ChemComm

# A novel stationary phase for chiral chromatography: poly-L-leucine supported on porous graphitic carbon and its application to the separation of the enantiomers of chiral epoxides

**Elizabeth J. Kelly,[a] David M. Haddleton,[a] David H. G. Crout,*[a] Paul Ross[b] and James Dutton[b]**

[a] *Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL. E-mail: d.h.g.crout@warwick.ac.uk*
[b] *Hypersil, Chadwick Road, Astmoor, Runcorn, Cheshire, UK WA7 7PR*

*Received (in Liverpool, UK) 17th February 1999, Accepted 19th May 1999*

**A novel chiral stationary phase consisting of poly-L-leucine supported on porous graphitic carbon has been shown to be effective in the separation of the enantiomers of epoxides 1-5 by chiral high performance liquid chromatography.**

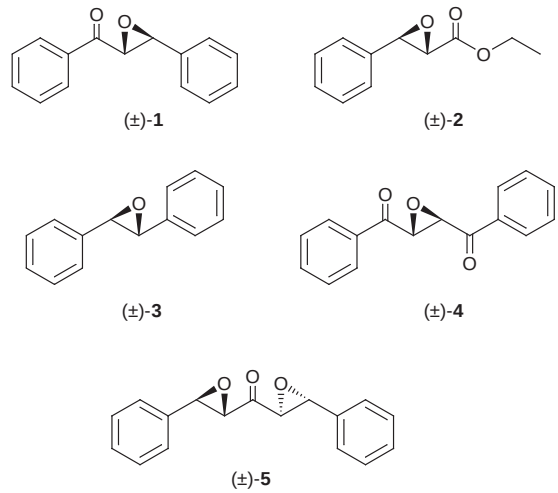
The separation of enantiomers using chiral stationary phases in high performance liquid chromatography is an important technique for both the analysis and preparation of chiral compounds.[1] The use of polysaccharide derivatives, first described by Okamoto *et al*.,[2] has been primarily responsible for the increase in accessibility of this technique. Polysaccharides, cellulose and amylose, in particular, are derivatised through the formation of carbamate residues which results in highly crystalline polymers. The recognition of chirality in analytes is attributable not only to the inherent chirality of the carbohydrate components of the polymer, but also on the substantial helical nature of the tertiary structure.[3]

Polymers are finding wide-spread use as chiral catalysts in asymmetric synthesis. For example, Julia[4] and Roberts[5] have shown that poly-L-leucine functions as an asymmetric catalyst for the epoxidation of certain α,β-unsaturated ketones. The use of polymeric α-amino acids in synthesis has been reviewed.[6] However, the basis of enantiorecognition in this system has not been conclusively determined.[7] The factors involved are no doubt similar to those responsible for the enantioselectivity of chiral stationary phases in adsorption chromatography. An understanding of the mechanism of enantiorecognition in both systems would undoubtedly enhance the development of these techniques.[8] Colonna *et al*. have suggested that enantiorecognition is attributable to hydrogen bonding between the ketone functionality and the amide NH groups of the polymer backbone.[8] More recently Roberts and co-workers discussed the chiral architecture of poly-L-leucine and suggested that the enantiorecognition was induced mainly by the amino terminus of the peptide.[9]

The remarkable selectivity generated by poly-L-leucine in the Julia–Colonna system led us to explore the use of poly-L-leucine supported on porous graphitic carbon (PLL-PGC) as a chiral stationary phase for HPLC. Poly-L-leucine with derivatised end groups has been previously described in the enantioseparation of D- and L-leucine dipeptides.[10] The separation of a racemic mixture using PLL-PGC provides evidence for the level of interaction between poly-L-leucine and the analyte.

Poly-L-leucine was synthesised by ring opening polymerisation of L-leucine *N*-carboxyanhydride, using either 1,2-diaminoethane as initiator, resulting in polymer with amino functionality at both termini of the polymer chain, or water as initiator. Molecular mass information is difficult to obtain owing to the low solubility of this crystalline polymer. However, using highly acidic solutions (1% TFA and 1% formic acid respectively) both MALDI-TOF-MS and ESI-MS spectra of poly-L-leucine produced with 1,2-diaminoethane as initiator were obtained. In MALDI-TOF-MS the observed ions range from *m/z* 647.1 to 2116.1 (DP 5 to 18). The highest mass singly charged ion observed in electrospray ionisation had *m/z* 1759.3 (DP 15).

Poly-L-leucine was adsorbed onto the surface of porous graphitic carbon through the slow evaporation of a solution in 5% TFA in EtOH. The stationary phase was subsequently slurry packed under high pressure into high performance liquid chromatography columns. Columns giving the best performance were obtained using poly-L-leucine that had been continuously extracted (Soxhlet) with DMF–EtOH (5:1) for 48 h. The epoxides **1**-**5**, all but one of which correspond to the




epoxide of an α,β-unsaturated ketone or ester, were analysed by reversed-phase HPLC. The results are given in Table 1. In all cases complete resolution of the two enantiomers was achieved. Fig. 1 shows the chromatogram recorded for the separation of the enantiomers of chalcone α,β-epoxide **1**. When racemic mixtures of **1**–**5** were eluted on an uncoated porous graphitic column, no separation of enantiomers was observed. The retention times of epoxides **1**-**5** were much greater than those observed with PLL-PGC. For example, the elution of chalcone α,β-epoxide from the uncoated column did not begin until 15 min after injection. Elution was complete after 25 min.

