*Journal of Chromatography*, 366 (1986) 299–309
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 758

# PREPARATIVE LIQUID CHROMATOGRAPHY IN THE FIELD OF X-RAY CONTRAST AGENTS*

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(Received May 1st, 1986)

## SUMMARY

The use of preparative liquid chromatography (LC) for the effective purification of potential X-ray contrast agents or intermediates is described. Pure products in grams to kilogram quantities have been obtained by reversed-phase preparative LC of highly water-soluble non-ionic compounds, a weak aliphatic carboxylic acid and an isophthalic acid derivative. The mobile phases used were mixtures of methanol–water alone or with the addition of ammonium acetate buffers, tetrabutylammonium chloride and phosphate buffer.

## INTRODUCTION

Nycomed AS have been engaged in research on and the development of contrast agents for more than 30 years[1,2]. Products arising out of this research include the ionic medium metrizoic acid (Isopaque®) which was introduced in 1961, the first non-ionic medium to be marked, metrizamide (Amipaque®) which was introduced in 1974, and iohexol (Omnipaque®) which was introduced in 1982. The structures of these contrast agents are shown in Fig. 1.

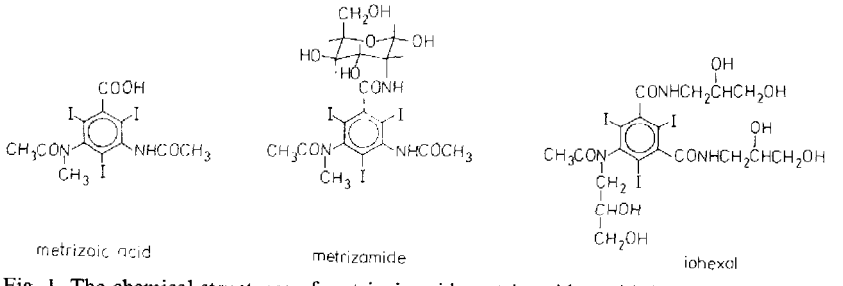



Fig. 1. The chemical structures of metrizoic acid, metrizamide and iohexol.

* Presented at the *1st International Symposium on Preparative and Up-Scale Liquid Chromatography, Paris, January 15–17, 1986*. Part of the papers presented at this meeting, have been published in *J. Chromatogr.*, Vol. 363, No. 1 (1986).

0021-9673/86/$03.50 

A diagnostically useful contrast agent should have the following characteristics[3]: opacity to X-rays, high iodine content and solubility, low viscosity and osmolality, chemical stability and biological safety. These criteria were nearly met by iohexol.

In the continuous search for new contrast agents preparative liquid chromatography (LC) has been an important tool. Some applications are described in this article.

## EXPERIMENTAL

*Purification of the highly water-soluble contrast agent iodixanol by reversed-phase preparative LC*

*Treatment of crude product prior to LC.* A 1.53-kg amount of the crude product was dissolved in the mobile phase A (methanol–water, 15:85) to a volume of 3.27 l. The pH of the solution was 3.4.

*Operating conditions.* The equipment used was a Jobin Yvon preparative system having a stainless-steel column (80 mm I.D.). A 1.0-kg amount of Merck LiChroprep RP-18, 60 Å, 15–25 μm was used as the stationary phase. Two different eluents were used; A, methanol–water (15:85); B, methanol–water (80:20). The elution pressure varied within the range 7.9–9.2 bar and the flow-rate was kept at 70 ml/min. Thirty cycles of automatic fractionation were carried out. Each repetitive loading contained 51 g of the crude product dissolved in 109 ml of eluent A, with an elution volume of 12.2 l and an elution time of 175 min. Dectection was performed by an IOTA refractive index (sensitivity = 8; IOTA RI, S = 8) and UV detector at 280 nm.

*Work-up and analysis of the separated fractions.* The product was isolated by removing the solvent at reduced pressure (15–20 mmHg, 60°C). Pure fractions were pooled to give the final product. The solvent-delivery system consisted of a Perkin-Elmer Series 3B liquid chromatograph. Detector: Perkin-Elmer LC 75 spectrophotometric detector (254 nm) with a Perkin-Elmer Sigma 10 chromatography data station. Injector: Perkin-Elmer LC-420 autosampler. The injected sample was eluted using a 60-min linear gradient of 3–17% acetonitrile in water. Column: 25-cm RP-18, 5 μm (Brownlee Labs.).

