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# ENANTIOMER SEPARATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON SILICA GEL WITH COVALENTLY BOUND MONOSACCHARIDES

JÜRGEN SCHULZE and WILFRIED A. KÖNIG*
*Institut für Organische Chemie der Universität, D-2000 Hamburg 13 (F.R.G.)*
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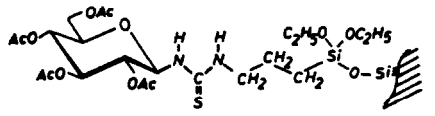
## SUMMARY

1-Isothiocyanato-D-glucopyranosides with different substituents were covalently bound to aminopropyl silica gel. The glucopyranosyl system was used as a chiral selector for the chromatographic separation of racemic compounds. By introducing different substituents at C-2 of the carbohydrate residue, enantioselectivity of the chiral support could be adapted to various types of compound.

## INTRODUCTION

In recent years a considerable number of chiral supports for liquid chromatographic enantiomer separation have been proposed[1–3]. Except for amino acid deriv-



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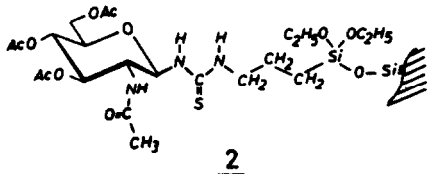

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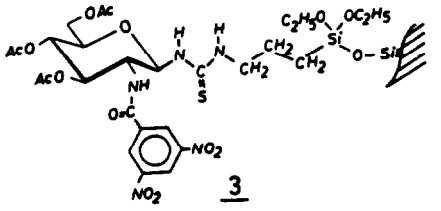

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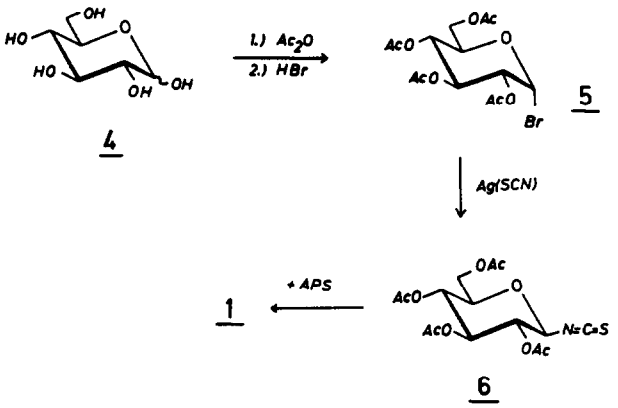
atives[4–6], polysaccharides proved to be particularly suitable as chiral selectors[7–10]. Although various derivatives of cellulose, *e.g.* the triacetate[7] and tribenzoate[9], and cyclodextrin bound to silica gel, were used successfully as chiral supports for enantiomer separation, there have been no reports on the use of monosaccharides covalently bound to silica gel for the resolution of racemates. We have synthesized the chiral supports (1–3) by connecting derivatives of D-glucose to silica gel and investigated the properties of these supports with respect to enantiomer separation.

## EXPERIMENTAL

### *Synthesis of chiral supports*

In the coupling reaction of the chiral selectors in 1 and 2, Nucleosil-$NH_2$ (Macherey & Nagel, Düren, F.R.G.) 10 μm particle size and 100 Å pore diameter was used, while 5 μm LiChrosorb-$NH_2$ (Merck, Darmstadt, F.R.G.) was applied for 3. Solvents were dried and distilled.

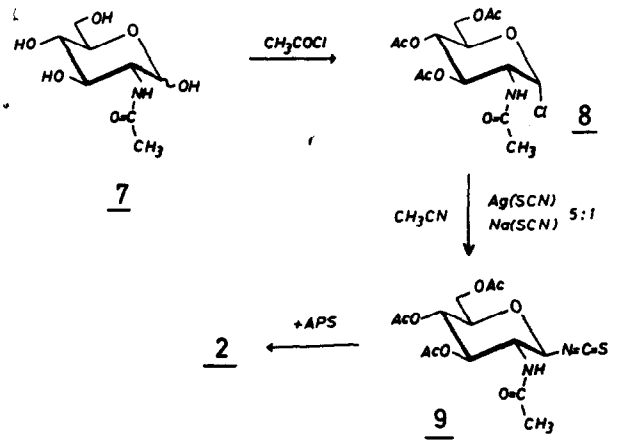
The synthesis of 1 is shown in Scheme 1. The peracetylated β-bromo derivative 5 was obtained according to Lemieux[11] and the isothiocyanate 6 as described by De *et al.*[12].




