US006391862B1

(12) **United States Patent**
Vigh

(10) **Patent No.: US 6,391,862 B1**
(45) **Date of Patent: May 21, 2002**

(54) **CHIRAL RESOLVING AGENTS FOR ENANTIOSEPARATIONS**

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(73) Assignee: **The Texas A&M University System**, College Station, TX (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/172,575**

(22) Filed: **Oct. 14, 1998**

Related U.S. Application Data

(60) Provisional application No. 60/063,998, filed on Oct. 14, 1997.

(51) **Int. Cl.**[7] **A61K 31/715**; C08B 37/16; C07H 15/04

(52) **U.S. Cl.** **514/58**; 536/103; 536/120; 536/122

(58) **Field of Search** 514/58; 536/103, 536/120, 122

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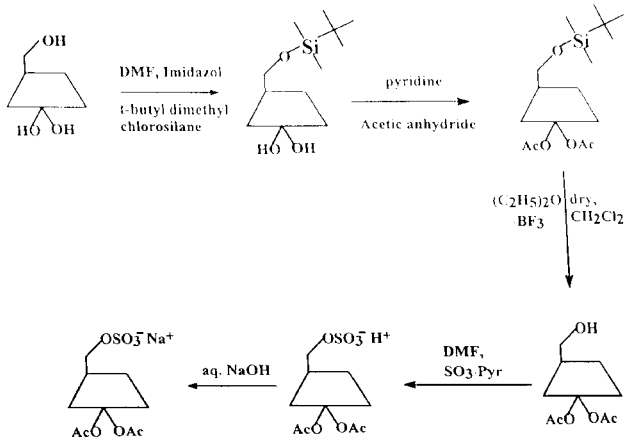
(57) **ABSTRACT**

A cyclodextrin composition of substantially pure, sulfated-cyclodextrin derivatives particularly suitable as chiral resolving agents for enantioseparation by electrophoresis. The cyclodextrin composition preferably have an isomeric purity of at least 80 mole %. Non-sulfato substituents for the substantially pure cyclodextrin derivatives are hydrogen, C_1–C_{12} alkyl groups, C_2–C_8 hydroxyalkyl groups, C_1–C_{12} alkylnitryl groups, C_2–C_{12} acyl groups, aryl groups, carbamate groups, thiocarbamate groups or combinations thereof.

17 Claims, 28 Drawing Sheets



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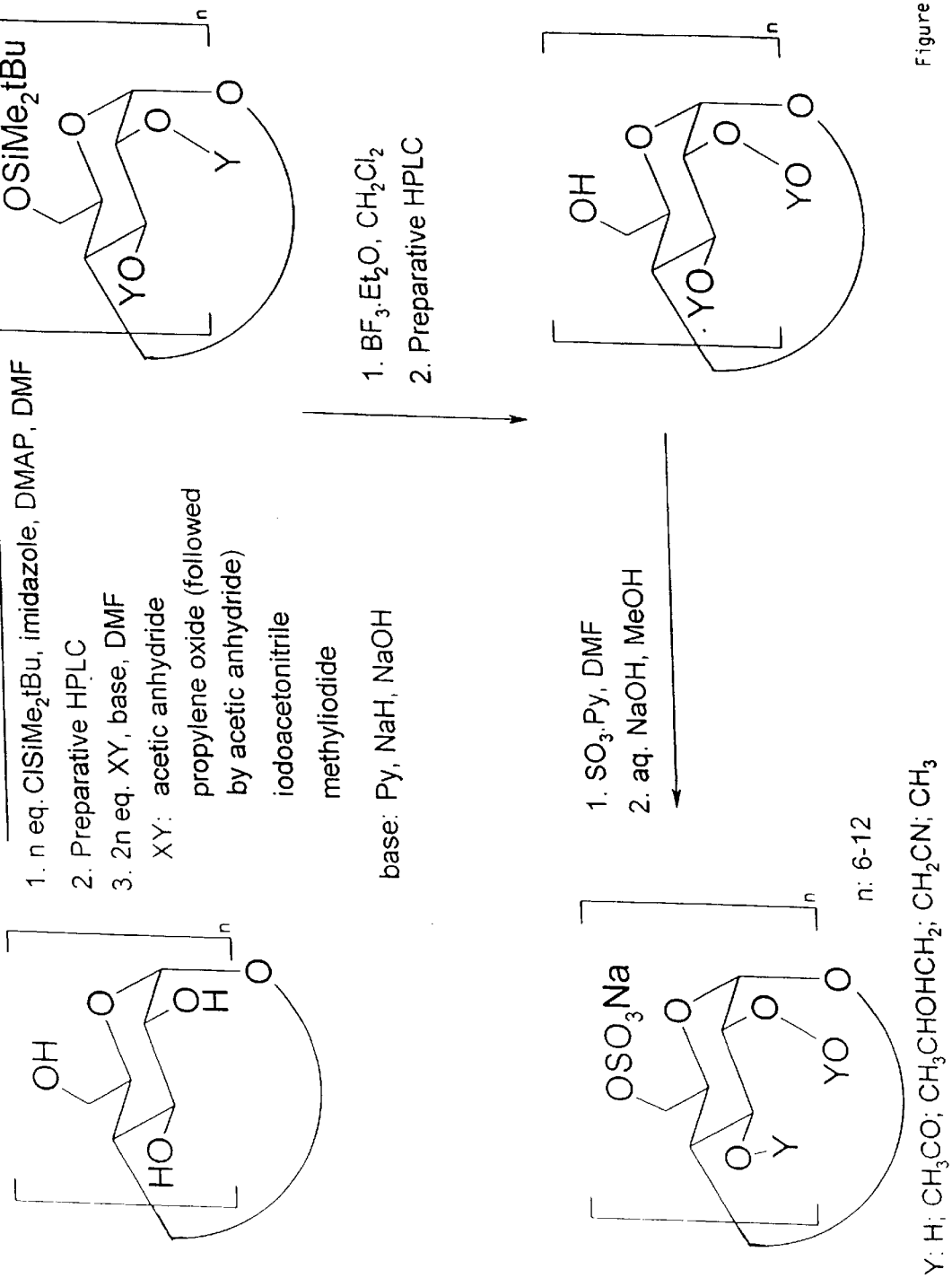

1. n eq. ClSiMe2tBu, imidazole, DMAP, DMF
2. Preparative HPLC
3. 2n eq. XY, base, DMF
XY: acetic anhydride
propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
base: Py, NaH, NaOH
1. BF3.Et2O, CH2Cl2
2. Preparative HPLC
1. SO3.Py, DMF
2. aq. NaOH, MeOH
OSiMe2tBu
OH
OSO3Na
YO
n: 6-12
Y: H; CH3CO; CH3CHOHCH2; CH2CN; CH3

Figure 1

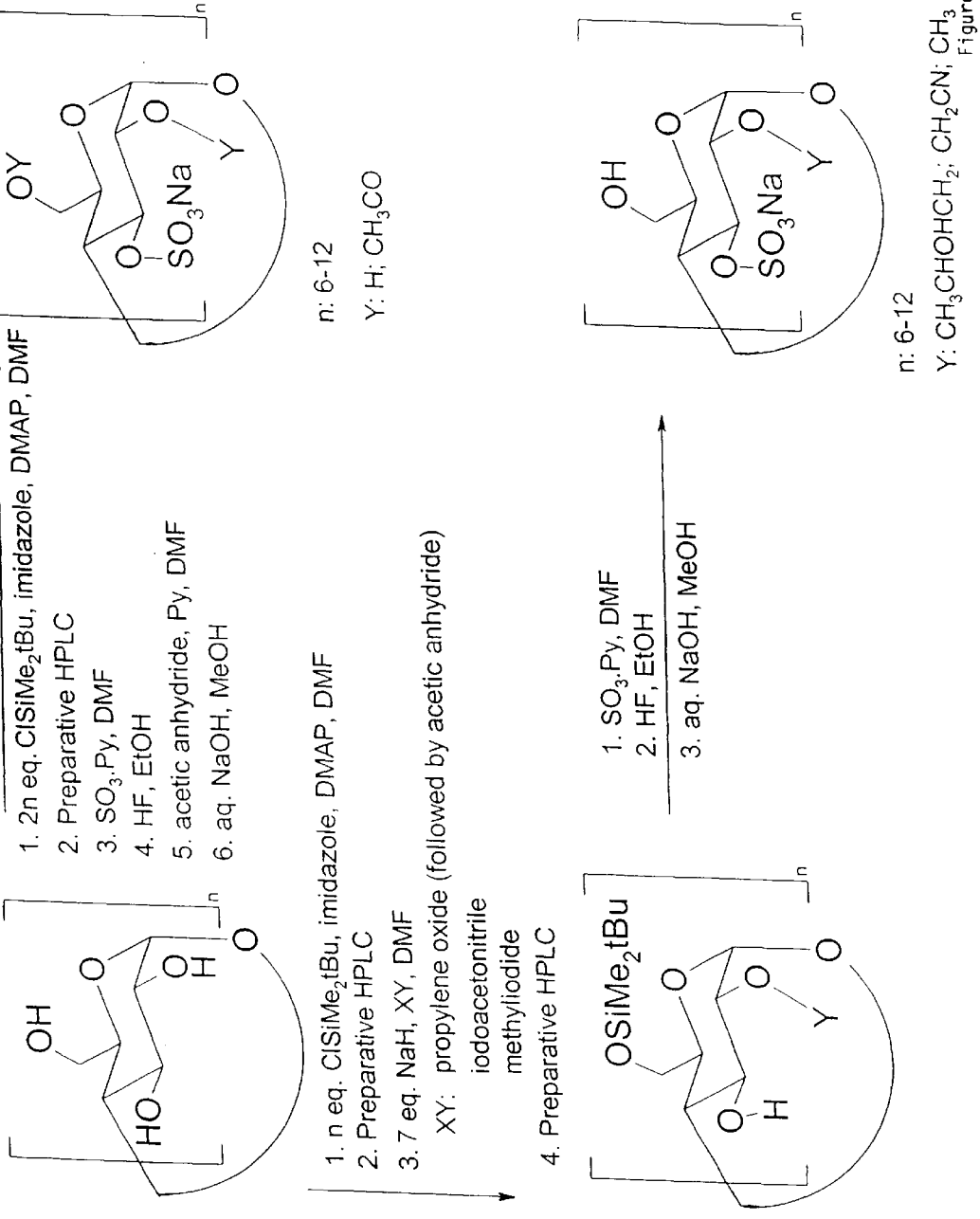

1. 2n eq. ClSiMe2tBu, imidazole, DMAP, DMF
2. Preparative HPLC
3. SO3.Py, DMF
4. HF, EtOH
5. acetic anhydride, Py, DMF
6. aq. NaOH, MeOH
n: 6-12
Y: H; CH3CO
1. n eq. ClSiMe2tBu, imidazole, DMAP, DMF
2. Preparative HPLC
3. 7 eq. NaH, XY, DMF
XY: propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
4. Preparative HPLC
1. SO3.Py, DMF
2. HF, EtOH
3. aq. NaOH, MeOH
n: 6-12
Y: CH3CHOHCH2; CH2CN; CH3

Figure 2

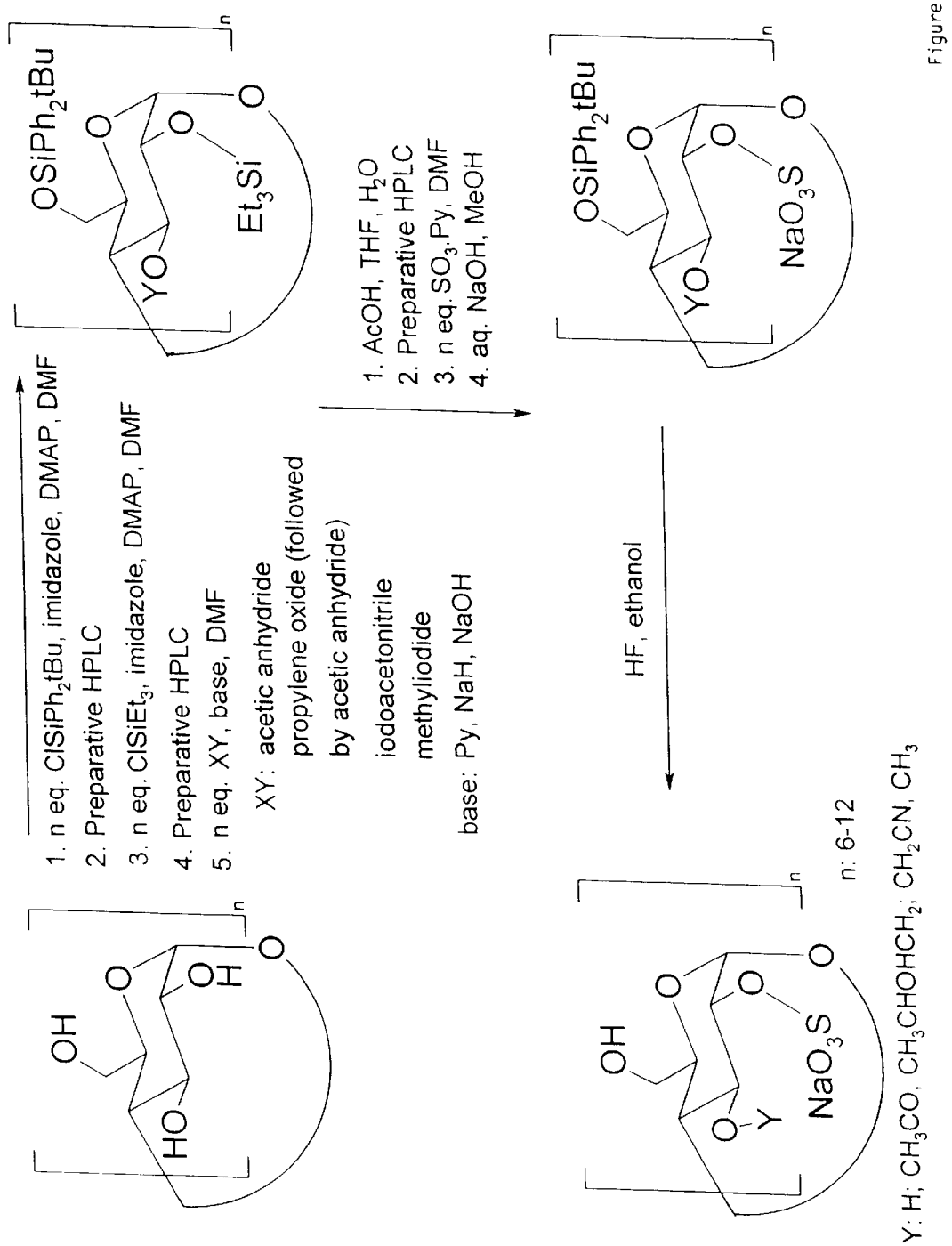

1. n eq. ClSiPh2tBu, imidazole, DMAP, DMF
2. Preparative HPLC
3. n eq. ClSiEt3, imidazole, DMAP, DMF
4. Preparative HPLC
5. n eq. XY, base, DMF
XY: acetic anhydride
propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
base: Py, NaH, NaOH
1. AcOH, THF, H2O
2. Preparative HPLC
3. n eq. SO3.Py, DMF
4. aq. NaOH, MeOH
HF, ethanol
OSiPh2tBu
Et3Si
YO
NaO3S
OH
HO
n
n: 6-12
Y: H; CH3CO, CH3CHOHCH2; CH2CN, CH3

Figure 3

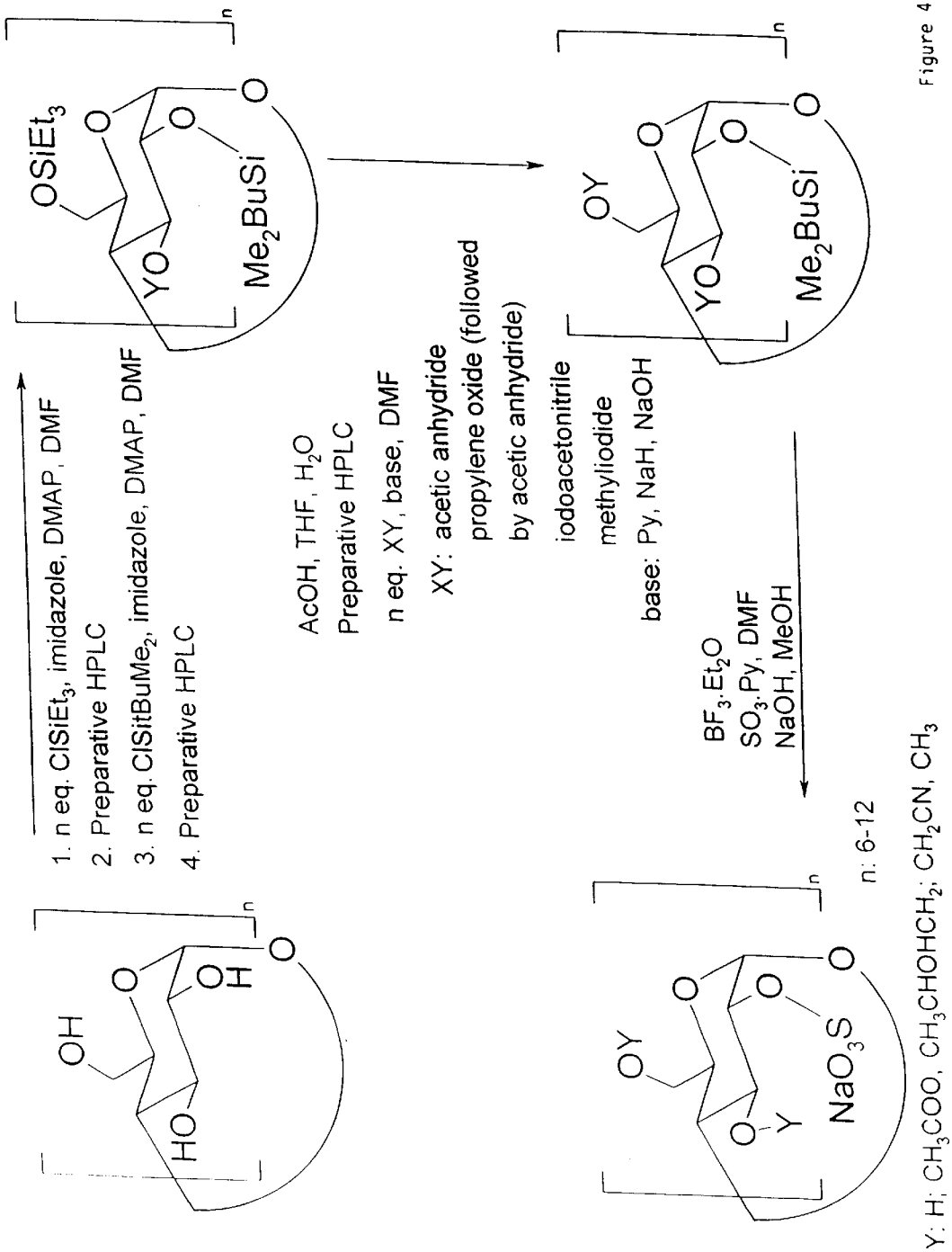

1. n eq. ClSiEt3, imidazole, DMAP, DMF
2. Preparative HPLC
3. n eq. ClSitBuMe2, imidazole, DMAP, DMF
4. Preparative HPLC
AcOH, THF, H2O
Preparative HPLC
n eq. XY, base, DMF
XY: acetic anhydride
propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
base: Py, NaH, NaOH
BF3.Et2O
SO3.Py, DMF
NaOH, MeOH
n: 6-12
Y: H; CH3COO, CH3CHOHCH2; CH2CN, CH3

