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Journal of Chromatography A 822 (1998) 11–18

JOURNAL OF CHROMATOGRAPHY A

Noncovalent linking of a chiral selector to unmodified silica gel through amine functional groups

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Received 23 April 1998; accepted 4 June 1998

Abstract

The noncovalent linking of Pirkle's dinitrobenzoyl–L-leucine chiral selector to unmodified silica gel by means of polar moieties such as amines and quaternary ammonium ions was studied. Selectors linked with amine groups provided resolution of racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol comparable to that of Pirkle's column having a covalently bonded selector. Non-covalent linking by means of the quaternary ammonium ion group was much less efficient in resolving optical isomers.

Keywords: Noncovalent linking; Kinetic coating; Chiral selectors; Chiral stationary phases, LC; Amines; Quaternary ammonium

1. Introduction

Bonded stationary phases on silica gel are usually immobilized onto the silica gel surface by covalent bonding using silicon–oxygen bonds, the chemistry of which is well established and documented. While the covalent linkage is chemically stable, noncovalent immobilization (kinetic coating) is easier to carry out and can have advantages in some cases. For example, the noncovalent immobilization process can be reversible, the selectivity of a noncovalent coated column can be varied by simply washing off the original coating and re-coating the column with a different stationary phase. Noncovalent coating has been utilized to prepare a number of silica gel-based stationary phases [1]. For examples, many polymeric stationary phases (including the commercially available polysaccharide and chiral synthetic polymer columns) are coated onto silica gel (or modified silica gel) through noncovalent linking [2,3]. In terms of noncovalent linking of monomeric stationary phases, metal–ligand interaction has been widely used to immobilize stationary phases in ligand-exchange chromatography [4]. In reversed-phase chromatography, hydrophobic anchors have been utilized to immobilize the stationary phase onto the reversed-phase silica gel [5]. In the normal-phase mode, a carboxylic functional group was used successfully as the anchoring group to link chiral selectors to aminopropyl silica gel [6]. In this paper, we would like to report our studies using polar amine or quaternary ammonium moieties as the anchoring group(s) to functionalize unmodified silica gel.

Quaternary ammonium compounds and amines adsorb strongly to unmodified silica gel; therefore, these compounds have the potential to act as anchor groups to immobilize a desired stationary phase

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0021-9673/98/$19.00
PII: S0021-9673(98)00491-9

directly to unmodified silica gel. To test such an idea, we chose to modify Pirkle's dinitrobenzoyl–L-leucine selector with such functional groups. This selector is easy to synthesize and has been used to resolve various kinds of optical isomers successfully [7]. Furthermore, the interactions involved in chiral recognition are relatively well understood [8]. Consequently, three compounds with this chiral selector were prepared: selector **1** with a single amino group as the anchor point, selector **2** with a quaternary ammonium ion group, and selector **3** with three amino groups as anchor points to silica gel.

2. Experimental

2.1. Chemicals

N-(3,5-Dinitrobenzoyl)–L-leucine (DNB–L-leu), and all other chemicals and solvents were purchased from either Aldrich, Fluka or Fisher Scientific unless otherwise noted. HPLC-grade Allsphere silica gel (particle size 5 μm, pore size 80 Å and surface area 220 m^2/g) was purchased from Alltech (Deerfield, IL, USA). Selecto silica gel (32–63 μm) from Fisher Scientific was used for flash column chromatographic purification of target compounds. Thin-layer chromatography was completed using EM silica gel 60 F-254 TLC plates (0.25 mm). D-Leucine and S-N1N-naphthylleucine columns of the Pirkle design were purchased from Regis Technologies (Morton Grove, IL, USA). ^{1}H-NMR spectra were recorded with a Bruker 300 MHz instrument. Elemental analyses were conducted by Atlantic Microlab, Norcross, GA, USA.

2.2. Apparatus

A Spectra-Physics HPLC system equipped with an SP 8800 ternary pump, an SP 8500 dynamic mixer, a Spectromonitor 3200 UV detector and a Hitachi D-2500 chromato-integrator was used in this study.

A standard column packer was purchased from Alltech. Empty stainless HPLC column hardware was from Separation Technologies (Hopedale, MA, USA).