Scheme 1. Synthetic pathway for chiral support 1.

For the coupling reaction, 1.5 g of 6 was added to a suspension of 2.5 g of aminopropyl silica gel in 25 ml of dichloromethane and 1 ml of pyridine, and kept for 3 days at room temperature under shaking. The product was centrifuged, washed with methanol, dichloromethane and ether, and dried in high vacuum. The yield of 1 was 2.94 g. IR spectroscopy (1% in KBr): $\nu$ = 1750 $cm^{-1}$ (C=O, ester); 1550 $cm^{-1}$ (NH–CS–NH). Elemental analysis of $C_{18}H_{27}N_2O_9S \cdot (SiO_2)_x$: (found) C = 12.30 (0.48 mmol/g); H = 1.50 (0.57 mmol/g); N = 1.48 (0.54 mmol/g); S = 1.52 (0.48 mmol/g). Residual analysis: 75.7% ashes (0.54 mmol/g).

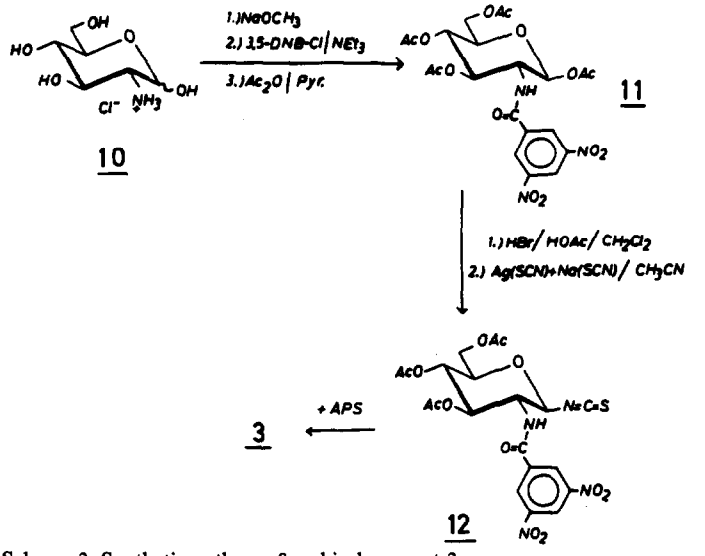
The synthesis of 2 is shown in Scheme 2. Compound 8 was prepared from D-glucosamine according to Horton[13] and 9 according to F. Micheel *et al.*[14]. The coupling to aminopropyl silica gel proceeded as described above. IR spectroscopy (1% in KBr): $\nu$ = 1750 $cm^{-1}$ (C=O, ester); 1660 $cm^{-1}$ (amide); 1550 $cm^{-1}$ (NH–




Scheme 2. Synthetic pathway for chiral support 2.

CS–NH). Elemental analysis of $C_{18}H_{28}N_3O_8S \cdot (SiO_2)_x$: (found) C = 9.81 (0.45 mmol/g); H = 1.60 (0.57 mmol/g); N = 1.96 (0.47 mmol/g); S = 1.27 (0.40 mmol/g). Residual analysis: 76.5% ashes (0.52 mmol/g).

The synthesis of 3 is shown in Scheme 3. To a suspension of 6.47 g (30 mmol) of D-glucosamine · HCl in 60 ml of methanol a solution of 0.69 g (30 mmol) of sodium in 10 ml of methanol was added. After 10 min the sodium chloride was filtered off and 4.17 g (30 mmol) of triethylamine was added to the filtrate. A solution of 7.61 g (33 mmol) of 3,5-dinitrobenzoylchloride in 60 ml of anhydrous dioxane was added to the filtrate. After 16 h at 20°C the solution was taken to dryness and the residue taken up in 200 ml of anhydrous pyridine. Acetic anhydride (120 ml) was




Scheme 3. Synthetic pathway for chiral support 3.

added at 0°C and kept at 20°C for 10 h under stirring. The solvent was evaporated and the product was dissolved in chloroform. The solution was washed with water and solutions of sodium hydrogen sulphate and sodium hydrogen carbonate. After recrystallization from chloroform–hexane, 11 was obtained as a slightly red product. Yield: 10.3 g (63%); m.p. 218°C; $[\alpha]_D^{20} = +54.2$ ($c = 0.875$ in dichloromethane).

