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(54) Title: PREPARATION, USES AND SOLID FORMS OF OBETICHOLIC ACID

(57) Abstract: The present invention relates to obeticholic acid: or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof. Obeticholic acid is useful for the treatment or prevention of a FXR mediated disease or condition, cardiovascular disease or cholestatic liver disease, and for reducing HDL cholesterol, for lowering triglycerides in a mammal, or for inhibition of fibrosis. The present invention also relates to processes for the synthesis of obeticholic acid.

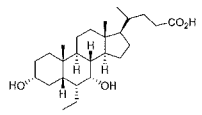
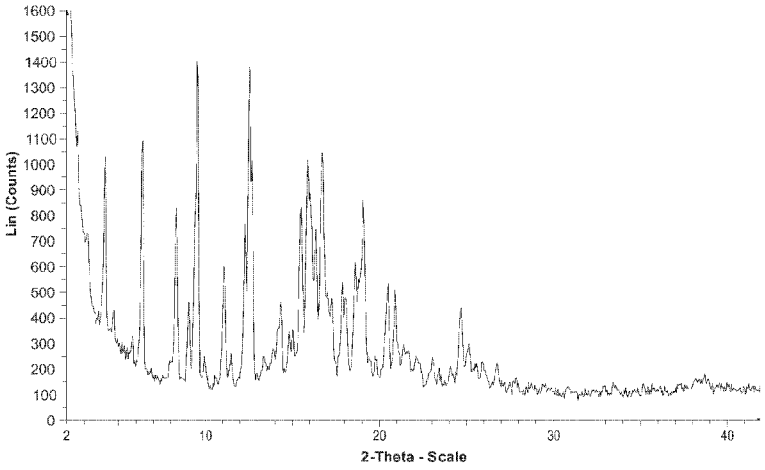


FIG. 5



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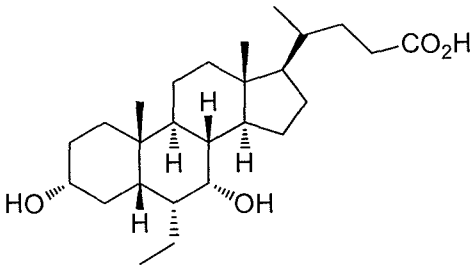
PREPARATION, USES AND SOLID FORMS OF OBETICHOLIC ACID

SUMMARY OF THE INVENTION

The present invention relates to obeticholic acid, an agonist for FXR, processes of preparation for obeticholic acid, pharmaceutical formulations comprising obeticholic acid, and the therapeutic use of the same.



obeticholic acid
(also known as INT-747)

The present invention relates to a crystalline obeticholic acid Form C characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 9.5, 12.5, and 16.7 degrees 2-Theta. The crystalline obeticholic acid Form C is characterized by an X-ray diffraction pattern substantially similar to that set forth in Figure 5 and further characterized by a Differential Scanning Calorimetry (DSC) thermogram having an endotherm value at about 98±2 °C.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the step of converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid; reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid; and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid; reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid; reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester; reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid; reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester; reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester; reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid; reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid; reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and converting crystalline obeticholic acid to obeticholic acid Form 1.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein converting crystalline obeticholic acid Form C to obeticholic acid Form 1 comprises the step of dissolving crystalline obeticholic acid Form C in aqueous NaOH solution and adding HCl.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein in reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid is carried out at a temperature at about 85 °C to about 110 °C in a basic aqueous solution.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid is carried out at a temperature at about 100 °C to about 105 °C and at a pressure at about 4 to about 5 bars.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid is carried out at a temperature at about 20 °C to about 60 °C.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with

CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester is carried out in a polar aprotic solvent at a temperature at about -50 °C to about -70 °C in the presence of BF_3.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester is carried out in a polar aprotic solvent at a temperature at about -10 °C to about -30 °C.

The present invention relates to a process for preparing obeticholic acid Form 1, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester is heated for about 3 hours and the pH of the reaction mixture is adjusted with an aqueous basic solution to a pH-value of about 6.5 to about 8.0.

The present invention relates to a obeticholic acid, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof, having a potency of greater than about 98%, greater than about 98.5%, greater than about 99.0%, or greater than about 99.5%. The present invention relates to a pharmaceutical composition comprising obeticholic acid Form 1 produced by a process of the invention and a pharmaceutically acceptable carrier.

The present invention relates to a method of treating or preventing an FXR mediated disease or condition in a subject comprise of administering an effective amount of obeticholic acid Form 1. The disease or condition is selected from biliary atresia, cholestatic liver disease, chronic liver disease, nonalcoholic steatohepatitis (NASH), hepatitis C infection, alcoholic liver disease, primary biliary cirrhosis (PBC), liver damage due to progressive fibrosis, liver fibrosis, and cardiovascular diseases including atherosclerosis, arteriosclerosis, hypercholesteremia, and hyperlipidemia. The present invention relates to a method for lowering triglycerides in a subject comprise of administering an effective amount of obeticholic acid Form 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a HPLC-UV/MS chromatogram of crude compound 5 of Step 4 of Example 1 injected at 1 mg/mL, injection volume 3µl. The chromatogram is obtained according to the method described in Example 2.

Figure 2 is a HPLC-UV/MS chromatogram of compound 5 of Step 4 of Example 1, purified reference injected at 1 mg/mL, injection volume 20 µL. The chromatogram is obtained according to the method described in Example 2.

Figure 3 is a UV chromatogram of crude compound 5 of step 4 of Example 1 using HPLC method. The chromatogram is obtained according to the method described in Example 2.

Figure 4A is an accurate ion trace of m/z 850.61914 ± 3ppm from the main peak fraction (RT 29.0 min) of compound 5 of Step 4 of Example 1, purely isolated with HPLC method (see Example 2).

Figure 4B is an accurate ion trace of m/z 850.61914 ± 3ppm from the minor peak fraction (RT 29.9 min) of compound 5 of Step 4 of Example 1, purely isolated with HPLC method (see Example 2).

Figure 4C is an accurate ion trace of m/z 850.61914 ± 3ppm from crude compound 5 of Step 4 of Example 1 (see Example 2).

Figure 4D is an accurate ion trace of m/z 850.61914 ± 3ppm from compound 5 of Step 4 of Example 1, purified reference (see Example 2).

Figure 5 is an XRPD diffractogram of crystalline obeticholic acid Form C (see Example 3).

Figure 6 shows TGA and DSC Thermograms of crystalline obeticholic acid Form C (see Example 3).

Figure 7 shows VT-XRPD diffractograms of crystalline obeticholic acid at 25 °C, 110 °C, and 120 °C (see Example 3).

Figure 8A is a GVS isotherm plot of crystalline obeticholic acid Form C (see Example 3).

Figure 8B is a GVS kinetic plot of crystalline obeticholic acid Form C (see Example 3).

Figure 8C shows XRPD diffractograms of crystalline obeticholic acid Form C before and after GVS analysis (see Example 3).

Figure 9 shows XRPD diffractograms of crystalline obeticholic acid Form C before and after storage at 40 °C/75% RH (see Example 3).

Figure 10 is an XRPD diffractogram of batch 1 of obeticholic acid Form 1(see Example 5).

Figure 11 shows the XRPD diffractorgraphs for batches 1, 2, 3, 4, 5 and 6 of obeticholic acid Form 1 (see Example 5).

Figure 12 is a NMR spectrum of batch 1 of obeticholic acid Form 1 in d_6-DMSO (see Example 5).

Figure 13 shows the ^{1}H NMR spectra for batches 1, 2, 3, 4, 5 and 6 of obeticholic acid Form 1 (see Example 5).

Figure 14 is an expansion of ^{13}C DEPTQ NMR spectrum of obeticholic acid Form 1 from region 10-75 ppm (see Example 5).

Figure 15 is an expansion of ^{13}C DEPT135 NMR spectrum of obeticholic acid Form 1 supressing quaternary carbons from region 0-75 ppm (see Example 5).

Figure 16 is a quantitative ^{13}C NMR of obeticholic acid Form 1 (see Example 5).

Figure 17 is an expanded view of peaks at 32.3 ppm of Figure 16 (see Example 5).

Figure 18 is a FT-IR spectrum of batch 1 of obeticholic acid Form 1 (see Example 5).

Figure 19 shows TGA and DSC thermograms of batch 1 of obeticholic acid Form 1 (see Example 5).

Figure 20 shows modulated DSC thermograms of batch 1 of obeticholic acid Form 1 (see Example 5).

Figure 21 shows the TGA traces of batches 1, 2, 3, 4, 5, and 6 of obeticholic acid Form 1 (see Example 5).

Figure 22 shows the DSC traces of batches 1, 2, 3, 4, 5, and 6 of obeticholic acid Form 1 (see Example 5).

Figure 23A is a picture of batch 1 of obeticholic acid Form 1 under polarized light microscopy. Figure 23B is a picture of batch 2 of obeticholic acid Form 1 under polarized light microscopy. Figure 23C is a picture of batch 3 of

obeticholic acid Form 1 under polarized light microscopy. Figure 23D is a picture of batch 4 of obeticholic acid Form 1 under polarized light microscopy. Figure 23E is a picture of batch 5 of obeticholic acid Form 1 under polarized light microscopy. Figure 23F is a picture of batch 6 of obeticholic acid Form 1 under polarized light microscopy.

Figure 24 shows GVS isotherm plot of batch 1 of obeticholic acid Form 1 (see Example 5).

Figure 25 shows GVS kinetics plot of batch 1 of obeticholic acid Form 1 (see Example 5).

Figure 26 shows XRPD diffractograms of batch 1 of obeticholic acid Form 1 before and after GVS (see Example 5).

Figure 27 is a graph of the measurement of pKa at three different methanol/water ratios for obeticholic acid Form 1 (see Example 5).

Figure 28 is a Yasuda-Shedlovsky plot for obeticholic acid Form 1 (see Example 5).

Figure 29 is a graph showing the distribution of the species depending on pH for obeticholic acid Form 1 (see Example 5).

Figure 30 is a graph showing the difference curve obtained by potentiometry for obeticholic acid Form 1 (see Example 5).

Figure 31 shows the lipophilicity profile of obeticholic acid Form 1 (see Example 5).

Figure 32 shows the XRPD diffractograms of batch 1 of obeticholic acid Form 1 after storage at 40 °C/75% RH (see Example 5).

Figure 33 shows the XRPD diffractograms of batch 1 of obeticholic acid Form 1 after storage at 25 °C/97% RH (see Example 5).

Figure 34 shows a view of the molecule of obeticholic acid Form G from the crystal structure showing anisotropic atomic displacement ellipsoids for the non-hydrogen atoms at the 50% probability level (see Example 6).

Figure 35 shows a view of the intermolecular hydrogen bonds of the crystal structure of obeticholic acid Form G where hydrogen bondings are shown in dashed lines (See Example 6).

Figure 36 shows an XRPD overlay of the simulated powder pattern, experimental patterns of the collected crystal, and obeticholic acid Form G (see Example 6).

Figure 37 shows a graph of the plasma obeticholic acid profile vs. time after oral administration of 20 mg/kg of obeticholic acid Form 1 and crystalline Form F (see Example 7).

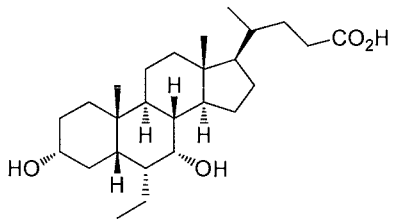
Figure 38 shows a graph of the plasma concentration of tauro conjugate of obeticholic acid Form 1 and crystalline Form F at different time interval after the administration (see Example 7).

Figure 39 shows the DSC curve of Form 1 (see Example 7).

Figure 40 shows the DSC curve of Form F (see Example 7)

DETAILED DESCRIPTION OF THE INVENTION

The present application is directed to obeticholic acid, a pharmaceutically active ingredient (also known as INT-747) having the chemical structure:



obeticholic acid
(also known as INT-747)

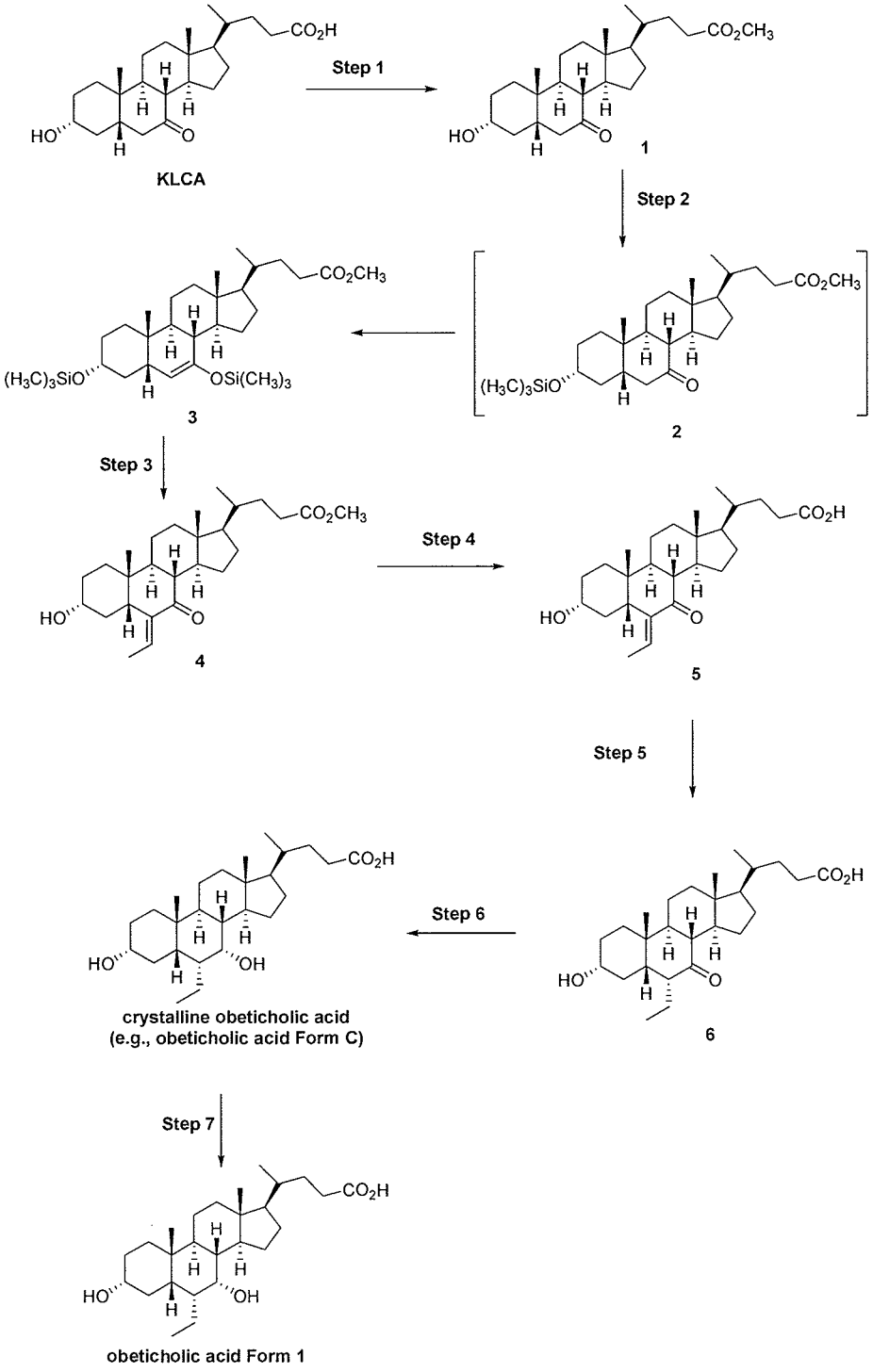
including, substantially pure obeticholic acid, a process for the preparation of obeticholic acid comprising crystalline obeticholic acid as a synthetic intermediate, and analytical methods for confirming the presence and purity of obeticholic acid and synthetic intermediates in the process to prepare obeticholic acid. The present application also describes pharmaceutical compositions and formulations of obeticholic acid and uses for such compositions.

Process to prepare obeticholic acid

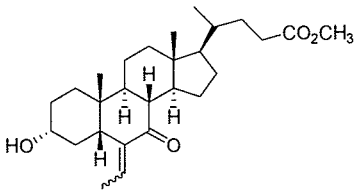
The present application is directed to a process for preparing highly pure obeticholic acid. The process of the present application is shown in Scheme 1. The

process is a 6-step synthesis followed by one purification step to produce highly pure obeticholic acid.

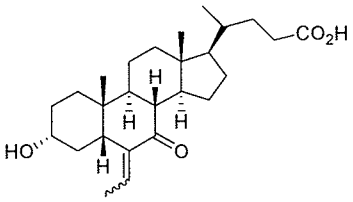
Scheme 1



The process of the present invention also includes a process according to Scheme 1 where compounds 4 and 5 are each comprised of a mixture of the E and Z isomers as illustrated by the structures of compounds 4A and 5A below:



4A



5A

In one embodiment, the E/Z isomer ratio of E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) is about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 90%, greater than about 93%, greater than about 95%, or greater than about 99%. In one embodiment, the E/Z ratio is determined by HPLC. In one embodiment, the ratio is greater than about 80%. In one embodiment, the ratio is greater than about 83%. In one embodiment, the ratio is greater than about 85%. In one embodiment, the ratio is greater than about 90%. In one embodiment, the ratio is greater than about 93%. In one embodiment, the ratio is greater than about 95%. In one embodiment, the ratio is greater than about 99%.

In one embodiment, the E/Z isomer ratio of E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) is about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 90%, greater than about 93%, greater than about 95%, or greater than about 99%. In one embodiment, the E/Z ratio is determined by HPLC. In one embodiment, the ratio is greater than about 80%. In one embodiment, the ratio is greater than about 83%. In one embodiment, the ratio is greater than about 85%. In one embodiment, the ratio is greater than about 90%. In one embodiment, the ratio is greater than about 93%. In one embodiment, the ratio is greater than about 95%. In one embodiment, the ratio is greater than about 99%.

The process of the present application has never been reported in the art. The process is a 6-step synthesis followed by one purification step. Step 1 is the esterification of the C-24 carboxylic acid of 7-keto lithocholic acid (KLCA) using methanol in the presence of acidic catalysis and heat to produce the methyl ester compound 1. Step 2 is silylenol ether formation from compound 1 using a strong base followed by treatment

with chlorosilane to produce compound 3. Step 3 is an aldol condensation reaction of the silylenol ether compound 3 and acetaldehyde to produce compound 4 (or compound 4A). Step 4 is ester hydrolysis i.e., saponification of the C-24 methyl ester of compound 4 (or compound 4A) to produce the carboxylic acid compound 5 (or compound 5A). Step 5 is the hydrogenation of the 6-ethylidene moiety of compound 5 (or compound 5A) followed by isomerization to produce compound 6. Step 6 is the selective reduction of the 7-keto group of compound 6 to a 7α-hydroxy group to produce crystalline obeticholic acid. Step 7 is the conversion of crystalline obeticholic acid to obeticholic acid Form 1.

The process of the present invention relates to a process for preparing obeticholic acid Form 1, where the process utilizes a crystalline form of obeticholic acid as a synthetic intermediate.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the step of converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3),

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3),

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid (KLCA) with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1).

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3),

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A),

reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid (KLCA) with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1).

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3),

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5),

reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6),

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

In one embodiment, the present invention relates to a process for preparing obeticholic acid Form 1 using crystalline obeticholic acid. In another embodiment, the crystalline obeticholic acid is Form C. In one embodiment, the crystalline obeticholic acid Form C is characterized by an X-ray diffraction pattern similar to that set forth in Figure

5. In one embodiment, the crystalline obeticholic acid Form C is crystallized and recrystallized from n-butyl acetate.

Step 1

Step 1 is the reaction of 3α-hydroxy-7-keto-5β-cholan-24-oic acid (KLCA) with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1). In one embodiment of step 1, the reaction mixture is heated for about 3 hours and the pH of the reaction mixture is adjusted with an aqueous basic solution to a pH-value of about 6.5 to about 8.0. In one embodiment, the isolation of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) further comprises treatment with activated carbon. In one embodiment, the isolation of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) does not further comprise treatment with activated carbon. In one embodiment, isolation of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) without the treatment with activated carbon affords a higher yield. In one embodiment, reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid (1) with CH_3OH and H_2SO_4 is carried out in methanol. In one embodiment, the basic solution is an aqueous NaOH solution. In one embodiment, the pH-value is about 7.0 to about 7.5.

In one embodiment, the methyl alcohol acts as the methylating reagent as well as the reaction solvent. In one embodiment, the solution containing the product is treated with activated carbon for about 30 minutes and filtered to remove the carbon solids. In one embodiment, the solution containing the product is not treated with activated carbon. To precipitate the product, water at about 5 °C to about 20 °C and seeding material are added. In another embodiment, the water is at about 10 °C to about 15 °C. In one embodiment, the product is isolated with a centrifuge and washed with a mixture of methanol and water. In one embodiment, the water content of the wet material is quantified by Karl Fischer (KF). In one embodiment, the material is dried in a tumble dryer before use in the next step. In one embodiment, the material is not dried before use in the next step.

Step 2

Step 2 is the reaction of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3). In one embodiment, step 2 is carried out in a polar

aprotic solvent at a temperature at about -10 °C to about -30 °C. In one embodiment, the polar aprotic solvent is tetrahydrofuran. In one embodiment, the temperature is about -20 °C to about -25 °C. In one embodiment, reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1) with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ is stirred for about 2 hours.

In one embodiment, compound 1 is charged into the reactor under inert conditions. In another embodiment, residual water and methanol are removed by repeated azeotropic distillation at about 65 °C and normal pressure. In another embodiment, THF is added to the residue as necessary and the distillation is repeated about 4 times. In another embodiment, the distillation is repeated about 3 times, about 2 times, or about 1 time. In one embodiment, the remaining solution containing the product has a final water content of $\leq 0.05\%$ (Karl Fischer Titration). Water can hydrolyze chlorotrimethylsilane, which is added later in this step. In one embodiment, the solution of the product is pre-cooled to about -10 °C to about -30 °C and then chlorotrimethylsilane is added. In another embodiment, the solution is pre-cooled to about -20 °C to about -25 °C. In one embodiment, a strong base and THF are charged to a separate reactor and cooled to about -10 °C to about -30 °C. In one embodiment, the strong base is lithium diisopropylamide. In another embodiment, the reactor is inert, *e.g.*, under a nitrogen or argon atmosphere. In another embodiment, the solution of base and THF is cooled to about -20 °C to about -25 °C. In one embodiment, the dry, cooled solution of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester, THF, and chlorotrimethylsilane is charged into the basic solution at about -10 °C to about -30 °C. In another embodiment, the temperature is about -20 °C to about -25 °C. In one embodiment, the reaction mixture is stirred for about 2 hours. In one embodiment, for the workup, the reaction mixture is added to a pre-cooled acidic solution. In another embodiment, the acidic solution is an aqueous citric acid solution. In one embodiment, after the addition, the aqueous phase is separated and discarded. In one embodiment, the solvent is removed from the organic phase, by vacuum distillation at about 50 °C. In one embodiment, the isolated residue is 3α,7α -ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) is used 'as is' in the next step. Alternatively, compound 3 can be purified before Step 3.

Step 3

Step 3 is the reaction of 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3) with CH_3CHO to form 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4). In one embodiment, step 3 is carried out in a polar aprotic solvent at a temperature at about -50 °C to about -70 °C in the presence of BF_3. In one embodiment, the polar aprotic solvent is dichloromethane. In one embodiment, the BF_3 is a 16% wt. solution in acetonitrile. In one embodiment, the temperature is about -60 °C to about -65 °C.

In one embodiment, compound 3 in a polar aprotic solvent is charged into an inert reactor. In another embodiment, the polar aprotic solvent is the residual solvent from the previous step (e.g., THF). In one embodiment, THF is added to help distill off residual water and diisopropylamine. At a maximum temperature of about 50 °C, residual amounts of the polar aprotic solvent are distilled off under vacuum. The water content in the residue containing compound 3 is limited to $\leq 0.5\%$ (Karl Fischer titration). The residue containing compound 3 is then dissolved in a polar aprotic solvent and pre-cooled to about -50 °C to about -70 °C. The polar aprotic solvent is dichloromethane. In another embodiment, residue containing compound 3 in the polar aprotic solvent is pre-cooled to about -60 °C to about -65 °C. Acetaldehyde (CH_3CHO) is added. A polar aprotic solvent and boron trifluoride (BF_3) solvated complex are charged into a separate reactor and then cooled to about -50 °C to about -70 °C. In another embodiment, the polar aprotic solvent is dichloromethane. In another embodiment, the boron trifluoride solvated complex is a boron trifluoride acetonitrile complex. The temperature of the BF_3 solution is about -60 °C to about -65 °C. The solution containing compound 3 and acetaldehyde is added to the BF_3 solution at about -60 °C to about -65 °C. In another embodiment, the solution containing compound 3 and acetaldehyde is dry. In one embodiment, the reaction mixture is stirred for about two hours at about -60 °C to about -65 °C, heated up to about 23 °C to about 28 °C, stirred for another about 2 hours and cooled to about 2 °C to about 10 °C for the hydrolysis/work-up. In one embodiment, the total time for addition and stirring is about 4 hours. In one embodiment, for the workup, the cooled solution from the reactor is added to a pre-cooled aqueous basic solution. In another embodiment, the aqueous basic solution is about 50% wt. sodium hydroxide (NaOH; caustic soda). In one embodiment, the phases are separated and the (lower) organic layer is transferred to a separate reactor. In one embodiment, from the organic layer, the solvent is removed by distillation at not more than (NMT) 50 °C as far as possible. In one embodiment, the

residue comprises 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) and some remaining acetonitrile and dichloromethane. It is understood that step 4 may form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A). The product of Step 3 is taken on directly to Step 4.

Step 4

Step 4 is the reaction of 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH to form E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5). In one embodiment, prior to step 4, the residue from step 3 is heated to about 45 °C to about 60 °C to remove residual amounts of solvent. In one embodiment, the temperature is about 49 °C to about 55 °C. In one embodiment, the ester hydrolysis reaction reacting 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) with NaOH is carried out at about 20 °C to about 25 °C in methanol, water, and a NaOH solution.

In one embodiment, reacting 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4) is charged into a reactor. In another embodiment, the reactor is inert, *e.g.*, under a nitrogen or argon atmosphere. At a temperature of NMT 50 °C, residual amounts of solvent are distilled off under vacuum. In one embodiment, the residue is heated up to about 45 °C to about 60 °C. In another embodiment, the residue is heated up to about 49 °C to about 55 °C. In another embodiment, the residue from Step 3 (compound 4) is dissolved in methanol and water and an aqueous basic solution. In another embodiment, the aqueous basic solution is about 50% wt. sodium hydroxide (NaOH; caustic soda). The ester hydrolysis reaction of Step 4 is carried out at about 20 °C to about 60 °C and stirred until the hydrolysis reaction is complete. In one embodiment, the ester hydrolysis is carried out at about 20 °C to about 25 °C. The pH of the reaction mixture is checked to verify it is > 12. If the pH is < 12, then additional NaOH is added. The reaction mixture is diluted with water and the temperature is adjusted to about 20 °C to about 35 °C. In another aspect, the reaction mixture is diluted with water and the temperature is adjusted to about 25 °C to about 35 °C. In one embodiment, for the workup, the phases are separated and the lower aqueous layer is transferred into a separate reactor and the organic layer is discarded. Compound 5 is in the aqueous phase. In one embodiment, ethyl acetate and an acid were added to the aqueous phase containing compound 5 with intensive stirring to the aqueous layer. In

another embodiment, the acid is an aqueous citric acid solution. In one embodiment, the phases are separated and the lower aqueous layer is discarded. Compound 5 is in the organic layer. In one embodiment, ethyl acetate is distilled off from the organic layer and replaced with ethyl acetate. In one embodiment, the distillation is repeated until the water content of the distillate is NMT 1% or until a constant boiling point is reached. In one embodiment, the suspension is cooled to about 10 °C to about 30 °C and isolated and washed with ethyl acetate. In another embodiment, the resulting suspension containing compound 5 is cooled to about 20 °C to about 25 °C. In one embodiment, drying of the resulting product is done under vacuum (e.g, tumble dryer) at about 60 °C.

In one embodiment, crude E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) is crystallized using ethanol. In one embodiment, ethanol and crude compound 5 are charged into reactor. In another embodiment, the reactor is inert. In one embodiment, to dissolve the crude compound 5, the mixture is heated to reflux. In one embodiment, mixture is cooled in a controlled cooling ramp to about 15 °C to about 20 °C. In one embodiment, the crystalline compound 5 is isolated using a centrifuge and then washed with ethyl acetate. In one embodiment, drying of crystalline compound 5 is done under vacuum (e.g, tumble dryer) and at about 60 °C. A sample can be taken to measure assay, purity, and moisture of the purified compound 5. In one embodiment, purified compound 5 contains both E and Z isomers of 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid. In one embodiment, the E to Z ratio is about 99:1, about 98:2, about 95: 5, about 90:10, about 85:15, about 80:20, about 75:25, about 70:30, about 65:35, about 60:40, about 55:45, or about 50:50. See Example 2 for full details regarding the identification and characterization of purified compound 5.

Step 4 can also be carried out starting with a compound that is a mixture of E/Z isomer. For example, Step 4 is the reaction of E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A). In one embodiment, prior to step 4, the residue from step 3 is heated about 45 °C to about 60 °C to remove residual amounts of solvent. In one embodiment, the temperature is about 49 °C to about 55 °C. In one embodiment, the ester hydrolysis reaction involving reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) with NaOH is carried out at about 20 °C to about 25 °C in

methanol, water, and a NaOH solution. In one embodiment, the NaOH solution is a 50% wt. aqueous solution.

In one embodiment, reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4A) is charged into a reactor. In another embodiment, the reactor is inert, *e.g.*, under a nitrogen or argon atmosphere. At a temperature of NMT 50 °C, residual amounts of solvent are distilled off under vacuum. In one embodiment, the residue is heated up to about 45 °C to about 60 °C. In one embodiment, the temperature is about 49 °C to about 55 °C. In one embodiment, the residue from step 3 (compound 4A) is dissolved in methanol and water and an aqueous basic solution. In another embodiment, the aqueous basic solution is about 50% wt. sodium hydroxide (NaOH; caustic soda). The ester hydrolysis reaction of step 4 is carried out at about 20 °C to about 60 °C and stirred until the hydrolysis reaction is complete. In one embodiment, the ester hydrolysis is carried out at about 20 °C to about 25 °C. The pH of the reaction mixture is checked to verify it is > 12. If the pH is < 12, then additional NaOH is added. The reaction mixture is diluted with water and the temperature is adjusted to about 25 °C to about 35 °C. In one embodiment, for the workup, the phases are separated and the lower aqueous layer is transferred into a separate reactor and the organic layer is discarded. Compound 5A is in the aqueous phase. In one embodiment, ethyl acetate and an acid were added to the aqueous phase containing compound 5A with intensive stirring to the aqueous layer. In another embodiment, the acid is an aqueous citric acid solution. In one embodiment, the phases are separated and the lower aqueous layer is discarded. Compound 5A is in the organic layer. In one embodiment, ethyl acetate is distilled off from the organic layer and replaced with ethyl acetate. In one embodiment, the distillation is repeated until the water content of the distillate is NMT 1% or until a constant boiling point is reached. In one embodiment, the suspension is cooled to about 10 °C to about 30 °C and isolated and washed with ethyl acetate. In another embodiment, the resulting suspension containing compound 5A is cooled to about 20 °C to about 25 °C. In one embodiment, drying of the resulting product is done under vacuum (e.g, tumble dryer) at about 60 °C. Compound 5A can be carried on without purification to Step 5.

In one embodiment, crude E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) is crystallized using ethanol. In one embodiment, ethanol and crude compound 5A are charged into reactor. In another embodiment, the reactor is inert. In one

embodiment, to dissolve the crude compound 5A, the mixture is heated to reflux. In one embodiment, mixture is cooled in a controlled cooling ramp to about 15 °C to about 20 °C. In one embodiment, the crystalline compound 5A is isolated using a centrifuge and then washed with ethyl acetate. In one embodiment, drying of crystalline compound 5A is done under vacuum (e.g, tumble dryer) and at about 60 °C. In one embodiment, the isolated crystalline product of step 4 is compound 5.

Alternative Step 4

Compound 5 can be prepared according to an alternative method. In one embodiment, compound 4 is charged into the inert reactor. At about 50 °C (maximum) residual amounts of solvent (e.g., acetonitrile, dichloromethane) may be distilled off under vacuum. The residue is dissolved in methanol and cooled. Tap-water and caustic soda (50 % weight NaOH) are added. In one embodiment, the reaction mixture is stirred for about four hours at about 20 °C to about 25 °C. The solution is diluted with tap-water and toluene is added. After stirring, the phases are separated and the lower, aqueous layer is transferred into the inert reactor. The organic layer is discarded. Acetic acid ethylester and a solution of citric acid are added during intensive stirring to the aqueous layer. The phases are separated and the lower, aqueous layer is discarded. The organic layer is transferred into the inert reactor. From the organic layer acetic acid ethylester is distilled off and replaced with acetic acid ethyl ester. In one embodiment, this operation is repeated until the water content of the distillate is not more than about 1 % or until a constant boiling point is reached. The present suspension is cooled to about 20 °C to about 25 °C, and compound 5 is isolated and washed with acetic acid ethylester with the inert centrifuge. Drying is done in the tumble dryer under vacuum and approximately 60 °C.

This alternative Step 4 can also be carried out starting with a compound that is a mixture of E/Z isomer. In one embodiment, compound 4A is charged into the inert reactor. At about 50 °C (maximum) residual amounts of solvent (e.g., acetonitrile, dichloromethane) may be distilled off under vacuum. The residue is dissolved in methanol and cooled. Tap-water and caustic soda (50%wt, NaOH) are added. In one embodiment, the reaction mixture is stirred for approximately four hours at about 20 °C to about 25 °C. The solution is diluted with tap-water and toluene is added. After stirring, the phases are separated and the lower, aqueous layer is transferred into the inert reactor.

The organic layer is discarded. Acetic acid ethylester and a solution of citric acid are added during intensive stirring to the aqueous layer. The phases are separated and the lower, aqueous layer is discarded. The organic layer is transferred into the inert reactor. From the organic layer acetic acid ethylester is distilled off and replaced with acetic acid ethylester. In one embodiment, this operation is repeated until the water content of the distillate is not more than about 1 % or until a constant boiling point is reached. The present suspension is cooled to 20 °C to 25 °C, and compound 5A is isolated and washed with acetic acid ethylester with the inert centrifuge. Drying is done in the tumble dryer under vacuum and approximately 60 °C.

Step 5

Step 5 is the reaction of E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6). Step 5 can be carried out in one phase (hydrogenation and isomerization together) or in two phases (hydrogenation followed by isomerization). In one embodiment, Step 5 is carried out at a temperature at about 90 °C to about 110 °C and at a pressure at about 4 to about 5 bars. In one embodiment, during workup, the organic phase of the reaction mixture is treated with activated carbon. In one embodiment, the pressure is about 4.5 to about 5.5 bars. In another embodiment, the pressure is about 5 bars. In one embodiment, the hydrogenation reaction mixture is allowed to stir for about 1 hour. In one embodiment, reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 2 hour to about 5 hours. In one embodiment, reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 3 hours.

In one embodiment, reacting E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5) with Pd/C and hydrogen gas is carried out in the presence of a basic solution. In one embodiment, the basic solution is a 50% wt. sodium hydroxide (NaOH; caustic soda) solution. After the hydrogenation reaction, the reaction mixture is heated up to about 100 °C (to carry out the isomerisation of the C-6 position from beta configuration to alpha configuration) and then cooled to about 40 °C to about 50 °C. For the workup, the Pd/C is filtered off. In one embodiment, to the filtrate, n-butyl acetate and an acid are added. In another embodiment, the acid is hydrochloric acid (HCl). The aqueous phase is

separated and discarded after checking the pH-value to make sure that it was acidic. The organic phase containing the product is treated with activated carbon. In one embodiment, the activated carbon is filtered off and the resulting filtrate containing the product is condensed by distillation and the resulting suspension is cooled to about 10 °C to about 30 °C. In another embodiment, the suspension is cooled to about 15 °C to about 20 °C. The suspension containing compound 6 is isolated and washed with n-butyl acetate. Compound 6 is filtered using a pressure filter. In one embodiment, drying is done in the pressure filter under vacuum at about 80 °C.

In one embodiment in Step 5, E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5), water, NaOH solution (e.g. 50% wt.), and Pd/C are mixed at about 5 bar of H_2 gas and at a temperature at about 100 °C to about 105 °C until H_2 uptake has ceased. The reaction mixture is cooled to about 40 °C to about 50 °C and Pd/C is filtered off. Then n-butyl acetate and HCl are added to the solution containing compound 6. In one embodiment, the aqueous phase is separated and discarded. The organic phase containing compound 6 is treated with activated carbon. The carbon is filtered off and the filtrate is moved to another reactor where it is reduced down by distillation, and then the suspension is cooled to about 5 °C to about 20 °C. In one embodiment, compound 6 is isolated via filtration and the filtrate is dried on the pressure filter under vacuum at about 80 °C.

Step 5 can also be carried out starting with a compound that is a mixture of E/Z isomer. For example, Step 5 is the reaction of E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas and heat to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6). Step 5 can be carried out in one phase (hydrogenation and isomerization together) or in two phases (hydrogenation, followed by isomerization). In one aspect, step 5 is carried out at a temperature at about 90 °C to about 110 °C and at a pressure at about 4 to about 5 bars. In one embodiment, during workup, the organic phase of the reaction mixture is treated with activated carbon. In one embodiment, the pressure is about 4.5 to about 5.5 bars. In another embodiment, the pressure is about 5 bars. In one embodiment, the hydrogenation reaction mixture is allowed to stir for about 1 hour. In one embodiment, reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 2 hour to about 5 hours. In one embodiment, reacting

E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 3 hours.

In one embodiment, reacting E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A) with Pd/C and hydrogen gas is carried out in the presence of a basic solution. In one embodiment, the basic solution is a 50% wt. sodium hydroxide (NaOH; caustic soda) solution. After the hydrogenation reaction, the reaction mixture is heated up to about 100 °C (to carry out the isomerisation of the C-6 position from beta configuration to alpha configuration) and then cooled to about 40 °C to about 50 °C. For the workup, the Pd/C is filtered off. In one embodiment, to the filtrate, n-butyl acetate and an acid are added. In another embodiment, the acid is hydrochloric acid (HCl). The aqueous phase is separated and discarded after checking the pH-value to make sure that it was acidic. The organic phase containing the product is treated with activated carbon. In one embodiment, the activated carbon is filtered off and the resulting filtrate containing the product is condensed by distillation and the resulting suspension is cooled to about 10 °C to about 30 °C. In another embodiment, the suspension is cooled to about 15 °C to about 20 °C. The suspension containing compound 6 is isolated and washed with n-butyl acetate. Compound 6 is filtered using a pressure filter. In one embodiment, drying is done in the pressure filter under vacuum at about 80 °C.

In one embodiment in Step 5, E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5A), water, NaOH solution (e.g. 50% wt.), and Pd/C are mixed at about 5 bar of H_2 gas and at a temperature at about 100 °C to about 105 °C until H_2 uptake has ceased. The reaction mixture is cooled to about 40 °C to about 50 °C and Pd/C is filtered off. Then n-butyl acetate and HCl are added to the solution containing compound 6. In one embodiment, the aqueous phase is separated and discarded. The organic phase containing compound 6 is treated with activated carbon. The carbon is filtered off and the filtrate is moved to another reactor where it is reduced down by distillation, and then the suspension is cooled to about 5 °C to about 20 °C. In one embodiment, compound 6 is isolated via filtration and the filtrate is dried on the pressure filter under vacuum at about 80 °C.

In another embodiment, the hydrogenation/isomerization reactions described above to prepare compound 6 are carried out in two phases (starting from compound 5 or compound 5A). First, the hydrogenation is carried out at about 4 to 5 bars and then second, the reaction mixture is heated to about 20 °C to about 40 °C. Heating the reaction

mixture isomerizes the ethyl group at the 6-position to the desired alpha configuration. The reaction mixture is heated until the isomerization is complete.

Step 6

Step 6 is the reaction of 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6) with $NaBH_4$ to form crystalline obeticholic acid. In one embodiment, Step 6 is carried out at a temperature at about 85 °C to about 110 °C in a basic aqueous solution. In one embodiment, the temperature is about 90 °C to about 95 °C. In one embodiment, the basic aqueous solution is an aqueous NaOH solution. In one embodiment, the basic aqueous solution is a mixture of 50% wt. NaOH solution and water. In one embodiment, the reaction mixture of compound 6 and $NaBH_4$ was stirred for about 3 hours to about 5 hours. In another embodiment, the reaction mixture was stirred for about 4 hours.

For the workup, after the reaction is complete, the mixture is cooled to about 80 °C and transferred to a cooled reactor. In one embodiment, at about 20 °C to about 60 °C, n-butyl acetate and an acid are added. In one embodiment, the temperature is about 40 °C to about 45 °C. In another embodiment, the acid is citric acid. The aqueous phase is separated and discarded after checking the pH-value to make sure that it was acidic. The organic phase containing the product is concentrated by distillation. In one embodiment, n-butyl acetate is added to the residue and distilled off again. In one embodiment, n-butyl acetate is added again to the residue and then is slowly cooled down. In another embodiment the residue is seeded at about 50 °C. In another embodiment, after crystallization has occurred, the mixture is heated to 52 °C and then slowly cooled down to about 15 °C to about 20 °C. In another embodiment, the residue is cooled to about 15 °C to about 20 °C. In one embodiment, the resulting obeticholic acid is washed with n-butyl acetate. In one embodiment, the obeticholic acid is isolated and washed with n-butyl acetate (e.g, in a pressure filter). In another embodiment, the pressure filter is inert. The crystalline product is dried under vacuum at about 60 °C. In one embodiment, the resulting crystalline obeticholic acid is isolated from organic solvent (e.g., heptane). See example 3 for full details regarding the identification and characterization of crystalline obeticholic acid Form C.

Step 7

Step 7 is the conversion of crystalline obeticholic acid Form C to obeticholic acid Form 1. In one embodiment, Step 7 comprises the step of dissolving crystalline obeticholic acid Form C in aqueous NaOH solution and adding HCl.

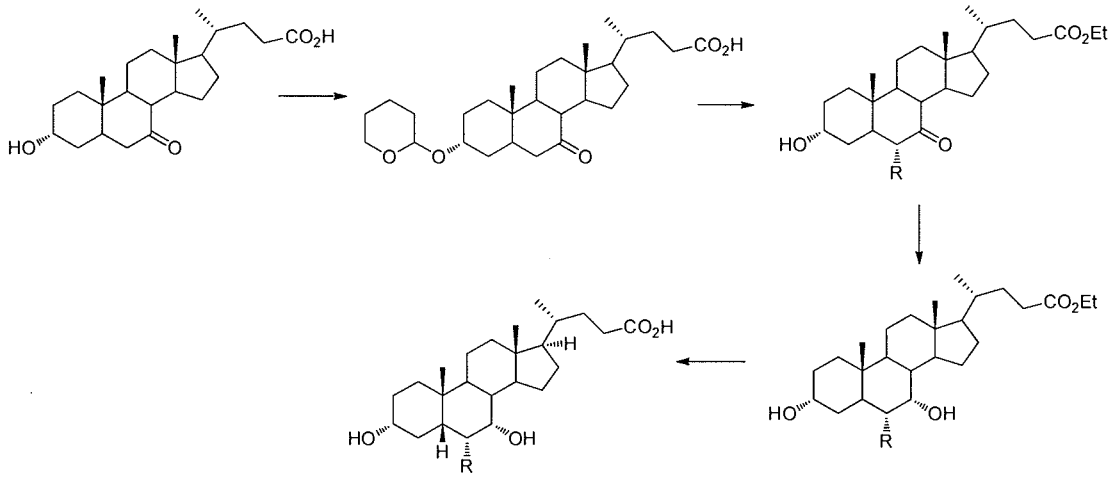
In one embodiment, crystalline obeticholic acid is dissolved in water and caustic soda solution (50% wt.) at about 20 °C to about 50 °C. In one embodiment, the temperature is about 30 °C to about 40 °C. In one embodiment, the crystalline obeticholic acid is Form C. In one embodiment, the resulting solution of crystalline obeticholic acid Form C is added to diluted acid at about 20 °C to about 50 °C. In another embodiment, the temperature is about 30 °C to about 40 °C. In another embodiment, the acid is hydrochloric acid (e.g., 37%). In one embodiment, the 37% hydrochloric acid solution is diluted with water to less than about 1% by volume. In one embodiment, the 37% hydrochloric acid solution is diluted with water to about 0.7% by volume. In one embodiment, the suspension of product in the diluted acid is stirred for about 30 minutes at about 20 °C to about 50 °C. In another embodiment, the temperature is about 30 °C to about 40 °C. In one embodiment, obeticholic acid Form 1 is isolated and washed with water (e.g., in the pressure filter) at NMT about 20 °C. In one embodiment, obeticholic acid Form 1 is isolated and washed with water (e.g., in the pressure filter) at NMT about 20 °C. In another embodiment, the pressure filter is inert. The product is dried on the pressure filter under vacuum at a temperature of NMT about 50 °C.

The process of the present application utilizes a crystalline intermediate in the preparation of obeticholic acid Form 1, which unexpectedly led to significant improvements in the overall preparation and purity of the final product. Specifically, Step 6 of the synthesis produces a novel crystalline form of obeticholic acid. The production of this crystalline form leads to substantially pure obeticholic acid Form 1.

The process of the present application is an improvement over the processes disclosed in the prior art. The preparation of obeticholic acid is disclosed in U.S. Publication No. 2009/0062526 A1 (herein referred to as the "'526 publication"), U.S. Patent No. 7,138,390 (referred to herein as the "'390 patent"), and WO 2006/122977 (referred to herein as the "'977 application").

The process to prepare obeticholic acid in the '390 patent (referred to herein as the "'390 process") is depicted in Scheme 3 (R is ethyl):

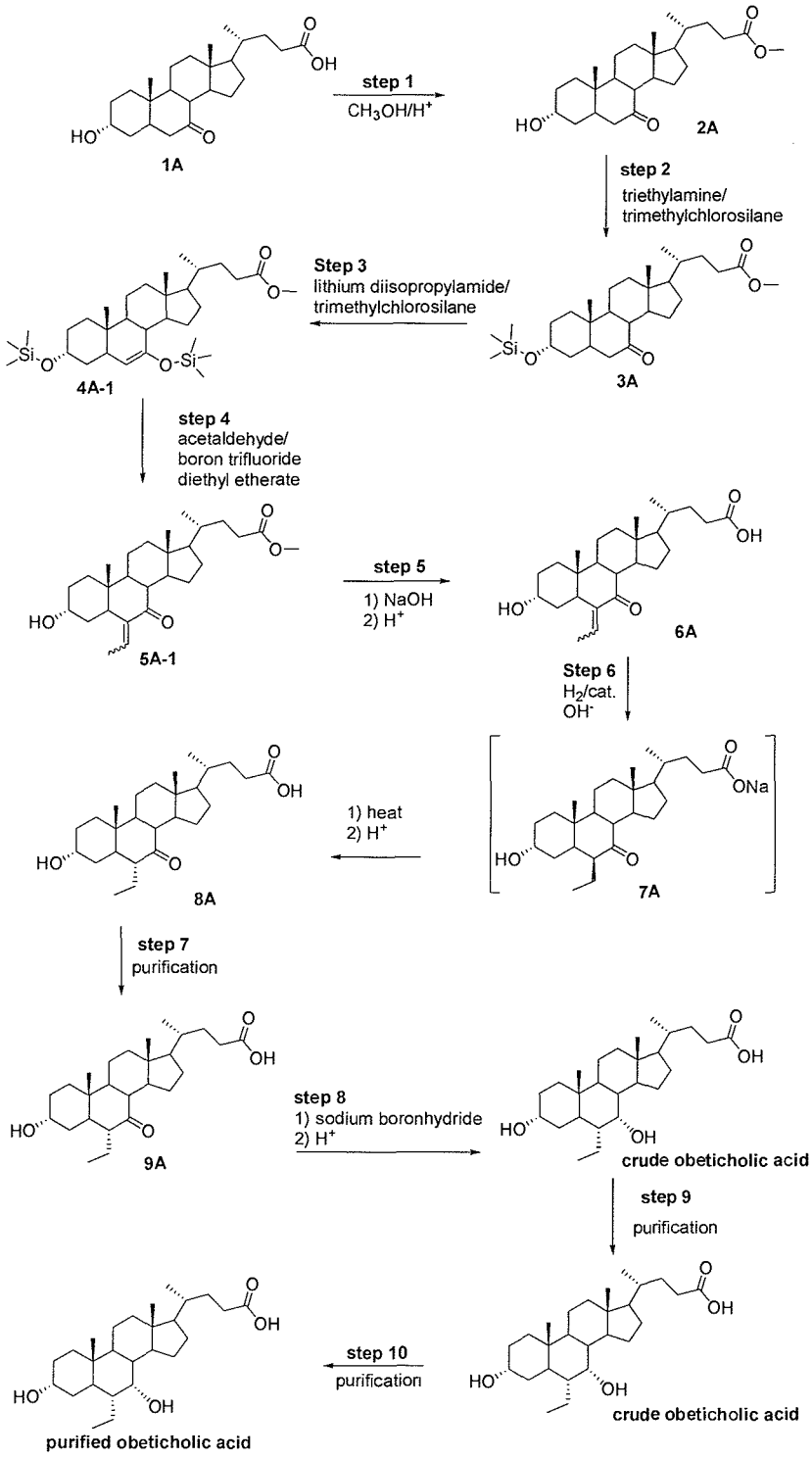
Scheme 3



Even though this process comprises a few steps, it presents a series of drawbacks. In all of the steps, the reaction products are purified on a chromatographic column, namely a very expensive separation method that cannot be used on an industrial scale. Moreover, the reaction yield in step 2 is extremely low (12-13%) with a considerable decrease in the global yield, which is lower than 3.5%. This process also uses hexamethylenphosphonamide as reactant, which is a known carcinogenic agent.

