Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 4267–4273

TETRAHEDRON:
ASYMMETRY

Chromatographic resolution of antihypertensive benzopyrans by closed-loop recycling enantioselective high pressure preparative chromatography and their X-ray analysis

Guy Nadler,[a,*] Catherine Dartois,[a] Drake S. Eggleston,[b] R. Curtis Haltiwanger[c] and Michel Martin[a]

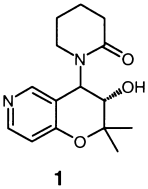
[a] *SmithKline Beecham Laboratoires Pharmaceutiques, BP58, 35762 Saint Grégoire Cedex, France*
[b] *SmithKline Beecham Pharmaceuticals, NFSP, The Pinnacles, Harlow, Essex, CM19 5AD, UK*
[c] *SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, Upper Merion, Pennsylvania, USA*

Received 31 October 1998; accepted 10 November 1998

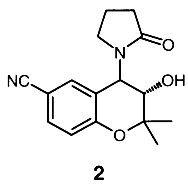
Abstract

The resolution of $(\pm)$-*trans*-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-2H-pyrano[3,2-c]pyridin-3-ol **1**,[1] a potassium channel opener, has been achieved using closed-loop recycling enantioselective preparative chromatography. The resolved enantiomers showed an ee of $>97\%$ based on analytical HPLC and ^{1}H NMR. The absolute configuration has been determined by X-ray analysis of the (*R*)-(+)-α-methylphenylcarbamoyl derivative of one of the enantiomers. © 1998 Elsevier Science Ltd. All rights reserved.

Cromakalim **2**,[2] a potent antihypertensive agent which relaxes smooth muscles by activating potassium channels, was the precursor of a long litany of 3,4-dihydro-2,2-dimethyl-2H-1-benzopyran derivatives. These compounds contain two stereogenic centres but the relative stereochemistry of these is fixed in the *trans* configuration, so that each compound exists as only one pair of enantiomers. As the antihypertensive activity of cromakalim resides principally in one enantiomer,[3] it is important to have suitable preparative methods to resolve the enantiomers of further derivatives of this chemical class.



1



2

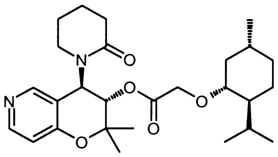
The resolution of the two enantiomers of *trans* **1** proved more difficult than for other 3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans with which we had experience. To investigate separation approaches, we

---

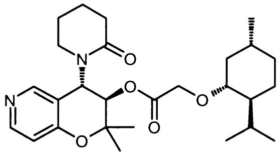
* Corresponding author.

0957-4166/98/$ - see front matter
PII: S0957-4166(98)00461-3

first treated (±)-**1** with (−)-menth-3-yl-oxyacetylchloride, a chiral auxiliary which has been shown to be efficient with similar structures,[4] to obtain the two diastereomeric esters **3** and **4**.[5]

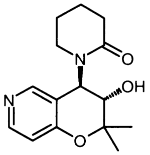


3



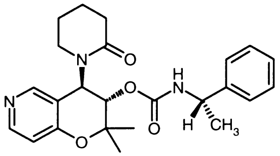
4

Trituration of the **3**+**4** mixture in petroleum ether followed by a re-crystallisation in diethyl ether afforded a sample of pure **3** as determined by HPLC on a Pirkle type chiral stationary phase (CSP). Cleavage of **3** under mild conditions afforded (−)-**1**.

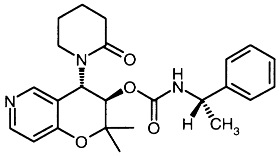


(-) **1**

The difference in retention time between these two diastereomers was sufficient for analytical purposes but did not inspire confidence in a preparative scale-up to isolate **4** from the mother liquor containing a mixture of **3** and **4,** now in an approximately 20:80 ratio. After regeneration of **1**, now enriched in (+)-**1**, from this mother liquor, we derivatized with *S*-(−)-1-phenylethyl isocyanate[3] to obtain a mixture of the two diastereomeric carbamates **5** and **6**.



