Effect of Multivalency on the Performance of Enantioselective Separation Media for Chiral HPLC Prepared by Linking Multiple Selectors to a Porous Polymer Support via Aliphatic Dendrons

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Chiral stationary phases (CSPs) containing L-proline indananilide chiral selectors attached through a multivalent dendritic linker to monodisperse macroporous poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads have been prepared using two different approaches. The convergent method involves the preparation of ligands in solution and their subsequent attachment to the support. The divergent approach is based on the stepwise "on-bead" formation of the linker using methods that are typical of solid-phase synthesis. While the convergent CSPs feature well-defined ligands, their loading is relatively low. In contrast, the divergent technique affords CSPs with higher loading but with more limited control over precise ligand architecture. Excellent enantioselectivities characterized by separation factors of up to 31 were achieved for the separation of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid alkyl amides with these new CSPs under normal-phase HPLC conditions.

Introduction

Following safety and regulatory suggestions, the vast majority of new chiral drugs are now administered as pure single enantiomers. Therefore, the development and manufacturing of single enantiomer drugs continues to be a prime target of the pharmaceutical industry.[1] It is well-known today that the enantiomers of a single compound may differ considerably in properties such as pharmacodynamics, pharmacokinetics, potency, toxicity, and metabolism.[2] Consequently, the testing of both enantiomers of new chiral drugs is made by drug companies and required by regulatory agencies for drugs administered as a racemate.[3]

Synthesis from chiral starting materials, asymmetric synthesis, and chromatographic separations are currently the methods most often used to obtain enantiomerically pure compounds.[3a,4] Liquid chromatography is typically carried out in columns packed with a chiral stationary phase (CSP) that can be used in the analytical, preparative, and even the production scales. For example, the recently implemented simulated moving bed preparative liquid chromatography process enables the annual production of up to 50 metric tons of pure enantiomers.[5] Since chromatographic separations are also used for process monitoring, quality control, as well as for the "polishing" of optical purity of products prepared using large scale asymmetric synthesis, current goals in chiral chromatography include both highly efficient analyses optimized for rapid, real time, monitoring and control, as well as large scale downstream processing of both racemic and enantiomerically enriched products.

By and large, the separation of racemates into pure enantiomers is a challenging task because the physical and chemical properties of both optical isomers are identical in an achiral environment. While the separation of enantiomers using a solid support was first suggested by Willstätter almost a century ago,[6] it was not until 1960 that the first chromatographic enantioseparations were realized.[7] The pioneering studies of Allenmark, Armstrong, Blaschke, Cram, Davankov, Hermansson, Okamoto, Pirkle, and others have led to a variety of stationary phases useful in the separation of enantiomers. A considerable number of CSPs for liquid chromatography have emerged during the last two decades and more than a hundred of them are now being actively used in industrial and academic laboratories.[8]

In addition to the intrinsic capability of the immobilized chiral selector to differentiate between the two enantiomers, the separation factor α or enantioselectivity is affected by a number of other factors such as the manner by which the selector is tethered to the support, the length and chemistry of the tethering arm, the nature of the underlying support, and the presence of variable amounts of undesired nonspecific interacting sites. All of these factors affect the overall chiral recognition

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(1) Stinson, S. C. *Chem. Eng. News* **1998**, *76*(38), 83.

(2) Sheldon, R. A. *Chirotechnology: Industrial Synthesis of Optically Active Compounds*; Marcel Dekker: New York, 1993.

(3) (a) Brown, J. R. In *A Practical Approach to Chiral Separations by Liquid Chromatography*; Subramanian, G., Ed.; VCH: Weinheim, 1994. (b) Caldwell, J. *J. Chromatogr.* **1996**, *719*, 3. (c) Branch S. K. In *Chiral Separation Techniques. A Practical Approach*; Subramanian, G., Ed.; Wiley-VCH: New York, 2001.

(4) (a) Jinno, K., Ed. *Chromatographic Separations Based on Molecular Recognition*; Wiley-VCH: New York, 1997. (b) Ahuja, S., Ed. *Chiral Separations: Applications and Technology*; American Chemical Society: Washington DC, 1997. (c) Gawley, R. E.; Aubé, J. *Principles of Asymmetric Synthesis*; Pergamon: New York, 1996. (d) Aboul-Enein, H. Y., Wainer, I. W., Eds. *The Impact of Stereochemistry on Drug Development and Use*; Wiley-VCH: New York, 1997.

(5) McCoy, M. *Chem. Eng. News* **2000**, *78*(25), 17.

(6) Willstätter, R. *Chem. Ber.* **1904**, *37*, 3758.

(7) Klemm, L. H.; Reed, D. *J. Chromatogr.* **1960**, *3*, 364.

(8) (a) Allenmark, S. *Chromatographic Enantioseparations: Methods and Applications*; Ellis Horwood, New York, 1991. (b) Siret, L.; Bargmann-Leyder, N.; Tambute, A.; Caude, M. *Analusis.* **1992**, *20*, 427. (c) Beesley, T. E.; Scott, R. P. *Chiral Chromatography*; Wiley-VCH: New York, 1998.

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process and, therefore, the performance of the chiral separation media.

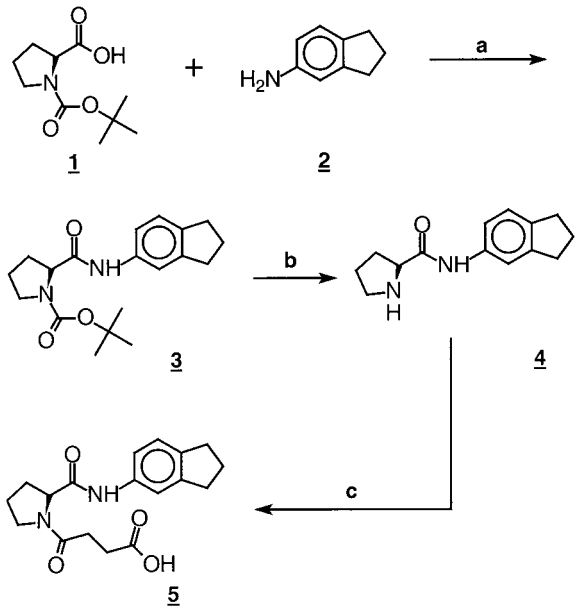
Current Pirkle-type CSPs rely mostly on the monovalent interactions of an analyte with a single isolated ligand attached to a support. In contrast, many recognition processes in nature are facilitated and significantly enhanced by multivalent ligands and multivalent receptors. In fact, multivalency is likely to emerge as the new frontier in the design and development of a broad range of biologically active compounds.[9] Although the source of the enhanced properties derived from multivalency has not yet been unambiguously elucidated, it is believed to originate either from the synergetic effect of specifically located multiple ligands or from an aggregation and precipitation process.[10] Regardless of the mechanism, a substantial increase in binding strength should prove valuable to the development of at least some specialized enantioselective separation media.

In 1986, Pirkle found that the separation factor for enantiomers of bivalent *N*-(3,5-dinitrobenzoyl)leucine bisamide analyte on monovalent CSP was higher by a factor of almost 20 when compared to that of the monovalent analyte.[11] Presumably, such enhancement in selectivities was due to the multiple binding interactions similar to those observed for chelates. Unfortunately, this approach is not very practical, as it would require the "dimerization" of the enantiomers onto a divalent linker, their separation on a column, and the subsequent cleavage of the desired single enantiomers from the linker. Therefore, other concepts leading to a considerable enhancement of the selectivity are desirable. One of these approaches may consist in the use of multivalent selectors. Indeed, we have recently observed in a preliminary study that attachment of a chiral selector to organic porous polymer beads through a branched linker increases the specific enantioselectivity over that of the equivalent separation medium with a traditional linear tether.[12]

Dendrons are monodisperse polymers that have a well-defined architecture and are made from branched repeat units. As a result of their highly branched structure, dendritic molecules are ideal for positioning a specific number of functional groups in precisely defined locations.[13] Unlike typical dendrimers, dendrons have two different functionalities: one located at the focal point and a multiplicity of functionalities of an orthogonal type located at the periphery. These features make dendrons very suitable for assembling multivalent ligands and their attachment to a solid support through a single reactive site.

