology of the carbohydrate functionalized monolithic column was studied using a Zeiss Gemini ultra-55 field emission scanning electron microscopy (SEM) (Deutschland, Germany) at an acceleration voltage of 5 kV. All of the micro-LC experiments were carried out using a lab-assembled nano-LC system which consists of a DiNa-S nano pump (Tokyo, Japan), a Shimadzu SPD-15C detector (Kyoto, Japan) with a laboratory-made online detection cell, and a Valco four port injection valve with 20 nL internal loop (Houston, USA). All pH values were measured by Sartorius PB-10 pH meter (Gottingen, Germany). Data acquisition and handling were done using a Unimico Trisep^{TM} workstation 2003 (Shanghai, China). All chromatograms were saved as txt files and redrawn using Microcal Origin 9.0. The enrichment experiment was carried out using a Longer TS-2A /L0107-2A syringe pump (Baoding, China). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) experiments were conducted in positive ion mode on a UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, USA) equipped with a delayed ion-extraction device and a pulsed nitrogen laser operated at 337 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV. Glycopeptides searching parameters were set the same as our previous work [26].

2.3. Synthesis of PMA-carbohydrate monomers

PMA-carbohydrates (PMA-glucose and PMA-maltose, **c1** and **c2**) were synthesized via the alkyne-azide reaction according to previous reported method [27,28] with minor modifications (Fig. 1), and the details are shown in **supporting information**. The structure of PMA-glucose and PMA-maltose were determined by ESI-MS, ^{1}H NMR and ^{13}C NMR spectra.

2.4. Preparation of the carbohydrate-functionalized monolithic columns

In order to provide anchoring sites for polymeric bed, the fused-silica capillaries were pretreated by γ-MAPS using previously reported method [29]. The poly(PMA-glucose-*co*-MBA) (**Column A**) and poly(PMA-maltose-*co*-MBA) (**Column B**) were then prepared through single-step *in-situ* copolymerization of monomer mixtures consisting of PMA-carbohydrate monomers (PMA-glucose or PMA-maltose), porogens (H_2O and MeOH), crosslinker (MBA), and the initiator (AIBN) (Fig. 2). The polymerization mixtures were mixed in 2 mL vials and sonicated for 10 min to form homogenous solutions. The mixtures were introduced into the pretreated capillaries with a total length of 20 cm, respectively. Both the ends of capillaries were blocked by rubber plugs and the capillaries were submerged into a water bath at 60°C for 12 h. After completing the copolymerization, MeOH was pumped through the columns to remove the unreacted residuals. An effective length of capillary column was cut for micro-LC and glycopeptide enrichment

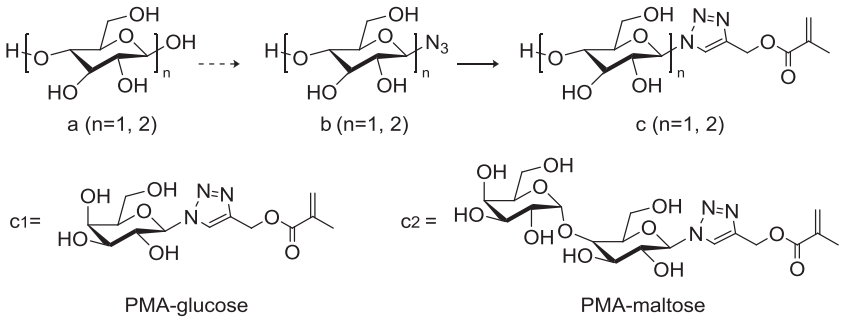

Fig. 1. Scheme of the synthesis of PMA-carbohydrates.