The process to prepare obeticholic acid in the '977 application is depicted in Scheme 4.

Scheme 4



The '977 process to prepare obeticholic acid is an 8-step synthetic process which includes one purification step (step 7) followed by 2 additional purification steps. There are a significant number of differences between the '977 process and the process of the

present application. Table A below describes at least some of the differences between the two processes:

Table A: Differences Between '977 Process and Process of the Application

Synthetic Step	Changes		Advantages of the Change
	'977 Process	Process of the application	
Step 1	Methanesulfonic acid	Sulfuric acid	Scale and safety (mesylate)
	30% ammonia (aqueous)	NaOH (aqueous)	Scale-up
	No purification/treatment	Use of activated carbon treatment	Improve purity/color
Step 2 (Process of application step 2 combines '977 Process Steps 2 and 3)	Triethylamine	Lithium diisopropylamide (LDA)	LDA is a suitable alternative reagent for this step
	Toluene	Tetrahydrofuran (THF)	THF is a suitable alternative reagent for this step
	No acidic quench	Quench into citric acid solution	Scale-up
Step 3 (Process of application step 3 same as '977 Step 4)	Boron trifluoride diethyl etherate	Boron trifluoride acetonitrile complex	Safety concerns of handling etherate (explosion hazard with ether)
Step 4 (Process of application step 4 same as '977 Step 5)	Toluene	Methanol	Safety (toluene); scale
	Phosphoric acid (aqueous) quench	Citric acid (aqueous) quench	Scale-up
	No purification/treatment	Crystallization step is part of workup	Improve purity
Step 5 (Process of application step 5 combines '977 Process steps 6 and 7)	Phosphoric acid (aqueous) quench	Hydrochloric acid (aqueous) quench	Scale-up
	No purification/treatment	Use of activated carbon treatment	Improve purity/color
	Purification carried out as Step 7	Crystallization step is part of workup	Scale-up
Step 6 (Process of application step 6 combines '977 Process steps 8 and 9)	Dichloromethane	n-Butylacetate	Safety (dichloromethane)
	Phosphoric acid (aqueous) quench	Citric acid (aqueous) quench	Scale-up
	Purification carried out as Step 9 – using dichloromethane /ethyl acetate	Crystallization step is part of workup – using n-butylacetate	Scale and safety (dichloromethane)
Step 7 (Process of application step 7 same as '977 step 10)	Ammonia solution	NaOH solution	Scale-up
	Phosphoric acid (aqueous) quench	Hydrochloric acid (aqueous) quench	Scale-up

The differences in the process of the present application as compared to the '977 process result in significant improvements to the process, including improvements related to scale-up optimization, safety, as well as purity and improvements in the overall process. The purity of obeticholic acid produced by the processes of the present application is substantially pure. Specifically, obeticholic acid produced by the processes of the present application is substantially more pure than obeticholic acid produced by processes in the prior art, including the '390 process and the '977 process. For example, a comparison of the results presented in the Certificate of Analysis of obeticholic acid produced by a process of the present application and obeticholic acid produced by the '977 process are shown in the Table B below. The percentages of impurities were determined using HPLC methods.

Table B: Comparison of Impurities of Obeticholic Acid Generated from Process of the Application and '977 Process

Parameter	Specification limit	Process of the application	'977 process
Water (KF)	NMT 4.5%	1.0%	2.1%
Impurity 1 and Impurity 4	NMT 0.15%	<0.05%	<0.05%
Impurity 2	NMT 0.15%	<0.05%	<0.1%
Impurity 3	NMT 0.15%	<0.05%	<0.1%
Impurity 5	NMT 3.0%	0.2%	1.0%
Impurity 6	NMT 0.15%	<0.05%	<0.05%

Impurity 1 is 6-ethylursodeoxycholic acid.
Impurity 2 is 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid.
Impurity 3 is 6β-ethylchenodeoxycholic acid.
Impurity 4 is 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid.
Impurity 5 is chenodeoxycholic acid.
Impurity 6 is 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid (6ECDCA dimer).
NMT refers to "not more than".

Crystalline Obeticholic Acid as a Synthetic Intermediate

Obeticholic acid is currently being developed as an active pharmaceutical ingredient as a non-crystalline solid. In order to facilitate the development of obeticholic acid, an initial crystallization and polymorphism study was carried out in order to determine if crystalline forms were accessible and if so, if they were suitable for development. After a preliminary solubility screen designed to give a better understanding of the behavior of the material in various solvents, it appeared that the

material had a tendency to form gels and could possibly be crystallized. An extensive polymorph screen was then carried out, exposing the material to a large range of solvents and crystallization conditions in order to identify and characterize as many relevant polymorphs as possible. Five different solid forms were found during this screen.

Three forms (A, C, and D) of obeticholic acid were mixed hydrates/solvates containing 0.25 mol eq of water and varying amounts of a range of organic solvents. On heating, these solids lost crystallinity and the solvent at the same time and unfortunately, these solvated forms were not suitable for further development as a pharmaceutical ingredient due to their low melting temperatures and high solvent content. It is also noted that similar "unsuitable" forms of this type exist. For example, a low-melting solvated form was found in later experiments, as well as single crystals of another form, which was shown to be a monohydrate/anisole solvate by SCXRD (Single crystal X-ray diffraction).

The two remaining forms were higher melting and potentially more promising, but one of them (Form G) could not be reproduced on scale-up, nor repeated despite many attempts. The difficulty in producing this form alone makes it unsuitable for development. The remaining non-solvated Form F was reproducibly prepared, but it required extensive recrystallization procedures and the use of nitromethane, which is a toxic solvent and may detonate if sensitized by amines, alkalis, strong acids, or high temperatures or adiabatic compression. Concerns about the residual levels of nitromethane deemed Form F also to be unsuitable for development.

The overall results of the initial crystallization and polymorph study revealed that the material could form various forms of crystalline materials, but none of the crystalline materials or forms were considered suitable for development.

It was not until much later that it was discovered the importance of producing crystalline obeticholic acid as an intermediate in the penultimate step of the process of the present application. Crystalline obeticholic acid could readily be isolated on large scale using the process of the application. This crystalline obeticholic acid was determined to be consistent with Form C from the initial crystallization and polymorph study. The formation, ease of isolation, and highly pure crystalline obeticholic acid produced as a synthetic intermediate in step 7 in the process of the present application is indeed critical to the preparation of substantially pure obeticholic acid.

In one embodiment, the present invention relates to a crystalline obeticholic acid Form C characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 9.5, 12.5, and 16.7 degrees 2-Theta. In one embodiment, the X-ray diffraction pattern includes characteristic peaks at about 4.2, 6.4, 9.5, 12.5, 12.6, 15.5, 15.8, 16.0, 16.7 and 19.0 degrees 2-Theta. In one embodiment, the X-ray diffraction pattern includes characteristic peaks at about 4.2, 6.4, 8.3, 9.5, 11.1, 12.2, 12.5, 12.6, 15.5, 15.8, 16.0, 16.3, 16.7, 18.6 and 19.0 degrees 2-Theta. In one embodiment, the X-ray diffraction pattern includes characteristic peaks at about 4.2, 6.4, 8.3, 9.5, 11.1, 12.2, 12.5, 12.6, 15.5, 15.8, 16.0, 16.3, 16.7, 17.0, 17.8, 18.6, 18.8, 19.0, 20.5 and 20.9 degrees 2-Theta. In one embodiment, the present invention relates to a crystalline obeticholic acid Form C characterized by an X-ray diffraction pattern substantially similar to that set forth in Figure 5. In one embodiment, the X-ray diffraction pattern is collected on a diffractometer using Cu Kα radiation (40 kV, 40 mA). In one embodiment, the X-ray diffraction pattern includes characteristic peaks at about 12.0 to about 12.8 and about 15.4 to about 21.0.

In one embodiment, the present invention relates to a crystalline obeticholic acid Form C characterized by a Differential Scanning Calorimetry (DSC) thermogram having an endotherm value at about 98±2 °C, as measured by a Mettler DSC 823e instrument. In one embodiment, the Differential Scanning Calorimetry (DSC) thermogram has an endotherm value at about 98±2 °C, as measured by a Mettler DSC 823e instrument.

In one embodiment, the present invention relates to a crystalline obeticholic acid, wherein said crystalline obeticholic acid is Form C and has a purity greater than about 90%. In one embodiment, the purity of said crystalline obeticholic acid Form C is determined by HPLC. In one embodiment, the present invention relates to a crystalline obeticholic acid Form C, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof. In one embodiment, the solvate is a hydrate. In one embodiment, the purity is greater than about 92%. In one embodiment, the purity is greater than about 94%. In one embodiment, the purity is greater than about 96%. In one embodiment, the purity is greater than about 98%. In one embodiment, the purity is greater than about 99%.

In one embodiment, the present invention relates to a crystalline obeticholic acid, wherein said crystalline obeticholic acid is Form C and has a potency greater than about 90%. In one embodiment, the purity of said crystalline obeticholic acid Form C is

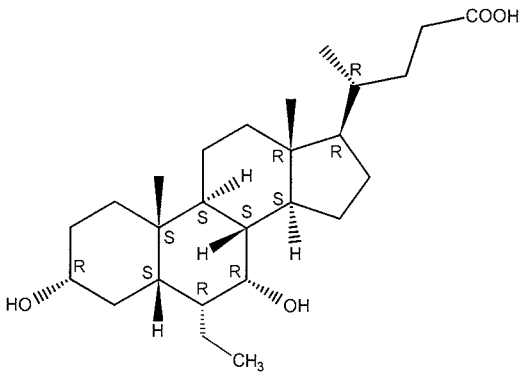
determined by HPLC and/or other analytical procedures known in the art. In one embodiment, the present invention relates to a crystalline obeticholic acid Form C, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof. In one embodiment, the solvate is a hydrate. In one embodiment, the potency is greater than about 92%. In one embodiment, the potency is greater than about 94%. In one embodiment, the potency is greater than about 96%. In one embodiment, the potency is greater than about 98%. In one embodiment, the potency is greater than about 99%.

In one embodiment, the present invention relates to a crystalline obeticholic acid Form C that contains a total of less than about 4% of one or more impurities selected from 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, the total impurities is less than about 3.8%. In one embodiment, the total impurities is less than about 3.6%.

Example 3 of the application provides full characterization of this novel crystalline form of obeticholic acid.

The single crystal X-ray structure of obeticholic acid was obtained and the absolute stereochemistry assigned. For example, the single crystal X-ray structure of crystalline obeticholic acid Form G was determined from a crystal obtained from the recrystallization of obeticholic acid from an acetonitrile solution after cooling to 5°C at 0.1°C/min followed by maturation at RT/50°C 8 h cycles for 1 week.

The structure is orthorhombic, space group $P2_12_12_1$, and contains one molecule of obeticholic acid in the asymmetric unit. Final R1 [$I>2\sigma(I)$] = 3.22 %. The absolute stereochemistry of the molecule was determined as shown below with a Flack parameter = -0.01 (13). The structure had no disorder.

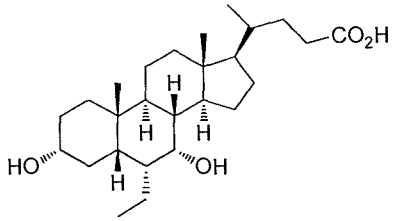


A bioavailability study of obeticholic acid Form 1 (non-crystalline) vs. crystalline obeticholic acid Form F was carried out (Example 7). The results of the study show that that physical state of a solid obeticholic acid can play a role in the bioavailability of the molecule when administered orally to a subject. The plasma kinetics after oral administration and the efficiency of the intestinal absorption and the pharmacokinetics of solid obeticholic acid Form 1 (non-crystalline) and crystalline Form F were evaluated according to methods known in the art. Example 8 of the present invention shows the profiles of obeticholic acid plasma concentration vs time, the t_{max}, C_{max} and AUC after administration of Form 1 or Form F of obeticholic acid (see Figures 37-38). Crystalline Form F has a higher bioavailability than obeticholic acid Form 1 (non-crystalline). The plasma profiles show that the Form F is absorbed more efficiently (higher AUC) and even the kinetics is more regular, reflecting an optimal distribution of the drug in the intestinal content.

The water solubility of obeticholic acid Form 1 (non-crystalline) is slightly higher than that of Form F. Form F appears to be stable as the thermo gravimetric analysis (TGA) did not show any weight loss in the temperature range studied.

Substantially Pure Obeticholic Acid

The present application provides substantially pure obeticholic acid and pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof:



obeticholic acid
(also known as INT-747)

Other names for the pharmaceutically active ingredient obeticholic acid are INT-747, 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid, 6α-ethyl-chenodeoxycholic acid, 6-ethyl-CDCA, 6ECDCA, and cholan-24-oic acid,6-ethyl-3,7-dihydroxy-,(3α,5β,6α,7α)-.

The present application provides compositions comprising obeticholic acid Form 1 and processes for the synthesis of highly pure obeticholic acid Form 1 which are safe and which produce obeticholic acid on a large scale. In one aspect, obeticholic acid Form 1 is produced on a commercial scale process. The term "commercial scale process" refers to a process which is run as a single batch of at least about 100 grams. In one

aspect, the process of the present application produces obeticholic acid Form 1 in high yield (>80%) and with limited impurities.

The term "purity" as used herein refers to the amount of obeticholic acid based on HPLC. Purity is based on the "organic" purity of the compound. Purity does not include a measure of any amount of water, solvent, metal, inorganic salt, etc. In one aspect, the purity of obeticholic acid is compared to the purity of the reference standard by comparing the area under the peak. In another aspect, the known standard for purity is an obeticholic acid reference standard. In one aspect, obeticholic acid has a purity of greater than about 96%. In one aspect, obeticholic acid has a purity of greater than about 98%. For example, the purity of obeticholic acid Form 1 is 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9 %, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. For example, the purity of obeticholic acid Form 1 is 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. For example, the purity of obeticholic acid is 98.0%, 98.5%, 99.0%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. For example, the purity of obeticholic acid is 98.5%, 99.0%, or 99.5%. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, the present invention relates to obeticholic acid having a purity greater than about 98%. In one embodiment, the purity is determined by HPLC. In another embodiment, the present invention relates to obeticholic acid, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof. In one embodiment, the purity is greater than about 98.5%. In one embodiment, the purity is greater than about 99.0%. In one embodiment, the purity is greater than about 99.5%. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

The term "potency" as used herein is a measure of the amount of obeticholic acid based on that of a known standard (e.g., acceptance criteria of about 95% to about 102%). Potency takes into account all possible impurities including water, solvents, organic, and inorganic impurities. In one aspect, the known standard is obeticholic acid. In one aspect, obeticholic acid has a potency of greater than about 96%. In one aspect, obeticholic acid has a potency of greater than about 98%. In one aspect, the known standard is obeticholic acid. In another aspect, potency is 100% minus the amounts of

water, sulphated ash, residual solvents, and other impurity contents such as 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In another embodiment, potency accounts for impurities due to water, solvent, metals, inorganic salts, and other inorganic or organic impurities. For example, the potency of obeticholic acid Form 1 is 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9 %, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. In one aspect, the potency of obeticholic acid Form 1 is 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. For example, the potency of obeticholic acid is 98.0%, 98.5%, 99.0%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. For example, the potency of obeticholic acid is 98.5%, 99.0%, or 99.5%. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, the present invention relates to obeticholic acid containing a total of less than about 2% of one or more impurities selected from 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, the total of impurities is less than about 1.5%. In one embodiment, the total of impurities is less than about 1.4%. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, obeticholic acid contains less than about 10% of water, less than about 9% of water, less than 8% of water, less than 7% of water, less than 6% of water, less than 5% of water, less than 4% of water, less than 3% of water, less than 2% of water, or less than 1% of water. In one embodiment, obeticholic acid contains less than about 1.2% of water. In one embodiment, obeticholic acid contains less than about 1.0% of water. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In another embodiment, obeticholic acid contains not more than (NMT) 0.15 % of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid.

In another embodiment, obeticholic acid contains a total of less than about 0.07% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains a total of less than about 0.06% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains a total of less than about 0.05% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, obeticholic acid contains not more than (NMT) 0.15 % of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.07% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.06% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.05% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, obeticholic acid contains not more than (NMT) 0.15% of 6β-ethylchenodeoxycholic acid. In one embodiment, obeticholic acid contains less than about 0.07% of 6β-ethylchenodeoxycholic acid. In one embodiment, obeticholic acid contains less than about 0.06% of 6β-ethylchenodeoxycholic acid. In one embodiment, obeticholic acid contains less than about 0.05% of 6β-ethylchenodeoxycholic acid. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, obeticholic acid contains no more than (NMT) 3% of chenodeoxycholic acid (CDCA). In one embodiment, obeticholic acid contains less than about 1% of CDCA. In one embodiment, obeticholic acid contains less than about 0.5% of CDCA. In one embodiment, obeticholic acid contains less than about 0.3% of CDCA. In one embodiment, obeticholic acid contains less than about 0.2% of CDCA. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

In one embodiment, obeticholic acid contains no more than (NMT) 4% of CDCA and 6-ethylursodeoxycholic acid.

In one embodiment, obeticholic acid contains no more than (NMT) 1.5 % of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 1% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.07% of

3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.06% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, obeticholic acid contains less than about 0.05% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. In one embodiment, the obeticholic acid is obeticholic acid Form 1.

Oral Formulation and Administration

Obeticholic acid is for oral administration. In one embodiment, the formulation is oral administration for the prevention and treatment of FXR mediated diseases and conditions. In one embodiment, the formulation comprises of obeticholic acid Form 1. In another embodiment, the formulation comprises of substantially pure obeticholic acid.

Formulations suitable for oral administration may be provided as discrete units, such as tablets, capsules, cachets (wafer capsule used by pharmacists for presenting a drug), lozenges, each containing a predetermined amount of obeticholic acid; as powders or granules; as solutions or suspensions in aqueous or non-aqueous liquids; or as oil-in-water or water-in-oil emulsions.

Formulations of the invention may be prepared by any suitable method, typically by uniformly and intimately admixing obeticholic acid with liquids or finely divided solid carriers or both, in the required proportions and then, if necessary, shaping the resulting mixture into the desired shape.

For example a tablet may be prepared by compressing an intimate mixture comprising a powder or granules of obeticholic acid and one or more optional ingredients, such as a binder, lubricant, inert diluent, or surface active dispersing agent, or by moulding an intimate mixture of powdered active ingredient and inert liquid diluent.

For example, one or more tablets may be administered to get to a target dose level based on the subject's weight, e.g., a human between about 30 kg to about 70 kg.

In one embodiment, the subject is a child and the formulation is used to treat biliary atresia. Biliary atresia, also known as "extrahepatic ductopenia" and "progressive obliterative cholangiopathy" is a congenital or acquired disease of the liver and one of the principal forms of chronic rejection of a transplanted liver allograft. In the congenital form, the common bile duct between the liver and the small intestine is blocked or absent.

The acquired type most often occurs in the setting of autoimmune disease, and is one of the principal forms of chronic rejection of a transplanted liver allograft.

Infants and children with biliary atresia have progressive cholestasis with all the usual concomitant features: jaundice, pruritus, malabsorption with growth retardation, fat-soluble vitamin deficiencies, hyperlipidemia, and eventually cirrhosis with portal hypertension. If unrecognized, the condition leads to liver failure—but not kernicterus, as the liver is still able to conjugate bilirubin, and conjugated bilirubin is unable to cross the blood–brain barrier. The cause of the condition is unknown. The only effective treatments are certain surgeries such as the kasai procedure, or liver transplantation

In one embodiment, the human child has had a Kasai procedure, where the Kasai procedure effectively gives them a functional bile duct when they born either without a bile duct of its completely blocked at birth.

In addition to the ingredients specifically mentioned above, the oral formulations of the present invention may include other agents known to those skilled in the art of pharmacy, having regard for the type of formulation in issue. Oral formulations suitable may include flavoring agents.

In one embodiment, the present invention relates to a pharmaceutical formulation of obeticholic acid or a pharmaceutically acceptable salt, solvate, or amino acid conjugate thereof, wherein obeticholic acid is produced by a process of the invention (obeticholic acid Form 1). In another embodiment, the formulation is administered orally.

In one embodiment, the formulation is in tablet form. In another embodiment, the formulation comprises obeticholic acid and one or more components selected from microcrystalline cellulose, sodium starch glycolate, magnesium stearate, coating material, or colloidal silicon dioxide. In one embodiment, the coating material is an Opadry® coating material.

In another embodiment, the formulation comprises about 0.1 mg to about 1500 mg of obeticholic acid per tablet. In another embodiment, the formulation comprises about 1 mg to about 100 mg. In another embodiment, the formulation comprises about 1 mg to about 50 mg. In another embodiment, the formulation comprises about 1 mg to about 30 mg. In another embodiment, the formulation comprises about 4 mg to about 26 mg. In another embodiment, the formulation comprises about 5 mg to about 25 mg. In one embodiment, the formulation comprises about 1 mg to about 2 mg. In one embodiment, the formulation comprises about 1.2 mg to about 1.8 mg. In one embodiment, the

formulation comprises about 1.3 mg to about 1.7 mg. In one embodiment, the formulation comprises about 1.5 mg.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 1 mg of obeticholic acid, about 180 to about 190 mg of microcrystalline cellulose, about 10 to about 15 mg of sodium starch glycolate, about 1 to about 3 mg of magnesium stearate, and about 5 mg to about 10 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 1 mg of obeticholic acid, about 185.0 mg of microcrystalline cellulose, about 12.0 mg of sodium starch glycolate, about 2.0 mg of magnesium stearate, and about 8.0 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 5 mg of obeticholic acid, about 175 to about 190 mg of microcrystalline cellulose, about 10 to about 15 mg of sodium starch glycolate, about 1 to about 3 mg of magnesium stearate, and about 5 mg to about 10 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 5 mg of obeticholic acid, about 181.0 mg of microcrystalline cellulose, about 12.0 mg of sodium starch glycolate, about 2.0 mg of magnesium stearate, and about 8.0 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 10 mg of obeticholic acid, about 170 mg to about 180 mg of microcrystalline cellulose, about 10 mg to about 15 mg of sodium starch glycolate, about 1 mg to about 3 mg of magnesium stearate, and about 5 mg to about 10 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 10 mg of obeticholic acid, about 176.0 mg of microcrystalline cellulose, about 12.0 mg of sodium

starch glycolate, about 2.0 mg of magnesium stearate, and about 8.0 mg of coating material. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 25 mg of obeticholic acid, about 150 mg to about 160 mg of microcrystalline cellulose, about 10 mg to about 15 mg of sodium starch glycolate, about 1 mg to about 3 mg of magnesium stearate, about 5 to about 10 mg of coating material, and about 1 to about 10 mg of colloidal silicon dioxide. In one embodiment, the coating material is an Opadry® coating material.

In one embodiment, the formulation comprises of about 1 mg to about 25 mg of obeticholic acid per tablet. In one embodiment, the formulation comprises about 25 mg of obeticholic acid, about 157.0 mg of microcrystalline cellulose, about 12.0 mg of sodium starch glycolate, about 2.0 mg of magnesium stearate, about 8.0 mg of coating material, and about 4.0 mg of colloidal silicon dioxide. In one embodiment, the coating material is an Opadry® coating material.

All percentages and ratios used herein, unless otherwise indicated, are by weight. The percent dimeric impurity is on an area percent basis, typically as quantified by analytical HPLC.

Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

Formulation of Tablets

Film Coated Tablet			
Component	**Quantity per Tablet**	**Function**	**Reference to Standard**
1 mg tablet			
Obeticholic acid	1.0 mg*	API	HSE
Microcrystalline cellulose	185.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP

Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green, white, or yellow	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
5 mg tablet			
Obeticholic acid	5.0 mg*	API	HSE
Microcrystalline cellulose	181.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green, white, or yellow	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
10 mg tablet			
Obeticholic acid	10.0 mg*	API	HSE
Microcrystalline cellulose	176.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green, white, or yellow	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
25 mg tablet			
Obeticholic acid	25.0 mg*	API	HSE
Microcrystalline cellulose	157.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Collodial silicon dioxide	4.0 mg	Glidant	USP-NF/EP/JP
Opadry® II green, white, or yellow	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		

API: Active pharmaceutical ingredient
HSE = In house specification
USP-NF = US Pharmacopeia National Formulary
Ph Eur = European Pharmacopeia
JP =Japanese Pharmacopeia
* obeticholic acid quantity presented assumes API is anhydrous and 100% pure; actual amount is adjusted based on the potency of the drug substance Lot used, and amount of microcrystalline cellulose is correspondingly decreased.

In one embodiment, the tablet comprises yellow Opadry®. In another embodiment, the tablet comprises white Opadry®. In another embodiment, the tablet comprises green Opadry®.

Pharmaceutical Compositions

Obeticholic acid, including obeticholic acid Form 1, substantially pure forms of obeticholic acid and crystalline forms of obeticholic acid, or a pharmaceutically acceptable salt, solvate, or amino acid conjugate thereof is useful for a variety of medicinal purposes. Obeticholic acid may be used in methods for the prevention or treatment of FXR mediated diseases and conditions. In one embodiment, the disease or condition is selected from biliary atresia, cholestatic liver disease, chronic liver disease, nonalcoholic steatohepatitis (NASH), hepatitis C infection, alcoholic liver disease, primary biliary cirrhosis (PBC), liver damage due to progressive fibrosis, liver fibrosis, and cardiovascular diseases including atherosclerosis, arteriosclerosis, hypercholesteremia, and hyperlipidemia. In one embodiment, obeticholic acid Form 1 may be used in methods for lowering triglycerides. In one embodiment, crystalline obeticholic acid may be used in methods for lowering triglycerides. Obeticholic acid Form 1 or crystalline obeticholic acid may increase HDL. Other effects of obeticholic acid Form 1 or crystalline obeticholic acid include lowering of alkaline phosphatase (ALP), bilirubin, ALT, AST, and GGT.

In one embodiment, the present invention relates to a pharmaceutical composition comprising obeticholic acid and a pharmaceutically acceptable carrier, wherein the obeticholic acid is produced by a process of the invention, *e.g.*, obeticholic acid Form 1. In one embodiment, the pharmaceutical composition comprises of substantially pure obeticholic acid and a pharmaceutically acceptable carrier. In another embodiment, the pharmaceutical composition comprises of crystalline obeticholic acid and a pharmaceutically acceptable carrier. In another embodiment, the crystalline obeticholic acid is the Form C.

In one embodiment, the present invention relates to a method of treating or preventing an FXR mediated disease or condition in a subject comprising administering an effective amount of obeticholic acid Form 1 produced by a process of the invention or a pharmaceutical composition thereof. In one embodiment, the present invention relates to a method of treating or preventing an FXR mediated disease or condition in a subject comprising administering an effective amount of substantially pure obeticholic acid produced by a process of the invention or a pharmaceutical composition thereof. In one embodiment, the present invention relates to a method of treating or preventing an FXR mediated disease or condition in a subject comprising administering an effective amount of crystalline obeticholic acid or a pharmaceutical composition thereof. In another

embodiment, the crystalline obeticholic acid is Form C. In one embodiment, the crystalline obeticholic acid is Form A. In one embodiment, the crystalline obeticholic acid is Form C. In one embodiment, the crystalline obeticholic acid is Form D. In one embodiment, the crystalline obeticholic acid is Form F. In one embodiment, the crystalline obeticholic acid is Form G.

In another embodiment, the disease or condition is cardiovascular disease or cholestatic liver disease and for lowering triglycerides. In another embodiment, the cardiovascular disease is atherosclerosis or hypercholesteremia. In another embodiment, the subject is a mammal. In another embodiment, the mammal is human.

In another embodiment, the compound or pharmaceutical composition is administered orally, parenterally, or topically. In another embodiment, the compound or pharmaceutical composition is administered orally.

In one embodiment, the present invention relates to a method for inhibiting fibrosis in a subject who is suffering from a cholestatic condition, the method comprising the step of administering to the subject an effective amount of obeticholic acid or a pharmaceutical composition thereof, wherein obeticholic acid is produced by the process of the invention. In one embodiment, the present invention relates to a method for inhibiting fibrosis in a subject who is not suffering from a cholestatic condition, the method comprising the step of administering to the subject an effective amount of obeticholic acid or a pharmaceutical composition thereof, wherein obeticholic acid is produced by the process of the invention. In embodiment, the fibrosis to be inhibited occurs in an organ where FXR is expressed.

In one embodiment, the cholestatic condition is defined as having abnormally elevated serum levels of alkaline phosphatase, 7-glutamyl transpeptidase (GGT), and 5' nucleotidase. In another embodiment, the cholestatic condition is further defined as presenting with at least one clinical symptom. In another embodiment, the symptom is itching (pruritus). In another embodiment, the fibrosis is selected from the group consisting of liver fibrosis, kidney fibrosis, and intestinal fibrosis. In another embodiment, the cholestatic condition is selected from the group consisting of primary biliary cirrhosis, primary sclerosing cholangitis, drug-induced cholestasis, hereditary cholestasis, and intrahepatic cholestasis of pregnancy. In another embodiment, the subject is not suffering from a cholestatic condition associated with a disease or condition

selected from the group consisting of primary liver and biliary cancer, metastatic cancer, sepsis, chronic total parenteral nutrition, cystic fibrosis, and granulomatous liver disease.

In another embodiment, the subject has liver fibrosis associated with a disease selected from the group consisting of hepatitis B; hepatitis C; parasitic liver diseases; post-transplant bacterial, viral and fungal infections; alcoholic liver disease (ALD); non-alcoholic fatty liver disease (NAFLD); non-alcoholic steatohepatitis (NASH); liver diseases induced by methotrexate, isoniazid, oxyphenistatin, methyldopa, chlorpromazine, tolbutamide, or amiodarone; autoimmune hepatitis; sarcoidosis; Wilson's disease; hemochromatosis; Gaucher's disease; types III, IV, VI, IX and X glycogen storage diseases; α_1-antitrypsin deficiency; Zellweger syndrome; tyrosinemia; fructosemia; galactosemia; vascular derangement associated with Budd-Chiari syndrome, veno-occlusive disease, or portal vein thrombosis; and congenital hepatic fibrosis.

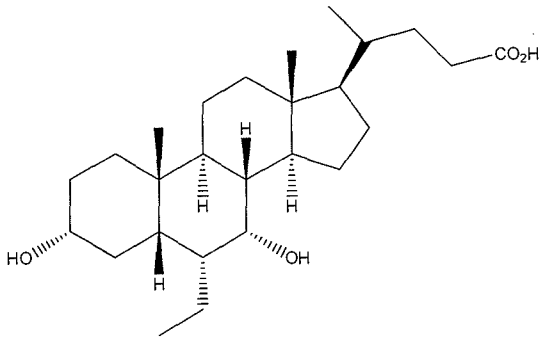
In another embodiment, the subject has intestinal fibrosis associated with a disease selected from the group consisting of Crohn's disease, ulcerative colitis, post-radiation colitis, and microscopic colitis.

In another embodiment, the subject has renal fibrosis associated with a disease selected from the group consisting of diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, chronic transplant glomerulopathy, chronic interstitial nephritis, and polycystic kidney disease.

Definitions

For convenience, certain terms used in the specification, examples and appended claims are collected here.

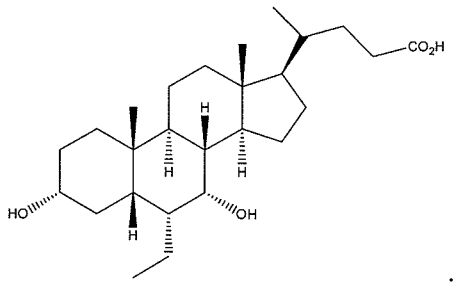
As used herein the term "obeticholic acid" or "OCA" refers to a compound having the chemical structure:



. Other chemical names for obeticholic acid include: 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid, 6α-ethyl-chenodeoxycholic

acid, 6-ethyl-CDCA, 6ECDCA, cholan-24-oic acid,6-ethyl-3,7-dihydroxy-,(3α,5β, 6α,7α)- and INT-747. The CAS registry number for obeticholic acid is 459789-99-2. This term refers to all forms of obeticholic acid, e.g., non-crystalline, crystalline and substantially pure.

As used herein the term "crystalline obeticholic acid" refers to any crystalline form of a compound having the chemical structure:



. Crystalline obeticholic acid means that the compound is crystallized into a specific crystal packing arrangement in three spatial dimensions or the compound having external face planes. The crystalline form of obeticholic acid (or a pharmaceutically acceptable salt, amino acid conjugate, solvate thereof) can crystallize into different crystal packing arrangements, all of which have the same elemental composition of obeticholic acid. Different crystal forms usually have different X-ray diffraction patterns, infrared spectral, melting points, density hardness, crystal shape, optical and electrical properties, stability and solubility. Recrystallization solvent, rate of crystallization, storage temperature, and other factors may cause one crystal form to dominate. Crystals of obeticholic acid can be prepared by crystallization under different conditions, *e.g.*, different solvents, temperatures, etc.

As used herein, the term "crystalline obeticholic acid Form C" refers to a crystalline form of obeticholic acid with an X-ray diffraction pattern that is substantially similar to that set forth in Figure 5, e.g., the crystalline form as characterized in Example 3.

As used herein, the term "substantially pure obeticholic acid" refers to obeticholic acid that has a potency of greater than about 95%. The potency of the obeticholic acid takes into account impurities including e.g., water, solvents, and other organic and inorganic impurities that are in a sample of obeticholic acid. In another embodiment, the known standard for potency is 100% obeticholic acid, and the potency is determined by subtracting percentages of impurities such as solvent, water, and other organic and inorganic impurities from 100% of the known standard. In one aspect, the inorganic impurities include e.g., inorganic salts and sulphated ash. In one aspect, the organic

impurities include 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid. The amounts of the impurities can be determined by procedures known in the art, e.g., HPLC, NMR, or methods from US Pharmacopeial, or European Pharmacopeia, or a combination of two or more of these methods.

As used herein, the term "purity" refers to a chemical analysis of a compound obtained from e.g., HPLC. In one embodiment, the purity of a compound is compared to the purity of the reference standard, e.g., obeticholic acid, via the area under their respective peak for comparisons. In one embodiment, purity accounts for the organic impurities in a sample.

As used herein, the term "reaction mixture" refers a mixture of one or more substances combined together. In one embodiment, the mixing or combining of the substances causes a chemical transformation or change in one or more of the original substances.

As used herein, the term "obeticholic acid Form 1" refers to non-crystalline obeticholic acid. In one embodiment, this form of obeticholic acid is produced via a crystalline obeticholic acid as a synthetic intermediate. For example, this form of obeticholic acid is produced by the process of the application via crystalline obeticholic acid Form C as the synthetic intermediate. In one embodiment, obeticholic acid Form 1 is the form that it used as the pharmaceutically active ingredient. See Example 5 for more details.

"Treating", includes any effect, *e.g.*, lessening, reducing, modulating, or eliminating, that results in the improvement of the condition, disease, disorder, etc. "Treating" or "treatment" of a disease state includes: inhibiting the disease state, *i.e.*, arresting the development of the disease state or its clinical symptoms; or relieving the disease state, *i.e.*, causing temporary or permanent regression of the disease state or its clinical symptoms.

"Preventing" the disease state includes causing the clinical symptoms of the disease state not to develop in a subject that may be exposed to or predisposed to the disease state, but does not yet experience or display symptoms of the disease state.

"Disease state" means any disease, disorder, condition, symptom, or indication.

The term "effective amount" as used herein refers to an amount of obeticholic acid (*e.g.*, an FXR-activating ligand) that produces an acute or chronic therapeutic effect upon appropriate dose administration. The effect includes the prevention, correction, inhibition, or reversal of the symptoms, signs and underlying pathology of a disease/condition (*e.g.*, fibrosis of the liver, kidney, or intestine) and related complications to any detectable extent.

"A therapeutically effective amount" means the amount of obeticholic acid that, when administered to a mammal for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on obeticholic acid, the disease and its severity and the age, weight, etc., of the mammal to be treated.

A therapeutically effective amount of obeticholic acid can be formulated with a pharmaceutically acceptable carrier for administration to a human or an animal. Accordingly, obeticholic acid or its formulations can be administered, for example, via oral, parenteral, or topical routes, to provide an effective amount of the compound. In alternative embodiments, obeticholic acid prepared in accordance with the present invention can be used to coat or impregnate a medical device, *e.g.*, a stent.

"Pharmacological effect" as used herein encompasses effects produced in the subject that achieve the intended purpose of a therapy. In one embodiment, a pharmacological effect means that primary indications of the subject being treated are prevented, alleviated, or reduced. For example, a pharmacological effect would be one that results in the prevention, alleviation or reduction of primary indications in a treated subject. In another embodiment, a pharmacological effect means that disorders or symptoms of the primary indications of the subject being treated are prevented, alleviated, or reduced. For example, a pharmacological effect would be one that results in the prevention or reduction of primary indications in a treated subject.

The invention also comprehends isotopically-labeled obeticholic acid, or pharmaceutically acceptable salts, solvate, or amino acid conjugates thereof, which are identical to those recited in formulae of the invention and following, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number most commonly found in nature. Examples of isotopes that can be incorporated into obeticholic acid, or pharmaceutically

acceptable salts, solvate, or amino acid conjugates thereof include isotopes of hydrogen, carbon, nitrogen, fluorine, such as ^{3}H, ^{11}C, ^{14}C and ^{18}F.

Obeticholic acid, or pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of the present invention. Isotopically-labeled obeticholic acid, or pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof, for example those into which radioactive isotopes such as ^{3}H, ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, *i.e.*, ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances, isotopically labeled obeticholic acid, or pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples of the invention, by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent. In one embodiment, obeticholic acid, or pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof are not isotopically labelled. In one embodiment, deuterated obeticholic acid is useful for bioanalytical assays. In another embodiment, obeticholic acid, or pharmaceutically acceptable salts, solvates, or amino acid conjugates thereof are radiolabelled.

"Geometric Isomers" means the diastereomers that owe their existence to hindered rotation about double bonds. These configurations are differentiated in their names by the prefixes cis and trans, or Z and E, which indicate that the groups are on the same or opposite side of the double bond in the molecule according to the Cahn-Ingold-Prelog rules.

"Solvates" means solvent addition forms that contain either stoichiometric or non stoichiometric amounts of solvent. Obeticholic acid may have a tendency to trap a fixed molar ratio of solvent molecules in the crystalline solid state, thus forming a solvate. If the solvent is water the solvate formed is a hydrate, when the solvent is alcohol, the solvate formed is an alcoholate. Hydrates are formed by the combination of one or more molecules of water with one of the substances in which the water retains its molecular state as H_2O, such combination being able to form one or more hydrate. Additionally, the

compounds of the present invention, for example, the salts of the compounds, can exist in either hydrated or unhydrated (the anhydrous) form or as solvates with other solvent molecules. Nonlimiting examples of hydrates include monohydrates, dihydrates, etc. Nonlimiting examples of solvates include ethanol solvates, acetone solvates, etc.

"Tautomers" refers to compounds whose structures differ markedly in arrangement of atoms, but which exist in easy and rapid equilibrium. It is to be understood that obeticholic acid may be depicted as different tautomers. It should also be understood that when obeticholic acid and synthetic intermediates of the invention have tautomeric forms, all tautomeric forms are intended to be within the scope of the invention, and the naming of obeticholic acid does not exclude any tautomer form. Obeticholic acid and synthetic intermediates of the invention can exist in several tautomeric forms, including the keto-enol. For example, in keto-enol tautomerism a simultaneous shift of electrons and a hydrogen atom occurs. Tautomers exist as mixtures of a tautomeric set in solution. In solid form, usually one tautomer predominates. Even though one tautomer may be described, the present invention includes all tautomers of the present compounds.

It is to be understood accordingly that the isomers arising from asymmetric carbon atoms (*e.g.*, all enantiomers and diastereomers) are included within the scope of the invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis. Furthermore, the structures and other compounds and moieties discussed in this application also include all tautomers thereof. Alkenes can include either the E- or Z-geometry, where appropriate. Obeticholic acid and synthetic intermediates may exist in stereoisomeric form, and therefore can be produced as individual stereoisomers or as mixtures.

A "pharmaceutical composition" is a formulation containing obeticholic acid in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. It is can be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active reagent calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the

invention are dictated by and directly dependent on the unique characteristics of the active reagent and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active agent for the treatment of individuals.

The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler, or a vial. The quantity obeticholic acid (*e.g.*, a formulation of obeticholic acid, or a pharmaceutically acceptable salt, solvate, or amino acid conjugate thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, inhalational, buccal, sublingual, intrapleural, intrathecal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, obeticholic acid is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

The term “flash dose” refers to obeticholic acid formulations that are rapidly dispersing dosage forms.

The term "immediate release" is defined as a release of obeticholic acid from a dosage form in a relatively brief period of time, generally up to about 60 minutes. The term "modified release" is defined to include delayed release, extended release, and pulsed release. The term "pulsed release" is defined as a series of releases of drug from a dosage form. The term “sustained release” or “extended release” is defined as continuous release of obeticholic acid from a dosage form over a prolonged period.

A “subject” includes mammals, *e.g.*, humans, companion animals (*e.g.*, dogs, cats, birds, and the like), farm animals (*e.g.*, cows, sheep, pigs, horses, fowl, and the like) and laboratory animals (*e.g.*, rats, mice, guinea pigs, birds, and the like). In one embodiment, the subject is human. In one embodiment, the subject is human child (e.g., between about 30 kg to about 70 kg). In one embodiment, the human child has had a Kasai procedure, where the Kasai procedure effectively gives them a functional bile duct when they born either without a bile duct of its completely blocked at birth.

As used herein, the phrase "pharmaceutically acceptable" refers to those compounds, materials, compositions, carriers, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

While it is possible to administer obeticholic acid directly without any formulation, obeticholic acid is usually administered in the form of pharmaceutical formulations comprising a pharmaceutically acceptable excipient and obeticholic acid. These formulations can be administered by a variety of routes including oral, buccal, rectal, intranasal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Oral formulation of obeticholic acid are described further herein under the section entitled "Oral Formulation and Administration".

In one embodiment, obeticholic acid can be administered transdermally. In order to administer transdermally, a transdermal delivery device ("patch") is needed. Such transdermal patches may be used to provide continuous or discontinuous infusion of a compound of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, *e.g.*, U.S. Patent No. 5,023,252. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

In one embodiment of the present invention, there is provided a pharmaceutical formulation comprising at least obeticholic acid as described above in a formulation adapted for buccal and/or sublingual, or nasal administration. This embodiment provides administration of obeticholic acid in a manner that avoids gastric complications, such as first pass metabolism by the gastric system and/or through the liver. This administration route may also reduce adsorption times, providing more rapid onset of therapeutic benefit. The compounds of the present invention may provide particularly favorable solubility profiles to facilitate sublingual/buccal formulations. Such formulations typically require

relatively high concentrations of active ingredients to deliver sufficient amounts of active ingredients to the limited surface area of the sublingual/buccal mucosa for the relatively short durations the formulation is in contact with the surface area, to allow the absorption of the active ingredient. Thus, the very high activity of obeticholic acid, combined with its high solubility, facilitates its suitability for sublingual/buccal formulation.

Obeticholic acid is preferably formulated in a unit dosage form, each dosage containing from about 0.1 mg to about 1500 mg. In another embodiment, the formulation comprises about 1 mg to about 100 mg. In another embodiment, the formulation comprises about 1 mg to about 50 mg. In another embodiment, the formulation comprises about 1 mg to about 30 mg. In another embodiment, the formulation comprises about 4 mg to about 26 mg. In another embodiment, the formulation comprises about 5 mg to about 25 mg. In one embodiment, the formulation comprises about 1 mg to about 2 mg. In one embodiment, the formulation comprises about 1.2 mg to about 1.8 mg. In one embodiment, the formulation comprises about 1.3 mg to about 1.7 mg. In one embodiment, the formulation comprises about 1.5 mg. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient as described above.

Obeticholic acid is generally effective over a wide dosage range. For examples, dosages per day normally fall within the range of about 0.0001 to about 30 mg/kg of body weight. In the treatment of adult humans, the range of about 0.1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. In embodiment, the formulation comprises about 0.1 mg to about 1500 mg. In another embodiment, the formulation comprises about 1 mg to about 100 mg. In another embodiment, the formulation comprises about 1 mg to about 50 mg. In another embodiment, the formulation comprises about 1 mg to about 30 mg. In another embodiment, the formulation comprises about 4 mg to about 26 mg. In another embodiment, the formulation comprises about 5 mg to about 25 mg. In one embodiment, the formulation comprises about 1 mg to about 2 mg. In one embodiment, the formulation comprises about 1.2 mg to about 1.8 mg. In one embodiment, the formulation comprises about 1.3 mg to about 1.7 mg. In one embodiment, the formulation comprises about 1.5 mg. However, it will be understood that the amount of obeticholic acid actually administered will be determined by a

physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the form of obeticholic acid administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

"Process of the invention" refers to a method for preparing obeticholic acid as described herein, wherein the method comprises of crystalline obeticholic acid.

"Fibrosis" refers to a condition involving the development of excessive fibrous connective tissue, *e.g.*, scar tissue, in a tissue or organ. Such generation of scar tissue may occur in response to infection, inflammation, or injury of the organ due to a disease, trauma, chemical toxicity, and so on. Fibrosis may develop in a variety of different tissues and organs, including the liver, kidney, intestine, lung, heart, *etc.*

The term "inhibiting" or "inhibition," as used herein, refers to any detectable positive effect on the development or progression of a disease or condition. Such a positive effect may include the delay or prevention of the onset of at least one symptom or sign of the disease or condition, alleviation or reversal of the symptom(s) or sign(s), and slowing or prevention of the further worsening of the symptom(s) or sign(s).

As used herein, a "cholestatic condition" refers to any disease or condition in which bile excretion from the liver is impaired or blocked, which can occur either in the liver or in the bile ducts. Intrahepatic cholestasis and extrahepatic cholestasis are the two types of cholestatic conditions. Intrahepatic cholestasis (which occurs inside the liver) is most commonly seen in primary biliary cirrhosis, primary sclerosing cholangitis, sepsis (generalized infection), acute alcoholic hepatitis, drug toxicity, total parenteral nutrition (being fed intravenously), malignancy, cystic fibrosis, and pregnancy. Extrahepatic cholestasis (which occurs outside the liver) can be caused by bile duct tumors, strictures, cysts, diverticula, stone formation in the common bile duct, pancreatitis, pancreatic tumor or pseudocyst, and compression due to a mass or tumor in a nearby organ.

Clinical symptoms and signs of a cholestatic condition include: itching (pruritus), fatigue, jaundiced skin or eyes, inability to digest certain foods, nausea, vomiting, pale stools, dark urine, and right upper quadrant abdominal pain. A patient with a cholestatic

condition can be diagnosed and followed clinically based on a set of standard clinical laboratory tests, including measurement of levels of alkaline phosphatase, γ-glutamyl transpeptidase (GGT), 5' nucleotidase, bilirubin, bile acids, and cholesterol in a patient's blood serum. Generally, a patient is diagnosed as having a cholestatic condition if serum levels of all three of the diagnostic markers alkaline phosphatase, GGT, and 5' nucleotidase, are considered abnormally elevated. The normal serum level of these markers may vary to some degree from laboratory to laboratory and from procedure to procedure, depending on the testing protocol. Thus, a physician will be able to determine, based on the specific laboratory and test procedure, what is an abnormally elevated blood level for each of the markers. For example, a patient suffering from a cholestatic condition generally has greater than about 125 IU/L alkaline phosphatase, greater than about 65 IU/L GGT, and greater than about 17 NIL 5' nucleotidase in the blood. Because of the variability in the level of serum markers, a cholestatic condition may be diagnosed on the basis of abnormal levels of these three markers in addition to at least one of the symptoms mentioned above, such as itching (pruritus).

