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A comparison of silica and porous graphitic carbon as support materials for a chiral selector in HPLC

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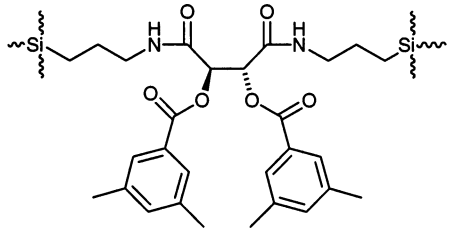
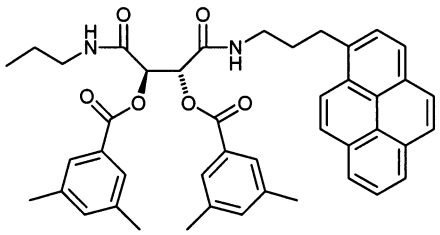
Abstract—The tartaramide based chiral selector **2** was synthesized, coated onto porous graphitic carbon (PGC), and evaluated as a chiral stationary phase for HPLC. Its performance was compared to the silica based sorbent **1**, containing the same chiral moiety. The retention and separation characteristics of the two columns were found to be correlated, but separation factors and column efficiencies were constantly lower on the PGC column. Coating of PGC as a novel and simple means to evaluate chiral selectors was evaluated. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The pharmaceutical industry's need to separate enantiomers, on both analytical and preparative scales, has been one of the major driving forces in the development of chiral stationary phases for HPLC. To date, the commercially available enantioselective sorbents are silica based, having either a coated surface or a covalently bound chiral selector. The need for improved chemical stability as well as general selectivity of these columns has led researchers to focus not only on developing new selectors, but also to examine new support materials. Over recent years the relatively novel materials zirconium oxide[1] and porous graphitic carbon[2–6] have been examined as supports for chiral selectors. The interest in polymeric supports and polymeric chiral selectors also continues[7–9] and more innovative materials can be expected as materials science evolves. The surface of porous graphitic carbon (PGC) is non-polar, and it is usually used in reversed phase chromatography, having a distinctly different selectivity than a C-18 column. The PGC surface has been dynamically as well as permanently coated with different chiral selectors.[2–6,10–15] The possibility of permanently coating the PGC surface stems from its extremely high affinity for polyaromatic systems. By linking a chiral selector to such an anchor it is possible to permanently modify the PGC surface. A few such anchors have been used, e.g. pyrene, chrysene and tetrabenzofluorene. They have been reported to adsorb to the PGC surface so strongly that they stay there under all common chromatographic conditions, showing no tendency to leak or degrade.[3–5] With this in mind, it was of interest to examine the effect of PGC on the chromatographic performance of a chiral selector, by comparing a PGC-based and a silica-based column containing the same selector. Kromasil-DMB was used as the silica-based reference column. It contains a network copolymer **1** of a benzoylated tartaric amide and a multifunctional hydrosilane covalently attached to silica.[16] Compound **2** was synthesized to resemble the Kromasil selector closely, but a pyrenyl anchor was used to allow immobilization on the PGC surface by a facile coating procedure.



1



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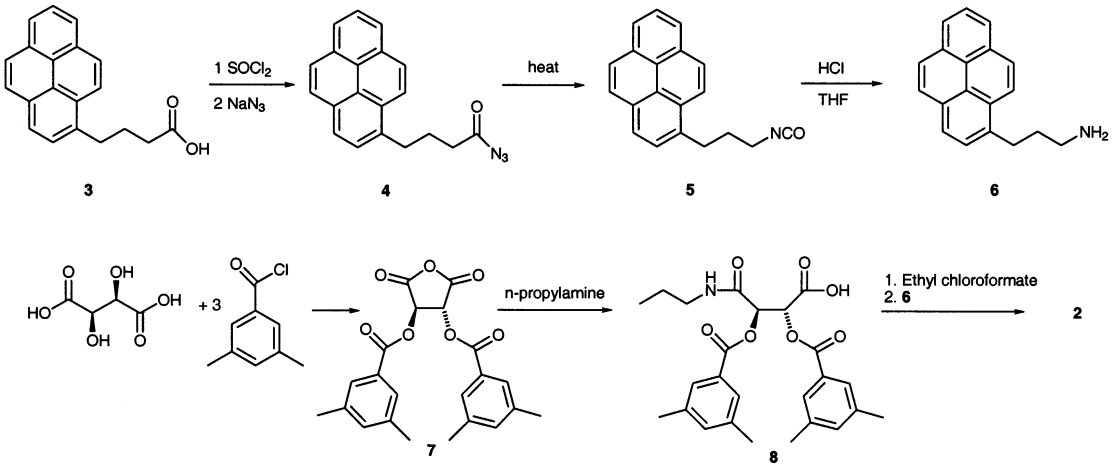
Since it is possible to wash selector **2** from the PGC with neat THF, a further question was whether the procedure comprising attachment of a selector to an anchor followed by coating on PGC could serve as a substitute for immobilization on silica and packing into columns. If so, it seems that coating on PGC would be a straightforward way to evaluate new selectors, avoiding the rather difficult and time-consuming step of column packing.