2.3. Column packing

All normal phase HPLC columns required for this study were packed using the standard slurry packing method at 7000 p.s.i. (1 p.s.i.=6894.76 Pa) with ethanol as both the slurry and pressurizing solvent. Allsphere silica gel (5 μm, 80 Å) was acid-washed prior to use following a procedure from the literature [9,10].

2.4. Preparation of compound *1*

To a mixture of DNB–L-leu (0.95 g, 2.9 mmol), *N*-Boc-1,3-diaminopropane (0.50 ml, 2.9 mmol) and *N*-methylmorpholine (0.64 ml, 5.8 mmol) in dimethylformamide (DMF) (5 ml) was added pentafluorophenyl diphenylphosphinate (FDPP; 1.3 g, 3.4 mmol) at 0°C. After warming to and stirring at room temperature for 4 h, the mixture was diluted with CH_2Cl_2 (50 ml) and then washed with 10% citric acid, 10% $NaHCO_3$ and water. After removal of organic solvents, the residue was purified by flash column chromatography (first 10% then 30% ethyl acetate in CH_2Cl_2) to yield amide **4** (0.70 g, 50%). The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3$ δ 0.93–1.00 (m, 6H), 1.41 (s, 9H), 1.63–1.78 (m, 5H), 3.20 (t, J=5.9 Hz, 2H), 3.32 (t, J=5.9 Hz, 2H), 4.71 (m, 1H), 4.76 (m, 1H), 7.20 (m, 1H), 7.84 (d, J=8.2 Hz, 1H), 9.00 (d, J=2.0 Hz, 2H), 9.15 (t, J=2.2 Hz, 1H).

Amide **4** (0.70 g, 1.5 mmol) was stirred with 10 ml of 4 M HCl in dioxane for 30 min. Upon removal of excess HCl and solvent, the residue was dissolved in 10% methanol in CH_2Cl_2 and passed through an amberlyst A21 weakly basic ion-exchange resin to yield the free amine, compound **1** (0.53 g, 95%). The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3/CD_3OD$ (10/1): δ 0.93–1.05 (m, 6H), 1.62–1.80 (m, 5H), 2.73 (t, J=6.0 Hz, 2H), 3.32 (t, J=5.9 Hz, 2H), 4.65 (m, 1H), 9.15 (m, 3H).

2.5. Preparation of compound *2*

Selector **1** (0.15 g, 0.39 mmol) was dissolved in ethanol (5 ml); subsequently, methyl iodide (1.0 ml, 16 mmol) and $NaHCO_3$ (0.5 g, 6 mmol) were added. The mixture was refluxed at 80°C for 24 h. After

evaporation of ethanol and excess methyl iodide, the residue was purified by a method we developed recently [11] to give compound **2** (0.10 g, 50%). The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3/CD_3OD$ (10/1): δ 0.95–1.05 (m, 6H), 1.70–2.10 (m, 5H), 3.22 (s, 9H), 3.25–3.70 (m, 4H), 4.55 (m, 1H), 9.18 (m, 3H).

2.6. Preparation of compound 3

To tris(2-aminoethyl)ethylamine (5.2 ml, 35 mmol) in CH_2Cl_2 (600 ml) at −78°C, di-*tert.*-butyl dicarbonate (Boc_2O; 7.6 g, 35 mmol) in CH_2Cl_2 (200 ml) was added dropwise with vigorous stirring. After the addition was complete, the mixture was warmed to room temperature and stirred for 1 h. Upon solvent removal, the residue was purified by flash chromatography (first with 5% methanol in CH_2Cl_2, then 1% ammonium hydroxide in methanol) to give compound **5** (4.1 g, 34%). The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3$: δ 1.45 (s, 18H), 2.46–2.58 (m, 6H), 2.73 (t, J=6.0 Hz, 2H), 3.18 (m, 4H), 5.28 (broad s, 2H).

To a mixture of DNB–L-leu (1.30 g, 4.0 mmol) and **5** (1.39 g,4.0 mmol) in methylene chloride (20 ml), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ; 1.19 g, 4.8 mmol) was added. After stirring at room temperature for 18 h, the solvent was removed and the residue was purified by flash chromatography (first with 10% and then 30% ethyl acetate in CH_2Cl_2) to give **6** (1.4 g, 54%). The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3/CD_3OD$ (10/1): δ 0.98–1.02 (m, 6H), 1.44 (s, 18H), 1.69–1.77 (m, 3H), 2.50–2.63 (m, 6H), 3.10 (m, 4H), 3.27 (m, 2H), 4.74 (m, 1H), 9.16 (s, 3H).