The term "organ" refers to a differentiated structure (as in a heart, lung, kidney, liver, *etc.*) consisting of cells and tissues and performing some specific function in an organism. This term also encompasses bodily parts performing a function or cooperating in an activity (*e.g.*, an eye and related structures that make up the visual organs). The term "organ" further encompasses any partial structure of differentiated cells and tissues that is potentially capable of developing into a complete structure (*e.g.*, a lobe or a section of a liver).

All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

In the specification, the singular forms also include the plural, unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms

used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present specification will control.

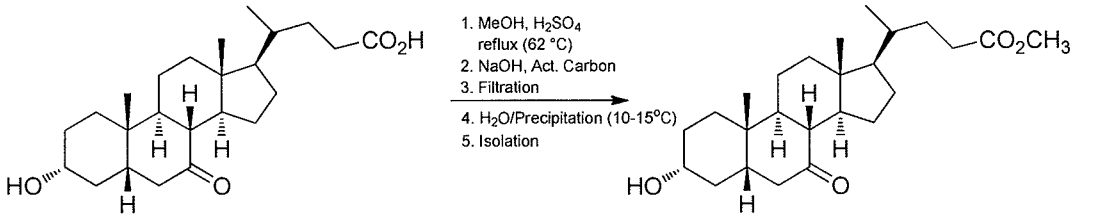
All percentages and ratios used herein, unless otherwise indicated, are by weight.

EXAMPLES

EXAMPLE 1: Synthesis of obeticholic acid

The compound numbers referred to in this synthetic procedure refer to those found in Scheme 1 and the reaction that correspond to each of the steps.

Step 1 – Preparation of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1):



3α-hydroxy-7-keto-5β-cholan-24-oic acid
KLCA

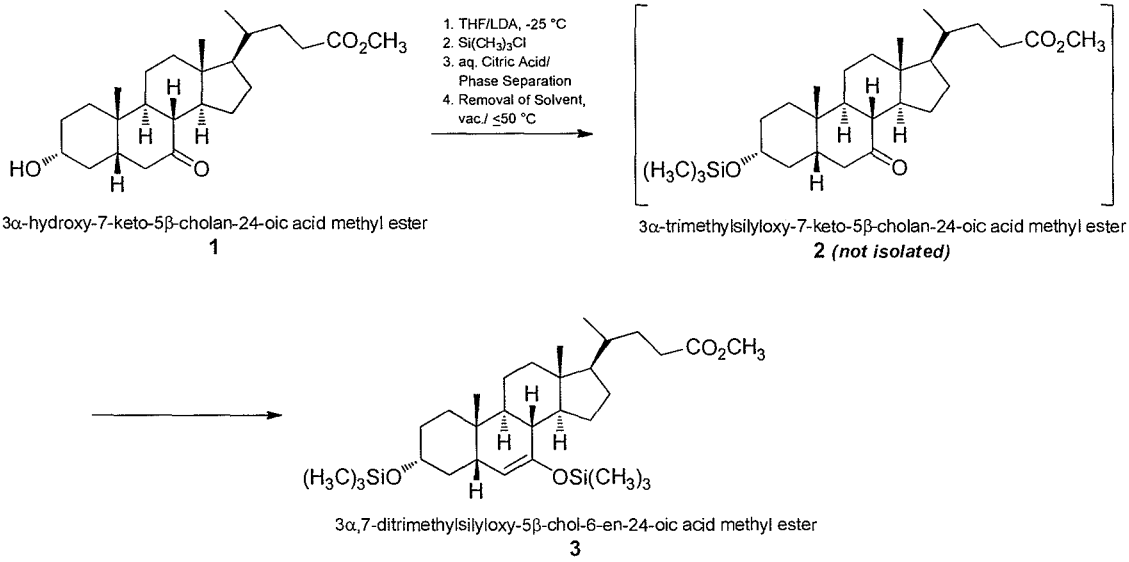
3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester
1

Reaction 1: Esterification of C-24 carboxylic acid of 7-keto lithocholic acid (KLCA)

3α-hydroxy-7-keto-5β-cholan-24-oic acid (KLCA; 500.0 g, 1.28 mol) was esterified using methyl alcohol (2500 mL), in the presence of acidic catalysis (sulfuric acid, 1.0 mL) and was heated up to 62 °C to 64 °C for approximately 3 hours, to yield 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester (1). In this reaction, the methyl alcohol acts as the methylating reagent as well as the reaction solvent. For the work-up, the pH-value was adjusted with sodium hydroxide solution (2N) to pH 7.0 to 7.5. The solution was treated with activated carbon (25 g) for approximately 30 minutes and filtered to remove the carbon solids. Alternatively, the solution was not treated with activated carbon. To precipitate the product, water (625 mL) at 10 °C to 15 °C was added over 15 minutes and seeding material was added. The reaction mixture is stirred for 1 hour at 10 °C to 15 °C. Another portion of water (1875 mL) was added over about 20 to 25 minutes. The product suspension was stirred for 30 minutes at 10 °C to 15 °C. The product was isolated with a centrifuge and washed with a mixture of methanol and water

(1:1, 350 mL). The water content of the wet material was quantified by Karl Fischer (KF). The material was dried in a tumble dryer under vacuum at NMT 70 °C. The material can also be used in the next step without drying. The yield (calculated on dried product) is 501.4 g (1.24 mol, 96.8%).

Step 2 – Preparation of 3α,7α -ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester (3):



3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester
1

3α-trimethylsilyloxy-7-keto-5β-cholan-24-oic acid methyl ester
2 *(not isolated)*

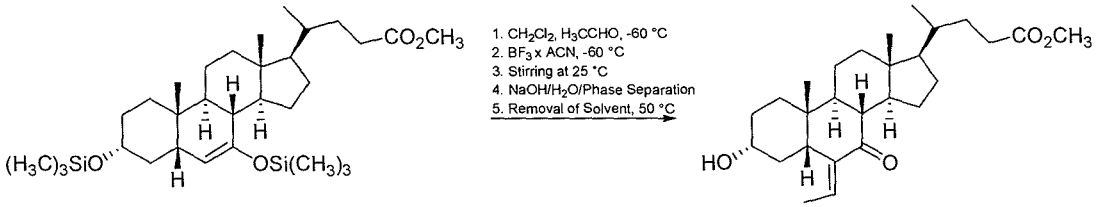
3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester
3

Reaction 2: Silylenol ether formation from 7-keto lithocholic methyl ester

Compound 1 (60.69 g, 150 mmol, calculated as dry substance), containing residual water and methanol, was charged into the reactor under inert conditions and was dissolved in tetrahydrofuran (THF, 363 mL). Water and methanol were removed by repeated azeotropic distillation at approximately 65 °C and normal pressure. THF was added to the residue as necessary and the distillation was repeated approximately 4 times. The remaining solution must have a final water content of ≤ 0.05% (Karl Fischer Titration). This solution was pre-cooled to -20 °C to -25 °C and then chlorotrimethylsilane (73.33 g, 675 mmol, 4.5 equivalents)was added in about 30 to 45 minutes. Under nitrogen atmosphere, lithium diisopropyl amide (28% LDA solution, 900 mmol) and THF (504 mL) were charged to a separate inert reactor and cooled to -20 °C to -25 °C. The dry, cooled solution of compound 1, THF (84 mL), and chlorotrimethylsilane was charged into the LDA solution at -20 °C to

-25 °C. Then, the reaction mixture was stirred for approximately 2 hours. For the workup, the reaction mixture was added to a pre-cooled aqueous solution of citric acid (34.6 g in 300 mL) at 2 °C to 8 °C. After the addition, the aqueous phase was separated and discarded. From the organic phase, the liquid was removed by vacuum distillation at maximum 50 °C. The isolated residue contained compound 3 and some residual solvents and was used 'as is' in the next step.

Step 3 – Preparation of 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester (4):



3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester
3

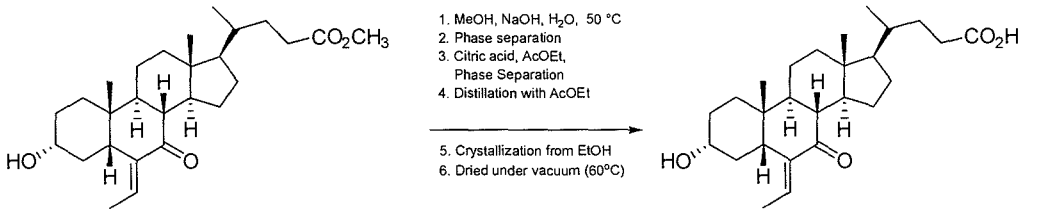
3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester
4

Reaction 3: Aldol condensation of the silylenol ether and acetaldehyde

Compound 3 (164.68 g, 300 mmol, calculated as dried substance) solution in THF was charged into an inert reactor. At a maximum temperature of 50 °C, residual amounts of THF were distilled off under vacuum. The water content in the residue was limited to ≤ 0.5% (Karl Fischer titration) in order to proceed. The residue was then dissolved in dichloromethane (200 mL) and pre-cooled to -60 °C to -65 °C. Acetaldehyde (33.8 mL, 600 mmol) was then added. Under nitrogen atmosphere, dichloromethane (700 mL) and boron trifluoride (16 wt% solution in acetonitrile, 318 g, 750 mmol) acetonitrile complex were charged into a separate reactor and then cooled to -60 °C to -65 °C. At -60 °C to -65 °C, the dry compound 3 solution was added. The reaction mixture was stirred for approximately two hours at -60 °C to -65 °C, heated up to 23 °C to 28 °C, stirred for another approximately 3 hours and cooled to approximately 2 °C to 10 °C for the hydrolysis/work-up. For the workup, the cooled solution from the reactor was added to a pre-cooled aqueous solution of 50% wt. caustic soda (40 mL) and 660 mL of water. After about 10 minutes of intensive stirring, the phases were separated and the (lower) organic layer was transferred to a separate reactor. From the organic layer, the solvent was

removed by distillation at NMT 50 °C as far as possible. The residue, consisting of compound 4 and some remaining acetonitrile and dichloromethane, was discharged into drums. Compound 4A, a mixture of E/Z-isomers can also be prepared by the procedure described above for Step 3.

Step 4 – Preparation of 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5):



3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester
4

3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid
5

Reaction 4: Saponification of C-24 ester

Compound 4 (258.37 g, 600 mmol, calculated as dried substance) was charged into an inert reactor. At a temperature of NMT 50 °C, residual amounts of solvent were distilled off under vacuum. The residue was dissolved in methanol (360 mL) and water (54 mL) and caustic soda 50% wt. (54 mL) were added. The reaction mixture was heated up to 49 °C to 53 °C and stirred at this temperature for at least 2 hours. The pH of the reaction mixture is checked and verified to be > 12. If the pH is < 12, additional NaOH is added and the 2 hour reaction time is repeated. The solution was diluted with water (1000 mL) and the temperature was adjusted to 25 °C to 35 °C. For the workup, reaction mixture was allowed to rest for at least 30 minutes. The phases were separated and the lower aqueous layer was transferred into a separate reactor and the organic layer was discarded. Ethyl acetate (1400 mL) and aqueous citric acid (244 g in 480 mL) were added with intensive stirring to the aqueous layer. The reaction mixture was stirred at 25 °C to 35 °C for 10 minutes. The phases were separated and the lower aqueous layer was discarded. Ethyl acetate was distilled off from the organic layer and replaced with ethyl acetate (800 mL). This operation was repeated until the water content of the distillate was NMT 1% or until a constant boiling point was reached. The suspension was cooled to 20 °C to 25 °C, stirred for 30 minutes, and then the product was isolated and washed with ethyl acetate (100 mL, 3 to 4 times). Drying was done in a tumble dryer under vacuum at

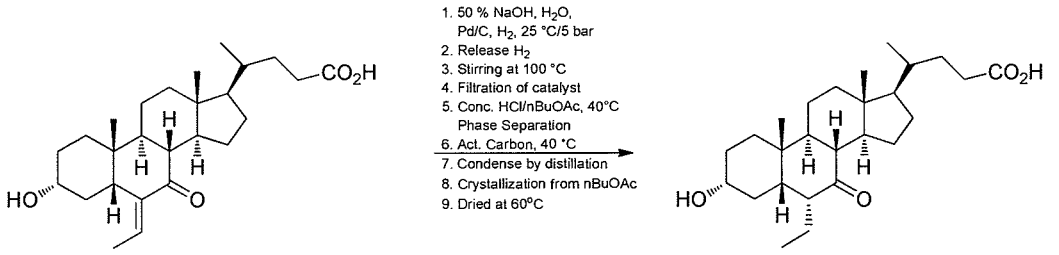
approximately 60 °C. The yield is 118.71 g (47.5% from KLCA) of crude compound 5. Compound 4A, a mixture of E/Z isomers also can be used as starting material to produce compound 5A, a mixture of E/Z isomers.

Crude compound 5 was then crystallized using ethanol. The crude compound for crystallization can also be a mixture of E/Z isomers, compound 5A. Ethanol (390 to 520 mL) and crude compound 5 (130 g) were charged into an inert reactor. To dissolve the crude compound 5, the reaction mixture was heated to reflux. Then, the reaction mixture was cooled in a controlled cooling ramp to 15 °C to 20 °C within 3 to 5 hours by a linear profile. The crystalline compound 5A was isolated using a centrifuge and then washed with ethyl acetate (50-100 mL, 2 times). Drying was done in the tumble dryer under vacuum and at approximately 60 °C. This leads to 85.8 g (66%) yield. A sample was taken to measure assay, purity, and moisture of the purified compound 5. Purified compound 5 is the E isomer of 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid. See example 2 for full details regarding the identification and characterization of purified compound 5. Isolation of the purified compound 5, the E isomer, can be optional. The E isomer and Z isomers have different solubilities. The E isomer is less soluable and crystallizes such that the Z isomer can be washed away.

An alternative method to prepare compound 5 is as follows. Compound 4 (111.96 g) was charged into the inert reactor. At maximum 50 °C residual amounts of solvent (e.g., acetonitrile, dichloromethane) were distilled off under vacuum. The residue was dissolved in methanol (156 mL) and cooled to about 10 °C. Tap-water (23.4 mL) and caustic soda 50 % (23.4 mL) were added. The reaction mixture was stirred for about four hours at about 20 °C to about 25 °C. The solution was diluted with tap-water (433 mL) and toluene (144 mL) was added. After stirring, the phases were separated and the lower, aqueous layer was transferred into the inert reactor. The organic layer was discarded. Acetic acid ethylester (607 mL) and a solution of citric acid (105.7 g in 208 mL of water) were added during intensive stirring to the aqueous layer. The phases were separated and the lower, aqueous layer was discarded. The organic layer was transferred into the inert reactor. From the organic layer acetic acid ethylester was distilled off and replaced with acetic acid ethylester (347 mL). In one embodiment, this operation was repeated with acetic acid ethylester (173 mL) until the water content of the distillate was not more than about 1 % or until a constant boiling point was reached. The present suspension was cooled to 20 °C to 25 °C. Compound 5 was isolated and washed with acetic acid

ethylester (3 to 4 times each 43 mL) with inert centrifuge. Drying was done in the tumble dryer under vacuum and approximately 60 °C (64.8% yield based on compound 1). Compound 4A (a mixture of E/Z isomers) can also be used as starting material for Step 4 to produce Compound 5A (a mixture of E/Z isomers).

Step 5 – Preparation of 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (6):



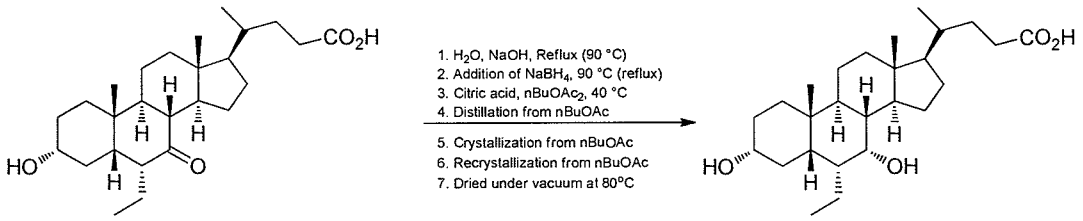
3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid
5

3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid
6

Reaction 5: Hydrogenation of the 6-ethylidene moiety

A mixture of purified compound 5 (110 g, 264 mmol), water (1100 mL), caustic soda solution (35.8 mL, 682 mmol) at 50% and palladium catalyst (Pd/C, 11 g) were charged to a hydrogenation reactor. The temperature was adjusted to 25 °C to 35 °C and the reactor was flushed three times with nitrogen (2 bar) and three times with hydrogen (1 bar). These pressure values were given relative to ambient pressure (= 0 bar). A hydrogen pressure of 5 bar was applied and the reaction mixture was heated up to 100 °C (for isomerisation to the alpha position) over a period of 1.5 hours and then stirred for 3 hours while maintaining the hydrogen pressure at 4.5 to 5 bar. The reaction mixture is then cooled to 40 °C to 50 °C. For the workup, the Pd/C is filtered off. To the filtrate, n-butyl acetate (1320 mL) and hydrochloric acid (67.8 mL, 815 mmol, 37%) were added. The aqueous phase was separated and discarded. The organic phase was treated with activated carbon (5.5 g) for about 10 minutes at 40 to 50 °C. The activated carbon was filtered off and the filtrate was condensed by distillation and the resulting suspension was cooled to 15 °C to 20 °C within 2 to 3 hours. The precipitated compound 6 was isolated and washed with n-butyl acetate (160 mL). The product was filtered using a pressure filter. Drying was done in the pressure filter under vacuum at approximately 60 °C. This leads to 89.8 g (81.2%) of Compound 6. Compound 5A, a mixture of E/Z isomers, can be used in step 5 to prepare Compound 6.

Step 6 – Preparation of 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid (obeticholic acid):



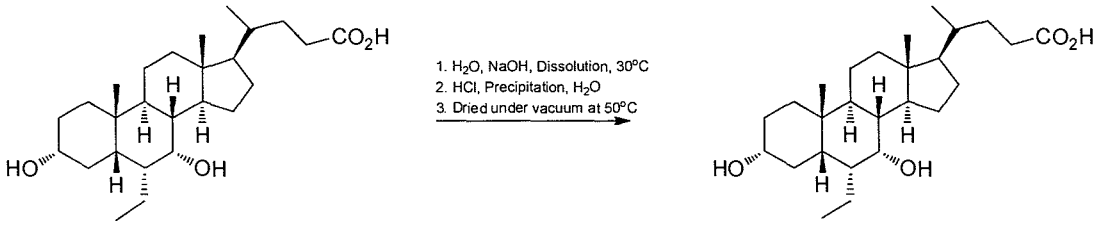
3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid
6

3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid
crystalline obeticholic acid Form C

Reaction 6: Selective reduction of 7-keto group to 7α-hydroxy group

A mixture of compound 6 (86 g, 205.4 mmol), water (688 mL) and 50% sodium hydroxide solution (56.4 mL) was reacted with sodium borohydride (7.77 g, 205.4 mmol) in a mixture of 50% wt. sodium hydroxide solution (1.5 mL) and water (20 mL) at 90 °C to 105 °C. The reaction mixture was heated to reflux and stirred for at least 3 hours. For the workup, after the reaction was complete, the reaction mixture was cooled to approximately 80 °C and transferred to a cooled reactor. At 30 °C to 50 °C, n-butyl acetate (860 mL) and citric acid (320.2 g, anhydrous) in water (491 mL) were added. The aqueous phase was separated and discarded after checking the pH-value to make sure that it was acidic. The organic phase was transferred and distilled. The residue is diluted with n-butyl acetate and was slowly cooled to 15 °C to 20 °C and the crude obeticholic acid was filtered using a centrifuge. The wet product was crystallized from n-butyl acetate. The product obeticholic acid was isolated and washed with n-butyl acetate (43 mL, 4 times) in an inert pressure filter. Drying was done in the pressure filter under vacuum at approximately 80 °C. This led to 67.34 g (77.9%) of crystalline obeticholic acid. See example 3 for full details regarding the identification and characterization of crystalline obeticholic acid.

Step 7 – Preparation of obeticholic acid Form 1:



3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid
cystalline obeticholic acid Form C

obeticholic acid Form 1

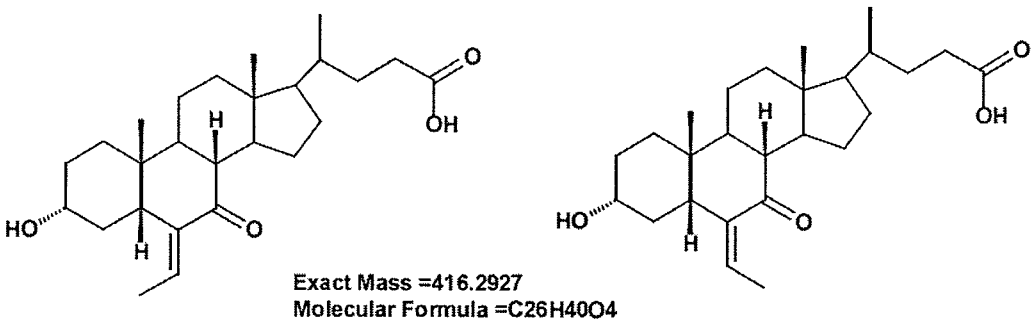
Reaction 7: Preparation of obeticholic acid Form 1 from crystalline obeticholic acid Form C

Crystalline obeticholic acid Form C (58 g) was dissolved in water (870 mL) and caustic soda solution (50%, 8.7 mL, 166 mmol) at 30 °C to 40 °C. The mixture was stirred until all solid has dissolved. The product was precipitated using the following workup. The obeticholic acid solution was slowly added via a filter to diluted hydrochloric acid (37%, 16.05 mL, 193 mmol) in water (870 mL) at 30 °C to 40 °C. The suspension was stirred for approximately 30 minutes at 30 °C to 40 °C and then cooled to not more than (NMT) 20 °C. The product was isolated and washed with water (465 mL, 6 times) in the inert pressure filter. Drying was done in the pressure filter under vacuum at a temperature of NMT 50°C. This led to 53.2 g (91.7%) of obeticholic acid Form 1.

EXAMPLE 2: Characterization of E-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid (5)

Compound 5 is the key intermediate for the process of the application. The compound was isolated from ethyl acetate and was then crystallized from ethanol. The highly pure compound 5 allows for efficient and high yielding production of compound 6 and subsequently crystalline obeticholic acid Form C and obeticholic acid Form 1, including substantially pure obeticholic acid.

The structure of compound 5 from step 4 in example 1 was confirmed using ^{1}H NMR, ^{13}C NMR, and mass spec. Crude product from step 4 resulted in a major product at retention time (RT) 27.457 min and a minor product at RT 28.078 min in the UV chromatogram generated by quality control method 1 by means of LC/MS-coupling. The two products are the E/Z isomers of compound 5:



Exact Mass =416.2927
Molecular Formula =C26H40O4

These two isomers show the same accurate mass and the same fragmentation in the MS/MS spectrum. They cannot be distinguished by the mass spectrometric data.

Using a semi-preparative method to isolate the E/Z isomer peaks, the structures of the E/Z isomers were confirmed using a two stage approach. The HPLC quality control method 1 used a non-volatile phosphoric acid buffer and thus, direct LC/MS coupling with the non-volatile buffer was not possible. Preliminary tests for adjustment of the method showed that only a UPLC method allowed for very high plate numbers for adequate separation of the E/Z isomers. The two stage approach was the following: Step A was identification of the E/Z isomers in two samples with the new developed UPLC/MS method and Step B was isolation of the fraction of the E/Z isomer peaks with the HPLC method 2 and subsequent identification with the UPLC/MS method 1. The experimental details of the methods were as follows:

Table C

1. MS compatible UPLC method (method 1)	
Instrument:	Accela UPL Ccoupling with LTQ FTSpectrometer (ThermoScientific)
Column:	200 x 2mm Hypersil Gold 1.9 µm
Eluent:	A: Water + 10 mM Ammoniumformiat + 0.1% Formic acid B: Acetonitrile
Gradient:	45% B in 20 minutes to 60% B (10 min isocratic)
Flow:	0.4 ml/min, 40 °C column temperature
Detection:	MS: ESI positive and negative ions; UV: PDA 200.600 nm
Mass resolution:	R=100000 ICR
Sample:	1 mg / ml in water/acetonitrile (1:1), 3µl/20µl injected
2. HPLC (method 2)	
Instrument:	Agilent1100 HPLC (Agilent Technologies)
Column:	125 x 4mm Purospher STAR C18 5 Lm
Eluent:	A: Water pH 2.6 with phosphoric acid B: Acetonitrile

Gradient:	30 % B in 10 minutes to 35% B in 30 minutes to 60% B In 1 minutes to 90% B (9 min isocratic)
Flow:	1 ml/min, 35 °C column temperature
Detection:	UV: DAD 200 – 400 nm (UVA 200 nm)
Sample:	10 mg / ml in water/acetonitrile (9:1), 25µl injected

The results are shown in Figures 1 and 2. Figures 1 and 2 are UPLC UV/MS chromatograms for "crude compound 5" (Figure 1) and compound 5 "purified reference" (Figure 2) obtained on a high performance UPLC column. For Figure 1, the sample was dissolved at 1 mg/mL in ACN/H_20 1:1; 200x2mm Hypersil GOLD R122; LMA:H20+10mM AF + 0.1%HFo; LMB:ACN; 45%-20-60%(10); 0.4mL/min; 40°C; UVA=200nm; 3µL injection volume. For Figure 2, the sample was dissolved at 1 mg/ml in ACN/H20; 200x2mm Hypersil GOLD R122; A: 10mM AF + 0.1%HFo; B:ACN; 45%-20-60%B(10); 0.4mL/min; 20µL injection volume. In both samples, the molecular weight of the main component (RT 9.92 min) and of the minor component (RT 10.77 min) was the same as expected and the accurate masses of the two compounds were consistent with the structures provided as shown in Tables D and E of data of the positive and negative ion measurement show below:

Table D: Data of the positive ion measurement

RT(min)	Ion m/z	Formula	Structure proposal
9.98	417.30008	$C_{26}H_{41}O_4$ ΔM 0.35 ppm	M+H E Isomer
	833.59381	$C_{52}H_{81}O_8$ ΔM 1.45 ppm	2M +H E Isomer
	850.61938	$C_{52}H_{84}O_8N$ ΔM 0.28 ppm	2M + NH4 E Isomer
10.77	417.30023	$C_{26}H_{41}O_4$	M+H Z Isomer
	833.59409	$C_{52}H_{81}O_8$	2M +H Z Isomer
	850.61984	$C_{52}H_{84}O_8N$	2M + NH4 Z Isomer

Table E: Data of the negative ion measurement

RT(min)	Ion m/z	Formula	Structure proposal
9.98	415.28520	$C_{26}H_{39}O_4$ ΔM -0.44 ppm	M-H Z Isomer
	461.29051	$C_{27}H_{41}O_6$ ΔM -0.76 ppm	M + Formiat Z Isomer
	831.57683	$C_{52}H_{79}O_8$ ΔM -1.46 ppm	2M - H Z Isomer
10.77	415.28545	$C_{26}H_{39}O_4$	M-H E Isomer
	461.29069	$C_{27}H_{41}O_6$	M + Formiat E Isomer
	831.57739	$C_{52}H_{79}O_8$	2M - H E Isomer

To ensure the portability of the quality control HPLC method 2, the original separation was repeated exactly under the prescribed conditions. The main peak and the minor peak were isolated as semipreparative. The resulting UV chromatogram with the marked positions of the trapped fractions is shown in Figure 3. Figure 3 is a UV chromatogram of crude compound 5 using HPLC method 2; 125x4mm Purospher STAR C18 5µm AG; LMA:H2O pH 2.6mit H_3PO_4; LMB:ACN; 30%B-10-35%-30-60%-1-90%(9); 1 mL/min; 35°C; UVA=200nm; ohne MS; 25 mL. Subsequently, the isolated fractions were separately analyzed with the newly developed UPLC/MS method. For the evaluation of the accurate ion trace of the quasimolecular ion [2M+NH4] at 850.61914±3ppm was used. The resulting chromatograms of the main peak fraction, the minor peak fraction and of the two samples are shown in Figure 4 (A-D). The MS studies showed that the two peaks generated by quality control method 2 at RT 27.457 min and at RT 28.078 min are two isomers with the formula $C_{26}H_{40}O_4$. This formula is consistent with the structure proposed for the E/Z isomers. Thus, the development of the UPLC-MS method has shown that the E/Z isomers of 3α-hydroxy-ethyliden-7-keto-5β-cholic-24 acid are chromatographically separable with high resolution. The accurate MS data from the FR-ICR mass spectrometer are consistent with the structure proposed for the E/Z isomers. For both isomers, the same formula $C_{26}H_{40}O_4$ was derived.

Due to the semi-preparative isolation of the E/Z-isomer peaks with HPLC method 2 and subsequent identification with the UPLC-MS method we can show that the two peaks generated by the quality control method 2 (RT 27.457 minutes and RT 28.078 minutes, see figure 1) are the two isomers with the formula $C_{26}H_{40}O_4$. This formula is consistent with the structure proposal of the E/Z-isomers. In conjunction with the NMR results described below the following assignments were obtained: RT 27.457 minutes belongs to the E-isomer and RT 28.078 minutes to the Z-isomer.

The assignment of the ^{1}H and ^{13}C shifts for the E isomer of 3α-hydroxy-ethyliden-7-keto-5β-cholic-24 acid are shown below. Shifts were estimated according to "L. Bettarello et al., II Farmaco 55 (2000), 51-55 (substance 3α-hydroxy-7-keto-5β-cholan-24-oic acid).

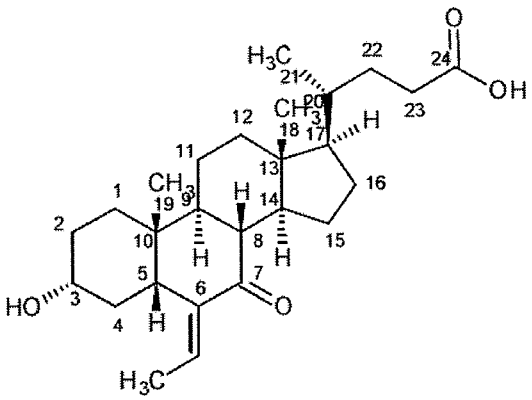


Table F: ^{1}H Shift Assignment (^{1}H-NMR, 500 MHz, 303K, CD_3OD)

Chemical shift [ppm]	Intensity [H]	Multiplicity	Assignment
6.10	1	Q	25
3.61	1	M	3
2.69	1	DD	5
2.28	2	DT	23
1.72	3	D	26
1.05	3	S	19
0.99	3	D	21
0,70	3	S	18

Table G: ^{13}C Shift Assignment (^{13}C-NMR, 125 MHz, 303K, CD_3OD)

Chemical shift [ppm]	Multiplicity	Assignment
207.5	S	7
178.1	S	24
145.3	S	6
130.4	D	25
71.0	D	3
56.0	S	17
52.0 and 50.1	D each	8 and 14
46.9	D	5
44.7	S	13
40.7	D	9
40.3	T	12*
38.3	T	4*
36.5	D	20
35.8	S	10
35.4	T	1
32.3 and 32.0	T each	22 and 23
30.5	T	2*
29.4	T	16*
27.0	T	15*
23.2	Q	19
22.4	T	11
18.9	Q	21
12.7	Q	26
12.5	Q	18

S = singlet
D = doublet
T = triplet
Q = quartet
M = multiplet
DD = doublet of doublets
DT = doublet of triplets

EXAMPLE 3: Characterization of crystalline obeticholic acid Form C

Full solid-state characterization of the product from step 6 of Scheme 1 and Example 1 showed that the obeticholic acid is crystalline. This crystalline form is labeled Form C. Below is a table that summarizes the characterization of crystalline obeticholic acid Form C:

Table G: Summary of Crystalline Obeticholic Acid Form C Characteristics

Technique	Crystalline Obeticholic Acid Form C
appearance	White powder
NMR	Consistent with supplied structure ca. 3.5% w/w heptane
XRPD	Crystalline

TGA	Weight losses between r.t. to 85 °C (0.4%) and 85-115 °C (4.1%)
DSC	Endotherm with onset of 97.9 °C
GVS	Slightly hygroscopic, 1.2 % water uptake at 90% RH
Karl Fisher Water Determination	1.5 % w/w
Stability at 40 °C/75% RH	No change in form or crystallinity

Thermal Analysis

DSC (Differential Scanning Calorimetry) data were collected on a Mettler DSC 823e equipped with a 34 position auto-sampler. The instrument was calibrated for energy and temperature using certified indium. Typically 0.5-1 mg of each sample, in a pin-holed aluminium pan, was heated at 10 °C·min^{-1} from 25 °C to 350 °C. A nitrogen purge at 50 ml·min^{-1} was maintained over the sample. The instrument control and data analysis software was STARe v 9.20.

TGA (Thermo-Gravimetric Analysis) data were collected on a Mettler TGA/SDTA 851e equipped with a 34 position auto-sampler. The instrument was temperature calibrated using certified indium. Typically 5-10 mg of each sample was loaded onto a pre-weighed aluminium crucible and was heated at 10 °C·min^{-1} from ambient temperature to 300 °C. A nitrogen purge at 50 ml·min^{-1} was maintained over the sample. The instrument control and data analysis software was STARe v 9.20.

Two weight loss steps were observed by TGA of crystalline obeticholic acid Form C. The first took place between room temperature (r.t.) and 85 °C (0.41 %) and the second occurred between 85 °C-115 °C (4.10 %). The first weight loss step can be attributed to water loss with the second step being attributed to the loss of the remaining water (water responsible for around 1.2 % weight loss) and the loss of bound heptane (ca. 3.4 % weight loss). Crystalline obeticholic acid Form C contained between 0.15 and 0.2 moles solvent (heptane) and ca. 1.5 % w/w (0.3 moles). The DSC thermogram of crystalline obeticholic acid Form C contained one endotherm. This was fairly sharp and had an onset of around 98 °C. See Figure 6. Different solvents would have different boiling points and therefore would evaporate at different temperatures within the DSC and TGA experiments.

X-Ray Powder Diffraction (XRPD) Analysis

Bruker AXS C2 GADDS

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu Kα radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for auto sample positioning and a HiStar 2-dimensional area detector. X-ray optics consisted of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm. A weekly performance check was carried out using a certified standard NIST 1976 Corundum (flat plate).

The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 4 mm. A θ-θ continuous scan mode was employed with a sample - detector distance of 20 cm which gives an effective 2θ range of 3.2 ° – 29.7 °. Typically the sample was exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analyzed and presented using Diffrac *Plus* EVA v 9.0.0.2 or v 13.0.0.2.

Ambient conditions: Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface.

Non-ambient conditions: Samples run under non-ambient conditions were mounted on a silicon wafer with heat-conducting compound. The sample was then heated to the appropriate temperature at ca. 10 °C·min^{-1} and subsequently held isothermally for ca. 1 minute before data collection was initiated.

Bruker AXS/Siemens D5000

X-Ray Powder Diffraction patterns were collected on a Siemens D5000 diffractometer using Cu Kα radiation (40 kV, 40 mA), θ-θ goniometer, divergence of V20 and receiving slits, a graphite secondary monochromator and a scintillation counter. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was Diffrac *Plus* XRD Commander v2.3.1 and the data were analyzed and presented using Diffrac *Plus* EVA v 11,0.0.2 or v 13.0.0.2.

Samples were run under ambient conditions as flat plate specimens using powder as received. Approximately 20 mg of the sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to 42 °2θ
- Step size: 0.05 °2θ
- Collection time: 4 s·step^{-1}

Bruker AXS D8 Advance

X-Ray Powder Diffraction patterns were collected on a Bruker D8 diffractometer using Cu Kα radiation (40 kV, 40 mA), θ-2θ goniometer, and divergence of V4 and receiving slits, a Ge monochromator and a Lynxeye detector. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was Diffrac *Plus* XRD Commander v 2.5.0 and the data were analyzed and presented using Diffrac *Plus* EVA v 11.0.0.2 or v 13.0.0.2.

Samples were run under ambient conditions as flat plate specimens using powder as received. Approximately 5 mg of the sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to 42 °2θ
- Step size: 0.05 °2θ
- Collection time: 0.5 $s \cdot step^{-1}$

XRPD showed the powder of isolated from step 6 of the process of the invention was collected on Bruker AXS D8 Advance. See Figure 5. The corresponding data for the X-ray diffractogram is presented in the table below.The software used for data collection was Diffrac *Plus* XRD Commander v2.6.1 and the data were analysed and presented using Diffrac *Plus* EVA v13.0.0.2 or v15.0.0.0. Samples were run under ambient conditions as flat plate specimens using powder as received. The sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to 42 °2θ
- Step size: 0.05 °2θ
- Collection time: 0.5 $s \cdot step^{-1}$

Table H: X-ray Diffractogram Data of Crystalline Obeticholic Acid Form C

peak	Angle 2-Theta (deg)	d value (Angstrom)
1	4.2	21.0203
2	6.35	13.90839
3	8.298	10.64718
4	9.5	9.30229
5	11.05	8.00042
6	12.246	7.22192
7	12.498	7.07692
8	12.647	6.99367

9	15.497	5.71337
10	15.843	5.5895
11	15.998	5.53561
12	16.346	5.41836
13	16.695	5.30601
14	16.996	5.21251
15	17.849	4.96547
16	18.593	4.76844
17	18.798	4.71689
18	19.047	4.65579
19	20.493	4.33041
20	20.894	4.24808

VT-XRPD (Variable Temperature-X-ray Diffraction) revealed that the endotherm seen in the DSC thermogram corresponded to the desolvation of the sample as no form changes were observed on heating. A temperature difference exists between the DSC and the VT-XRPD data as the VT-XRPD experiment was carried out in a large space with the sample exposed whereas the DSC experiment was carried out in a confined, closed space. This difference is around 20 °C and explains why the sample melted at a much lower temperature in the DSC experiment and the sample still appears crystalline at 110 °C in the VT-XRPD experiment. VT-XRPD shows that drying of the solvent from the material resulted in loss of crystallinity which is consistent with the material being in a solvated form. See Figure 7.

Gravimetric Vapour Sorption (GVS)

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyzer, controlled by DVS Intrinsic Control software v 1.0.0.30. The sample temperature was maintained at 25 °C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 $ml \cdot min^{-1}$. The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0-100% RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of % RH (relative humidity) was constantly monitored by the microbalance (accuracy ±0.005 mg).

5 to 20 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40% RH and 25 °C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25 °C at 10 % RH intervals over a 0.5-90 % RH range. Data analysis was undertaken in Microsoft Excel

using DVS Analysis Suite v6.0.0.7. Method Parameters for SMS DVS Intrinsic Experiments are as follows:

Parameters	Values
Adsorption - Scan 1	40 – 90 %
Desorption / Adsorption - Scan 2	90 - 0, 0 – 40 %
Intervals (% RH)	10
Number of Scans	2
Flow rate ($ml \cdot min^{-1}$)	200
Temperature (°C)	25
Stability ($°C \cdot min^{-1}$)	0.2
Sorption Time (hours)	6 hour time out

The sample was recovered after completion of the isotherm and re-analyzed by XRPD.

Analysis of crystalline obeticholic acid Form C showed that the sample was slightly hygroscopic as a mass increase of 1.18% was noted between 0-90% RH. This uptake of water was steady throughout the analysis and equilibrium was reached for all steps. The hysteresis of the curve was small indicating that the sample readily lost the water it had taken up. XRPD analysis after the GVS analysis showed that the sample was unchanged. See Figures 8A, 8B, and 8C.

Water Determination by Karl Fischer Titration (KF)

The water content of each sample was measured on a Mettler Toledo DL39 Coulometer using Hydranal Coulomat AG reagent and an argon purge. Weighed solid samples were introduced into the vessel on a platinum TGA pan which was connected to a subaseal to avoid water ingress. Approx 10 mg of sample was used per titration and duplicate determinations were made.

Karl Fischer analysis showed that crystalline obeticholic acid Form C contained 1.5% water which corresponds to about 0.3 moles water.

One Week Stability at 40 °C and 75% RH

The stability of obeticholic acid at 40 °C and 75% RH (relative humidity) was determined as follows. A sample of obeticholic acid was stored in a humidity chamber for one week at 40 °C/75 % RH. The sample was re-analyzed by XRPD and was found to have been unchanged.

The solid state study has shown that the presence of a relatively large amount of organic solvent is required to crystallize obeticholic acid Form C. It is highly unlikely that a sample of obeticholic acid Form 1 will spontaneously crystallize to form crystalline obeticholic acid Form C on storage.

5

EXAMPLE 4: Obeticholic Acid Tablet formulation

The table below shows the quantitative composition of obeticholic acid tablets. The 5 mg, 10 mg, and 25 mg formulations have been used as phase 3 clinical trial material.

Table I: Film Coated Tablet

Film Coated Tablet			
Component	**Quantity per Tablet**	**Function**	**Reference to Standard**
1 mg tablet			
Obeticholic acid	1.0 mg*	API	HSE
Microcrystalline cellulose	185.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green or white	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
5 mg tablet			
Obeticholic acid	5.0 mg*	API	HSE
Microcrystalline cellulose	181.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green or white	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
10 mg tablet			
Obeticholic acid	10.0 mg*	API	HSE
Microcrystalline cellulose	176.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Opadry® II green or white	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		
25 mg tablet			
Obeticholic acid	25.0 mg*	API	HSE
Microcrystalline cellulose	157.0 mg*	Filler/Binder	USP-NF/EP/JP
Sodium starch glycolate	12.0 mg	Disintegrant	USP-NF/EP/JP
Magnesium stearate	2.0 mg	Lubricant	USP-NF/EP/JP
Collodial silicon dioxide	4.0 mg	Glidant	USP-NF/EP/JP

Opadry® II green or white	8.0 mg	Coating Material	HSE
Total weight	208.0 mg		

API: Active pharmaceutical ingredient
HSE = In house specification
USP-NF = US Pharmacopeia National Formulary
Ph Eur = European Pharmacopeia
JP =Japanese Pharmacopeia
* obeticholic acid quantity presented assumes API is anhydrous and 100% pure; actual amount is adjusted based on the potency of the drug substance Lot used, and amount of microcrystalline cellulose is correspondingly decreased.

EXAMPLE 5: Characterization of Obeticholic Acid Form 1

Obeticholic acid Form 1 refers to the non-crystalline form of obeticholic acid. This form of obeticholic acid can be produced via a crystalline obeticholic acid as a synthetic intermediate. Obeticholic acid Form 1 can be used as the pharmaceutically active ingredient. Obeticholic acid Form 1 was characterized and analyzed as follows.

Batch 1 of obeticholic acid form 1 was characterized using the following techniques: assessment by X-ray powder diffraction (XPRD) for crystallinity, ^{1}H and ^{13}C nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FT-IR), optical assessment (e.g., particle shape/size), thermal properties (e.g., differential scanning calorimetry (DSC) and thermo-gravimetric analysis (TGA)), water determination by Karl Fischer (KF), storage at 40°C and 75 %RH and reanalysis after 2 weeks by XRPD, pKa by potentiometric method, Log P/D (octanol/water) by potentiometry, and stability to moisture using gravimetric vapour sorption (GVS; e.g., complete sorption-desorption cycle with analysis of solid collected by XRPD). Five other batches (e.g., batch 2, 3, 4, 5, and 6) of obeticholic acid Form 1 were also characterized and compared using the following techniques: assessment by XRPD and comparison to main batch 1 pattern, ^{1}H and ^{13}C NMR, FT-IR, optical assessment (e.g., particle shape/size), thermal properties (e.g., DSC, TGA, and hot-stage microscopy), and water determination by KF.

X-Ray Powder Diffraction (XRPD) Analysis

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu Kα radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for auto-sample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm. The beam divergence, i.e. the effective size of the X-ray beam on the sample,

was approximately 4 mm. A θ-θ continuous scan mode was employed with a sample – detector distance of 20 cm which gives an effective 2θ range of 3.2 ° – 29.7 °. Typically the sample was exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analyzed and presented using Diffrac *Plus* EVA v 9.0.0.2 or v 13.0.0.2.

Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a silicon wafer to obtain a flat surface. The diffractograms show that obeticholic acid Form 1 is non-crystalline (See, Figure 10 and Figure 11).

NMR Characterization

NMR spectra were collected on a Bruker 400 MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICONNMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 8) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone. Samples were prepared in *d6*-DMSO, unless otherwise stated. Off-line analysis was carried out using ACD SpecManager v 9.09 (build 7703).

Figure 12 shows the ^{1}H NMR spectrum for batch 1. ^{1}H NMR spectra of batches 2-6 were also recorded and compared with the spectrum of batch 1. See Figure 13. The spectra are all similar, but with varying amounts of water. Some differences are noted in the integration of the large group of protons between 0.75 ppm and 2 ppm, where peaks overlap and cannot be integrated separately. Table J shows the total number of protons integrated in the spectra of batches 1-6, taking into account the variation in the 0.75 – 2 ppm region.

Table J

Batch number	Number of H by integration (excluding COOH)
1	43
2	42
3	40
4	41
5	42
6	41-42

The carboxylic acid proton has been excluded, so the number of protons should be 43, but it actually varies from 40 to 43 between the 6 spectra. However, the area where the variation comes from (0.75-2 ppm) is quite wide, and due to the quality of the baseline, this integration cannot be relied upon.

As the spectrum could not be fully assigned and the integration varied, a ^{13}C NMR spectrum of batch 2 was recorded. Figure 14 shows the DEPTQ spectrum, where CH_2 and quaternary carbons peaks point up, while CH_3 and CH groups point down. There are thirteen peaks pointing down, which correspond to nine CHs and four CH_3 groups. This is consistent with the structure. The peak of the carbon of the carboxylic acid was seen at 175 ppm. It has been excluded from this expanded view for clarity of the area of interest. However, there are only eleven peaks pointing up, whereas there should be twelve, as there are ten CH_2 groups and two quaternary carbons in the molecule (excluding the carbonyl). One carbon appears to be overlapping with another signal. Therefore, a DEPT135 spectrum was collected, suppressing the quaternary carbon signals, which could show whether the overlapping signal is quaternary. See Figure 15. A comparison of the DEPT135 spectrum with the DEPTQ spectrum shows that one peak (at 42.5 ppm) disappears. There are two quaternary carbons in the molecule, which should correspond to two peaks disappearing. Therefore the overlapping carbon signal is a quaternary one.

Further, an experiment to determine the relaxation time of the carbons was carried out to determine where the missing quaternary carbon signal is overlapping with another carbon signal. See Figure 16. This ^{13}C spectrum contains peaks that were integrated. This showed that peak at 32.3 ppm accounts for two carbons. See Figure 17 for an expanded view of the peak at 32.3 ppm. Thus, twenty-six carbons are now accounted for by integrations (including the carboxylic acid), which is consistent with the structure.

FT-IR by ATR

Data were collected on a Perkin-Elmer Spectrum One fitted with a Universal ATR sampling accessory. The data were collected and analyzed using Spectrum v5.0.1 software. See Figure 18.

Thermal Analysis by Differential Scanning Calorimetry (DSC) and Thermo-Gravimetric Analysis (TGA)

DSC data were collected on a TA Instruments Q2000 equipped with a 50 position autosampler. The instrument was calibrated for energy and temperature calibration using certified indium. Typically 0.5-3 mg of each sample, in a pin-holed aluminium pan, was heated at 10 °C.min^{-1} from 25 °C to 300 °C. A nitrogen purge at 50 ml.min^{-1} was maintained over the sample. The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3 and the data were analyzed using Universal Analysis v4.3A. For modulated DSC, the sample was prepared as before, and the pan was heated at 2 °C.min^{-1} from 25 °C to 200 °C. Modulator conditions were an amplitude of 0.20 °C and a periodicity of 40 s. The sampling interval was 1 sec/pt.

TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position autosampler. The instrument was temperature calibrated using certified Alumel. Typically 5-10 mg of each sample was loaded onto a pre-tared platinum crucible and aluminium DSC pan, and was heated at 10 °C.min^{-1} from ambient temperature to 350 °C. A nitrogen purge at 60 ml.min^{-1} was maintained over the sample. The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3.

Thermal analysis of batch 1 was performed by DSC and TGA. The TGA trace (see Figure 19) shows a weight loss of 1.7% between ambient temperature and 121 °C, which is likely to be loss of water. The DSC trace (see Figure 19) shows a broad low temperature endotherm, probably corresponding to the loss of water, followed by a small endotherm with onset at 94 °C.