In this paper, we wish to describe in detail two general strategies that afford CSPs with dendritic polyester linkers connecting a model selector to the porous polymer support: a divergent scheme where the linker is grown from the surface of the support followed by functionalization with selector (ligand) and a convergent scheme where a well-defined selector-functionalized polyester dendron is anchored onto the solid support. In assessing the efficacy of each strategy, spacer inclusion, dendron generation, and intermediate endcapping were examined for their effects on the selector loading and the performance of these CSPs.

Scheme 1. Synthesis of Chiral Selector[a]



[a] Key: (a) EEDQ, CH_2Cl_2; (b) TFA, AcOH, CH_2Cl_2; (c) succinic anhydride, DMAP, 1:1 THF/Et_3N.

Results and Discussion

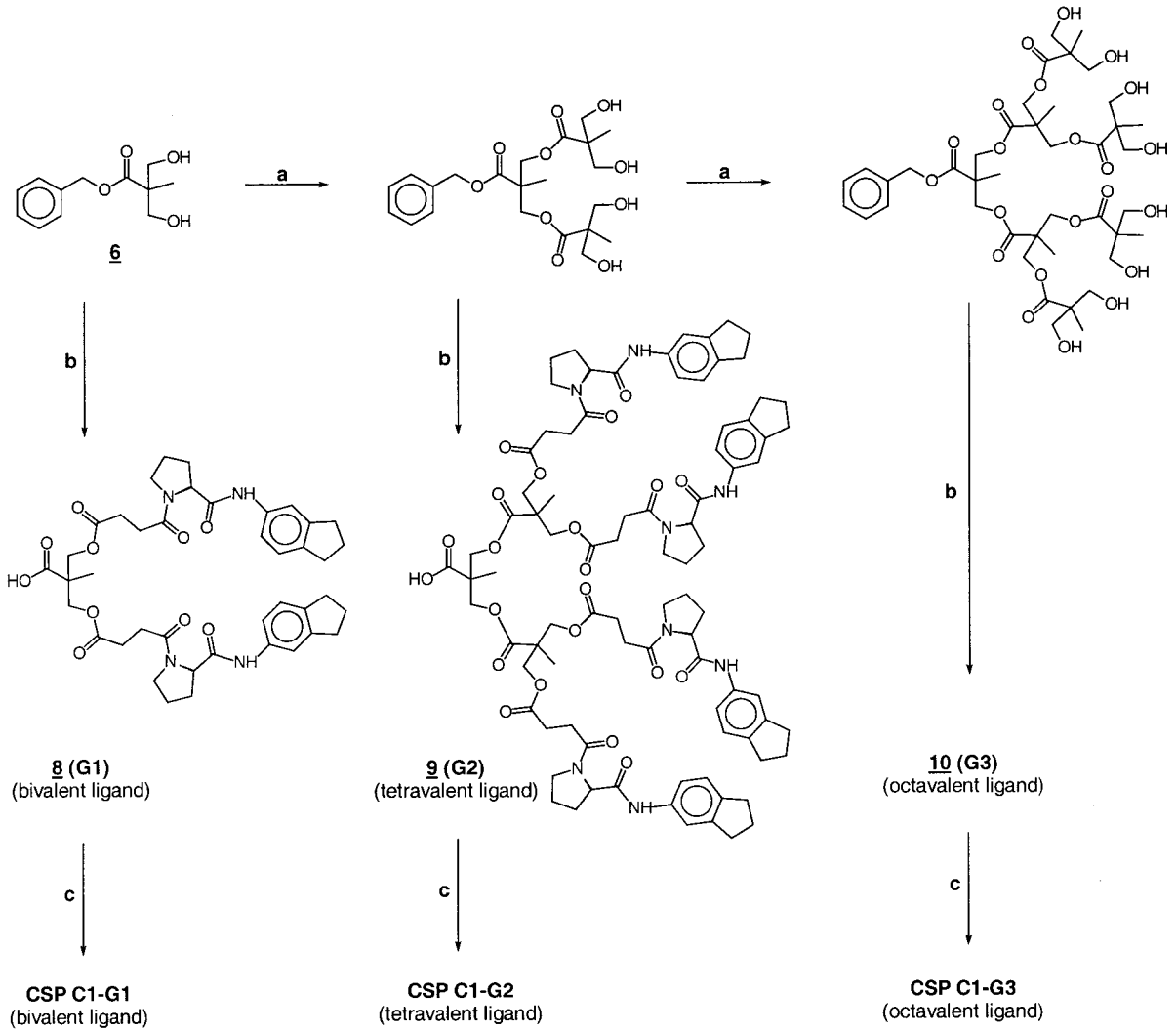
Preparation of Chiral Selector. All chiral stationary phases in this study contained a model chiral selector **4** derived from L-proline characterized by very high selectivity toward the dinitrobenzoyl derivatives of leucine and alanine that we have previously discovered using methods of combinatorial chemistry.[14] This selector was derivatized with succinic anhydride to afford compound **5** containing a linker with carboxylic acid functionality that is suitable for coupling with hydroxyl functionalities via ester linkage (Scheme 1).

Preparation of Monovalent Chiral Stationary Phase. In contrast to the CSP with same proline-based selector linked to the support via carbamate that we have developed earlier,[14] the monovalent (nondendritic) CSP **G0** was prepared by direct esterification of hydroxyl groups of the poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) support beads with selector **5** using DIC-coupling chemistry shown in Scheme 4. The chemistry of this CSP matched that of our CSPs with multivalent selectors and served as a benchmark for the evaluation of enantioselectivity.

Preparation of Multivalent Chiral Stationary Phases. The current synthesis of dendritic molecules[15] employs one of two basic approaches, convergent[16] or divergent.[17] In the convergent strategy, the synthesis begins at what will eventually become the periphery of

(9) (a) Henry, C. W.; McCarroll, M. E.; Warner, I. M. *J. Chromatogr.* **2001**, *905*, 319. (b) Liapis, A. I.; Grimes, B. A. *J. Coll. Interface Sci.* **2000**, *229*, 540.
(10) Fung, Y. S.; Long, Y. H. *J. Chromatogr.* **2001**, *907*, 301.
(11) Pirkle, W. H.; Pochapsky, S. *J. Chromatogr.* **1986**, *369*, 175.
(12) Liu, Y.; Juneau, K. N.; Svec, F.; Fréchet, J. M. J. *Polym. Bull.* **1998**, *41*, 183.
(13) (a) Archut, A.; Vögtle, F. *Chem. Soc. Rev.* **1998**, *27*, 233. (b) Vögtle, F.; Gestermann, S.; Hesse, R.; Schwierz, H.; Windisch, B. *Prog. Polym. Sci.* **2000**, *25*, 987.
(14) Murer, P.; Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1999**, *71*, 1278.
(15) (a) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Molecules: Concepts, Synthesis, Perspectives*; VCH: Weinheim, 1996. (b) Chow, H.-F.; Mong, T. K.-K.; Nongrum, M. F.; Wan, C.-W. *Tetrahedron* **1998,** *54*, 8543.
(16) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990,** *112*, 7638.

Scheme 2. Preparation of CSPs with Dendritic Linkers via the Convergent Approach[a]



[a] Key: (a) (i) **21,** DMAP, CH_2Cl_2, (ii) 0.3 M H_2SO_4, 1:2 H_2O/MeOH; (b) (i) **5**, DIC, DPTS, CH_2Cl_2, (ii) H_2, Pd/C, EtOAc; (c) (i) HEMA–EDMA **20**, DPTS (0.5 equiv with respect to the selector), DIC (2 equiv with respect to the selector), CH_2Cl_2, room temperature, 72 h, (ii) acetic anhydride, DMAP, CH_2Cl_2.

the dendrimer and converge toward a core. Given a monomer of the form $A-B_2$, the "B" sites are protected and the reactive "A" site, located at the focal point, is used for further linkage to another $A-B_2$ unit in which the A functionality is protected. The resulting composite, a dendron, is then deprotected at its focal point and reacted with another "A" protected monomer. This sequence of deprotection and coupling is successively repeated until the dendron of desired generation is obtained, at which point, the peripheral "B" functionalities are deprotected for functionalization with ligands. In contrast, the divergent approach begins with growth from a core toward the periphery. Starting with monomer protected only at the focal "A" site, the dendron is extended by linkage of the active "B" functionalities with monomers protected only at their peripheral "B" sites. Higher generation dendrons are prepared by successive deprotection of the periphery followed by linkage with "B" protected monomers. Both of these two methods are amenable toward the preparation of CSPs with branched linkers.