*Purification of N-α-(3-acetamido-2,4,6-triiodobenzoyl)-N-ε-[3-acetamido-5-(N-methylacetamido)-2,4,6-triiodobenzoyl]-L-lysine (2)*

*Treatment of crude product prior to LC.* A 34.0-g amount of the crude product as its sodium salt was dissolved on heating in a mixture of 120 ml methanol–water (40:60). The pH was adjusted to 7.6 with 1 *M* hydrochloric acid.

*Operating conditions.* The equipment used was a Jobin Yvon prepamatic LC 2 system having a stainless-steel column (80 mm I.D.). An 800-g amount of Merck LiChroprep RP-18, 15–25 μm was used as the stationary phase. The eluent was methanol–water (30:70) containing 10 m*M* ammonium acetate, pH 6.4. The elution pressure varied within the range 11.5–12 bar and the flow-rate was kept at 60 ml/min. A 34-g amount of the crude lysine derivative (2) dissolved in 120 ml of methanol–water (40:60) at pH 7.6 was loaded on the column. Detection was performed by an IOTA RI, S = 8 and UV detector at 280 nm.

*Work-up and analysis of the separated fractions.* Each fraction was concen-

trated at reduced pressure. The pH was adjusted to 2.0–2.5 with hydrochloric acid. The precipitate formed was filtered off, washed with water and dried *in vacuo* at 50°C. Each fraction was analyzed by high-performance liquid chromatography (HPLC) with the same solvent-delivery system as described for iodixanol (1). The sample was injected and eluted using a 60-min linear gradient of 10–40% acetonitrile in water, containing 10 m*M* ammonium acetate buffer[4]. Column: 25-cm RP-18, 5 μm (Brownlee Labs.).

*Purification of 5-[N-(5-hydroxy-2-methoxymethyl-3,7-dioxaoctyl)acetamido]-2,4,6-triiodoisophthalic acid (4b)*

*Operating conditions.* The equipment used was a Jobin Yvon prepamatic LC 2 system having a stainless-steel column (80 mm I.D.). A 1.0-kg amount of Merck LiChroprep RP 18, 60 Å, 25–40 μm was used as the stationary phase. The eluent was methanol–water (20:80) containing 10 m*M* phosphate buffer and 1 m*M* tetrabutylammonium chloride (TBA)[5].

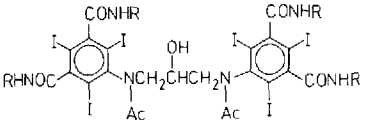
The elution pressure was about 10 bar and the flow-rate was 74 ml/min. The instrument was operated manually and eight separate injections were done. Each injection consisted of 37.5 g of the crude isophthalic acid derivative (4; see Fig. 11) dissolved in 100 ml of water. The pH was adjusted to 7.0 with 6 *M* hydrochloric acid. Each cycle required 70 min with an elution volume of 5.2 l.

*Work-up and analysis of the separated fractions.* Each fraction was concentrated at reduced pressure. On adjustment of the pH to about 0.5 with concentrated hydrochloric acid, precipitation occurred. The water was decanted off and the sticky product was dried *in vacuo* at 50°C overnight. Each fraction was analyzed by HPLC with the same solvent-delivery system as described for iodixanol. The sample was eluted using a 60-min linear gradient of 5–30% acetonitrile in water, containing 30 m*M* TBA and 10 m*M* phosphate buffer pH 7.7. Column: 25-cm RP-18, 5 μm (Brownlee Labs.).

*Purification of N,N'-bis(2,3-dihydroxypropyl)-5-[N-(5-hydroxy-2-methoxymethyl-3,7-dioxaoctyl)acetamido]-2,4,6-triiodoisophthalamide (6b)*

*Operating conditions.* The Jobin Yvon prepamatic system was used. The steel column (80 mm I.D.) was packed with 950 g of Merck LiChroprep RP-18, 15–25 μm as the stationary phase. The eluent was methanol–water (18:82), and the elution pressure was 11 bar. Each injection consisted of about 30 g of compounds 6a–6b (1:5.3) dissolved in 90 ml water. Two manual injections were done. The fractions collected were concentrated to dryness at reduced pressure and dried *in vacuo* at 50°C. Each fraction was analyzed by HPLC using the same solvent-delivery system as described for iodixanol. The sample was eluted using a 60-min linear gradient of 3–17% acetonitrile in water.