2. Results and discussion

The amount of selector **2** (prepared according to Scheme 1) adsorbed on the PGC column (6.2×10^{-5} mol) is in good agreement with previously found values,[3,5] roughly corresponding to 50% monolayer coverage. PGC, having wide pores and low surface area (5 μm particles, 250 Å pore size and 120 m^2/g surface area) contributes to the significantly lower amount of selector immobilized compared to the silica-based column (100 Å pore size and 320 m^2/g surface area). The amount of selector per 100 mm column is roughly 3.5 times higher for the silica-based column. Because the surface area is 2.7 times greater for the silica column, an important reason for greater selector loading is probably larger accessible surface. The observed retention of analytes can be expressed as a sum of selective and non-selective retention, $k' = k'_{sel} + k'_{ns}$, where the selective part stems from interactions with the selector, and the non-selective part stems from interactions with the matrix. This model predicts a linear dependence of retention factors on surface coverage, which has been verified experimentally with the Kromasil-DMB column. If non-selective retention is negligible and selective retention is similar on both columns we would observe k' values three to four times greater on the silica column.

It can be seen in Fig. 1 that this is not the case. However, it is evident that retention factors obtained



Scheme 1. Synthesis of chiral selector **2**.

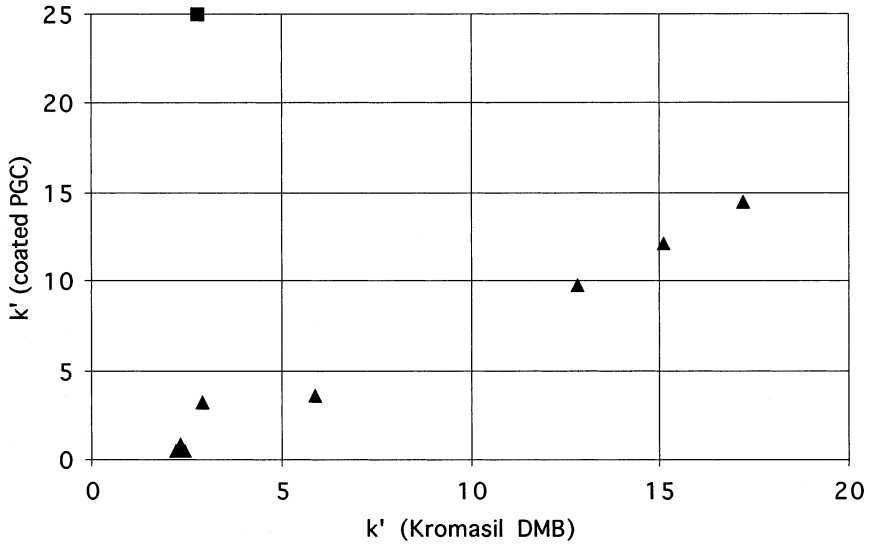


Figure 1. Correlation of the retention factors (k') determined for the Kromasil DMB column and the coated PGC column. The anomalous value marked with ■ corresponds to trifluoroanthrylethanol. Mobile phase: 95/5 hexane/IPA.