This second endotherm might indicate a glass transition and was further investigated by modulated DSC (see Figure 20). This technique enables reversible events, such as a glass transition, to be separated from irreversible ones, such as loss of solvent or a melt of a crystalline form. The reversible heat flow trace in modulated DSC shows the glass transition as a step with an inflexion point (Tg) at 95 °C. This is high for a glass transition and suggests that Form 1 is stable. The small endotherm with onset at 89 °C on the non-reversible heat flow trace corresponds to molecular relaxation of the bulk material at the glass transition temperature.

The DSC trace (see Figure 19) shows decomposition starting around 220 °C, which also corresponds to the TGA trace curving down.

The TGA traces of batches 1, 2, 3, 4, 5, and 6 are of similar shape (Figure 21). The weight losses measured between ambient and 120°C are shown in Table K. They are consistent with the varying amounts of water observed by NMR. These amounts were

further quantified by Karl Fischer (KF) water titration. See water determination by FK.

Table K: Summary of TGA weight losses of received samples

Batch number	Weight loss by TGA
1	1.7%
2	0.6%
3	1.2%
4	0.9%
5	1.5%
6	1.6%

Figure 22 shows the DSC traces of the six batches for comparison. The traces are similar, with a broad low temperature endotherm of varying size, consistent with varying amounts of water, followed by a small endotherm around the glass transition temperature as seen in section DSC and TGA. The results are summarized in Table L.

Table L: Summary of DSC results of received samples

Batch number	1st endotherm, broad	2nd endotherm, small	Start of decomposition
1	28.3J/g, Tmax = 64°C	1.2J/g, Tonset =94°C	220°C
2	7.4J/g, Tmax =48°C	1.4J/g, Tonset =94°C	220°C
3	none	2.0J/g, Tonset =89°C	175°C
4	14.5J/g, Tmax = 58°C	1.3J/g, Tonset =94°C	200°C
5	12.2J/g, Tmax = 59°C	1.2J/g, Tonset =94°C	175°C
6	28.7J/g, Tmax =59°C	1.5J/g, Tonset =94°C	200°C

Polarized Light Microscopy (PLM)

Samples were studied on a Leica LM/DM polarized light microscope with a digital video camera for image capture. A small amount of each sample was placed on a glass slide, mounted in silicone oil and covered with a glass slip, the individual particles being separated as well as possible. The sample was viewed with appropriate magnification and partially polarized light, coupled to a λ false-color filter.

Figures 23A-23F show that batches 1, 2, 3, 4, 5, and 6 are material made up of large hard agglomerates of small irregular particles. Batches 1, 2, 3, 4, 5, and 6 all look similar. No birefringence was observed under plane polarized light, which is consistent with the material being non-crystalline. Particle size ranges from less than 1μm to 3 μm. The small size of these particles suggests that they have been precipitated out very quickly.

Gravimetric Vapour Sorption (GVS)

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyzer, controlled by SMS Analysis Suite software. The sample temperature was maintained at 25 °C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 $ml.min^{-1}$. The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0-100 % RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly monitored by the microbalance (accuracy ± 0.005 mg).

Typically 5-20 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40% RH and 25 °C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25 °C at 10% RH intervals over a 0.5-90 %RH range.

Table M

Parameters	Values
Adsorption - Scan 1	40 - 90
Desorption / Adsorption - Scan 2	85 - Dry, Dry - 40
Intervals (%RH)	10
Number of Scans	2
Flow rate ($ml.min^{-1}$)	200
Temperature (°C)	25
Stability ($°C.min^{-1}$)	0.2
Sorption Time (hours)	6 hour time out

The Gravimetric Vapour Sorption (GVS) isotherm was obtained for batch 1 at 25 °C and is shown in Figure 24. The sample appears to be moderately hygroscopic, with a total weight change of 3.8% from 0 to 90% relative humidity (RH). The hysteresis (area between adsorption and desorption curves) is small, indicating that the solid releases quite readily the water adsorbed. No formation of hydrate is observed. There was no significant weight change after the whole experiment (0.3%).

The kinetics plot of the GVS (Figure 25) shows that the adsorption of the water occurred mostly at very high humidities and the desorption at very low humidities. On the adsorption phase, the sample reached equilibrium quite quickly up to 80% RH and took longer to equilibrate at 90% RH. On desorption, the mass stabilized at all steps.

After completion of the GVS, the sample was recovered and reanalyzed by XRPD, which showed that the material was still non-crystalline (Figure 26).

Water Determination by Karl Fischer (KF)

The water content of each sample was measured on a Mettler Toledo DL39 Coulometer using Hydranal Coulomat AG reagent and an argon purge. Weighed solid samples were introduced into the vessel on a platinum TGA pan, which was connected to a subaseal to avoid water ingress. Approximately 10 mg of sample was used per titration and duplicate determinations were made.

Titration of water by coulometric Karl Fischer gave a result of 2.4 wt% water. This is slightly higher than the weight loss observed by TGA. It could mean that some of the water is not released from the material on heating, but it is likely to be due to the different experimental procedures for these two techniques.

The water content of each batch was determined by coulometric Karl Fischer. Table N shows these results and compares them with earlier Karl Fischer results obtained and with the weight losses observed by TGA. Data are consistent as the trend is the same in all three analyses. The Karl Fischer data obtained earlier show lower amounts of water than the results obtained here. This is consistent with the material being hygroscopic, although some samples have taken up more water than others. TGA weight loss is consistently lower than the results obtained by Karl Fischer titration, which might mean that some water stays trapped in the material and is not released on heating but might also be due to the experimental procedure.

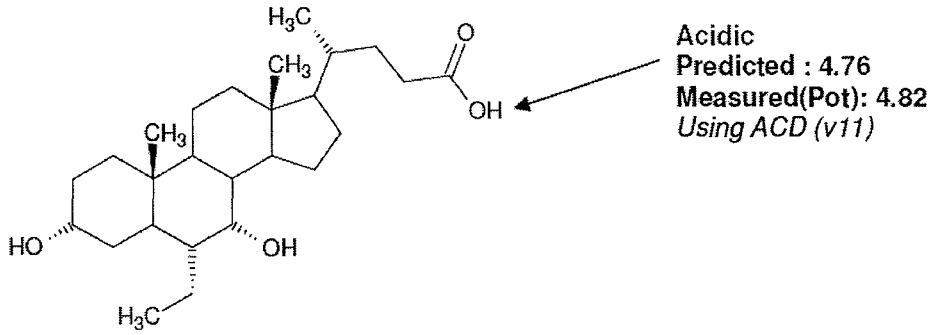
Table N: Karl Fischer (KF) results and summary of water content data

Batch number	KF water content	Earlier KF results	TGA weight loss
1	2.4%	2.1%	1.7%
2	1.9%	0.4%	0.6%
3	2.5%	1.4%	1.2%
4	2.2%	0.92%	0.9%
5	2.3%	0.53%	1.5%
6	2.8%	2.1%	1.6%

pKa Determination and Prediction

pKa determination data were collected on a Sirius GlpKa instrument with a D-PAS attachment. Measurements were made at 25 °C in aqueous solution by UV and in methanol water mixtures by potentiometry. The titration media was ionic-strength adjusted (ISA) with 0.15 M KCl (aq). The values found in the methanol water mixtures were corrected to 0 % co-solvent via a Yasuda-Shedlovsky extrapolation. The data were

refined using Refinement Pro software v1.0. Prediction of pKa values was made using ACD pKa prediction software v9.



The pKa of obeticholic acid was measured by potentiometry using methanol as a cosolvent (Figure 27) and extrapolated to 0% co-solvent using a Yasuda-Shedlovsky extrapolation (Figure 28). The pKa enables determination of the proportion of the neutral and the ionized form of the compound at a given pH. Figure 29 shows the distribution of the species depending on pH.

Log P Determination

Data were collected by potentiometric titration on a Sirius GlpKa instrument using three ratios of octanol: ionic-strength adjusted (ISA) water to generate Log P, Log P_{ion}, and Log D values. The data were refined using Refinement Pro software v1.0. Prediction of Log P values was made using ACD v9 and Syracuse KOWWIN v1.67 software.

Table O: Predicted and measured LogP

ACD (V9) Predicted LogP	5.81
Measured LogP	5.54
Measured LogPion	1.58
Measured LogD7.4	2.98

LogP was predicted using ACD software then measured by potentiometry. Three titrations were performed at three different octanol / ISA water ratios, giving the difference curve plotted in Figure 30. The black curve is the pure aqueous pKa titration and the 3 colored curves correspond to the three octanol / ISA water ratios. The shifts in pKa enable determination of LogP.

The lipophilicity curve (logD as a function of pH) is shown in Figure 31. Log D is the distribution coefficient, representing the combined lipophilicity of all species present at a specific pH. LogP is a compound constant, which corresponds to the partition coefficient of the pure neutral species, while LogPion is that of the pure ionized species. LogP and LogPion can be determined from the lipophilicity curve, as the intersection of the Y axis with respectively the tangent at the start of the pH scale (when the molecule is purely in its neutral form) and the tangent at the end of the pH scale (when the molecule is completely ionized).

Two Weeks Stability at 40°C & 75% RH and 25°C & 97% RH

A sample of batch 1 was stored at 40°C and 75% relative humidity (RH) in an accelerated stability testing of the solid form. Another sample was stored at 25°C and 97% relative humidity to check the effect of very high humidity. Both samples were re-analyzed by XRPD after five days and after two weeks. Both samples remained non-crystalline under the two storage conditions for up to two weeks, showing that Form 1 is stable to these conditions. See Figure 32 and Figure 33.

The six batches analyzed were all non-crystalline. The glass transition temperature was measured at 95°C with a modulated DSC experiment. The six batches appeared very similar with all analytical techniques used, the only difference between them being their water content, which varied from 1.9% to 2.8% by Karl Fischer titration. Thermal analysis showed the varying amount of water and indicated decomposition starting around 175-220°C. Measured pKa was 4.82 and LogP is 5.54. Microscopic evaluation showed large hard agglomerates of very small irregular particles.

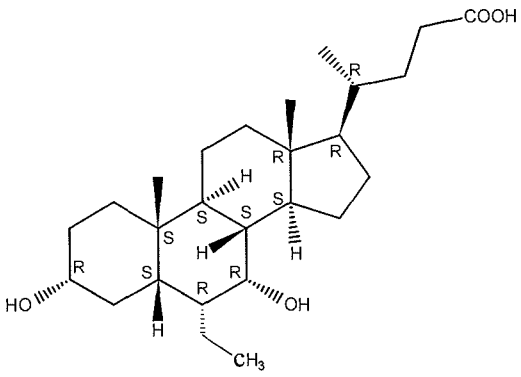
Stability testing showed that the material was still non-crystalline after two weeks under accelerated conditions (40°C / 75% RH) or under high humidity (25°C / 97% RH). Gravimetric Vapour Sorption (GVS) analysis showed the material is only moderately hygroscopic, with a total weight gain of 3.8% from 0 to 90% relative humidity (RH). No hydrate formation was observed under GVS. The sample re-analyzed by XRPD after GVS was still non-crystalline. The high glass transition temperature and the stability testing results suggest that the non-crystalline form is stable.

EXAMPLE 6: Single Crystal X-ray Structure and Absolute Stereochemistry

The single crystal X-ray structure of obeticholic acid was determined from a crystal obtained from the recrystallization of obeticholic acid from an acetonitrile solution after cooling to 5°C at 0.1°C/min followed by maturation at RT/50°C 8 h cycles for 1 week (see Figure 34). The structure is consistent with Form G and a simulated XRPD pattern has been generated as a reference pattern for this material. Form G can be prepared by cooling a solution of obeticholic acid in e.g., acetonitrile.

The structure is orthorhombic, space group $P2_12_12_1$, and contains one molecule of obeticholic acid in the asymmetric unit. Final R1 [$I>2\sigma(I)$] = 3.22 %. The crystal exhibited prism morphology of approximate dimensions 0.4 x 0.4 x 0.3mm. The absolute stereochemistry of the molecule was determined as *S* at chiral centres C5, C9, C10 and C14 and *R* at chiral centres C3, C6, C7, C8, C13, C17 and C22 with a Flack parameter = -0.01 (13). For the inverted structure with chiral centres C5, C9, C10 and C14 in the *R* configuration and chiral centres C3, C6, C7, C8, C13, C17 and C22 in the *S* configuration, the Flack parameter = 1.01(13), confirming the assignation mentioned above.

Overall, the structure had a strong data set and no disorder.



The software used to assign the stereochemistry (PLATON) determines the chiral centre (C8) as an *R* stereocentre, whereas ACD software (and the Cahn-Ingold-Prelog) assignment for (C8) is *S*. However, the assignment of the trans ring junction for B/C ring system is absolutely defined from the crystal structure.

Determination of the absolute structure using Bayesian statistics on Bijvoet differences, (Hooft *et al.*, *J. Appl. Cryst.*, (2008), 41, 96-103), reveals that the probability of the absolute structure as presented being correct is 1.000, while the probabilities of the

absolute structure being a racemic twin or false are 0.000 and 0.000 respectively. The Flack equivalent and its uncertainty are calculated through this program to be -0.019(17).

The structure of obeticholic acid contains one 5 membered ring and 3 six membered rings which are fused together. Conformational analysis on the 5 membered ring (C13, C14, C15, C16 and C17)) reveals that the closest puckering descriptor for this ring is a half-chair. Conformational analysis on the three 6 membered rings (C1, C2, C3, C4, C5 and C10); (C5, C6, C7, C8, C9 and C10) and (C 8, C9, C11, C12 C13 and C14) reveals that the closest puckering descriptor for these rings is a chair.

Two unique intermolecular hydrogen bonds are observed in the crystal structure. Each molecule of obeticholic acid forms a hydrogen bond to two different symmetry related molecules of obeticholic acid, with the oxygens, O1 and O4, acting as donors to the oxygens, O3 and O1 respectively, acting as acceptors, O1— H1C---O3 [D···A = 2.7419(12)Å] and O4— H4C---O1 [D···A = 2.6053(13)Å (see Figure 35). These interactions result in a complex 3 dimensional hydrogen bonded network. The final Fourier difference map shows maximal and minimal electron densities of 0.402 and -0.176 $eÅ^{-3}$, respectively.

An overlay of the calculated XRPD pattern for the structure with the experimental batches shows that the crystal is consistent with the bulk and is obeticholic acid Form G (see Figure 36).

Table 1. Crystal data for obeticholic acid Form G

Crystallization solvents	Acetonitrile	
Crystallization method	Maturation at RT/50°C	
Empirical formula	$C_{26}H_{44}O_4$	
Formula weight	420.63	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal size	0.40 x 0.40 x 0.30 mm	
Crystal habit	Colourless Prism	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.72510(10) Å	α = 90°
	b = 12.69860(10) Å	β = 90°
	c = 22.5408(2) Å	γ = 90°
Volume	2497.44(4) $Å^3$	

Z	4
Density (calculated)	1.119 Mg/m^3
Absorption coefficient	0.574 mm^{-1}
F(000)	928

Table 2. Data collection and structure refinement for obeticholic acid Form G

Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Radiation source	SuperNova (Cu) X-ray Source, CuKα
Data collection method	omega scans
Theta range for data collection	9.15 to 74.49°
Index ranges	$-10 \leq h \leq 10$, $-15 \leq k \leq 15$, $-28 \leq l \leq 26$
Reflections collected	50001
Independent reflections	5073 [R(int) = 0.0220]
Coverage of independent reflections	99.4 %
Variation in check reflections	N/A
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.78605
Structure solution technique	direct
Structure solution program	SHELXTL (Sheldrick, 2001)
Refinement technique	Full-matrix least-squares on F^2
Refinement program	SHELXTL (Sheldrick, 2001)
Function minimized	$\Sigma w(F_o^2-F_c^2)^2$
Data / restraints / parameters	5073 / 0 / 286
Goodness-of-fit on F^2	1.060
Δ/σ_{max}	0.001
Final R indices	
5039 data; I>2σ(I)	R1 = 0.0320, wR2 = 0.0859
all data	R1 = 0.0322, wR2 = 0.0861
Weighting scheme	calc $w=1 / [\sigma^2 (F_o^2)+(0.0503P)^2+0.5520P]$ where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	-0.01(13)
Largest diff. peak and hole	0.402 and -0.176 $eÅ^{-3}$

Refinement summary of the structure is as follows:

Ordered Non-H atoms, XYZ	Freely refining
Ordered Non-H atoms, U	Anisotropic

H atoms (on carbon), XYZ	Idealized positions riding on attached atoms
H atoms (on carbon), U	Appropriate multiple of U(eq) for bonded atom
H atoms (on heteroatoms), XYZ	Freely refining
H atoms (on heteroatoms), U	Isotropic
Disordered atoms, OCC	No disorder
Disordered atoms, XYZ	No disorder
Disordered atoms, U	No disorder

Example 7: Bioavailability Difference Between Obeticholic Acid Form 1 (non-crystalline) and Crystalline (Form F) Forms

The physical state of a solid obeticholic acid can play a role in the bioavailability of the molecule when administered orally to a subject (e.g., rats). The study described below was carried out to evaluate the plasma kinetics after a single oral administration and the efficiency of the intestinal absorption and the pharmacokinetics of solid non-crystalline and crystalline forms of obeticholic acid. The profiles of obeticholic acid plasma concentration vs time, the t_{max}, C_{max} and AUC after administration of obeticholic acid Form 1 (non-crystalline) or Form F were compared (see Figures 37-38)

Obeticholic acid Form 1 (non-crystalline) and Form F were administered to rats and in each animal blood was collected at different period of times for at least 3 hours. Six animals were studied for each form of obeticholic acid.

Experimental protocol:

The test substance used was obeticholic acid Form 1 (non-crystalline) and crystalline Form F. Form F can be prepared by maturation from acetonitrile or nitromethane. The formulation was prepared as a suspension in water at pH 4. The study model is adult male Sprague Dawley rats about 225 to about 250 g (Harlan Laboratories). Six animals were used per dosage route. The dosage is PO 20 mg/kg/5 mL. The animals were fasted overnight before treatment with the formulation of obeticholic acid. Oral administration was performed by gastric gavage.

On day one animals were be fitted with a cannula implanted in the left jugular vein (SOP VIVO/SAF6), anaesthesia was obtained by Isoflurane. The experiment was started after one day of recovery from surgery. About 500 μL of blood (250μL of plasma) was taken via cannula in an heparinised syringe (Na Heparin) and collected immediately

in microtubes in an ice/water bath. Within 1 hour, samples were centrifuged at 10000xg for 5 minutes at 4 °C. Plasma was immediately transferred in microtubes and stored at – 20 °C. Samples of blood were collected 30 minutes, 1 hour, 1.3 hour, 2 hours, and 3 hour post-dose. Plasma samples were analyzed using the HPLC-ES/MS/MS quantitative method. Pharmacokinetics study was preformed using non-compartmental methods.

Results:

The mean plasma concentrations of obeticholic after 20 mg/Kg b.w oral single dose administration of the two solid forms are reported in Figure 37. Values are the mean of six set of experiments for each formulation. The standard deviations are reported in the graph.

After administration of the crystalline form the Cmax is achieved after 1.5 hours and the plasma obeticholic acid concentration follows a regular kinetics with one maximum value and after 3 hours the dose is almost half of the Cmax.

The kinetics profile after the administration of obeticholic acid Form 1 (non-crystalline) Form 1 is different from that of the crystalline Form F. An early plasma concentration peak is obtained after 30 minutes and a second one after 2 hours. The variability of the data in the 6 rats is very low and this behaviour is statistically different from that of the crystalline form. The AUC for the three hours studied is higher for the crystalline form. The kinetics suggest that the obeticholic acid is still present in plasma after 3 hours. It has previously been demonstrated that the passage of obeticholic acid through the liver produce the hepatic metabolite tauro conjugate, which is secreted into bile and accumulate in the enterohepatic circulation. Thus, the measurement of the tauro conjugate can be used to determine the passage of the amount of obeticholic acid through the liver. The rate of tauro conjugate formation is reported in Figure 38, which shows that the tauro conjugate formation is faster and a higher concentration is achieved after administration of the crystalline form.

Melting point and glass transition

The melting point of obeticholic acid Form 1 (non-crystalline) Form 1 and crystalline Form F were measured using a conventional method. The melting point of Chenodeoxycholic acid and Ursodeoxycholic acid were measured as reference compounds. Measurements have been performed in triplicate. For the crystalline form the transition from the solid to liquid state is defined as melting temperature(T_m) while for the

non-crystalline form is defined as glass temperature transition (T_g), In the table are reported the measured values expressed in both Celsius °C and Kelvin °K.

Table 3: Melting points of obeticholic acid (Form 1 and Form F) and CDCA and UDCA

	Experimental data		Literature data	
Compound	T_m (°C)	T_g (°C)	T_m (°C)	T_g (°C)
CDCA	136-140	-	119 143 163	98
UDCA	203-207	-	203	105
Obeticholic acid	120-124 235-237	108-112	-	-

	Experimental data			Literature data		
Compound	T_m (°K)	T_g (°K)	T_g/T_m (°K)	T_m (°K)	T_g (°K)	T_g/T_m (°K)
CDCA	409-413	-	-	392 416 436	371	0,85
UDCA	476-480	-	-	477	378	0,79
Obeticholic acid	393-397 508-510	381-385	0,75	-	-	0,75

Results:

The values obtained for CDCA and UDCA agree with those previously reported, where the melting point of UDCA is higher than that of CDCA. The transition glass temperature Tg of Form 1 (102-112 °C) is lower than the melting point temperature Tm of Form F (120-124 °C). This observed pattern agrees with previous reported data when the two solid state forms are compared. Form F has an additional transition at a higher temperature (235-237 °C).

The ratio between the highest meting point temperature and the glass transition temperature expressed in Kelvin degree is quite similar to other drugs and other bile acids. (J. Kerc *et al.* Thermochim. Acta, 1995 (248) 81-95).

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) analysis was carried out to better define the melting points and the physical state of obeticholic acid crystalline and non-crystalline forms. The instrument used was a Mettler Toledo DSC model 821e. Approximately 4-5 mg of each Form 1 and Form F were submitted to analysis. The compounds were exposed to the temperature range of 30-300 °C at 10 °C/min heating rate.

Figure 39 shows the DSC curve obtained for obeticholic acid crystalline Form F. One endothermic transition at 120.04 °C was detected corresponding to the melting point of the compound. This result was confirmed also by hot stage microscopy (HSM); in the range 30°-240 °C the solid-liquid transition observed was at 122-124 °C. In the DSC trace, the peak shape and intensity obtained for Form F are in agreement with typical behaviour showed by crystalline forms. However, the peak width is rather broad; this can be due to not homogeneous crystals. Thermo gravimetric analysis (TGA) did not show any weight loss in the 30-300 °C temperature range.

Figure 40 shows the DSC curve obtained for obeticholic acid non-crystalline Form 1. One endothermic transition at 79.95 °C was observed. Peak shape and intensity are in agreement with behaviour expected for non-crystalline compounds. For these substances energy required for solid-liquid transition (glass transition) is less than for crystalline compounds. The thermogram did not show any weight loss in the 30-300 °C temperature range.

Water solubility

The water solubility of obeticholic acid Form 1 (non-crystalline) Form 1 and crystalline Form F was measured following procedures known in the art. Briefly, the solid was suspended in water at a low pH (HCl 0.1 mol/L) and left to equilibrate at 25 °C for one week under slightly mixing. The saturate solution was filtered and the concentration of the compound in solution measured by HPLC-ES-MS/MS.

Results:

	Water solubility (µmol/L)
Form 1	17.9
Form F	9.1

Form 1 present a higher solubility 17.9 μmol/L vs. 9.1 μmol/L for Form F.

According to the bioavailability data of the obeticholic acid, crystalline Form F is higher than the obeticholic acid Form 1 (non-crystalline). Despite an earlier plasma concentration peak after administration of the Form 1, the plasma profiles show that the Form F is absorbed more efficiently (higher AUC) and even the kinetics is more regular, reflecting an optimal distribution of the drug in the intestinal content. Form 1 shows this early peak then a later second one with a Cmax lower that than of Form F.

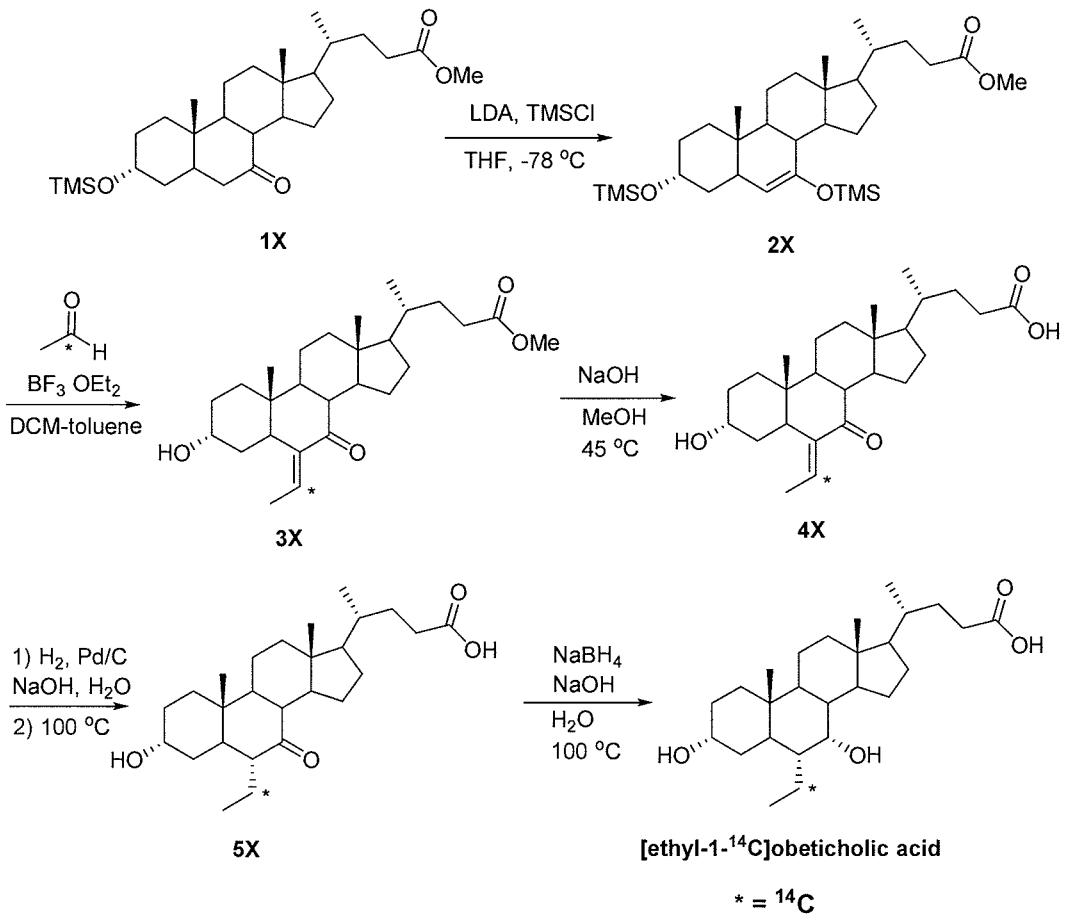
The water solubility of the Form 1 is higher than that of Form F. Form F appears to be stable as the thermo gravimetric analysis (TGA) did not show any weight loss in the temperature range studied.

According to these results, Form F when administered orally appears more efficiently absorbed by the intestine and taken up by the liver. The rate of formation of the main hepatic metabolite tauro conjugate is almost twice for Form F compared to Form 1, suggesting a more efficient transport and accumulation in the enterohepatic circulation and the plasma concentration after 3 hours.

Example 8: Preparation of Radiolabelled Obeticholic Acid

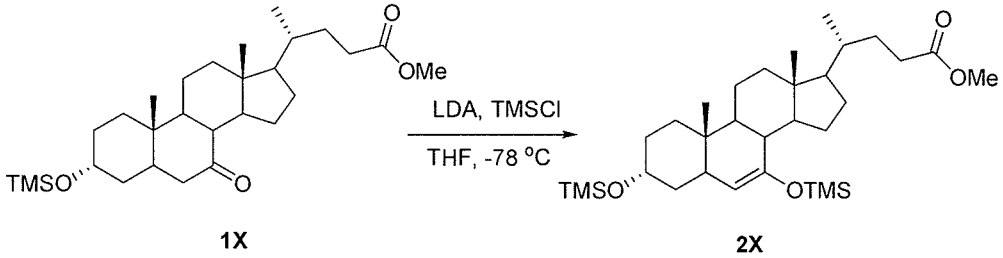
Radiolabelled obeticholic acid was prepared according to the scheme below.

Scheme 5



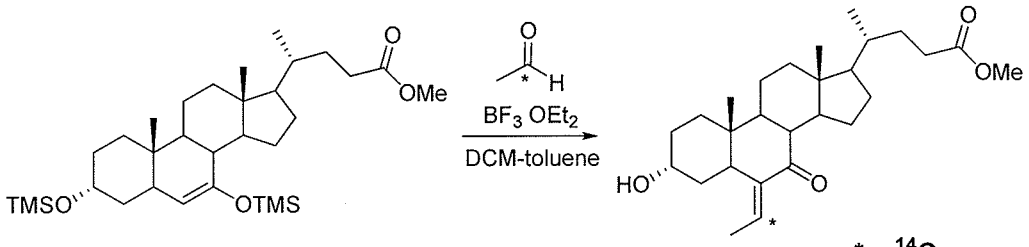
NMR spectra were recorded in $CDC1_3$ and MeOD-d_4 solution in 5-mm o.d. tubes (Norell, Inc. 507-HP) at 30 °C and were collected on Varian VNMRS-400 at 400 MHz for ^1H. The chemical shifts (δ) are relative to tetramethylsilane (TMS = 0.00 ppm) and expressed in ppm. LC-MS/MS was taken on Ion-trap Mass Spectrometer on Accela-Thermo Finnigan LCQ Fleet operating EST (-) ionization mode. HPLC was taken on Agilent 1200 series (Column: Xterra MS C8, 250 x 4.6 mm, 5 µm, 40 °C) in line β-Ram. Specific activity was taken on LSA (Liquid Scintillation Analyzer, Perkin Elmer, Tri-Carb 2900TR).

Preparation of compound 2X



To a solution of diisopropylamine (1.59 g, 15.8 mmol) in dry THF (6.0 mL) was added n-BuLi (6.30 mL, 2.5 M, 15.8 mmol) at -20 °C. After stirring the reaction mixture for 1 h at -20 °C, cooled to -78 °C and TMSCl (1.72 g, 15.8 mmol) was added followed by compound 1X (3.00 g, 6.29 mmol) in dry THF (6.0 mL). The reaction mixture was stirred for 1 h at -78 °C, quenched by addition of $NaHCO_3$ and stirred for 30 min at room temperature. The organic layer was separated and concentrated *in vacuo* to give the compound 2X (3.29 g, 95%) and used for next step without further purification.

Preparation of compound 3X

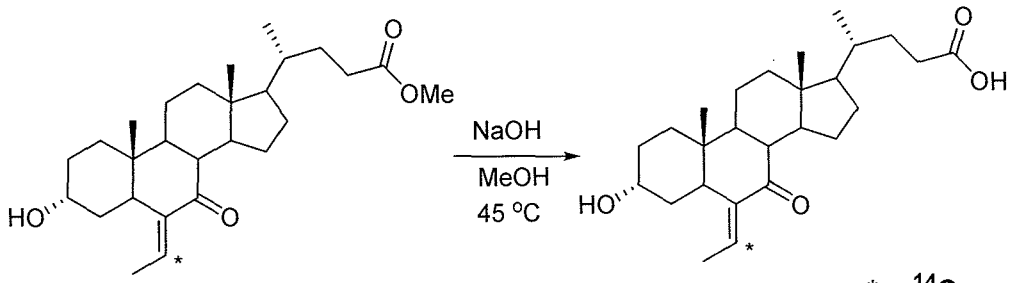


2X 3X * = ^{14}C

The [1-^{14}C]actaldehyde (330 mCi, 5.63 minol) (prepared from [^{14}C]$BaCO_3$, SA = 58.6 mCi/mmol) in toluene (1.0 mL) and acetaldehyde (130 mg, 2.95 mmol) in DCM (2.0 mL) were mixed at -78 °C and then transferred to a solution of compound 2X (3.29 g, 6.00 mmol) in DCM (13.0 mL) followed by addition of $BF_3 \cdot OEt_2$ (1.05 g, 7.40 mmol) at -78 °C. After stirring for 1 h at -78 °C, the reaction mixture was allowed to warm up to 35 °C and stirred for 1 h at the above temperature. The reaction was quenched by addition of water (10 mL), the aqueous layer was extracted with DCM, the combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* The residue was purified by column chromatography on SiO_2 (Hexane: EtOAc = 5:1 to 3:1) to give the compound 3X (102 mCi, 31%, SAW 37.0 mCi/mmol) as a white solid.

^{1}H-NMR ($CDCl_3$, Varian, 400 MHz): 8 0.65 (3H, s); 0.93 (3H, d, *J*= 6.0 Hz), 1.01 (3H, s), 1.06-1.49 (12H, m), 1.62-2.04 (7H, m), 1.69 (3H, d, J= 6.8 Hz), 2.18-2.28 (2H, m), 2.32-2.43 (2H, m), 2.58 (1H, dd, *J*= 12.8, 4.0 Hz), 3.62-3.70 (1H, m), 3.67 (3H, s), 6.18 (1H, q, *J*= 6.8 Hz).

Preparation of compound 4X

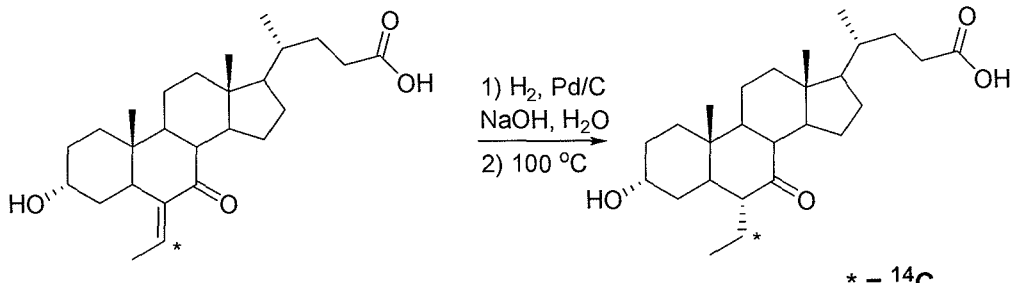


3X 4X

To a solution of compound 3X (102 mCi, 2.75 mmol) in MeOH (6.0 mL) was added NaOH (220 mg, 5.50 mmol) in H_2O (3.0 mL) at room temperature. After stirring the reaction mixture for 1 h at 45 °C, cooled to room temperature, MeOH was removed under reduced pressure and diluted with H_2O (12 mL). The aqueous layer was acidified with H_3PO_4, extracted with DCM and the organic layer was concentrated *in vacuo*. The residue was suspended in Et_2O and the precipitate was collected by filtration to give the compound 4X (86.3 mCi, 85%) as a white solid.

^{1}H-NMR ($CDCl_3$, Varian, 400 MHz); 8 0.63 (3H, s), 0.92 (3H, d, *J*= 6.0 Hz), 0.99 (3H, s), 1.04-1.50 (13H, m), 1.61-2.01 (7H, m), 1.67 (3H, d, *J*= 7.2 Hz), 2.21-2.28 (2H, m), 2.35-2.41 (2H, m), 2.56 (1H, dd, *J*= 12.8, 4.0 Hz), 3.58-3.69 (1H, m), 6.16 (1H, q,J= 7.2 Hz).

Preparation of compound 5X

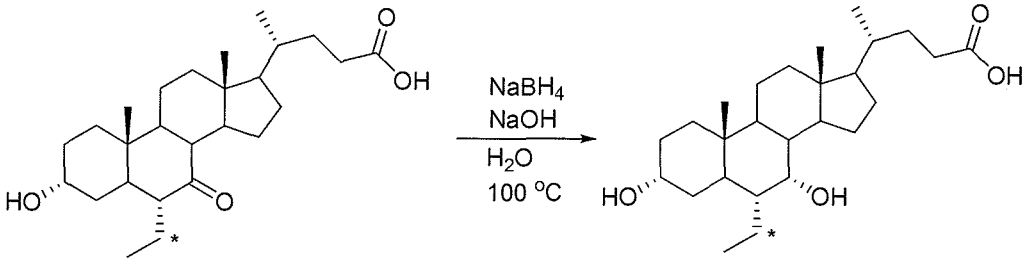


4X 5X

The mixture of compound 4X (86.3 mCi, 2.35 mmol) and 5% - Pd/C (100 mg) in aq. 0.5 M NaOH (10 mL, 5.0 mmol) was stirred for 10 h at room temperature under H_2 atmosphere (balloon) and then stirred for 14 h at 100 °C. The catalyst was removed by filtration, washed with water and the filtrate was acidified with H_3PO_4. The precipitates was collected by filtration, the solid was dissolved in EtOAc, washed with brine, filtered through a short pad of SiO_2 and concentrated *in vacuo*. The residual solid was recrystallization with EtOAc to give the compound 5X (67.7 mCi, 78%) as a white solid.

^{1}H-NMR (MeOD-d_4, Varian, 400 MHz): 8 0.71 (311, s), 0.75-0.84 (1H, m), 0.81 (3H, t, J = 7.4 Hz), 0.921.01 (1H, m), 0.96 (3H, d, J= 6.4 Hz), 1.06-1.38 (7H, m), 1.25 (3H, s), 1.41-1.96 (1211, m), 2.01-2.05 (1H, m), 2.11-2.24 (2H, m), 2.30-2.37 (1H, m), 2.50 (1H, t, J= 11.4 Hz), 2.80-2.85 (1H, m), 3.42-3.49 (1H, m).

Preparation of [ethyl-1-^{14}C]obeticholic acid



5X

[ethyl-1-^{14}C]obeticholic acid

* = ^{14}C

To a solution of compound 5X (67.7 mCi, 1.83 mmol) in aq. 2 M NaOH (4.50 mL, 9.00 mmol) was added a solution of $NaBH_4$ (416 mg, 11.0 mmol) in 1120 (2.0 ml) at 80 °C. After stirring the reaction mixture for 2 h at 100 °C, water (6.0 mL) was added at room temperature and acidified with H_3PO_4. The aqueous layer was extracted with DCM, dried over anhydrous Na_2SO_4, filtered through a short pad of SiO_2 and concentrated *in vacuo,* The residue was purified by column chromatography on SiO_2 (Hexane:EtOAc = 1:1 to 1:3) to give the product (44.0 mCi, 65%) as a white solid. The product (44.0 mCi, 1.19 mmol) and obeticholic acid (120 mg, 0.285 mmol) were dissolved in EtOAc (4 mL), the solution was stirred for 2 h at 50 °C and then concentrated *in vacuo.* The residual oil suspended in Et_2O, the precipitate was collected by filtration to give the [ethyl-1-^{14}C]obeticholic acid (560 mg, 38.5 mCi, SA = 29 mCi/ mmol) as a white solid.

^{1}H-NMR ($CDCl_3$, Varian, 400 MHz): 8 0.66 (3H, s), 0.88 (311, s), 0.93 (3H, t, J = 7.2 Hz), 0.93 (3H, d, I = 6.4 Hz), 0.96-1.04 (1H, m), 1.08-1.52 (14H, m), 1.51-1.60 (1011, m), 2.22-2.30 (111, m), 2.36-2.44 (1H, m), 3.38-3.45 (111, m), 3.71 (IH, s).

LC-MS/MS (MS: LCQ Fleet): MS Calcd.: 421.56; MS Found: 421.07 $[M-H]^-$.

Radio TLC: TLC plate of silica 60 F_{254}, and mobile phase is EtOAc. Radiochemical purity is 98.90%, Rf = 0.675

HPLC (Agilent 1200 series): Mobile phase; acetonitrile: 5 mM Phosphate buffer (pH = 3):MeOH = 450:450:100. Radiochemical purity is 98.19% (β-ram), Rt = 20.00 min.

[Ehtyl-1-^{14}C]obeticholic acid has a molecular formula of $^{14}C_1C_{25}H_{44}O_4$ and a molecular weight of 421.46 at the specific activity of 29 mCi/mmol by LSC.

WE CLAIM:

1. A crystalline obeticholic acid Form C characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 9.5, 12.5, and 16.7 degrees 2-Theta.

2. A crystalline obeticholic acid Form C characterized by an X-ray diffraction pattern substantially similar to that set forth in Figure 5.

3. The crystalline obeticholic acid Form C according to claim 1 characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 9.5, 12.5, 12.6, 15.5, 15.8, 16.0, 16.7 and 19.0 degrees 2-Theta.

4. The crystalline obeticholic acid Form C according to claim 1 or claim 3 characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 8.3, 9.5, 11.1, 12.2, 12.5, 12.6, 15.5, 15.8, 16.0, 16.3, 16.7, 18.6 and 19.0 degrees 2-Theta.

5. The crystalline obeticholic acid Form C according to any one of claims 1 or 3-4 characterized by an X-ray diffraction pattern including characteristic peaks at about 4.2, 6.4, 8.3, 9.5, 11.1, 12.2, 12.5, 12.6, 15.5, 15.8, 16.0, 16.3, 16.7, 17.0, 17.8, 18.6, 18.8, 19.0, 20.5 and 20.9 degrees 2-Theta.

6. The crystalline obeticholic acid Form C according to any one of claims 1-5, wherein the X-ray diffraction pattern is collected on a diffractometer using Cu Kα radiation.

7. The crystalline obeticholic acid Form C according to any one of claims 1-6 characterized by an X-ray diffraction pattern including characteristic peaks at about 12.0 to about 12.8 and about 15.4 to about 21.0.

8. A crystalline obeticholic acid Form C characterized by a Differential Scanning Calorimetry (DSC) thermogram having an endotherm value at about 98±2 °C.

9. The crystalline obeticholic acid Form C according to any one of claims 1-8, further characterized by a Differential Scanning Calorimetry (DSC) thermogram having an endotherm value at about 98±2 °C.

10. A process for preparing obeticholic acid Form 1, comprising a crystalline form of obeticholic acid as a synthetic intermediate.

11. A process for preparing obeticholic acid Form 1, comprising converting crystalline obeticholic acid to obeticholic acid Form 1.

12. The process according to claim 11, comprising the steps of

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

13. The process according to any one of claims 11-12, comprising the steps of

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid,

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

14. The process according to any one of claims 11-13, comprising the steps of

reacting 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid,

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

15. The process according to any one of claims 11-14, comprising the steps of

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid,

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

16. The process according to any one of claims 11-15, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester,

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid,

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

17. The process according to any one of claims 11-16, comprising the steps of

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester,

reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester,

reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid,

reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid,

reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid, and

converting crystalline obeticholic acid to obeticholic acid Form 1.

18. The process according to any one of claims 10-17, wherein the crystalline obeticholic acid is Form C.

19. The process according to claim 18, wherein crystalline obeticholic acid Form C is characterized by an X-ray diffraction pattern similar to that set forth in Figure 5.

20. The process according to any one of claims 18 or 19, wherein the crystalline obeticholic acid Form C is crystallized from n-butyl acetate.

21. The process according to any one of claims 11-17, wherein converting crystalline obeticholic acid Form C to obeticholic acid Form 1 comprises the step of dissolving crystalline obeticholic acid Form C in aqueous NaOH solution and adding HCl.

22. The process according to claim 21, wherein the isolated crystalline obeticholic acid Form C is dried under vacuum at about 80 °C.

23. The process according to any one of claims 12-17, wherein reacting 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid with $NaBH_4$ to form crystalline obeticholic acid is carried out at a temperature at about 85 °C to about 110 °C in a basic aqueous solution.

24. The process according to claim 23, wherein the temperature is about 90 °C to about 105 °C.

25. The process according to any one of claims 23-24, wherein the basic aqueous solution is an aqueous NaOH solution.

26. The process according to any one of claims 25, wherein the basic aqueous solution is a mixture of 50% wt. NaOH solution and water.

27. The process according to any one of claims 13-17, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas to form 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid is carried out at a temperature at about 20 °C to about 40 °C and at a pressure at about 4 to about 5 bars.

28. The process according to claim 27, wherein, the organic phase of the reaction mixture is treated with activated carbon.

29. The process according to any one of claims 27-28, wherein the temperature is about 25 °C to about 35 °C.

30. The process according to any one of claims 27-29, wherein the pressure is about 4.5 to about 5.5 bars.

31. The process according to any one of claims 27-30, wherein the hydrogenation reaction mixture is allowed to stir for about 1 hour.

32. The process according to any one of claims 27-31, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 2 hour to about 5 hours.

33. The process according to any one of claims 27-32, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid with Pd/C and hydrogen gas is heated to about 100 °C and stirred for about 3 hours.

34. The process according to any one of claims 14-17, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH to form E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid is carried out at a temperature at about 20 °C to about 60 °C.

35. The process according to claim 34, wherein the temperature is about 20 °C to about 25 °C.

36. The process according to any one of claims 34-35, wherein reacting E- or E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester with NaOH is carried out in methanol, water, and a NaOH solution.

37. The process according to any one of claims 34-36, wherein the NaOH solution is a 50% wt. aqueous solution.

38. The process according to any one of claims 15-17, wherein reacting 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester with CH_3CHO to form E- or E/Z- 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid methyl ester is carried out in a polar aprotic solvent at a temperature at about -50 °C to about -70 °C in the presence of BF_3.

39. The process according to claim 38, wherein the polar aprotic solvent is dichloromethane.

40. The process according to any one of claims 38-39, wherein the BF_3 is a 16% wt. solution in acetonitrile.

41. The process according to any one of claims 38-40, wherein the temperature is about
-60 °C to about -65 °C.

42. The process according to any one of claims 16-17, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ to form 3α,7-ditrimethylsilyloxy-5β-chol-6-en-24-oic acid methyl ester is carried out in a polar aprotic solvent at a temperature at about -10 °C to about -30 °C.

43. The process according to claim 42, wherein the polar aprotic solvent is tetrahydrofuran.

44. The process according to any one of claims 42-43, wherein the temperature is about
-20 °C to about -25 °C.

45. The process according to any one of claims 42-44, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester with $Li[N(CH(CH_3)_2)_2]$ and $Si(CH_3)_3Cl$ is stirred for about 2 hours.

46. The process according to claim 17, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid with CH_3OH and H_2SO_4 to form 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester is heated for about 3 hours and the pH of the reaction mixture is adjusted with an aqueous basic solution to a pH-value of about 6.5 to about 8.0.

47. The process according to claim 46, wherein the isolation of 3α-hydroxy-7-keto-5β-cholan-24-oic acid methyl ester further comprises the addition of activated carbon.

48. The process according to any one of claims 46-47, wherein reacting 3α-hydroxy-7-keto-5β-cholan-24-oic acid with CH_3OH and H_2SO_4 is carried out in methanol.

49. The process according to any one of claims 46-48, wherein the basic solution is an aqueous NaOH solution.

50. The process according to any one of claims 46-49, wherein the pH is about 7.0 to about 7.5.

51. A compound E/Z-3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oic acid produced by the process according to any one of claims 10-50, wherein the E/Z isomer ratio is greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 90%, greater than about 93%, greater than about 95%, or greater than about 99%.

52. The compound according to claim 51, wherein the E/Z ratio is determined by HPLC.

53. The compound according to any one of claims 51-52, wherein the ratio is greater than about 80%.

54. The compound according to any one of claims 51-53, wherein the ratio is greater than about 83%.

55. The compound according to any one of claims 51-54, wherein the ratio is greater than about 85%.

56. The compound according to any one of claims 51-55, wherein the ratio is greater than about 90%.

57. The compound according to any one of claims 51-56, wherein the ratio is greater than about 93%.

58. The compound according to any one of claims 51-57, wherein the ratio is greater than about 95%.

59. The compound according to any one of claims 51-58, wherein the ratio is greater than about 99%.

60. A obeticholic acid, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof, having a potency of greater than about 98%.

61. The obeticholic acid of claim 60, wherein said obeticholic acid has a potency of greater than about 98%.

62. The obeticholic acid according to claim 60, wherein the potency of said obeticholic acid is determined by subtracting the amount of water, sulphated ash, residual solvents, and other organic impurities.

63. The obeticholic acid according to any one of claims 61-62 having a potency of greater than about 98.5%.

64. The obeticholic acid according to any one of claims 61-63 having a potency of greater than about 99.0%.

65. The obeticholic acid according to any one of claims 61-64 having a potency of greater than about 99.5%.

66. The obeticholic acid according to any one of claims 61-65, wherein said obeticholic acid contains a total of less than about 2% of one or more impurities selected from 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid.

67. The obeticholic acid according to claim 66, wherein said obeticholic acid contains a total of less than about 1.5% of impurities.

68. The obeticholic acid according to any one of claims 66-67, wherein said obeticholic acid Form 1 contains a total of less than about 1.4% of impurities.

69. The obeticholic acid according to any one of claims 66-68, wherein said obeticholic acid contains less than about 1.2% of water.