To a solution of 4.2 g (7.8 mmol) of 11 in 100 ml of anhydrous dichloromethane, 0.9 ml of acetic acid anhydride and 1.87 ml (11 mmol) of a 33% solution of hydrogen bromide in acetic acid were added and stirred for 2 h at 20°C. The solution of the intermediate 1-bromo derivative was concentrated at 10–20°C and the excess of hydrogen bromide was removed in high vacuum over sodium hydroxide. The residue was taken up in 50 ml of anhydrous acetonitrile and, after addition of 6 g of silver thiocyanate and 0.5 g of sodium thiocyanate, stirred for 20 h at 20°C in the dark. After filtration over celite–silica gel, 12 was recrystallized from ethyl acetate–hexane. Yield: 3.1 g (73%); m.p. 174°C; $[\alpha]_D^{20} = +28.4$ ($c = 1.31$ in ethyl acetate).

To a suspension of 2.6 g of aminopropyl silica gel in 30 ml of anhydrous dichloromethane, 0.1 ml of pyridine and a solution of 2.36 g (4.37 mmol) of 12 in 30 ml of dichloromethane was added. The reaction proceeded for 2 days under continuous shaking. After centrifugation and washing with dichloromethane, methanol and ether 3.01 g of 3 was obtained. IR spectroscopy (1% in KBr): $\nu = 1740\ cm^{-1}$ (C=O, ester); 1620–1640 $cm^{-1}$ (amide); 1550 $cm^{-1}$ (NH–CS–NH). Elemental analysis of $C_{23}H_{28}N_5O_{12}S \cdot (SiO_2)_x$: (found) C = 10.05 (0.36 mmol/g); H = 11.44 (0.51 mmol/g); N = 2.75 (0.39 mmol/g); S = 1.10 (0.24 mmol/g). Residual analysis: 75.89% ashes (0.4 mmol/g).

*Preparation of derivatives*

Amino acids were converted into N-phenylcarbamates with phenyl isocyanate in 1 *N* sodium hydroxide and esterified with a solution of diazomethane in ether. Reaction of the N-phenylcarbamates with a 1.25 *N* solution of hydrogen chloride gas in methanol at 100°C yielded the hydantoins. Amino alcohols (β-blockers) were converted into phenylurethanes by dissolving 0.1–0.5 mg of sample in 250 μl of dichloromethane and 10 μl of triethylamine, adding 10 μl of phenyl isocyanate, and keeping this mixture for 20 min at room temperature.

*Packing of columns*

The stainless-steel columns (25 × 0.46 cm I.D.) were slurry-packed with carbon tetrachloride–methanol (95:5).

*Chromatography*

A Spectra-Physics SP 8000 HPLC system with a Schöffel UV detector was used. The solvents used as mobile phase were dried and distilled prior to use.

## RESULTS AND DISCUSSION

Tetra-O-acetyl-D-glucopyranosyl isothiocyanate (6, Scheme 1) was been used as a chiral reagent to form diastereoisomers of racemic amino acids[15], amines[16] and amino alcohols[17] for liquid chromatographic separation. Since the isothiocyanates are known for fast and almost quantitative reactions with amino groups to form