5



6

This mixture, analysed on a CSP, gave a satisfactory peak separation on an analytical column (Fig. 1 A and B). The partial separation obtained on a preparative column filled with exactly the same phase, under overload conditions, suggested that a closed-loop recycling and peak-shaving chromatography approach could be successfully applied.

This technique, suggested by Martin[6] in 1958, was first realised in practice by Porter and Johnson[7,8] and offers a satisfactory solution in preparative chromatographic separation of enantiomers, especially when the products are only partially resolved. Surprisingly, and despite its inherent advantages and different published industrial versions,[9,10] recycling chromatography has not become very popular.

Using a Prochrom® column filled with 100 g of CSP and operated under 100 bar, we applied this technique to batches of ≈80 mg and recovered, after 6 cycles (Fig. 2), an average of 70% of **6** present in the initial mixture with a minimal ee ranging from 97 to 98%.

It is noteworthy that in the overload conditions used there is no separation of the first peak. The purity of the enantiomer **6** was confirmed by analytical HPLC and ^{1}H NMR and was satisfactory for biological testing. Nevertheless, we preferred to use a purer sample for the absolute configuration studies. Therefore we purified, by the same procedure, a sample which already had 90% ee in **5**. Since its peak was the first to appear it was easier to purify (Fig. 3). Pure **5** (ee >99%) was obtained in only 4 cycles.

The configuration of each stereogenic centre on the pyran ring of **5** has been determined on the basis of the known absolute stereochemistry of the chiral substituent, using three-dimensional X-ray diffraction data collected at reduced temperature. The configurations have been assigned as *R* for the piperidone

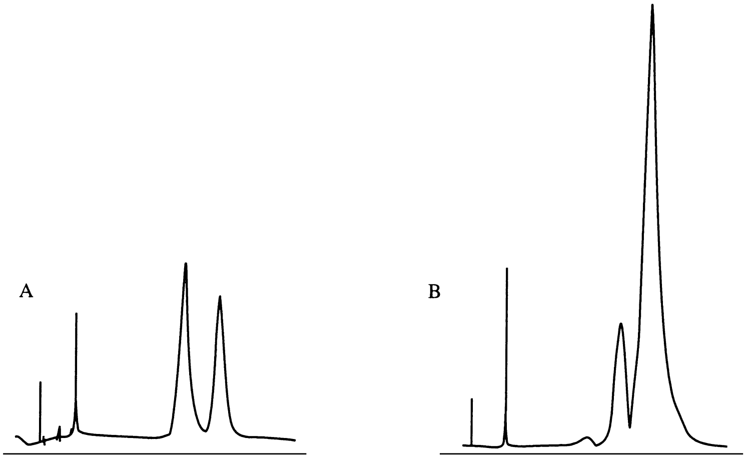


Figure 1. Analytical HPLC separation of a 1:1 mixture of **5** and **6** (A), and the 17:83 mixture used for preparative separation of **5** and **6** (B). For chromatographic conditions see the experimental section

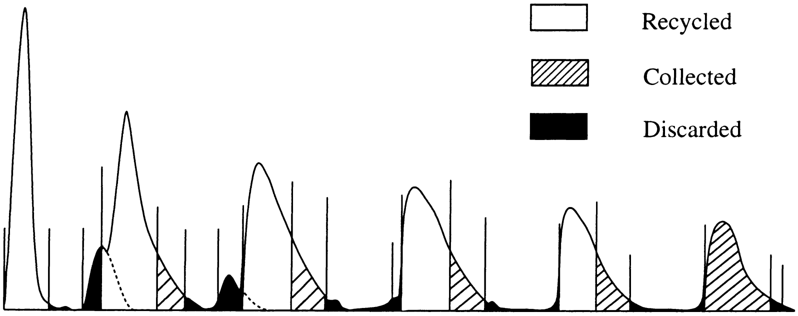


Figure 2. Closed loop recycling of a mixture of **5** and **6**. The empty part of the peak was recycled, the filled part discarded and the hatched part collected to recover **6**. For chromatographic conditions, see the experimental section