Compound **6** was converted to the free amine **3** following the same procedure as that used for the preparation of compound **1**. The compound was pure, as judged by ^{1}H-NMR. In $CDCl_3/CD_3OD$ (10/1): δ 0.90–1.05 (m, 6H), 1.60–1.80 (m, 3H), 2.46–2.64 (m, 6H), 2.75 (t, J=6.0 Hz, 4H), 4.68 (m, 1H), 9.16 (s, 3H).

2.7. Determination of enantiomeric purity

The enantiomeric purity of the synthetic intermediates **4** and **6** was determined by HPLC using Pirkle's S-N1N-naphthylleucine column (mobile phase: CH_3OH–CH_2Cl_2, 55:45, v/v). For control experiments, the corresponding racemic mixtures were synthesized in each case starting from racemic DNB–leu following the same synthetic sequence. The enantiomeric purity (enantiomeric excess) of DNB–L-leu was determined to be 95% (mobile phase: 80% isopropanol in hexane) using the same column.

2.8. Column coating procedure

To coat a normal phase silica gel HPLC column, the chiral selector of interest (**1**, **2** or **3**) was dissolved in methylene chloride (100 mg in 50 ml). If solubility problems occurred, a small amount of methanol (<5%) was added. The solution was pumped through the column until the column was saturated with the coating molecule. Saturation was determined by monitoring column elution using an UV detector. Usually less than 25 ml of solution was needed to coat one short column (5×0.46 cm I.D.). Subsequent washing with two column volumes of the same solvent as used to dissolve the selector ensured removal of nonbonded selector molecules. The column was then conditioned with the desired mobile phase. After baseline stabilization, column testing commenced.

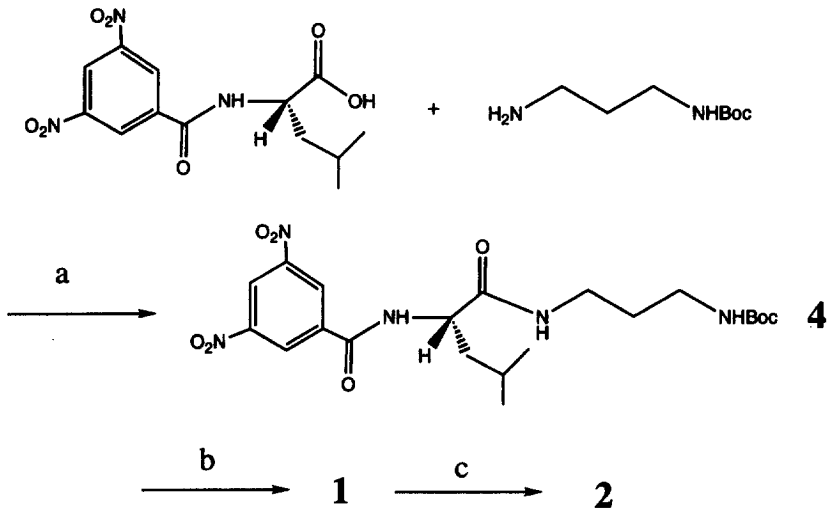
2.9. Surface coverage determination of columns 1, 2 and 3

The surface DNB–leu coverage was determined by elemental analysis. Columns **1–3** were unpacked after coating and column evaluation, the coated silica gel stationary phases were thoroughly mixed and the percentage nitrogen of each coated stationary phase was determined by elemental analysis. The percentages of nitrogen in stationary phases **1–3** were 2.07, 0.98 and 1.51%, respectively.