**Table 1** HPLC data for the resolution of the enantiomers of epoxides **1**-**5** by poly-L-leucine supported on porous graphitic carbon[a]

| Compound | $k_1$ | $k_2$ | $\alpha$ |
|---|---|---|---|
| **1** | 4.35 | 10.68 | 2.56 |
| **2** | 4.33 | 5.82 | 1.34 |
| **3** | 2.05 | 2.42 | 1.18 |
| **4** | 5.58 | 12.45 | 2.13 |
| **5** | 6.47 | 12.30 | 1.90 |

[a] Solvent system: MeCN–$H_2O$ (90 : 10 v/v), 0.5 cm$^3$ min$^{-1}$; column: 250 × 4.8 mm, $k = (t_R - t_0)/t_0$; $k_1$ = capacity factor for the first eluted peak; $k_2$ = capacity factor for the second eluted peak; $\alpha$ is the separation factor and is defined as $\alpha = k_2/k_1 = (t_{R2} - t_0)/(t_{R1} - t_0)$.

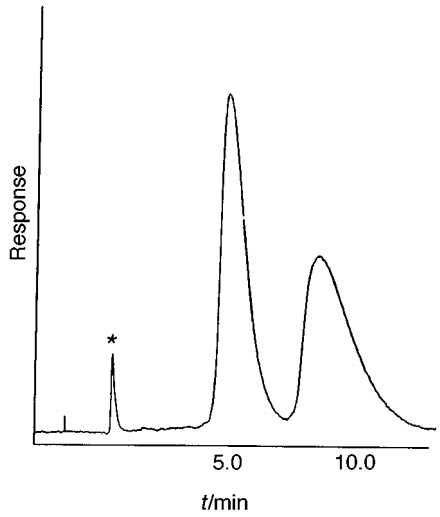
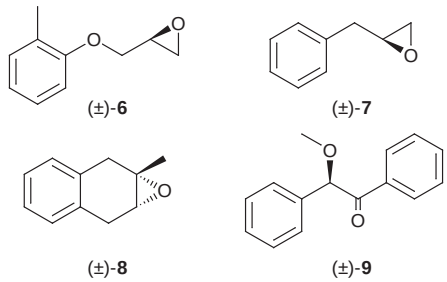



**Fig. 1** Enantioseparation of epoxide **1** using PGC-PLL. Mobile phase: MeCN–$H_2O$ (90 : 10 v/v), flow rate = 1.0 $cm^3$ $min^{-1}$; $t_0$ is marked with acetone (*).

The use of poly-L-leucine as an asymmetric catalyst for epoxidation has been largely confined to α,β-unsaturated ketones. The separation of *trans*-stilbene oxide **3** suggests that the mechanism of enantiorecognition is dominated by the chirality of the epoxide and its interaction with the polymer, and not by the presence of the carbonyl group. Compounds **6**-**9** were




not separated on the PLL-PGC column. Comparison between this group and compounds **1**-**5** suggests the presence of an epoxide group with an attached phenyl or benzoyl substituent is necessary for successful separation of enantiomers. Although the number of examples is limited, these results do suggest that there is a parallel between the ability of poly-L-leucine to catalyse asymmetric epoxidation of α,β-unsaturated ketones and its ability to resolve the corresponding epoxides on PLL-PGC columns.

In summary we have demonstrated the use of a poly(amino acid)-coated porous graphitic carbon as an effective stationary phase in chiral HPLC. Poly-L-leucine has been shown to be an excellent alternative to cellulose as a stationary phase for the separation of the enantiomers of chiral epoxides. The parallel between the effectiveness of poly-L-leucine in catalysing the epoxidation of α,β-unsaturated ketones with high stereodiscrimination, and its effectiveness in resolving racemic mixtures of the same epoxides in the chiral HPLC system described above, suggests that the same factors are responsible for the stereoselectivity exhibited by both systems. It also suggests that the degree of separation of enantiomers of a given epoxide in the PLL-PGC system might be used to predict the effectiveness of poly-L-leucine as a catalyst for the epoxidation of the corresponding α,β-unsaturated ketones.

We thank Hypersil for financial support (to E. J. K.) and Professor K. R. Jennings and Dr A. Millar for electrospray ionisation mass spectrometry.

## Notes and references

1 *A Practical Approach to Chiral Separations by Liquid Chromatography*, ed. G. Subramanian, VCH, New York, 1994.
2 Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 1984, **106**, 5357.
3 T. D. Booth, W. J. Lough, M. Saeed, T. A. G. Nocter and I. W. Wainer, *Chirality*, 1997, **9**, 173.
4 S. Banfi, S. Colonna, H. Molinari, S. Julia and J. Guixer, *Tetrahedron*, 1984, **40**, 5207.
5 W. Kroutil, M. E. Lasterra-Sanhez, S. J. Maddrell, P. Mayon, P. Morgan, S. M. Roberts, S. R. Thornton, C. J. Todd and M. Tuter, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2837.
6 S. Ebrahim and M. Wills, *Tetrahedron: Asymmetry*, 1997, **8**, 3163.
7 L. Pu, *Tetrahedron: Asymmetry*, 1998, **9**, 1457.
8 S. Colonna, H. Molinari, S. Banfi, S. Julia, J. Masana and A. Alvarez, *Tetrahedron*, 1983, **39**, 1635.
9 P. A. Bentley, W. Kroutil, J. A. Littlechild and S. M. Roberts, *Chirality*, 1997, **9**, 198.
10 C. Hirayama, H. Ihara and K. Tanaka, *J. Chromatogr.*, 1988, **450**, 271.

*Communication 9/01367I*