Figure 4

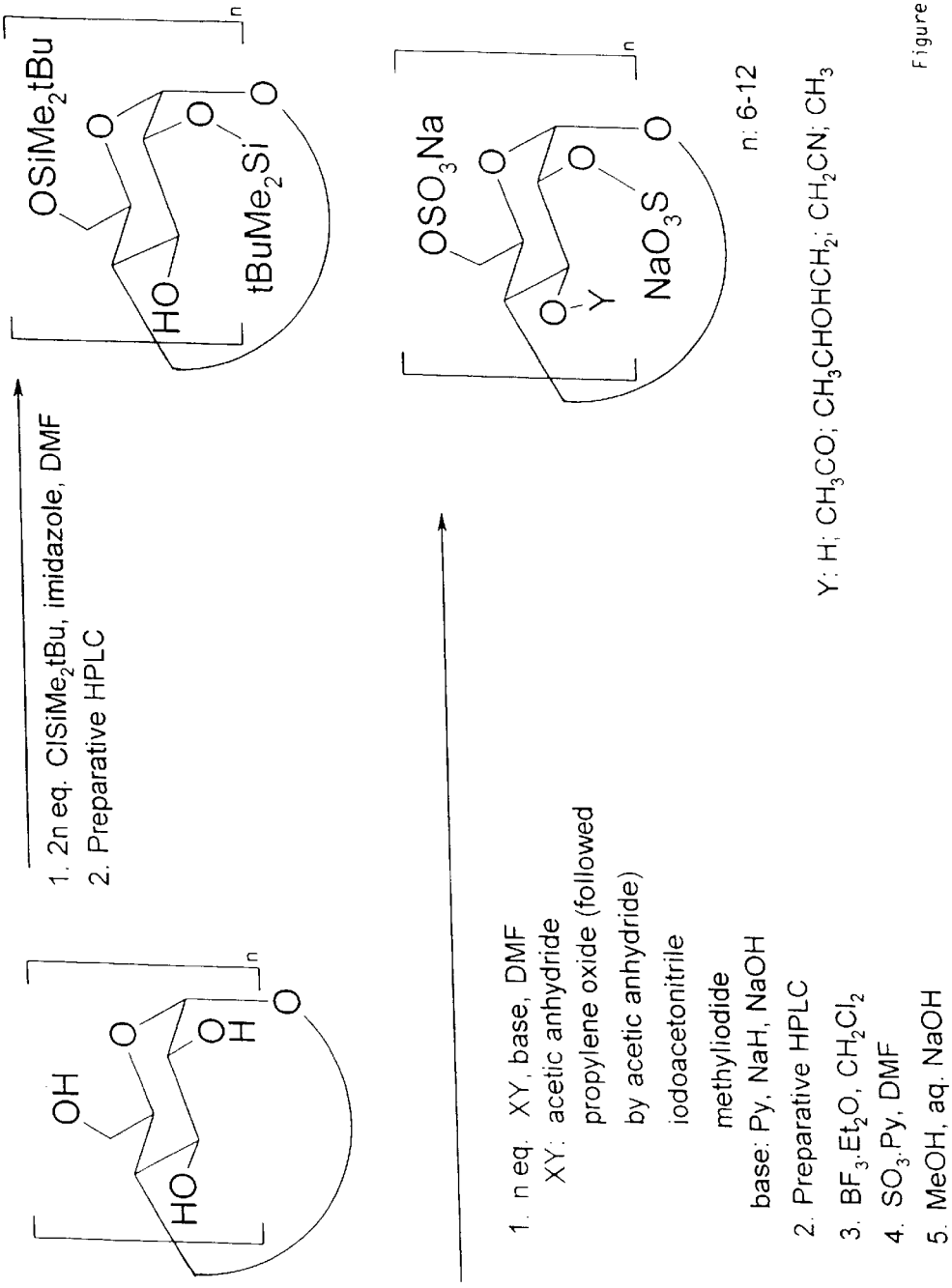

1. 2n eq. ClSiMe2tBu, imidazole, DMF
2. Preparative HPLC
OSiMe2tBu
tBuMe2Si
HO
OH
1. n eq. XY, base, DMF
XY: acetic anhydride
propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
base: Py, NaH, NaOH
2. Preparative HPLC
3. BF3.Et2O, CH2Cl2
4. SO3.Py, DMF
5. MeOH, aq. NaOH
OSO3Na
NaO3S
Y
n: 6-12
Y: H; CH3CO; CH3CHOHCH2; CH2CN; CH3

Figure 5

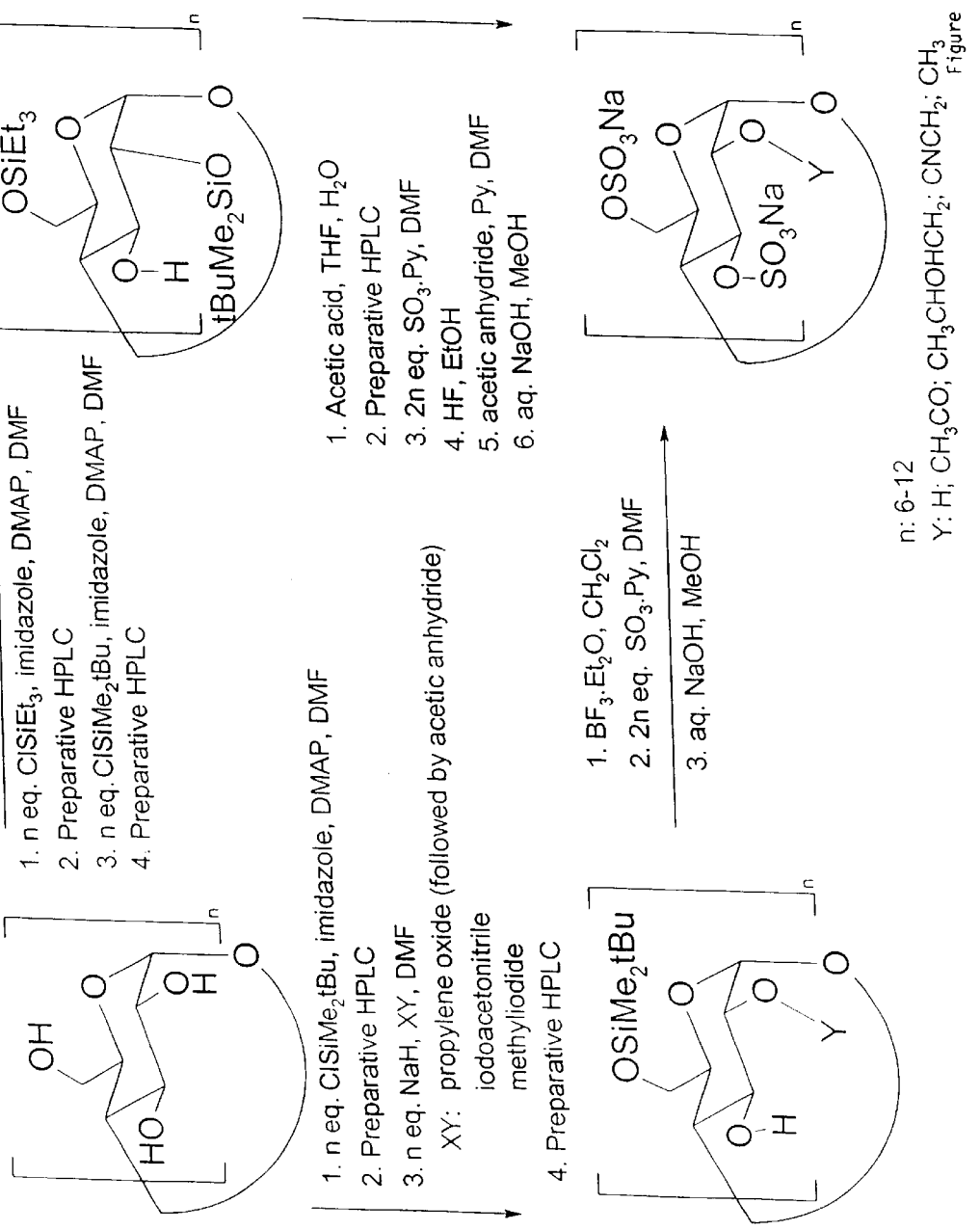

1. n eq. ClSiEt3, imidazole, DMAP, DMF
2. Preparative HPLC
3. n eq. ClSiMe2tBu, imidazole, DMAP, DMF
4. Preparative HPLC
1. n eq. ClSiMe2tBu, imidazole, DMAP, DMF
2. Preparative HPLC
3. n eq. NaH, XY, DMF
XY: propylene oxide (followed by acetic anhydride)
iodoacetonitrile
methyliodide
4. Preparative HPLC
1. Acetic acid, THF, H2O
2. Preparative HPLC
3. 2n eq. SO3.Py, DMF
4. HF, EtOH
5. acetic anhydride, Py, DMF
6. aq. NaOH, MeOH
1. BF3.Et2O, CH2Cl2
2. 2n eq. SO3.Py, DMF
3. aq. NaOH, MeOH
n: 6-12
Y: H; CH3CO; CH3CHOHCH2; CNCH2; CH3

Figure 6

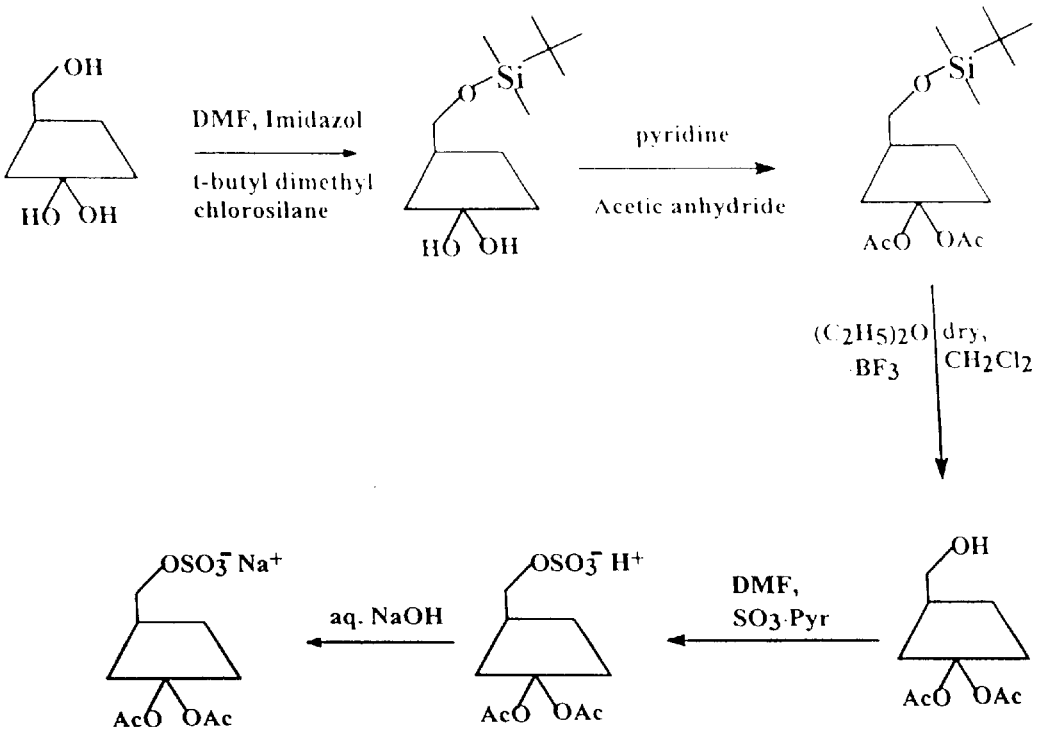

OH
HO OH
DMF, Imidazol
t-butyl dimethyl chlorosilane
Si
O
HO OH
pyridine
Acetic anhydride
AcO OAc
$(C_2H_5)_2O \cdot BF_3$
dry, CH_2Cl_2
OH
AcO OAc
DMF, SO_3-Pyr
$OSO_3^- H^+$
AcO OAc
aq. NaOH
$OSO_3^- Na^+$
AcO OAc

Figure 7

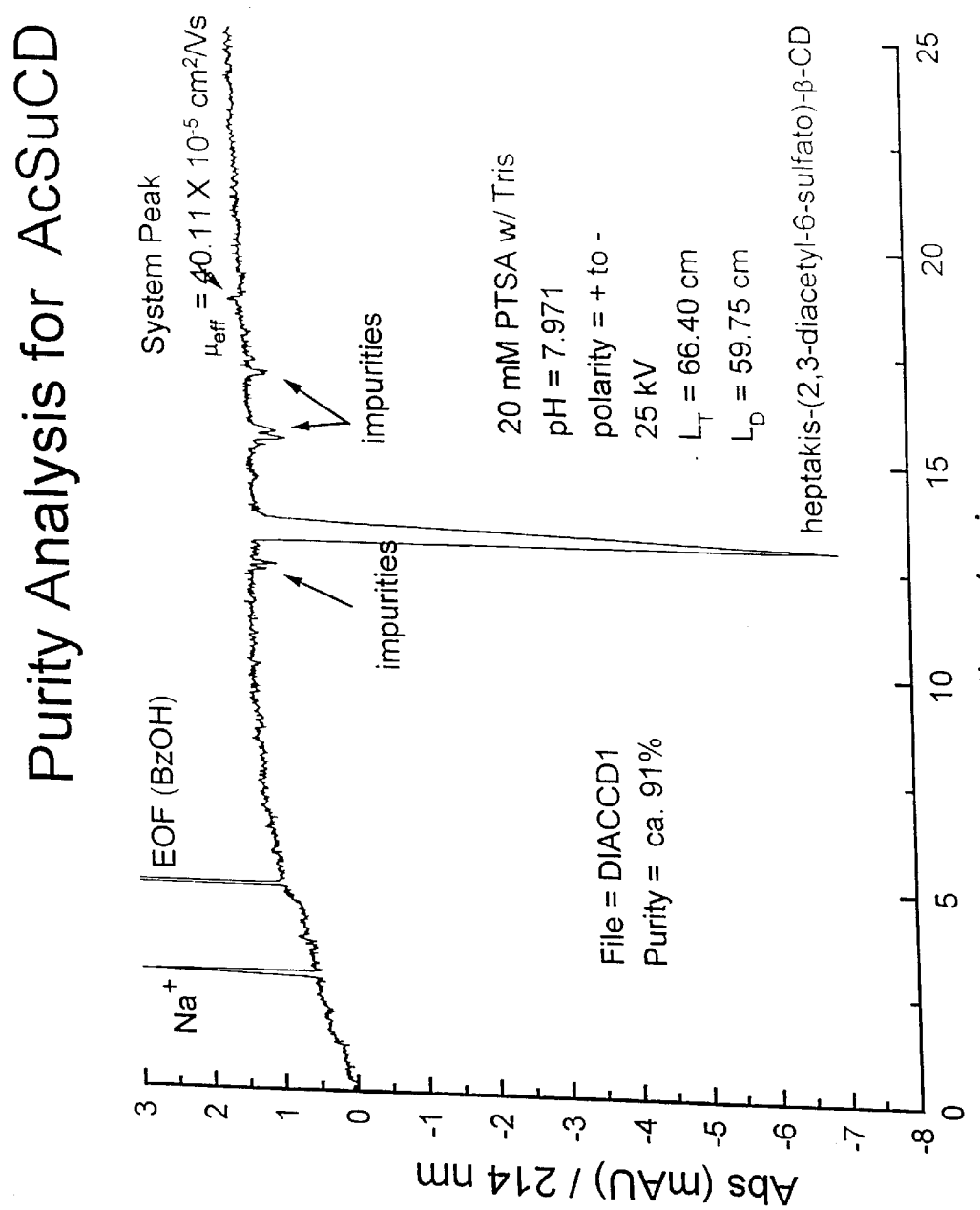

Purity Analysis for AcSuCD
System Peak
μ_{eff} = 40.11 X 10^{-5} cm^2/Vs
impurities
impurities
EOF (BzOH)
Na^+
20 mM PTSA w/ Tris
pH = 7.971
polarity = + to -
25 kV
L_T = 66.40 cm
L_D = 59.75 cm
heptakis-(2,3-diacetyl-6-sulfato)-β-CD
File = DIACCD1
Purity = ca. 91%
Abs (mAU) / 214 nm
time / min.

Figure 8

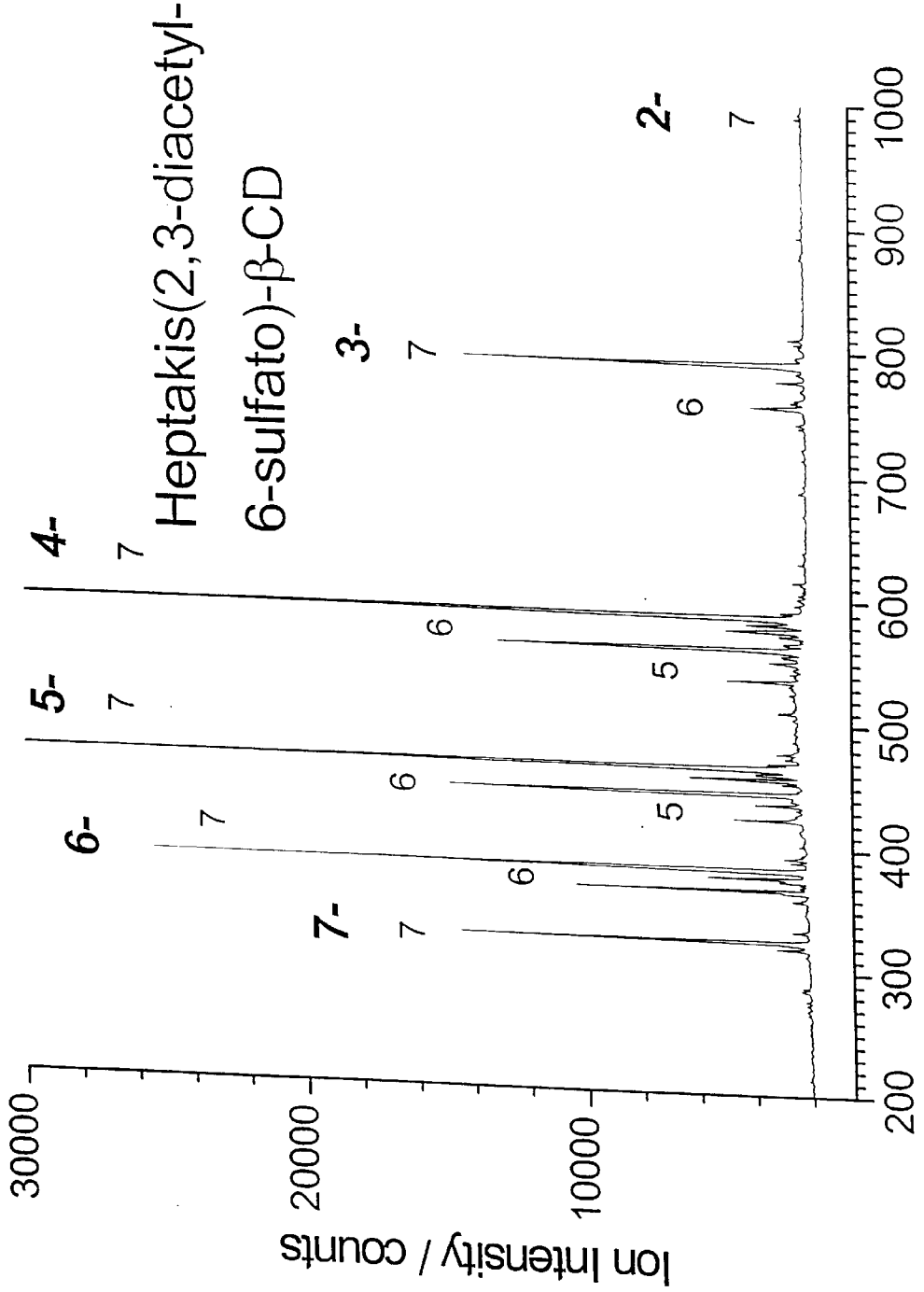

Heptakis(2,3-diacetyl-6-sulfato)-β-CD
Ion Intensity / counts
Mass (m/z)
30000
20000
10000
200
300
400
500
600
700
800
900
1000
7-
6-
5-
4-
3-
2-