All the analytical HPLC systems were developed by K. Skinnemoen, Nycomed AS, Department of Chemical Analysis[4]. All the compounds described have been characterized by spectroscopic data.




1, R = $CH_2CH(OH)CH_2OH$

Fig. 2. The chemical structure of iodixanol (1).

## RESULTS AND DISCUSSION

### *Iodixanol (1)*

The chemical structure of iodixanol and the composition of the crude product which was loaded on the column are shown in Figs. 2 and 3 respectively. The crude product contained 82% of iodixanol. In the chromatogram, two isomeric forms are seen due to *exo–endo* isomerism (Fig. 4)[6]. A total of 1530 g of crude product was purified by preparative LC. Under the operating conditions applied, 10 g/h of pure iodixanol were obtained. The preparative chromatogram is shown in Fig. 5 and the yield and purity of each fraction are given in Table I.

Fractions 1–5 contained 76% of the total amount of substance loaded on the

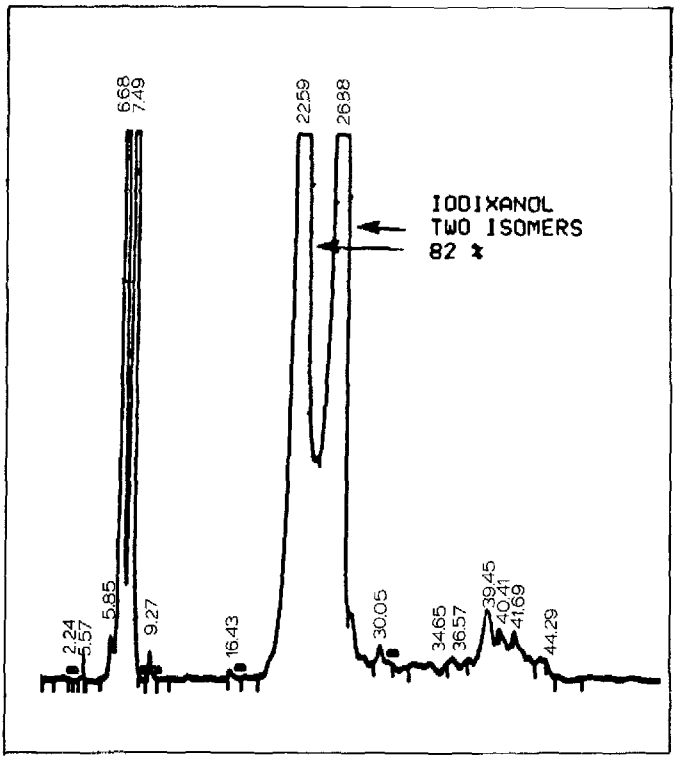



Fig. 3. HPLC of crude iodixanol. Column: 25-cm RP-18, 5 μm (Brownlee Labs.). Eluent: 60-min linear gradient of 3–17% acetonitrile in water. Loading: 15 μg.

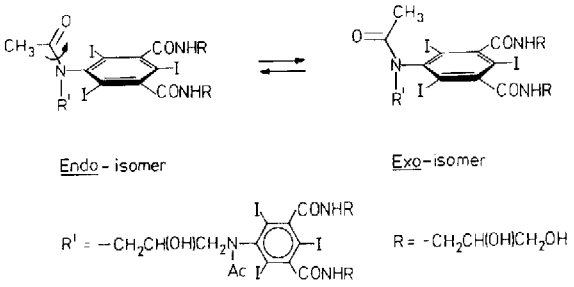



Fig. 4. *exo–endo* Isomerism of iodixanol (I).

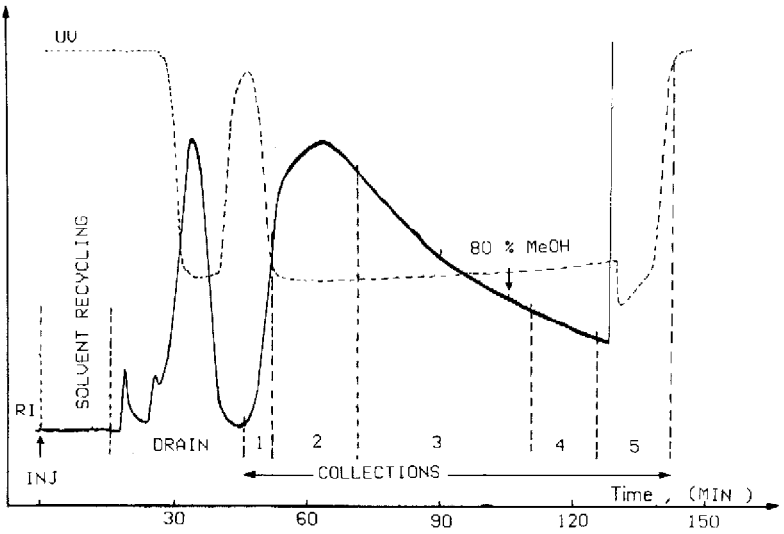



Fig. 5. Preparative chromatogram from the purification of iodixanol on a Jobin Yvon preparative system.

