Polymer Bulletin 41, 183–189 (1998)

Polymer Bulletin


# Use of branched aliphatic linkers for the preparation of selective chiral media for the HPLC separation of enantiomers

**Yuelong Liu[1], Kathleen N. Juneau[2], Frantisek Svec[1], Jean M. J. Fréchet[1,*]**

[1] Department of Chemistry, University of California, Berkeley, CA 94720-1460, USA
[2] Hoechst Celanese Corporation Technical Center, Corpus Christi, TX 78469, USA

Received: 31 March 1998/Revised version: 27 May 1998/Accepted: 27 May 1998

**Summary**
Highly branched aliphatic molecules have been used for the first time as tethers in the preparation of polymeric chiral stationary phases. Attachment of a specifically designed chiral selector to organic porous polymer beads through a branched linker substantially increases the enantioselectivity compared to that of the equivalent separation medium with a traditional linear tether.

## Introduction

The separation of mixtures of enantiomers to obtain pure chiral compounds is difficult because the physical and chemical properties of both optical isomers are identical in an achiral environment. Since substantial improvements are expected from the use of single enantiomers in applications such as drugs, pesticides, flavors, and pheromones, their direct preparation or the separation of individual enantiomers from their mixtures is desirable and requires the development of new, efficient, technologies. The separation of individual enantiomers trough processes such as the crystallization of diastereomers dates back more than 100 years (1). The separation of enantiomers using a solid support was also suggested a long time ago (2). However, it was not until 1960 that the first chromatographic enantioseparation was reported (3). Currently, chromatography is frequently the method of choice for chiral separations because of its simplicity, high efficiency, and easy scale-up (4). Numerous chiral stationary phases (CSP) for both gas and liquid chromatography are now commercially available (4,5).

Several families of chiral selectors that include π-π donor-acceptor complex forming functionalities (6), proteins (7), antibiotics (8), synthetic polymers and polysaccharides (9), cyclodextrins and crown ethers (10), and transition metal complexes (11) have been attached to solid matrix and used for the chromatographic separation of enantiomers. Two general mechanisms for chiral recognition with these selectors are described: (i) the recognition results from the difference in stability of two labile diastereomeric selector-enantiomer complexes formed in 1:1 ratio which is typical of well-defined single molecule selectors (6,11); (ii) cooperative interactions and inclusion phenomena characteristic of selectors with several asymmetric centers and cavities (7-10).

"Brush" type CSPs for the HPLC separations that were pioneered by Pirkle (6) typically involve a chiral selector, a tether, and a solid porous support. Much has been already done, in particular by Pirkle and his coworkers, in the area of selectors that may

* Corresponding author

be designed specifically for the required separation (6). Selectors in most of commercial CSPs for HPLC are attached to porous silica beads. The use of silica as a chromatographic support benefits from some obvious advantages such as its ready availability, resistance to swelling, and good efficiency. However, care must be taken to eliminate residual silanol groups on the surface of silica to avoid non-specific interactions with analyte enantiomers that would deteriorate the separation (6,12). Though long neglected, synthetic polymer beads offer certain features such as stability over the entire range of pH, and a wealth of well controlled surface chemistries that make them alternative for the preparation of chiral separation media. Today, only a few types of chromatographic packings based on a synthetic polymer are available for chiral HPLC separations (13). We have recently demonstrated, that the use of a porous polymer matrix substantially increases enantioselectivity and decreases the retention times when compared to the analogous silica-based brush-type chiral stationary phase (14).