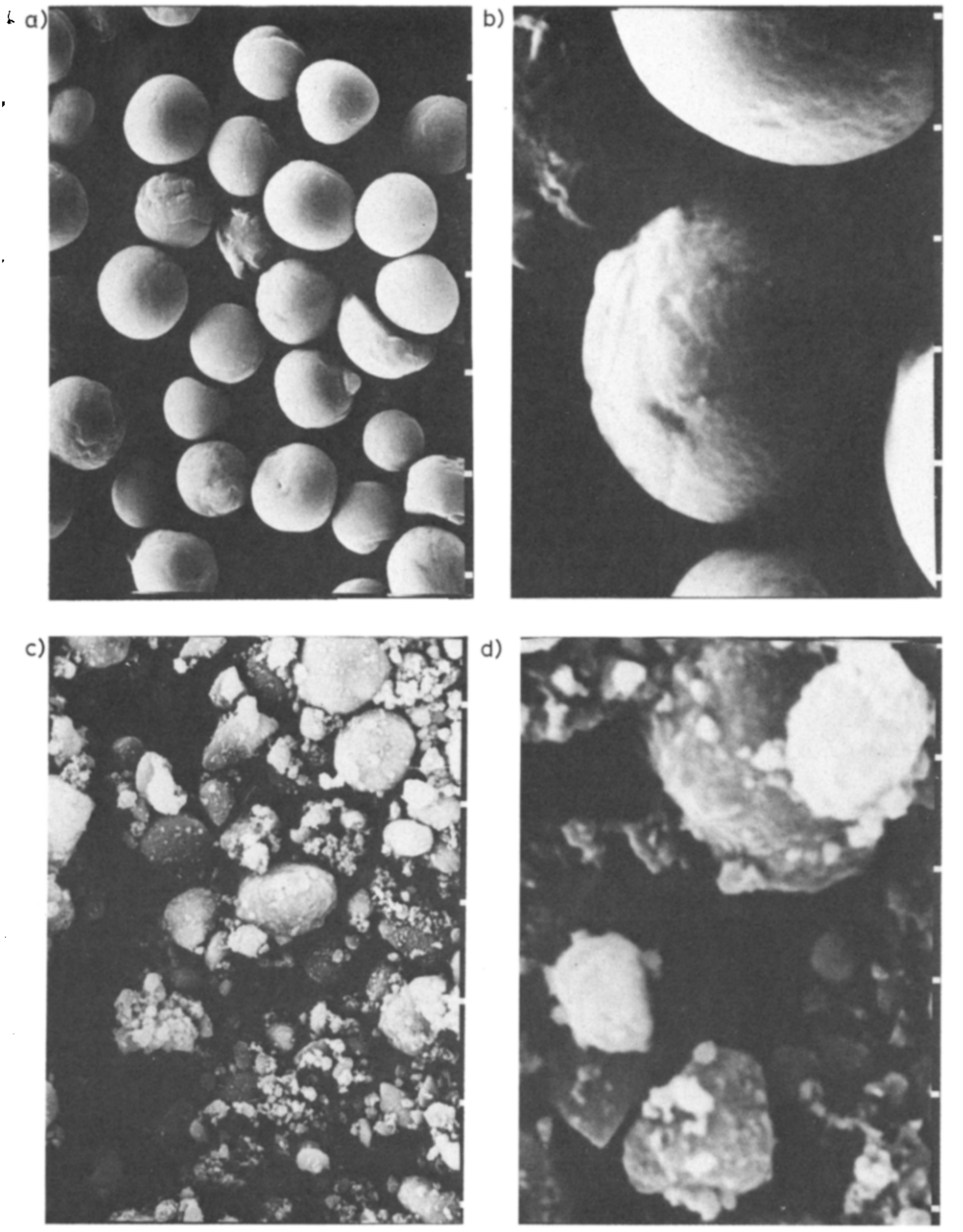



Fig. 1. Electron micrographs of Nucleosil-$NH_2$ silica gel: (a) and (b) original particle shape (1500 and 5625 times enlarged); (c) and (d) after stirring with magnetic bar (1500 and 5625 times enlarged). (We are indebted to Mrs. E. Mehrling, University of Hamburg, for these pictures.)