Preparation by Convergent Approach. Scheme 2 shows the reaction sequence used for the preparation of multivalent CSPs in the convergent way. Since our target was the preparation of enantioselective stationary phases, we chose not to use the architecture based on the established poly(benzyl ether) dendron chemistry as the aromatic units may contribute to nonspecific $\pi-\pi$ interactions. Instead, we used dendrons with aliphatic ester chemistry prepared from derivatives of 2,2-bis(2-methoxy)propionic acid (bis-MPA) **6** and **21** (Figure 1) that have been recently developed in our group.[18] The peripheral 1,3-diol groups of the dendrons were protected with isopropylidene groups while their carboxylic acid functionality at the focal point was protected by a benzyl ester group. These units can be selectively deprotected and subjected to further functionalization: the peripheral diols are liberated by treatment with dilute sulfuric acid solution in methanol–water mixture while the focal point is deprotected by hydrogenolysis under atmospheric pressure using palladium catalyst in ethyl acetate.

For the synthesis of the bivalent (G1) selector, both hydroxyl groups of **6** were esterified with the selector

(17) (a) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117. (b) Newkome, G. R.; Yao, Z.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985,** *50*, 2003.

(18) Ihre, H.; Hult, A.; Fréchet, J. M. J.; Gitsov, I. *Macromolecules* **1998**, *31*, 4061.

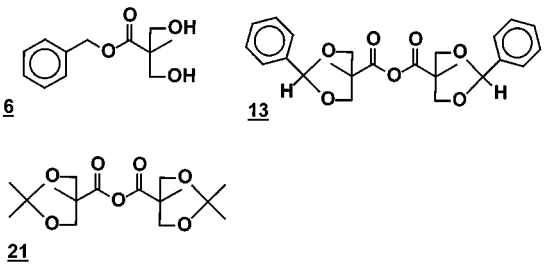
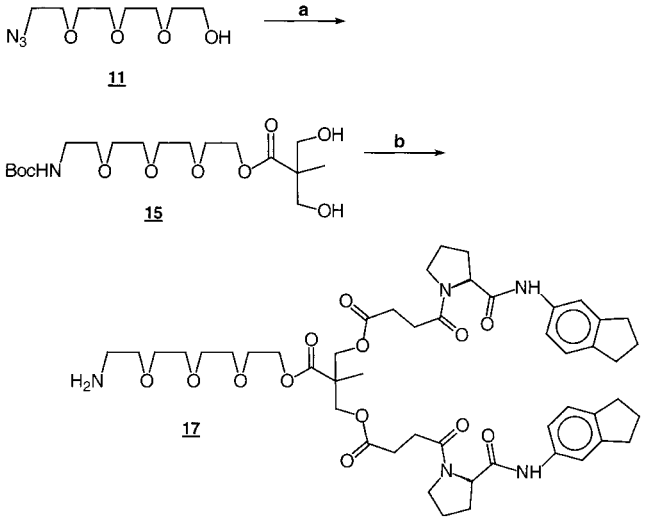


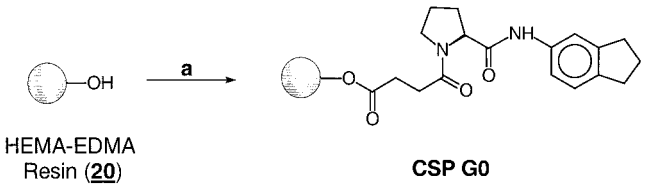
Figure 1. Structures of the bis-MPA derivatives used as building blocks for dendritic linkage.

Scheme 3. Synthesis of Dendritic Selector with Tetraethylene Glycol Linker[a]



[a] Key: (a) (i) H_2 (g), Pd/C, di-*tert*-butyl dicarbonate, EtOAc, (ii) **13,** DMAP, CH_2Cl_2, (iii) H_2 (g), Pd/C, EtOAc; (b) (i) **5**, DCC, DPTS,CH_2Cl_2, (ii) TFA, HOAc, CH_2Cl_2.

Scheme 4. Preparation of Monovalent Chiral Stationary Phase[a]



[a] Key: (a) **5**, DIC, DPTS, CH_2Cl_2.

moieties **5** to afford intermediate **7**, which is subsequently subjected to hydrogenolysis to deprotect the carboxylic acid, giving **8** (Scheme 2). Similarly, second- and third-generation dendritic selectors **9** and **10** were prepared by deprotection of peripheral diols followed by their functionalization with **5** and completed by liberation of the focal point functionality.

Three series of CSPs (**C1**, **C2**, and **C3**) were prepared by reaction of multivalent selectors of the first, second, and third generations (**G1**, **G2**, and **G3**), respectively, with HEMA beads. In the **C1** series, the dendritic selectors **8**−**10** were attached to the solid support directly using DIC coupling chemistry to afford CSPs **C1-G1**, **C1-G2**, and **C1-G3** (Scheme 2). In an attempt to increase the surface coverage, the HEMA beads were first treated with isopropylidene-2,2-bis(methoxy)propionic anhydride **21** followed by deprotection of the diol functionalities using mild acid conditions. Presumably, this approach should increase the number of reactive hydroxyl functionalities located at the pore surface of the beads by a factor of 2. The multivalent selectors **8**−**10** were then immobilized onto these pretreated beads to afford CSPs **C2-G1**, **C2-G2**, and **C2-G3.** In the last series, tetraethylene glycol (TEG)[19] spacer was inserted between the hydroxyethyl site at the solid surface and the dendritic moieties in order to provide the selector with enhanced mobility and ease the steric constraints encountered during the immobilization of these bulky branched selectors. Our initial attempt to exploit tetraethylene glycol as a spacer by attaching the functionalized dendrons to the HEMA beads through its TEG hydroxyl terminus failed. In contrast, a TEG spacer with a nucleophilic amine end group shown in Scheme 3 facilitated the coupling. For example, dendritic selectors **17** (G1, Scheme 3), **18** (G2), and **19** (G3) were prepared from the TEG derivative **15** that was coupled with **5**, **8**, and **9**, respectively, followed by Boc-deprotection of the terminal amine functionality. These selectors were then attached to HEMA beads activated with 4-nitrophenyl chloroformate to afford CSPs **C3-G1**, **C3-G2**, and **C3-G3**, respectively. The unreacted 4-nitrophenyl groups were then displaced by diethylamine. This approach enabled a certain increase in the loading levels at higher generations of dendritic selectors (Table 1).

Overall, our efforts to increase the selector loading were not very successful. This is probably due to a combination of factors that include the bulkiness of the dendritic selector as well as the difficulty in coupling the focal point of the dendron to the pendant hydroxyl groups of the resin located at the rigid pore surface.

Preparation by Divergent Approach. While the preparation of dendrons is generally carried out in solution, the preparation of dendrimers and dendritic ligands on resin has also gained some attention recently.[20] Since the divergent solid-phase supported approach to dendritic structures obviates the need for tedious repetitive purification steps and allows the use of excess reagents to drive the reaction to completion, we used this approach to prepare CSPs with dendritic linkers. This preparation requires an iterative three-step procedure: growth of the linker by addition of protected bis-MPA anhydride **21**, capping of unreacted hydroxyl groups with acetic acid anhydride, and selective deprotection of the isopropylidene groups with dilute sulfuric acid (Scheme 5)[21] For example, the first generation CSP **D1-G1** is prepared by reacting a slurry of HEMA beads **20** with an excess of anhydride **21** in the presence of a catalytic amount of DMAP. After the mixture reacts for 24 h at room temperature, acetic anhydride is added to the mixture to cap the unreacted hydroxyl groups. The capping ensures that the selector moieties are attached to the linker and not to the hydroxyl functionalities originating from the support. Following capping, the isopropylidene protecting group are removed by treatment with dilute sulfuric acid solution in methanol−water mixture to liberate the peripheral hydroxyl groups.