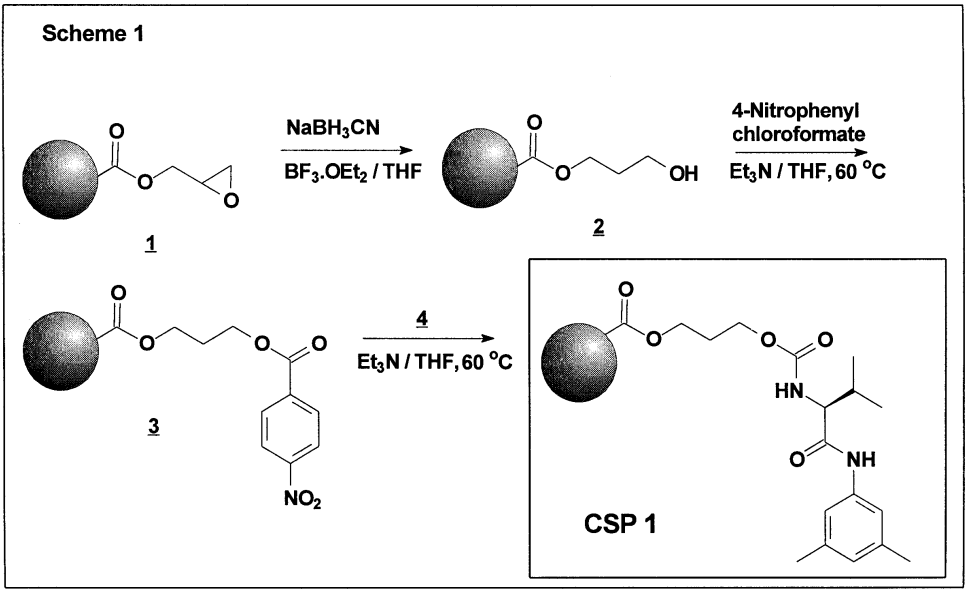
In the case of chiral stationary phases that have to exhibit selectivity toward compounds differing only in geometry, the effect of residual surface chemistry (e.g. silanol groups in silica beads) is claimed to decrease as the distance between ligand and surface is increased (15). While the use of linear spacer arms is currently well-established (15), we now report our preliminary work on the application of novel highly branched spacers that make use of some of the three dimensional space within pores for the placement of chiral selector groups.

## Experimental

*Uniformly sized macroporous poly(glycidyl methacrylate-co-ethylene dimethacrylate) beads*

The monodisperse porous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads **1** (Scheme 1) were prepared by a staged templated suspension polymerization process reported elsewhere (16).

The specific surface area of the porous beads calculated from the BET isotherm of nitrogen was 84 $m^2/g$; the pore volume of 1.12 ml/g and the median pore size of 33 nm were determined in dry state by mercury intrusion porosimetry using a custom-made combined BET-Sorptometer and Mercury Porosimeter from Porous Materials (Ithaca,



NY). The content of epoxide groups (1.53 mmol/g) was determined by volumetric titration.

*Reduced beads,* **2**

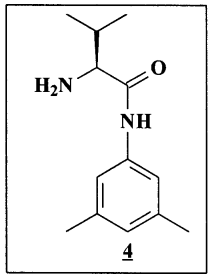
To a slurry of 2.0 g of beads **1** in 25 ml of dry tetrahydrofuran was added 10 ml of 1 mol/l $NaBH_3CN$ in tetrahydrofuran (THF), and a small amount of bromocresol green indicator. Boron trifluoride diethyl etherate was then introduced dropwise into the reaction mixture with gentle stirring until the color changed to yellow. The reaction was maintained at 50 °C for 5 h with occasional stirring while additional $BF_3$ $OEt_2$ was added periodically to maintain the acidity. Upon completion, the beads were washed successively with 1 mol/l NaOH, water, methanol, tetrahydrofuran, and ethyl ether, and dried under vacuum. The content of epoxide groups remaining in the beads was 0.28 mmol/g. This small amount of epoxide moieties was deemed to be unreactive.

*Activated beads* **3**

To 2.0 g of the reduced beads **2** suspended in 30 ml of dry tetrahydrofuran were added 1.2 g of 4-nitrophenyl chloroformate and 0.4 g of triethylamine. After the addition, the reaction mixture was heated at 60 °C with stirring overnight. The modified beads were then washed repeatedly with tetrahydrofuran and ethyl ether, and dried under vacuum. Nitrogen analysis indicated that the resulting beads contained 0.89 mmol/g of 4-nitrophenyl carbonate groups on their surface.

*Chiral stationary phase CSP 1 (Scheme 1)*

The preparation of *L*-valine-3,5-dimethylanilide selector **4** has been described elsewhere (14). This compound (1 g) and triethylamine (0.15 g) were added to 1.2 g of the polymer beads **3** suspended in 10 ml of dry tetrahydrofuran. The resulting slurry was heated at 60 °C with stirring overnight. The beads so modified with the chiral selector were then washed thoroughly with methanol and tetrahydrofuran. The surface coverage of the beads was calculated to be 0.44 mmol/g based on elemental analysis assuming that all of the nitrogen originates from the chiral selector functionalities.