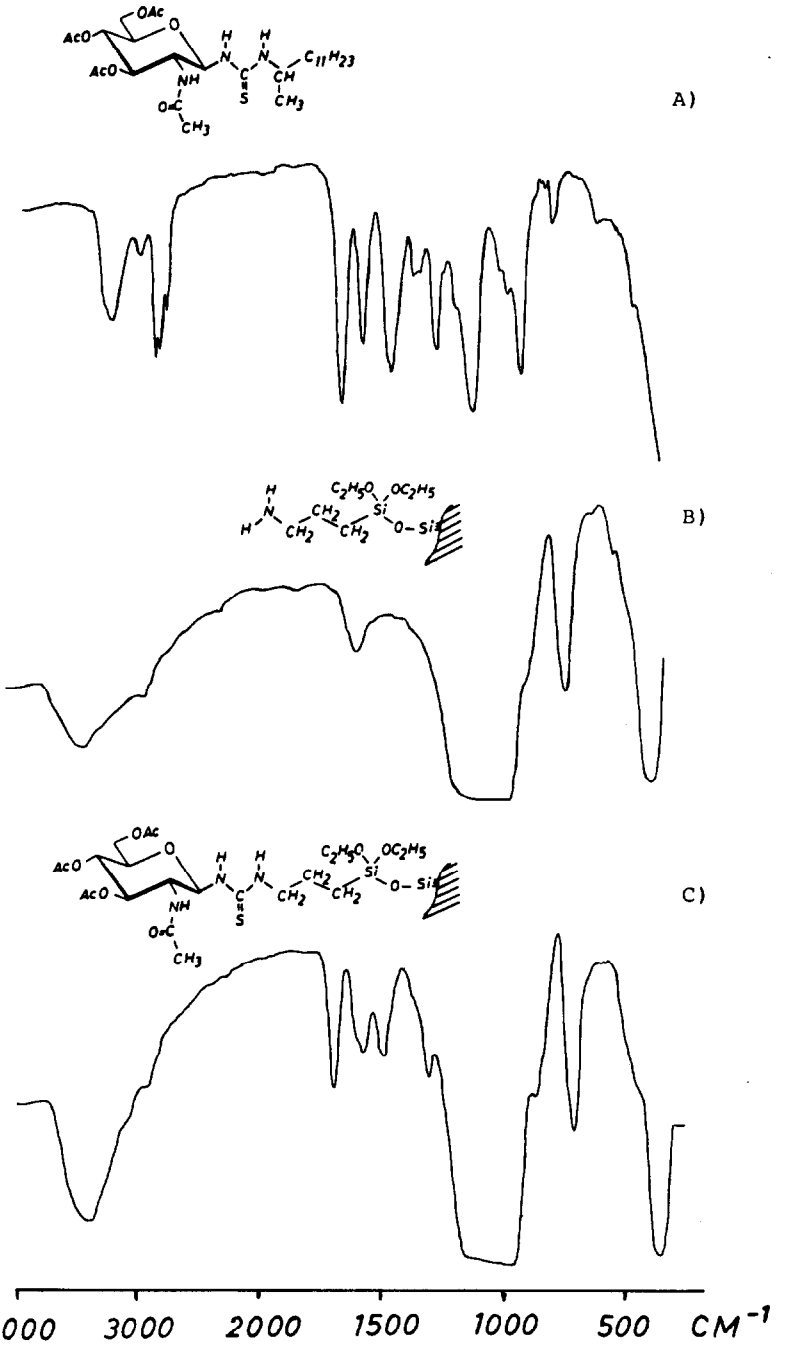



Fig. 2. IR spectra of (A) reaction product of 9 with 2-aminotridecane, (B) 1-aminopropyl silica gel and (C) chiral support 2.

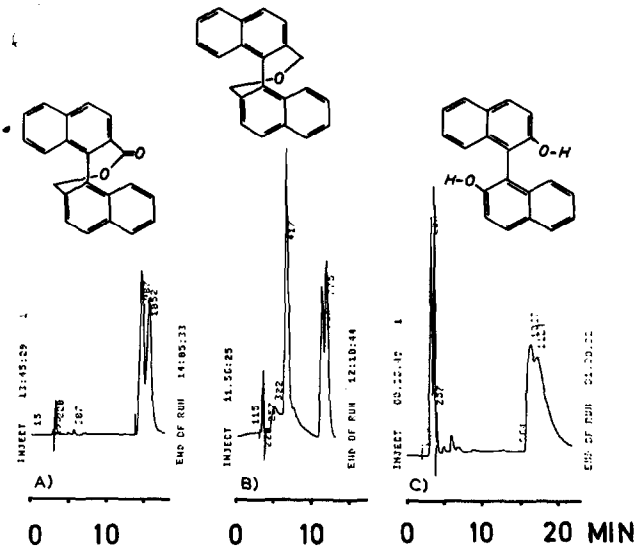



Fig. 3. Enantiomer separation of some atropisomeric binaphthyl derivatives on chiral support 1. (We thank Prof. A. deMeijere and Dr. D. Bruchmann, University of Hamburg, for providing these samples.) Mobile phase *n*-hexane–dichloromethane: (A) 80:20; (B) 93:7; (C) 60:40; flow-rate, 1 ml/min; temperature, 20°C.