70. The obeticholic acid according to any one of claims 66-69, wherein said obeticholic acid contains less than about 1.0% of water.

71. The obeticholic acid according to any one of claims 66-70, wherein said obeticholic acid contains a total of less than about 0.15% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid.

72. The obeticholic acid according to any one of claims 66-71, wherein said obeticholic acid contains a total of less than about 0.06% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid.

73. The obeticholic acid according to any one of claims 66-72, wherein said obeticholic acid contains a total of less than about 0.05% of 6-ethylursodeoxycholic acid and 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid.

74. The obeticholic acid according to any one of claims 66-73, wherein said obeticholic acid contains less than about 0.15% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid.

75. The obeticholic acid according to any one of claims 66-74, wherein said obeticholic acid contains less than about 0.06% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid.

76. The obeticholic acid according to any one of claims 66-75, wherein said obeticholic acid contains less than about 0.05% of 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid.

77. The obeticholic acid according to any one of claims 66-76, wherein said obeticholic acid contains less than about 0.15% of 6β-ethylchenodeoxycholic acid.

78. The obeticholic acid according to any one of claims 66-77, wherein said obeticholic acid contains less than about 0.06% of 6β-ethylchenodeoxycholic acid.

79. The obeticholic acid according to any one of claims 66-78, wherein said obeticholic acid contains less than about 0.05% of 6β-ethylchenodeoxycholic acid.

80. The obeticholic acid according to any one of claims 66-79, wherein said obeticholic acid contains less than about 3% of chenodeoxycholic acid.

81. The obeticholic acid according to any one of claims 66-80, wherein said obeticholic acid contains less than about 1% of chenodeoxycholic acid.

82. The obeticholic acid according to any one of claims 66-81, wherein said obeticholic acid contains less than about 0.2% of chenodeoxycholic acid.

83. The obeticholic acid according to any one of claims 66-82, wherein said obeticholic acid contains less than about 0.15% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid.

84. The obeticholic acid according to any one of claims 66-83, wherein said obeticholic acid contains less than about 0.06% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid.

85. The obeticholic acid according to any one of claims 66-84, wherein said obeticholic acid contains less than about 0.05% of 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid.

86. The obeticholic acid according to any one of claims 61-85, wherein the obeticholic acid is obeticholic acid Form 1.

87. A crystalline obeticholic acid, or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof, wherein said crystalline obeticholic acid is Form C.

88. The crystalline obeticholic acid according to claim 87, wherein said crystalline obeticholic acid has a potency greater than about 90%.

89. The crystalline obeticholic acid Form C according to claim 88, wherein the potency of said obeticholic acid Form C is determined by subtracting the amount of water, sulphated ash, residual solvents, and other organic impurities.

90. The crystalline obeticholic acid Form C according claim 87, wherein the solvate is a hydrate.

91. The crystalline obeticholic acid Form C according to any one of claims 88-90 having a potency of greater than about 92%.

92. The crystalline obeticholic acid Form C according to any one of claims 88-91 having a potency of greater than about 94%.

93. The crystalline obeticholic acid Form C according to any one of claims 88-92 having a potency of greater than about 96%.

94. The crystalline obeticholic acid Form C according to any one of claims 88-93 having a potency of greater than about 98%.

95. The crystalline obeticholic acid Form C according to any one of claims 88-94 having a potency of greater than about 99%.

96. The crystalline obeticholic acid Form C according to any one of claims 87-95, wherein the crystalline obeticholic acid Form C contains a total of less than about 4% of one or more impurities selected from 6-ethylursodeoxycholic acid, 3α-hydroxy-6α-ethyl-7-cheto-5β-cholan-24-oic acid, 6β-ethylchenodeoxycholic acid, 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oic acid, chenodeoxycholic acid, and 3α(3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oyloxy)-7α-hydroxy-6α-ethyl-5β-cholan-24-oic acid.

97. The crystalline obeticholic acid Form C according to claim 96, wherein the total impurities is less than about 3.8 %.

98. The crystalline obeticholic acid Form C according to any one of claims 96-97, wherein the total impurities is less than about 3.6%.

99. A pharmaceutical composition comprising obeticholic acid Form 1 produced by the process of any one of claims 10-50 and a pharmaceutically acceptable carrier.

100. A pharmaceutical composition comprising of crystalline obeticholic acid and a pharmaceutically acceptable carrier.

101. The pharmaceutical composition according to claim 100, wherein the crystalline obeticholic acid is Form C.

102. A method of treating or preventing an FXR mediated disease or condition in a subject comprise of administering an effective amount of obeticholic acid Form 1 according to any one of claims 60-86 or produced by a process according to any one of claims 10-50, or a pharmaceutical composition according to claim 99.

103. A method of treating or preventing an FXR mediated disease or condition in a subject comprise of administering an effective amount of crystalline obeticholic acid according to any one of claims 1-9 or 87-98 or a pharmaceutical composition according to any one of claims 100-101.

104. The method according to any one of claims 102-103, wherein the disease or condition is selected from biliary atresia, cholestatic liver disease, chronic liver disease, nonalcoholic steatohepatitis (NASH), hepatitis C infection, alcoholic liver disease, primary biliary cirrhosis (PBC), liver damage due to progressive fibrosis, liver fibrosis, and cardiovascular diseases including atherosclerosis, arteriosclerosis, hypercholesteremia, and hyperlipidemia.

105. A method for lowering triglycerides in a subject comprise of administering an effective amount of obeticholic acid Form 1 according to any one of claims 60-86 or produced by a process according to any one of claims 10-50, or a pharmaceutical composition according to claim 99.

5

106. A method for lowering triglycerides in a subject comprise of administering an effective amount of crystalline obeticholic acid according to any one of claims 1-9 or 87-98 or a pharmaceutical composition according to any one of claims 100-101.

FIG. 1

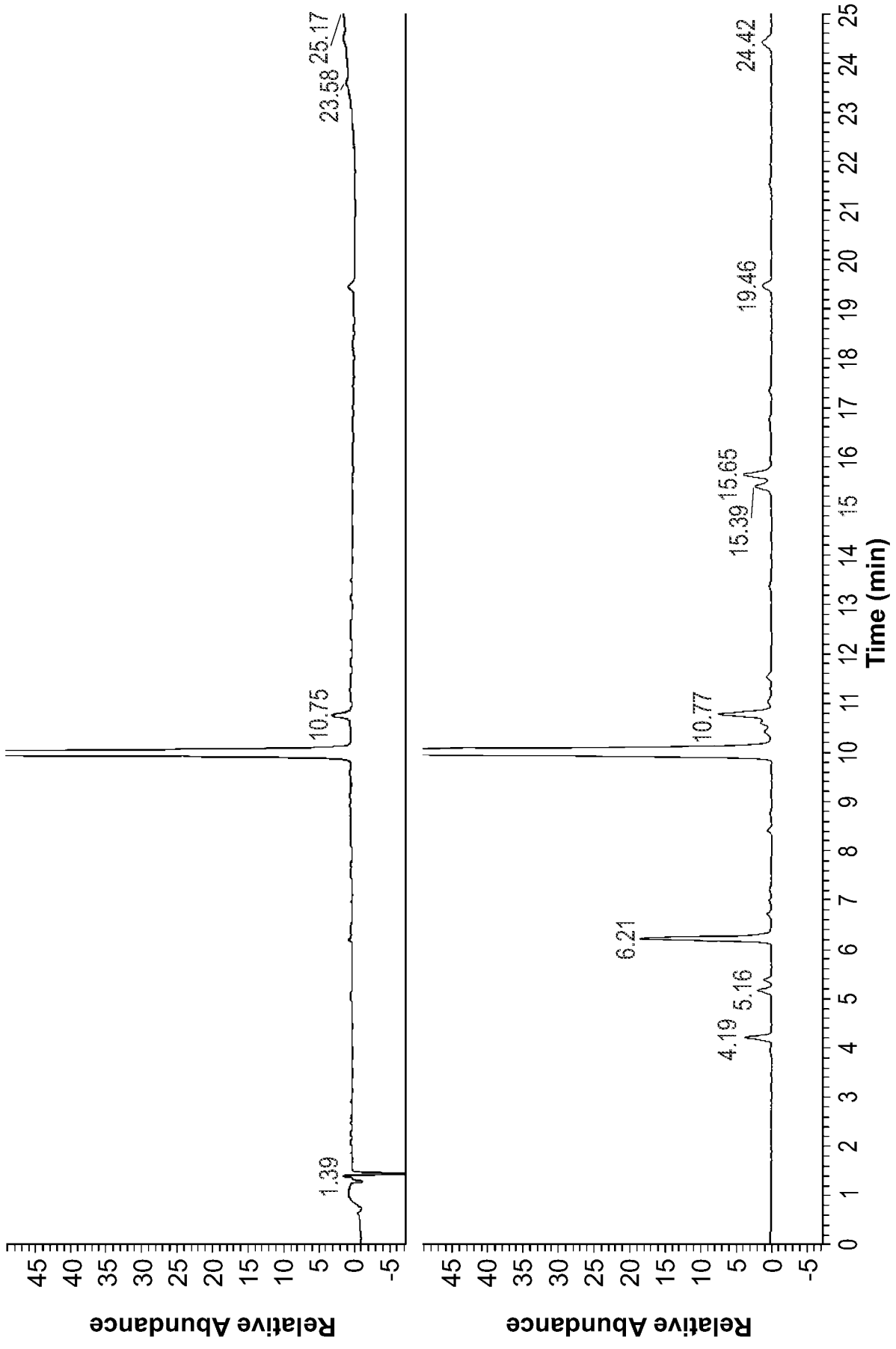

Relative Abundance
45
40
35
30
25
20
15
10
5
0
-5
1.39
10.75
23.58
25.17
Relative Abundance
4.19
5.16
6.21
10.77
15.39
15.65
19.46
24.42
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Time (min)

FIG. 2

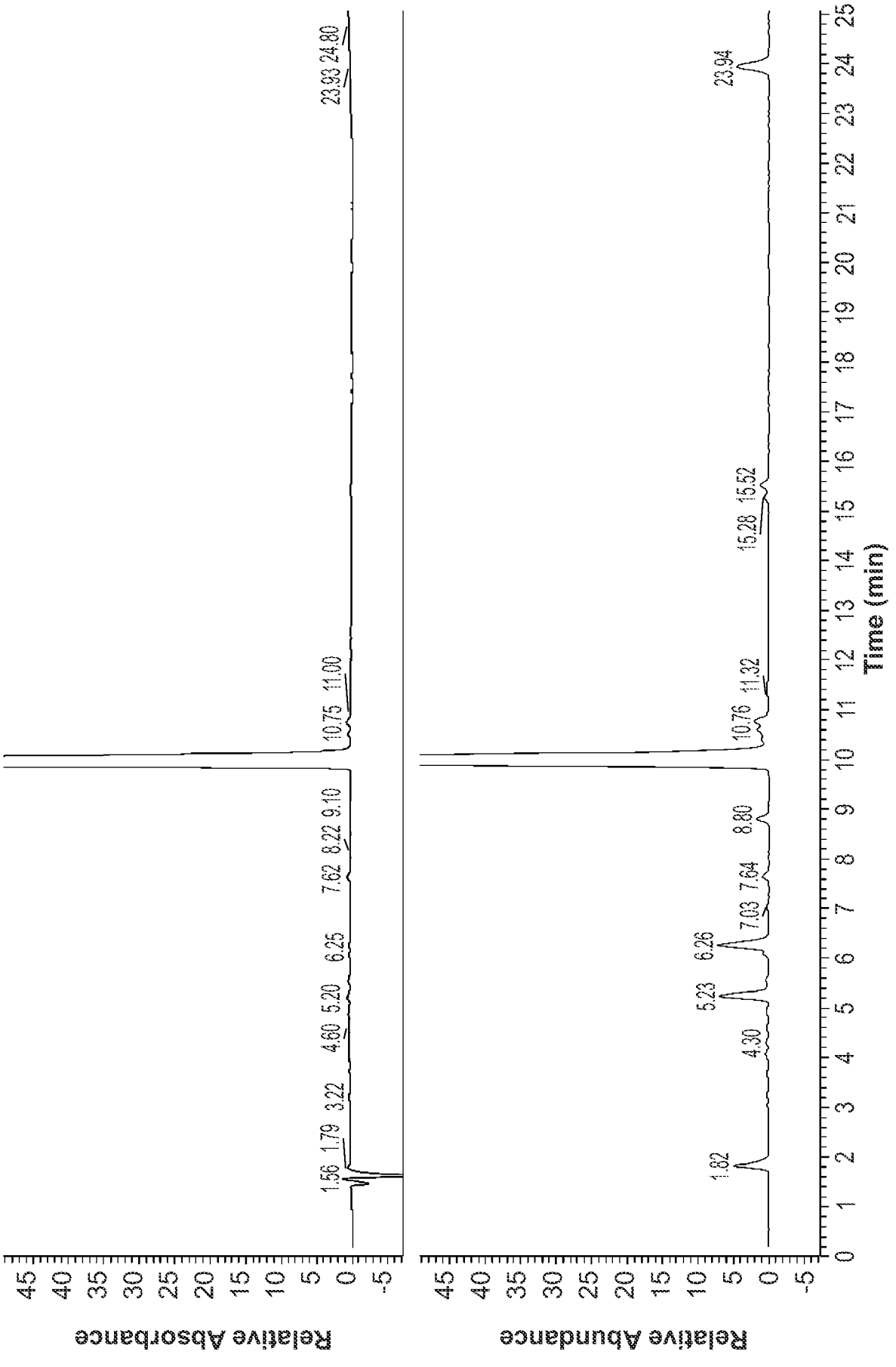

Relative Absorbance
Relative Abundance
Time (min)
1.56
1.79
3.22
4.60
5.20
6.25
7.62
8.22
9.10
10.75
11.00
23.93
24.80
1.82
4.30
5.23
6.26
7.03
7.64
8.80
10.76
11.32
15.28
15.52
23.94

FIG. 3

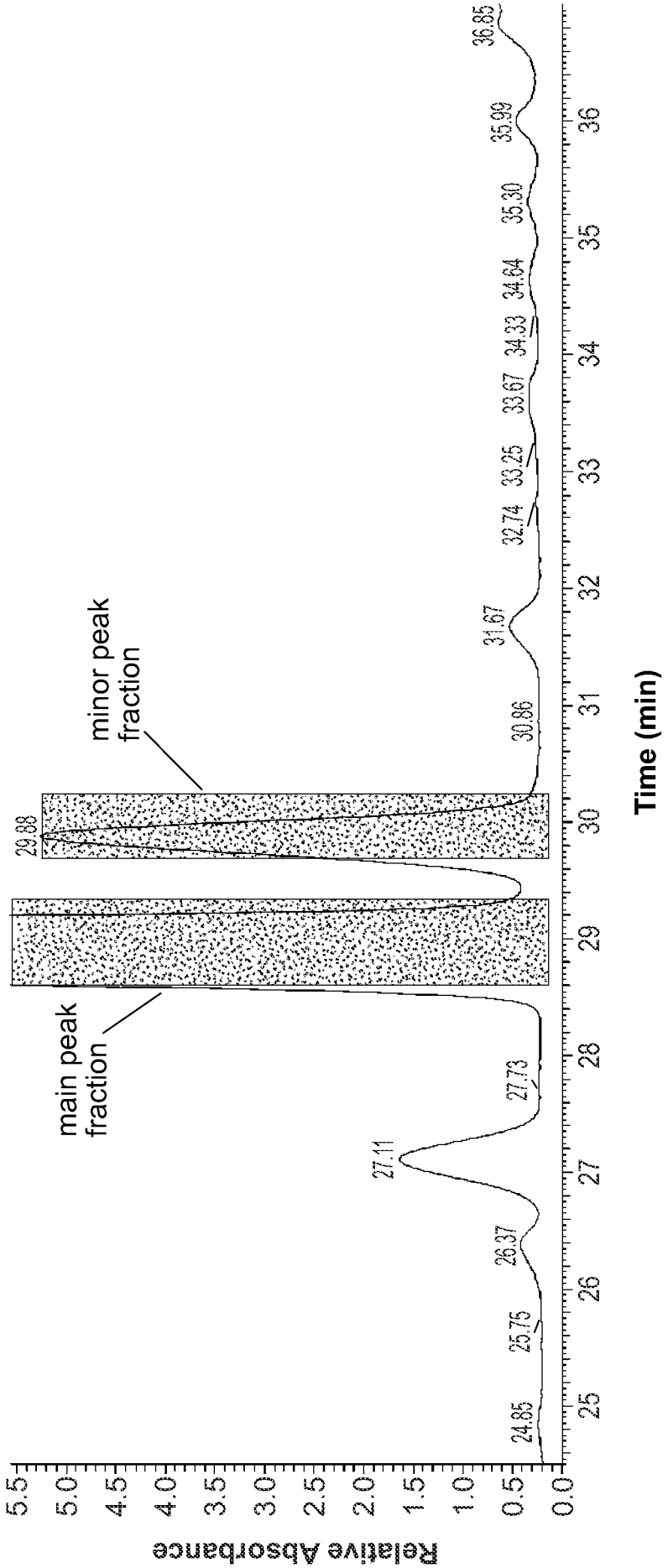

Relative Absorbance
Time (min)
main peak fraction
minor peak fraction
24.85
25.75
26.37
27.11
27.73
29.88
30.86
31.67
32.74
33.25
33.67
34.33
34.64
35.30
35.99
36.85

FIG. 4A

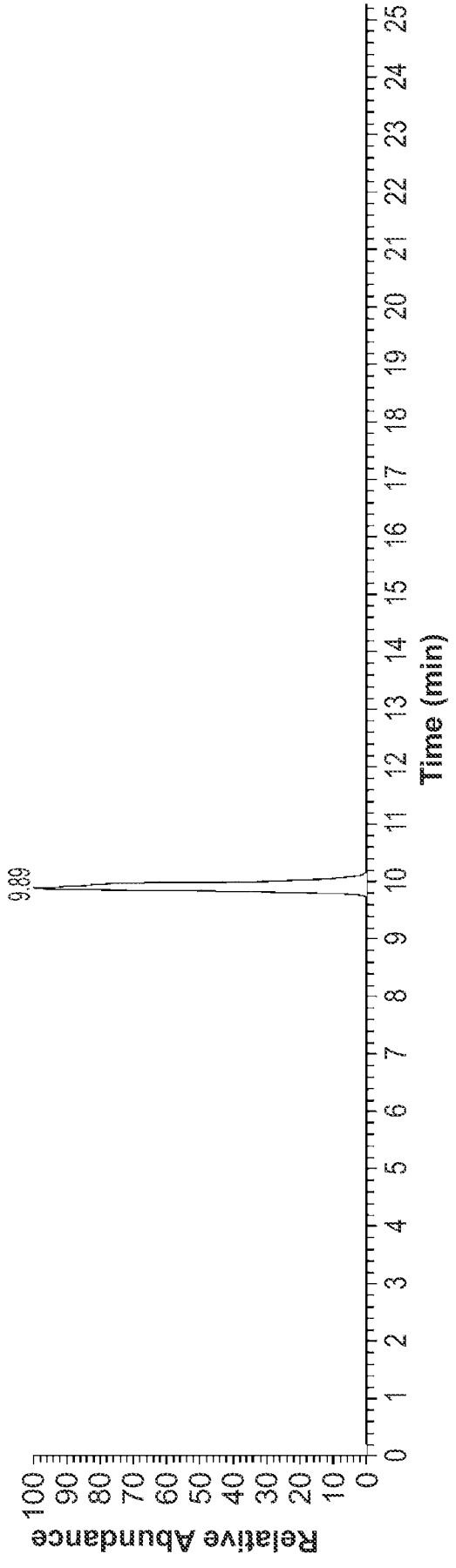

Relative Abundance
Time (min)
9.89

FIG. 4B

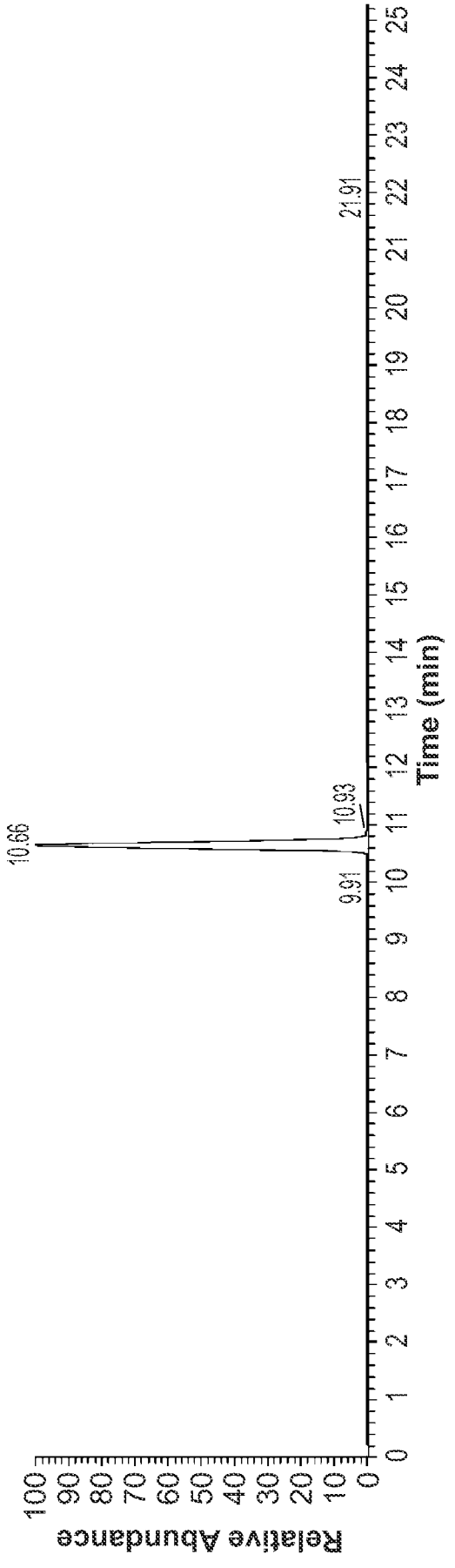

Relative Abundance
Time (min)
10.66
10.93
9.91
21.91

FIG. 4C

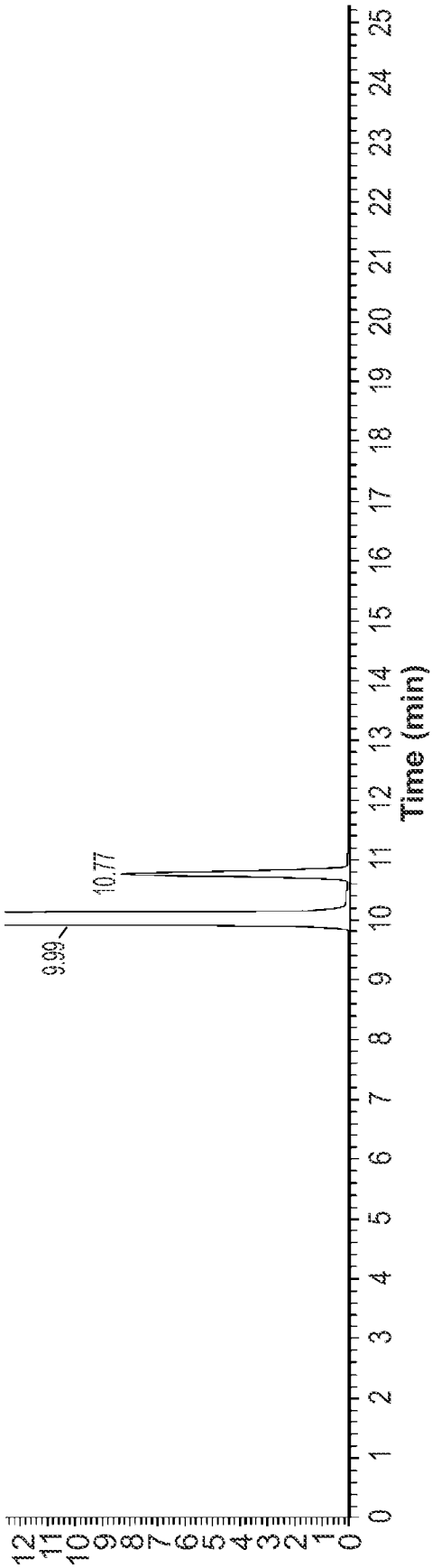

Relative Abundance
Time (min)
9.99
10.77

FIG. 4D

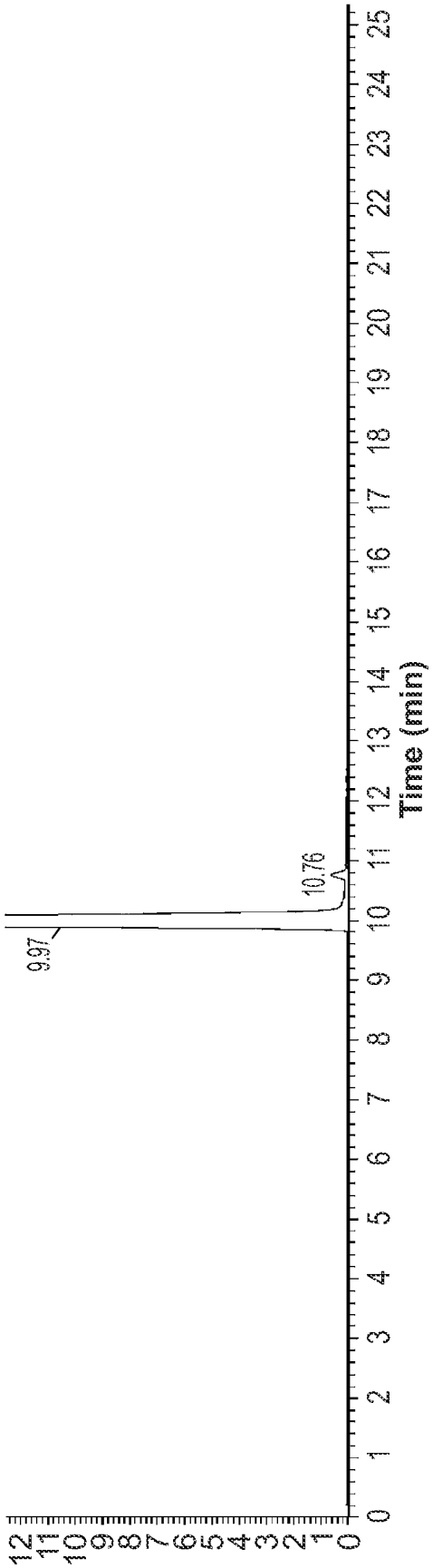

Relative Abundance
Time (min)
9.97
10.76

FIG. 5

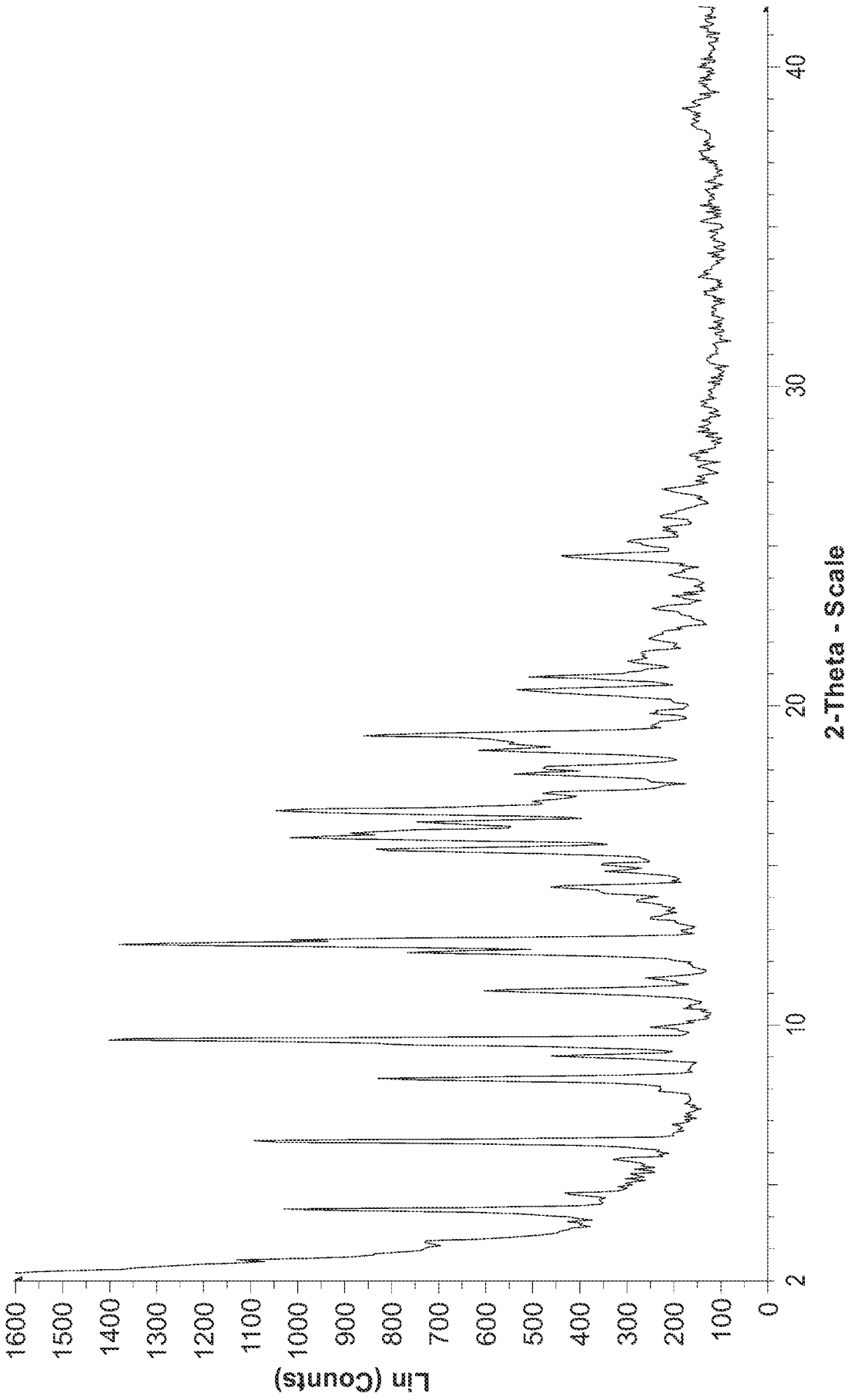

Lin (Counts)
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2
10
20
30
40
2-Theta - Scale

FIG. 6

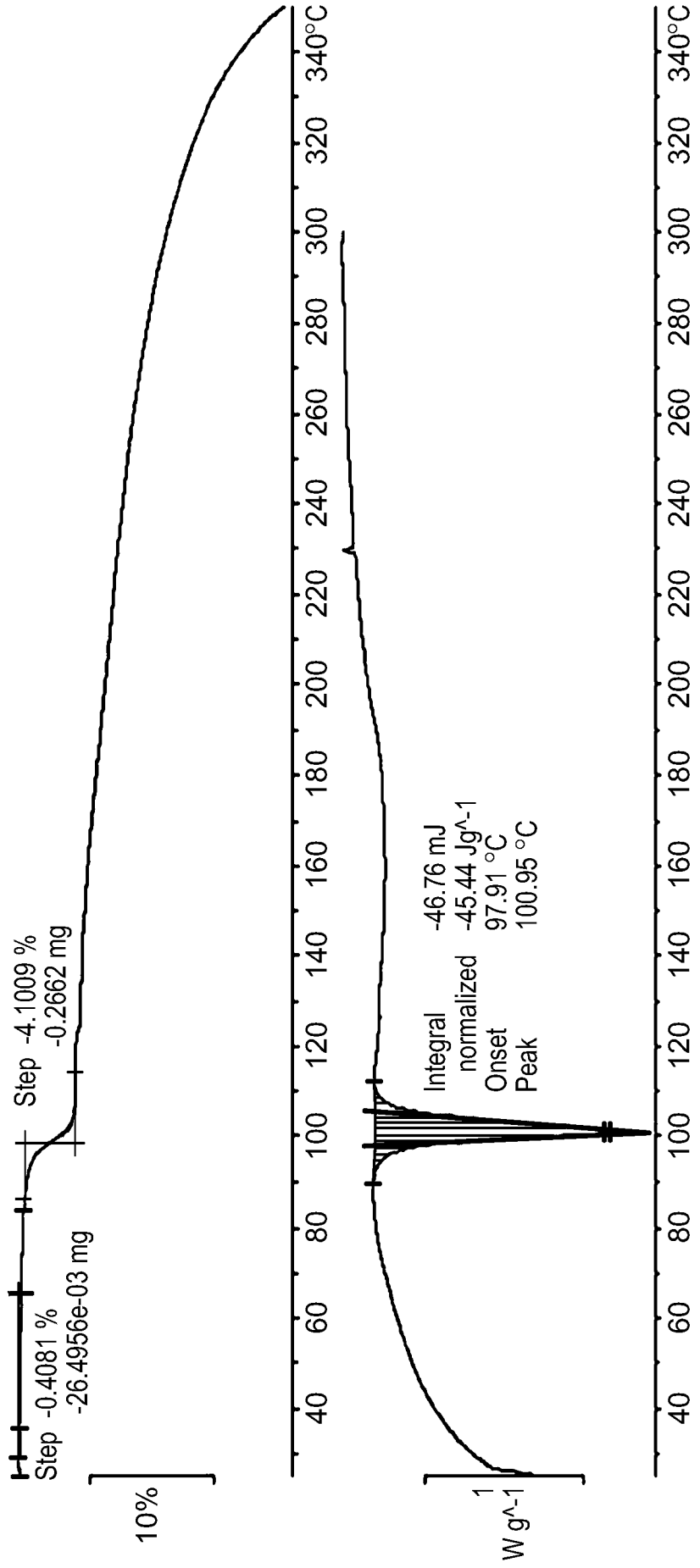

Step -0.4081 %
-26.4956e-03 mg
Step -4.1009 %
-0.2662 mg
10%
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340°C
Integral -46.76 mJ
normalized -45.44 Jg^-1
Onset 97.91 °C
Peak 100.95 °C
1
W g^-1

FIG. 7

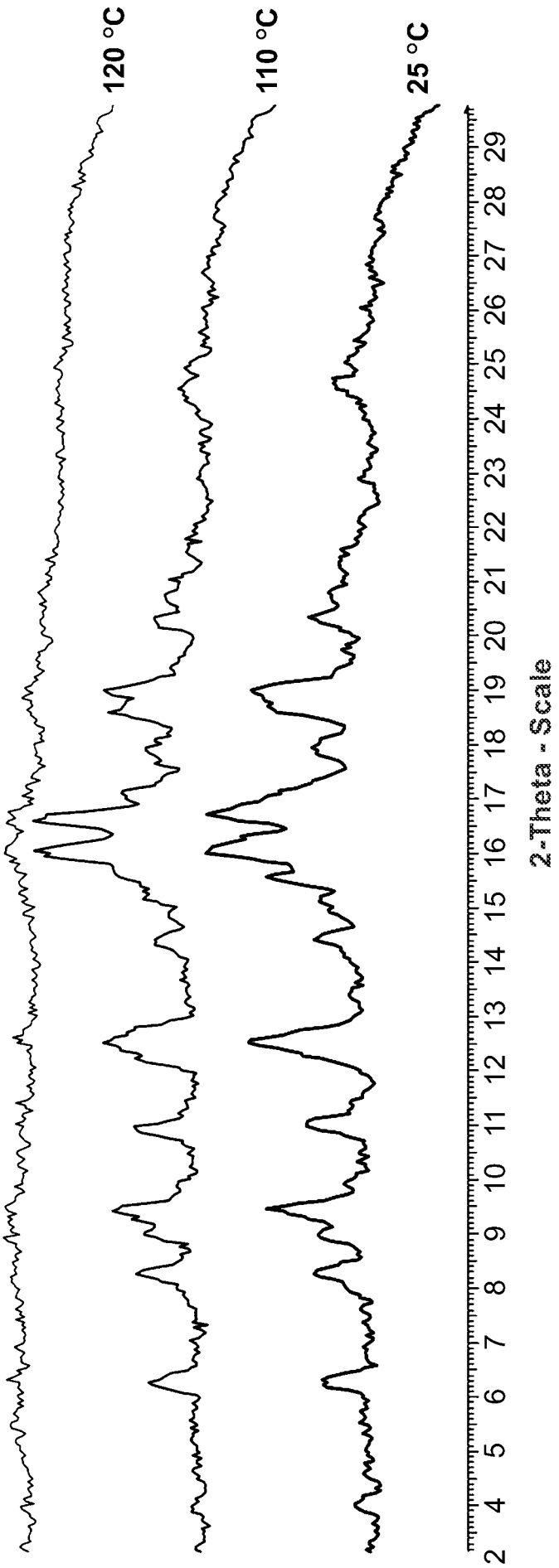
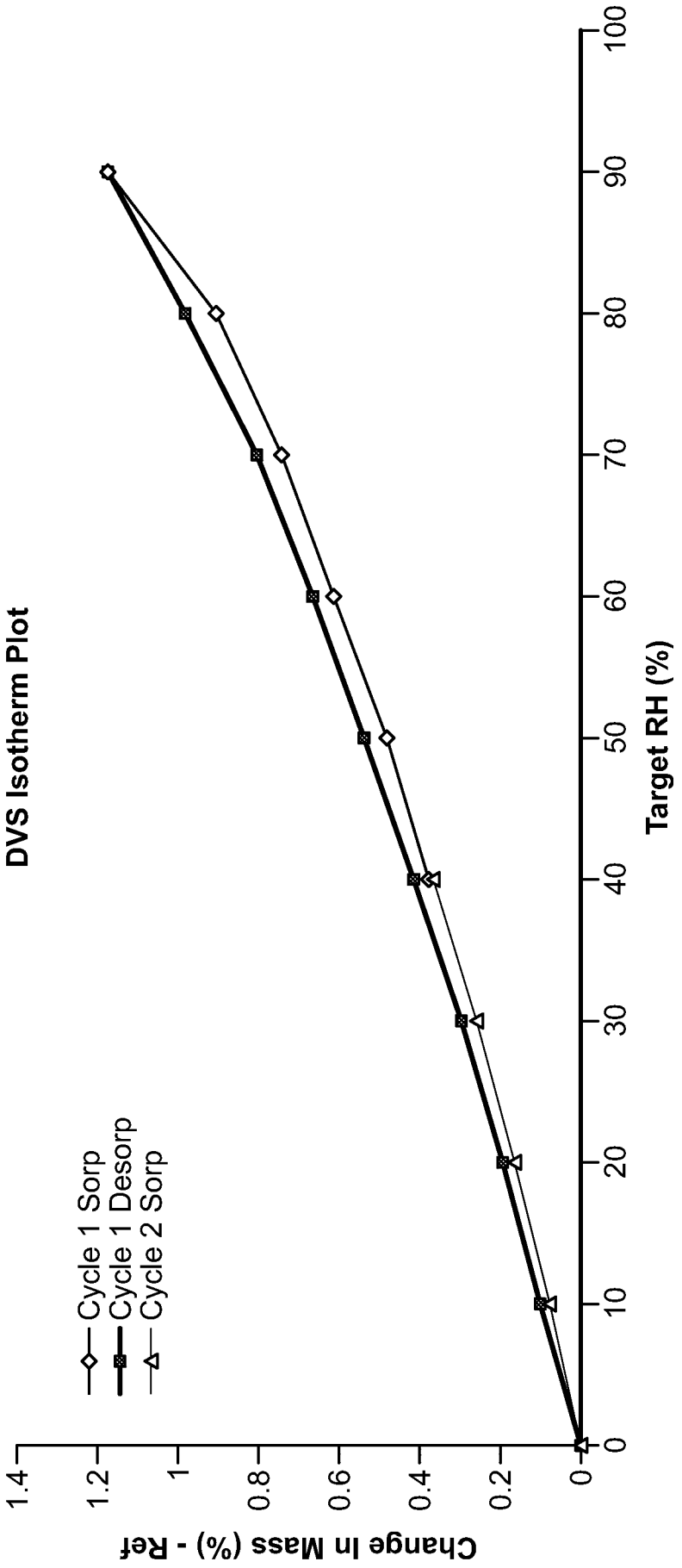

120 °C
110 °C
25 °C
2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
2-Theta - Scale

FIG. 8A


DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Cycle 2 Sorp
Change In Mass (%) - Ref
Target RH (%)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
10
20
30
40
50
60
70
80
90
100

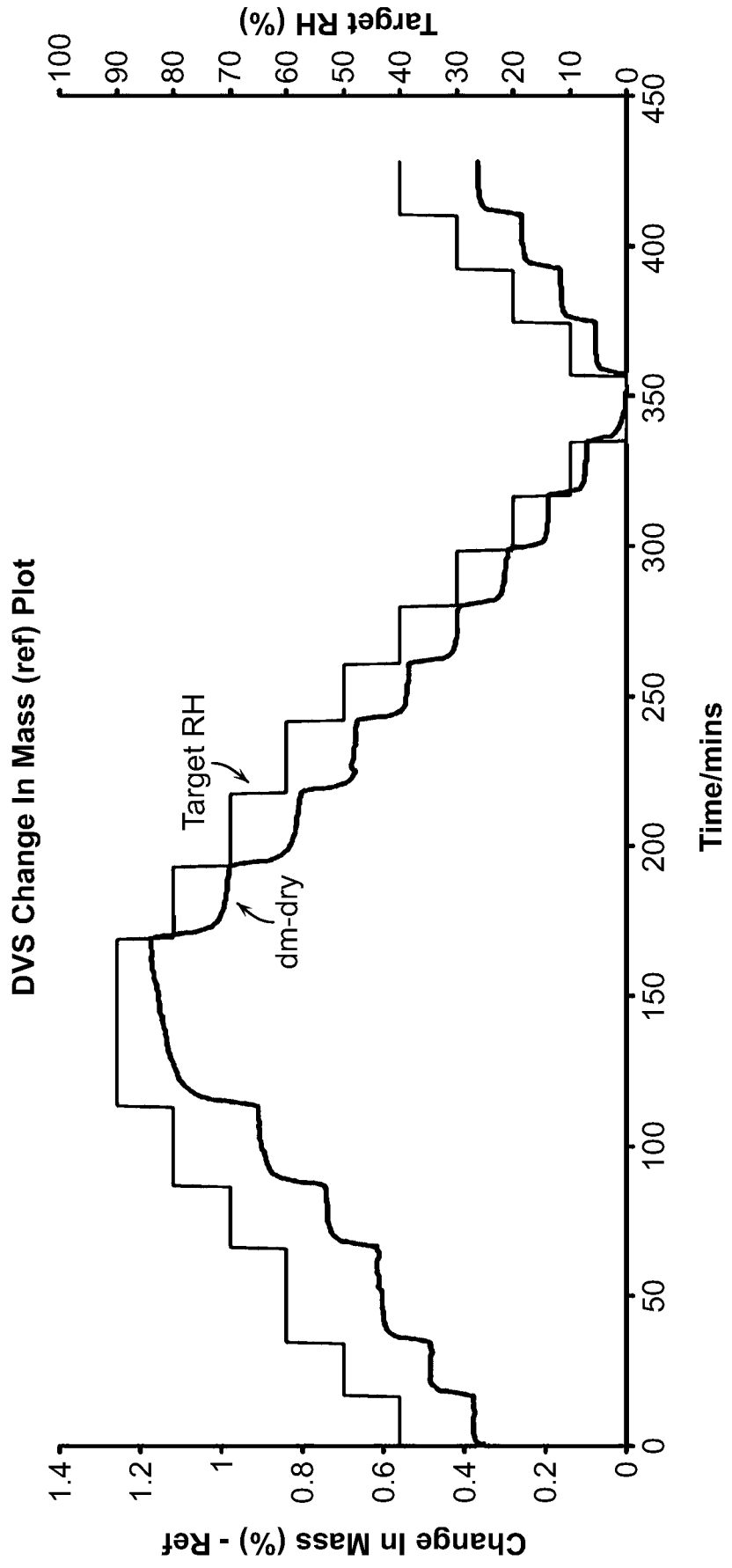

DVS Change In Mass (ref) Plot
Target RH
dm-dry
Change In Mass (%) - Ref
Target RH (%)
Time/mins
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450

FIG. 8B

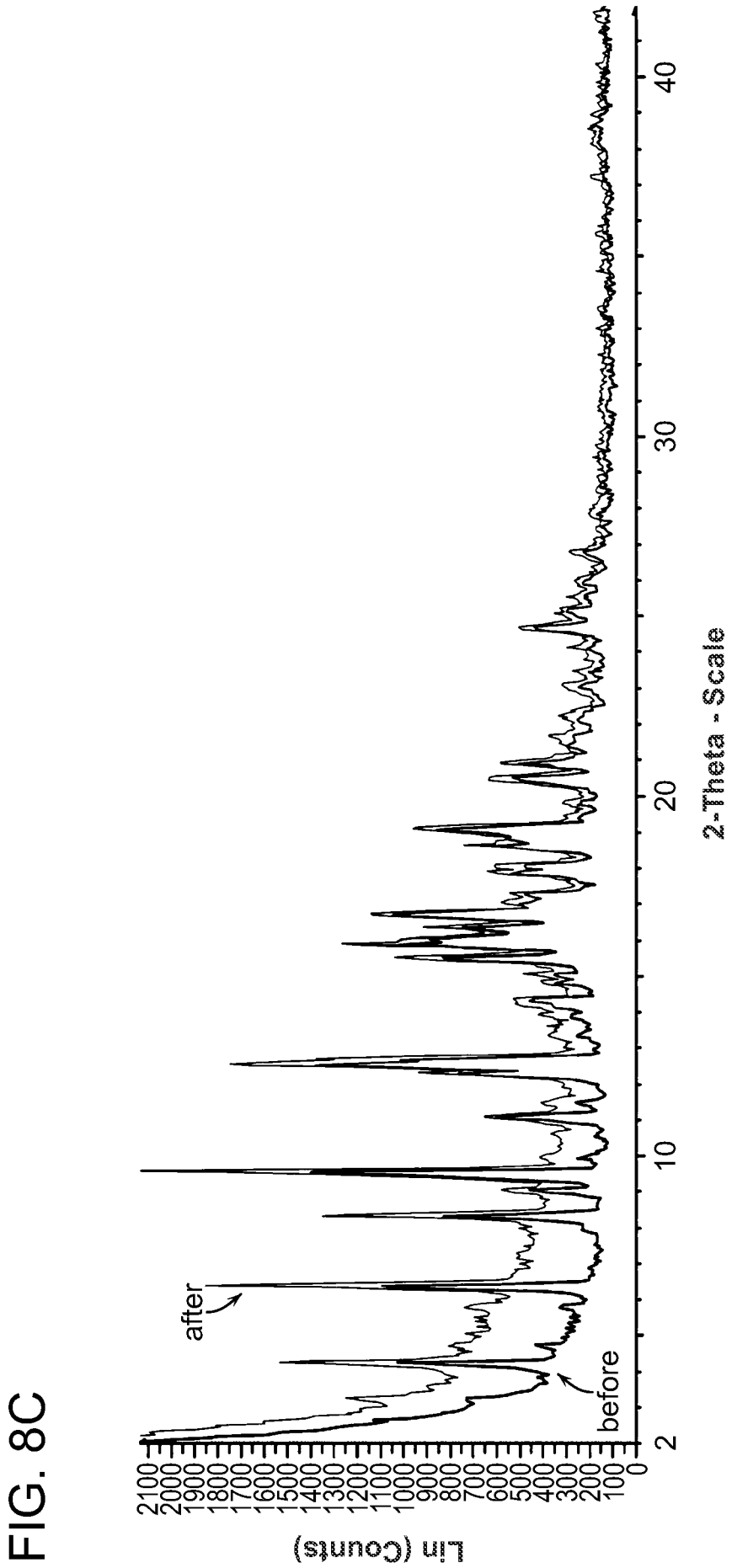

Lin (Counts)
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2
10
20
30
40
2-Theta - Scale
after
before