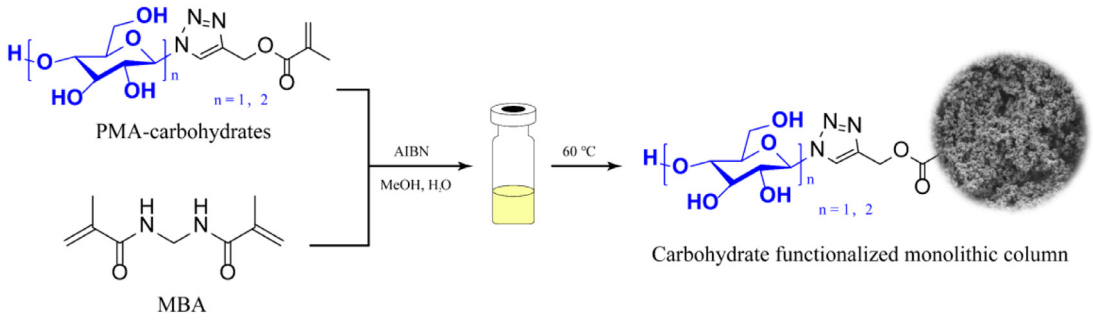

Fig. 2. Preparation of carbohydrate functionalized monolithic columns.

experiments, while another 1 cm of capillary column was cut for SEM analysis.

2.5. Enrichment of glycopeptides

In order to evaluate the enrichment capability of the carbohydrate-functionalized monolithic columns, the tryptic digest of human IgG was used as model sample. A 10 cm of monolithic columns (100 μm I.D.) were first equilibrated with loading buffer (ACN/H_2O/TFA, 80/19/1, % v/v/v). Then 20 μL of sample solution (0.5 mg·mL^{-1} IgG tryptic digestion in loading buffer) was injected on column, and the column was washed with 100 μL of loading buffer to remove unbound compounds. Finally, the trapped peptides were eluted from the column using 20 μL of elution buffer (ACN/H_2O/TFA, 50/49.9/0.1, % v/v/v), and detected by MALDI-TOF MS.

3. Results and discussion

3.1. Synthesis of the PMA-carbohydrates

Two carbohydrates, including glucose and maltose, were employed to evaluate the applicability of the proposed production procedure as presented in Fig. 1. Briefly, the hydroxyl group at the C1' position on carbohydrate was replaced by azido group, and then PMA was attached on host by being an azole ring (click reaction). According the results of ESI-MS, ^{1}H NMR and ^{13}C NMR spectra, all intermediates and products were successfully synthesized. In this procedure, it seems the click reaction with high structural selectivity favored the selective 1,4-conversion, according the ^{1}H NMR spectra of regioisomeric proportion [12,13]. And each step of synthesis procedure can be carried out under mild reaction conditions with high yield (> 70%), further indicating that this procedure could be widely applied to other mono- and oligo- saccharides. Herein, the polymerizable PMA-carbohydrates used as functional monomers in the next fabrication procedure, providing good possibilities to prepare the corresponding monolithic columns.

3.2. Preparation and optimization of carbohydrate-functionalized monolithic columns

In this procedure, because of good solubility for PMA-glucose and PMA-maltose, a mixture of MeOH and H_2O was selected as porogen for preparation of poly(PMA-glucose-*co*-MBA) and poly(PMA-maltose-*co*-MBA) monoliths. The effects of the amount of carbohydrate monomer, weight ratio of porogens and crosslinker in the polymerization mixture were systematically studied and optimized (Tables S1 and S2). For poly(PMA-glucose-*co*-MBA), the effect of monomer content on column performance was firstly investigated by varying the weight content of PMA-glucose at three different percentages, i.e. 15% (**Column A1**), 20% (**Column A2**) and 25% (**Column A3**), respectively. The results showed that increasing the monomer content led to higher column back-pressure. Good permeability was obtained on **Column A2** (0.2 MPa at a flow rate of 300 nL/min). The influence of the composition of porogens (MeOH and H_2O) was also examined. As shown in Table S1 (**Columns A2, A4** and **A5**), the increase of the H_2O content from 30% to 40% caused a significant increase of the back-pressure of monolithic column, even making it hard to pump through the stationary phase. It seems that higher percentage of H_2O in polymerization mixture results in more narrow flow channels in monolithic bed, and then the back-pressure of column increase. Thus, the ratio of MeOH to H_2O was selected as 65/35 (w/w) for further experiments (**Column A4**). Finally, the content of crosslinker MBA was investigated as it can influence permeability and separation ability of monolithic column. As stated in Table S1 (**Columns A4, A6** and **A7**), when the weight content of MBA increased from 18% to 28%, the back pressure of column significantly rose to 5.5 MPa. An MBA weight content of 35% (**Column A4**) can provide lower column pressure (0.5 MPa) and higher efficiencies (about 71,000 plates·m^{-1}). To summarize, **Column A4** was adopted as optimal.