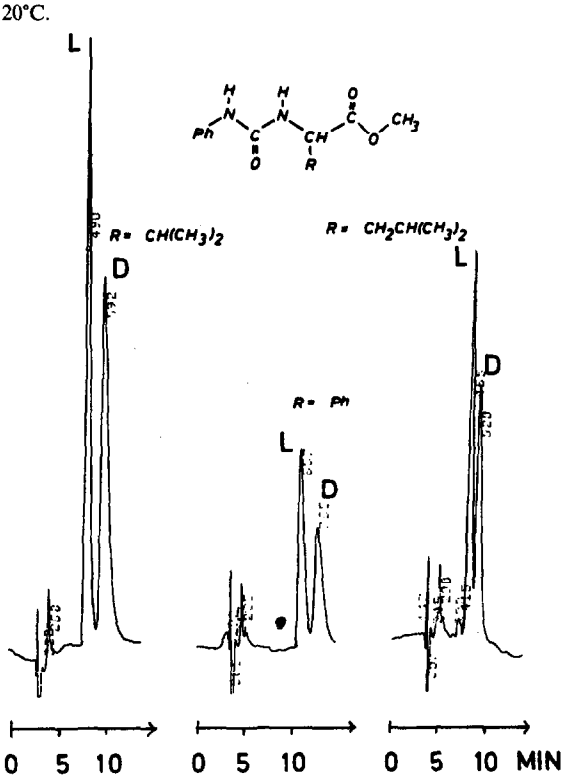
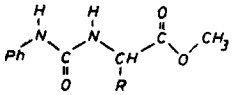



Fig. 4. Enantiomer separation of some N-phenylcarbamoyl amino acid methyl esters on chiral support 2. Mobile phase, *n*-hexane–2-propanol–dichloromethane (80:2:18); flow-rate, 1 ml/min; temperature, 25°C.

TABLE I

SEPARATION OF ENANTIOMERS ON CHIRAL SUPPORT 2

Conditions: column temperature, 20°C; flow-rate, 1 ml/min; mobile phases *n*-hexane–dichloromethane–2-propanol: (A) 75:22:3; (B) 85:15; (C) 80:19:1.

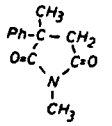


13 a) R = $CH(CH_3)_2$

b) R = Ph

c) R = $CH_3$

d) R = $CH_2CH(CH_3)_2$



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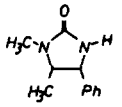

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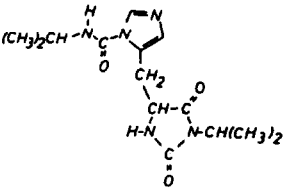

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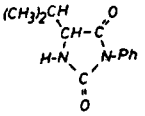

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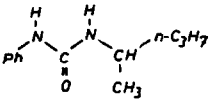

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| *Compound* | *Mobile phase* | $k_1'$* | $k_2'$* | $\alpha$** |
|---|---|---|---|---|
| 13a | A | 1.30 (L) | 1.76 (D) | 1.354 |
| 13b | A | 1.38 (L) | 1.68 (D) | 1.217 |
| 13c | A | 1.07 | 1.25 | 1.168 |
| 13d | A | 1.56 (L) | 1.86 (D) | 1.192 |
| 14 | B | 5.79 | 5.88 | 1.016 |
| 15 | A | 1.38 | 1.57 | 1.137 |
| 16 | A | 7.03 | 7.63 | 1.085 |
| 17 | A | 1.73 | 1.85 (D) | 1.069 |
| 18 | C | 8.26 | 8.44 | 1.022 |

* $k_1'$, $k_2'$ = capacity factors.

** $\alpha$ = separation factor.

thiourea derivatives, the 1-isothiocyanato derivative of D-glucose was used for the coupling reaction with 1-aminopropyl silica gel.

Except for the tetra-O-acetyl-glucose derivatives, the 2-acetamido- and the 2-(3,5-dinitro)benzamido-glucose derivatives 9 and 12 (Schemes 2 and 3) were connected to silica gel. In this way additional sites for hydrogen-bond interactions and a charge-transfer acceptor system were introduced, which was already proposed by Pirkle *et al.*[5] as a very suitable chiral selector in combination with amino acid derivatives.

The isothiocyanates 6, 9 and 12 were obtained as pure $\beta$-anomers as concluded from the H-1 coupling constants in the proton NMR spectra. No anomerisation during the reaction with aliphatic amines was observed and, consequently, the coupling reaction with aminopropyl silica gel can also be considered to proceed with retention of configuration. The coupling reaction was performed in a flask under continuous automatic shaking. Stirring with a magnetic bar over prolonged periods resulted in partial degradation of silica gel particles (Fig. 1) and should be avoided.

