Flash Chiral Chromatography using Carbohydrate Carbamate-coated Silica

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Enantiomers of many chiral compounds are resolved rapidly on a preparative scale by passage through a column packed with flash chromatography silica which has been physically coated with a carbohydrate carbamate.

Demand for optically pure samples of enantiomers for biological investigation in modern drug development has led to extensive research in asymmetric synthesis and separation technology. Chromatographic columns containing a chiral stationary phase chemically bonded to, or physically coated on, an inert support have proved to be extremely successful for the analysis of enantiomeric composition by HPLC.[1] Preparative HPLC columns with chiral phases are available,[2] but their use is limited by the technical difficulty of high-pressure slurry packing long, wide-bore tubes and by the expense of such columns and the associated equipment.

The chemically-bonded chiral phases developed by Pirkle's group[3] have been shown[4] to have potential for preparative applications using flash chromatography in a limited range of cases where enantiomers possess large ($\alpha > 2$) separation factors. However, this approach was reported to be of reduced utility for obtaining high recoveries of pure enantiomers from a racemate with a lower (*e.g.* $\alpha = 1.2$) separation factor.[4]

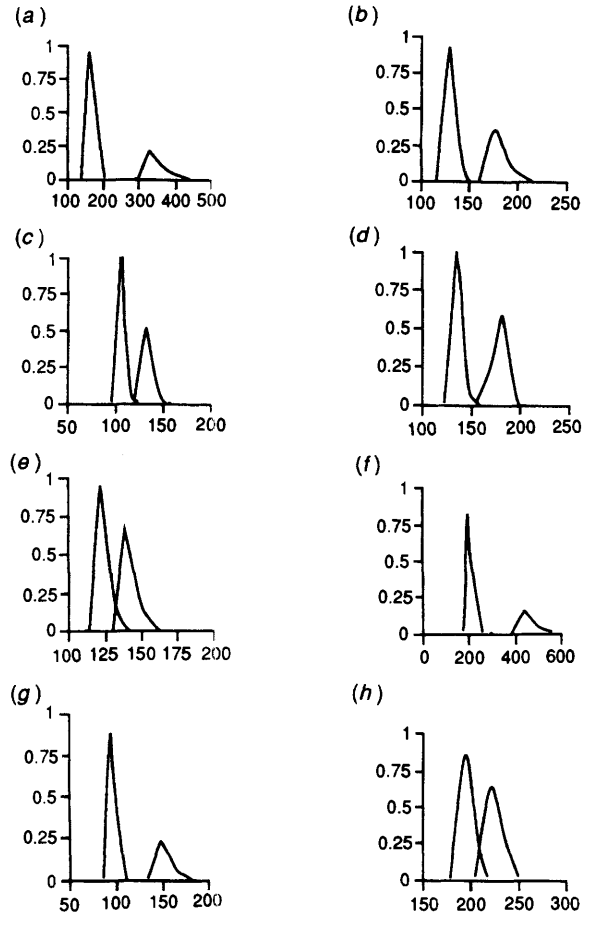
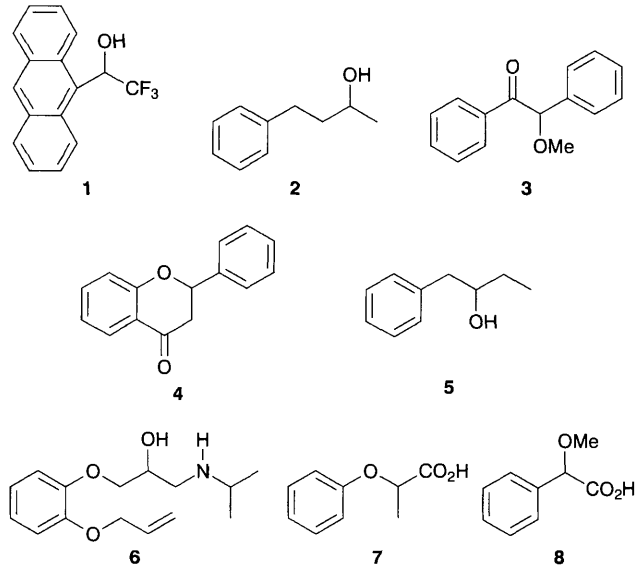


Fig. 1 Chromatographic resolutions of enantiomers of neutral (**1–5**), basic (**6**) and acidic (**7–8**) compounds on cellulose tris(3,5-dimethylphenyl carbamate) coated on flash chromatography silica (for elution conditions, see Table 1): (*a*) **1** 1-(9-anthryl)-2,2,2-trifluoroethanol; (*b*) **2** 4-phenylbutan-2-ol; (*c*) **3** benzoin methyl ether; (*d*) **4** flavanone; (*e*) **5** 1-phenylbutan-2-ol; (*f*) **6** oxprenolol; (*g*) **7** 2-phenoxypropionic acid; (*h*) **8** α-methoxyphenyl acetic acid. *x*-axes = volume/ml; *y*-axes = relative response.

During the last decade, the chiral HPLC phases developed by Okamoto's group and commercialised by Daicel have proved to be outstandingly successful for the chromatographic resolution of a wide range of chiral compounds.[5] These phases are prepared by the silylation (*e.g.* with aminopropyl triethoxysilane) of a wide-pore (1000–4000 Å) silica (typically 7 μm diameter, spherical beads) onto which a carbohydrate carbamate layer (*ca.* 20% *m/m*) is coated by evaporation of a solution. The carbohydrate carbamates are synthesised by reaction of cellulose or amylose with an aryl isocyanate such as phenyl, 3,5-dimethylphenyl or (*S*)-α-methylbenzyl isocyanate and Okamoto has reported[5] that about 80% of all racemates examined were resolved on such phases.