Figure 9

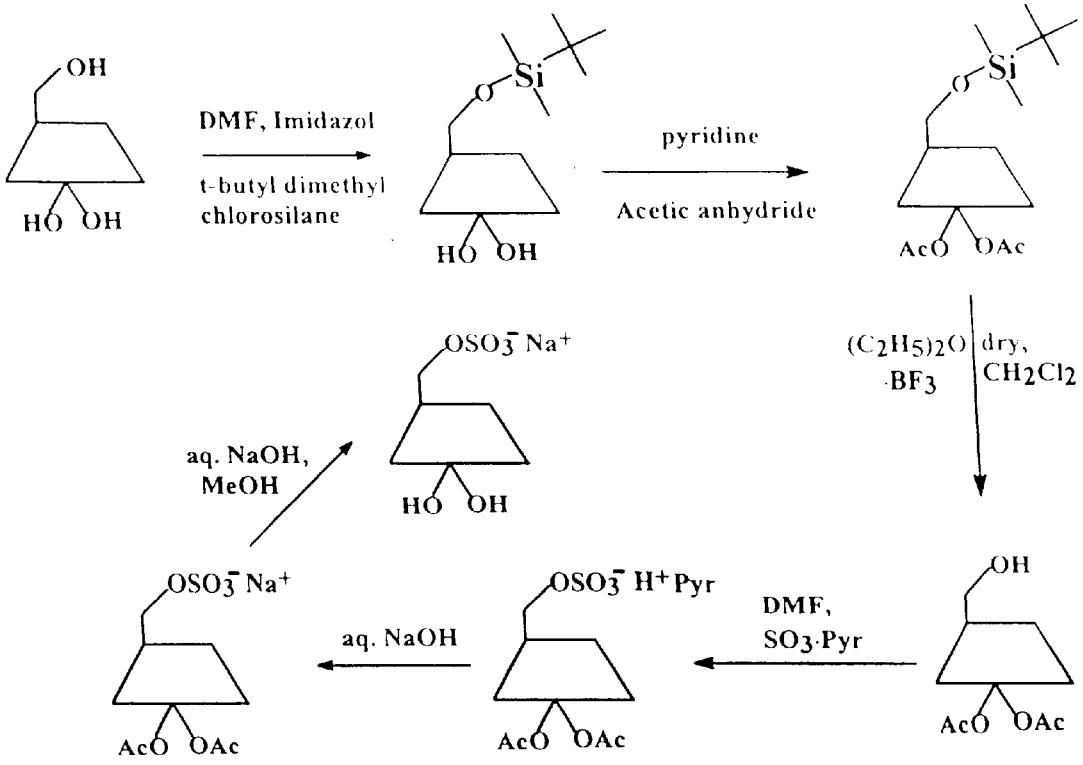

OH
HO OH
DMF, Imidazol
t-butyl dimethyl chlorosilane
Si
O
HO OH
pyridine
Acetic anhydride
AcO OAc
$(C_2H_5)_2O$ dry, CH_2Cl_2
BF_3
$OSO_3^- Na^+$
aq. NaOH, MeOH
$OSO_3^- H^+ Pyr$
DMF, SO_3·Pyr
aq. NaOH

Figure 10

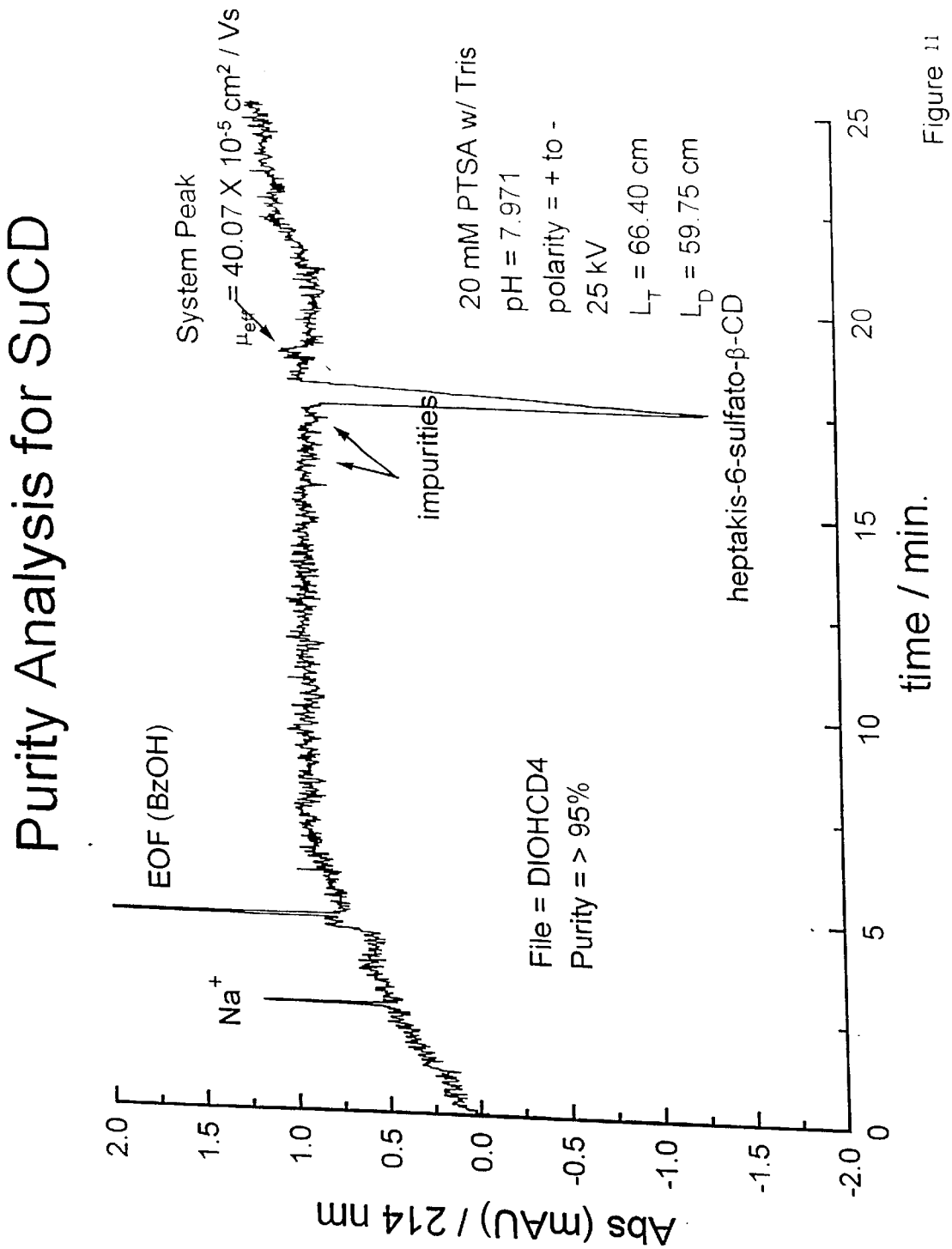

Purity Analysis for SuCD
EOF (BzOH)
Na+
System Peak
μep = 40.07 X 10-5 cm2 / Vs
impurities
File = DIOHCD4
Purity = > 95%
20 mM PTSA w/ Tris
pH = 7.971
polarity = + to -
25 kV
LT = 66.40 cm
LD = 59.75 cm
heptakis-6-sulfato-β-CD
Abs (mAU) / 214 nm
time / min.
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
0
5
10
15
20
25

Figure 11

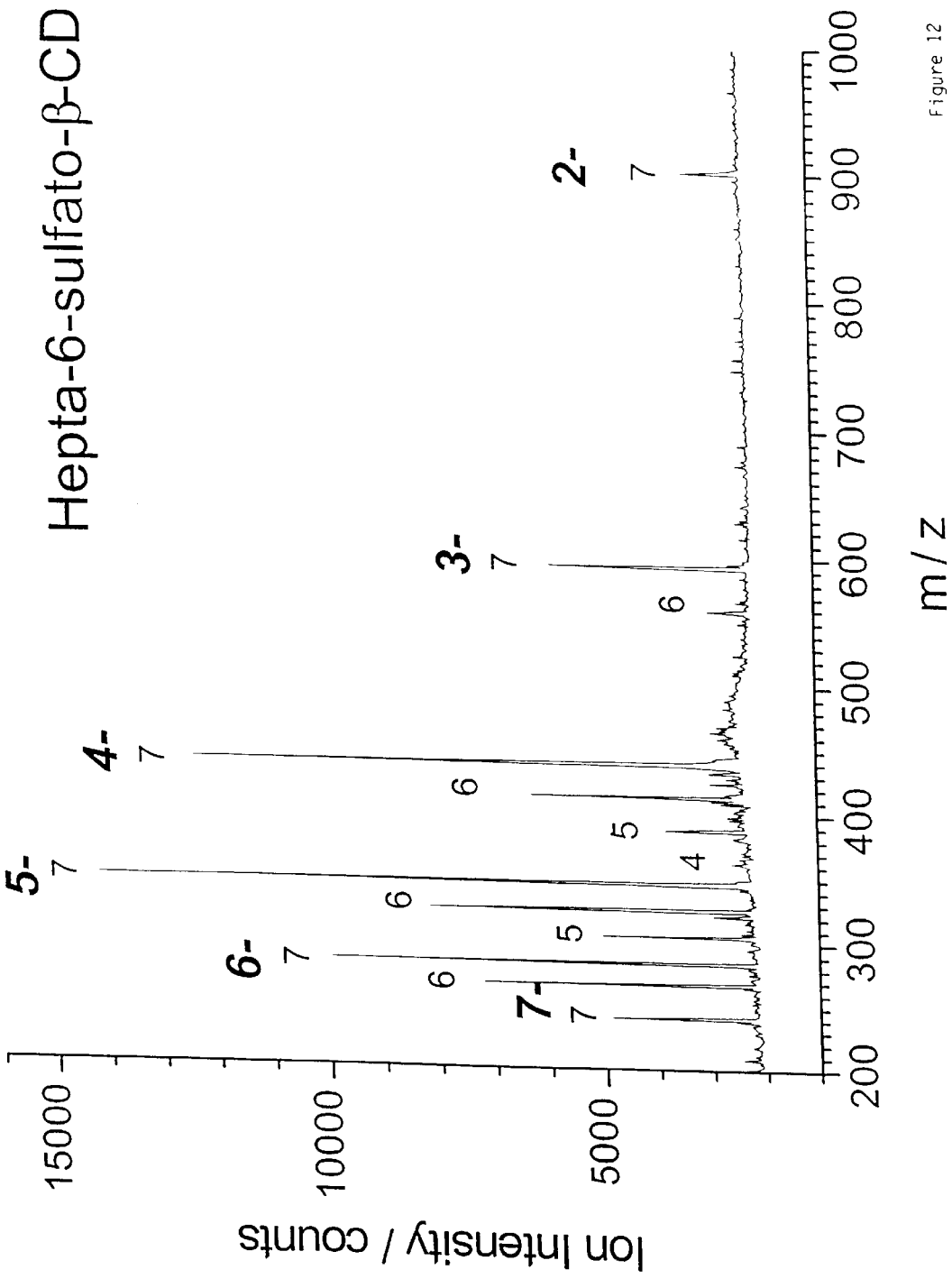

Hepta-6-sulfato-β-CD
Ion Intensity / counts
m/z
15000
10000
5000
200
300
400
500
600
700
800
900
1000
5-
4-
6-
7-
3-
2-

Figure 12

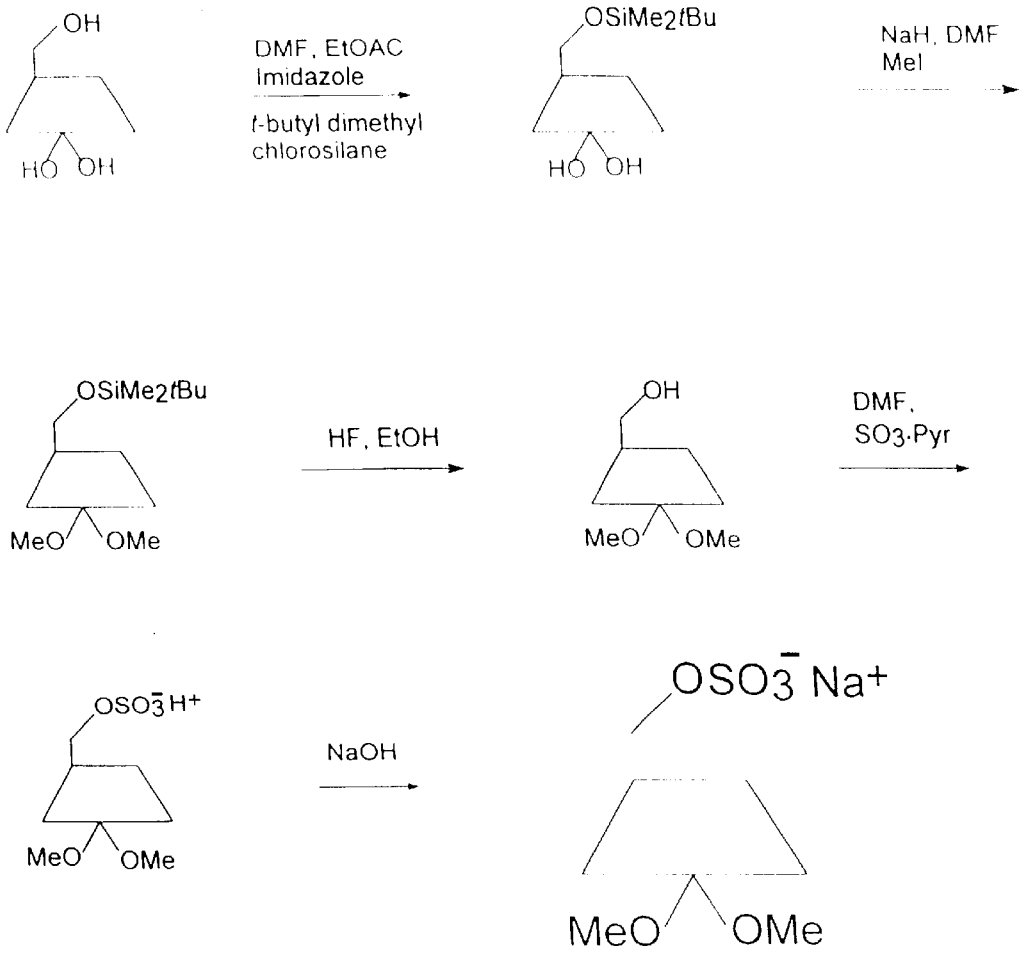

OH
HO OH
DMF, EtOAC
Imidazole
t-butyl dimethyl
chlorosilane
OSiMe2tBu
HO OH
NaH, DMF
MeI
OSiMe2tBu
MeO OMe
HF, EtOH
OH
MeO OMe
DMF,
SO3·Pyr
OSO3 H+
MeO OMe
NaOH
OSO3 Na+
MeO OMe

Figure 13

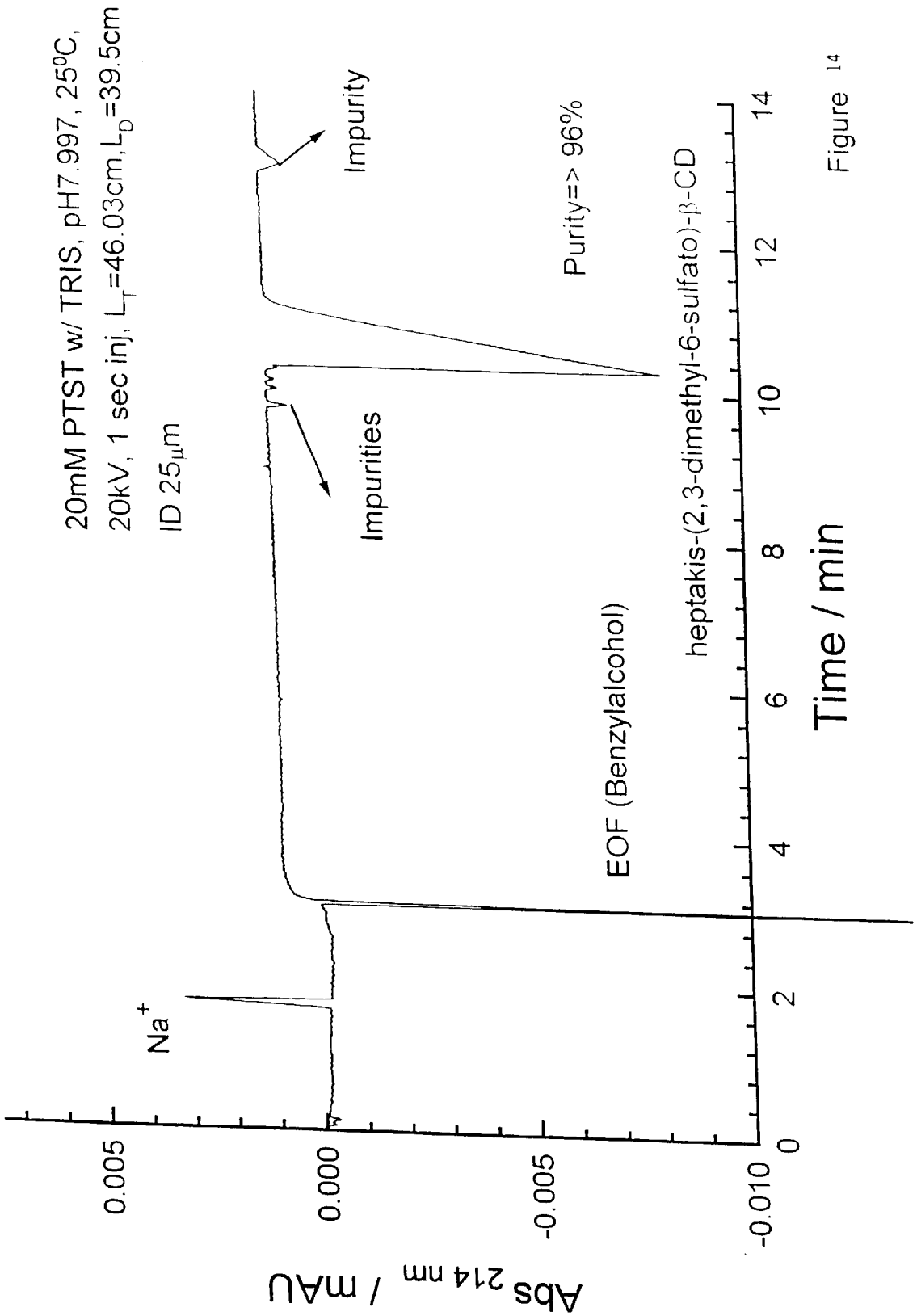

20mM PTST w/ TRIS, pH7.997, 25°C,
20kV, 1 sec inj, L_T=46.03cm, L_D=39.5cm
ID 25μm
Na^+
Impurities
EOF (Benzylalcohol)
heptakis-(2,3-dimethyl-6-sulfato)-β-CD
Purity=> 96%
Impurity
Abs 214 nm / mAU
Time / min
0.005
0.000
-0.005
-0.010
0
2
4
6
8
10
12
14

Figure 14

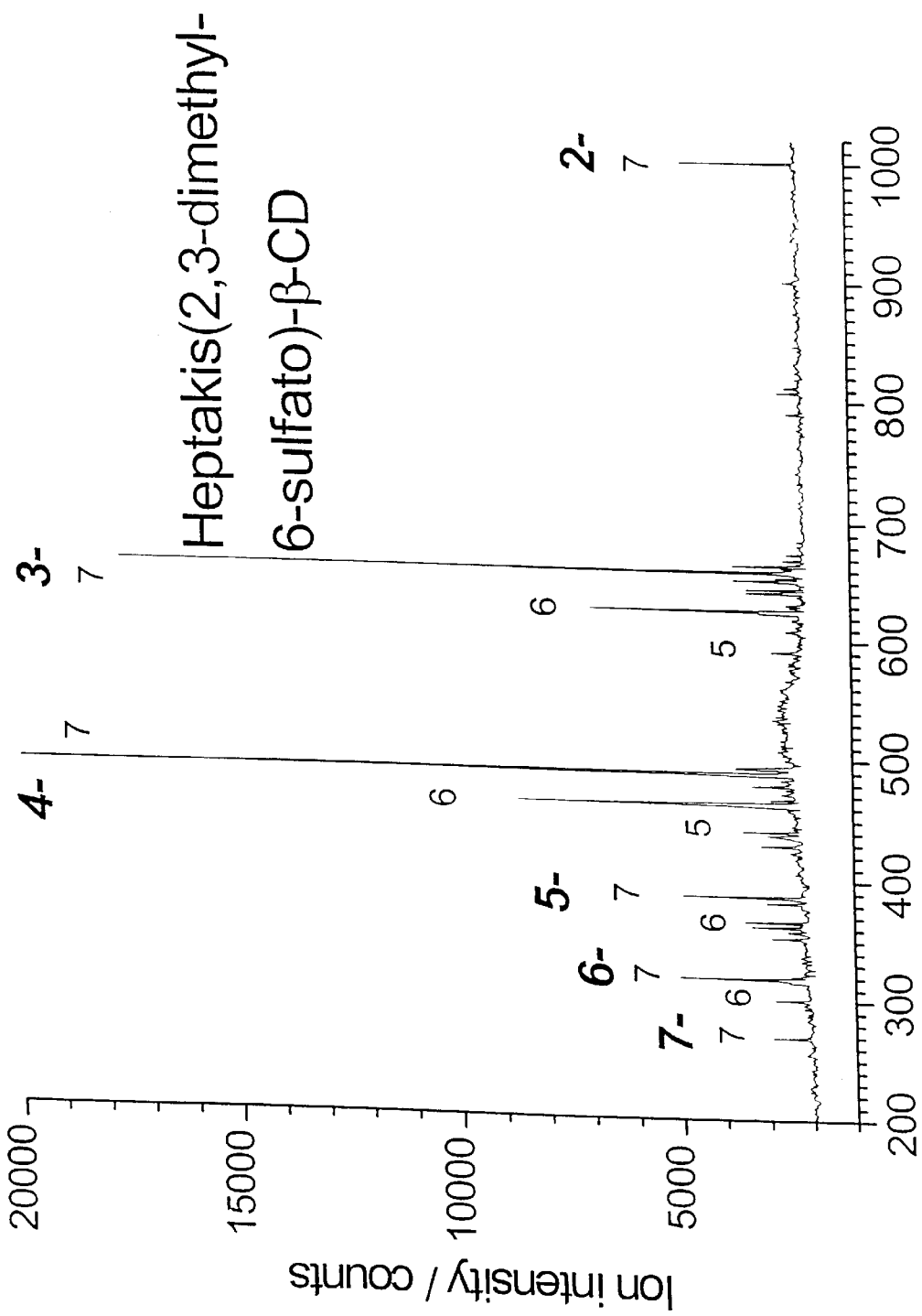

Heptakis(2,3-dimethyl-
6-sulfato)-β-CD
4-
3-
2-
5-
6-
7-
Ion intensity / counts
Mass (m/z)
20000
15000
10000
5000
200
300
400
500
600
700
800
900
1000