TABLE I

YIELDS AND PURITY OF FRACTIONS 1–5 FROM PREPARATIVE LC OF CRUDE IODIXANOL

| *Fraction No.* | *Average amount from each cycle (g)* | *Total amount isolated (g)* | *Purity* according to HPLC (%)* |
|---|---|---|---|
| 1 | 0.8 | 24 | 98.6 |
| 2 | 10.5 | 315 | 99.6 |
| 3 | 15.3 | 459 | 99.6 |
| 4 | 4.2 | 126 | 99.2 |
| 5 | 8.0 | 240 | 61.6 |

* Column: 25-cm RP-18, 5 μm (Brownlee Labs.). Eluent: 60-min linear gradient of 3–17% acetonitrile in water.

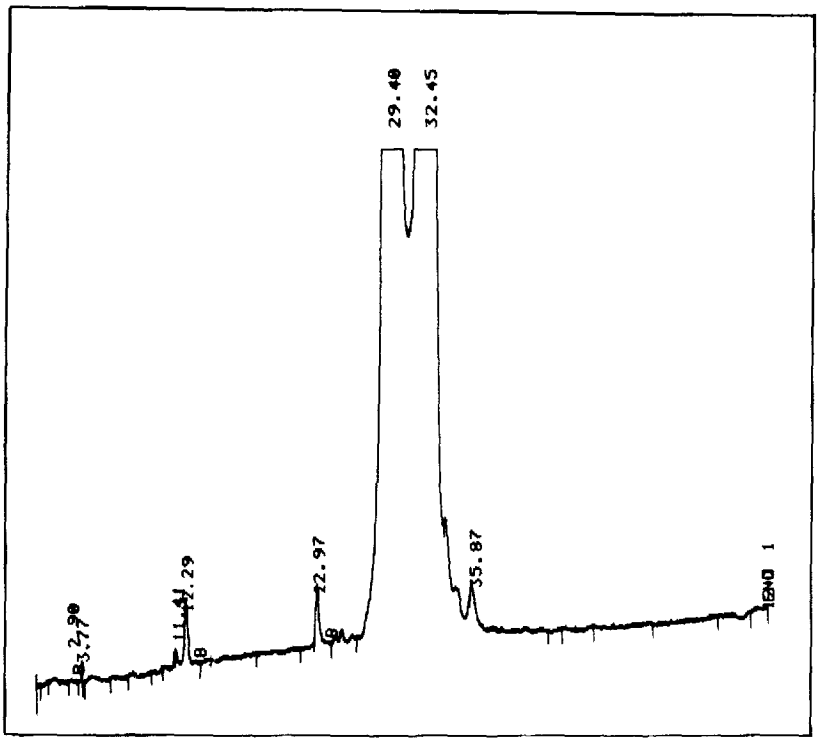


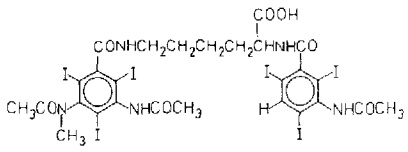

Fig. 6. Iodixanol purified by preparative LC. Yield 72%, purity 99.6%. Eluent: 60-min linear gradient of 3–17% acetonitrile in water.

column. Fractions 2–4 were pooled to give 900 g of iodixanol. The yield was 72% based on the amount of iodixanol present in the crude product, and the purity was 99.6% (Fig. 6).

*N-α(3-Acetamido-2,4,6-triiodobenzoyl)-N-ε-[3-acetamido-5-(N-methylacetamido)-2,4,6-triiodobenzoyl]-L-lysine (2)*

The chemical structure of compound 2 and the composition of the crude product which was loaded on the column are shown in Figs. 7 and 8, respectively. The content of compound 2 in the mixture was 45%. Both the *exo-* and *endo*-forms of the product were detected[6]. A total of 34 g of crude product was loaded on the column in one experiment. The preparative chromatogram is shown in Fig. 9 and the yield and purity of each fraction are given in Table II.