(19) Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem.* **1991**, *56*, 4326.

(20) (a) Arya, P.; Rao, N. V.; Singkhonrat, J.; Alper, H.; Bourque, S. C.; Manzer, L. E. *J. Org. Chem.* **2000**, *65*, 1881. (b) Basso, A.; Evans, B.; Pegg, N.; Bradley, M. *Tetrahedron Lett.* **2000**, *41*, 3763. (c) Marsh, A.; Carlisle, S. J.; Smith, S. C. *Tetrahedron Lett.* **2001**, *42*, 493. (d) Ulrich, K. E.; Boegeman, S.; Fréchet, J. M. J.; Turner, S. R. *Polym. Bull.* **1991**, *25*, 551.

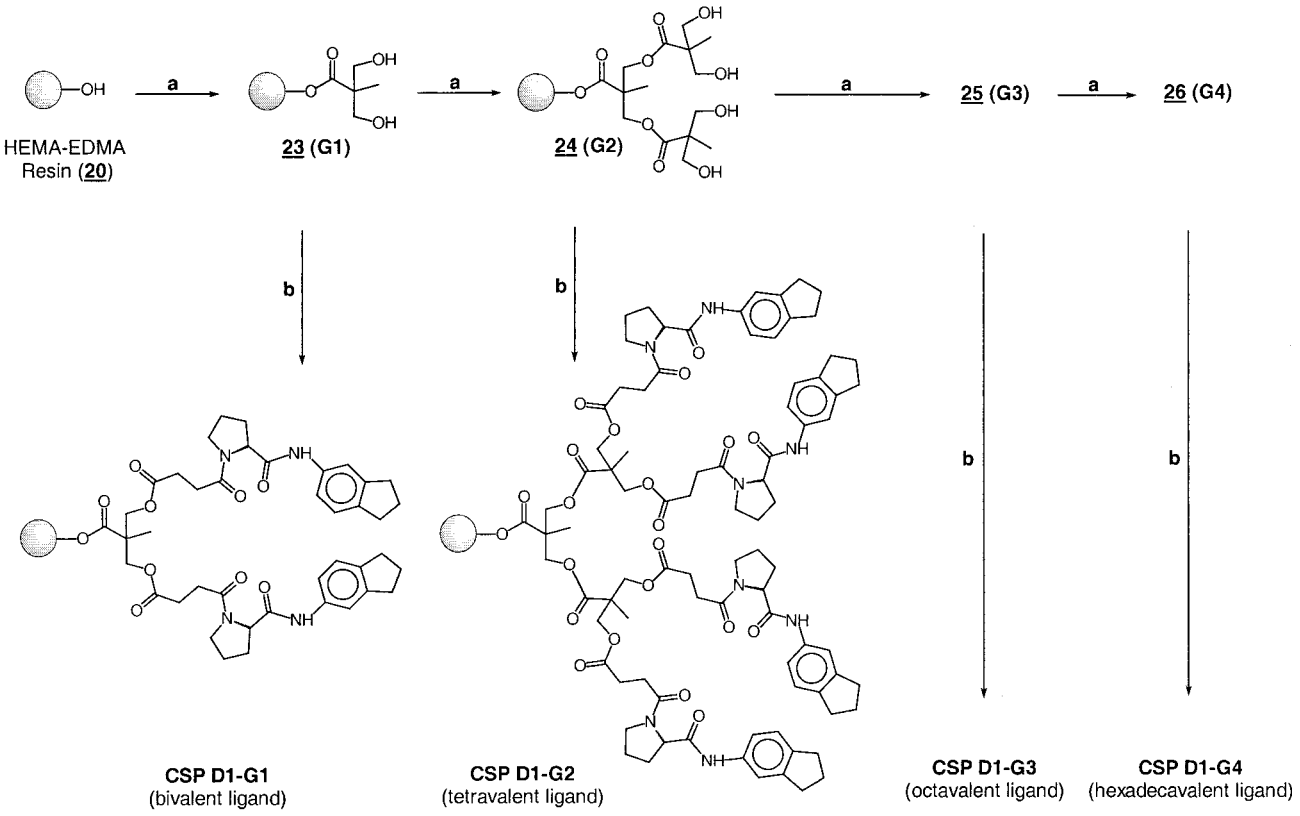
(21) (a) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *Chem. Mater.* **1998**, *10*, 385. (b) Petro, M.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1997**, *69*, 3131.

Table 1. Loadings and Separation Factors α Obtained for Enantioselective Separation of Racemic Compounds on CSP G0 and Chiral Stationary Phases Prepared by the Convergent Approach[a]

CSP	G0	C1-G1	C1-G2	C1-G3	C2-G1	C2-G2	C2-G3	C3-G1	C3-G2	C3-G3
selector loading,[b] mmol/g	0.27	0.27	0.12	0.13	0.28	0.08	0.12	0.14	0.14	0.23
analyte[c]					separation factor, α					
DNB-Leu-diallyl	21.1	17.5	7.4	9.1	21.9	9.0	12.9	12.4	12.2	16.6
DNB-Leu-diethyl	20.6	17.9	6.9	8.6	22.3	8.2	14.1	10.6	12.6	15.7
DNB-Ala-diallyl	18.6	15.5	6.7	8.5	20.1	7.2	12.4	11.4	16.2	15.6
DNB-Ala-diethyl	17.4	14.9	5.9	7.4	20.0	8.7	15.8	9.4	12.0	14.7

[a] Chromatographic conditions: column, 150 × 4.6 mm i.d.; mobile phase, 20% hexane in CH_2Cl_2; flow rate, 1.0 mL min^{-1}; void marker, 1,3,5-tri-*tert*-butylbenzene; UV detection, 254 nm. [b] Determined by elemental analysis of nitrogen. [c] For structures of the analytes, see Figure 2.

Scheme 5. Preparation of CSPs with Dendritic Linkers via the Divergent Approach[a]



[a] Key: (a) (i) **21,** DMAP, CH_2Cl_2, room temperature, 24 h, (ii) add acetic anhydride, room temperature,12 h; (b) (i) **5**, DIC, DPTS, CH_2Cl_2, room temperature, 24 h, (ii) acetic anhydride, DMAP, CH_2Cl_2.

Table 2. Loadings and Separation Factors α Obtained for Enantioselective Separation of Racemic Compounds on Chiral Stationary Phases Prepared by the Divergent Approach[a]

CSP	D1-G1	D1-G2	D1-G3	D1-G4	D2-G1	D2-G2	D2-G3	D2-G4
selector loading,[b] mmol/g	0.56	0.73	0.61	0.46	0.61	0.84	0.75	0.64
analyte[c]				separation factor, α				
DNB-Leu-diallyl	27.8	23.3	19.4	18.3	25.7	24.0	23.4	22.6
DNB-Leu-diethyl	30.7	25.8	21.7	20.2	28.1	28.0	28.2	26.3
DNB-Ala-diallyl	25.8	20.4	20.6	18.4	23.6	24.4	23.6	23.6
DNB-Ala-diethyl	26.7	21.3	21.6	19.1	24.4	26.0	26.2	26.8

[a] Chromatographic conditions: column, 150 × 4.6 mm i.d.; mobile phase, 20% hexane in CH_2Cl_2; flow rate, 1.0 mL min^{-1}; void marker, 1,3,5-tri-*tert*-butylbenzene; UV detection, 254 nm. [b] Determined by elemental analysis of nitrogen. [c] For structures of the analytes, see Figure 2.

Finally, the selector is linked to the modified beads. Reacting the deprotected hydroxyl groups with anhydride **21** instead of selectors leads to branched linkers of higher generations.