FIG. 8C

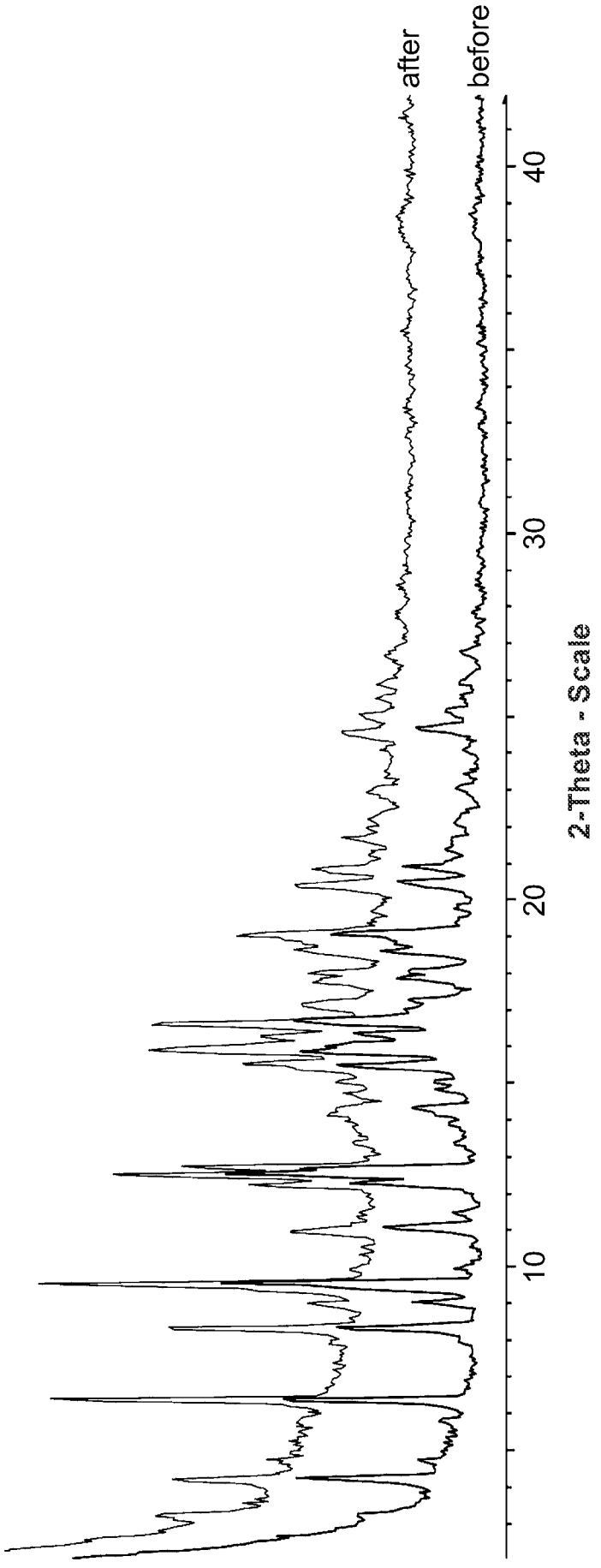


FIG. 9

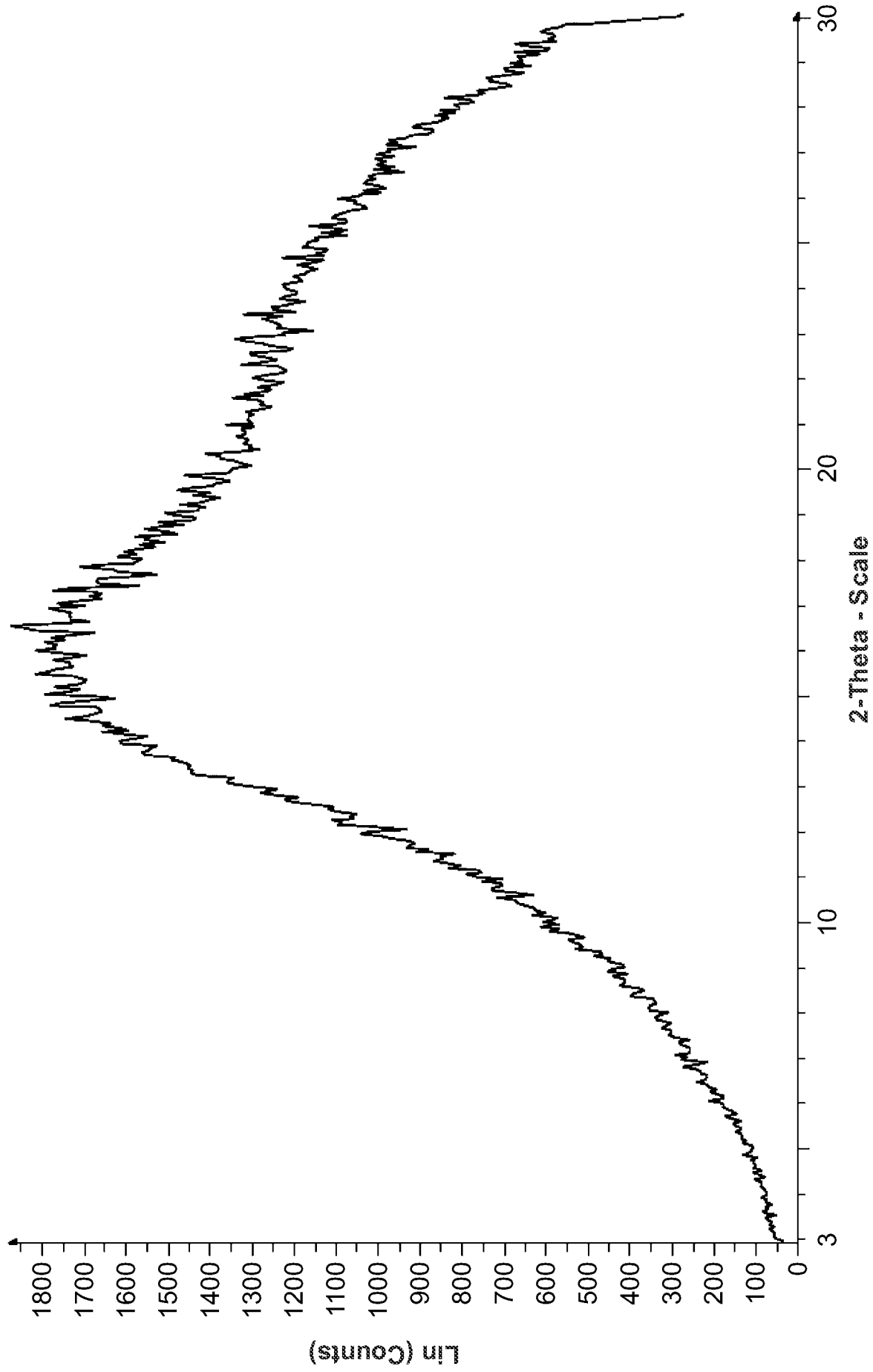

Lin (Counts)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
3
10
20
30
2-Theta - Scale

FIG. 10

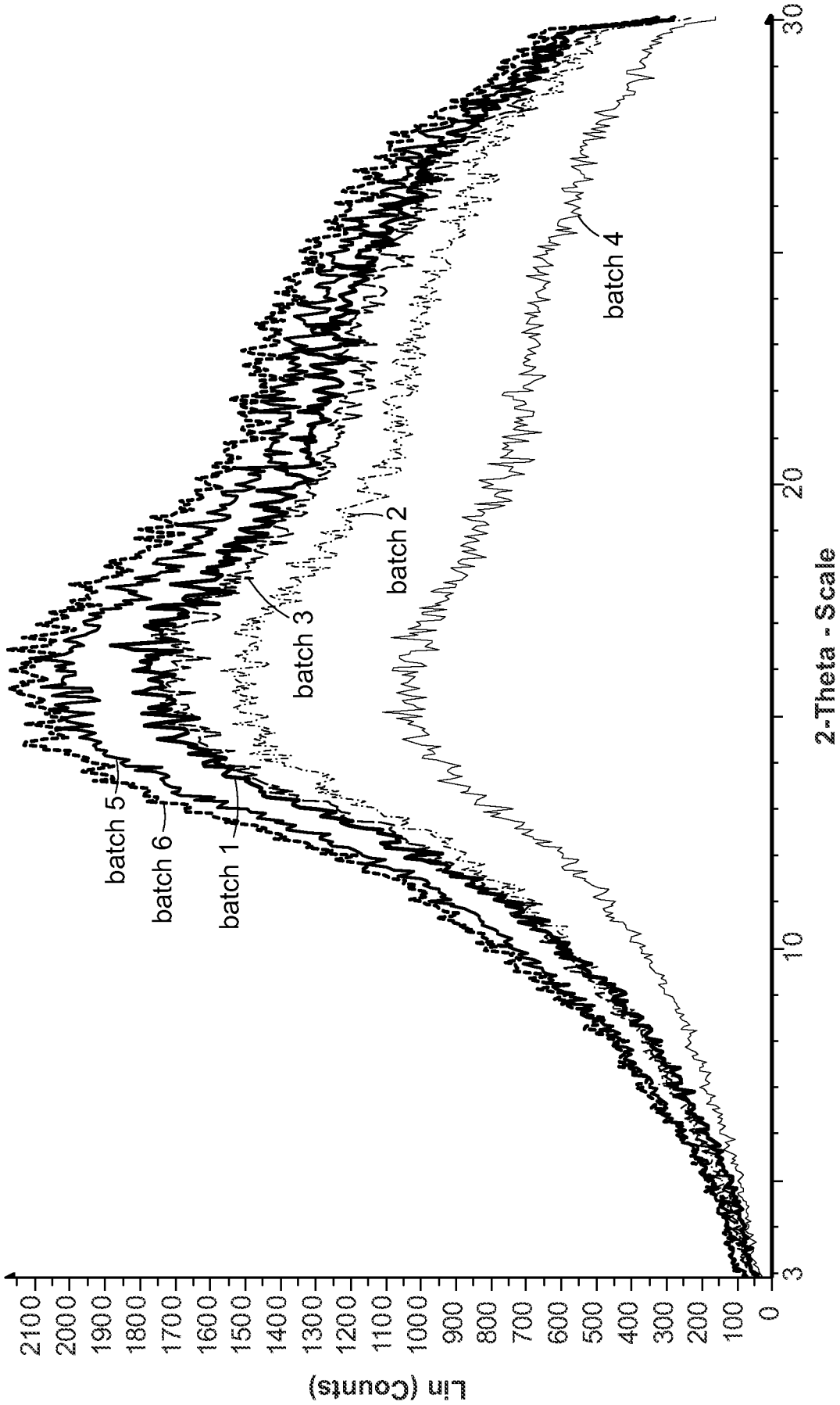

Lin (Counts)
2-Theta - Scale
batch 1
batch 2
batch 3
batch 4
batch 5
batch 6

FIG. 11

FIG. 12

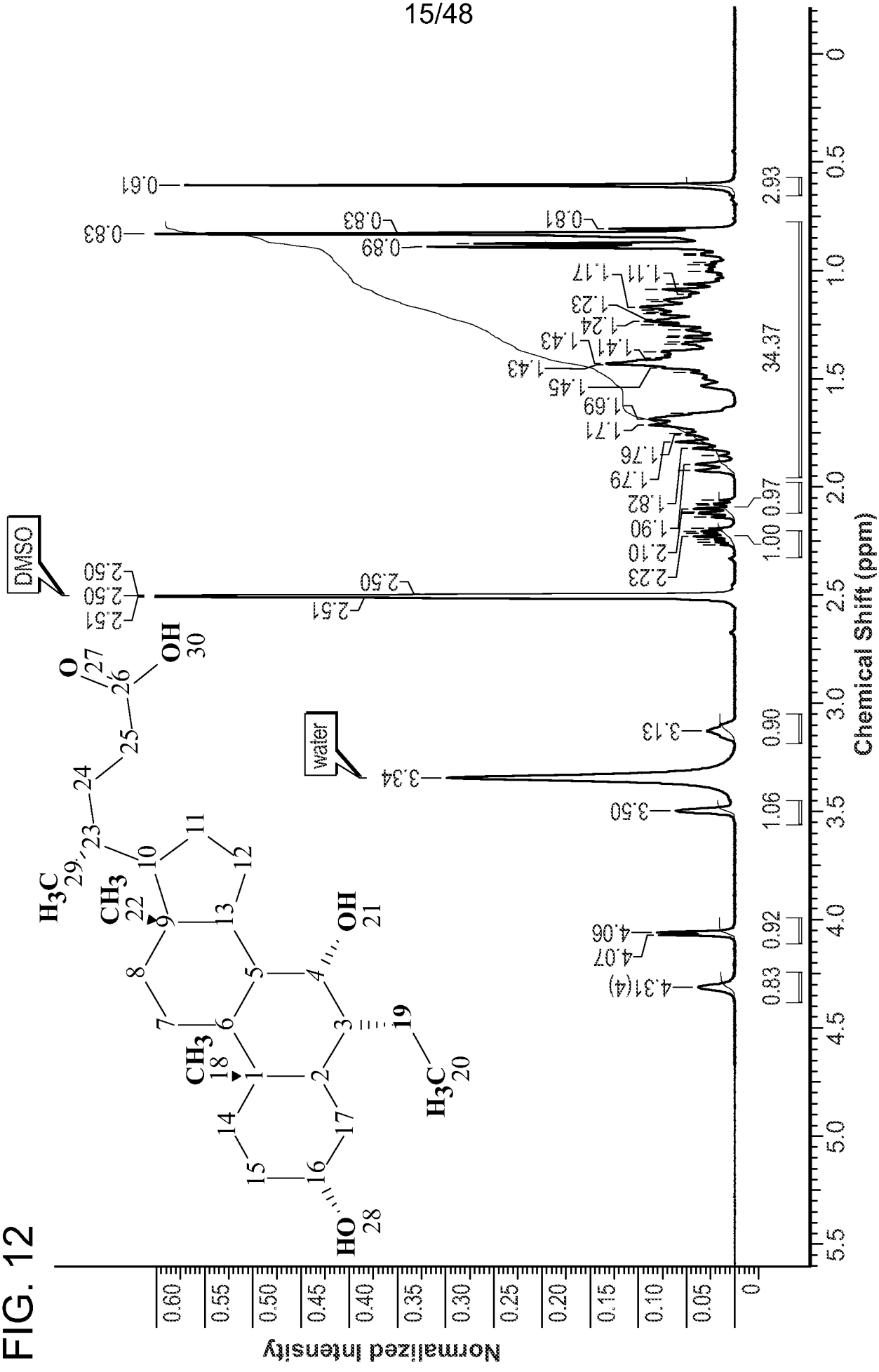
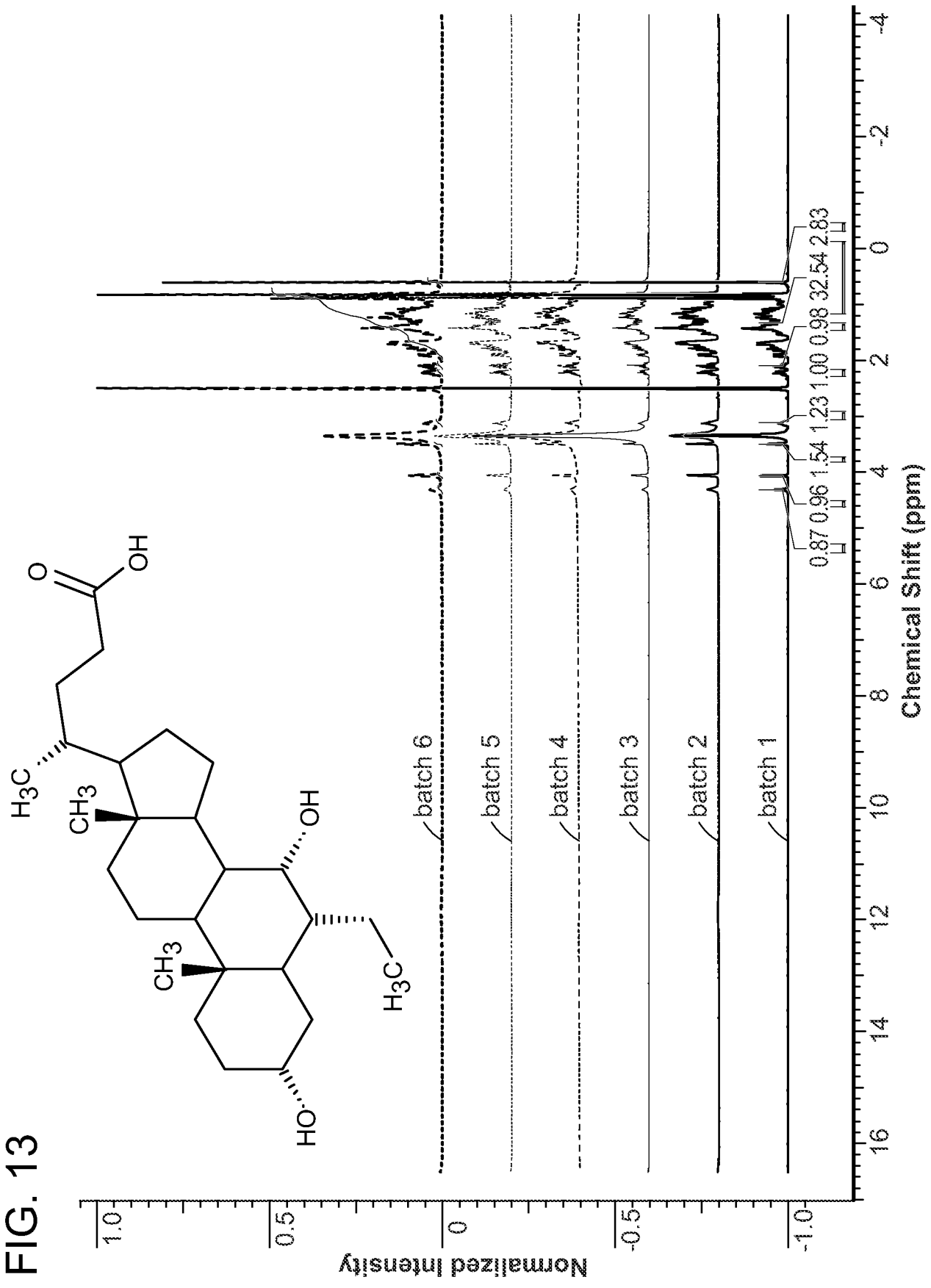

Normalized Intensity
Chemical Shift (ppm)
DMSO
water
0.61
0.83
0.89
0.81
0.83
1.11
1.17
1.23
1.24
1.41
1.43
1.43
1.45
1.69
1.71
1.76
1.79
1.82
1.90
2.10
2.23
2.50
2.50
2.50
2.51
3.13
3.34
3.50
4.06
4.07
4.31(4)
2.93
34.37
0.97
1.00
0.90
1.06
0.92
0.83
OH 30
O 27
26
25
24
23
29 H3C
CH3 22
10
11
12
9
13
OH 21
8
5
4
7
6
3
19
CH3 18
1
2
H3C 20
14
17
15
16
HO 28

FIG. 13


Normalized Intensity
1.0
0.5
0
-0.5
-1.0
Chemical Shift (ppm)
16
14
12
10
8
6
4
2
0
-2
-4
0.87 0.96 1.54 1.23 1.00 0.98 32.54 2.83
batch 6
batch 5
batch 4
batch 3
batch 2
batch 1
O
OH
H3C
CH3
CH3
OH
HO
H3C

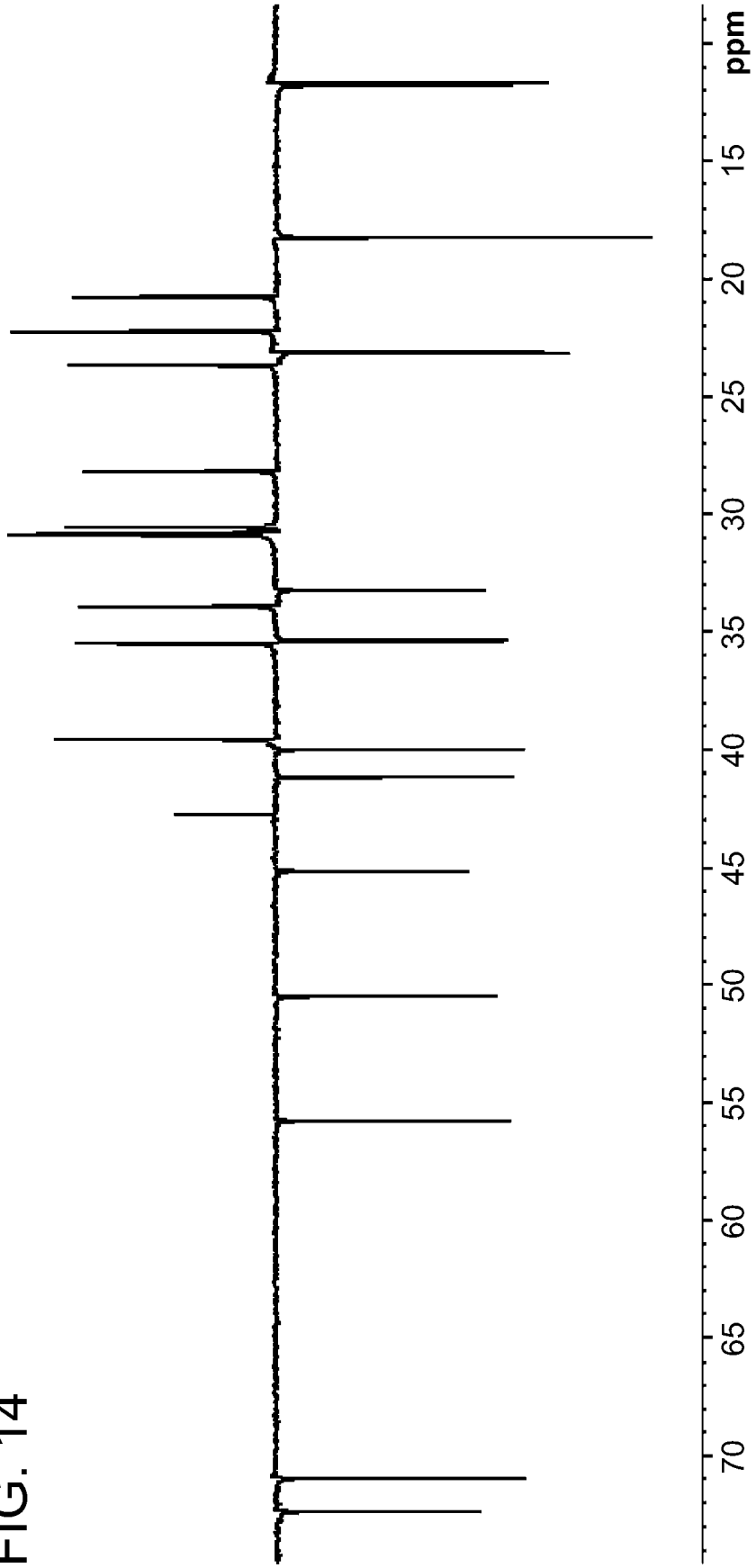


FIG. 14

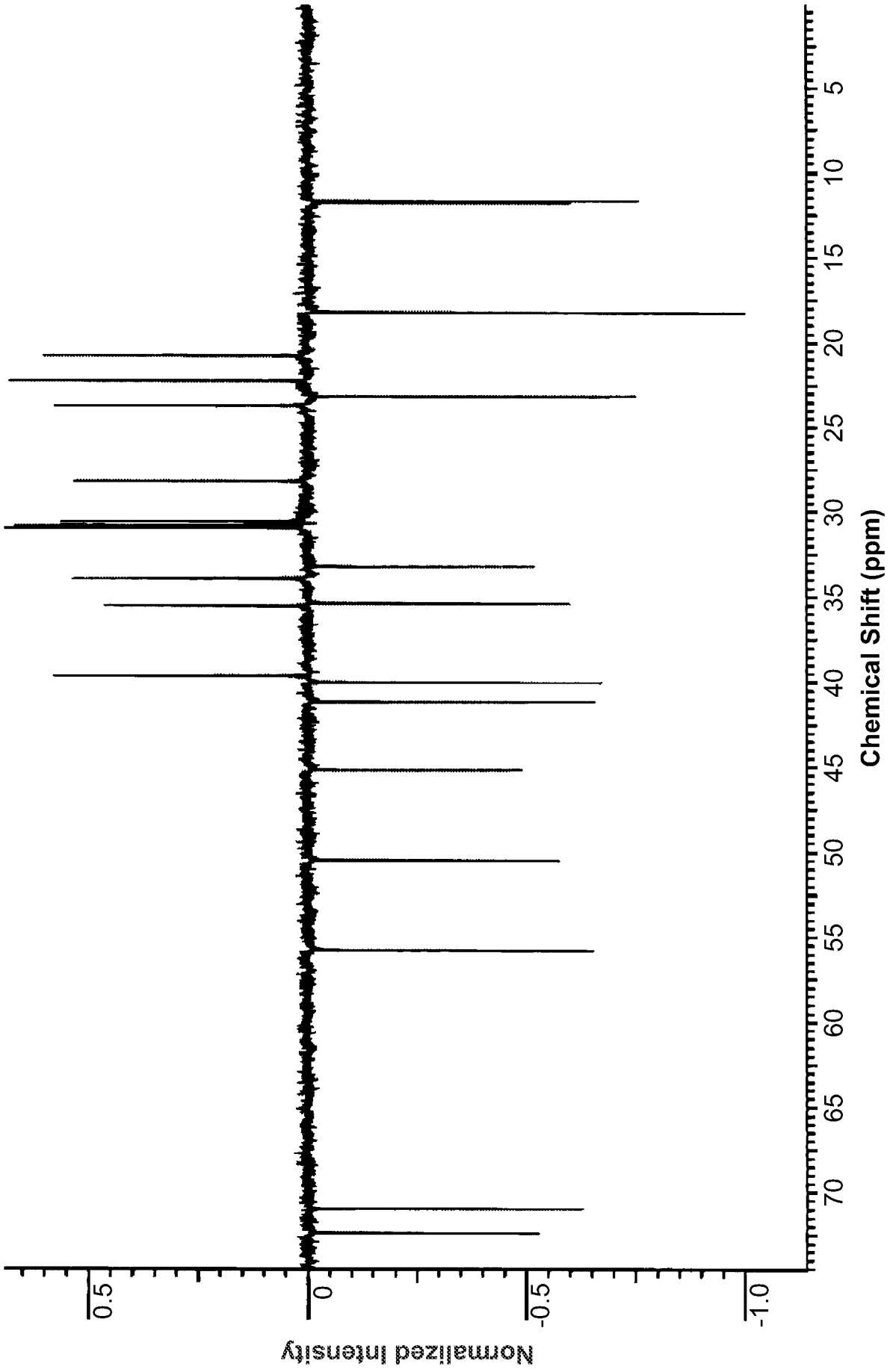

Chemical Shift (ppm)
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Normalized Intensity
0.5
0
-0.5
-1.0

FIG. 15

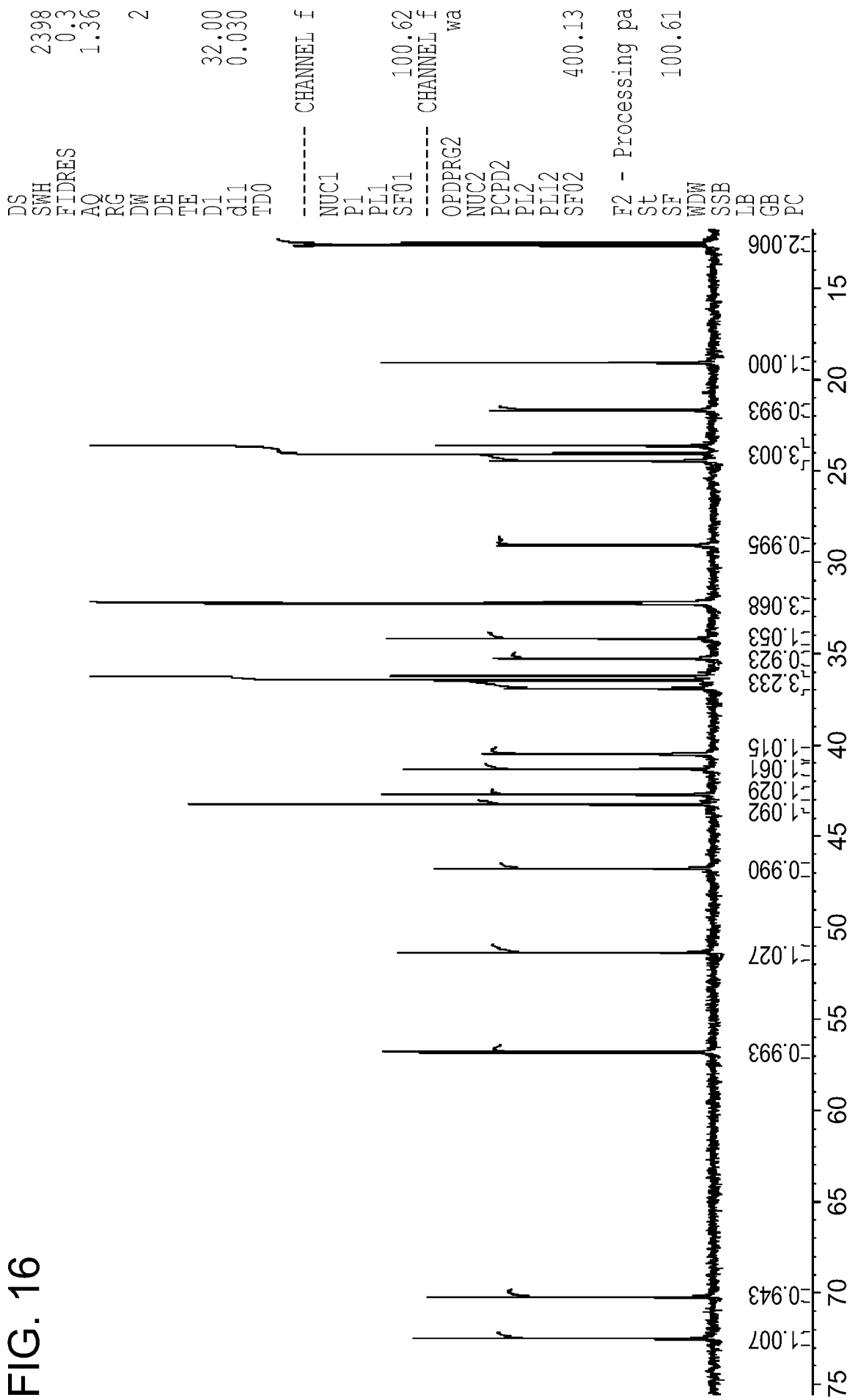

DS 2398
SWH 0.3
FIDRES 1.36
AQ
RG 2
DW
DE
TE
D1 32.00
d11 0.030
TD0
-------- CHANNEL f
NUC1
P1
PL1 100.62
SF01 -------- CHANNEL f
OPDPRG2 wa
NUC2
PCPD2
PL2
PL12
SF02 400.13
F2 - Processing pa
St
SF 100.61
WDW
SSB
LB
GB
PC
2.006
1.000
0.993
3.003
0.995
3.068
1.053
0.923
3.233
1.015
1.061
1.029
1.092
0.990
1.027
0.993
0.943
1.007
15
20
25
30
35
40
45
50
55
60
65
70
75

FIG. 16

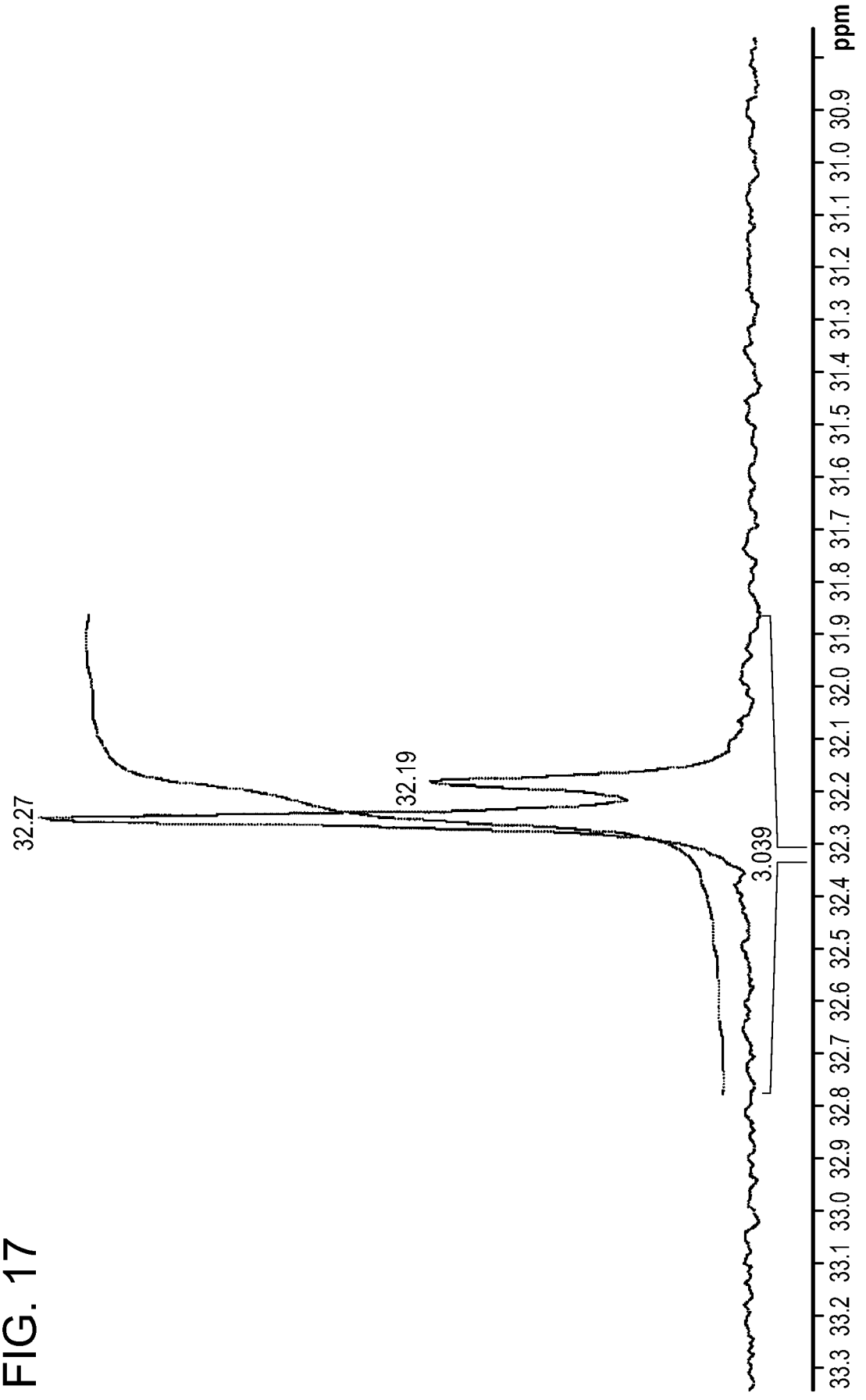

32.27
32.19
3.039
33.3 33.2 33.1 33.0 32.9 32.8 32.7 32.6 32.5 32.4 32.3 32.2 32.1 32.0 31.9 31.8 31.7 31.6 31.5 31.4 31.3 31.2 31.1 31.0 30.9
ppm

FIG. 17

FIG. 18

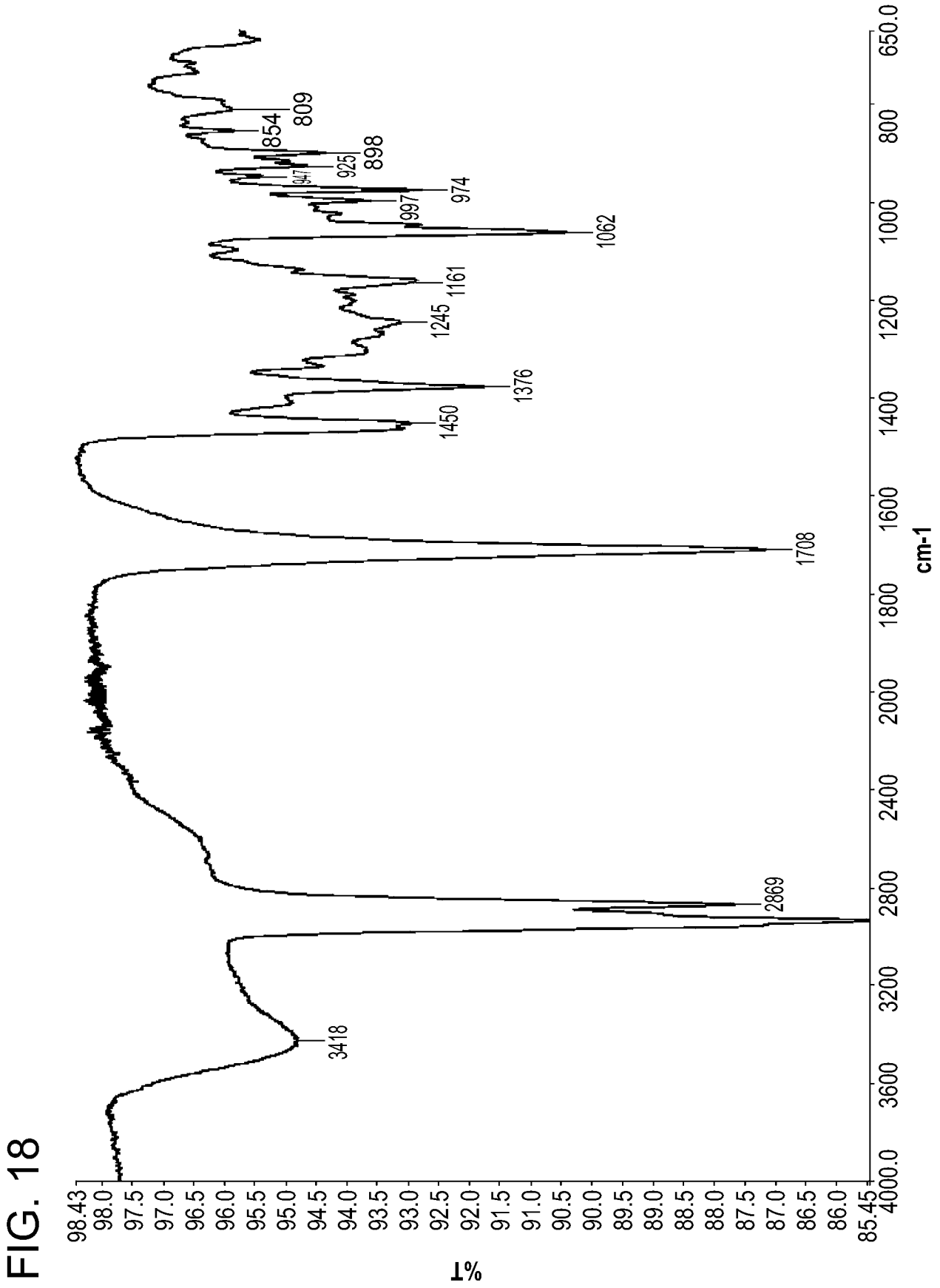
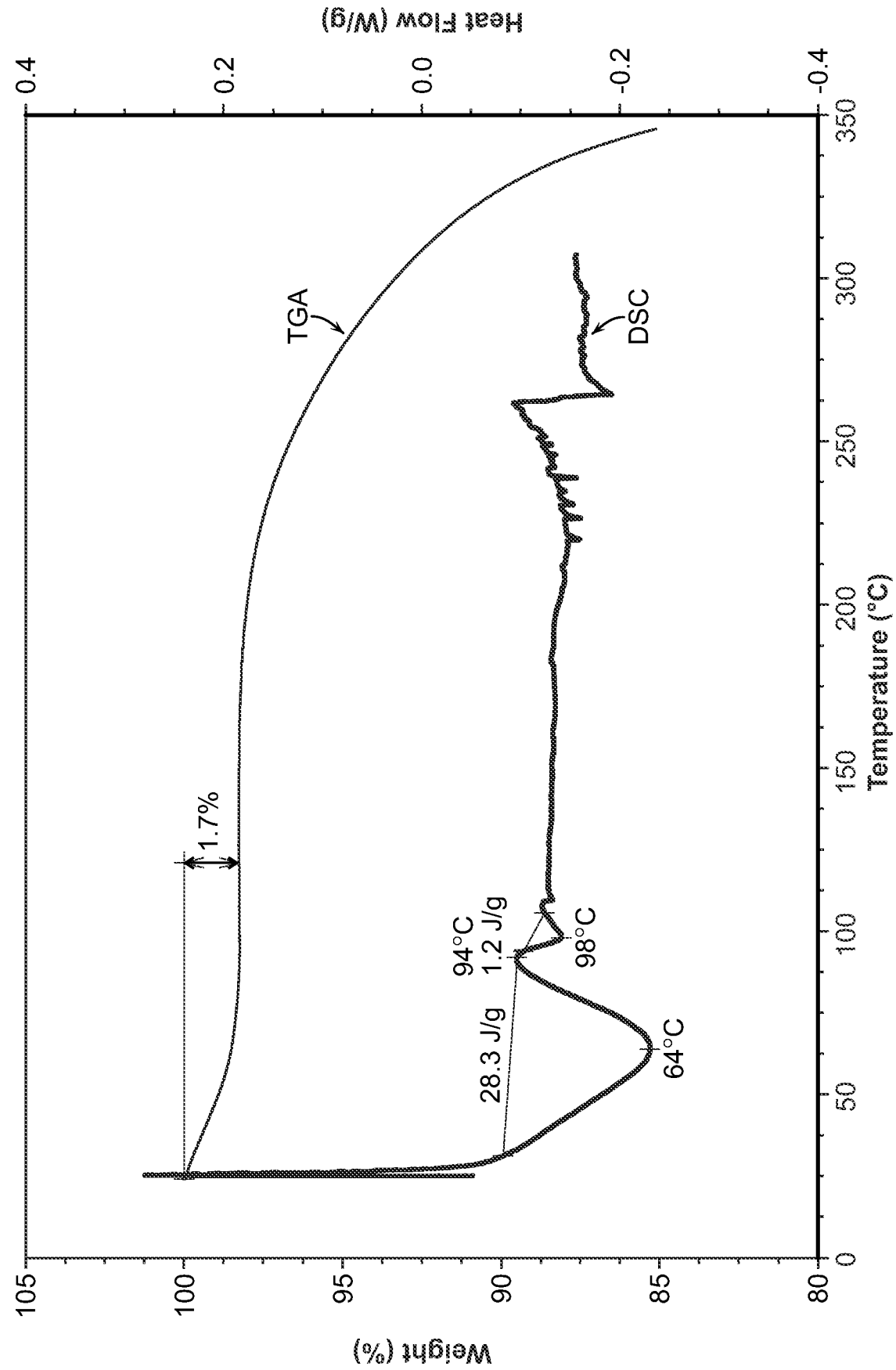

%T
cm-1
98.43
98.0
97.5
97.0
96.5
96.0
95.5
95.0
94.5
94.0
93.5
93.0
92.5
92.0
91.5
91.0
90.5
90.0
89.5
89.0
88.5
88.0
87.5
87.0
86.5
86.0
85.45
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650.0
3418
2869
1708
1450
1376
1245
1161
1062
997
974
947
925
898
854
809

FIG. 19


Heat Flow (W/g)
0.4
0.2
0.0
-0.2
-0.4
TGA
DSC
1.7%
94°C
1.2 J/g
98°C
28.3 J/g
64°C
Weight (%)
105
100
95
90
85
80
0
50
100
150
200
250
300
350
Temperature (°C)

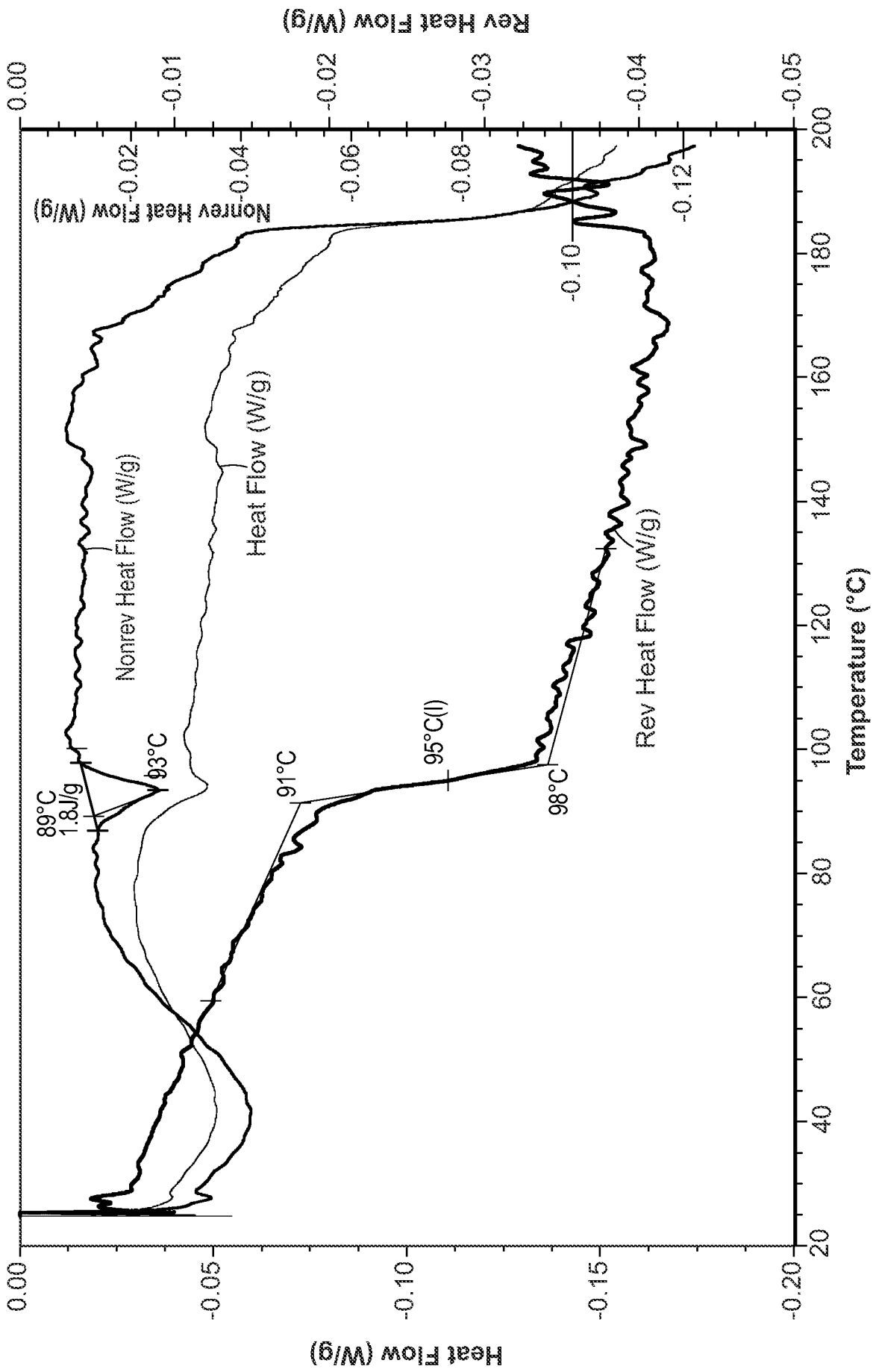
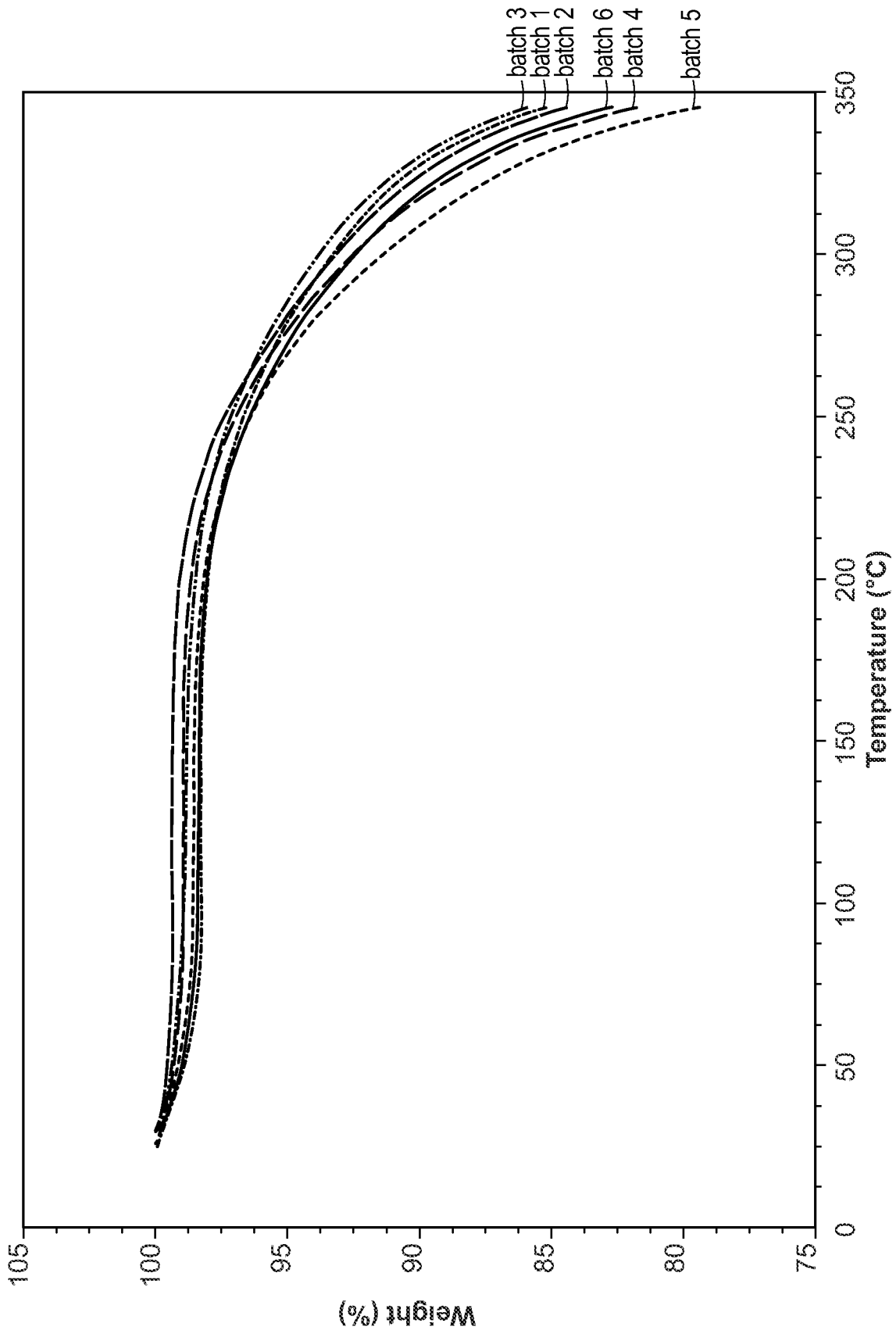