For the poly(PMA-maltose-*co*-MBA), a similar optimization procedure was conducted (Table S2), and it was found that **Column B9** gave most satisfactory back pressure (1.2 MPa) and column efficiencies (39,586 plates·m^{-1}) at a flow rate of 300 nL/min.

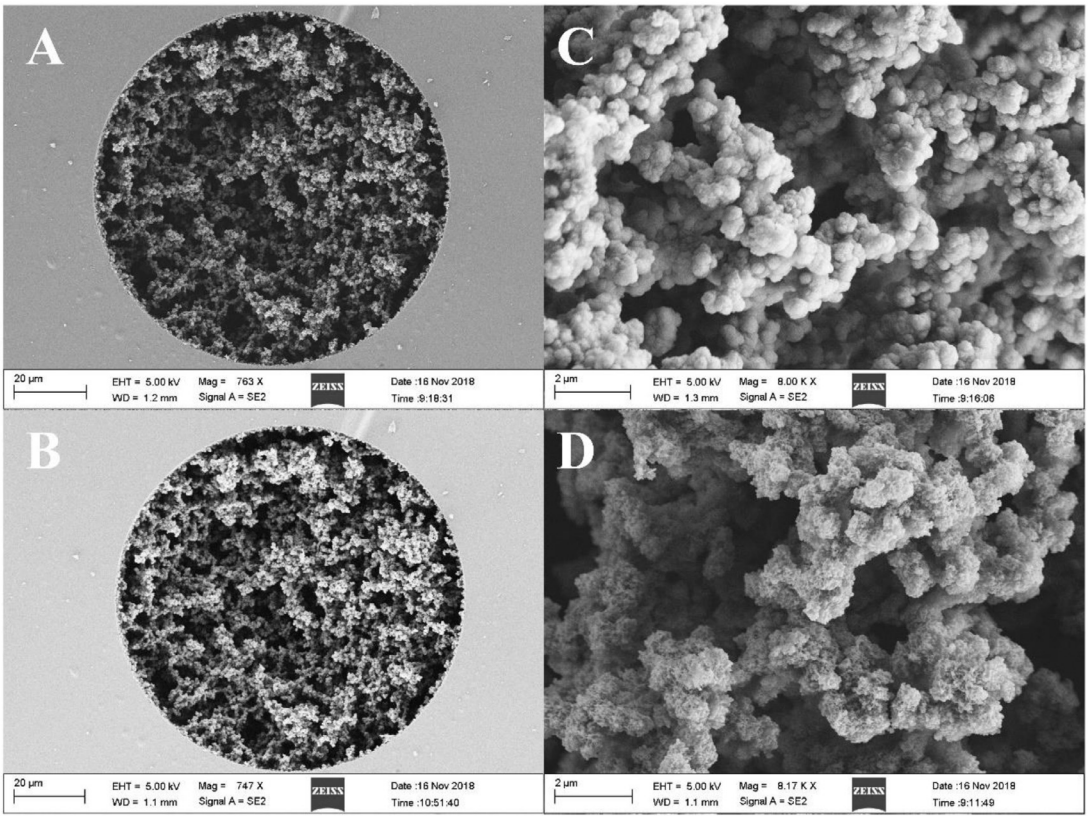

Fig. 3. Scanning electron microphotographs of Column A4 (A, C) and Column B9 (B, D).