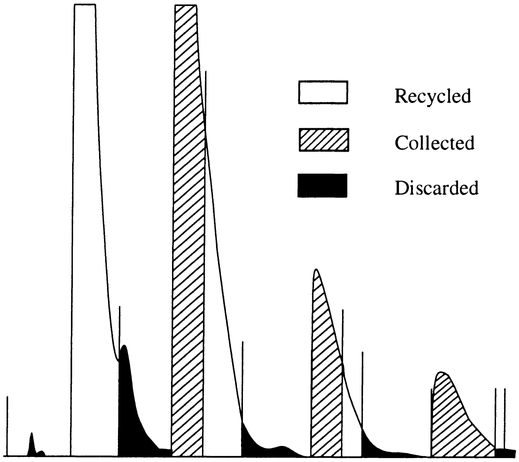


Figure 3. Closed loop recycling of a mixture of **5** and **6**. The empty part of the peak was recycled, the filled part discarded and the hatched part collected to recover **5**. For chromatographic conditions, see the experimental section

substituted carbon and *S* for the adjacent carbon carrying the carbamate substituent. The molecule is shown with the labelling scheme in Fig. 4.

1. Experimental

1.1. General

Optical rotations were determined on a Perkin–Elmer Model 241 digital polarimeter. Melting points were determined with a Gallenkamp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker AC 200 spectrometer in $CDCl_3$ or DMSO-d_6. Preparative high pressure chromatography was performed on a Prochrom LC.50 column (column ID: 5 cm, length: 50 cm, maximum bed length: 35 cm, maximum working pressure: 100 bar (1450 psi)) equipped with two Lewa pumps, a Spectra 100 variable wavelength detector and a Hoke Selecto-mite three way valve as manual injector. Analytical HPLC was performed using a Waters 600E Multisolvent Delivery System and a Waters 996 Photodiode Array Detector. Both the analytical and the preparative columns were filled with the same Pirkle type CSP (10 μm spherical Nucleosil amino silicon polymer covalently bound with *N*-(3,5-dinitrobenzoyl)-(*R*)-phenylglycine) prepared by Interchim (Montluçon, France).

*1.2. (±)-*trans*-3,4-Dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)2H-pyrano-[3,2-c]-pyridin-3-ol* ***1***

The racemic compound was synthesised following the procedure[1] described in Scheme 1, from the appropriate 2,2-dimethyl-2*H*-pyranopyridine **7**.[11]

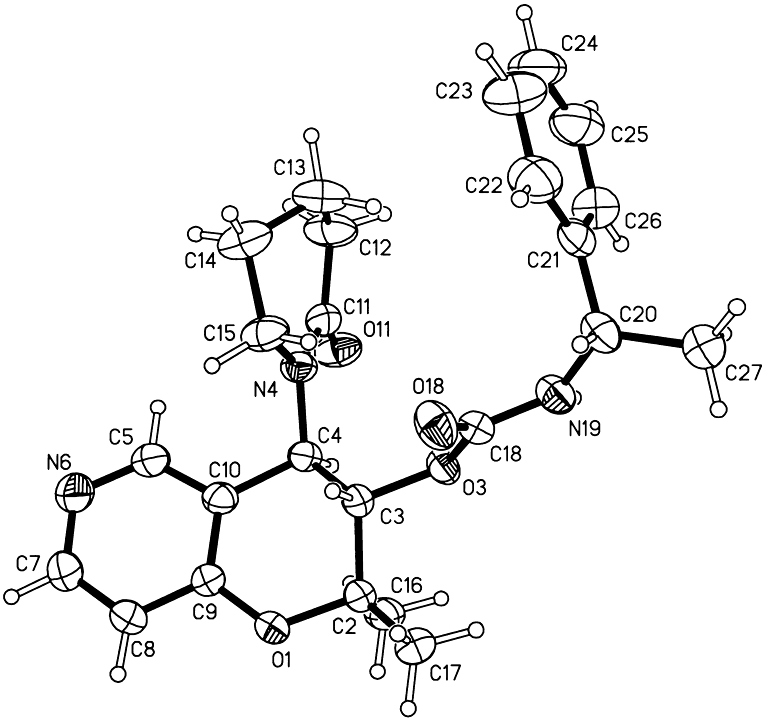
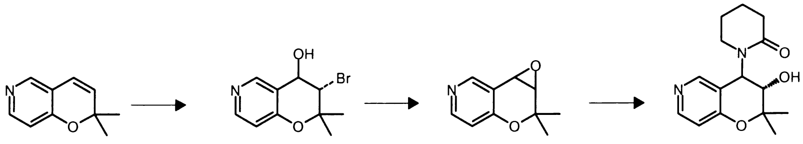


Figure 4. A view of **5** from its crystal structure showing the numbering scheme employed. Anisotropic displacement ellipsoids for non-hydrogen atoms are shown at 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius



Scheme 1.