**4**

*Chiral stationary phase CSP 2 (Scheme 2)*

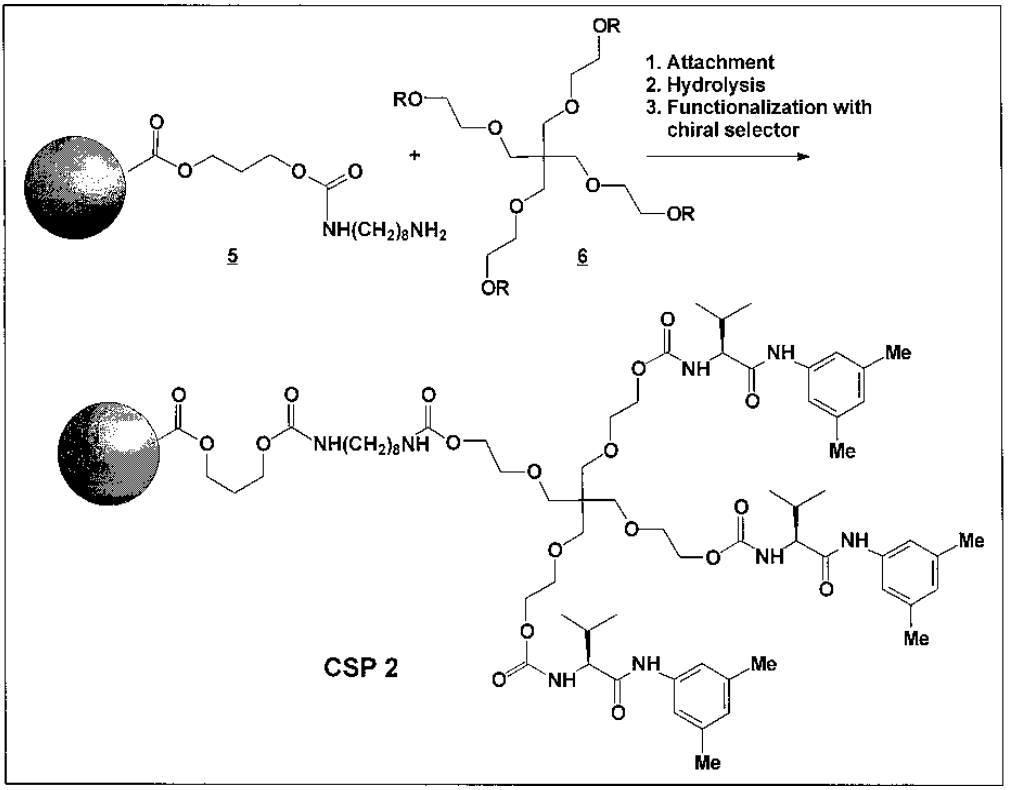
Activated beads **3** (2.4 g) were suspended in 25 ml THF and modified with a large excess of 1,8-diaminooctane (6.91 g) under reflux for 24 h to afford support **5** with 0.41 mmol/g attached diamine. To ethoxylated pentaerythritol (Perstorp Polyols, Sweden, 1 g) dissolved in dry THF (10 ml) 3.4 g of 4-nitrophenylchloroformate were added and 10 ml of pyridine were admixed at a temperature of 0 °C. The temperature was slowly increased and the mixture held under reflux for 24 h. After cooling to the ambient temperature, the solid was filtered, the mixture diluted with 20 ml ethyl acetate and washed twice with water. The organic phase was collected and dried over anhydrous magnesium sulfate, the solvent was evaporated *in vacuo* to afford 2.66 g of the activated compound **6**. This compound (1.4 g) was dissolved in a mixture of THF (15 ml) and triethylamine (0.4 g) added to 1.2 g of aminated beads **5** and the reaction was allowed to proceed under stirring for 24 h at a temperature of 75 °C. The beads were washed by decantation with a few portions of THF, dichloromethane, THF/ethanol (1:1), and dried. *L*-Valine-3,5-dimethylanilide **4** (0.8 g, 2.5 equiv.) was added to 1.2 g of the beads in 20 ml dichloromethane and the reaction allowed to proceed for 32 h. The surface coverage of the selector was less than 0.11 mmol/g according to results of nitrogen analysis.