To demonstrate the effect of endcapping of unreacted hydroxyl groups, we also prepared a second series of CSPs **D2** by omitting the capping between the extension and deprotection steps. Although this approach affords CSPs with somewhat higher selector loading (Table 2), the exclusivity of their peripheral location is not guaranteed.

Evaluation of CSPs. For chromatographic evaluation, all CSPs were slurry packed into 150 × 4.6 mm i.d. columns and used for the separation of *N*-(3,5,-dinitrobenzoyl)-α-amino acid derivatives shown in Figure 2 in isocratic mode using 20% hexane/dichloromethane as

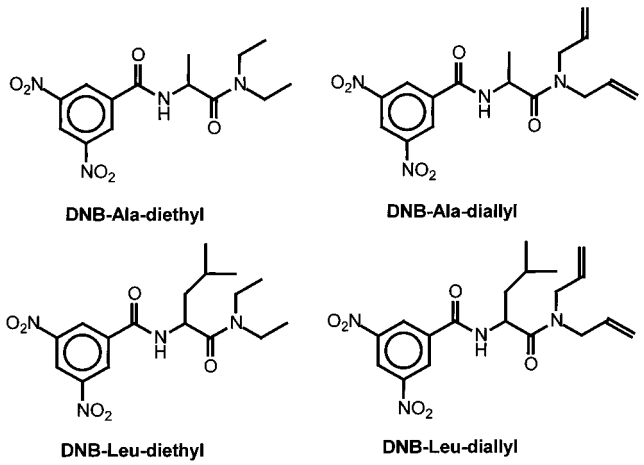


Figure 2. Structures of the *N*-(3,5-dinitrobenzoyl)-α-amino acid dialkyl amide analytes used in the study.

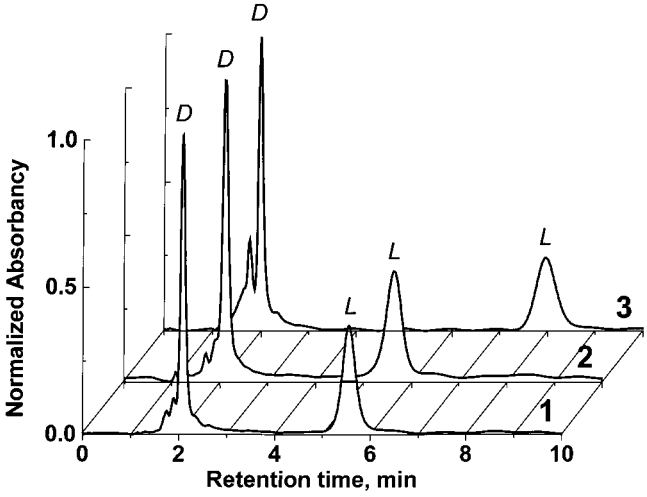


Figure 3. HPLC chromatogram of the enantioseparation of *N*-(3,5-dinitrobenzoyl)leucine diallyl amide via CSPs: (1) **C2-G1**, (2) **C2-G2**, and (3) **C2-G3**. Mobile phase: 20% hexane in CH_2Cl_2. Flow rate: 1.0 mL/min.

the mobile phase. Because of very high selectivities of all of our CSPs, the racemates were separated with excellent resolution.

First, the enantioselectivity of monovalent CSP **G0** with a loading of 0.26 mmol/g was examined as a benchmark. The separation factors attained for all four probe racemates are large with values ranging from 17.4 to 21.1.

Evaluation of Convergent CSPs. Selector loadings and results of the separations for both monovalent CSP **G0** and the CSPs assembled in the convergent way are shown in Table 1. The loading of CSPs with branched linkers of both second and third generations attached in a single step rapidly decreases and typically reaches values in the range of 0.12−0.14 mmol/g.

These CSPs exhibited high selectivities in the range of 5.9−22.3 toward our model analytes. As shown in Table 1, most of these CSPs with branched linkers have lower apparent selectivities compared to the monovalent CSP **G0**. The enantioselectivity of these CSPs clearly depends on the loading. The higher the selector loading, the better the separation observed within each series. Figure 3 demonstrates the separations of racemic *N*-(3,5-dinitrobenzoyl)-leucine diallyl amide on CSPs of the **C2** series. Clearly, CSP **C2-G3** retains L-enantiomer more strongly (retention time 8 min) than either CSP **C2-G1** or **C2-G2** for which retention times of only 5.5 min were observed. Although the retention times of the D-enantiomer do not vary much from CSP to CSP under our experimental conditions, its modest increase on the **C2-G3** results in separation factors that are similar to that of **C2-G2**, but much lower than that for the first generation **C2-G1**.

Clearly, the enantioselectivity depends on the selector loading and since it is difficult to prepare CSPs of different generation linkage that have identical loading, we compared our CSPs using a "normalized" value of specific selectivity α′ defined as the ratio of separation factor α per unit of the selector loading that we introduced in our previous studies.[22] The specific selectivity enables better assessment of the effects of branched linkers on selectivity. For example, the α′ value for all studied racemates is in the range of 60 to 80 for CSP **G0**. CSPs **C2-G2** exhibits α′ values between 90 and 110 while α′ values of 100 to 130 were observed for **C2-G3**. This demonstrates that the selectivity per selector moiety is enhanced for CSPs at higher generations. Although this may be an indication of desired synergistic interactions of selectors that are confined in a close proximity, it can also be attributed to the lengthening of the distance between the individual selectors and the surface of the matrix.

To improve the reaction of the sterically bulky dendron with the support for increased loading, we also prepared CSPs containing branched selectors with a tetraethylene glycol unit linked to the focal point of the dendron through an ester bond on one side and terminated with the more nucleophilic primary amine functionality on the other side (**C3** series). Table 1 shows that the apparent separation factors are higher than those of the **C1** and **C2** series with G1 and G2 linkers. This increase appears to result from their higher loading since their specific selectivities (65 to 90) do not reveal any significant enhancement due to the branching. However, the evaluation of these CSPs cannot be fairly compared to those achieved with CSPs of **C1** and **C2** series because the selectors are attached to the support through a carbamate group rather than an ester group.

Evaluation of Divergent CSPs. Two series of CSPs were prepared using the divergent methodology: (i) **D1** with endcapping of unreacted hydroxyl groups after each extension step and (ii) **D2** in which the endcapping step was omitted and all hydroxyl groups were allowed to compete in further reaction steps. Provided that the capping steps used in **D1** series completely eliminates all undesired hydroxyl groups, it ensures that all ligand moieties are anchored only at the periphery of the dendritic linker. In contrast, the individual selectors for the **D2** series may lie at varying distances from the reactive site of the bead since the "internal" hydroxyl groups that were not previously capped could participate in the selector loading. Data from Table 2 support this hypothesis since the loading levels for the **D2** series are higher for each dendritic generation. Obviously, capping with anhydride **21** after linker extension diminishes the amount of selectors that can be attached to CSPs of the **D1** series as compared to the **D2** series, but this also

(22) Lewandowski, K.; Murer, P.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1998**, *70*, 1629.

implies that the structure of the dendrimer grown from the solid surface contains defects.

The divergent approach enables preparation of CSPs with much higher loading levels (Table 2). For example, the amount of selector is 0.56 mmol/g for **D1-G1** and 0.61 mmol/g for **D2-G1**, both first generation ligands. This is more than twice as much compared to **G0** and several times higher than the loadings of CSPs in the **C** series. In theory, each increase in generation of the dendritic linker should result in doubled loading. Although G2 exhibits a higher loading than G1, this increase represents only 30% for **D1-G2** and 38% for **D2-G2** confirming that the peripheral hydroxyl groups were partially functionalized. Moreover, a simple calculation shows intrinsic limitations for the growth of perfect dendrimers on solid support. Assuming that 65% of the hydroxyl groups originating from the HEMA (3.0 mmol/g) are available for coupling,[21a] then 0.24 g (0.20 mL) of bis-MPA are immobilized in the first step. Clearly, ideal growth beyond the first generation is unlikely, as this would imply that the volume taken up by the linker exceeds that available within the pores.