The yields of the coupling reactions was calculated from elemental and residual analyses data, and amounted to ca. 0.35–0.5 mmol/g of support. In the IR spectra of the modified silica gels 1–3, as well as the vibrations of the Si–O–Si and Si–OH bonds the vibrational absorptions typical for esters, amides and thiourea groups in the range between 1500 and 1800 $cm^{-1}$ were observed (Fig. 2).

The chiral supports 1–3 exhibited unexpectedly strong differences in enantioselectivity towards different substance classes. On 1 exclusively some chiral binaphthyl derivatives could be separated (Fig. 3). As mobile phases for these separations aprotic mixtures of hexane, diethyl ether, dichloromethane or dioxane were used, while only 1% of 2-propanol caused complete breakdown of enantiomer resolution. No enantioselectivity was observed for racemates with a chiral centre.

The chiral silica gel 2, with an amide group at C-2 of the glucose residue instead of the ester group, does not show any enantioselectivity towards the atropisomers separated on 1. However, enantiomeric separation was possible for compounds with carbamate or amide functions. Fig. 4 shows the separation of some N-phenylcarbamoyl amino acid esters. Additional results are given in Table I. In the case of 2,

TABLE II

SEPARATION OF N-PHENYLCARBAMOYL DERIVATIVES OF AMINO ACID METHYL ESTERS, TOCAINIDE AND PHENYLETHYLAMINE ON CHIRAL SUPPORT 3

Conditions: see Table I; mobile phases *n*-hexane–dichloromethane–2-propanol: (A) 75:20:5; (B) 93:6:1.

| *Compound* | *Mobile phase* | $k'_1$* | $k'_2$* | $\alpha$** |
|---|---|---|---|---|
| DL-Valine | A | 1.16 (L) | 1.60 (D) | 1.38 |
| DL-Phenylalanine | A | 2.26 (L) | 2.85 (D) | 1.26 |
| DL-Alanine | A | 0.98 | 1.15 | 1.17 |
| DL-Leucine | A | 1.38 (L) | 2.03 (D) | 1.47 |
| DL-N-Benzoylalanine | B | 1.57 | 1.71 | 1.09 |
| DL-Alanine-(2,6-dimethyl)anilide (tocainide) | A | 1.19 | 1.79 | 1.50 |
| (*R*,*S*)-$\alpha$-Phenylethylamine | B | 1.37 | 1.52 | 1.11 |

* $k'_1$, $k'_2$ = capacity factors.
** $\alpha$ = separation factor.

in contrast to 1, mixtures of *n*-hexane and 2-propanol could also be used as mobile phases.

In order to increase further the capability for enantioselective interactions the modified silica gel 3 was synthesized. In addition to hydrogen-bonding interactions, 3 displays charge-transfer properties.

Again N-phenylcarbamoyl amino acid esters could be separated (Table II), but in addition compounds not separated on 2 could now be separated (Table II). Common to these compounds is the presence of carbamoyl or amide functions. On 3 also the N-phenylurethane derivatives of some amino alcohols with pharmaceutical relevance ($\beta$-blockers) were separated. The urethane derivatives of N-*tert.*-butyl substituted amino alcohols showed $\alpha$-values of 1.17 to 1.21 (Fig. 5, Table III), while the N-phenylcarbamoyl derivatives failed to be separated.

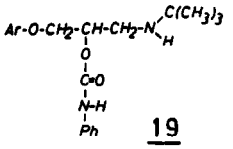
The results obtained with the chiral supports 1–3 demonstrate the suitability of the $\beta$-D-glucopyranosyl system as a chiral selector for liquid chromatographic enantiomer separation. The introduction of an amide function at C-2 facilitates chiral recognition. This effect is even more pronounced if an additional charge-transfer interaction is introduced.

In conclusion, it can be stated that derivatives of monosaccharides, connected to silica gel, form a new type of chiral support for enantiomer separation. Except for many variations in the substitution numerous stereoisomers of sugars are easily accessible for optimization of chiral recognition.