*Chiral stationary phase CSP 3*

Using a very unoptimized method that essentially duplicates that used in the preparation of CSP 2, a hyperbranched aliphatic polyester said to be terminated with 32 hydroxyl groups (MW ca. 3 600, G-3, Perstorp Polyols) was activated, attached to beads **5**, and reacted with the chiral selector **4** to afford CSP 3 with a surface coverage of only 0.025 mmol/g.

*Chromatography*

The chiral stationary phases were slurry packed at the constant pressure of 15 MPa into 4.6 x150 mm stainless steel HPLC column using methanol as the dispersion liquid. A Waters HPLC system consisting of two 510 HPLC pumps, a 717plus autosampler, and a 486 UV detector, and Millenium 2010 software was used throughout. 1,3,5-Tri-tert.-butylbenzene was used to determine column void volumes under normal phase conditions. Selectivity $\alpha$ was calculated as a ratio of retention factors *k'* for the more and less retained enantiomer. If no separation is observed, the $\alpha$ value is 1.




*Racemic analytes*

*N*-(3,5-Dinitrobenzoyl)-$\alpha$-amino acid methyl ester and alkyl amides **7, 8,** and **9** were prepared by methods similar to those previously reported (17).

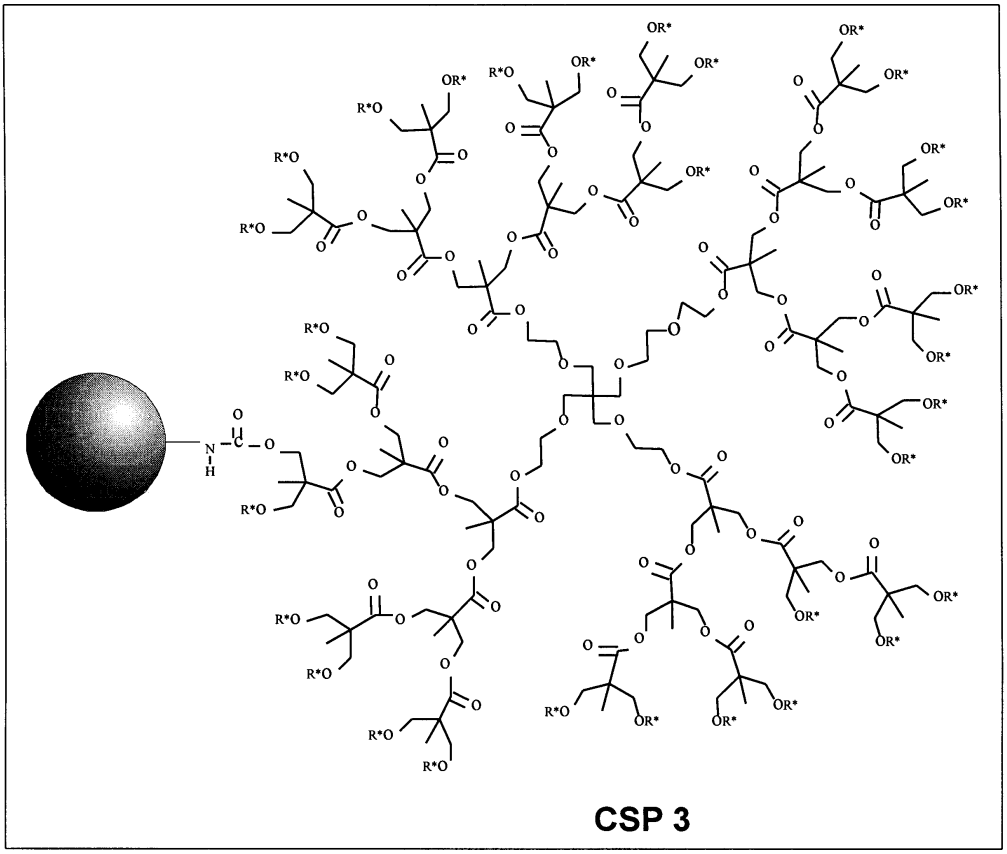
## Results and discussion

*Model CSPs*

First, the model "brush"-type CSP 1 was prepared attaching the selector moiety **4** to the polymeric support through an alkyl chain using reaction steps shown in Scheme 1. CSP 1

has a surface coverage of 0.44 mmol/g and exhibits remarkably high selectivities in the range 2.19-7.11 (Table 1) that exceed those obtained with a CSP bearing the same selector but based on silica beads (14). A column efficiency in excess of 30 000 plates/m was easily achieved with these 5 µm beads although the packing procedure was not optimized. This number translates into the reduced plate height of 4.4 bead diameters per plate.