The higher loadings of these CSPs also endow them with selectivities higher than those obtained for the convergently prepared CSPs. Table 2 also summarizes results of chromatographic evaluations of CSPs of both series **D1** and **D2.** The separations factors are in the range of 18−31. For example, an α value of 30.7 was obtained for the separation of racemic dinitrobenzoylleucine diethylamide on CSP **D1-G1**. This selectivity is about 50% higher than that observed for CSP **G0** and also significantly higher than the α values that we reported previously for the separation of these racemates.[14] However, the higher loading does not necessarily lead to higher selectivity. While the separation factors remain rather high for CSPs with higher generation dendrons (G2 through G4), the α values diminish with the increase in generation of the linker. The separation factors of CSP **D1-G1** are higher than those of CSP **D2-G1** even with a lower selector loading of the former. This indicates that the selectivity increases with the distance between the selector moiety and the solid support surface.

Surprisingly, the separation factors do not follow the trend of the increased loading even within each series. The α values decrease despite the higher content of selector moieties. Despite both high loading and selectivity, the actual α′ values for these divergent CSPs are only in the range of 30−50, considerably lower than those of CSP **G0** and CSPs of the convergent series. We deduce that this could result from an incomplete random substitution of the periphery of the dendritic linker that is not favorable for synergistic interactions.

Enantioseparations under Overload Conditions. Although an increase in throughput of chromatographic separations can be achieved by simply using larger columns scaled to the desired size operating under the conditions typical of analytical scale separations, this approach is resource intensive. This linear scale-up does not require any specifically developed CSPs as it typically involves a method that has already been developed on the analytical scale. In contrast, processes that use smaller columns and CSPs tailored for high enantioselectivity and high loading of a specific analyte can be operated under overload conditions, and thus, enable high throughput operations at lower capital costs. Since the preparation of CSPs using branched linkers affords high loading of selector moieties, these stationary phases are also attractive for the preparation of CSPs to be used for large-scale chromatography. Figure 4 shows a series of enantioseparations of 0.1 to 10 mg of racemic dinitrobenzoylleucine diallylamide using an analytical 150 × 4.6 mm i.d. column packed with CSP **D1-G1** and isocratically eluted with a mixture of 20% hexane in dichloromethane as the mobile phase at 1.0 mL/min. While a valley between eluted bands of both enantiomers is observed for injections of up to 5.0 mg, the absorption exceeds the limits of the UV detector and no apparent separation is observed for injection of 10 mg. In contrast, the less sensitive polarimetric chiral detector always monitors well-separated peaks of both enantiomers and enables a more accurate cut between the fractions needed to collect single enantiomers in the highest degree of purity (Figure 5). In addition, this detector confirms that the peaks contain opposite enantiomers. The peak areas are equal within the range of experimental error indicating that no other chiral compound is coeluted.

The identical chemical shifts in NMR spectra of the materials recovered by collecting two fractions of the eluent cut in the valley between the peaks monitored by the chiral detector also confirm the purity of both enantiomers. Another check of purity of the separated enantiomers was obtained by re-injecting them separately in the same column (Figure 6). Only very small peaks of the undesired enantiomers representing 0.8% and 0.5% of the total peak areas are visible in the trace together with large peaks containing 99.2% and 99.5% of the desired D and L enantiomers, respectively. The

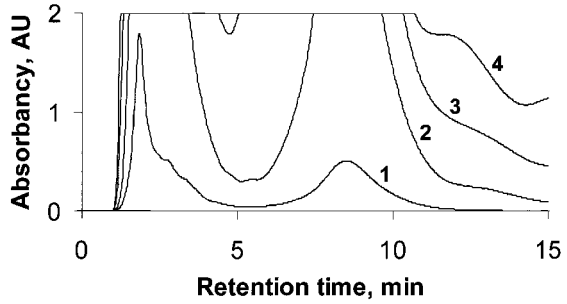


Figure 4. Chiral separation of *N*-(3,5-dinitrobenzoyl)leucine diallyl amide on CSP **D1-G1** under various overloading conditions: Mobile phase: 20% hexane in CH_2Cl_2. Flow rate: 1.0 mL/min. Injection: (1) 0.10 mg, (2) 1.0 mg, (3) 5.0 mg, and (4) 10.0 mg.

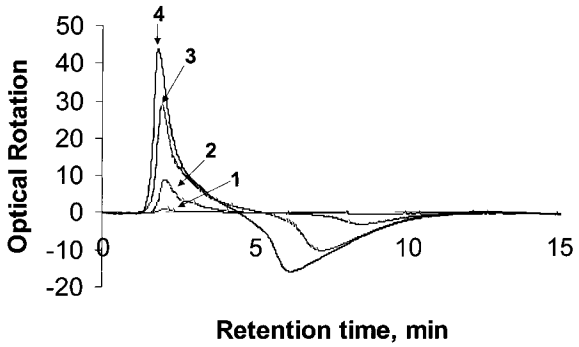


Figure 5. Separation of *N*-(3,5-dinitrobenzoyl)-leucine diallyl amide under various overloading conditions on CSP **D1-G1** visualized under chiral detector: Mobile phase: 20% hexane in CH_2Cl_2. Flow rate: 1.0 mL/min. Injection: (1) 0.10 mg, (2) 1.0 mg, (3) 5.0 mg, and (4) 10.0 mg.

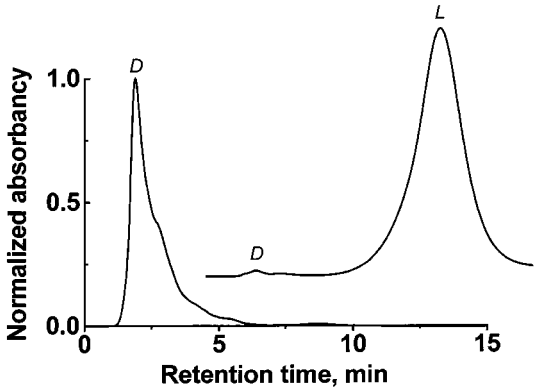


Figure 6. Reinjection of fractions from separated peaks collected from the 10.0 mg overload separation of *N*-(3,5-dinitrobenzoyl)leucine diallyl amide. Separation conditions: column CSP **D1-G1**; mobile phase, 20% hexane in CH_2Cl_2; flow rate, 1.0 mL/min.

separation in column packed with unbranched CSP **G0** under identical conditions afforded peak purities of only 92.9% and 99.4%. This again demonstrates the positive effect of the branched linker. A simple calculation reveals that even with an unoptimized system, 1.0 kg per day of the racemate could be separated using 1.2 kg of the branched linker containing CSP **D1-G1** packed into a column and carried out under standard normal-phase chromatographic mode.

Conclusions

Our results indicate that dendritic linkers are a beneficial feature while engineering a chiral stationary phase. In contrast to expectations arising from Pirkle's observation of a vast increase in selectivity factors for bivalent analytes,[11] we do not observe an increase in selectivity of such magnitude for our separations in the "reciprocal" mode. This could result from the differences in kinetics of the selector/analyte interactions since in our case the polyvalent counterpart is immobilized onto a rigid matrix while the monovalent target molecules are in the solution. While in Pirkle's case each end of the bis-analyte could independently and simultaneously interact with two different chiral selectors on the CSP, in our system, the analytes cannot associate with more than one selector in an identical configuration. Despite this limitation, very high separation factors of up to $\alpha = 31$ have been achieved with our CSPs containing branched linkers. These are considerably higher than those seen for the **G0** counterpart containing only monovalent selector moieties. This validates the positive synergistic effect of multiple selectors located in well-defined positions within in the dendritic CSP. This synergism may arise from the intercalation of the analyte between two selectors located in precise positions. The preparation of dendrons bearing chiral peripheral moieties can also be easily combined with the screening of combinatorial libraries of potential selectors for chiral recognition in order to accelerate the discovery of selectors targeted toward a specific racemate. Although much remains to be done, this study opens a new avenue for the preparation and application of tailor-made CSPs in large-scale high throughput enantioseparations.