3. Results and discussion

3.1. Synthesis of target compounds

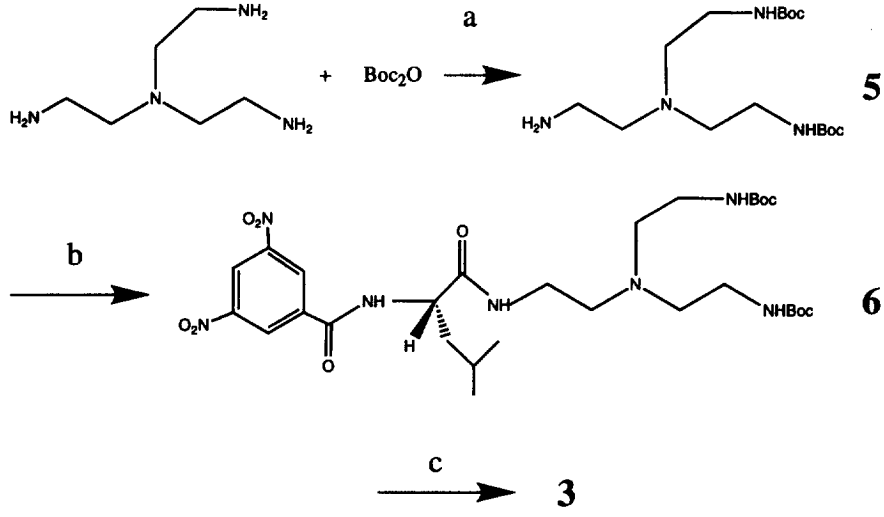
Compounds **1** and **2** were synthesized according to Scheme 1. The coupling between DNB–L-leu and



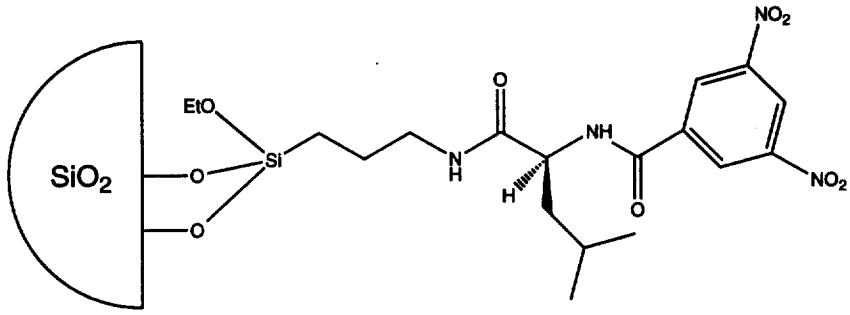
Scheme 1. Reagents, conditions and yields: (a) FDPP, 50%; (b) (1) HCl, (2) anion exchange resin, 95%; (c) MeI, $NaHCO_3$, 50%.

N-Boc-1,3-diaminopropane was completed with FDPP [12]. The enantiomeric purity (enantiomeric excess: ee%) of the resulting amide **4** was determined to be 95%, the same as that of the commercially available starting material.

Compound **3** was synthesized according to Scheme 2. The protection of tris(2-aminoethyl)ethylamine by two Boc groups required low temperature and high dilution [13]. The separation of amine **5** from both mono-Boc-protected and tris-Boc-protected amines was accomplished easily by flash column chromatography. Amide coupling between DNB-L-leu and amine **5** was initially carried out with FDPP; however, noticeable racemization was observed, with the ee of the resulting compound being 89%. Changing the coupling conditions to EEDQ, the coupling reagent used by Pirkle for the preparation of his covalent DNB-L-leu column [14], yielded the desired compound **6** with no detectable racemization (ee 95%).



Scheme 2. Reagents, conditions and yields: (a) CH_2Cl_2, −78°C, 65%; (b) EEDQ, 54%; (c) (1) HCl, (2) anion exchange resin.



Scheme 3. Pirkle's covalently linked L-leucine column.

3.2. Chromatographic evaluation of noncovalently linked chiral stationary phases

The chromatographic behavior of these three chiral selectors was examined by coating each selector on a separate normal phase silica gel column. Each column (column 1, 2 and 3 for selectors **1**, **2** and **3**, respectively) was evaluated using racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol as the analyte. For comparison, the covalent counterpart of the same selector (Scheme 3) was prepared following a published procedure [14], hereafter designated the noncommercial Pirkle column. Comparison was also made with a commercially available D-leucine column. The capacity factors and separation factors of these columns in two mobile phases are listed in Table 1.