TABLE III

ENANTIOMER SEPARATION OF SOME PHENYLURETHANE DERIVATIVES OF $\beta$-ADRENORECEPTOR BLOCKING DRUGS

Conditions: column temperature, 25°C; flow-rate, 2.3 ml/min; mobile phase, *n*-hexane–2-propanol (94:6).

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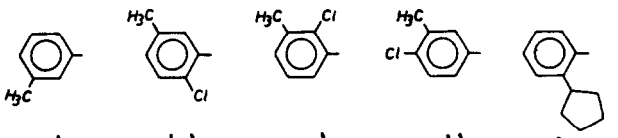
Ar =




a) b) c) d) e)

| *Compound* | $k'_1$* | $k'_2$* | $\alpha$** |
|---|---|---|---|
| 19a | 5.59 | 6.71 | 1.20 |
| 19b | 6.07 (+) | 7.28 (−) | 1.20 |
| 19c | 5.87 | 7.08 | 1.21 |
| 19d | 5.81 | 6.94 | 1.19 |
| 19e | 4.15 (+) | 4.85 (−) | 1.17 |

* $k'_1$, $k'_2$ = capacity factors.
** $\alpha$ = separation factor.

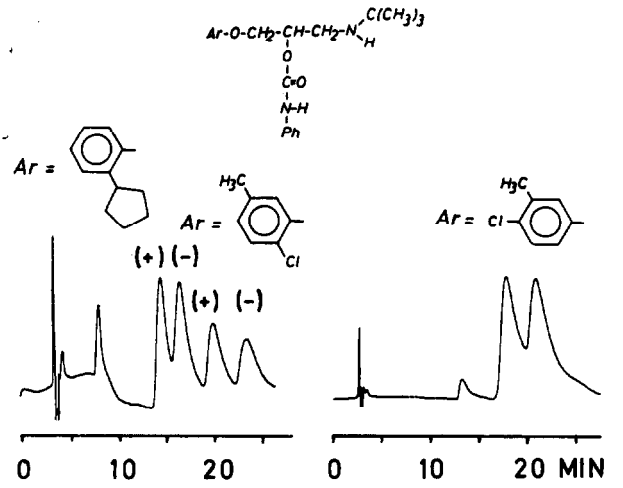



Fig. 5. Enantiomer separation of some β-adrenoreceptor blocking drugs (β-blockers) on chiral support 3. Mobile phase, *n*-hexane-2-propanol (94:6); flow-rate, 2.3 ml/min; temperature, 25°C.

ACKNOWLEDGEMENTS

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REFERENCES

1 S. Allenmark, *J. Biochem. Biophys. Methods*, 9 (1984) 1.
2 I. S. Krull, *Adv. Chromatogr.*, 16 (1978) 175.
3 G. Blaschke, *Angew. Chem.*, 92 (1980) 14.
4 A. Dobashi, K. Oka and S. Hara, *J.Am. Chem. Soc.*, 102 (1980) 7122.
5 W. H. Pirkle, J. M. Finn, B. C. Hamper, J. Schreiner and J. Pribish, in E. L. Eliel and S. Otsuka (Editors), *Asymmetric Reactions and Processes in Chemistry*, ACS Symposium Series, No. 185, American Chemical Society, Washington, DC, 1982, p. 245.
6 N. Ôi and H. Kitahara, *J. Chromatogr.*, 285 (1984) 198.
7 A. Mannschreck, H. Koller and R. Wernicke, *Kontakte*, 1 (1985) 40.
8 Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Letters*, (1984) 739.
9 Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 106 (1984) 5327.
10 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. D. Mond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
11 R. V. Lemieux, in R. L. Whistler and M. L. Wolfrom (Editors), *Methods of Carbohydrate Chemistry*, Vol. 2, Academic Press, New York, London, 1963, p. 221.
12 K. K. De, G. T. Shiau and R. E. Harmon, *J. Carbohydr., Nucleosides, Nucleotides*, 2 (1975) 171.
13 D. Horton, *Org. Chem.*, 46 (1966) 1.
14 F. Micheel, H. Petersen and H. Köchling, *Chem. Ber.*, 93 (1960) 1.
15 T. Kinoshita, Y. Kasahara and N. Nimura, *J. Chromatogr.*, 210 (1981) 77.
16 K. J. Miller, J. Gal and M. M. Ames, *J. Chromatogr.*, 307 (1984) 335.
17 J. Gal, *J. Chromatogr.*, 307 (1984) 220.