The presence of PMA-carbohydrates in the prepared monolith was confirmed by FTIR. As shown in Figure S1c and d, the bands at 3358 cm^{-1} represent the stretching vibration of O-H, and the characteristic absorbance at 1450 cm^{-1}, 1050 cm^{-1} are assigned to -N=N- stretching vibration, -C-N- stretching vibration in azole ring. These results indicated the PMA-glucose and PMA-maltose were successfully anchored on the **Column A4** and **Column B9**, respectively [30].

3.3. Characterization of carbohydrate-functionalized monolithic columns

3.3.1. Column morphology

SEM was employed to characterize the morphology of the optimal monoliths (**Column A4** and **Column B9**). As shown in Fig. 3, the monolith was attached to the inner wall of the capillary, and consisted of large through-pores and tightly connected microsphere clusters. These resulted in a polymer-based monolithic matrix with good permeability and stability for both monoliths. Notably, there is no substantial difference between **Column A4** and **Column B9** (Fig. 3A and B), probably because the same crosslinker and porogens were used, and a slight difference in content of the compounds in the polymerization mixture was insufficient to cause a difference in morphology. When the images were closely inspected (Fig. 3C and D), it became clear that the through-pores on **Column B9** were somewhat larger.

3.3.2. Column permeability, stability and reproducibility

According to Darcy's equation, the permeability ($\boldsymbol{K}$, m^2) of carbohydrate functionalized monolith was calculated according to the following equation [31]:

$$\boldsymbol{K} = \frac{\boldsymbol{uL\eta}}{\boldsymbol{\Delta P}}$$

Table 1
Permeability of Column A4 and Column B9.

Mobile phase	Visocosity η ($\cdot 10^{-3}$ Pa·s)	Permeability K ($\cdot 10^{-14}$ m^2)	
		Column A4	Column B9
ACN	0.369	6.54	12.0
ACN/H_2O (50/50, % v/v)	0.820	3.78	7.32
H_2O	0.890	1.57	6.98

Conditions: Column A4 and Column B9, all are 10 cm × 100 µm I.D.; mobile phase: ACN, ACN/H_2O (50/50, % v/v) and H_2O; UV detection wavelength: 254 nm; temperature: 25°C; injection: 20 nL; sample: toluene.

In this equation, $\boldsymbol{u}$ ($m \cdot s^{-1}$) represent linear velocity of mobile phase, $\boldsymbol{L}$ (m) represent the length of monolithic column, $\boldsymbol{\eta}$ ($Pa \cdot s^{-1}$) represent the viscosity of mobile phase, $\Delta\mathbf{P}$ represent the back pressure drop across the monolithic column. To determine the permeability of monolithic column, ACN, H_2O and ACN/H_2O (50/50, % v/v) were pumped through the column, and toluene was used as column dead-time marker to calculate $\boldsymbol{u}$.

As can be seen in Table 1, when using ACN, ACN/H_2O (50/50, % v/v) and H_2O as mobile phase, the resulting $\boldsymbol{K}$ value for **Column A4** were 6.54×10^{-14}, 3.78×10^{-14} and 1.57×10^{-14} m^2, respectively, and for the **Column B9** were 12.0×10^{-14}, 7.32×10^{-14} and 6.98×10^{-14} m^2, respectively. The lack of significant difference between the observed $\boldsymbol{K}$ values indicated that all monolithic materials remained rigid, showing no significant swelling or shrinkage in different solvents.