with the two columns show reasonably good correlation. The obvious exception is trifluoroanthrylethanol, which exhibits extremely high retention on the coated PGC column, due to its extended aromatic ring system. It is also clear that the correlation is complex, because the y-intercept is not zero. At low retention, k' values are roughly four times higher on the Kromasil-DMB column, but with higher retention, k' values are only about 20% greater. Thus, the coated PGC column shows a stronger retention of analytes than expected based on its lower surface coverage. This can be attributed to two factors: firstly, the low amount of selector adsorbed makes the non-selective contribution relatively large. It should also be emphasized that the PGC surface, having a strong affinity for aromatic systems, is only coated to 50%. The residual non-coated surface contributes with retention and leads to the very strong retention of trifluoroanthrylethanol. Trifluoroanthrylethanol is the only substance displaying a significantly stronger retention on PGC than on silica. Secondly, it is reasonable that the selective retention cannot be fully realized in the PGC column, because the anchor and the solid phase contribute to steric blocking of the selector. On the silica column, the selector is immobilized via a network polysiloxane copolymer **1**, which increases the distance between the selector and the solid phase, resulting in better accessibility.

It was found that the separation factors (α-values) are lower on the PGC column. This might be explained much in the same way as the observed retention differences, i.e. as a combined effect of the steric blocking effect from the anchor/solid phase and the strong non-selective retention. One further factor that might decrease the chiral discrimination is adsorption of the selector dimethyl benzoate groups on the PGC surface. Since the adsorption of isolated aromatic rings is relatively weak, restricting access to the selector by this mechanism is probably of less significance. However, it is not anticipated that lower surface coverage per se should impair separation. The loadability, though, is dependent on the surface coverage, wherefore it was ascertained that the PGC column was not run under overload conditions. From Fig. 2 it is evident that a correlation of separation factors can be established, though some exceptions exist. A good separation on Kromasil-DMB is a necessary, but not sufficient precondition for separation on the coated PGC column. Lopirazepam, for example, separates well on the Kromasil DMB column, but shows no separation on the coated PGC column. A probable reason is that the dominating docking mode on the Kromasil-DMB column cannot be achieved for steric reasons on the coated PGC column.

While it is often found that *tert*-butyl methyl ether (*t*-BME)/hexane systems afford somewhat better separations on the Kromasil-DMB column compared to 2-propanol/hexane, this is not the case for binaphthol on the PGC column. As is seen in Table 1, the separation of binaphthol vanishes in 40% *t*-BME/hexane, and even when the retention is increased by using 20% *t*-BME/hexane ($k'=1.07$) no separation occurs. Increased separation factors are however achieved for baclofenlactam and mephenytoine in *t*-BME-modified mobile phases.

It is evident that the length of the spacer between the pyrenyl anchor and the tartaramide moiety will influence the behavior of the selector. The optimum length with a tetrabenzofluorene anchor was reported to be six methylene groups.[4] However, it is also evident that much of the increased selectivity in this case is caused by restricted access to the PGC surface, because the retention of the anthryl alcohols employed as analytes was strongly dependent on the spacer length. It should also be mentioned that coatings devoid of a spacer have been reported to separate a number of racemates,[2,3,5] which justifies the use of a propyl spacer in the present investigation.

The column efficiency of about 1000–1100 plates is low for a 10 cm column. In reversed phase the non-coated PGC column performs well, exhibiting ca. 5000 plates. At first, it was thought that the low efficiency was caused by slow adsorption–desorption kinetics on the PGC surface. However, the efficiency was found to be relatively independent of flow rate over the interval 0.1–2 ml/min. Thus, kinetics was ruled out as a possible