Figure 15

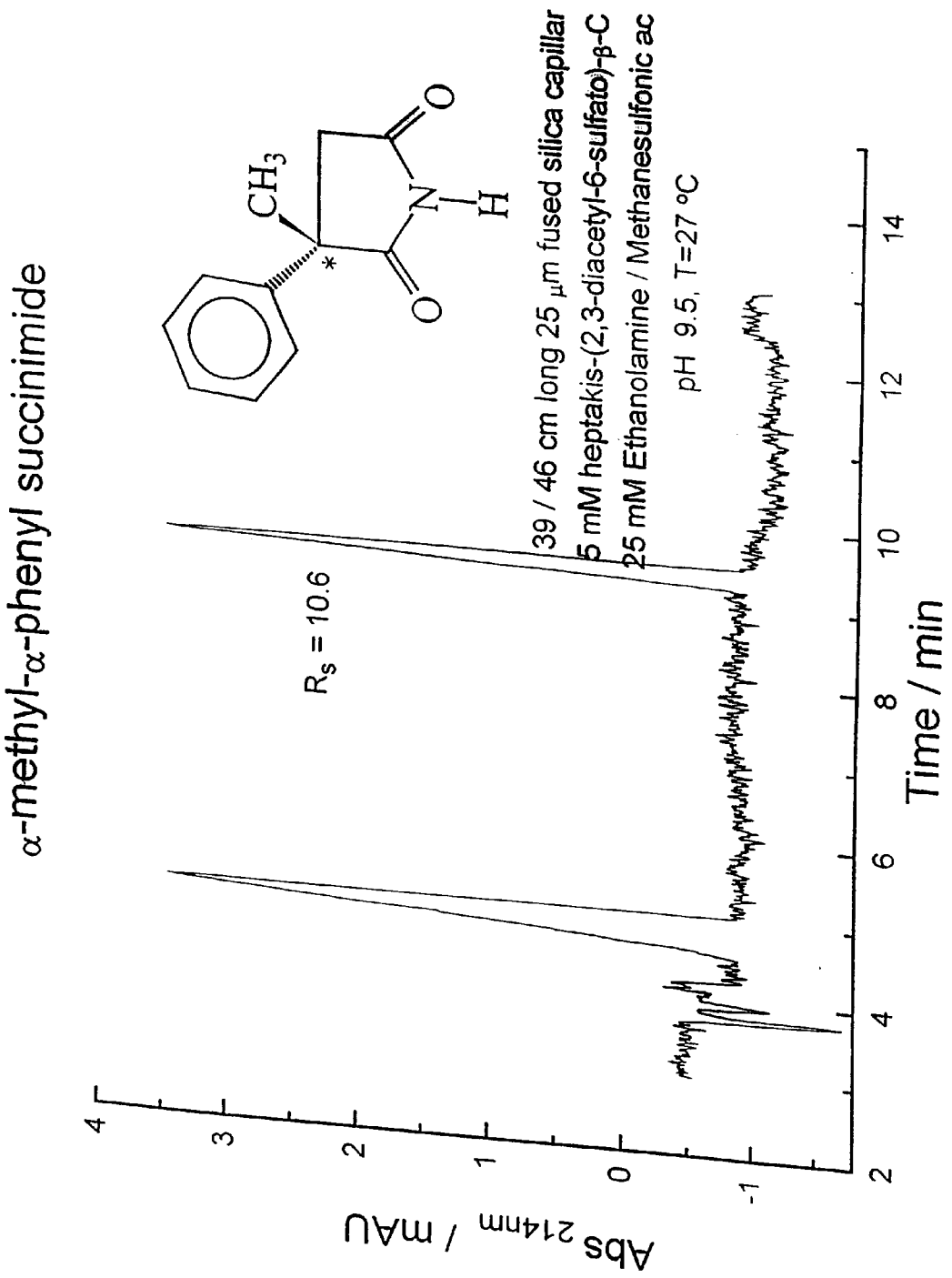

α-methyl-α-phenyl succinimide
39 / 46 cm long 25 μm fused silica capillar
5 mM heptakis-(2,3-diacetyl-6-sulfato)-β-C
25 mM Ethanolamine / Methanesulfonic ac
pH 9.5, T=27 °C
R_s = 10.6
Abs 214nm / mAU
Time / min

Figure 16

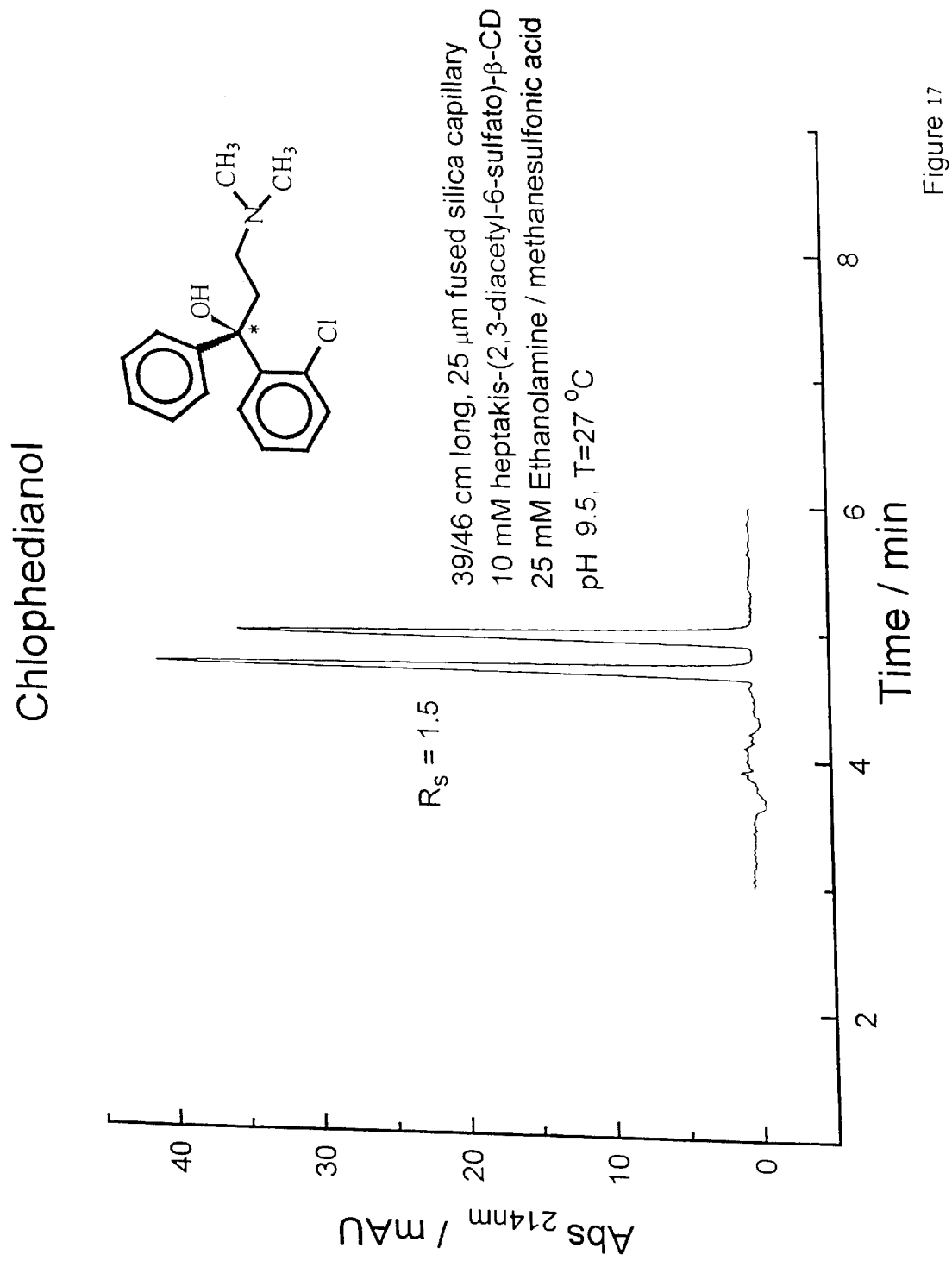

Chlophedianol
OH
CH3
CH3
N
Cl
39/46 cm long, 25 μm fused silica capillary
10 mM heptakis-(2,3-diacetyl-6-sulfato)-β-CD
25 mM Ethanolamine / methanesulfonic acid
pH 9.5, T=27 °C
Rs = 1.5
Abs 214nm / mAU
0
10
20
30
40
2
4
6
8
Time / min

Figure 17

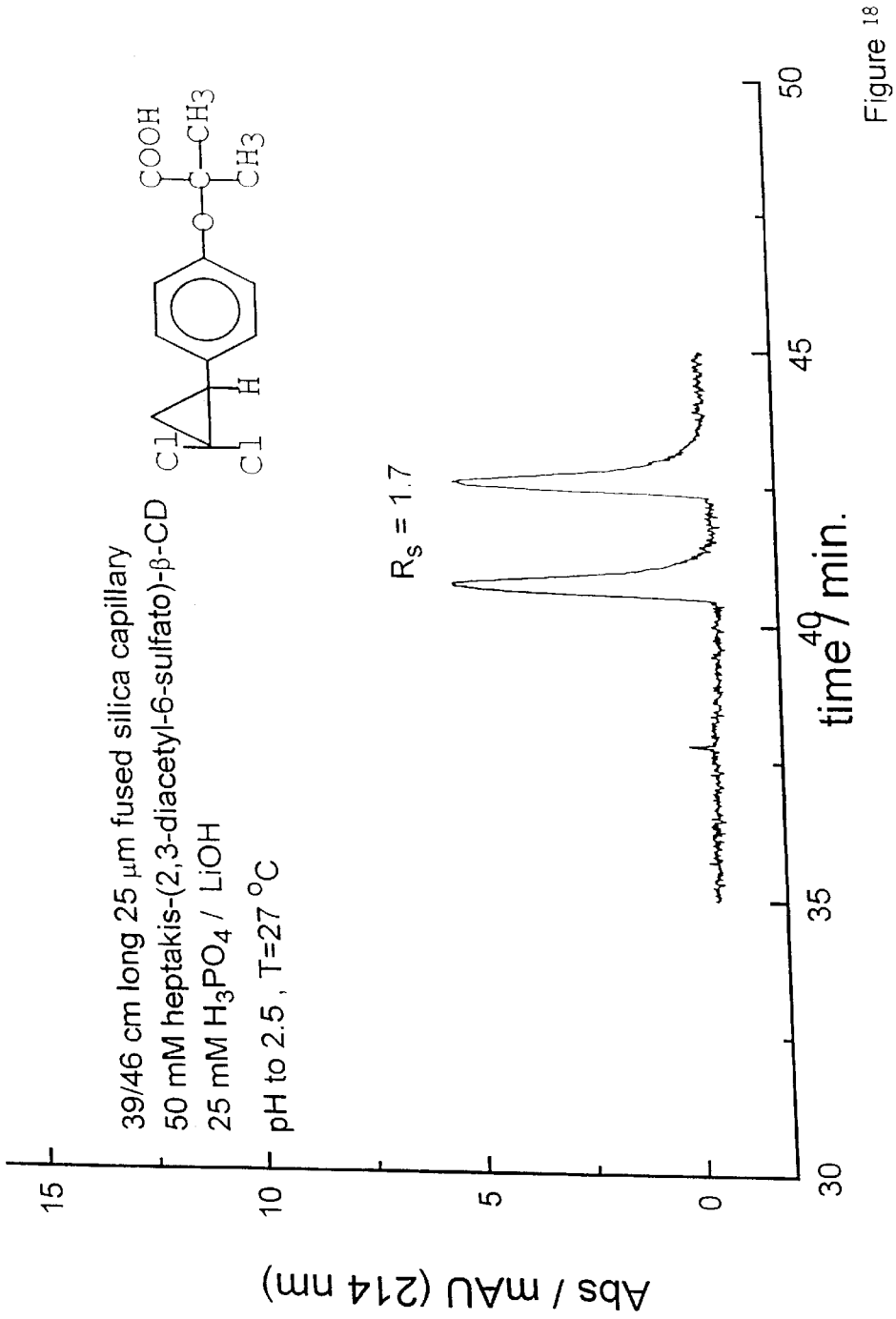

Ciprofibrate
39/46 cm long 25 µm fused silica capillary
50 mM heptakis-(2,3-diacetyl-6-sulfato)-β-CD
25 mM H_3PO_4 / LiOH
pH to 2.5 , T=27 °C
COOH
CH_3
CH_3
Cl
Cl
H
R_s = 1.7
Abs / mAU (214 nm)
15
10
5
0
30
35
40
45
50
time / min.

Figure 18

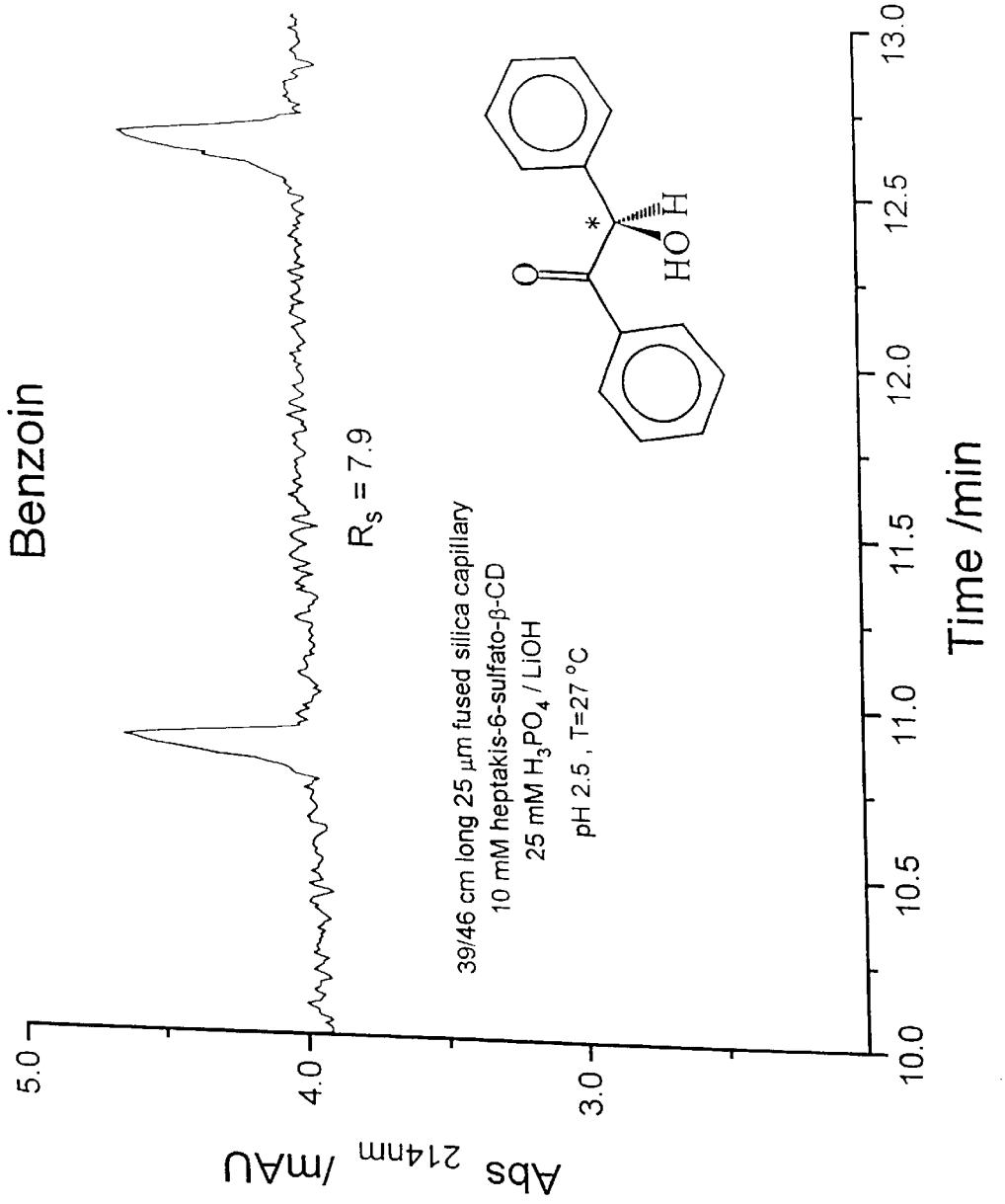

Benzoin
R_s = 7.9
39/46 cm long 25 µm fused silica capillary
10 mM heptakis-6-sulfato-β-CD
25 mM H_3PO_4 / LiOH
pH 2.5 , T=27 °C
Abs 214nm /mAU
5.0
4.0
3.0
10.0
10.5
11.0
11.5
12.0
12.5
13.0
Time /min

Figure 19

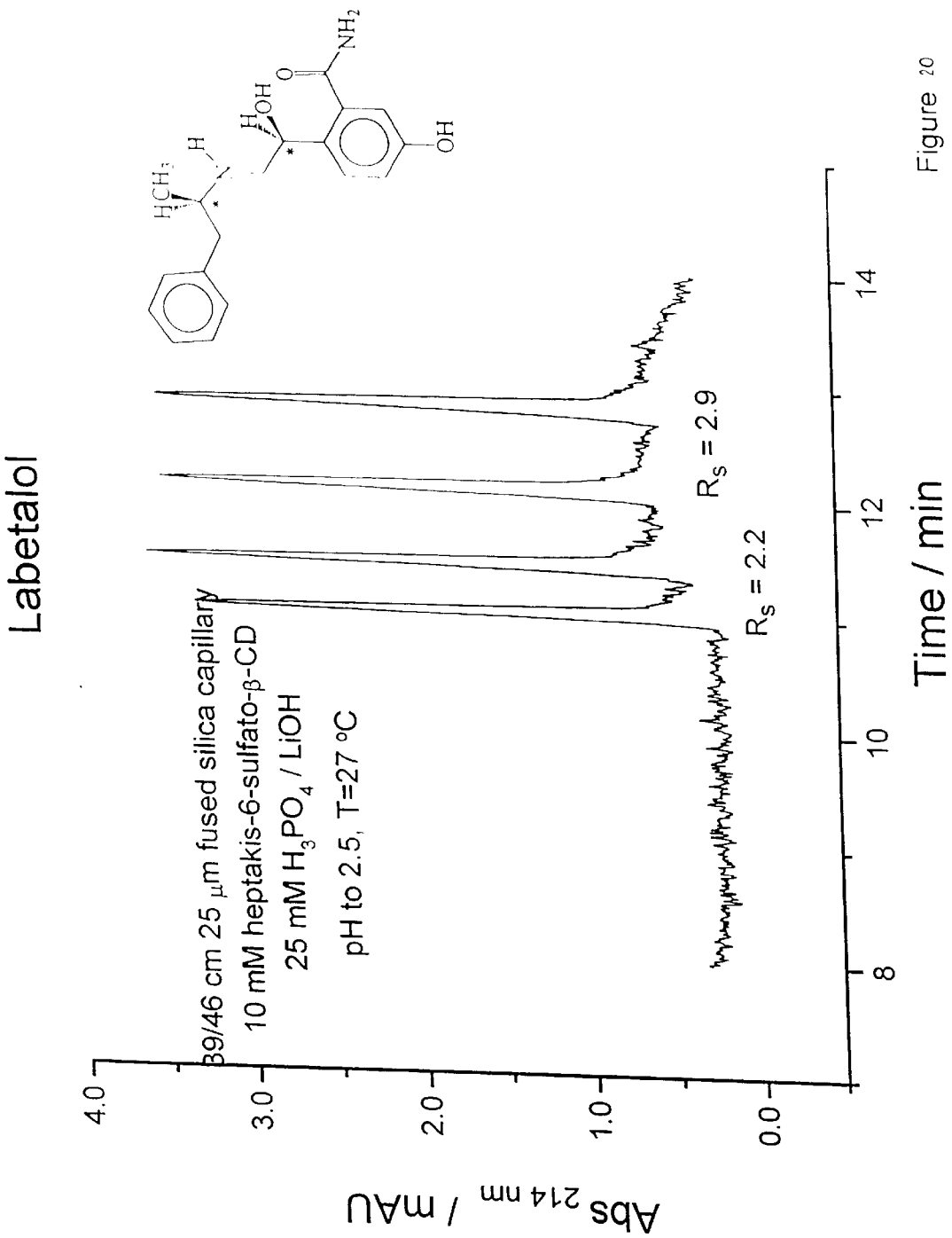

Labetalol
B9/46 cm 25 μm fused silica capillary
10 mM heptakis-6-sulfato-β-CD
25 mM H_3PO_4 / LiOH
pH to 2.5, T=27 °C
R_s = 2.2
R_s = 2.9
Abs 214 nm / mAU
Time / min
4.0
3.0
2.0
1.0
0.0
8
10
12
14
OH
O
NH_2
H
CH_3