The reproducibility of the carbohydrate-functionalized monoliths was determined by repeatedly analyzing thiourea using a mobile phase of ACN/H_2O (90/10, % v/v) and calculating the RSD% for the retention factor (k). Toluene was used as t_0 marker. The run to run (n = 10), day to day (n = 3), column to column (n = 3)

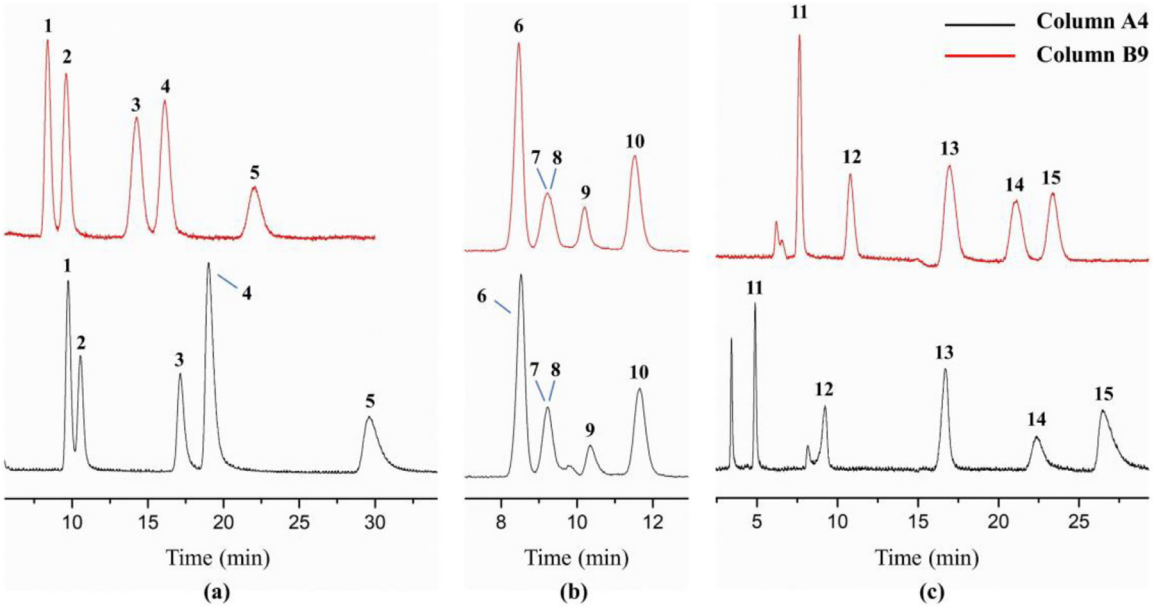

Fig. 4. Separation of nucleosides (a), phenols (b) and benzoic acid derivatives (c) on Column A4 and Column B9. Peaks: uridine (1), adenosine (2), inosine (3), cytidine (4), guanosine (5), phenol (6), hydroquinone (7), resorcinol (8), 4-aminophenol (9), phloroglucinol (10), 2,6-DHB (11), 2-HB (12), B (13), 4-HB (14) and 2,4-DHB (15). The details of whole chromatogram and chromatography conditions were showed in supporting information.

and batch to batch (n = 3) reproducibility of **Column A4** was 1.2, 3.5, 2.5 and 4.5, respectively. **Column B9** was also characterized in same condition, and the value were 2.3, 4.1 5.4 and 2.5, respectively. Those results indicted both prepared columns show satisfactory reproducibility for LC separation.

3.3.3. Retention mechanism

Because of having multiple polar groups, carbohydrates widely used as stationary phase for Hydrophilic Interaction Liquid Chromatography (HILIC) [5,10]. Hydrophilic interaction was considered to be the predominant reason for retention on carbohydrate-functionalized monolithic columns. In this study, the retention behavior was investigated on the **Column A4** and **Column B9** using a test mixture comprising the nonpolar compound toluene and the polar compound thiourea. The content of ACN was varied from 20% to 90% (mobile phase: ACN/H_2O). It was observed on **Column A4** (data not shown here), with the content of ACN increased, the retention factor of toluene still remained constant, while the retention time of thiourea rose dramatically from 60% to 90%. Similar retention was also observed on **Column B9**. These results further demonstrate that **Column A4** and **Column B9** show typical HILIC properties.