^{1}H NMR ($CDCl_3$): δ=8.29 (d, *J*=5.6 Hz, 1H), 8.16 (s, 1H), 6.75 (d, *J*=5.6 Hz, 1H), 5.99 (d, *J*=10.2 Hz, 1H), 3.78 (d, *J*=10.2 Hz, 1H), 3.05 (m, 2H), 2.57 (m, 2H), 1.84 (m, 4H), 1.53 (s, 3H), 1.29 (s, 3H).

*1.3. [3(*R*),4(*S*)-3,4-Dihydro-2,2-dimethyl-4-(2-oxopiperidine-1-yl)-2H-pyrano[3,2-c]pyridine-3-yl]-[1(*R*)-(1α,2β,5α)]-[[5-methyl-2-(1-methyl)cyclohexyl]oxy] acetate* ***3***

A solution of crude (−)-menth-3-yl-oxyacetylchloride (2.15 g, corresponding to 7.44 mmol) in CH_2Cl_2 (5 ml) was added dropwise to an ice cooled solution of (±) **1** (1.7 g, 6.15 mmol) and pyridine (0.59 g, 7.5 mmol) in CH_2Cl_2 (20 ml). After stirring for 4 h, CH_2Cl_2 (50 ml) was added and the solution was washed twice with water, dried over $MgSO_4$ and concentrated in vacuo. The residue (3.8 g) was purified by flash chromatography (SiO_2, CH_2Cl_2:MeOH 95:5). After concentration of the desired fractions, the residue was triturated with petrol ether affording 1.17 g of a white solid containing less than 4% of **4**. This solid was suspended in a mixture of petrol ether (25 ml) and diethylether (5 ml) and stirred for 30 min to afford **3** (1.02 g) containing less than 1.5% total impurities and an unmeasurable trace of **4** based on HPLC on a CSP column. M.p. 172°C; ^{1}H NMR ($CDCl_3$): δ=8.35 (d, *J*=5.8 Hz, 1H), 8.22 (s, 1H), 6.89 (d, *J*=5.8 Hz, 1H), 6.27 (d, *J*=9.5 Hz, 1H), 5.30 (d, *J*=9.5 Hz, 1H), 4.24 (d, *J*=16.9 Hz, 1H), 4.07 (d, *J*=16.9 Hz, 1H), 3.32–3.10 (m, 3H), 2.92–2.80 (m, 1H), 2.58–2.35 (m, 2H), 2.33–2.15 (m, 1H), 2.11–1.98 (m, 1H), 1.78–1.61 (m, 8H), 1.47 (s, 3H), 1.38 (s, 3H), 1.32–1.25 (m, 2H), 0.95–0.93 (m, 6H), 0.86 (d, *J*=13 Hz, 3H). Anal. calcd for $C_{27}H_{40}N_2O_5$: C, 68.62; H, 8.53; N, 5.93. Found: C, 68.33; H, 8.96; N, 5.99.

*1.4. (−)-*trans*-3,4-Dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-2H-pyrano-[3,2-c]-pyridin-3-ol (−)-* ***1***

A solution of **3** (1.36 g, 2.9 mmol) and sodium methylate (0.16 g, 3 mmol) in methanol (15 ml) was stirred for 3 h at room temperature (rt). The solvent was concentrated in vacuo and the residue purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1) to afford the title compound (0.71 g, 88,6% yield). M.p. 281°C; $[\alpha]_D^{20}$ −98.6 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$): identical to that of (±) **1**.