We have recently demonstrated[6] that it is not necessary to use silylated, wide-pore supports and that very efficient chiral separations can be achieved using HPLC columns containing a carbohydrate carbamate coated non-covalently onto a standard HPLC-grade silica (5 μm Hypersil, 120 Å pore diameter). In view of the wide separation scope of these phases, their ease and simplicity of preparation and their good loading capacity, these observations led us to explore their potential for preparative-scale application in a flash chromatography mode.

Flash chromatography[7] silica was coated at a loading of 20% *m/m* with cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC) by evaporation, and the dried material (40 g) was slurried in Pr^iOH–hexane (10:90 *v/v*) and packed into a glass chromatography column.† Racemic compounds (20 mg) were applied as solutions in 0.5–1 cm^3 of mobile phase and the column was run at 4 $cm^3\ min^{-1}$. Fractions were collected in 2–4 cm^3 ml aliquots and were analysed by HPLC on a column (15 cm × 0.46 cm i.d.) packed with Hypersil silica (5 μm, 120 Å, 180 $m^2\ g^{-1}$) coated with CDMPC (20% *m/m*). The results for a range of chiral compounds **1–8** are shown in Fig. 1. Whilst neutral compounds such as **1–5** can be eluted cleanly with alcohol–hexane mixtures, it is advisable to add diethylamine to the mobile phase for amines such as **6** and to add trifluoroacetic



acid for acids such as **7** and **8**, in order to minimise peak tailing.

Separation factors observed on the flash column were very similar to those obtained on the CDMPC-coated silica HPLC column (Table 1), so that the latter can be used to establish optimum preparative conditions, as well as to analyse the eluted fractions. For a given α value, the success of the separation, as measured by the recovery of each pure enantiomer, is determined by factors controlling peak shape. The resolution term R_s (required to be >1.5 for 100% peak separation) decreases with increasing peak width at the base and, as expected, is lower on the coarser flash silica than on the HPLC packing (Table 1). Peak tailing, particularly by the leading peak, can be a critical factor in reducing the recovery of the pure second enantiomer. For the four neutral compounds **1–4**, the amine **6** and the acid **7**, the good R_s values and limited tailing observed on the analytical HPLC column correlated with almost quantitative recovery of each enantiomer down to an analytical resolution of $\alpha = 1.43$. For the alcohol **5**, reduced α and R_s values and peak skewing were observed on the analytical column and the recoveries on the preparative column were correspondingly lower. Better recoveries were obtained for the acid **8** which, despite having a lower α and R_s than **5**, gave more symmetrical peaks which were just resolved to baseline on the analytical column.

The loading capacity of the flash chiral column depends on the extent of separation. A 100 mg sample of 4-phenylbutan-2-ol **2** gave 99% recovery of the first enantiomer and 82% recovery of the second enantiomer after one pass. A 100 mg sample of benzoin methyl ether **3** gave 94% recovery of the first enantiomer and 29% recovery of the second enantiomer after one pass; recycling of the intermediate fractions gave a net yield for the two passes of 98% of the first enantiomer and 93% of the second enantiomer. The same column can be retained and reused many times for separation of the same or different compounds, without appreciable loss of efficiency.

Further investigations of the scope and utility of flash chiral chromatography using carbohydrate carbamate phases are in progress.

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Table 1 Chromatographic parameters for analytical and preparative resolution of 20 mg samples of racemates

	HPLC column[a]		Flash preparative column[a]			
					Recovery of pure enantiomer (%)	
Compound	α	R_s	α	R_s	Peak 1	Peak 2
1	2.93	8.55	2.93	2.45	100	100
2	1.76	4.37	1.66	1.70	100	100
3	1.60	3.19	1.58	1.44	100	97
4	1.43	2.89	1.62	1.91	100	100
5	1.29	1.94	1.29	0.93	72	50
6	2.75	6.69	2.79	2.56	100	100
7	2.55	5.58	2.63	2.32	100	100
8	1.18	1.49	1.21	0.82	87	65

[a] *Mobile phases*: **1**, **3**, **4**: Pr^iOH–hexane 10:90 *v/v*; **2**: Bu^nOH–hexane 10:90 *v/v*; **5**: Bu^nOH–hexane 5:95 *v/v*; **6**: Et_2NH–Pr^iOH–hexane 1:20:80 *v/v*; **7**: CF_3CO_2H–Pr^iOH–hexane 0.5:10:90 *v/v*; **8** CF_3CO_2H–EtOH–hexane 0.5:5:95 *v/v*; eluted at 4 $cm^3\ min^{-1}$.

Footnote

† Flash chromatography silica (40 g, Davisil 40–63 μm, 150 Å pore diameter, surface area 300 $m^2\ g^{-1}$) was refluxed in dry THF (200 cm^3) for 30 min, cooled, and to the stirred suspension was added a solution of cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC) (10 g) in THF–*N*,*N*-dimethylacetamide (50 cm^3; 95:5 *v/v*). The solvent was removed on a rotary evaporator and the dry residue stood in a vacuum dessicator overnight over P_2O_5. A portion of this material (40 g) was slurried in Pr^iOH–hexane (10:90 *v/v*) and packed into a glass column (40 × 2 cm i.d.) fitted with a tap above which was inserted a plug of glass wool and a layer of fine sand. The packed column bed, with a height of *ca.* 25 cm, was topped with a further layer of sand.

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