As seen from the data, the commercial Pirkle column resolved racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol efficiently. The noncommercial Pirkle column could also resolve racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol efficiently. Both the monoamine selector-coated column (column 1) and the triamine selector-coated column (column 3) resolved racemic 2,2,2-trifluoro-1-(9-anthryl) ethanol with comparable efficiency to that of the noncommercial Pirkle column (Fig. 1). The selector bound with a quaternary ammonium group (column 2), however, proved to be much less efficient.

The preparation of the commercial Pirkle column was undisclosed, and the optical purity of this stationary phase was unknown. The optical purity of the noncommerical Pirkle column was determined to be 94% ee by a procedure that we had developed [15]. The optical purity of target molecules **1**, **2** and **3** in Scheme 4 was not directly measured; however,

Table 1
Separation of racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol on chiral columns[a]

Column (dimension)	Capacity factor (k') of least retained enantiomer		Separation factor (α)	
	Condition A	Condition B	Condition A	Condition B
Commercial Pirkle column (25×0.46 cm)	1.00	3.69	1.42	1.56
Noncommercial Pirkle column (5×0.46 cm)	1.09	4.26	1.39	1.49
Column 1 (10×0.46 cm)	0.67	3.06	1.39	1.43
Column 2 (5×0.46 cm)	1.14	na	1.15	na
Column 3 (5×0.46 cm)	1.06	4.06	1.29	1.33

[a]Column dimensions in length (cm)×I.D. (cm).
Condition A: 10% isopropanol in hexane as the mobile phase.
Condition B: 2% isopropanol in hexane as the mobile phase.
Flow rate: 1.2 ml/min.
The dead time, t_0, was measured with 1,3,5-tri-*tert.*-butylbenzene as the void volume marker [19].
Capacity factor (k') equals $(t_r - t_0)/t_r$ in which t_0 is the retention time and the separation factor (α) equals k'_2/k'_1.

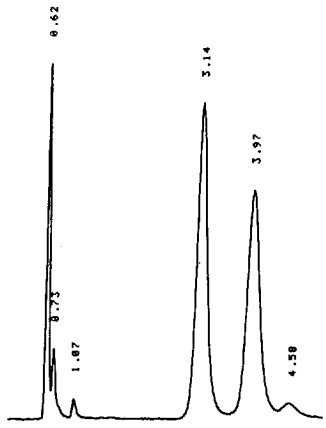
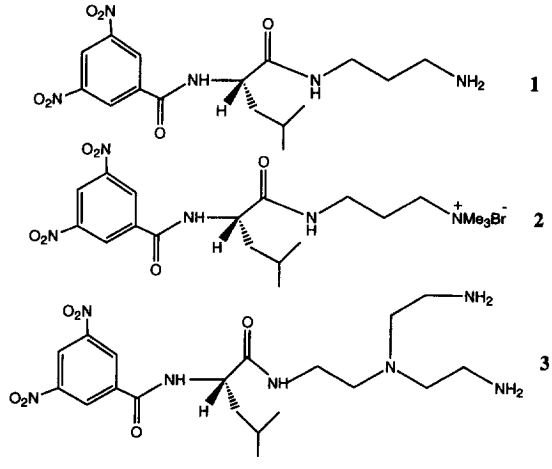


Fig. 1. Chiral resolution of racemic 2,2,2-trifluoro-1-(9-anthryl)-ethanol with column 3. Flow-rate, 1.2 ml/min. Mobile phase: 2% isopropanol in hexane. The peak at 0.62 min is from 1,3,5-tri-t-butylbenzene. The peak at 3.14 min is from the *S*-enantiomer and the peak at 3.97 min is that of the *R*-enantiomer. other peaks (0.73, 1.07, 4.58 min) are impurities.

the optical purity of the immediate precursors to each molecule was determined (see Section 2). Both the deprotection of Boc with HCl in dioxane and the conversion of amine hydrochloride to the free amine by a weak anion-exchange resin could not introduce racemization [16]. Permethylation with methyl iodide was unlikely to introduce racemization as well. Therefore, we believe that all of the stationary phases examined have an optical purity of very close to 95% ee, except the commercial Pirkle column