3.4. Applications

In order to assess the usefulness of the carbohydrate-functionalized monoliths for the separation of polar compounds, nucleosides, phenols and benzoic acid derivatives were analyzed. Nucleosides, structural subunits of nucleic acids, are the heredity-controlling components of all living cells. It is great challenge to obtain good separation and satisfied retention by using conventional RP-HPLC method for analyzing nucleosides.

Here, a mixture of uridine, adenosine, inosine, cytidine, and guanosine was injected on the **Column A4** and **Column B9** (Fig. 4a). As we can see, a good separation was achieved for the five closely related nucleosides with linear elution under optimized chromatographic conditions. The elution order of those samples is consistent with previous research on carbohydrate functionalized silica-based column [5], which further confirms that HILIC retention mechanism plays an important role in their separation.

A mixture of phenols with different numbers of hydroxy and/or amino substituents were also analyzed on these two monolithic columns. As shown in Fig. 4b, phenol, hydroquinone, resorcinol, 4-aminophenol and phloroglucinol are separated by **Column A4** and **Column B9** using ACN/H_2O (85/15, % v/v) with 0.2% formic acid (FA) as mobile phase. All test samples were eluted within 12 min. Similar to the above point, the retention increased with increasing analyte polarity. However, hydroquinone and resorcinol were not separated on either **Column A4** or **Column B9**. This observation could be explained by the almost same polarity of hydroquinone and resorcinol, the log D values of hydroquinone and resorcinol were 0.62 and 0.82 (Scifinder.cas.org) in this chromatographic condition.

To further evaluate the separation capability of these two monolithic columns, a mixture of five benzoic acid derivatives, including 2,6-DHB, 2-HB, B, 4-HB and 2,4-DHB, was analyzed. As shown in Fig. 4c, a good separation of the five compounds was observed on **Column A4** and **Column B9**, when using ACN/H_2O (85/15, % v/v) with 0.2% ammonium acetate (pH 4.5) as mobile phase. As mentioned above, these results demonstrate that the optimized poly(PMA-glucose-*co*-MBA) (**Column A4**) and poly(PMA-maltose-*co*-MBA) (**Column B9**) monolithic columns are suitable for the separation of polar compounds, as well as the success of our preparation method was further determined.

In particular, HILIC method has attracted increasing attention, owing to its broader glycan specificity, better reproducibility, and compatible with MS analysis [32,33]. In this study, the effectiveness of **Column A4** and **Column B9** in glycopeptide enrichment on HILIC mode was evaluated. Human immunoglobulin G (IgG) as standard sample. Fig. 5A showed the mass spectrum of the tryptic digest without treatment. No glycopeptides could be detected due to the ion suppression of the nonglycopeptides. After treatment of tryptic digest with **Column A4** or **Column B9**, the signal of non-glycosylated peptides in the mass spectra was significantly eliminated, while the signal of glycopeptides was extremely enhanced (Fig. 5B and C). Apparently, the carbohydrate-functionalized monoliths do not retain non-glycosylated peptides (such as 2546.087,