Figure 20

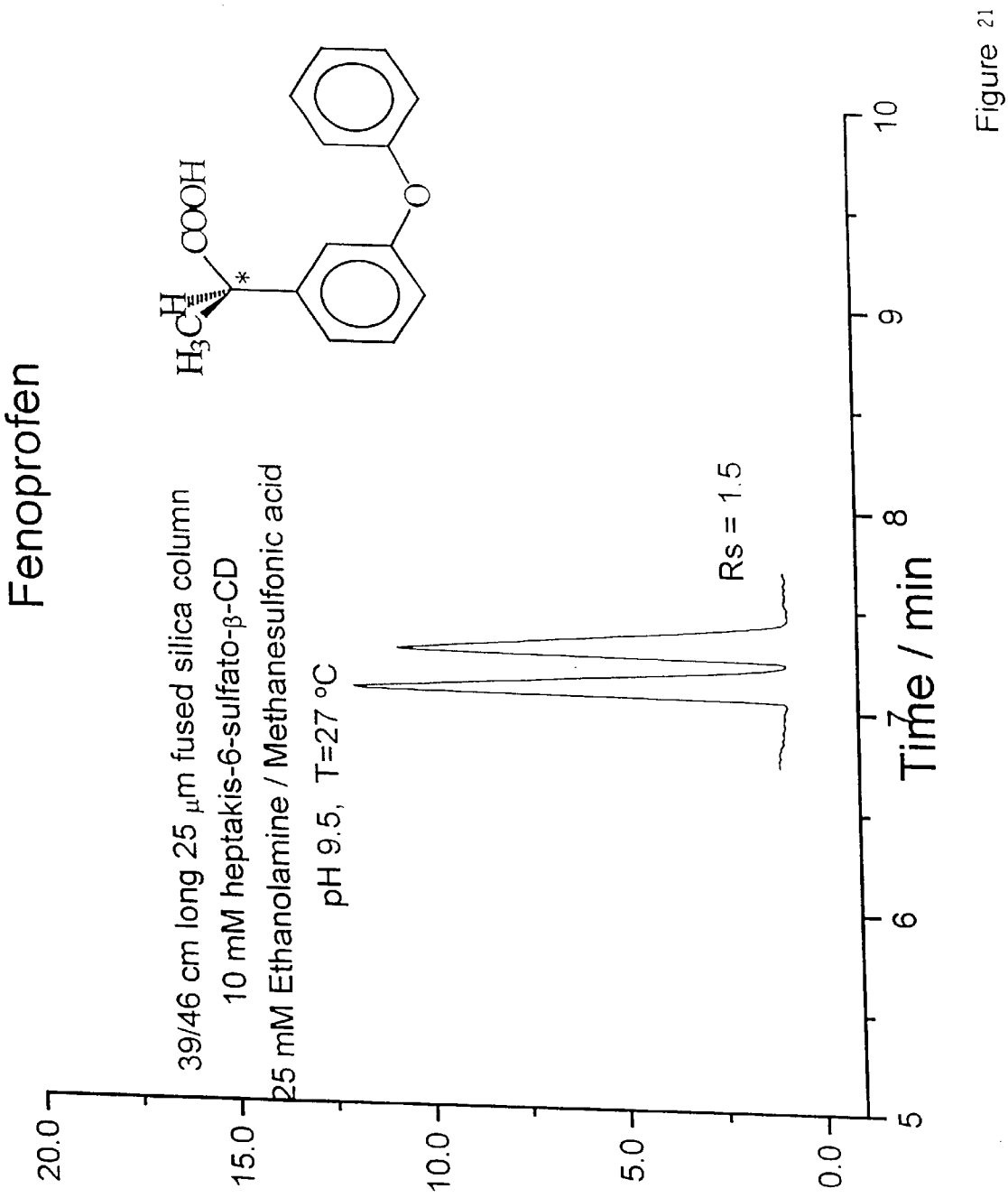

Fenoprofen
39/46 cm long 25 μm fused silica column
10 mM heptakis-6-sulfato-β-CD
25 mM Ethanolamine / Methanesulfonic acid
pH 9.5, T=27 °C
Rs = 1.5
Time / min
20.0
15.0
10.0
5.0
0.0
5
6
7
8
9
10
COOH
H3C
H
*
O

Figure 21

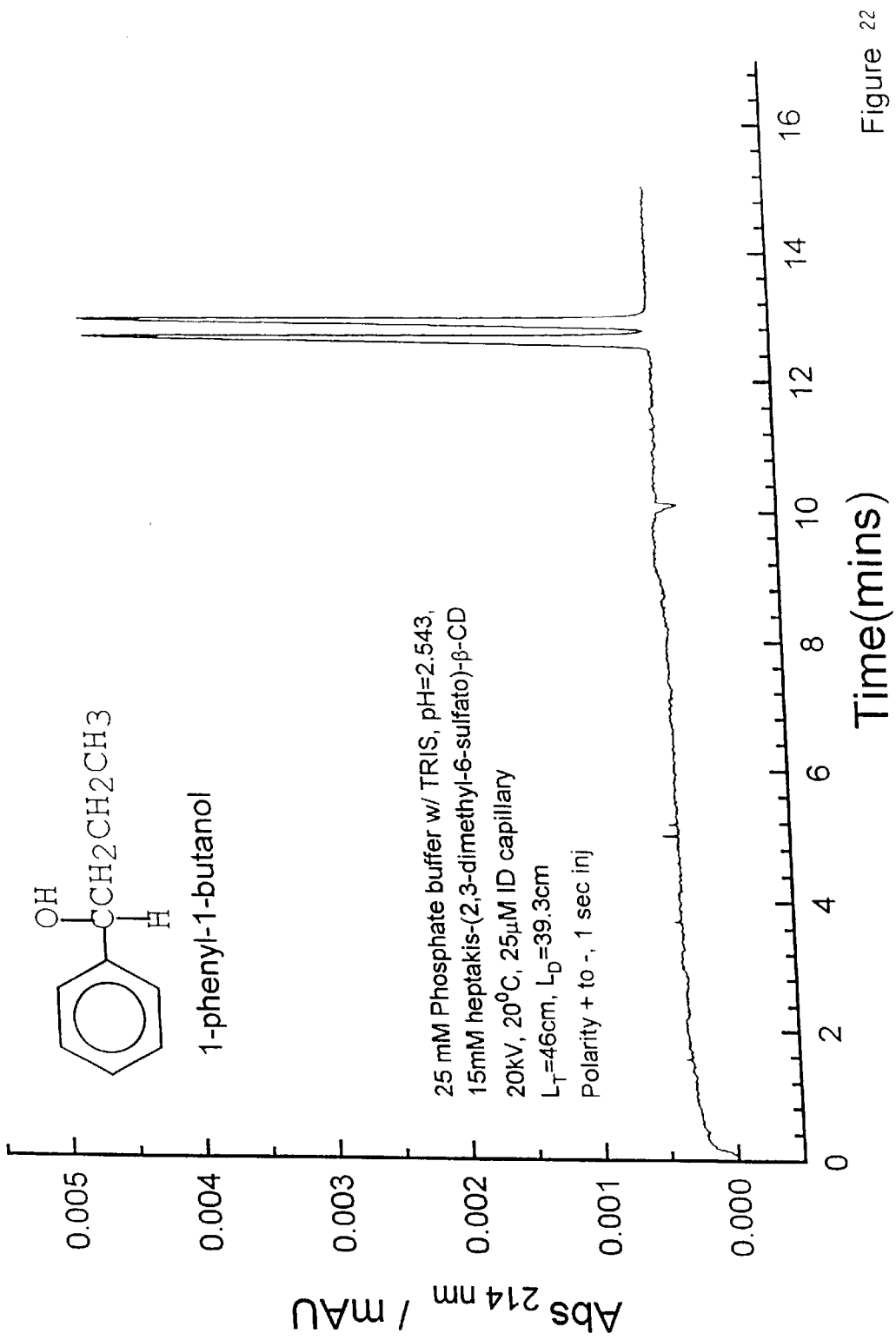

OH
CH2CH2CH3
H
1-phenyl-1-butanol
25 mM Phosphate buffer w/ TRIS, pH=2.543,
15mM heptakis-(2,3-dimethyl-6-sulfato)-β-CD
20kV, 20°C, 25μM ID capillary
L_T=46cm, L_D=39.3cm
Polarity + to -, 1 sec inj
Abs 214 nm / mAU
0.005
0.004
0.003
0.002
0.001
0.000
0
2
4
6
8
10
12
14
16
Time(mins)

Figure 22

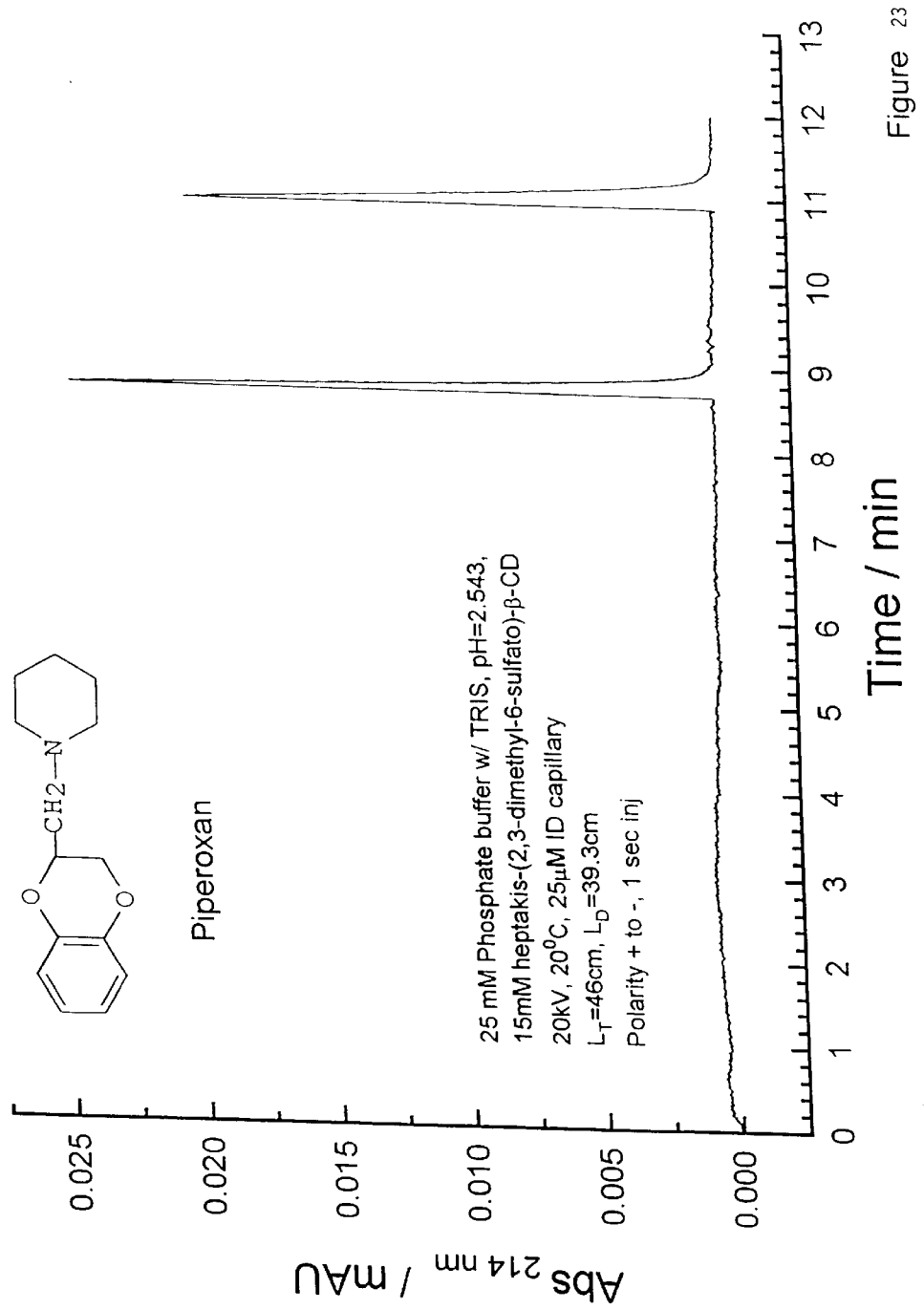

Piperoxan
25 mM Phosphate buffer w/ TRIS, pH=2.543,
15mM heptakis-(2,3-dimethyl-6-sulfato)-β-CD
20kV, 20°C, 25μM ID capillary
L_T=46cm, L_D=39.3cm
Polarity + to -, 1 sec inj
Abs 214 nm / mAU
0.025
0.020
0.015
0.010
0.005
0.000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Time / min

Figure 23

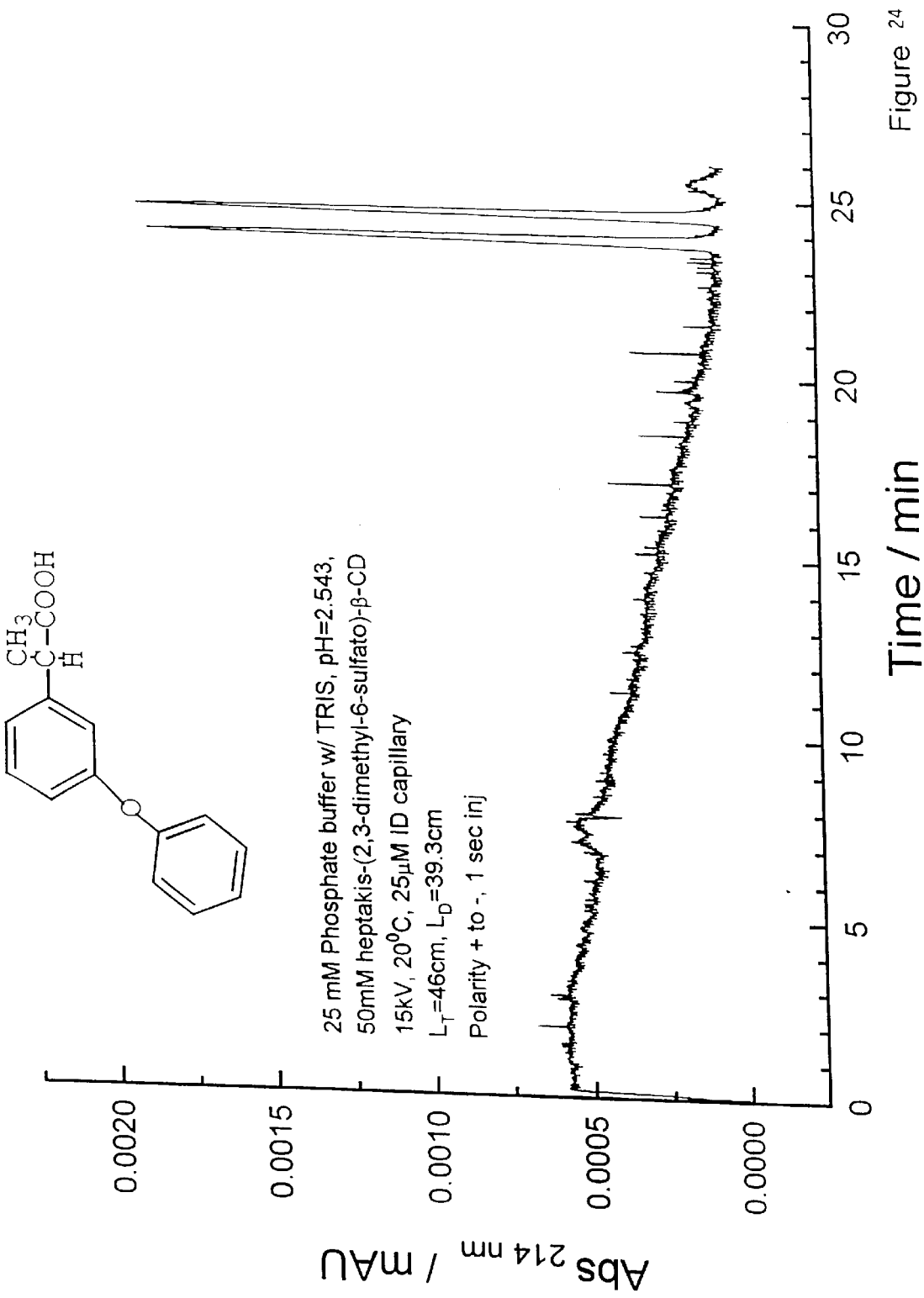

CH3
C
H
COOH
25 mM Phosphate buffer w/ TRIS, pH=2.543,
50mM heptakis-(2,3-dimethyl-6-sulfato)-β-CD
15kV, 20°C, 25μM ID capillary
L_T=46cm, L_D=39.3cm
Polarity + to -, 1 sec inj
Abs 214 nm / mAU
0.0020
0.0015
0.0010
0.0005
0.0000
0
5
10
15
20
25
30
Time / min

Figure 24

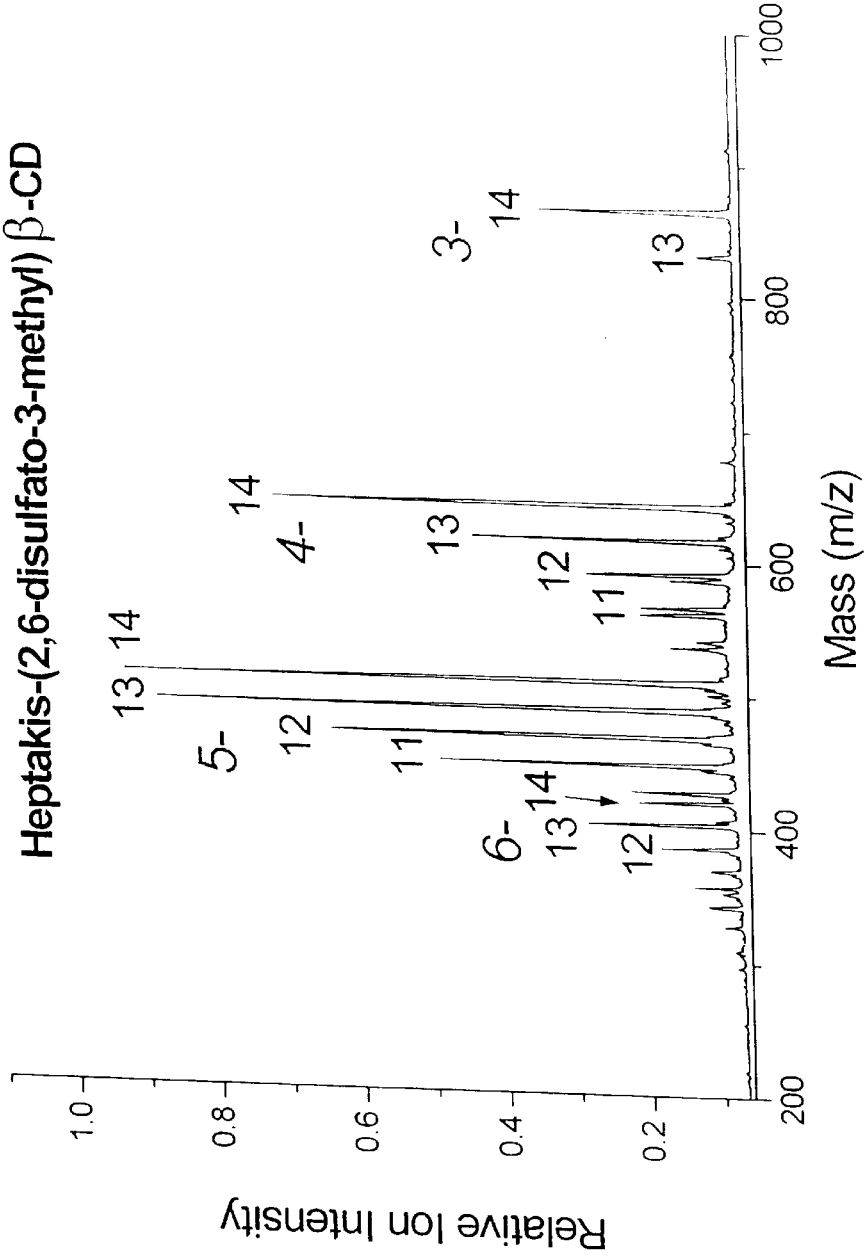

Heptakis-(2,6-disulfato-3-methyl) β-CD
Relative Ion Intensity
Mass (m/z)
1.0
0.8
0.6
0.4
0.2
200
400
600
800
1000
5-
6-
4-
3-
11
12
13
14