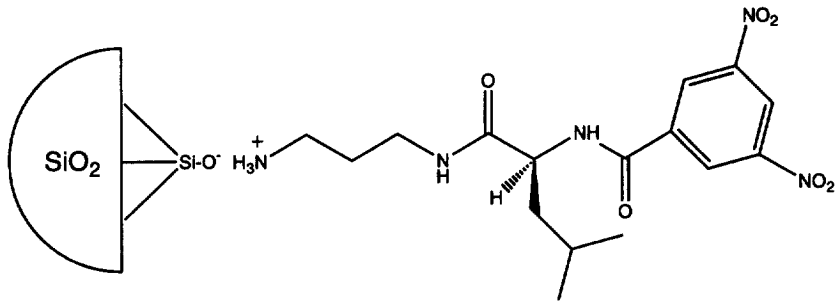
Scheme 4. Target chiral selectors.

which may have a higher optical purity. Regardless, in the range of separation factors observed, 5% ee or less should not introduce significant change in the separation factor [17]. We are inclined to believe that some type of endcapping was involved in making the commercial column. Previous studies indicated that endcapping had been shown to increase the separation efficiency of some of the Pirkle columns [18].

As stated above, column 1 had separation factors similar to those of the noncommercial Pirkle column, demonstrating the feasibility of noncovalent linking through an amino group to unmodified silica gel. The stability of this particular column, however, was less than desirable. The column, while stable with 2% isopropanol–hexane as the mobile phase, had a tendency to elute its coating when the mobile phase was changed to 10% isopropanol–hexane. The triamine-coated column was much more stable; elution of the coating was not observed even after prolonged washing with 10% isopropanol–hexane. The column coated with the quaternary ammonium ion selector proved to be stable at 10% isopropanol–hexane as well; however, as stated earlier, the separation factor was much worse.

The failure to obtain efficient separation with the quaternary ammonium ion-coated column indicates that the linkage between selector and stationary phase is of significant importance in chiral separations. As the silica gel surface consists of weakly acidic silanol groups, it is reasonable to say that an acid/base interaction is the foundation of the noncovalent immobilization (Scheme 5) with selector **1**. Consequently, the triamine-linked column (column 3) is more stable because three such interactions can exist. With a quaternary ammonium ion as the point of attachment, such acid–base interactions do not exist. Since the acidity of the surface silanol groups is not neutralized, they may participate in non-specific interactions with the analyte, thereby reducing the separation factor.

The surface concentration of the DNB–leu in stationary phases **1–3** was estimated from the nitrogen percentage of the stationary phases, which was determined by elemental analysis (see Section 2.9). For stationary phases **1–3**, the surface concentrations (per gram of resin) were 0.30, 0.14 and 0.15 mmol/g, respectively. Assuming that the coated stationary phase has approximately the same surface area as the



Scheme 5. Possible interactions between the amine anchor group and the silica gel surface.

parent silica gel (220 m^2/g, according to the supplier), the surface coverage can also be expressed as 1.4, 0.64 and 0.68 $\mu mol/m^2$, respectively. For comparison, the surface DNB–leu concentration of the noncommercial Pirkle column is 0.9 $\mu mol/m^2$ [15]. As pointed out by one reviewer, it is somewhat surprising that the surface coverage of stationary phase **3** is lower than that of stationary phase **1**. Under the conditions where the surface coverage was determined, both stationary phases **1** and **3** bind to the silica gel tightly. In stationary phase **3**, a larger anchoring group might be responsible for the lower coverage. The surface coverage of stationary phase **2** is also lower than that of stationary phase **1**. In this case, the surface binding is different and we cannot offer a reasonable explanation at this time.

4. Conclusion

Noncovalent linking of special stationary phases to unmodified silica gel through amine moieties is feasible. The column coated with a chiral selector having a monoamine functional group is as efficient as a noncommercial covalently linked Pirkle column in resolving racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol. The column coated with a selector possessing a triamine functional group is more stable and is still capable of efficient chiral resolution of racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol. The column coated with a selector possessing a quaternary ammonium functional group is much less efficient at resolving racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol. The generality and other applications of such an approach to functionalize unmodified silica gel will be studied.

Acknowledgements

Financial support from Vanderbilt University, in the form of a start-up fund, research council, and natural science fund is appreciated. The project is also supported in part with an ACS PRF grant (#31692-G1) and an American Cancer Society Institutional Research Grant (#IN-25-37, through Vanderbilt cancer center).

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