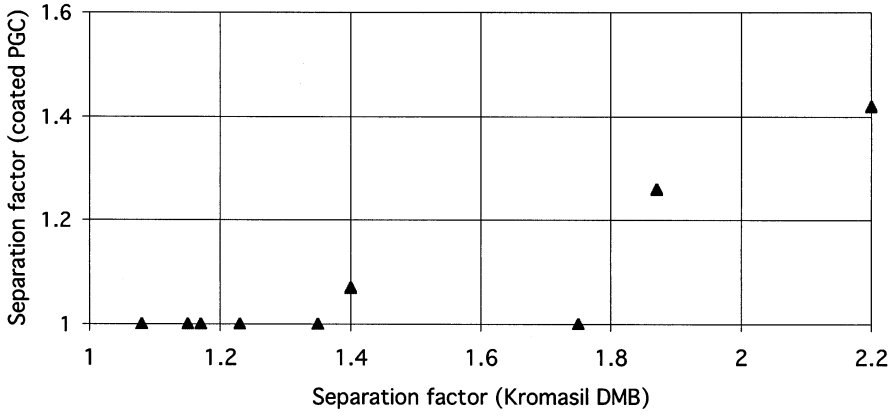
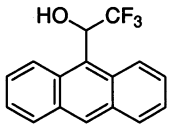
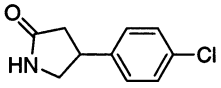
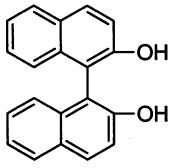
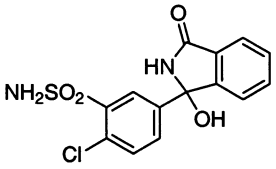
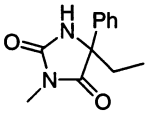
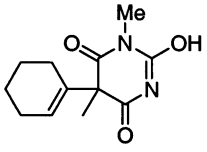
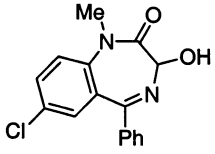
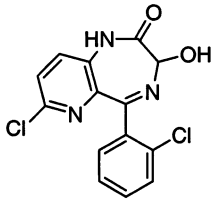
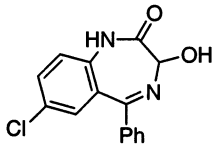


Figure 2. Correlation of separation factors (α-values) obtained on the Kromasil-DMB and coated PGC columns.

Table 1. Chromatographic results obtained on Kromasil-DMB and the coated PGC column

		Kromasil-DMB[A]		Coated PGC[A]		Coated PGC[B]	
		k'_1	α	k'_1	α	k'_1	α
	Trifluoro-anthrylethanol	2.80	1.08	25	1		
	Baclofenlactam	5.90	1.40	3.58	1.07	6.58	1.17
	Binaphthol	2.33	2.20	0.90	1.42	0.32	1
	Chlorthalidon	15.1	1.87	12.1	1.26		
	Mephenytoine	2.47	1.35	0.45	1	0.52	1.12
	Hexobarbital	2.20	1.23	0.48	1	0.18	1
	Temazepam	2.94	1.17	3.23	1	1.67	1
	Lopirazepam	17.2	1.75	14.5	1		
	Oxazepam	12.8	1.15	9.74	1		

A: Mobile phase: 5% 2-propanol in hexane
B: Mobile phase: 40% t-butylmethylether in hexane

cause for the low efficiency. A second idea, that the low efficiency was caused by multiple adsorption sites, was tested by attempting to 'deactivate' the PGC surface by adding benzene to the mobile phase. The addition of 0.3 and 1% benzene to the mobile phase did not affect the column efficiency, but on the other hand showed a pronounced effect on the retention of analytes. Addition of 1% benzene to 5% 2-propanol in hexane resulted in decreased retention, an effect also observed for Kromasil-DMB, though much less pronounced. This again indicates that the non-polar PGC surface contributes significantly to retention due to its high affinity for aromatic systems.

2.1. Long-term stability

As stated earlier, pyrenyl based coatings on PGC columns have been reported to be very stable under chromatographic conditions[3,5] and this is also the case in the present system. However, some problems with the coating were experienced. When running chromatography the retention and selectivity was found to be stable by repeatedly injecting samples of binaphthol. However, storing the column for a longer time at room temperature (i.e. several weeks to months) resulted in decreased retention and selectivity. This behavior is somewhat difficult to account for because leakage of the selector would leave an even greater part of the PGC surface uncoated, resulting in increased retention of the analytes.[3] It has been reported that the PGC surface adsorbs impurities, resulting in decreased retention and selectivity but the performance of the column could be restored by washing with 15% THF in hexane. When this remedy was applied in the present case, no significant improvement of selectivity or retention was found. Thus, it seems that the loss of retention and selectivity may be caused by degradation of the selector, leaving the pyrenyl group on the PGC surface, but washing the selector component off. Still, it should be noted that the commercial column with the tartaric amide **1** is very stable, which conflicts with this explanation.

3. Conclusions

The tartaramide based selector **2** coated onto porous graphitic carbon has been shown to effect enantioseparations. The retention and separation behavior were found to be correlated to those of the reference column Kromasil-DMB **1**, but the separation factors and column efficiencies are constantly lower on the PGC column. Consequently, the coating of selectors onto PGC as a relatively simple means to evaluate chiral selectors is of limited value, unless a longer linker between the anchor and the selector moiety leads to a significant increase in enantioseparation. It was also found that the chiral stationary phase was not entirely stable over time periods of months. Incomplete stability of similar phases has not been reported earlier, and stands in clear contrast to the high stability of Kromasil-DMB **1**.