Figure 25

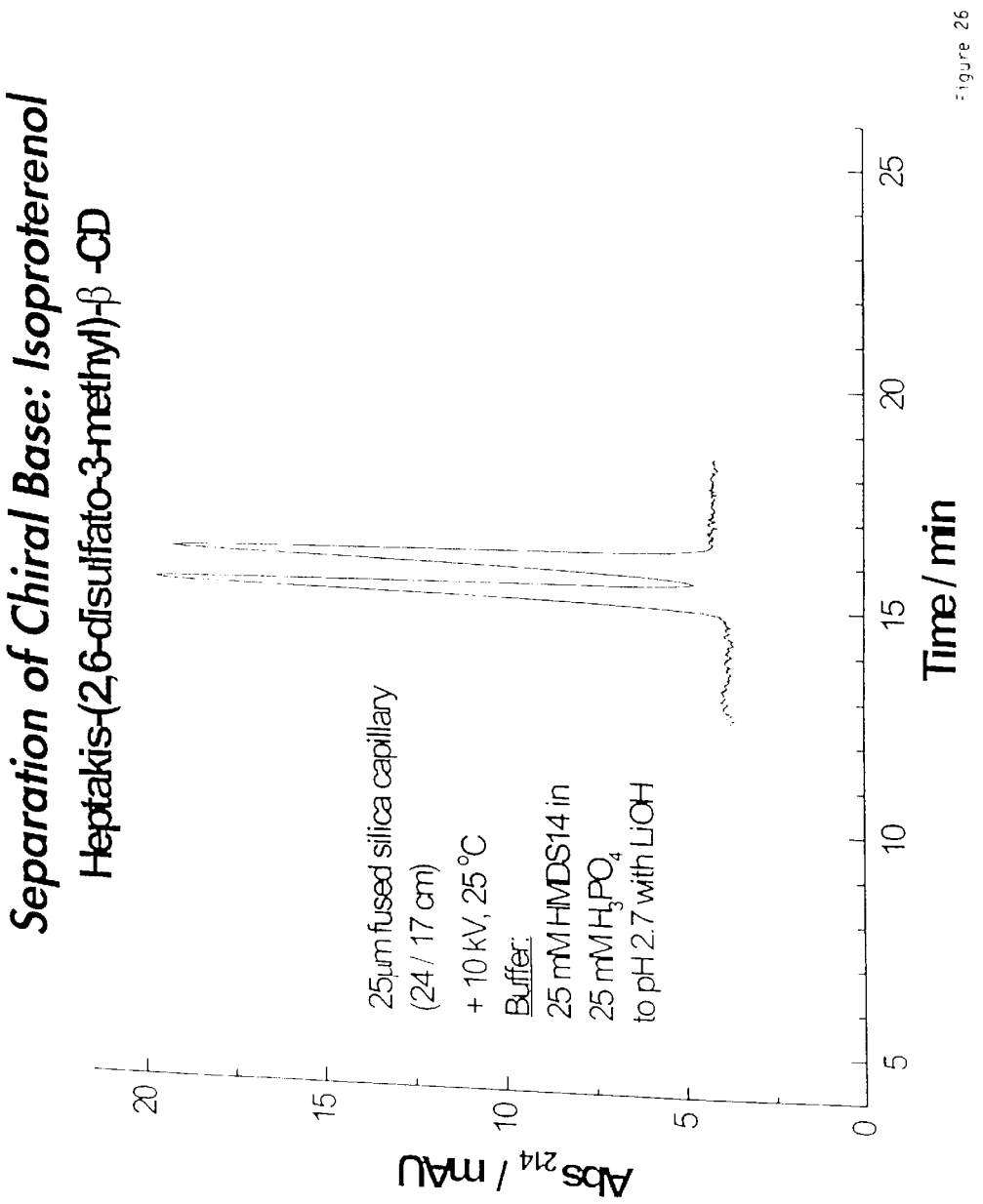

Separation of Chiral Base: Isoproterenol
Heptakis-(2,6-disulfato-3-methyl)-β -CD
25µm fused silica capillary
(24 / 17 cm)
+ 10 kV, 25 °C
Buffer:
25 mM HMDS14 in
25 mM H_3PO_4
to pH 2.7 with LiOH
Abs_{214} / mAU
0
5
10
15
20
Time / min
5
10
15
20
25

Figure 26

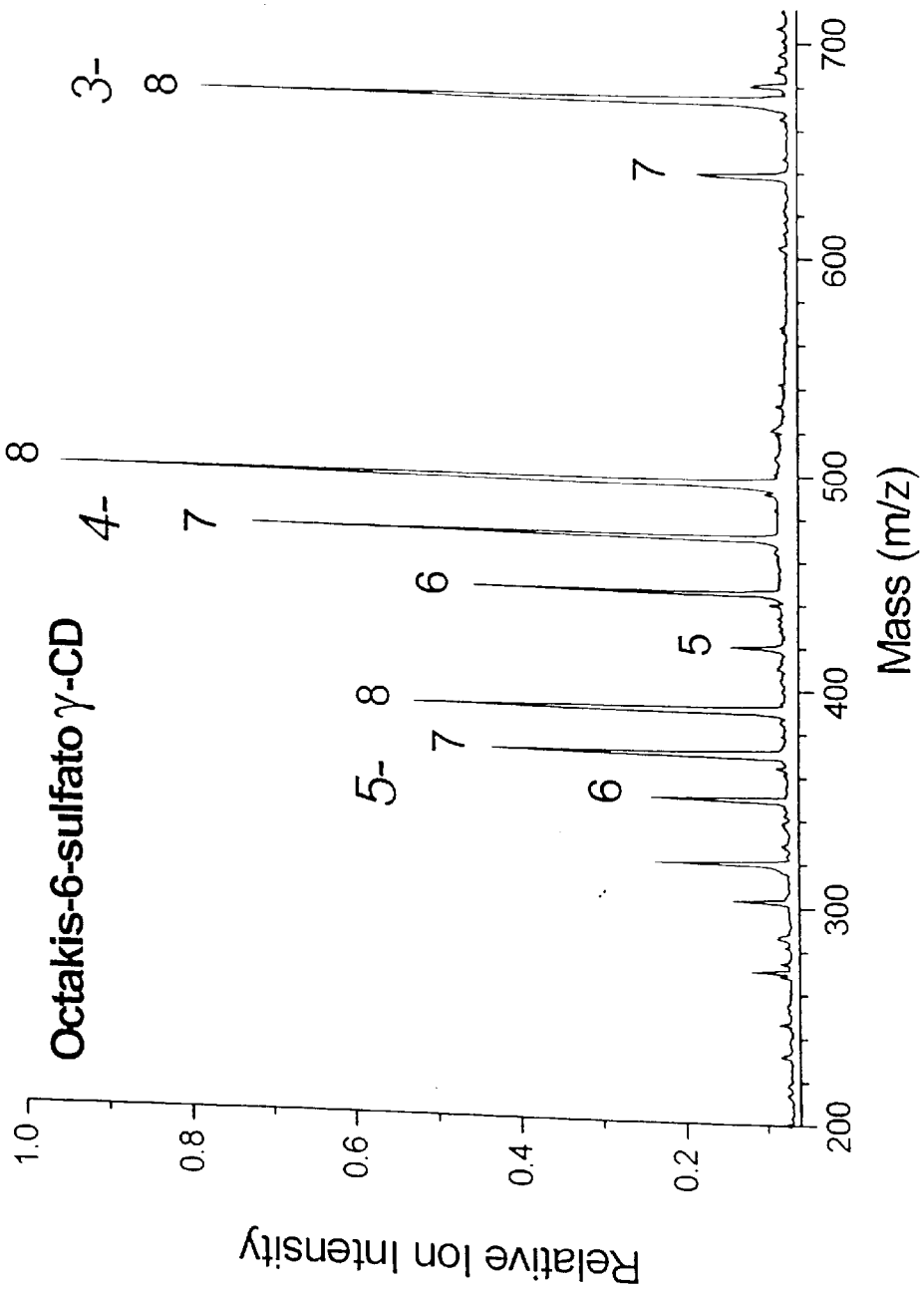

Octakis-6-sulfato γ-CD
Relative Ion Intensity
Mass (m/z)
1.0
0.8
0.6
0.4
0.2
200
300
400
500
600
700
8
7
6
5
4-
5-
3-

Figure 27

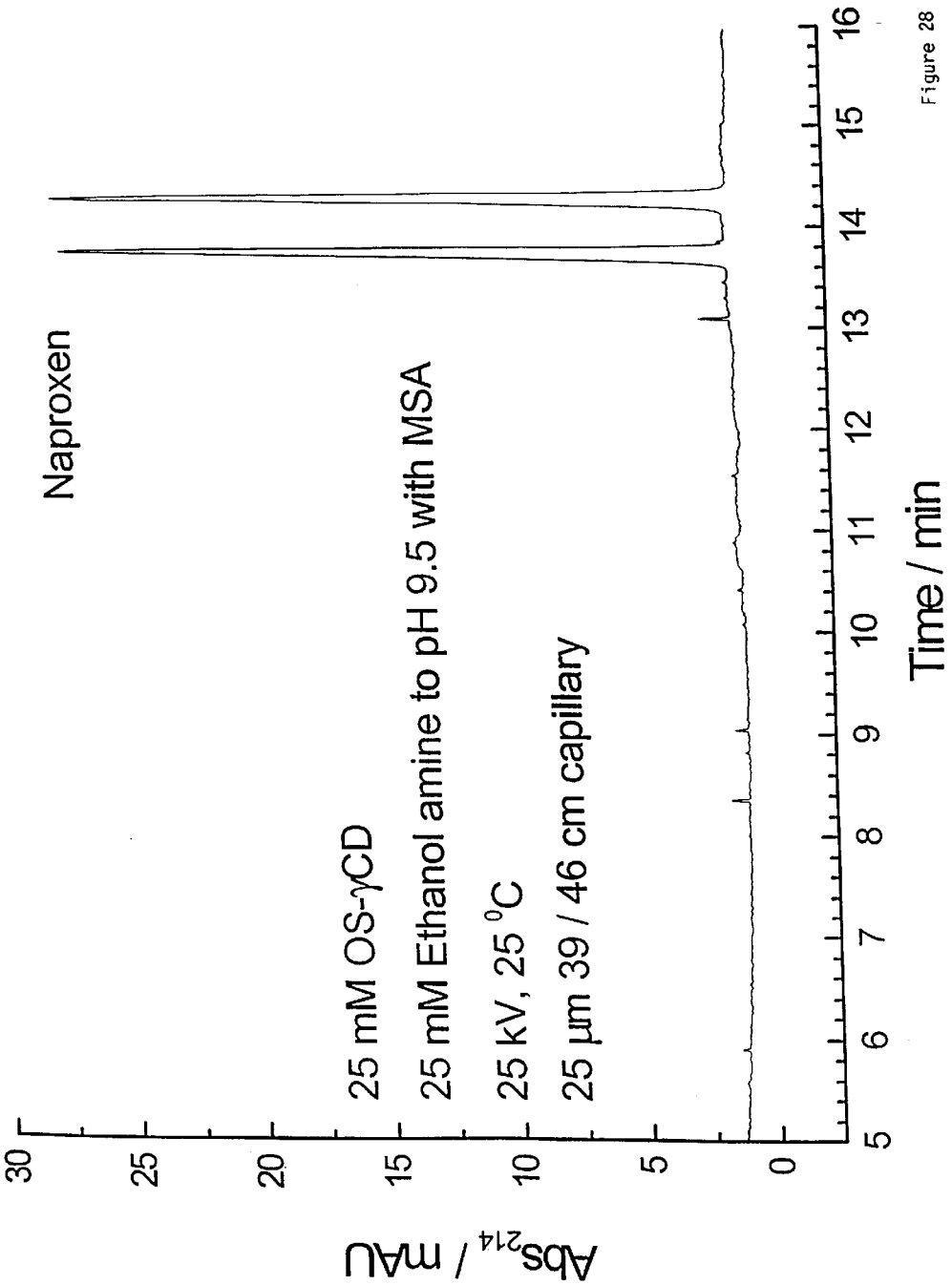

Naproxen
25 mM OS-γCD
25 mM Ethanol amine to pH 9.5 with MSA
25 kV, 25 °C
25 µm 39 / 46 cm capillary
Abs214 / mAU
Time / min
30
25
20
15
10
5
0
5
6
7
8
9
10
11
12
13
14
15
16

Figure 28

CHIRAL RESOLVING AGENTS FOR ENANTIOSEPARATIONS

This application claims priority under 35 U.S.C. §119(e) from U.S. Provisional Application No. 60/063,998, filed Oct. 14, 1997.

FIELD OF THE INVENTION

The present invention relates to single-isomer chiral resolving agents for separation stereoisomers and, more particularly, to functionalized single-isomer charged cyclodextrins for separations of stereoisomers.

BACKGROUND OF THE INVENTION

The separation of stereoisomers (e.g., enantiomers) is generally considered to be one of the more difficult tasks in analytical chemistry since chiral compounds exhibit identical physical properties in non-chiral environments. As a result, conventional separation techniques such as gas chromatography (GC), high pressure liquid chromatography (HPLC) and capillary electrophoresis (CE) have been modified to provide a chiral environment to facilitate enantiomer separation.

One such approach in providing a chiral environment has been through the use of chiral resolving agents, such as cyclodextrins. In fact, electrophoresis has become established as a powerful method for the separation of enantiomers (St. Claire, R. L., *Anal. Chem.* 1996, 68, 569R) due in part to the versatility of various cyclodextrins as resolving agents in both acidic and alkaline background electrolytes (BE). Although significant improvements in enantiomeric separations have been achieved with native as well as derivatized neutral cyclodextrins, as recently reviewed in Fanali, S., *J. Chromatogr. A*, 1996, 735, 77, the analysis of noncharged enantiomers only became possible when charged cyclodextrins entered the stage (Terabe, *S., Trends Anal. Chem.* 1989, 8, 129).

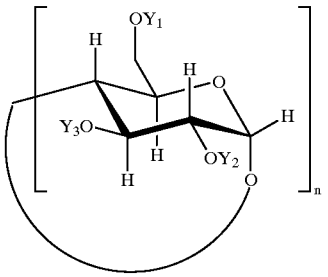
However, charged cyclodextrins used so far are complex mixtures that contain a large number of isomers differing both in their degree of substitution (the number of charges per cyclodextrin molecule) and the loci of substitution. As a result, the use of these resolving agent mixtures is fraught with at least four distinct problems in any given separation. First, the number and loci of substituents on the cyclodextrin greatly effect the chiral selectivity of the system, in which the direction and magnitude of these changes cannot be predicted a priori (Weseloh, et al., *J. Microcolumn Sep.* 1995, 7, 355). As a result, when mixtures of different isomers of substituted cyclodextrins are used, the overall separation selectivity of the system can be reduced or eliminated (Weseloch, et al., *J. Microcolumn Sep.* 1995, 7, 355; Szeman, et al., *J. Chromatogr. A* 1996, 728, 423; Stalcup, et al., *Anal. Chem.* 1995, 67, 19). Mixtures of charged cyclodextrins also present the problem of kinetic band broadening when the finite complexation rates of the different cyclodextrin isomers are slightly different, which unavoidably decreases separation efficiency. Likewise, fundamental molecular level studies through nuclear magnetic resonance (NMR) spectroscopy (Endresz, et al., *J. Chromatogr., A*, 1996, 732, 132) or crystallographic analysis (Harata, et al., *Carbohydr. Research* 1991, 222, 37) or molecular modeling (Lipkowitz, et al., *J. Am. Chem. Soc.*, 1997,114, 15540), which are aimed at improving the level of understanding of the chiral recognition process, are rendered impossible with mixtures of resolving agent. Finally, resolving agent mixtures (commercial or otherwise) often differ between batches and thus compromise the reproducibility of difficult separations.

Accordingly, there is a need in the art for resolving agents that do not exhibit the deficiencies associated with charged cyclodextrin mixtures.

It is, therefore, an object of the present invention to provide alternative resolving agents for use in chiral separations, inter alia, that do not exhibit the deficiencies commonly associated with charged cyclodextrin mixtures.

SUMMARY OF THE INVENTION

The present invention provides a single-isomer cyclodextrin composition of substantially pure cyclodextrin derivatives having the formula:



where "n" is 6–12, and at least one of Y_1, Y_2 and Y_3 is SO_3^-, and where Y_1, Y_2 and Y_3, being other than SO_3^-, are independently hydrogen, a C_1–C_{12} alkyl group, a C_2–C_8 hydroxyalkyl group, a C_2–C_{12} acyl group, an aryl group, a carbamate group, a thiocarbamate group or a combination thereof Preferably, the cyclodextrin composition has an isomeric purity of at least 80 mole %, with an isomeric purity of at least 90 mole % being more preferable, and an isomeric purity of at least 95 mole % being even more preferable.

In one embodiment, a single-isomer cyclodextrin composition is provided with Y_1 being SO_3^-, and Y_2 and Y_3 being preferably H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$. Examples of these single-isomer cyclodextrin derivatives are hepta-6-sulfato-β-cyclodextrin, heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin, heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin, octa-6-sulfato-γ-cyclodextrin, octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin, and octakis-(2,3-dimethyl-6-sulfato)-γ-cyclodextrin.

In another embodiment, a single-isomer cyclodextrin composition is provided with Y_2 being SO_3^-, and Y_1 and Y_3 being preferably H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$. In other embodiments, the present invention provides single-isomer cyclodextrin compositions with: Y_3 being SO_3^- and Y_1 and Y_2 being preferably H, CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; Y_1 and Y_2 being SO_3^- and Y_3 being H, CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; Y_1 and Y_3 being SO_3^- and Y_2 being preferably H, CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; Y_2 and Y_3 being SO_3^- and Y_1 being preferably H, CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$.

The present invention also provides a method for electrophorectically separating stereoisomers of a chiral analyte using the above-described single-isomer cyclodextrin compositions. The chiral analyte is separated into its respective stereoisomers by first introducing into an electrophoretic separation chamber a sample of the chiral analyte and of a background electrolyte containing at least one single-isomer

cyclodextrin composition and thereafter applying an electric potential across the electrophoretic separation chamber thereby inducing differential migration of the stereoisomers of the chiral analyte.

The present invention provides a method for chromatographically separating stereoisomers of a chiral analyte using the above-described single-isomer cyclodextrin compositions. The chiral analyte is separated into its respective stereoisomers by first introducing into a chromatographic separation chamber the chiral analyte, and a mobile phase containing at least one single-isomer cyclodextrin composition and thereafter applying pressure across the separation chamber thereby inducing differential displacement of the stereoisomers of the chiral analyte.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a reaction scheme depicting the synthesis of the single isomer 6-sulfato-cyclodextrin derivatives.

FIG. **2** is a reaction scheme depicting the synthesis of the single isomer 3-sulfato-cyclodextrin derivatives.

FIG. **3** is a reaction scheme depicting the synthesis of the single-isomer 2-sulfato-cyclodextrin derivatives.

FIG. **4** is an alternative reaction scheme depicting the synthesis of the single-isomer 2-sulfato-cyclodextrin derivatives.

FIG. **5** are reaction schemes depicting the synthesis of the single-isomer 2,6-disulfato-cyclodextrin derivatives.

FIG. **6** is a reaction scheme depicting the synthesis of the single-isomer 3,6-disulfato-cyclodextrin derivatives.

FIG. **7** is a reaction scheme depicting the synthesis of the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **8** is an indirect UV-detection electropherogram of the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **9** is an electrospray-ionization mass spectrum of the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **10** is a reaction scheme depicting the synthesis of the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **11** is an indirect UV-detection electropherogram of the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **12** is an electrospray-ionization mass spectrum of the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **13** is a reaction scheme depicting the synthesis of the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **14** is an indirect UV-detection electropherogram of the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **15** is an electrospray-ionization mass spectrum of the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **16** is an electropherogram of racemic α-methyl-α-phenyl-succinimide separated with the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **17** is an electropherogram of racemic chlophedianol separated with the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **18** is an electropherogram of racemic ciprofibrate separated with the single-isomer heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin.