*1.5. (±)-[*trans*-3,4-Dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-2H-pyrano-[3,2-c]-pyridin-3-yl]-*N*-[(*S*)-1-phenylethyl]-carbamate* ***5+6***

1.5.1. Purification of ***6***

Mother liquors from preparations of **3** were pooled and cleaved giving a mixture of (+)-**1** and (−)-**1**, now significantly enriched in (+)-**1**. A solution of this mixture (2.8 g, 10 mmol) and *S*-(−)-1-phenylethyl isocyanate (1.79 g, 12 mmole) in anhydrous toluene (80 ml) was refluxed for 24 h. The solvent was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2:MeOH 95:5), affording 4 g (94.4%) of a light tan solid. This mixture, analysed on a Pirkle type CSP column (4.6×250 mm), gave a 17:83 ratio for **5**:**6** (Fig. 1B).

Batches of 76 mg were dissolved in 2.5 ml of the mobile phase (heptane:CH_2Cl_2:iPrOH 90:12:2) and injected in the preparative column filled with 100 g of CSP.

The working pressure was 85 bars and the flow rate 107 ml/min. Each run was recycled 6 times (Fig. 2). A fraction in front of the main peak (corresponding to **5**) as well as a small fraction at the very end (corresponding to an impurity) were discarded (filled surface in Fig. 2). An increasing portion at the end of the main peak (hatched surface) was collected and represents the majority of the peak in the last cycle. The open surface (nearly the whole peak in the first cycle) was recycled.

A total of 1.09 g was injected during the seventeen runs and 0.75 g of **6** containing less than 1.5% of **5** was collected. ^{1}H NMR ($CDCl_3$): δ=8.30 (d, J=5.6 Hz, 1H), 8.17 (s, 1H), 7.40–7.24 (m, 5H), 6.75 (d, J=5.6 Hz, 1H), 6.23 (d, J=10.4 Hz, 1H), 5.28 (d, 1H, exch. D_2O), 5.07 (d, J=10.4 Hz, 1H), 4.81 (m, 1H), 3.31 (m, 1H), 3.43–3.21 (m, 1H), 2.99–2.80 (m, 1H), 2.71–2.48 (m, 2H), 1.96–1.71 (m, 4H), 1.50 (d, J=6.8 Hz, 3H), 1.42 (s, 3H), 1.31 (s, 3H). (A trace of the exchangeable proton of **5** could be detected at 5.39 ppm but not quantified.)

*1.5.2. Purification of **5***

For the X-ray analysis we wanted to submit a very pure sample. We derivatized an earlier, less satisfactory batch of (−)-**1** which afforded a batch of **5** with approximately 90% ee. This batch was chromatographed on CSP in the same conditions. Since the main peak appeared first it was not necessary to discard a front fraction, and as the starting purity was reasonably high, only four cycles were needed for each run (Fig. 3). A total of 1.29 g was purified in eleven runs affording 0.94 g of **5** as white crystals with no detectable **6** by analytical HPLC. M.p. 196°C. ^{1}H NMR ($CDCl_3$): δ=8.29 (d, J=5.6 Hz, 1H), 8.11 (s, 1H), 7.34–7.26 (m, 5H), 6.74 (d, J=5.6 Hz, 1H), 6.19 (d, J=10.4 Hz, 1H), 5.39 (d, 1H, exch. D_2O), 5.03 (d, J=10.4 Hz, 1H), 4.77 (m, 1H), 3.29–3.01 (m, 1H), 2.85–2.71 (m, 1H), 2.47–2.26 (m, 1H), 2.13–1.96 (m, 1H), 1.7–1.5 (m, 2H), 1.47 (d, J=6.7 Hz, 3H), 1.46 (s, 3H), 1.34 (s, 3H), 0.99 (s, 0.5H), 0.76 (s, 0.5H). Anal. calcd for $C_{24}H_{29}N_3O_4$: C, 68,07; H, 6.90; N, 9.92. Found: C, 68.16; H, 7.03; N, 9.86.