4. Experimental

4.1. Equipment

All NMR spectra were run in $CDCl_3$ with $CHCl_3$ as internal standard (7.26 ppm) on a Varian (Palo Alto, CA) VXR 400 instrument. The pre-packed porous graphitic carbon column used (100×4.6 mm) contains 5μ Hypercarb (Hypersil, Runcorn, UK).

4.2. Column coating

A coated PGC column was prepared by the following procedure. A solution of **2** in dichloromethane/acetonitrile (50.2 mg/100 ml) was pumped through the PGC column (100×4.6 mm, 5μ Hypercarb Thermohypersil) at 1 ml/min. UV monitoring revealed a clear-cut breakthrough time of 87.8 min, corresponding to 44.3 mg (6.2×10^{-5} mol) selector adsorbed. After initial washing with 10% 2-propanol in hexane, the column showed a remarkably high retention of binaphthol and very low column efficiency. This behavior was attributed to excessive adsorption of selector, and the performance of the column stabilized after washing with 300 ml of 15% THF in hexane, after which the retention times of binaphthol were reproducible and the column efficiency significantly improved. This treatment has previously been used for washing without deteriorating the coating.[3]

4.3. Pyrenebutyrylazide, 4

A suspension of pyrenylbutyric acid **3** (2.00 g, 6.93 mmol) in benzene (3 ml) and thionylchloride (1.65 g, 2 equiv.) was heated slowly to 80°C. After ca. 1.5 h the clear brown solution was evaporated under reduced pressure. One portion of dry benzene (2 ml) was added and the evaporation repeated. The yellow/brown acyl chloride residue was dissolved in acetone (ca. 6 ml, dried over $CaCl_2$) and was added dropwise to a stirred sodium azide solution (0.54 g, 1.2 equiv. in 1 ml aq.) at 0°C. The reaction mixture was left 0.5 h at rt, and 20 ml of water was added. The precipitate was filtered off, washed with water and dried in vacuo (1.80 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.33–8.27 (m, 1H) 8.20–8.11 (m, 4H), 8.05–7.98 (m, 3H), 7.90–7.84 (m, 1H), 3.41 (t, 2H, $J=7.8$ Hz), 2.50 (t, 2H, $J=7.4$ Hz), 2.28–2.17 (m, 2H).

4.4. Pyrenepropylisocyanate, 5

A suspension of the dry acylazide **4** (1.80 g) in dry benzene (7 ml) was heated from 50 to 80°C over 1.5 h, by which time no more gas was evolved. The clear brown solution was evaporated in vacuo. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.27–8.12 (m, 5H) 8.06–8.00 (m, 3H), 7.88 (d, 1H, $J=8$ Hz), 3.47 (t, 2H, $J=7$ Hz), 3.42 (t, 2H, $J=7$ Hz), 2.17 (p, 2H, $J=7$ Hz).

4.5. Pyrenepropylamine, 6

Hydrochloric acid (6 ml) was added in one portion to a solution of isocyanate **5** (1.7 g) in THF (10 ml) at 60°C,

which resulted in vigorous gas evolution. The organic solvent was evaporated in vacuo. The resulting aqueous solution was made alkaline with aqueous NaOH, and the solution was extracted with $CHCl_3$. The amine was precipitated from the dried and filtered solution with HCl (g), and after filtration liberated by treatment with aq. NaOH and $CHCl_3$. Drying over $MgSO_4$, filtration and evaporation in vacuo yielded the amine (0.69 g, 38% yield based on the acid). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, 1H, $J=9.6$ Hz) 8.20–7.98 (m, 7H), 7.89 (d, 1H, $J=8$ Hz), 3.41 (t, 2H, $J=7.8$ Hz), 2.89 (t, 2H, $J=7$ Hz), 2.03 (p, 2H, $J=7.4$ Hz); GC–MS: M+ 1 = 260.