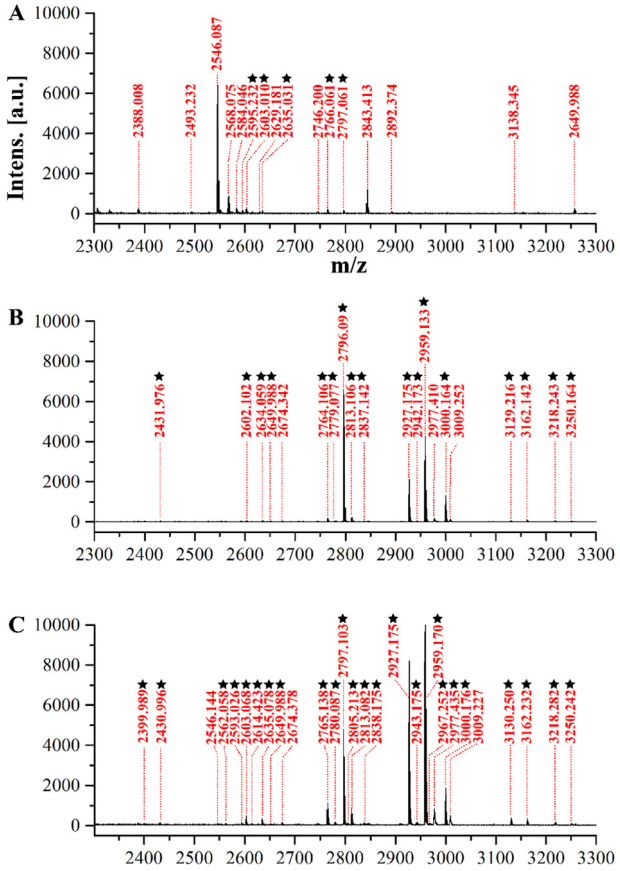

Fig. 5. MALDI-TOF mass spectrum of tryptic digested IgG (2 pmol) in different conditions. Mass spectrum of tryptic digested IgG without enrichment (A); Mass spectrum of tryptic digested IgG after enriched by Column A4 (B); Mass spectrum of tryptic digested IgG after enriched by Column B9 (C); Conditions: loading buffer (ACN/H_2O/TFA, 80/19/1, % v/v/v; 100 μL); elution buffer (ACN/H_2O/TFA, 50/49.9/0.1, % v/v/v; 20 μL); Glycopeptides are labeled with ⋆.

2568.075 and 2843.413), whereas glycopeptides are significantly retained and therefore enriched. In total, 17 and 24 glycopeptides were detected after enrichment by **Column A4** and **Column B9** respectively, which are much better than other work achieved on a zwitterionic hydrophilic monolith [26] and glucose-6-phosphate functionalized magnetic microspheres [34]. Considering the number and signal intensity of the observed glycopeptides in Fig. 5B and C, the enrichment ability of **Column B9** which comprises more hydroxyl groups at the functional monomer, seems to be better than trapping efficiency of **Column A4**. Overall, the results suggest that PMA-carbohydrate functionalized monolith has great potential for glycopeptides enrichment.

4. Conclusions

A new effective single-step strategy for the preparation of carbohydrate-functionalized methacrylate-based monolithic columns was developed in this work. Click chemistry approach (an alkyne-azide reaction) was used for synthesizing polymerizable carbohydrate functionalized monomers. The major advantage of our procedure is a simple *in-situ* single-step preparation of the monolithic column by directly crosslinking the novel carbohydrate monomers with a conventional crosslinker. This approach is a potential and promising method for synthesizing other polymerizable carbohydrate-functionalized monomers. Satisfactory column permeability, efficiency, and separation performance for polar test compounds were obtained for the optimized monolithic columns. Moreover, the monolithic columns also show great potential in glycopeptides enrichment from a tryptic digest of an IgG. We believe that the "PMA-carbohydrate" monomers, together with the fabrication strategy, will become more helpful for monolithic stationary phase preparation in many research fields.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Jincai Wang: Conceptualization, Methodology, Writing - original draft. **Jialiang Guo:** Data curation, Formal analysis. **Haowen Chen:** Investigation, Resources. **Xiaoling Huang:** Validation. **Govert W. Somsen:** Writing - review & editing. **Fenyun Song:** Project administration. **Zhengjin Jiang:** Supervision, Funding acquisition, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2020.461481.

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