Fractions 2 and 3 together contained 56% of the amount of compound 2




2

Fig. 7. The chemical structure of the lysine derivative 2.

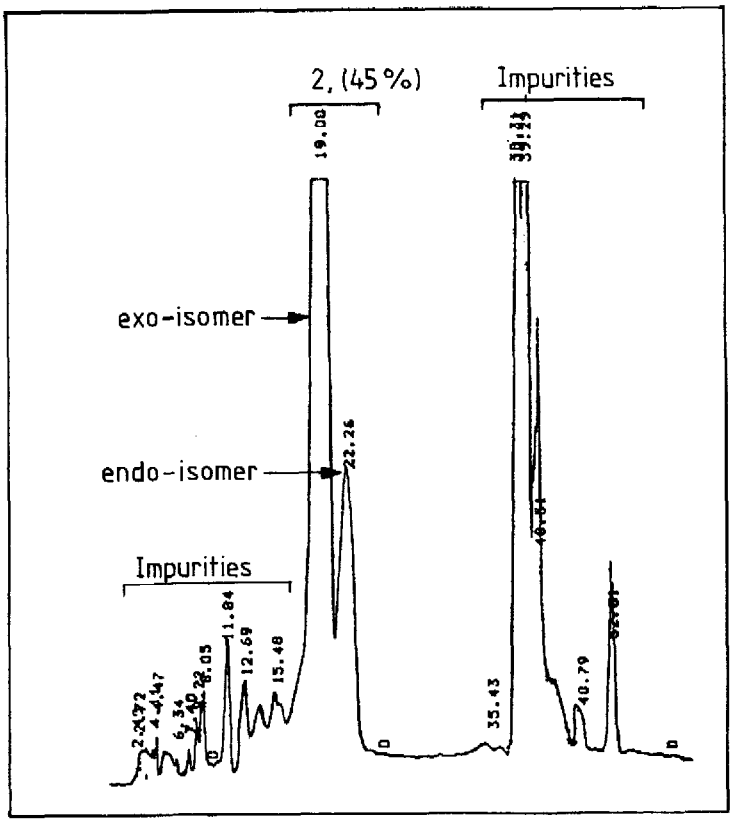



Fig. 8. HPLC of crude compound 2. Column: 25-cm RP-18, 5 $\mu$m (Brownlee Labs.). Eluent: acetonitrile–water containing 10 m*M* ammonium acetate; 15% acetonitrile for 30 min, 15–30% for 2 min, 30% for 28 min.

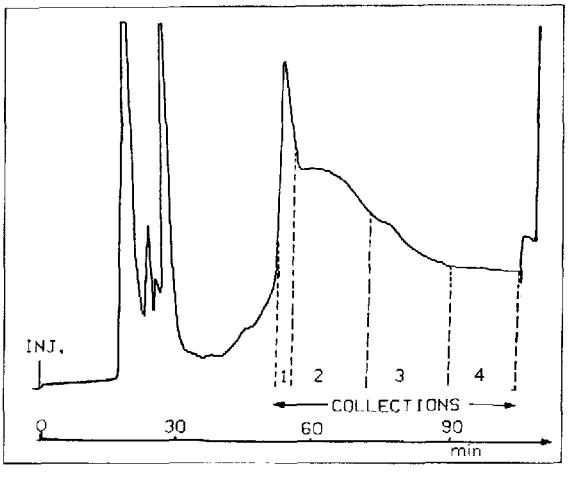



Fig. 9. Preparative chromatogram from the purification of the lysine derivative 2 on a Jobin Yvon prepamatic LC 2 system.

TABLE II

YIELDS AND PURITY OF FRACTIONS 1–4 FROM PREPARATIVE LC OF CRUDE LYSINE DERIVATIVE 2

| *Fraction No.* | *Yield** | | *Purity according to HPLC*** (%)* |
|---|---|---|---|
| | *g* | % | |
| 1 | 1.5 | 9.8 | 89.0 |
| 2 | 5.7 | 37.3 | 99.2 |
| 3 | 2.8 | 18.3 | 98.8 |
| 4 | 1.0 | 6.5 | 96.2 |

* Relative to the amount of compound 2 present in the crude product.