In the next preparation, a readily available branched molecule, ethoxylated pentaerythrytol, with four hydroxyl groups was used for the preparation of branched chiral stationary phase CSP 2 (Scheme 2). The hydroxyls of the polyol were activated with 4-nitrophenyl chloroformate. In parallel, the activated methacrylate beads **3** were modified with an excess of 1,8-diaminooctane to afford primary amino groups attached to the solid surface. Used in excess, the activated polyol **5** then reacts with the amino groups of the support, presumably leaving some of the carbonate groups available for the subsequent reaction with the selector **4**. Although no optimization of the preparation was attempted and the surface coverage of the selector was only 0.11 mmol/g, CSP 2 exhibits very good selectivity for all of the racemates tested with selectivities in the range 1.98-5.77 (Table 1).




*Attempted modification of the surface with selector terminated hyperbranched polyol linker*

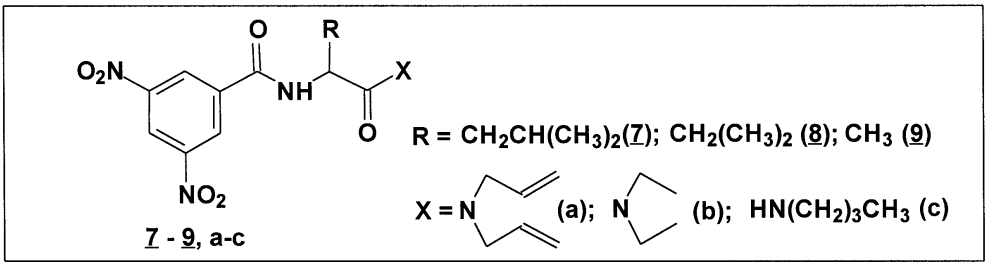
CSP 3 was obtained in a preliminary attempt to extend the concept of branched linkers

even further. Its unoptimized preparation mirrored that of CSP 2. Clearly, neither the dendritic polyol available commercially from Perstorp nor the preparative procedure tested are the best suitable for the preparation of a highly selective CSP. Surprisingly, and despite its extremely low loading of 0.05 mmol/g, even this CSP 3 displayed rather high selectivities of up to $\alpha$=4.09 (Table 1).

**Table 1.** Selectivities $\alpha$ achieved for the separation of racemates on columns packed with chiral stationary phases

| Analyte[a] | CSP 1 | CSP 1a | CSP 1b | CSP 2 | CSP 3 |
|---|---|---|---|---|---|
| | Surface coverage, mmol/g | | | | |
| | 0.44 | 0.05 | 0.10 | 0.11 | 0.05 |
| 7a | 7.11 | 1.44 | 1.68 | 5.77 | 4.09 |
| 7b | 7.25 | 1.27 | 1.43 | 5.15 | 3.26 |
| 7c | 3.57 | 1.16 | 1.37 | 2.97 | 2.36 |
| 8a | 3.06 | 1.00 | 1.00 | 2.09 | 1.59 |
| 8b | 3.34 | 1.00 | 1.00 | 2.26 | 1.55 |
| 8c | 2.37 | 1.00 | 1.00 | 1.98 | 1.44 |
| 9a | 6.17 | 1.00 | 1.31 | 3.63 | 2.45 |
| 9b | 5.54 | 1.00 | 1.39 | 3.52 | 1.84 |
| 9c | 2.19 | 1.00 | 1.18 | 2.46 | 1.71 |

Column 150 x 4.6 mm i.d.; mobile phase: 20% hexane in dichloromethane; flow rate: 1 mL/min; void marker: 1,3,5-tri-tert.-butylbenzene. [a] Racemic analytes:




A few more chromatographic experiments were performed to document the effect of the branched linker on the enantioselectivity of the separation medium. As expected, no separation was observed with the original beads 2 that contain only hydroxyl groups. Similarly, beads with attached branched polyol linker and hydrolyzed nitrophenyl ester groups (no selector is involved) do not exhibit any enantioseparation. These control experiments confirm the expected fact that only the selector functionalities afford the chiral recognition.

Overall our results show that the "brush"-type CSP 1 exhibits the highest selectivity ($\alpha \leq 7.11$) compared to all other CSPs in this study. However, the surface coverage of 0.44 mmol/g is much higher than that of both CSP 2 and CSP 3 and it is therefore difficult to compare directly the selectivities of the three CSPs reported herein. Clearly, much work remains to be done to prepare optimized chiral column with selectors anchored through a dendritic architecture.