Rev Heat Flow (W/g)
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
Nonrev Heat Flow (W/g)
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
Nonrev Heat Flow (W/g)
Heat Flow (W/g)
Rev Heat Flow (W/g)
89°C
1.8J/g
93°C
91°C
95°C(l)
98°C
-0.10
Heat Flow (W/g)
0.00
-0.05
-0.10
-0.15
-0.20
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 20

FIG. 21


batch 3
batch 1
batch 2
batch 6
batch 4
batch 5
Weight (%)
105
100
95
90
85
80
75
Temperature (°C)
0
50
100
150
200
250
300
350

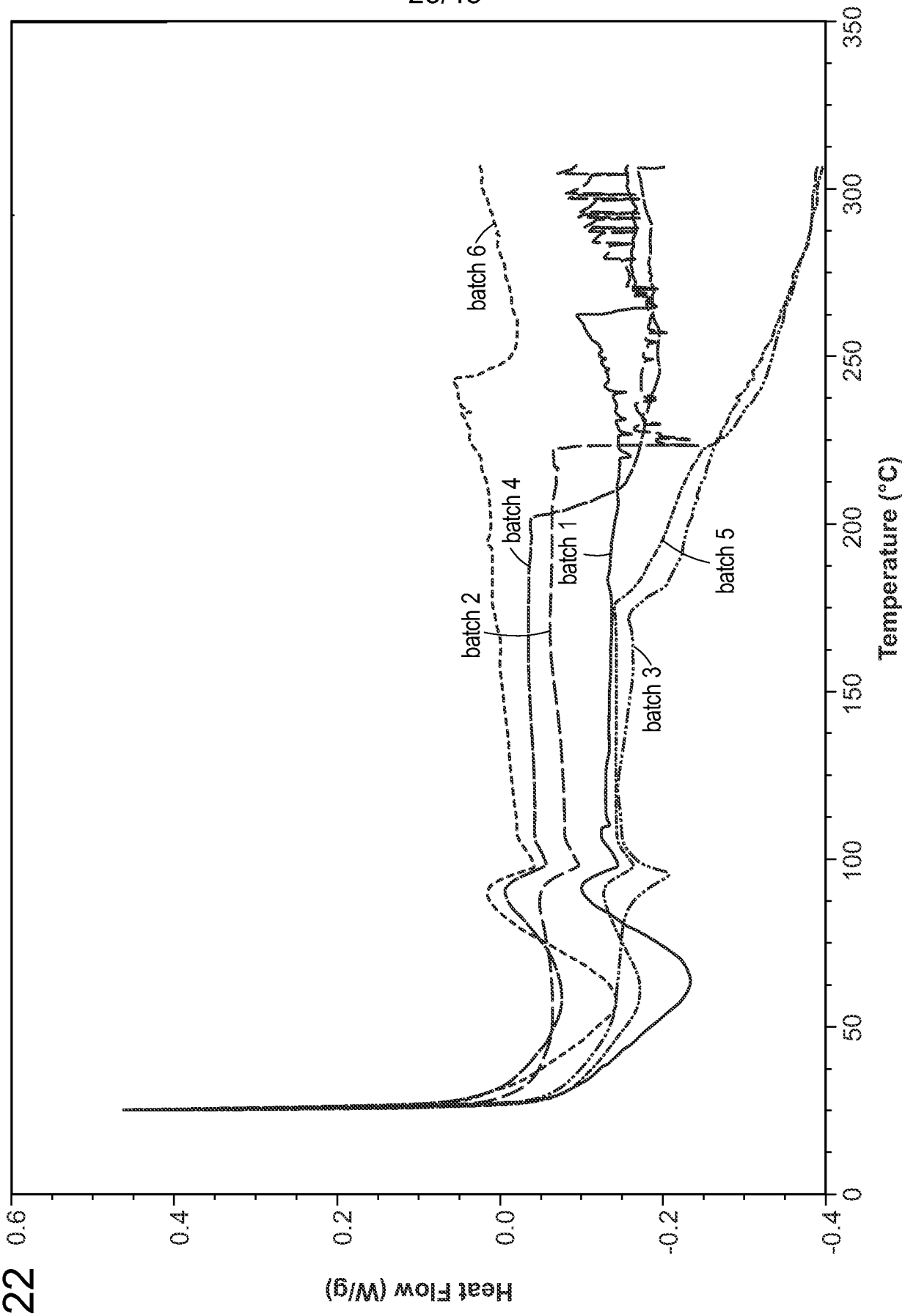

Heat Flow (W/g)
0.6
0.4
0.2
0.0
-0.2
-0.4
Temperature (°C)
0
50
100
150
200
250
300
350
batch 6
batch 4
batch 1
batch 5
batch 2
batch 3

FIG. 22

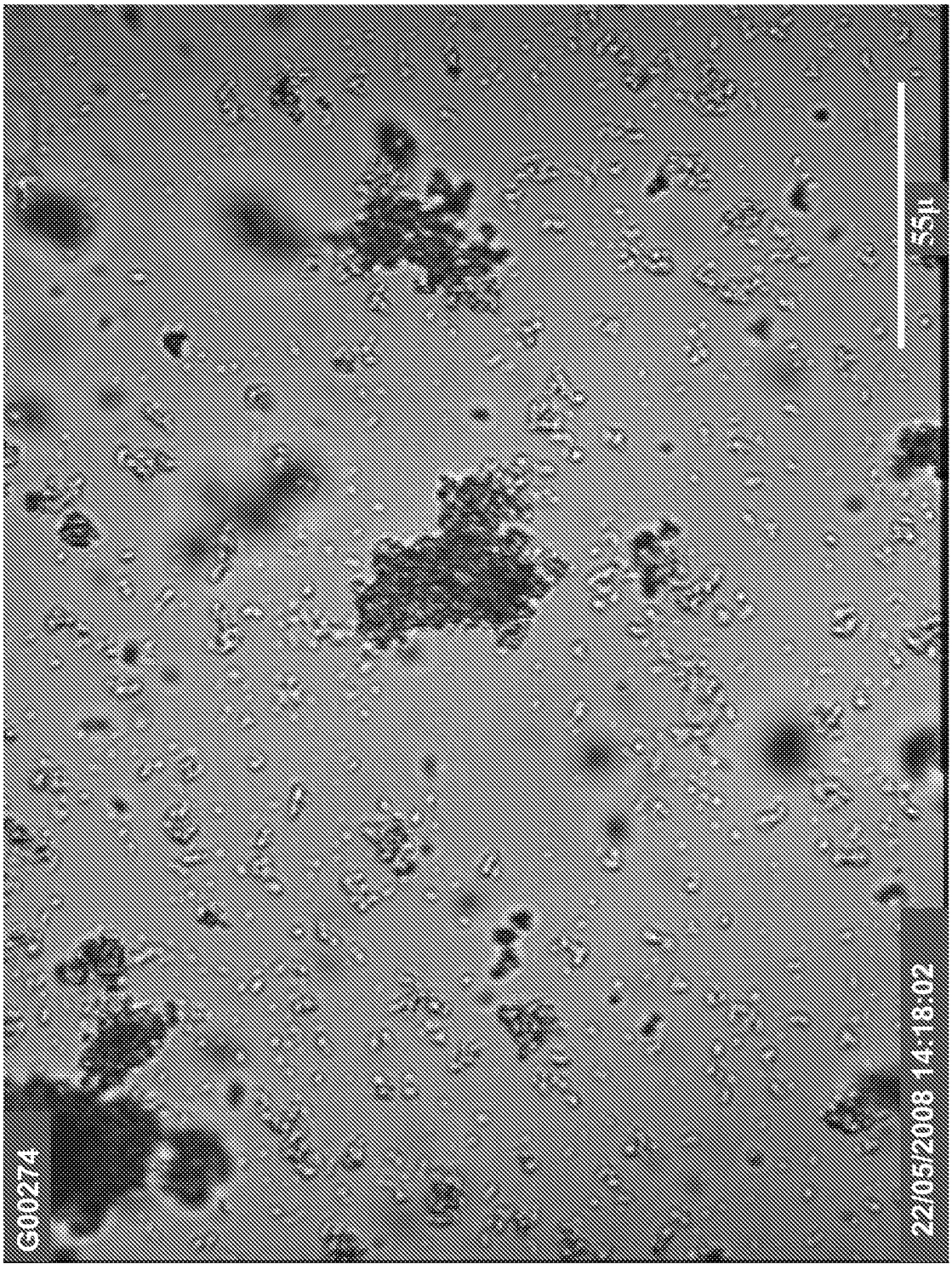


FIG. 23A

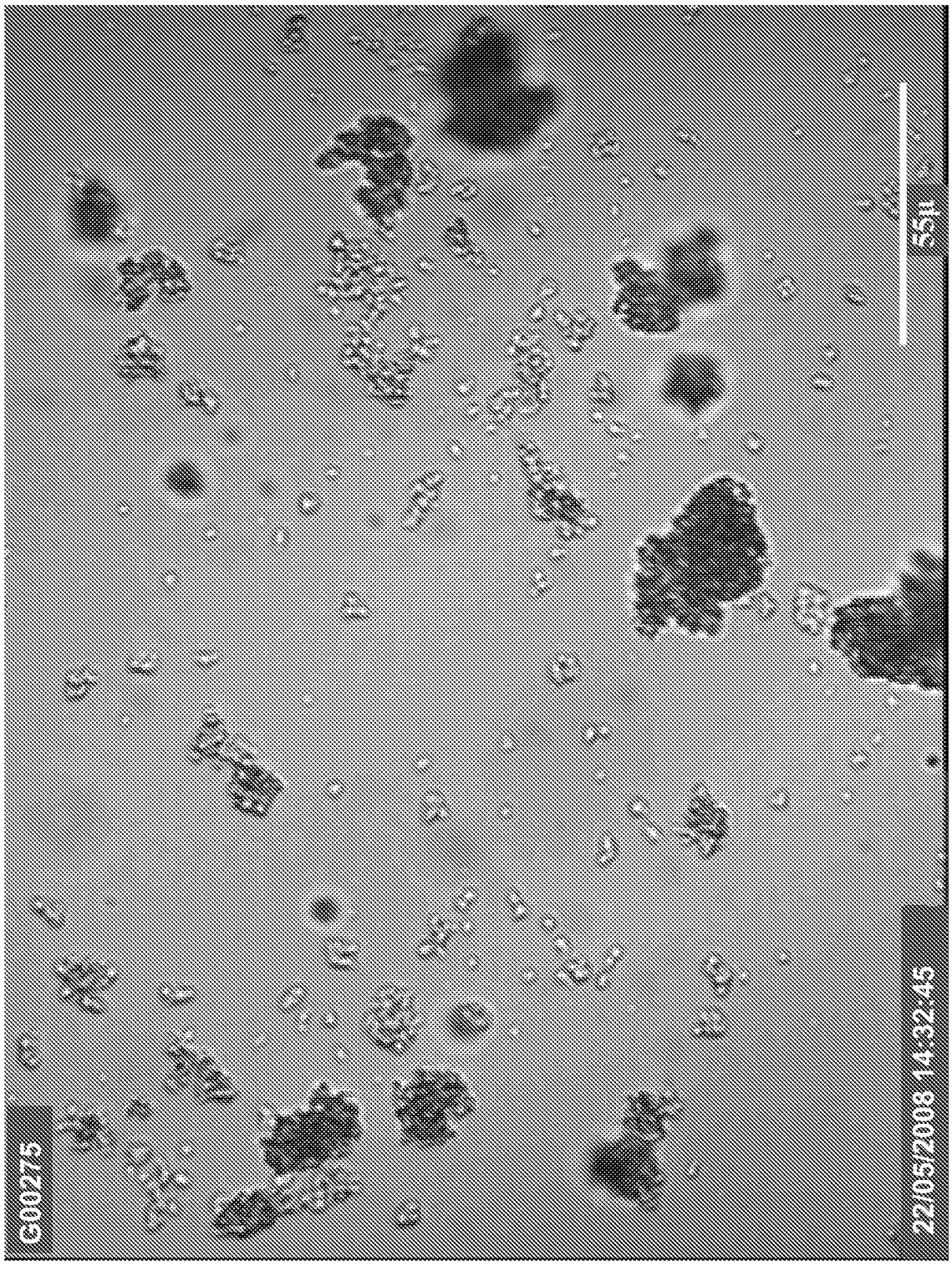


FIG. 23B

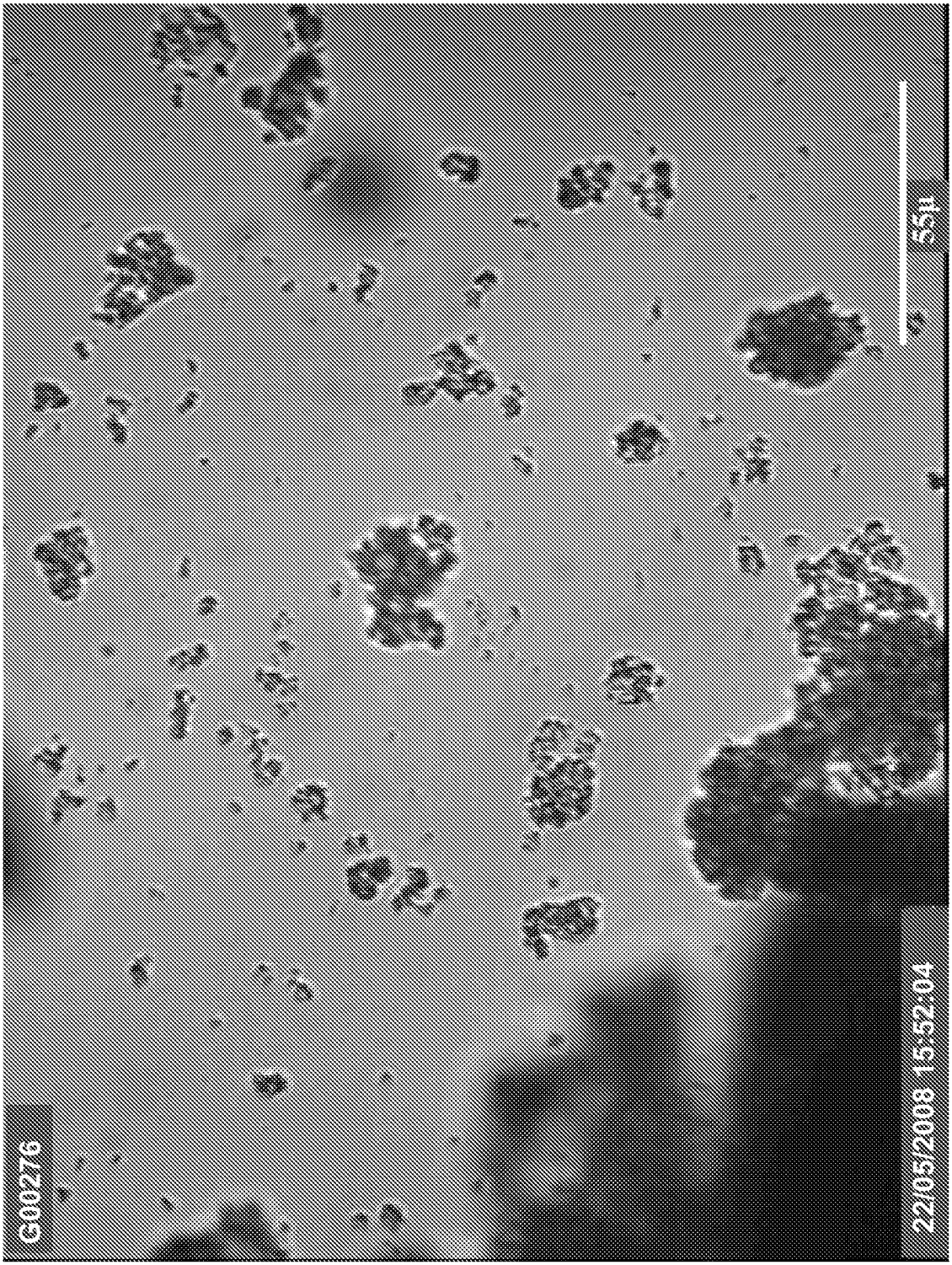


FIG. 23C

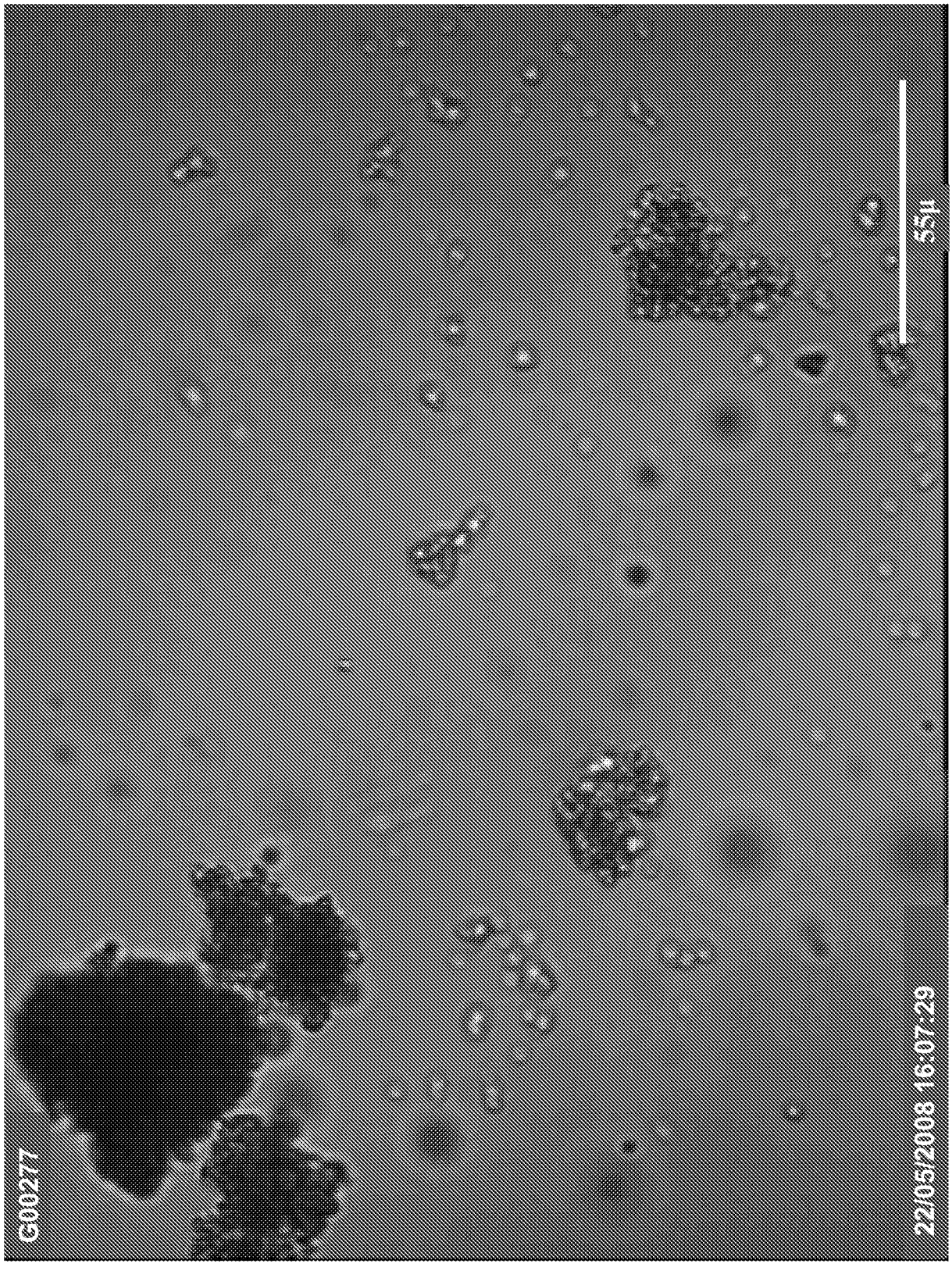


FIG. 23D

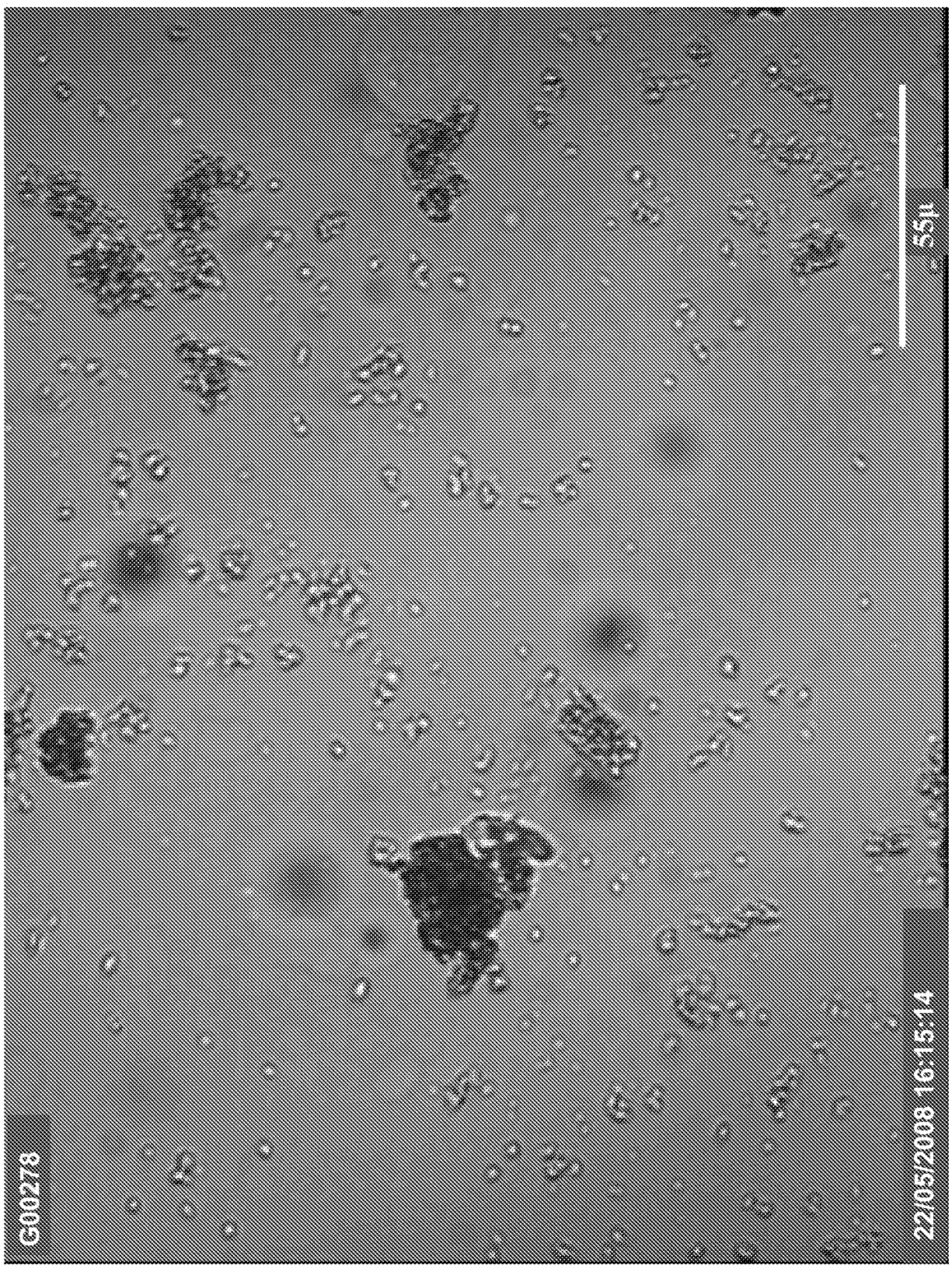


FIG. 23E

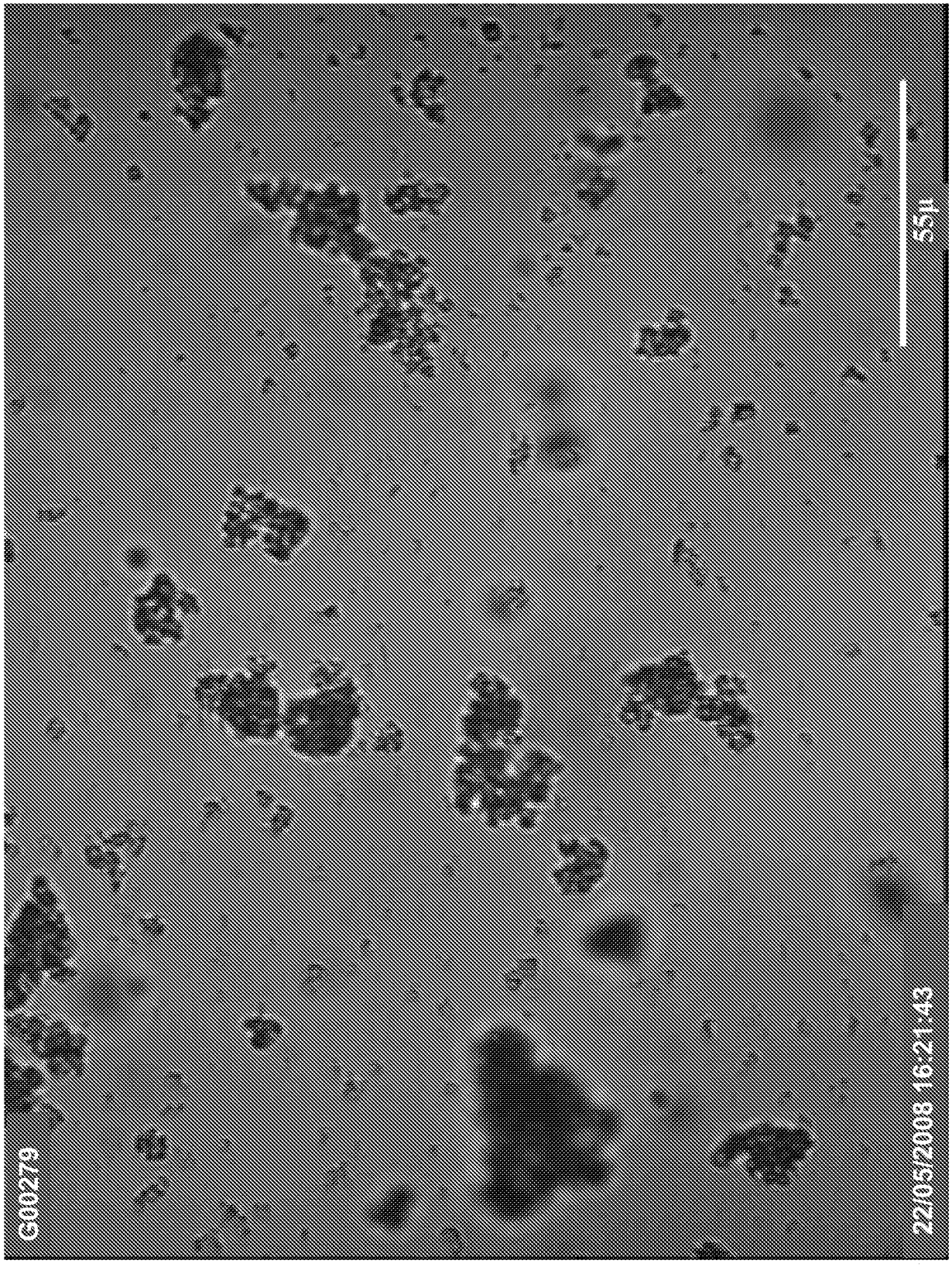


FIG. 23F

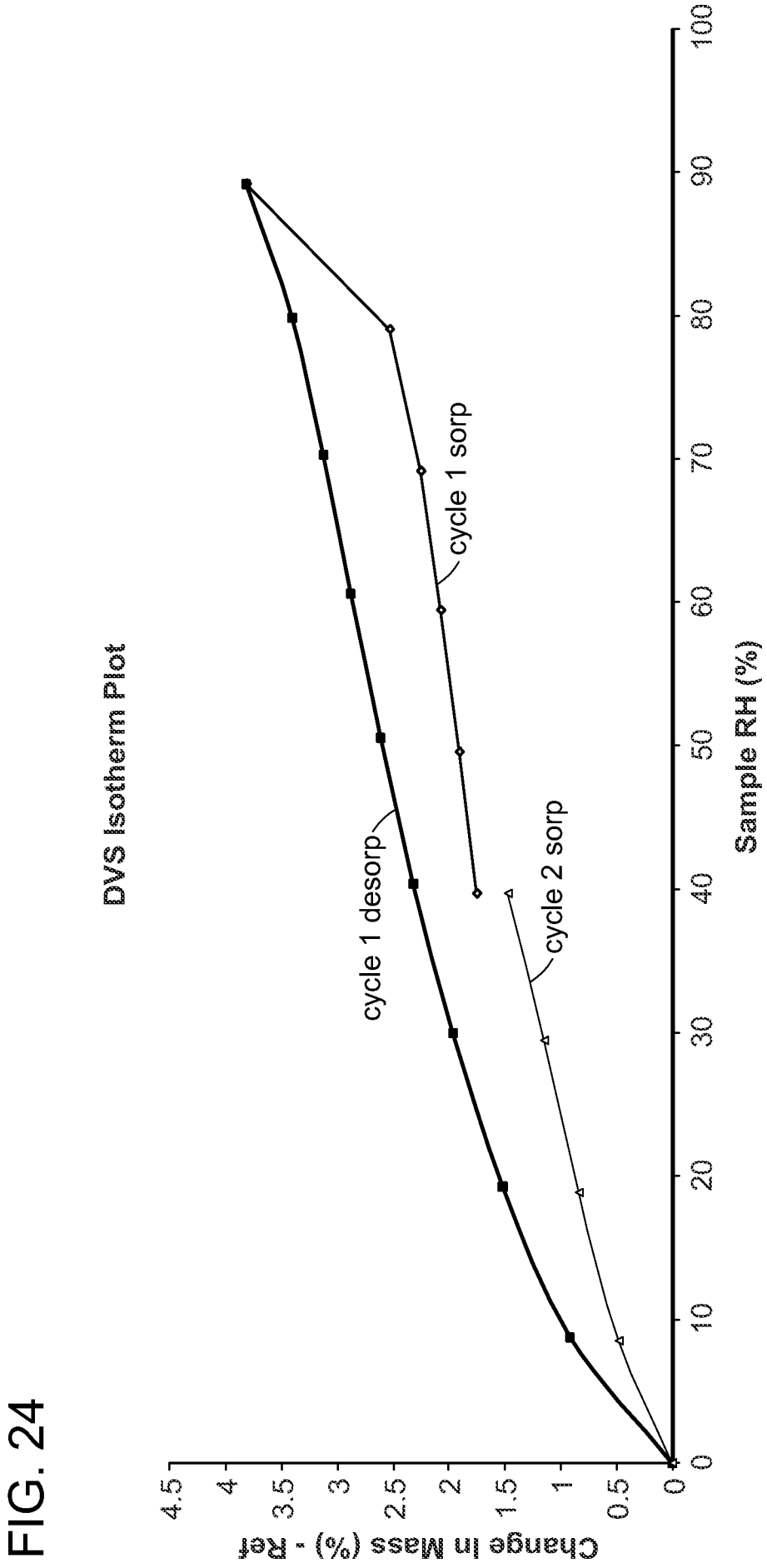

DVS Isotherm Plot
Change in Mass (%) - Ref
Sample RH (%)
cycle 1 sorp
cycle 1 desorp
cycle 2 sorp
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
10
20
30
40
50
60
70
80
90
100

FIG. 24

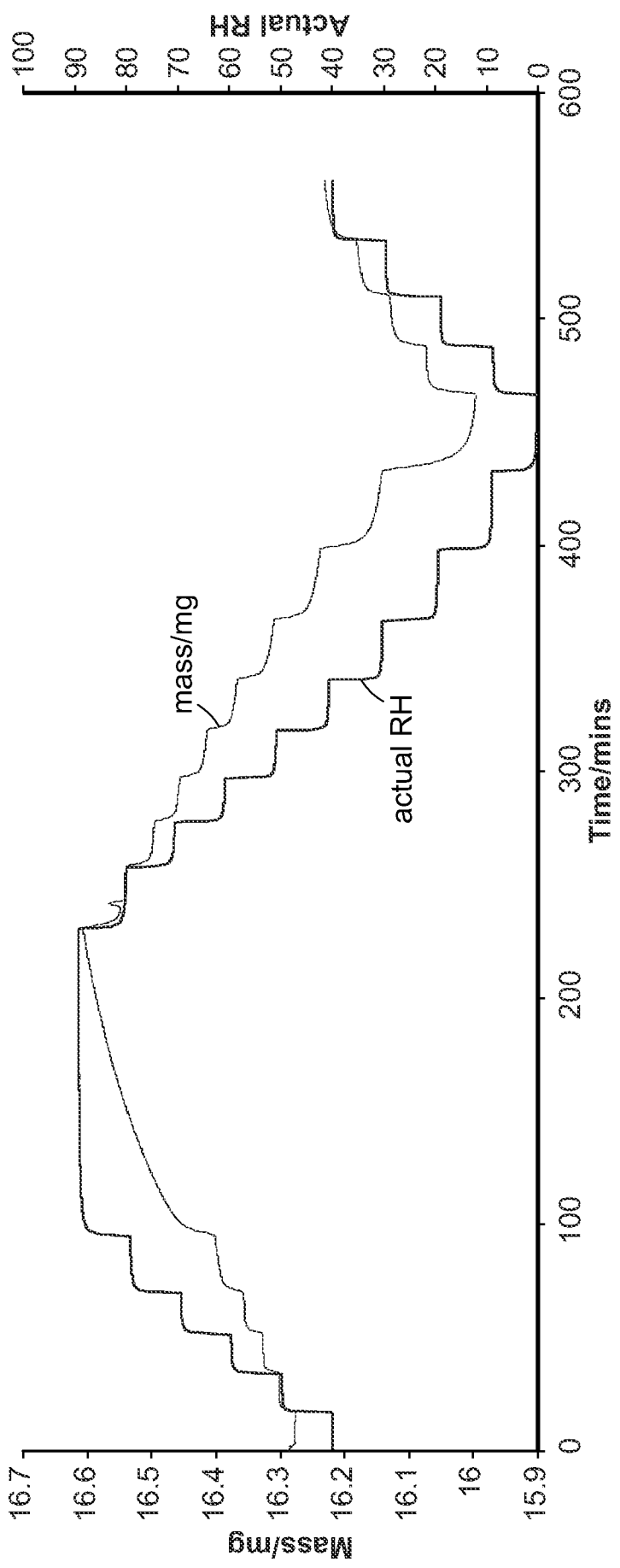

Actual RH
100
90
80
70
60
50
40
30
20
10
0
600
500
400
300
200
100
0
Time/mins
mass/mg
actual RH
16.7
16.6
16.5
16.4
16.3
16.2
16.1
16
15.9
Mass/mg

FIG. 25

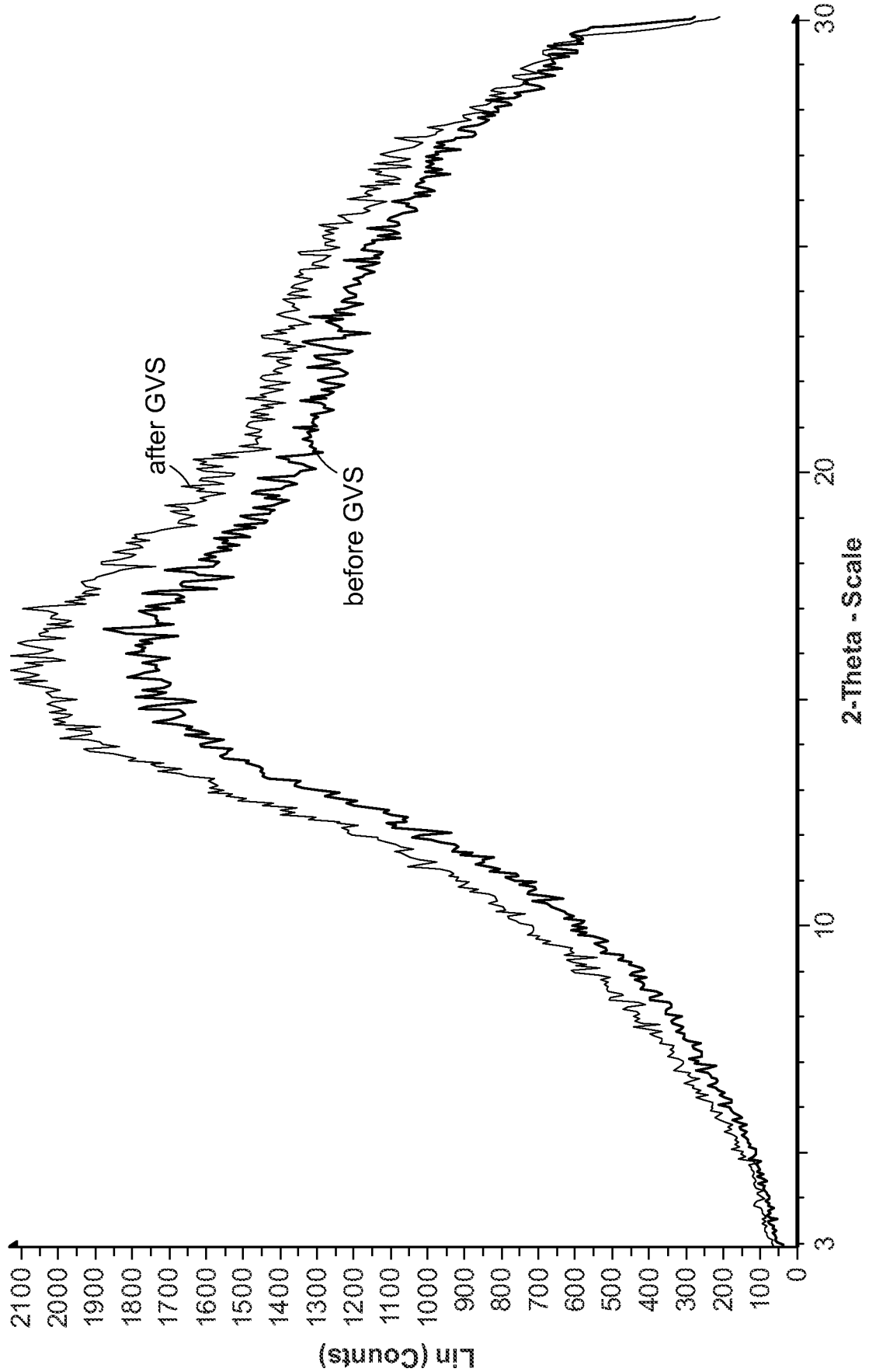

after GVS
before GVS
Lin (Counts)
2-Theta - Scale
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
3
10
20
30

FIG. 26

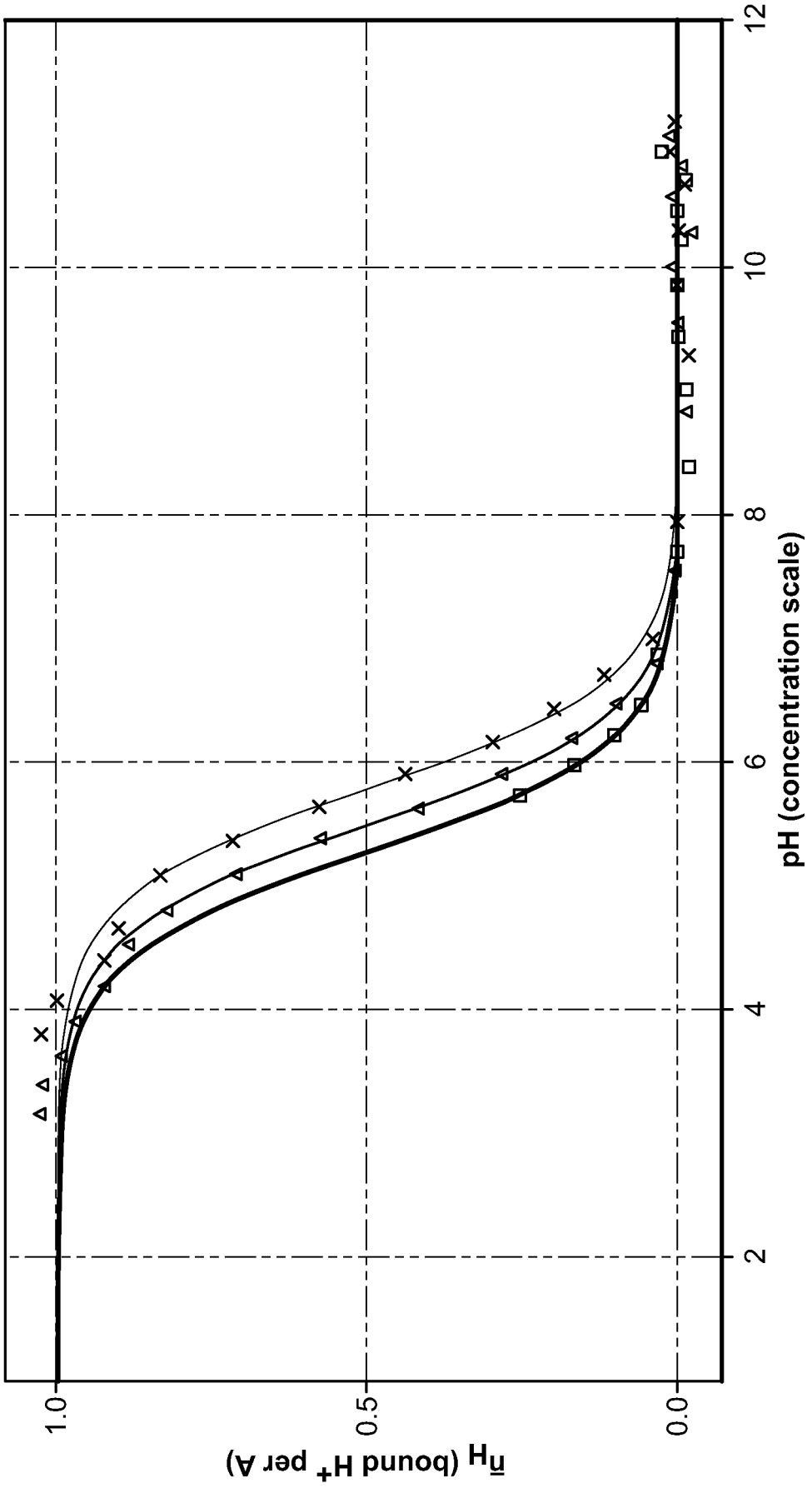
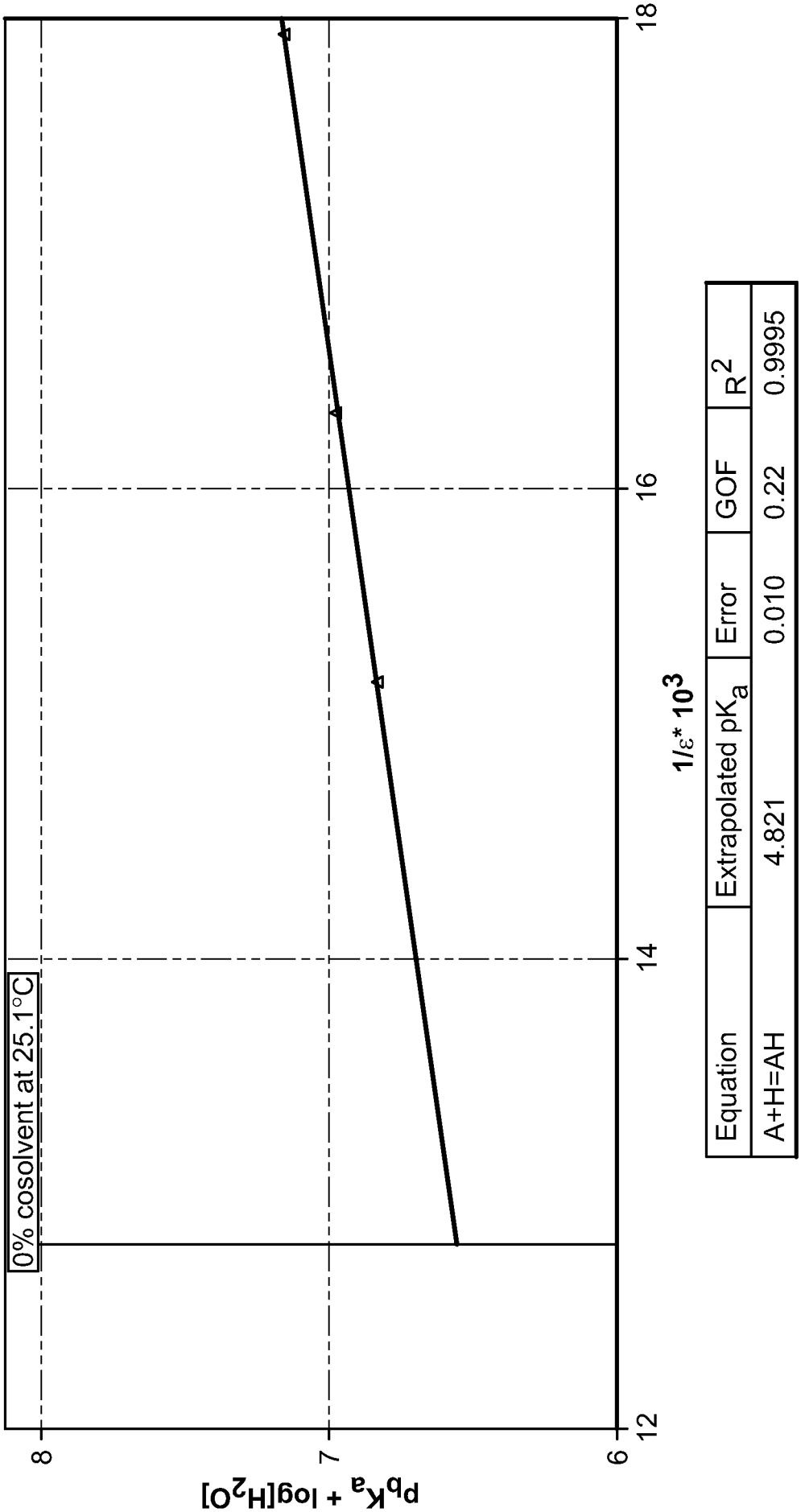


FIG. 27

FIG. 28


0% cosolvent at 25.1°C
$p_bK_a + \log[H_2O]$
$1/\varepsilon^* 10^3$
8
7
6
18
16
14
12
Equation
Extrapolated pK_a
Error
GOF
R^2
A+H=AH
4.821
0.010
0.22
0.9995