FIG. **19** is an electropherogram of racemic benzoin separated with the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **20** is an electropherogram of racemic labetalol separated with the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **21** is an electropherogram of racemic fenoprofen separated with the single-isomer hepta-6-sulfato-β-cyclodextrin.

FIG. **22** is an electropherogram of racemic 1-phenyl-1-butanol separated with the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **23** is an electropherogram of racemic piperoxan separated with the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **24** is an electropherogram of racemic fenoprofen separated with the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

FIG. **25** is an electrospray-ionization mass spectrum of the single-isomer heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin.

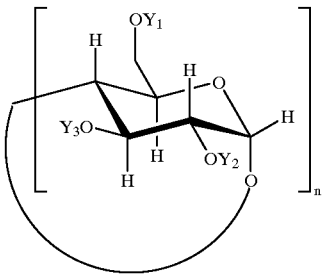
FIG. **26** is an electropherogram of racemic isoproterenol separated with the single-isomer heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin.

FIG. **27** is an electrospray-ionization mass spectrum of the single-isomer octakis-6-sulfato-γ-cyclodextrin.

FIG. **28** is an electropherogram of racemic naproxen separated with the single-isomer octakis-6-sulfato-γ-cyclodextrin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a new class of single-isomer charged cyclodextrin derivatives, which are particularly useful as chiral resolving agents in the separation of stereoisomers, and more particulary enantiomers. The single-isomer charged cyclodextrin derivatives of the present invention are substantially pure cyclodextrin derivatives having the formula:



where n is a single integer ranging from 6–12 and at least one of the substituents Y_1, Y_2 or Y_3 is a SO_3^-, and Y_1, Y_2 and Y_3, which are other than SO_3^-, are hydrogen, a C_1–C_{12} alkyl group, a C_2–C_8 hydroxyalkyl group, a C_1–C_{12} alkylnitryl group, a C_2–C_{12} acyl group, an aryl group, a carbamate group, a thiocarbamate group or a combination thereof. Advantageously, the functionalized cyclodextrin derivatives of the present invention due to their isomeric purity provide enantiomeric separations, e.g., using electrophoresis, with a consistency and resolution previously not achievable.

In the context of the present invention "substantially pure" means that the majority (i.e., greater than 50 mole %) of the cyclodextrin derivatives present in a given sample are in the form of the target single isomer. Preferably, the cyclodextrin derivatives have an isomeric purity of at least 80 mole %, with at least 90 mole % being more preferred, and at least 95 mole % being even more preferred.

Cyclodextrins to be used in accordance with the present invention are cyclodextrins in which "n", the number of D-(+)-glucopyranose units, is a single integer ranging from 6 to 12. Preferably, the cyclodextrins have a single "n" value from 6 to 9, which are commonly referred to α-, β-, γ-, and δ-cyclodextrins. Sources of commercially available cyclodextrins include Cerestar (Hammond, Ind.), Wacker GmbH (Munich, Germany), and Cyclolabs (Budapest, Hungary).

In accordance with the present invention, at least one of the substituents Y_1, Y_2, or Y_3 of the glucopyranose unit is a sulfato group, which provides the cyclodextrin derivatives with an ionic charge. Preferably, Y_1 is the sulfato group with the remaining non-sulfato Y_2 or Y_3 substituents being hydrogen, a C_1–C_{12} alkyl group, a C_2–C_8 hydroxyalkyl group, a C_1–C_{12} alkylnitryl group, a C_2–C_{12} acyl group, an aryl group, a carbamate group, a thiocarbamate group or a mixture thereof. However, if desired, Y_2 and Y_3 can also be sulfato groups to further increase the ionic charge and/or the binding selectivities of the cyclodextrin derivatives. As will be apparent to those skilled in the art following the teachings of the present invention, the Y substituents can be altered to vary the hydrophobicity or hydrophilicity of the substantially pure single-isomer cyclodextrin derivatives.

The alkyl substituents for Y_1, Y_2, or Y_3 preferably have one to eight carbon atoms, with one to five carbon atoms being preferred. The alkyl chain can be linear, branched, cyclic, saturated, or unsaturated. Examples of alky substituents include, but are not limited to, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl or cyclohexyl groups.

The hydroxyalkyl substituents preferably have two to six carbon atoms, with two to four carbon atoms being preferred. The alkyl chain can be linear, branched, cyclic, saturated, or unsaturated. Examples of hydroxyalkyl substituents include, but are not limited to, hydroxyethyl, hydroxypropyl, hydroxybutyl or cyclohexanol groups.

The alkylnitryl substituents preferably have an alkyl chain of one to eight carbon atoms, with one to 5 being more preferred. The alkyl chain can be linear, branched, cyclic, saturated, or unsaturated. Examples of alkylnitryl substituents include, but are not limited to, acetonitryl (i.e., methylcyano), ethylcyano or propylcyano groups.

The acyl substituents preferably have a carbon chain of one to eight carbon atoms, with one to six carbon atoms being more preferred. The carbon chain can be linear, branched, cyclic, saturated, unsaturated or aromatic. Examples of acyl substituents to be used include, but are not limited to, acetyl, propionyl, butyryl, pivaloyl, caproyl or benzoyl groups.

The aryl substituents preferably are monocyclic or bicyclic aromatics, optionally substituted with non-polar alkyl or polar groups. Examples of aryl substituents to be used include, but are not limited to, benzyl or napthenyl groups.

The carbamate and thiocarbamate substituents preferably have alkyl, aryl and substituted aryl groups. Examples of carbamate and thiocarbamate substituents to be used include, but are not limited to, methylcarbamate, ethylcarbamate, methylthiocarbamate, ethylthiocarbamate, phenylcarbamate, phenylthiocarbamate, 3,5-dimethylphenylcarbamate or 3,5-dimethylphenylthiocarbamate.

In one embodiment, the present invention provides substantially pure cyclodextrin derivatives with Y_1 (i.e., 6-position) being a sulfato group and Y_2 and Y_3 (i.e., 2- and 3-positions, respectively) preferably being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. In a more preferred embodiment, Y_2 and Y_3 of the 6-sulfato-cyclodextrin derivatives are the same substituents, thus providing substantially pure per-substituted 6-sulfato-cyclodextrin, 2,3-dimethyl-6-sulfato-cyclodextrin, 2,3-diacetyl-6-sulfato-cyclodextrin, 2,3-diacetonitryl-6-sulfato-cyclodextrin and 2,3-di-(2-hydroxypropyl)-6-sulfato-cyclodextrin. The reaction scheme for synthesizing the 6-sulfato-cyclodextrin derivatives is shown in FIG. **1**. For example, to synthesize 2,3-diacetyl-6-sulfato-cyclodextrin, the cyclodextrin starting material is first silylated with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is then purified by preparative HPLC. The pure intermediate is allowed to react with acetic anhydride using 4-N,N-dimethylamino pyridine as a catalyst, and pyridine as a base. The crude intermediate is purified by preparative HPLC. The pure acetylated, silylated intermediate is desilylated with borontrifluoride etherate and the crude intermediate is purified by preparative HPLC. The pure intermediate is then reacted with sulfur trioxide.pyridine, then with sodium hydroxide, to obtain the sodium salt of the single-isomer 2,3-diacetyl-6-sulfato-cyclodextrin.

In another embodiment, the present invention provides substantially pure cyclodextrin derivatives with Y_3 being a sulfato group and Y_1 and optionally Y_2 preferably being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. In a more preferred embodiment, Y_1 and Y_2 of the 3-sulfato-cyclodextrin derivatives are same substituent, thus providing substantially pure per-substituted 3-sulfato-cyclodextrin, 2,6-dimethyl-3-sulfato-cyclodextrin, 2,6-diacetyl-3-sulfato-cyclodextrin, 2,6-diacetonitryl-3-sulfato-cyclodextrin and 2,6-di-(2-hydroxypropyl)-3-sulfato-cyclodextrin. The reaction schemes for synthesizing 3-sulfato-cyclodextrin and 6-methyl-3-sulfato-cyclodextrin, among others, are shown in FIG. **2**. For example, to synthesize 3-sulfato-cyclodextrin, the cyclodextrin starting material is first silylated with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The 2,6-disilylated product is purified by preparative HPLC. The pure intermediate is reacted with sulfur trioxide.pyridine, then with hydrogen fluoride to remove the silyl protecting groups. The desilylated intermediate is reacted with sodium hydroxide to obtain the sodium salt of the 3-sulfato-cyclodextrin. Likewise, to synthesize 6-methyl-3-sulfato-cyclodextrin, the cyclodextrin starting material is first silylated with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The 6-silylated product is purified by preparative HPLC. The pure intermediate is reacted with methyliodide, with sodium hydride as base, to obtain the methylated and silylated crude intermediate. After purification by preparative HPLC, the intermediate is reacted with sulfur trioxide.pyridine, then with hydrogen fluoride to remove the silyl protecting groups. The desilylated intermediate is reacted with sodium hydroxide to obtain the sodium salt of the 6-methyl-3-sulfato-cyclodextrin.

The present invention also provides substantially pure cyclodextrin derivatives with Y_2 being a sulfato group, and preferably Y_1 being hydrogen and Y_3 being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. Examples of these 2-sulfato-cyclodextrin derivatives are 2-sulfato-cyclodextrin, 3-methyl-2-sulfato-cyclodextrin, 3-acetyl-2-sulfato-cyclodextrin, 3-acetonitryl-2-sulfato-cyclodextrin and 3-(2-hydroxypropyl)-2-sulfato-cyclodextrin. The reaction scheme for synthesizing these particular single-isomer cyclodextrin derivatives is shown in FIG. **3**. For example, to synthesize 3-acetyl-2-sulfato-cyclodextrin, the cyclodextrin starting material is first silylated with t-butyldimethylsilyl chloride using 4-N,N-

dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is purified by preparative HPLC. The pure intermediate is further reacted with triethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is purified by preparative HPLC. The pure intermediate then is reacted with acetic anhydride using 4-N,N-dimethylamino pyridine as a catalyst, and pyridine as a base. The crude intermediate is purified by preparative HPLC. The triethylsilyl group is removed from the pure acetylated, silylated intermediate with acetic acid and the new intermediate is purified by preparative HPLC. The purified intermediate is reacted with sulfur trioxide.pyridine, then with HF and sodium hydroxide, to obtain the sodium salt of the single-isomer 3-acetyl-2-sulfato-cyclodextrin.

The present invention also provides substantially pure cyclodextrin derivatives with Y_2 being a sulfato group, and Y_1 and Y_3 preferably being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. Examples of the per-substituted 2-sulfato-cyclodextrin derivatives are 2-sulfato-cyclodextrin, 3,6-dimethyl-2-sulfato-cyclodextrin, 3,6-diacetyl-2-sulfato-cyclodextrin, 3,6-diacetonitryl-2-sulfato-cyclodextrin and 3,6-di(2-hydroxypropyl)-2-sulfato-cyclodextrin. The reaction scheme for synthesizing these particular single-isomer cyclodextrin derivatives is shown in FIG. **4**. For example, to synthesize 3,6-dimethyl-2-sulfato-cyclodextrin, the cyclodextrin starting material is first silylated with triethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is purified by preparative HPLC. The pure intermediate is reacted with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is purified by preparative HPLC. The pure intermediate is then reacted with acetic acid to remove the triethylsilyl protecting groups and the crude intermediate is again purified. The pure 2-silylated intermediate is reacted with methyliodide, with sodium hydride as a base, yielding the crude methylated silylated intermediate which is purified by preparative HPLC. After removal of the t-butyldimethylsilyl protecting groups with HF, and repeated purification by preparative HPLC, the pure final intermediate is reacted with sulfur trioxide pyridine, then with sodium hydroxide, to obtain the sodium salt of the 3,6-dimethyl-2-sulfato-cyclodextrin.

As described above, more than one of the Y substituents can be a sulfato group. One such embodiment is substantially pure cyclodextrin derivatives with Y_1 and Y_2 being sulfato groups, and Y_3 preferably being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. Examples of these cyclodextrin derivatives are 2,6-disulfato-cyclodextrin, 3-methyl-2,6-disulfato-cyclodextrin, 3-acetyl-2,6-disulfato-cyclodextrin, 3-acetonitryl-2,6-disulfato-cyclodextrin and 3-(2-hydroxypropyl)-2,6-sulfato-cyclodextrin. The reaction scheme for synthesizing these particular single-isomer cyclodextrin derivatives is shown in FIG. **5**. For example, to synthesize 3-methyl-2,6-disulfato cyclodextrin, the cyclodextrin starting material is first silylated with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base. The silylated product is purified by preparative HPLC. The pure intermediate is then reacted with methyliodide, with sodium hydride as base, yielding the crude methylated silylated intermediate which is purified by preparative HPLC. After removal of the t-butyldimethylsilyl protecting groups with HF, and repeated purification, the pure final intermediate is further reacted with sulfur trioxide.pyridine, then with sodium hydroxide, to obtain the sodium salt of the 3-methyl-2,6-disulfato cyclodextrin.

The present invention also provides substantially pure cyclodextrin derivatives with Y_1 and Y_3 being sulfato groups, and Y_2 preferably being either hydrogen, a methyl, an acetyl, an acetonitryl or a 2-hydroxypropyl group. Examples of these cyclodextrin derivatives are 3,6-disulfato-cyclodextrin, 2-methyl-3,6-disulfato-cyclodextrin, 2-acetyl-3,6-disulfato-cyclodextrin, 2-acetonitryl-3,6-disulfato-cyclodextrin and 2-(2-hydroxypropyl)-3,6-sulfato-cyclodextrin. The reaction scheme for synthesizing these particular single-isomer cyclodextrin derivatives is shown in FIG. **6**. For example, to synthesize 3,6-disulfato cyclodextrin, the cyclodextrin starting material is first silylated with triethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as base. The silylated product is then purified with preparative HPLC. The pure intermediate is further reacted with t-butyldimethylsilyl chloride using 4-N,N-dimethylamino pyridine as a catalyst, and imidazole as a base, and purified by HPLC. The pure intermediate then is reacted with acetic acid to remove the triethylsilyl protecting groups and the crude intermediate again is purified. The pure 2-silylated intermediate is reacted with sulfur trioxide.pyridine, then with HF, then with sodium hydroxide, to obtain the sodium salt of 3,6-disulfato-cyclodextrin.

The single-isomer charged cyclodextrin derivatives of the present invention are particularly useful as chiral resolving agents for the separation of enantiomers via conventional separation techniques such as high pressure liquid chromatography and electrophoresis. As will be apparent to those skilled in the art, the single-isomer cyclodextrin derivatives of the present invention are also useful for other applications, in which cyclodextrins are typically used.

Enantiomers to be separated using the cyclodextrin derivatives of the present invention include neutral, basic, acidic and zwitterionic analytes. Protocols for using cyclodextrins as resolving agents in electrophoresis or high pressure liquid chromatography are well known in the art. For example, according to the charged resolving agent migration model (Williams et al. *J. Chromatogr. A*. 776 (1997) 295), in capillary electrophoresis, the charged single-isomer cyclodextrin derivative is added to both a low pH (e.g., pH 2.2) or a high pH (e.g., pH 9.5) buffer to obtain background electrolytes (BE). The electrode vials and the separation chamber of the electrophoretic system are then filled with the background electrolyte. The sample containing the enantiomers to be separated is dissolved in the background electrolytes, and an aliquot of this solution is injected into the separation chamber. The separation potential is applied to move the analytes past the detector of the electrophoretic unit. Due to the differential interactions of the enantioners with the single-isomer charged cyclodextrins which alter their electrophoretic mobilities, the enantiomers arrive at the detector at different times, thus resulting in their separation.

The following non-limiting examples illustrate the synthesis of the single-isomer charged cyclodextrin derivatives of the present invention and their use as resolving agents in electrophoresis applications.

EXAMPLES

Example 1

Synthesis of Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin

Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin was prepared following the reaction scheme shown in FIG. **7**. All chemicals for the synthesis were obtained from Aldrich Chemical Company (Milwaukee, Wis., USA), except 13-cyclodextrin, which was obtained from Cerestar

(Hammond, Ind., USA). Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin was produced by first reacting β-cyclodextrin with t-butyldimethylsilyl chloride as described in Takeo et al., *Carbohydr. Res.* 1989, 187, 203, which is incorporated herein by reference. Briefly, 79 mmol imidazole and 7.9 mmol 4-N,N-dimethylamino pyridine were added to warm N,N-dimethylformamide, followed by 8.8 mmol dried β-cyclodextrin. 66 mmol t-butyldimethylsilyl chloride was dissolved in ethyl acetate and dropped into the β-cyclodextrin solution. Once the reaction was complete, excess t-butyldimethylsilyl chloride was quenched by the addition of methanol, the reaction mixture was poured into an HCl solution to protonate the excess imidazole and precipitate the silylated cyclodextrin. The crude product, dissolved in hexanes, was purified by preparative gradient elution column chromatography (HPLC) using the equipment set forth in Vigh et al., *J. Chromatogr.* 1989, 484, 237, incorporated herein by reference, a 50 mm I.D., 300 mm long preparative HPLC column packed with 30 nm pore size, 10 μm irregular silica (Merck, Darmstadt, Germany), and hexane:ethylacetate:ethanol as eluent as described in Takeo et al. The purified intermediate was then peracetylated with acetic anhydride as described in Takeo et al. Specifically, to 200 mL of dry pyridine was added 0.5 mmol 4-N,N-dimethylamino pyridine, 5 mmol silylated cyclodextrin intermediate and 17 mL acetic anhydride. The reaction mixture was heated for 20 hours at 90° C., then quenched in water; the crude precipitate was collected and again was purified by gradient elution preparative column chromatography on silica gel using hexane: ethylacetate: ethanol as eluent as previously described.

The purified heptakis-(2,3-diacetyl-6-t-butylsilyl-dimethyl)-β-cyclodextrin was then reacted with boron trifluoride etherate as described in Takeo et al. to remove the dimethyl-t-butylsilyl protecting groups. Specifically, 4 mmol of the acetylated silylated intermediate was added to 225 mL dry dichloromethane and 10 mL boron trifluoride etherate. After 3 hours, the reaction mixture was quenched with water, neutralized with sodium carbonate, the organic phase was repeatedly washed with water and the organic phase was evaporated to dryness. The crude product was repurified by gradient elution preparative column chromatography on silica gel using hexane:ethylacetate:ethanol as eluent, as described above. Finally, the purified heptakis-(2,3-diacetyl)-β-cyclodextrin was reacted with SO_3·pyridine in DMF as described in U.S. Pat. No. 4,020,160 to Bernstein et al., incorporated herein by reference, to completely sulfate the primary hydroxyl groups of the cyclodextrin. Specifically, to 200 mL dry N,N-dimethylformamide, was added 5 mL dry pyridine and 6 mmol purified heptakis-(2,3-diacetyl)-cyclodextrin, followed by 10 g SO_3·pyridine. Progress of the reaction was monitored by indirect UV-detection capillary electrophoresis as set forth in Nardi et al, *Electrophoresis* 1990, 11, 774, incorporated herein by reference, using a 20 mM p-toluenesulfonic acid background electrolyte, whose pH was adjusted to 8 with tris (hydroxymethyl)aminomethane.

Once complete, the reaction mixture was poured into acetone, the semi-solid material was filtered out and redissolved in water. The aqueous solution was neutralized with NaOH and the material was reprecipitated with ethanol. The solid material was filtered out, redissolved in water and the remaining sodium sulfate was removed by repeated addition of ethanol. Finally, the end product was dried in a vacuum oven at 80° C. to afford pure heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. The purity of the material was checked by indirect UV detection capillary electrophoresis as described above and calculated to be greater than 90 mole %. The electropherogram of the heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin sample is shown in FIG. **8**.

Electrospray ionization mass spectra were also obtained with a Vestec model 201-A single quadrupole mass spectrometer, which was equipped with the Vestec electrospray ion source (PerSeptive Biosystems, Vestec Products, Framingham, Mass.). Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin was dissolved in 75 25% (v/v) mixture of methanol water at a concentration of 4 mg/mL. The apparent pH of the sample solution, measured with a combination glass electrode calibrated with aqueous reference buffers, was 6.6. The heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin solution was introduced by a model 341B SAGE syringe pump (Orion Research, Boston, Mass.) via a Valco injector with a 1 μL internal loop (Valco, Houston, Tex.). The solution was loaded into the loop of the sampling valve and introduced into the ion source at a flow rate of 1.6 μL/min. The ion source block and the spray chamber were maintained at 250° C. and 50° C., respectively. Voltages on the needle, nozzle and collimator of the electrospray ion source were set at −2.4 kV, −0.2 kV and −10 V, respectively. The skimmer potential was −10 V generating a skimmer-collimator voltage bias value of 0 V. The mass range was limited to m/z 1100 and an average of five scans were taken. The electrospray-ionization mass spectrum is shown in FIG. **9**.