** Column: 25-cm RP-18, 5 μm (Brownlee Labs.). Eluent: 60-min linear gradient of 10–40% acetonitrile in water, containing 10 m*M* ammonium acetate buffer.

present in the crude product and showed a purity of 99.2 and 98.8% respectively. Fraction 2 (Fig. 10) was tested as a potential contrast agent for cholangiography.

*5-[N-(5-Hydroxy-2-methoxymethyl-3,7-dioxaoctyl)acetamido]-2,4,6-triiodoisophthalic acid (4b) and N,N'-bis(2,3-dihydroxypropyl)-5-[N-(5-hydroxy-2-methoxymethyl-3,7-dioxaoctyl)acetamido]-2,4,6-triiodoisophthalamide (6b)*

Treatment of 5-acetamido-2,4,6-triiodoisophthalic acid (3) with an excess of

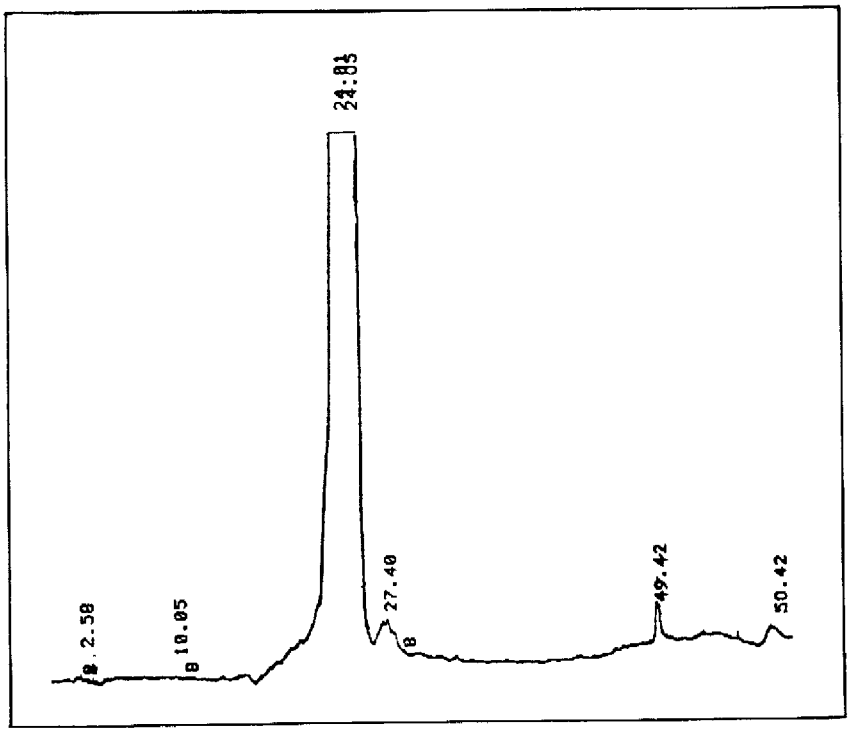
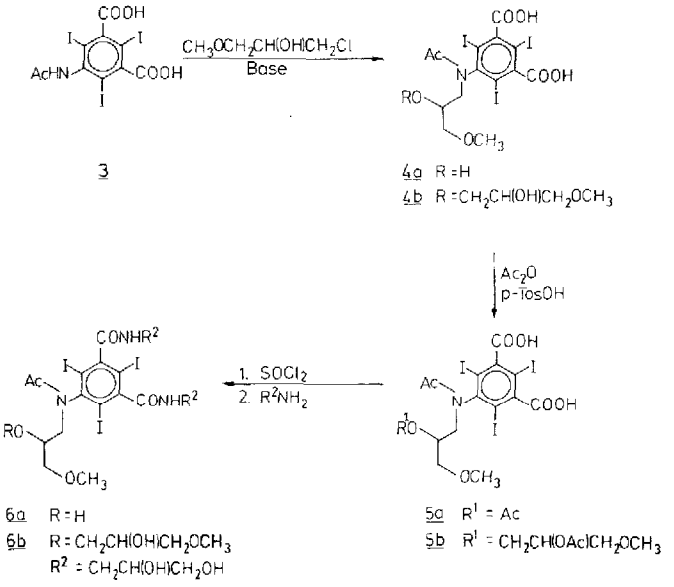



Fig. 10. Lysine derivative 2 purified by preparative LC. Fraction 2, 37% yield, purity 99.2%. Eluent: 60-min linear gradient of 10–40% acetonitrile in water, containing 10 m*M* ammonium acetate buffer.