In an effort to mitigate the effect of loading and obtain a crude preliminary comparison of the performance of brush and branched architecture, dilution experiments were carried out. The original beads of CSP 1 were "diluted" with unmodified beads **2** to achieve the overall selector coverage per column of 0.05 mmol/g (CSP 1a) and 0.1 mmol/g (CSP 1b). Although these columns do not match exactly those with branched spacers, the results in Table 1 show that the enantioselectivity decreases rapidly as the

overall content of selector functionalities in the column decreases. For example, in contrast to an $\alpha$ value of 4.09 for the analyte 7a and CSP 3, these values are only 1.44 and 1.68 for CSPs 1a and 1b, respectively.

This finding suggests that the presence of highly branched tether may be a desirable feature in the development of efficient chiral separation media. We now plan to develop more controlled processes for the attachment of chiral selector moieties onto dendritic tethers to optimize the use of the volume rather than only the surface of macroporous separation media.

**Acknowledgments**

Financial support of this research by Hoechst-Celanese Corporation is gratefully acknowledged. Partial support by the National Institute of General Medicinal Sciences of the National Institutes of Health (GM 44885) for F.S. is also acknowledged. Thanks are also due to Perstorp Polyols, Perstorp, Sweden for providing us with samples of branched polyhydroxyl compounds.

**References**

1. Theilacker W., Winkler HG (1954) Chem. Ber. 87: 690
2. Willstatter R (1904) Chem. Ber. 37: 3758
3. Klemm LH, Reed D (1960) J. Chromatogr. 3: 364
4. Allenmark S (1991) Chromatographic Enantioseparation: Methods and Applications, 2nd Ed., Horwood, New York; Ahuja S (1997) Chiral Separations. Applications and Technology, American Chemical Society, Washington, DC
5. Siret L, Bargmann-Leyder N, Tabute A, Caude M (1992) Analusis 20: 427
6. Pirkle WH, Pochapsky TC (1989) Chem. Rev. 89: 347
7. Allenmark S, Bomgren B (1983) J. Chromatogr. 264: 63; Hermansson J. (1983) J. Chromatogr. 269: 71; Miwa T, Miyazawa T, Kayano M., Miyake Y (1987) J. Chromatogr. 408: 316.
8. Armstrong DW, Tang Y, Chen S, Zhou Y, Bagwill C, Chen JR (1994) Anal. Chem. 66: 1473.
9. Okamoto Y, Suzuki K, Ohta K, Hatada K, Yuki J (1979) J. Am. Chem. Soc. 101: 4768; Okamoto Y, Kaida Y (1994) J. Chromatogr. 666: 403
10. Armstrong DW, DeMond W (1984) J. Chromatogr. Sci. 22: 411; Shinbo T, Yamaguchi T, Nishimura K, Suguira M (1987) J. Chromatogr. 405: 145; Sogah GDY, Cram DJ (1976) J. Am. Chem. Soc. 98: 3038; Hilton M, Armstrong DW (1991) J. Liquid Chromatogr. 14: 9
11. Davankov VA (1980) Adv. Chromatogr. 18: 139
12. Pirkle WH, Welch CJ (1992) J. Chromatogr. 589: 45
13. Hargitai T, Reinholdson P, Tornel B, Isakson R (1991) J. Chromatogr. 540: 145; Wulf G (1995) Angew. Chem., Int. Ed. Engl. 34: 1812; Sinibaldi M, Castellani L, Federici F, Messina A, Girelli AM, Lentini A, Tesarova E (1995) J. Liq. Chromatogr. 18: 3187; Mosbach K (1994) TIBS 19: 9
14. Liu Y, Svec F, Fréchet JMJ (1997) Anal. Chem. 69: 61
15. Pirkle WH, Hyun MH (1985) J. Chromatogr. 328: 1; Bargmann-Leyder N, Truffert JC, Tambuté A, Caude M (1994) J. Chromatogr. A 666: 27; Welch CJ (1994) J. Chromatogr. A 666: 3
16. Smigol V, Svec F (1992) J. Appl. Polym. Sci. 46:1439
17. Pirkle WH, Pochapsky TC (1986) J. Chromatogr. 369: 175