Experimental Section

Materials. Methylene chloride was distilled from CaH_2 under inert atmosphere. Tetrahydrofuran (THF) was distilled under N_2 from sodium benzophenone ketyl. Diisopropylcarbodiimide (DIC), dicyclohexylcarbodiimide (DCC), 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ), 2,2-bis(methoxy)-propionic acid (bis-MPA), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) were purchased from Aldrich and all other reagents and solvents were obtained from commercial suppliers and were used without further purification. Bis-MPA based building blocks and dendrons were prepared according to procedures described elsewhere.[18,24] All moisture or air-sensitive reactions were carried out under nitrogen in a dry solvent. The 7 mm monodisperse porous HEMA-EDMA beads consisting of 40 wt % 2-hydroxyethyl methacrylate (HEMA) and 60 wt % ethylene dimethacrylate (EDMA) were prepared using templated staged suspension polymerization that was developed by our group.[21a] These beads have a median pore size of 11 nm, a pore volume of 0.3 mL/g, and a specific surface area of 140 m^2/g.

Instrumentation. ^1H and ^{13}C NMR spectra were obtained with Fourier transform spectrometers Bruker AMX-300, Bruker AMX-400, Bruker AM-400, and Bruker DRX-500. Spectral data are reported as chemical shifts (multiplicity, coupling constant in Hz, number of protons, identity if possible) in ppm (δ units). Infrared spectra were recorded on a Nicolet Mattson Genesis II FT-IR spectrophotometer using KBr technique.

Synthesis of Selector with Carboxylic Acid Handle (*5*): (i) l-2-(Indan-5-ylcarbamoyl)pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (3).[14] To a solution of *N*-*t*-Boc-L-proline (**1**; 85 g, 0.40 mol) and EEDQ (100 g, 0.41 mol) in CH_2Cl_2 (200 mL) was added 5-aminoindan (**2**; 54 g, 0.41 mol) at 0 °C (Scheme 1). The reaction mixture was stirred at 0 °C for 2 h and then allowed to react overnight at room temperature. The reaction mixture was diluted with 100 mL of CH_2Cl_2 and washed with 1 M HCl (3 × 50 mL), saturated aqueous $NaHCO_3$ solution (2 × 50 mL), H_2O (50 mL), and brine (2 × 50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated to give a viscous oil. The crude product was then redissolved in a 1:1 mixture of CH_2Cl_2 and EtOAc. Cooling of the mixture to −10 °C affords **3** (116 g, 90%) as an off-white crystalline solid.

(ii) Selector. L-Pyrrolidine-2-carboxylic acid indan-5-ylamide (4). To a solution of **3** (20.2 g, 0.0611 mol) dissolved in CH_2Cl_2 (100 mL) at 0 °C was added 90 mL of a 1:1 TFA/HOAc mixture. The reaction mixture was stirred overnight at room temperature. Progress of the deprotection was followed by TLC (2:1 hexane/EtOAc). Upon complete deprotection, the volatile compounds were removed in vacuo and the product was diluted with H_2O (50 mL) and CH_2Cl_2 (50 mL). Then, 2 M KOH (aq) was added at 0 °C until the pH of the aqueous phase was between 9 and 10. The aqueous phase was then extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated to give selector **4** (14.0 g, 100%) as a waxy brown solid.

(iii) L-4-[2-(Indan-5-ylcarbamoyl)pyrrolidin-1-yl]-4-oxobutyric Acid (5). Compound **4** (1.0 g, 4.3 mmol) and succinic anhydride (0.6 g, 6 mmol) were dissolved in 1:1 THF/Et_3N (10 mL) solution, and DMAP (0.05 g, 0.4 mmol) was added. The reaction mixture was heated under reflux at 70 °C overnight. Upon completion, the crude product was diluted with 1 M HCl (100 mL) and extracted with EtOAc (4 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated to give **5** (1.3 g, 82%) as a light beige solid.

Synthesis of Bivalent Selector and a General Procedure for Synthesis of Dendritic Selectors: (Selector)$_2$-[G1]-CO_2H (8). To a mixture of benzyl-2,2-bis(methoxy)-propionate[18] **6** (0.50 g, 2.23 mmol), **5** (1.53 g, 4.62 mmol), and DPTS (0.37 g, 1.24 mmol) in CH_2Cl_2 (ca. 30 mL) was added DCC (1.38 g, 6.69 mmol) (Scheme 2). The reaction mixture was stirred under inert atmosphere at room temperature overnight,

(23) Hyun, M. H.; Choi, S. Y.; Um, B. H.; Han, S. C. *J. Chromatogr. A* **2001**, *922*, 119.

(24) (a) Freeman, A. W.; Fréchet, J. M. J. *Org. Lett.* **1999**, *1*, 685. (b) Liu, M.; Fréchet, J. M. J. *Pharmaceut. Sci. Technol. Today* **1999**, *2*, 393. (c) Grayson, S. M.; Jayaraman, M.; Fréchet, J. M. J. *Chem. Commun.* **1999**, 1329.

filtered, and concentrated. The crude reaction mixture was purified using flash chromatography to yield (selector)$_2$-[G1]-$CO_2CH_2C_6H_5$ **7** (1.90 g, 100% yield) as a beige crystalline solid that was then subjected to catalytic hydrogenolysis.

A mixture of **7** (1.00 g, 1.18 mmol) and Pd/C (0.096 g) in EtOAc (ca. 20 mL) was stirred under H_2 (g) overnight. The catalyst was removed by filtration and the filtrate was evaporated to afford **8** (0.89 g, 99.7%) as a white crystalline solid (Scheme 2). Second (**9**) and third (**10**) generation dendritic selectors were synthesized by methods similar to those affording **8**.

Synthesis of Bivalent Selector with TEG Linker and a General Procedure for Synthesis of Dendritic Selectors with TEG Linker: (i) (OH)$_2$-[G1]-[TEG]-NHBoc (15). To a suspension of Pd/C (10%, 240 mg) in EtOAc (20 mL) under H_2 (g) was added an EtOAc (10 mL) solution of monoazide derivate of tetraethylene glycol **11** (TEG)[19] (2.00 g, 9.12 mmol) and di-*tert*-butyl dicarbonate (1.2 equiv, 2.40 g, 11.0 mmol) (Scheme 3). The reaction mixture was stirred overnight. The catalyst was removed by filtration and the solvent evaporated to give crude product. Flash chromatography was carried out in two steps: 3:1 hexanes/EtOAc was used to remove residual di-*tert*-butyl dicarbonate and 1:20 MeOH/CH_2Cl_2 to afford [TEG]-NHBoc **12** as a colorless oil (1.40 g, 52%).

To a mixture of **12** (1.40 g, 4.76 mmol) in THF (ca. 20 mL) and pyridine (ca. 20 mL) were added DMAP (0.29 g, 2.37 mmol) and benzylidene-2,2-bis(methoxy)propionic anhydride **13** (4.10 g, 9.61 mmol). The reaction mixture was stirred under inert atmosphere at room temperature overnight before 5 mL of H_2O was added to quench unreacted **13**. The solution was extracted with $NaHSO_4$ (3 × 50 mL), Na_2CO_3 (saturated 3 × 20 mL), and brine (ca. 50 mL) and dried over $MgSO_4$. After 1 h of drying, the solution was filtered, concentrated, and redissolved in CH_2Cl_2 (ca. 4 mL). Addition of hexanes (ca. 20 mL) to the CH_2Cl_2 solution resulted instantly in a white precipitate. The solid was removed, and the filtrate was concentrated in vacuo to afford benzylidine-[G1]-[TEG]-NHBoc **14** (2.30 g, 97%) as a colorless oil. Compound **14** was deprotected using the hydrogenolysis procedure described above to yield **15** (1.80 g, 95%) as a colorless oil.