3

4a R = H
4b R = $CH_2CH(OH)CH_2OCH_3$

6a R = H
6b R = $CH_2CH(OH)CH_2OCH_3$
$R^2$ = $CH_2CH(OH)CH_2OH$

5a $R^1$ = Ac
5b $R^1$ = $CH_2CH(OAc)CH_2OCH_3$

Fig. 11. The synthetic route from compound 3 to 6b. *p*-TosOH = *p*-toluenesulphonic acid.

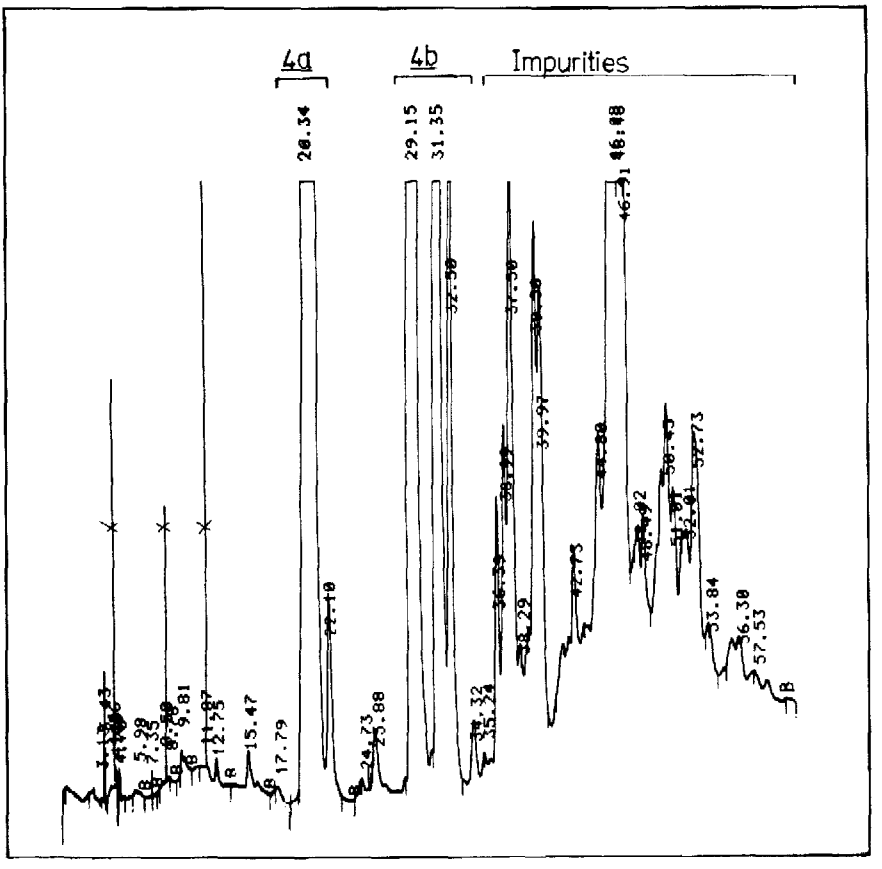



Fig. 12. HPLC of the crude isophthalic acid derivative 4b. Column: 25-cm RP-18, 5 μm (Brownlee Labs.) Eluent: 60-min linear gradient of 5–30% acetonitrile in water, containing 30 m*M* TBA and 10 m*M* phosphate buffer pH 7.7.

1-chloro-3-methoxy-2-propanol under basic conditions gave a mixture of the isophthalic acid derivatives 4a (62%) and 4b (19%) accompanied by products with longer retention times (19%) (see Figs. 11 and 12).

As our aim was to synthesize pure compound 6b we decided to isolate compound 4b by the use of preparative LC. The best LC eluent consisted of methanol–water containing phosphate buffer and TBA[5]. A separation of compounds 4a and 4b was not achieved under these conditions, but the amount of impurities with retention times longer than that of 4b was reduced to about 5% and the content of compound 4b increased to 32% (Fig. 13). As it was assumed that the separation of compounds 6a and 6b would be far easier than that of 4a and 4b, the next steps in the synthesis were carried out with the mixture (4a, 63%; 4b, 32%) (Fig. 11). Treatment of compounds 4a/4b with acetic anhydride in the presence of *p*-toluenesulphonic acid as a catalyst gave compunds 5a/5b. Reaction of compounds 5a/5b with thionyl chloride followed by 3-amino-1,2-propanediol gave compounds 6a/6b, which were separated by preparative LC to give pure 6b (Fig. 14).