Example 2

Synthesis of Heptakis-6-sulfato-β-cyclodextrin

Substantially pure hepta-6-sulfato-β-cyclodextrin was prepared following the reaction scheme shown in FIG. **10**. Briefly, heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin prepared as in Example 1 was deacetylated by dissolving 4 mmol heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin in 125 mL 50° C. water, adding 6 mL of 10 M aqueous NaOH to the solution, then adding, slowly, 5 mL methanol and stirring the mixture for 12 hours. Once indirect UV detection CE indicated that the reaction was complete following the procedure of Example 1, the reaction mixture was poured into ethanol, the solid was collected by filtration, washed with ethanol and dried in a vacuum oven at 80° C. yielding the end product, heptakis-6-sulfato-β-cyclodextrin. The purity of the final product was determined by indirect UV detection CE using 20 mM p-toluenesulfonic acid (PTSA) as a background electrolyte (BE), whose pH was adjusted to 8 with tris(hydroxymethyl)aminomethane (TRIS), and calculated to be greater than 95 mole %. The electropherogram of the heptakis-6-sulfato-β-cyclodextrin sample is shown in FIG. **11**. An electrospray-ionization mass spectrum of the final product is shown in FIG. **12**.

Example 3

Synthesis of Heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin

The sodium salt of heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin was prepared following the reaction scheme shown in FIG. **13**. First, heptakis-6-t-butyldimethylsilyl-β-cyclodextrin was obtained by reacting β-cyclodextrin with t-butyldimethylsilyl chloride as described in Example 1. The raw reaction mixture was purified by preparative gradient elution column chromatography using a silica gel column and the n-hexane: ethyl acetate: ethanol eluent system as described in Example 1.

The pure intermediate was methylated with iodomethane in the presence of NaH and purified as described in Takeo et al. Specifically, 220 mL anhydrous tetrahydrofuran and 5 g

NaH were added to a freshly flamed three-neck flask. A mixture of 7 mmol heptakis-6-t-butyldimethylsilyl-β-cyclodextrin, 35 mL methyliodide and 30 mL anhydrous tetrahydrofuran was added, dropwise, to the cool flask, allowed to react overnight, and was finally quenched with ethanol and mixed with butylacetate. The NaI precipitate was filtered off and the solvents were removed on a rotavap. The crude reaction mixture was extensively purified by preparative gradient elution column chromatography using the n-hexane:ethyl acetate:ethanol eluent system as in Example 1. 200 mL ethanol and 12 mmol of this second methylated, silylated cyclodextrin intermediate were added to a polyethylene beaker and reacted with 37 mL HF overnight, as described in Vigh et al., to remove the t-butyldimethylsilyl protecting group. The excess HF was carefully neutralized with $NaHCO_3$ and the inorganic components were removed from the reaction mixture by filtration. After removal of the alcohol-water solvent mixture on a rotavap, the crude reaction mixture containing heptakis-(2,3-dimethyl)-β-cyclodextrin was once again repurified by preparative gradient elution column chromatography using the n-hexane:ethyl acetate:ethanol eluent system as in Example 1.

The pure third intermediate was then sulfated with SO_3·pyridine as described in Example 1, but with N,N'-dimethylformamide as the solvent. The completeness of the sulfation reaction was monitored by indirect UV-detection CE using a 20 mM p-toluenesulfonic acid (PTSA) background electrolyte, whose pH was adjusted to 8 with tris (hydroxymethyl)aminomethane (TRIS).

Once the sulfation reaction was complete, the reaction mixture was poured into a tenfold excess of acetone, the gummy product was separated, redissolved in water and reacted with NaOH to liberate pyridine. The sodium sulfate byproduct was removed by repeated partial precipitation using ethanol. Finally, pure heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin was obtained by pouring the sodium sulfate-free aqueous solution into excess ethanol, collecting the precipitate and carefully drying it in the vacuum oven overnight. An indirect UV-detection electropherogram of the heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin sample is shown in FIG. **14**, in which purity was calculated to be greater than 96 mole %. An electrospray-ionization mass spectrum of the heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin sample is shown in FIG. **15**.

Example 4

Electrophoretic Separations using Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin

Two buffer stock solutions (a low pH buffer (pH 2.5) and a high pH buffer (pH 9.5)) were prepared according to the requirements of the charged resolving agent migration model (CHARM model) of CE enantiomer separations as described in Williams, B. A.; Vigh, Gy. *J. Chromatogr.* 1997, 776, 295, which is herein incorporated by reference. The low pH buffer was prepared by adding 0.0250 mole of concentrated (85% w/w) phosphoric acid to enough deionized water (Milli-Q, Millipore, Milford, Mass., USA) to obtain a solution of about 0.95 L. This solution was titrated to pH=2.5 with a saturated aqueous solution of LiOH using a combination glass electrode and a precision pH meter (both of them from Corning Science Products, Corning, N.Y., USA). Finally, the solution was quantitatively transferred to a 1 L volumetric flask, the volume was brought to mark with deionized water and the pH was remeasured. The high pH buffer was prepared similarly, except that 0.0250 mole of ethanolamine was titrated to pH=9.5 with an aqueous solution of methansulfonic acid, quantitatively transferred to a 1 L volumetric flask, the volume was brought to mark with deionized water and the pH was remeasured.

5, 10, 15, 30 and 50 mM heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-βCD) low pH and high pH background electrolytes (BE) were prepared by weighing out the required amounts of the sodium salt of heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin into 25 mL volumetric flasks and bringing the volumes to mark with the low pH and the high pH buffer stock solutions, respectively. 250 μL samples of three racemic analytes (α-methyl-(α-phenyl-succinimide (a neutral analyte), chlophedianol (a basic analyte), ciprofibrate (an acidic analyte)) were prepared at 0.5 mM concentrations in the P/ACE microvials with the respective HDAS-μCD BEs.

Separations were carried out on a UV detector-equipped P/ACE 2100 CE unit operated under the Gold Ver. 8.01 systems software (Beckman Instruments, Fullerton, Calif., USA). The detection wavelength was set at 214 nm, the cartridge coolant was thermostated at 20° C. The separations were carried out in 25 μm i.d. untreated fused silica capillaries (Polymicro Technologies, Phoenix, Ariz., USA) with a 45 cm total length and a 39 cm injector-to-detector length. The injection pressure was set at 5 p.s.i, the injection time was 1 second. The applied potential was varied between 12 kV and 20 kV in order to maintain a power dissipation of 500 to 700 mW/m.

Electropherograms illustrating the chiral separation of the above enantiomeric analytes are shown in FIGS. **16**, **17** and **18**. FIG. **16** shows the separation of the enantiomers of α-methyl-α-phenyl-succinimide in the high pH BE containing 5mM heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. FIG. **17** shows the separation of the enantiomers of chlophedianol in the high pH BE containing 10 mM heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. FIG. **18** shows the separation of the enantiomers of ciprofibrate in the high pH BE containing 10 mM heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. Apparent from all three electropherograms are the separate and well-defined peaks of the eluted analyte enantiomers, which demonstrate the ability of the functionalized cyclodextrins to alter the mobility of the analyte enantiomers.

Example 5

Electrophoretic Separations using Heptakis-(6-sulfato)-β-cyclodextrin

Following the procedure of Example 4, low pH and high pH BEs were prepared using the heptakis-(6-sulfato)-β-cyclodextrin of Example 2. 250 μL samples of three racemic analytes (benzoin (a neutral analyte), labetalol (a basic analyte), fenoprofen (an acidic analyte)) were prepared at 0.5 mM concentrations in the P/ACE microvials with the respective HS-βCD BEs.

Electropherograms illustrating the separation of the above enantiomers are shown in FIGS. **19**, **20** and **21**. FIG. **19** shows the separation of the enantiomers of benzoin in the low pH BE containing 10 mM hepta-6-sulfato-β-cyclodextrin. FIG. **20** shows the separation of the enantiomers of labetalol in the low pH BE containing 10 mM hepta-6-sulfato-β-cyclodextrin. FIG. **21** shows the separation of the enantiomers of fenoprofen in the high pH BE containing 10 mM hepta-6-sulfato-β-cyclodextrin. As in Example 4, the eluted analyte enantiomers can be seen as separate and well-defined peaks.

Example 6

Electrophoretic Separations using Heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin

Following the procedure of Example 4, low pH and high pH BEs were prepared using heptakis-(2,3-dimethyl-6-

sulfato)-β-cyclodextrin of Example 3. 250 μL samples of three racemic analytes (1-phenyl-1-butanol (a neutral analyte), piperoxan (a basic analyte), fenoprofen (an acidic analyte)) were prepared at 0.5 mM concentrations in the P/ACE microvials with the respective HS-βCD BEs. The electropherograms illustrating the separation of the above enantiomers are shown in FIGS. **22**, **23** and **24**. FIG. **22** shows the separation of the enantiomers of 1-phenyl-1-butanol in the low pH BE containing 15 mM heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin. FIG. **23** shows the separation of the enantiomers of piperoxan in the low pH BE containing 15 mM heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin. FIG. **24** shows the separation of the enantiomers of fenoprofen in the low pH BE containing 50 mM heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin.

Prophetic Example 7

Synthesis of Heptakis-(3,6-diacetyl-2-sulfato)-β-cyclodextrin

Heptakis-(3,6-diacetyl-2-sulfato)-β-cyclodextrin is prepared following the reaction scheme shown in FIG. **4**. Briefly, 79 mmol imidazole and 7.9 mmol 4-N,N-dimethylamino pyridine are added to warm N,N-dimethylformamide, followed by 8.8 mmol dried β-cyclodextrin. 66 mmol triethylsilyl chloride is diluted threefold in ethyl acetate and dropped into the β-cyclodextrin solution. Once the reaction is complete, excess triethylsilyl chloride is destroyed by methanol, the reaction mixture is poured into a fourfold volume of dichloromethane and the resulting solution is poured into a tenfold volume of water. The phases are separated, the aqueous wash is repeated and the crude product is purified by preparative gradient elution column chromatography as in Example 1. 8.8 mmol of the dried, purified intermediate is then dissolved in warm N,N-dimethylformamide which contains up to 50% tetrahydrofuran, 160 mmol imidazole and 16 mmol 4-N,N-dimethylamino pyridine and 140 mmol t-butyldimethylsilyl chloride. Once the silylation reaction is complete, excess t-butyldimethylsilyl chloride is destroyed by methanol, the reaction mixture is poured into a sixfold volume of hexane. The phases are separated and the hexane layer is poured into a tenfold volume of water. The phases are separated, the aqueous wash is repeated and the crude product is purified by preparative gradient elution column chromatography as in Example 1.

The purified heptakis-(2-t-butyldimethylsilyl-6-triethylsilyl)-β-cyclodextrin is then dissolved in dilute acetic acid-containing tetrahydrofuran to remove the triethylsilyl protecting groups. After work-up, the crude product is purified by preparative gradient elution column chromatography as in Example 1. The purified intermediate is then peracetylated with acetic anhydride as described in Example 1. The purified heptakis-(2-t-butyldimethylsilyl-3,6-diacetyl)-β-cyclodextrin is then reacted with boron trifluoride etherate as described in Example 1 to remove the t-butyldimethylsilyl protecting groups. The crude product is repurified by gradient elution preparative column chromatography on silica gel as in Example 1. Finally, the purified heptakis-(3,6-diacetyl)-β-cyclodextrin is reacted with SO_3·pyridine in DMF as in Example 1 to completely sulfate the exposed hydroxyl groups of the cyclodextrin as described in Example 1. Progress of the reaction is monitored and work-up is completed as in Example 1 to obtain pure heptakis-(3,6-diacetyl-2-sulfato)-β-cyclodextrin.

Prophetic Example 8

Synthesis of Hepta-3-sulfato-β-cyclodextrin

Hepta-3-sulfato-β-cyclodextrin is prepared following the reaction scheme shown in FIG. **2**. First, hepta-6-t-butyldimethylsilyl-β-cyclodextrin is prepared and purified as in Example 1. Next, 8.8 mmol of the dried, purified intermediate is then dissolved in warm N,N-dimethylformamide which contains up to 50% tetrahydrofuran, 160 mmol imidazole, 16 mmol 4-N,N-dimethylamino pyridine and 140 mmol t-butyldimethylsilyl chloride. Once the reaction is complete, excess t-butyldimethylsilyl chloride is destroyed by methanol, the reaction mixture is poured into a sixfold volume of hexane. The phases are separated and the hexane layer is poured into a tenfold volume of water. The phases are separated, the aqueous wash is repeated and the crude product is purified by preparative gradient elution column chromatography as in Example 1. The purified heptakis-(2,6-di(t-butyldimethylsilyl))-β-cyclodextrin is then sulfated with SO_3·pyridine as in Example 1. Finally, the t-butyldimethylsilyl protecting groups are removed with aqueous ethanolic HF as in Example 3 to yield pure hepta-3-sulfato-β-cyclodextrin.

Example 9

Synthesis of Heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin

Heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin was prepared following the reaction scheme shown in FIG. **5**. First, heptakis-(2,6-di(t-butyldimethylsilyl))-β-cyclodextrin was prepared and purified as in Example 8. Next, 7 mmol of heptakis-(2,6-di(t-butyldimethylsilyl))-β-cyclodextrin was methylated with NaH and methyliodide as in Example 3. 4 mmol of the purified intermediate was dissolved in tetrahydrofuran, to which 60 mmol tetrabutylammonium fluoride was added, and the solution refluxed for two hours to remove the t-butyldimethylsilyl protecting groups. After work-up, the crude reaction mixture containing hepta-3-methyl-β-cyclodextrin was once again repurified by preparative gradient elution column chromatography using the n-hexane:ethyl acetate:ethanol eluent system as in Example 1 and the pure final cyclodextrin intermediate was sulfated with SO_3·pyridine as described in Example 1. An electrospray ionization mass spectrum of the heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin sample is shown in FIG. **25**. An electropherogram demonstrating the separation of the enantiomers of isoproterenol using heptakis-(3-methyl-2,6-disulfato)-β-cyclodextrin, following the procedure described above, is shown in FIG. **26**.

Prophetic Example 10

Synthesis of Heptakis-(3,6-disulfato)-β-cyclodextrin

Heptakis-(3,6-disulfato)-β-cyclodextrin is prepared following the reaction scheme shown in FIG. **6**. First, hepta-6-triethylsilyl-β-cyclodextrin is prepared and purified as in Example 7. Heptakis-(2-t-butyldimethylsilyl-6-triethylsilyl)-β-cyclodextrin is prepared and purified as in Example 7. Next, the triethylsilyl protecting groups are removed from the purified intermediate as in Example 7 to yield hepta-2-t-butyldimethylsilyl-β-cyclodextrin, which is purified as in Example 7. The intermediate is then sulfated with SO_3·pyridine as described in Example 1 and, finally, the t-butyldimethylsilyl protecting groups are removed with aqueous ethanolic HF as in Example 3 yielding the desired product, heptakis-(3,6-disulfato)-β-cyclodextrin.

Prophetic Example 11

Synthesis of Heptakis-2,3-disulfato-β-cyclodextrin

Heptakis-(2,3-disulfato)-β-cyclodextrin is prepared by first synthesizing and purifying, as in Example 1, hepta-6-t-butyldimethylsilyl-β-cyclodextrin. This intermediate is then persulfated with SO_3·pyridine as in Examples 9 and 10,

followed by the removal of the t-butyldimethylsilyl protecting groups with aqueous ethanolic HF as in Example 3 yielding the desired product, heptakis-(2,3-disulfato)-β-cyclodextrin.

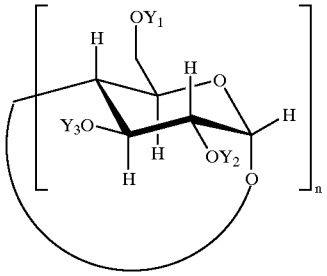
Example 12

Synthesis of Octakis-6-sulfato-γ-cyclodextrin

Octakis-6-sulfato-γ-cyclodextrin was prepared by first synthesizing and purifying octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin from y-cyclodextrin following the procedure described in Example 1. This intermediate was then deacetylated using the procedure described in Example 2 yielding the desired product, octakis-6-sulfato-γ-cyclodextrin. An electrospray-ionization mass spectrum of the octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin sample is shown in FIG. **27**. An electropherogram demonstrating the separation of the enantiomers of naproxen using octakis-6-sulfato-γ-cyclodextrin, following the procedure described above, is shown in FIG. **28**.

I claim:

1. A single-isomer cyclodextrin composition, which comprises substantially pure cyclodextrin derivatives having the formula:



wherein:

n is a single integer ranging from 6 to 12;

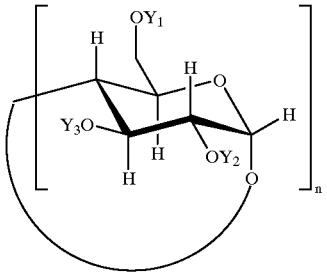
Y_1 is SO_3^-;

Y_2 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$;

Y_3 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; and

the cyclodextrin composition has an isomeric purity of at least 80 mole %.

2. A single-isomer cyclodextrin composition, which comprises substantially pure cyclodextrin derivatives having the formula:



wherein:

n is a single integer ranging from 6 to 12;

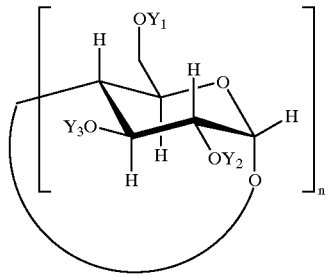
Y_2 is SO_3^-;

Y_1 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$;

Y_3 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; and

the cyclodextrin composition has an isomeric purity of at least 80 mole %.

3. A single-isomer cyclodextrin composition, which comprises substantially pure cyclodextrin derivatives having the formula:



wherein:

n is a single integer ranging from 6 to 12;

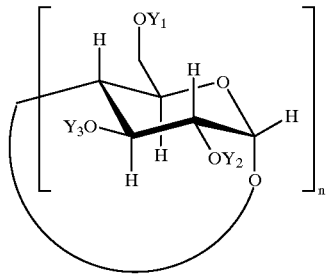
Y_3 is SO_3^-;

Y_1 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$;

Y_2 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$ and

the cyclodextrin composition has an isomeric purity of at least 80 mole %.

4. A single-isomer cyclodextrin composition, which comprises substantially pure cyclodextrin derivatives having the formula:



wherein

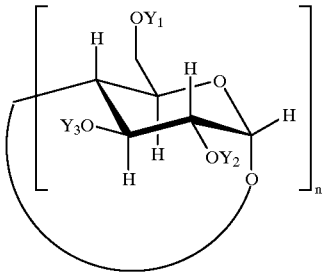
n is a single integer ranging from 6 to 12;

Y_1 and Y_2 is SO_3^-;

Y_3 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; and

the cyclodextrin composition has an isomeric purity of at least 80 mole %.

5. A single-isomer cyclodextrin composition, which comprises substantially pure cyclodextrin derivatives having the formula:



wherein:

n is a single integer ranging from 6 to 12;

Y_1 and Y_3 is SO_3^-;

Y_2 is H, CH_3, CH_2CH_3, $CH_2CHOHCH_3$, CH_2CN, or $OCCH_3$; and

the cyclodextrin composition has an isomeric purity of at least 80 mole %.

6. The composition of claim **1**, wherein Y_2 and Y_3 are the same.

7. The composition of claim **1**, wherein Y_2 and Y_3 are the different.

8. The composition of claim **2**, wherein Y_1 and Y_3 are the same.

9. The composition of claim **2**, wherein Y_1 and Y_3 are the different.

10. The composition of claim **3**, wherein Y_1 and Y_2 are the same.

11. The composition of claim **3**, wherein Y_1 and Y_2 are the different.

12. A single-isomer cyclodextrin composition, which comprises substantially pure hepta-6-sulfato-β-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

13. A single-isomer cyclodextrin composition, which comprises substantially pure heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

14. A single-isomer cyclodextrin composition, which comprises substantially pure heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

15. A single-isomer cyclodextrin composition, which comprises substantially pure octa-6-sulfato-γ-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

16. A single-isomer cyclodextrin composition, which comprises substantially pure octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

17. A single-isomer cyclodextrin composition, which comprises substantially pure octakis-(2,3-dimethyl-6-sulfato)-γ-cyclodextrin, wherein the cyclodextrin composition has an isomeric purity of at least 80 mole %.

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