*1.6. (+)-*trans*-3,4-Dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-2H-pyrano-[3,2-c]-pyridine-3-ol (+)-**1***

A solution of **6** (0.55 g, 1.3 mmol) and sodium methylate (76 mg, 1.37 mmol) in ethanol (25 ml) was stirred for 4 h at reflux. The solvent was concentrated in vacuo and the residue purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 95:5), to afford the title compound (0.28 g, 73.5% yield). M.p. 272°C; $[\alpha]_D^{20}$ +105 (c 0.88, MeOH). ^{1}H NMR ($CDCl_3$): identical to that of (±) **1**.

1.7. Crystal structure determination

The sample was recrystallized from methanol to provide crystals suitable for study. Lattice parameters were determined from the setting angles of 25 reflections, well distributed in reciprocal space, measured on an Enraf Nonius CAD-4 diffractometer. Intensity data were collected on the diffractometer using graphite monochromated copper radiation and an $\omega-2\theta$ variable speed scan technique. Three orientation controls were monitored to assess any crystal movement during the experiment. The intensities of three standard reflections measured at the beginning, end and every three hours of exposure time showed a variation of 3.4%. Data were corrected for this variation and for Lorentz and polarization effects. Equivalent reflections, but not Friedel mates, were averaged.

The structure was solved by direct methods using the SHELXS program and refined using the SHELXL-93 program. Positions for non-hydrogen atoms were eventually refined with anisotropic displacement parameters. The positional and isotropic displacement parameters for the carbamate hydrogen were refined. Other hydrogen atoms were included in idealized positions riding on the atom to which they are attached, with isotropic displacement factors assigned as a constant (1.2, 1.5 — methyl) multiplied by U_{eq} of the attached atom. The full-matrix least-squares refinement (on F^2) converged (Δ/σ_{max}=0.00) to values of the conventional crystallographic residuals. R=0.049 for the observed data and R=0.054 ($wR2$=0.160) for all data. The minimized function was $\sum w(F_o{}^2-F_c{}^2)^2$. Weights, w, were eventually assigned to the data as $w=1/[\sigma^2(F_o{}^2)+(0.1017P)^2+0.5478P]$ where $P=[\max(F_o{}^2,0)+2F_c{}^2]/3$. A final difference Fourier map showed residual density between +0.19 and −0.19 eÅ^{-3}. Values of the neutral atom scattering factors and the real and imaginary dispersion corrections were taken from the International Tables for X-Ray Crystallography.

Acknowledgements

The authors thank Mrs Isabelle Léger for performing the ^{1}H NMR spectra.

References

1. Burrel, G.; Cassidy, F.; Evans, J. M.; Lightowler, D.; Stemp, G. *J. Med. Chem.* **1990**, *33*, 3023–3027.
2. Hamilton, T.; Weir, S. W.; Weston, A. H. *Br. J. Pharmacol.* **1986**, *88*, 103–111.
3. Ashwood, V. A.; Buckingham, R. E.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C.; Nash, D. J.; Stemp, G.; Willcocks, K. *J. Med. Chem.* **1986**, *29*, 2194–2201.
4. Englert, H. C.; Lang, H.-J.; Mania, D.; Schölkens, B. *Eur. Pat. 277 612*, **1988**.
5. To facilitate the reading of this article, compounds **1** and **3**–**6** have been drawn with their absolute configuration, though unknown at this stage of the work. The absolute stereochemistry could only be assigned after interpretation of the X-ray diffraction data of **5**.
6. Martin, A. J. P. In *Gas Chromatography*; Coates, V. J.; Noebles, H. S.; Fagerson, I. S., Eds; Academic Press: New York, 1959; 237.
7. Porter, R. S.; Johnson, J. F. *Nature*, **1959**, *183*, 391–392.
8. Porter, R. S.; Johnson, J. F. *Nature*, **1959**, *184*, 978–979.
9. Nicoud, R. M.; Bailly, M. In *Proceedings of the 9th International Symposium on Preparative and Industrial Chromatography, Nancy, France, 6–7–8 April 1992*, Société Française de Chimie, **1992**; 205–220.
10. Dingenen, J.; Kinkel, J. N. *Journal of Chromatography A*, **1994**, *666*, 627–650.
11. Evans, J. M.; Stemp, G. *Synthetic Commun.* **1988**, *18*, 1111–1118.