4.6. (*R*,*R*)-*O*,*O*′-Bis(dimethylbenzoyl)tartaric anhydride, 7

A slurry of 3,5-dimethylbenzoic acid (8.65 g) in $SOCl_2$ (13.7 g, 2 equiv.) was heated with stirring at 60°C until no more gas evolved and a clear solution resulted (2 h). Evaporation under reduced pressure gave a clear oil to which tartaric acid (2.88 g, 0.3 equiv.) was added. The flask was fitted with an air cooler and a drying tube, and the mixture was heated from 80 to 140°C over 3 h with stirring, until the tartaric acid was dissolved. Benzene (85 ml dried over $CaCl_2$) was added, and the solution was left to crystallize. The precipitate was collected and dried in vacuo (4.49 g, 60%). Occasionally 3,5-dimethylbenzoic acid was found in the precipitate. A further recrystallization from dry benzene provided the pure anhydride in that case. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (s, 4H) 7.29 (s, 2H), 5.96 (s, 2H), 2.38 (s, 12H).

4.7. (*R*,*R*)-*N*-Propyl-*O*,*O*′-bis(dimethylbenzoyl)tartaric acid monoamide, 8

Propylamine (1.43 ml, 2.2 equiv.) was added dropwise to a stirred suspension of **7** (3.12 g) in CH_2Cl_2 (12 ml) at 0°C. The resulting white slurry was left at rt for 1 h, and evaporated in vacuo. The residue was taken up in ethyl acetate (100 ml) and washed with dilute HCl (2×10 ml), and brine (2×10 ml). After drying, filtration and evaporation of the organic phase, the residue was recrystallized from 2-propanol/hexane (1/1). Grinding and drying of the crystals (60°C at high vacuum for 24 h) provided the acid free of 2-propanol (3.7 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (s, 2H) 7.64 (s, 2H), 7.24 (s, 1H), 7.20 (s, 1H), 6.32 (br t, 1H, $J=5.8$ Hz, NH), 6.04 (d, 1H, $J=4$ Hz, CH), 5.99 (d, 1H, $J=4$ Hz, CH), 3.26–3.38 (m, 1H, $J=6.8$ Hz, NCH_2), 3.14–3.24 (m, 1H, $J=6.8$ Hz, NCH_2), 2.35 (s, 6H, $ArCH_3$), 2.33 (s, 6H, $ArCH_3$), 1.48 (p, 2H, $J=7.3$ Hz, CH_2), 0.85 (t, 3H, $J=7.4$ Hz, CH_3). Mp: 160°C (decomposes).

4.8. (*R*,*R*)-*N*-Propyl-*N*′-3(1-pyrenyl)propyl-*O*,*O*′-bis-(dimethylbenzoyl)tartaramide, 2

N-Methylmorpholine (68 μl, 1 equiv.) and ethylchloroformate (59 μl, 1 equiv.) was added dropwise to a stirred solution of **8** (282 mg) in dry THF (8 ml) at −21°C. After 20 min, a solution of pyrenpropylamine **6** (0.164 g, 1 equiv.) in dry THF (2 ml) was added dropwise at −21°C, and the solution stirred at 0°C for a further 6 h. The solvent was evaporated and the residue taken up in ethyl acetate (50 ml). The solution was washed with dilute HCl (3×5 ml), saturated sodium bicarbonate (3×5 ml), brine (3×5 ml) and dried ($MgSO_4$). Evaporation and trituration of the residue with diethyl ether yielded **2** (0.215 g, 48%). The product was purified further by flash chromatography, eluting with 5% isopropanol in chloroform. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.20–7.96 (m, 8H) 7.74 (d, 1H, $J=8$ Hz), 7.69 (s, 2H, *o*-PhH), 7.61 (s, 2H, *o*-PhH), 7.23 (s, 1H, *p*-PhH), 6.95 (s, 1H, *p*-PhH), 6.40 (br t, 1H, NH), 6.28 (br t, 1H, NH), 6.04–6.10 (m, 2H, CH), 3.56–3.10 (m, 6H), 2.34 (s, 6H, $ArCH_3$), 2.14 (s, 6H, $ArCH_3$), 1.90–2.02 (m, 2H), 1.50–1.38 (m, 2H), 0.81 (t, 2H, $J=7.6$ Hz). Elemental analysis: Found: C, 75.6; H, 6.6; N, 4.0; O, 13.5, expected: C, 75.8; H, 6.3; N, 4.0; O, 13.8%.

$[\alpha]^{589}=-69$ (*c* 0.26 dioxane). Mp: 232–234°C.

Acknowledgements

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