HPLC of the isophthalamide derivative 6b shows two diastereomers due to the proximity of the two chiral carbon atoms (Fig. 15). Each isomer exists as a mixture of *endo*- and *exo*-forms (similar to the equilibrium shown in Fig. 4). Peaks

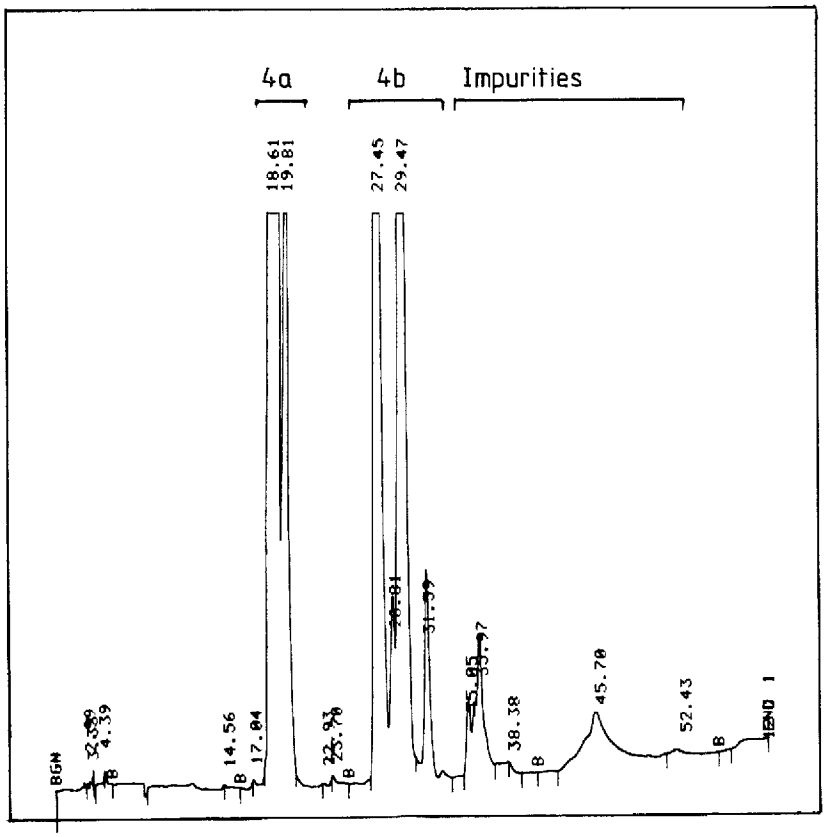



Fig. 13. HPLC of compounds 4a and 4b. Conditions as in Fig. 12.

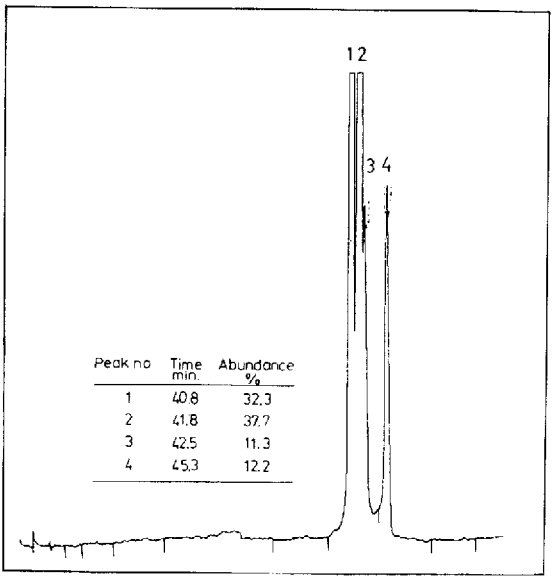
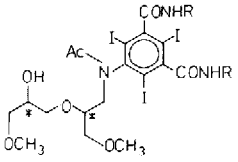



Fig. 14. HPLC of pure compound 6b. Column: 25-cm RP-18, 5 $\mu$m (Brownlee Labs.). Eluent: 60-min linear gradient of 3–17% acetonitrile in water.




6b R = $CH_2CH(OH)CH_2OH$

Fig. 15. Isomeric forms of compound 6b.

1 and 3 are most probably the *exo/endo* pair of one of the diastereomers (A), while peaks 2 and 4 are the *exo/endo* pair of the other (B). The ratio of the two diastereomers A:B is 1:1.2 and the *exo/endo* ratio is approximately 80:20.

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