(ii) (Selector)$_2$-[G1]-[TEG]-NH$_2$ (17). To a mixture of **15** (1.92 g, 4.69 mmol), **5** (3.40 g, 10.3 mmol), and DPTS (0.60 g, 2.04 mmol) in CH_2Cl_2 (ca. 60 mL) at 0 °C was added DCC (3.08 g, 14.9 mmol). The reaction mixture was allowed to slowly warm to room temperature and stirred overnight. The dicyclohexylurea was removed by filtration, and the filtrate was concentrated to give an orange crude. After column chromatography (10% MeOH in CH_2Cl_2), (selector)$_2$-[G1]-[TEG]-NHBoc **16** (5.00 g, quantitative) was obtained as a light orange solid. Deprotection of **16** was achieved again using hydrogenolysis to give **17** (4.50 g, quantitative) as a yellow solid.

Similarly, second- (**18**) and third-generation (**19**) dendritic selectors with tetraethylene glycol spacers coupled to the focal point were prepared by linking **15** to **8** and to **9**, respectively, using DCC coupling chemistry followed by removal of the Boc-protecting group.

Preparation of Chiral Stationary Phases. Since two different methods, convergent (**C**) and divergent (**D**), were used to prepare CSP with dendritic linkers of various generations (**G**), the CSPs used in this study are denoted with the letter indicating the method of preparation, followed by the enumeration of the CSP, and a numeric value of the dendron generation. For example, CSP **C3-G2** stands for chiral stationary-phase number three prepared by the convergent approach with a dendritic linker of the second generation.

Preparation of Monovalent CSP G0. To a slurry of HEMA-EDMA beads **20** (1.3 g) in CH_2Cl_2 (ca. 10 mL) were added **5** (0.5 g, 1.5 mmol), DPTS (1 equiv, 0.5 g, 1.5 mmol), and DIC (1.25 equiv, 0.25 g, 1.8 mmol) (Scheme 4). After the mixture was gently stirred overnight using rotation, the beads were filtered and washed with CH_2Cl_2 (20 mL), THF (20 mL), MeOH (2 × 20 mL), CH_2Cl_2 (20 mL), and Et_2O (20 mL). The residual hydroxyl groups were capped by reaction with acetic anhydride catalyzed by DMAP in 1:1 pyridine/THF mixture to give CSP **G0**.

Convergent Preparation of CSPs. CSPs **C1-G1, C1-G2,** and **C1-G3** were prepared according to Scheme 3 by adding dendritic selectors **8** (G1), **9** (G2), and **10** (G3), respectively, to a slurry of DPTS (0.5 equiv with respect to the selector) and DIC (2 equiv) and beads **20** in 30 mL of CH_2Cl_2 (Scheme 2). This reaction mixture was stirred at room temperature for 72 h. The modified beads were then filtered and washed with THF (1 × 20 mL), H_2O (2 × 20 mL), MeOH (2 × 20 mL), and CH_2Cl_2 (ca. 20 mL). The resulting beads were extracted for 24 h with each CH_2Cl_2 and THF. The residual hydroxyl groups were capped again by reaction with acetic anhydride catalyzed by DMAP in 1:1 pyridine/THF mixture. These beads were then filtered, washed with THF (60 mL) and triethylamine (40 mL) and H_2O (50 mL), and extracted with THF and CH_2Cl_2.

CSPs **C2-G1, C2-G2,** and **C2-G3** were prepared by heating a slurry of beads **20**, isopropylidene-2,2-bis(methoxy)propionic anhydride **21** (5 equiv), and DMAP (0.16 equiv) in THF/pyridine at 60 °C for 24 h. The modified beads were filtered and washed with THF and CH_2Cl_2. These beads were then treated with 0.4 M H_2SO_4 in MeOH to remove the isopropylidene protective group and regenerate the hydroxyl functionalities. The selectors **8** (G1), **9** (G2), and **10** (G3) were respectively immobilized onto these beads using methods similar to those described above for the preparation of CSP **C1** series.

CSPs **C3-G1, C3-G2,** and **C3-G3** were prepared by adding **17**, **18**, and **19,** respectively, to a slurry of HEMA-EDMA beads **22** activated with 4-nitrophenylchloroformate and DMAP in THF/pyridine. The reaction mixture was heated at 60 °C for 24 h. Then diethylamine was added to displace the unreacted 4-nitrophenyl groups. After the reaction was completed, the beads were washed repeatedly with THF, CH_2Cl_2, DMF, EtOH, H_2O, MeOH, and Et_2O. Extraction of these beads with THF and CH_2Cl_2, followed by drying in vacuo afforded CSPs **C3-G1, C3-G2,** and **C3-G3**.

Divergent Preparation of CSPs. This preparation of divergent CSPs was carried out in three major phases: (i) solid-phase synthesis of dendritic linker followed by (ii) capping with acetic anhydride and (iii) functionalization with selector (Scheme 5). To a suspension of HEMA beads **20** (6.0 g) in pyridine (24 mL) were added **21** (4.0 g, 12 mmol) and DMAP (2.0 g, 16 mmol). After the mixture was stirred at room temperature for 24 h, acetic anhydride (8.0 mL) was added and the reaction was continued for an additional 12 h to complete the capping of residual hydroxyl groups. The beads were then filtered and washed with several portions of CH_2Cl_2 and MeOH. Compared to the original support, IR spectra of these beads showed significantly smaller broad band centered around 3500^{-1} cm. Removal of the isopropylidene protecting group by an overnight treatment with 0.3 M H_2SO_4 in 1:2 H_2O/MeOH solution afforded the deprotected beads **23** (G1) which were then collected by filtration and washed with 0.3 M H_2SO_4, MeOH, THF, and CH_2Cl_2 and dried under vacuum. The deprotection was accompanied by the reappearance of the hydroxyl band in the IR spectrum. The preparation of beads with higher generation dendritic linkers **24** (G2), **25** (G3), and **26** (G4) was carried out by recursively repeating these procedures using reagents in similar proportions (Scheme 4).

Alternatively, we also prepared beads that were not end-capped after growth of the dendritic linker. The addition of acetic anhydride was omitted from the preparation to give beads **27**−**30** with G1, G2, G3, and G4 linkers, respectively.

Once the dendritic linkers were grown on the beads, they were subjected to coupling with selector. To a mixture of deprotected beads **23** (ca. 1.5 g), selector building block **5** (0.5 g, 1.5 mmol), and DPTS (0.5 g, 1.5 mmol) in CH_2Cl_2 (10 mL) was added DIC (1.2 equiv, 0.23 g, 1.8 mmol). After the mixture was stirred at room temperature for 24 h, the beads were filtered and washed with CH_2Cl_2, MeOH, and THF and air-dried. The functionalization was accompanied by appearance of amide N−H bending at 1547 cm^{-1}. Residual hydroxyl groups were capped by reaction of the beads with acetic anhydride (2 mL) and DMAP (0.5 g, 4.0 mmol) in CH_2Cl_2 (10 mL). After stirring overnight, the beads were filtered and washed with

CH_2Cl_2, MeOH, 0.2 M H_2SO_4 solution in MeOH/H_2O, and THF and then dried under high vacuum to afford CSP **D1-G1**. IR analysis showed the desired disappearance of the hydroxyl peak. Identical coupling procedures were applied to beads **24**–**30** to prepare CSPs **D1-G2, D1-G3, D1-G4, D2-G1, D2-G2, D2-G3,** and **D2-G4**, respectively.

Chromatographic Evaluation. All CSPs were treated with acetic anhydride before their packing into columns in order to cap all residual hydroxyl groups that could be the source of nonspecific interactions. After this treatment, the CSPs were dispersed in 80:20 CH_2Cl_2/hexane and packed at a constant pressure of 20 MPa into 150 × 4.6 mm i.d. stainless steel columns. A Waters Alliance (model 2690 XE) instrument with a 486 UV detector and a JASCO OR-990 chiral detector, or a system consisting of two Waters 510 HPLC pumps, a 717plus autosampler, and a 486 UV detector, were used alternatively for the chromatographic experiments. Both systems were controlled and data acquired by Waters Millennium software.

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Supporting Information Available: FT-IR spectra, NMR spectra, and elemental analysis of **3**, **4**, **5**, and dendritic selectors and their intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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