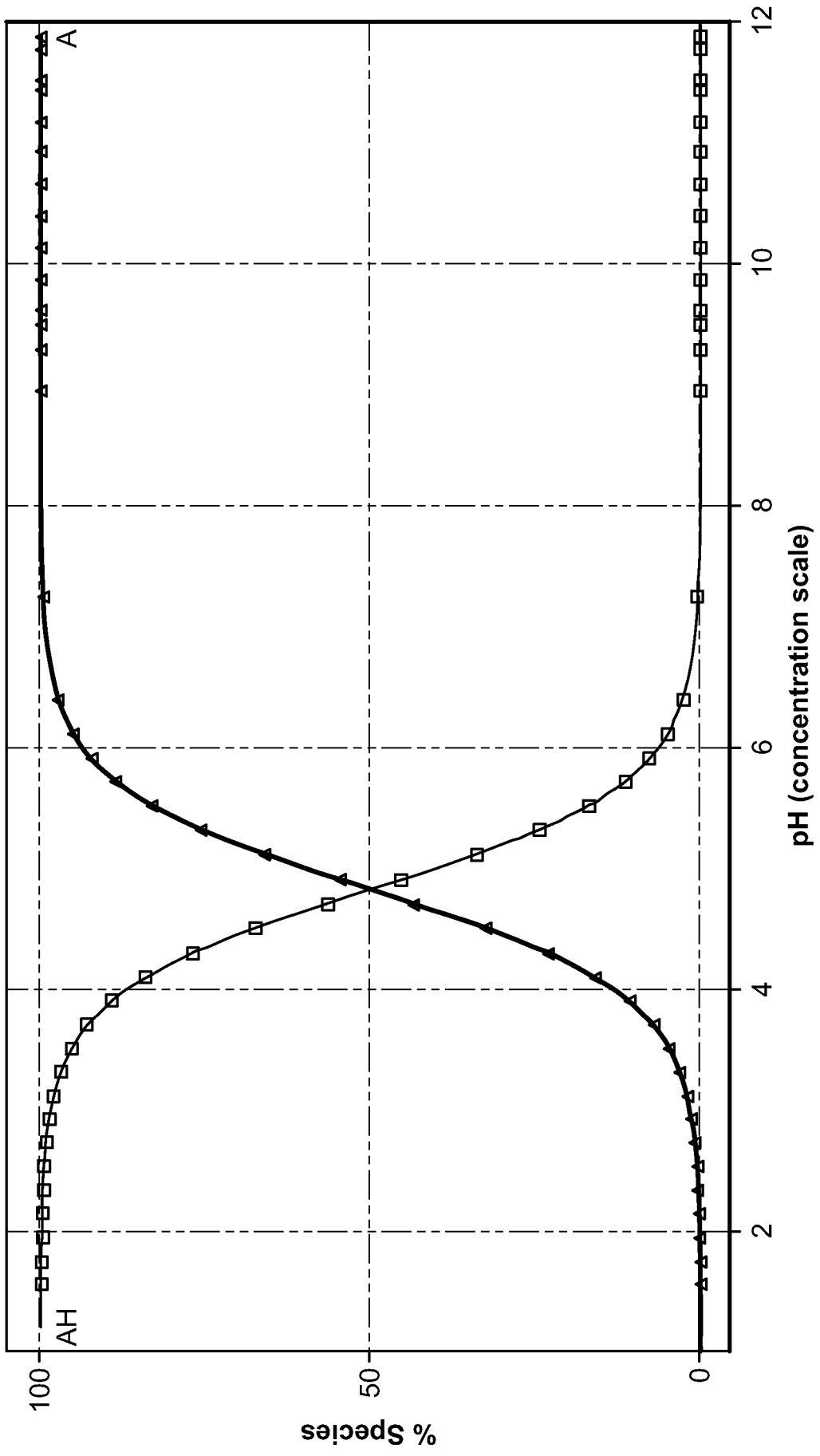


FIG. 29

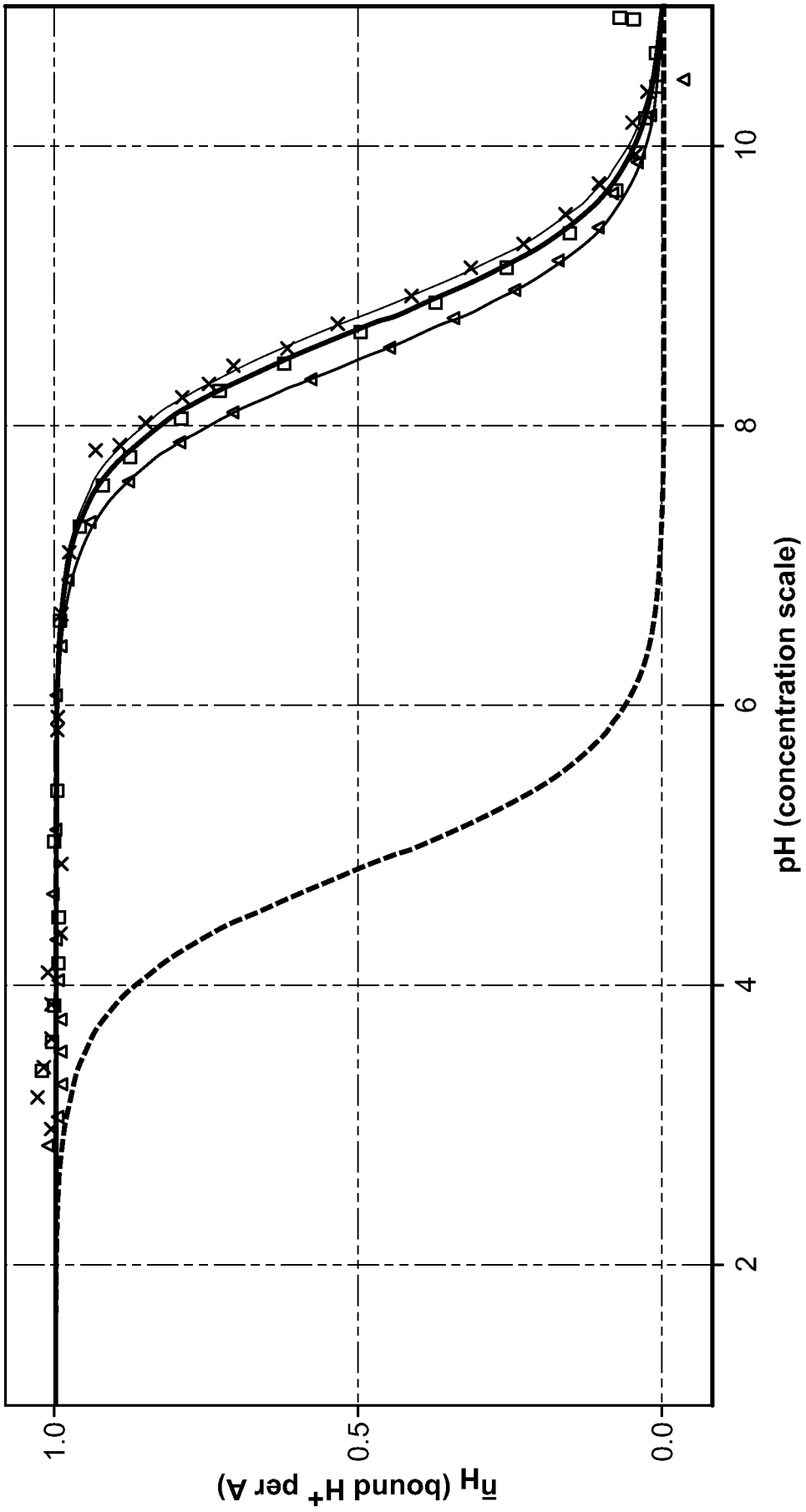


FIG. 30

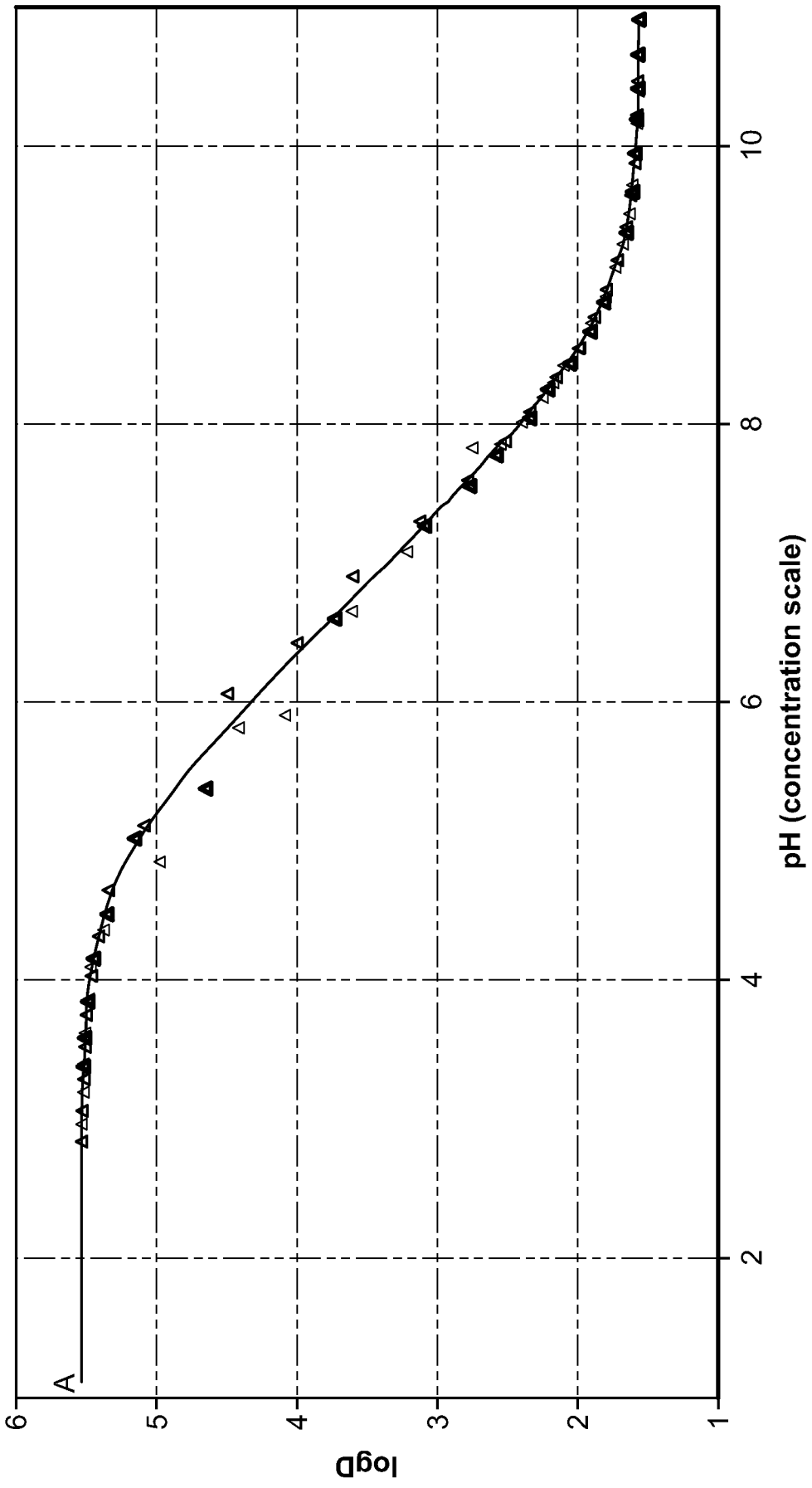


FIG. 31

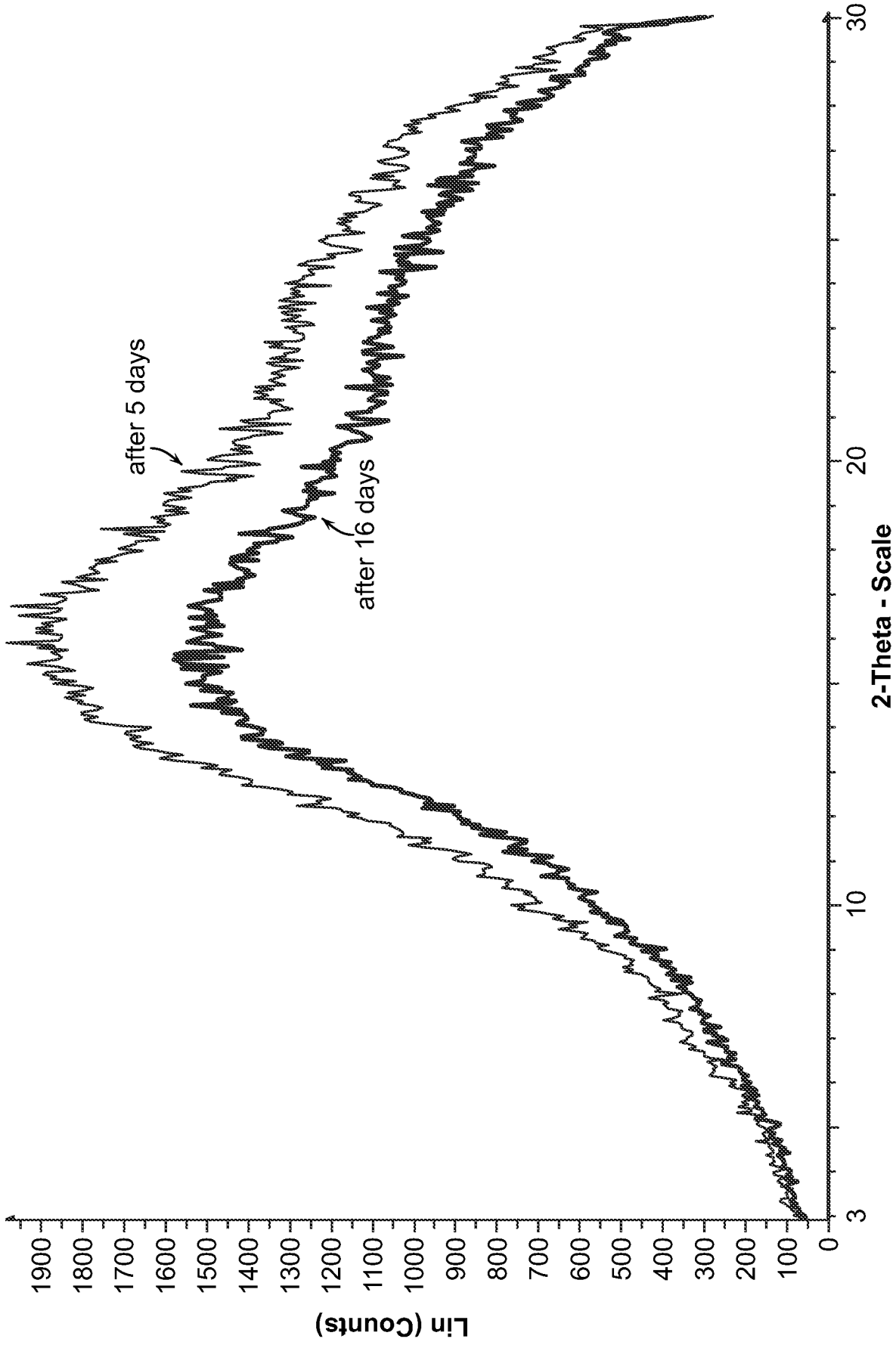
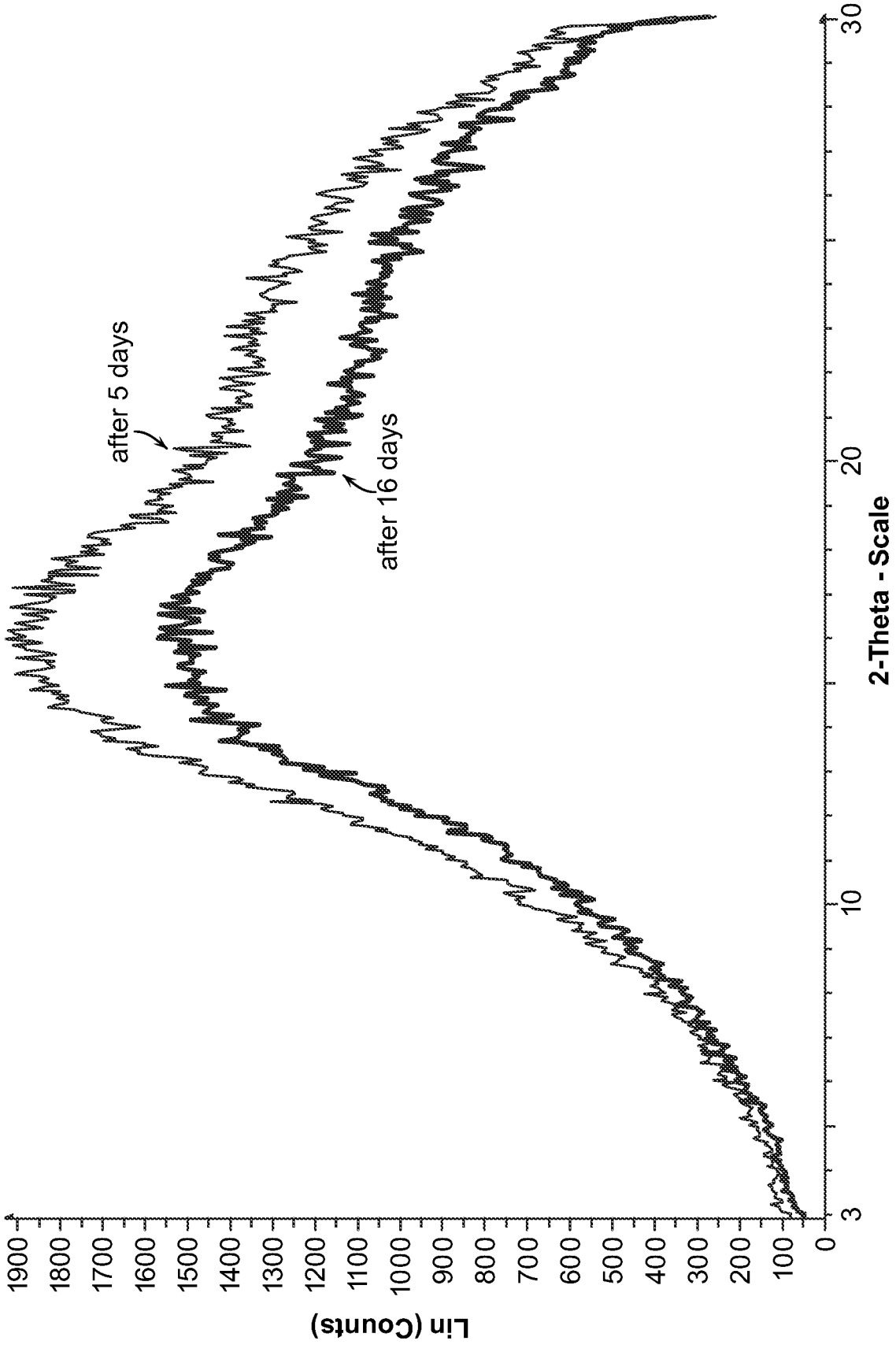

after 5 days
after 16 days
Lin (Counts)
2-Theta - Scale
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
0
3
10
20
30

FIG. 32

FIG. 33


after 5 days
after 16 days
Lin (Counts)
2-Theta - Scale
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
3
10
20
30

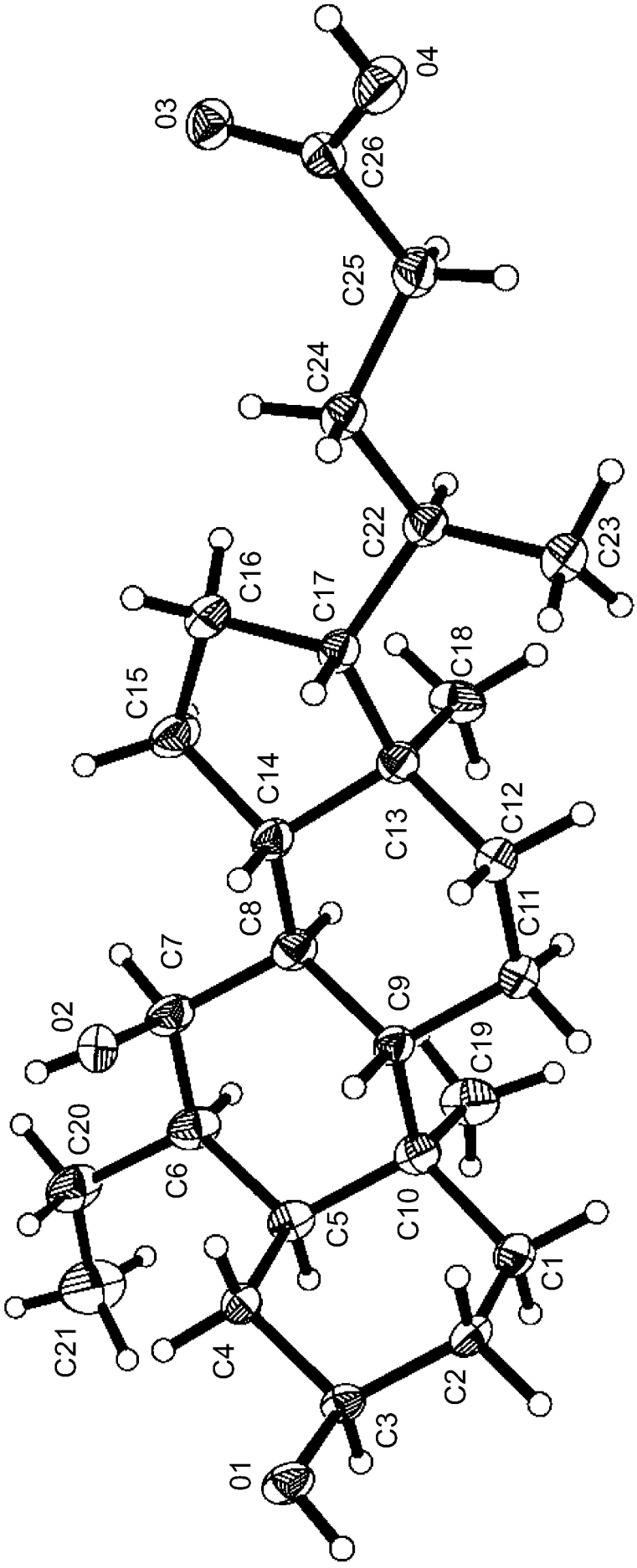

04
03
C26
C25
C24
C22
C23
C16
C17
C18
C15
C14
C13
C12
C8
C11
C7
C9
02
C19
C20
C6
C5
C10
C1
C21
C4
C2
C3
01

FIG. 34

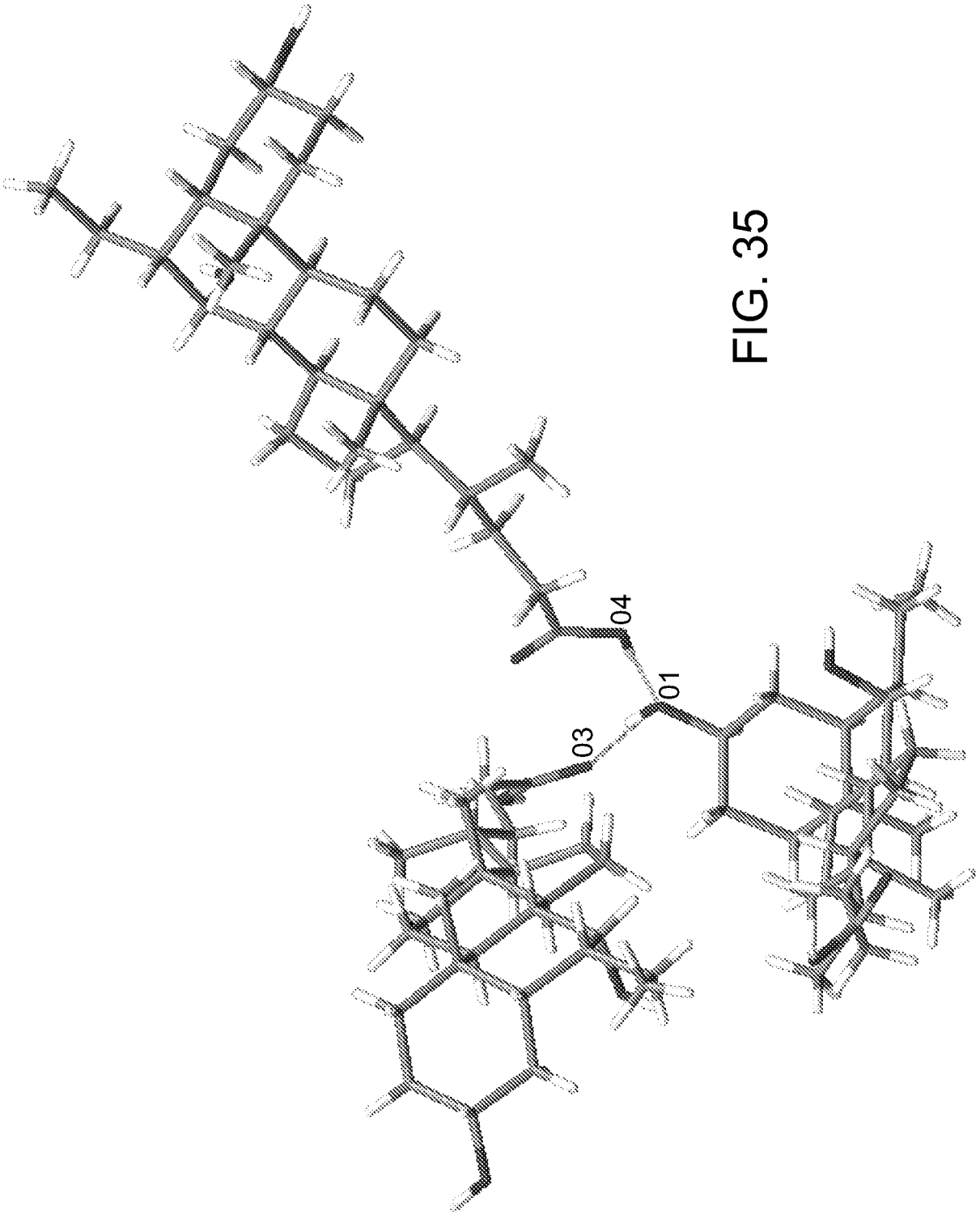


FIG. 35

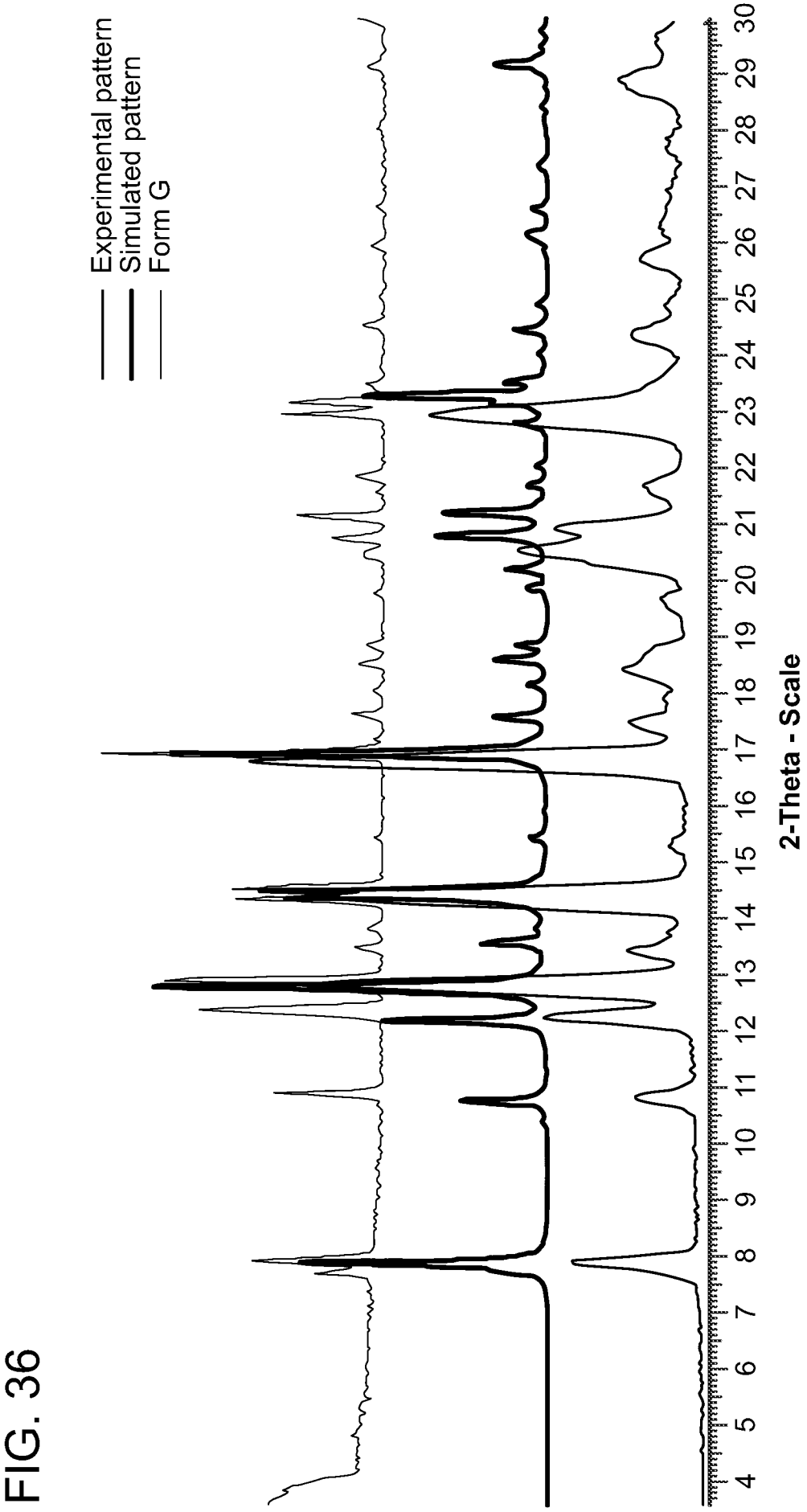

Experimental pattern
Simulated pattern
Form G
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2-Theta - Scale

FIG. 36

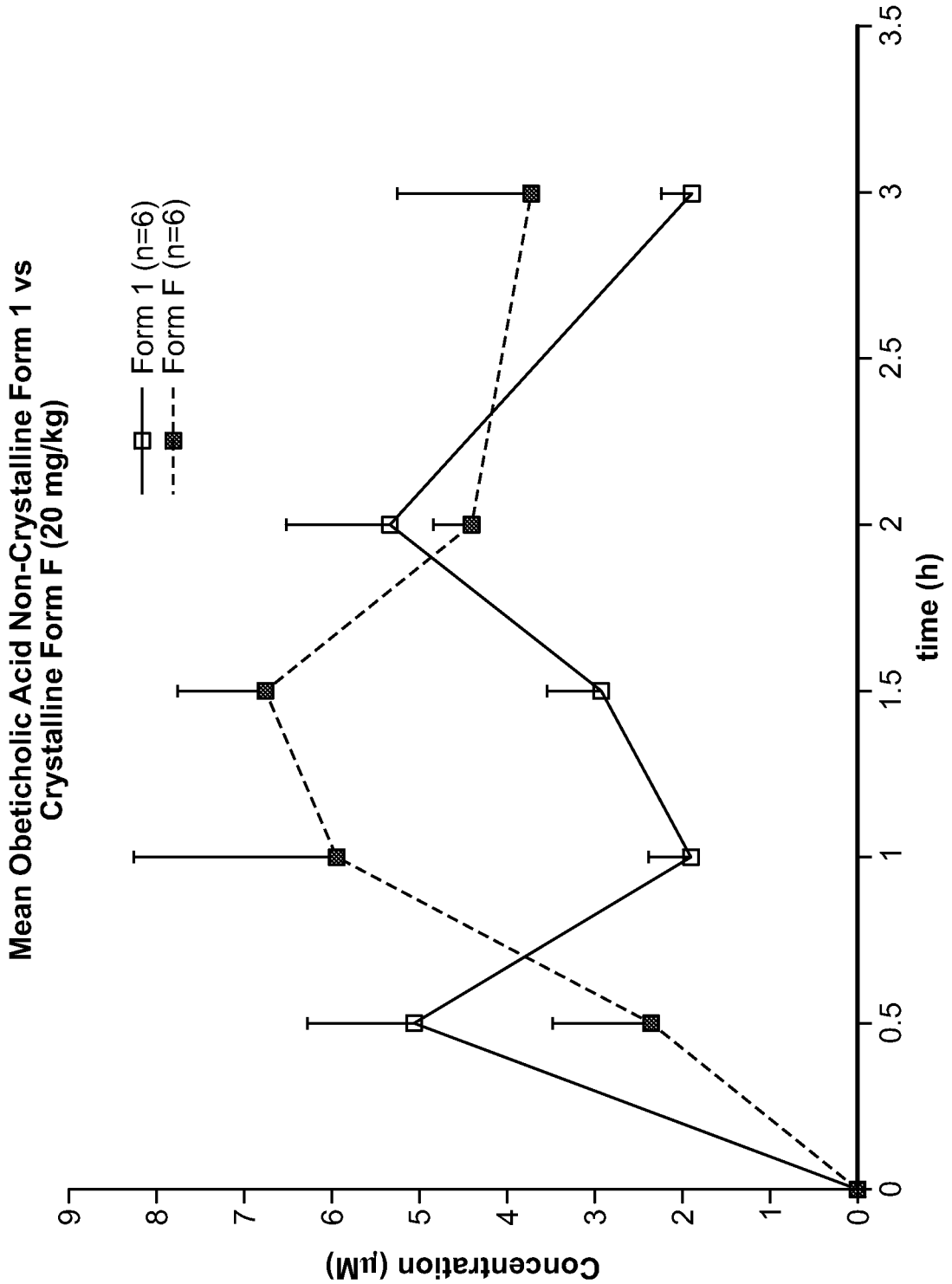

Mean Obeticholic Acid Non-Crystalline Form 1 vs
Crystalline Form F (20 mg/kg)
Form 1 (n=6)
Form F (n=6)
Concentration (μM)
time (h)
0
1
2
3
4
5
6
7
8
9
0
0.5
1
1.5
2
2.5
3
3.5

FIG. 37

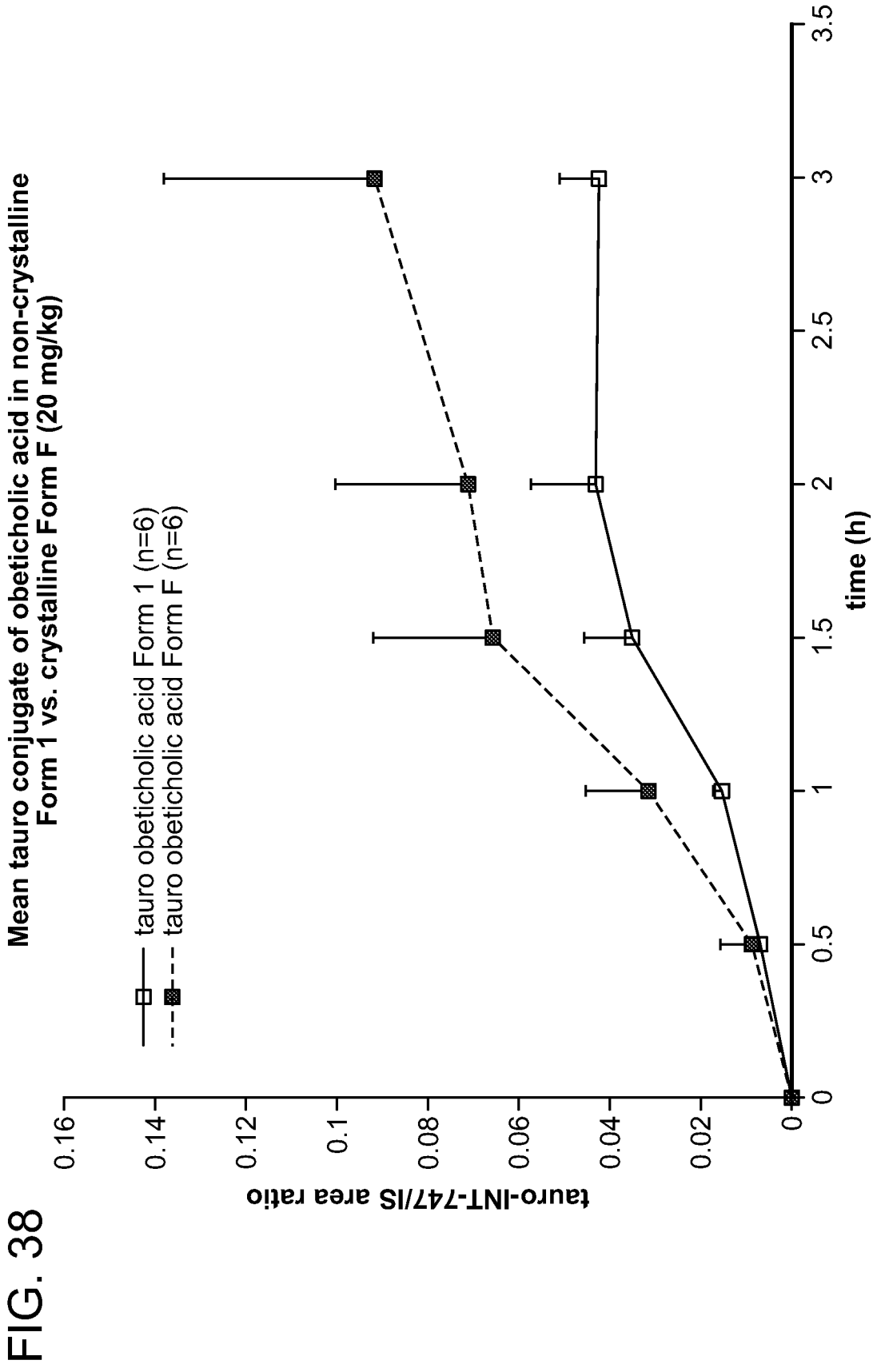

Mean tauro conjugate of obeticholic acid in non-crystalline
Form 1 vs. crystalline Form F (20 mg/kg)
tauro obeticholic acid Form 1 (n=6)
tauro obeticholic acid Form F (n=6)
tauro-INT-747/IS area ratio
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
time (h)
0
0.5
1
1.5
2
2.5
3
3.5

FIG. 38

FIG. 39

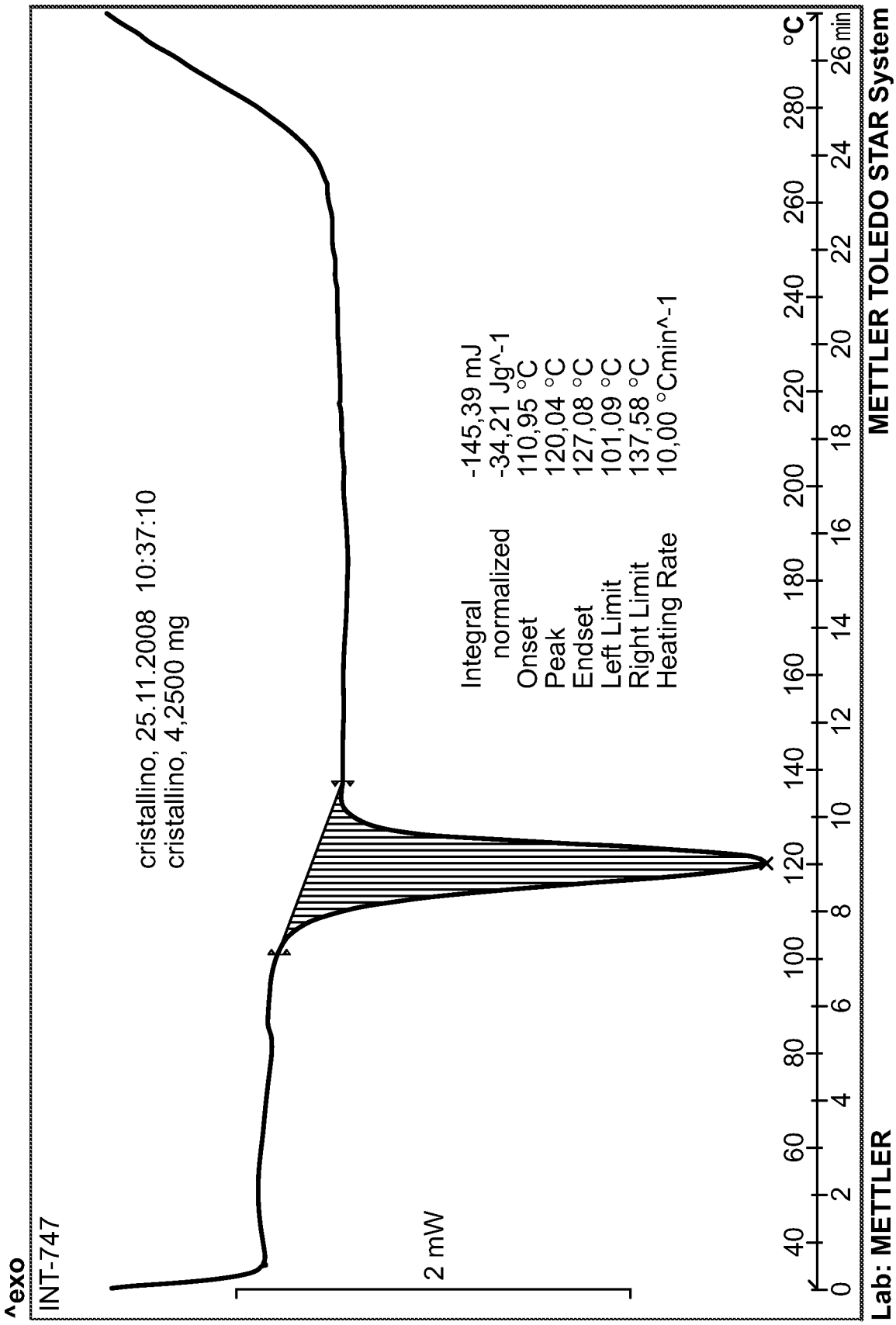
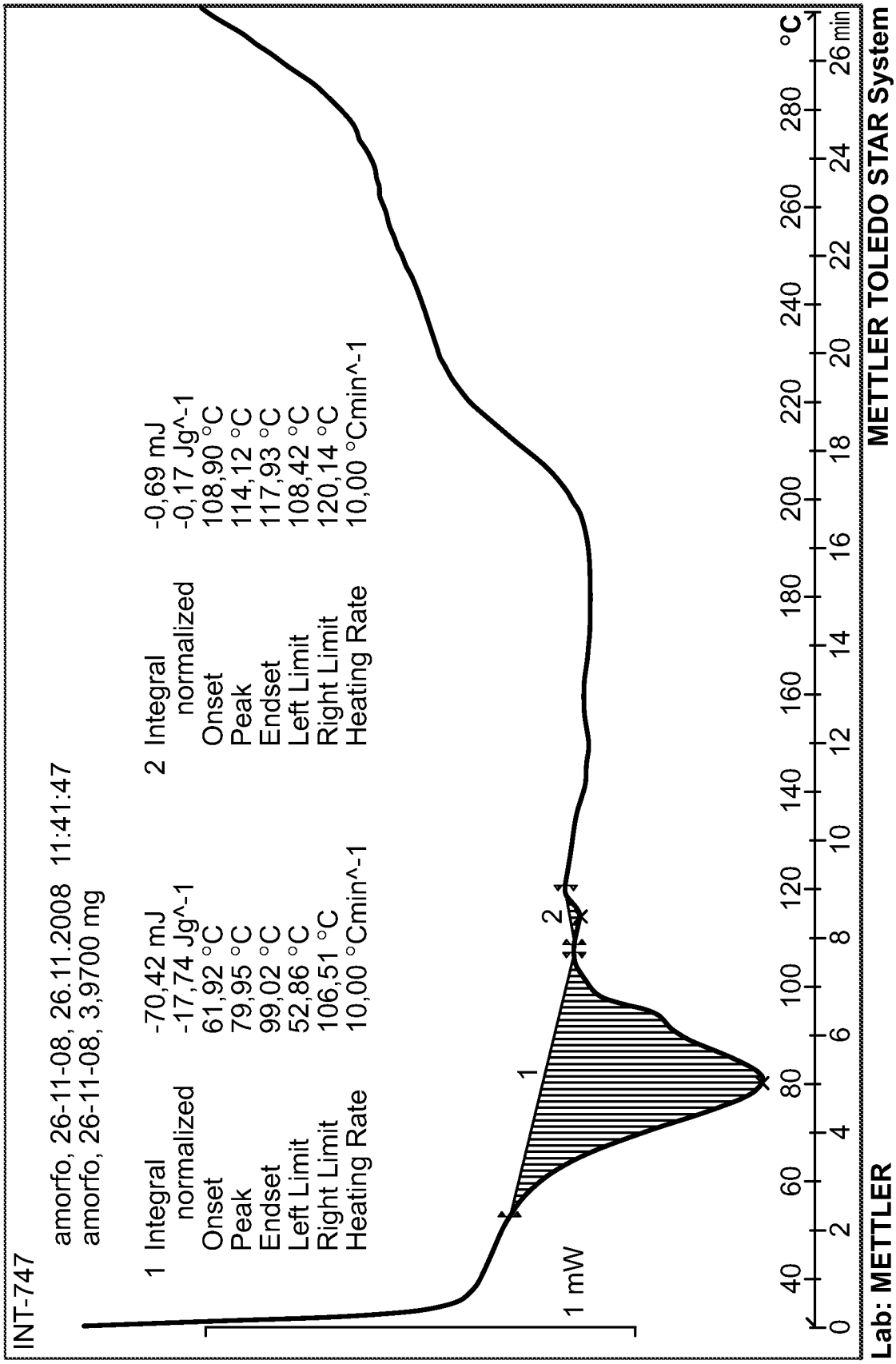

^exo
INT-747
cristallino, 25.11.2008 10:37:10
cristallino, 4,2500 mg
Integral -145,39 mJ
normalized -34,21 Jg^-1
Onset 110,95 °C
Peak 120,04 °C
Endset 127,08 °C
Left Limit 101,09 °C
Right Limit 137,58 °C
Heating Rate 10,00 °Cmin^-1
2 mW
°C
26 min
Lab: METTLER
METTLER TOLEDO STAR System

FIG. 40


^exo
INT-747
amorfo, 26-11-08, 26.11.2008 11:41:47
amorfo, 26-11-08, 3,9700 mg
1 Integral -70,42 mJ
normalized -17,74 Jg^-1
Onset 61,92 °C
Peak 79,95 °C
Endset 99,02 °C
Left Limit 52,86 °C
Right Limit 106,51 °C
Heating Rate 10,00 °Cmin^-1
2 Integral -0,69 mJ
normalized -0,17 Jg^-1
Onset 108,90 °C
Peak 114,12 °C
Endset 117,93 °C
Left Limit 108,42 °C
Right Limit 120,14 °C
Heating Rate 10,00 °Cmin^-1
1 mW
1
2
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
Lab: METTLER
METTLER TOLEDO STAR System

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046150

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07J9/00 A61K31/575 A61P1/16 C07J51/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07J A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/122977 A2 (ERREGIERRE SPA [IT]; FERRARI MASSIMO [IT]; PELLICCIARI ROBERTO [IT]) 23 November 2006 (2006-11-23) cited in the application page 1, lines 19-22; example 1 ----- -/--	1-106

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046150

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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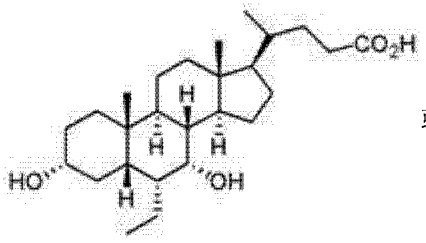
权利要求书6页 说明书62页 附图48页

(54)发明名称

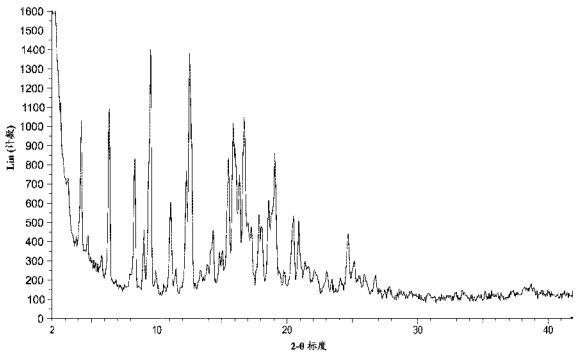
奥贝胆酸的制备、用途和固体形式

(57)摘要

本发明涉及奥贝胆酸：



或其药学上可接受的盐、溶剂合物或氨基酸缀合物。奥贝胆酸可用于治疗或预防FXR介导的疾病或病况、心血管疾病或胆汁郁积性肝病，并且可用于降低HDL胆固醇、降低哺乳动物的甘油三酯或抑制纤维变性。本发明还涉及奥贝胆酸的合成方法。



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1. 一种结晶奥贝胆酸 C 型，其特征在于包括在约 4.2、6.4、9.5、12.5 和 16.7° 2θ 处的特征峰的 X 射线衍射图。

2. 一种结晶奥贝胆酸 C 型，其特征在于基本类似于图 5 所示的 X 射线衍射图的 X 射线衍射图。

3. 权利要求 1 的结晶奥贝胆酸 C 型，其特征在于包括在约 4.2、6.4、9.5、12.5、12.6、15.5、15.8、16.0、16.7 和 19.0° 2θ 处的特征峰的 X 射线衍射图。

4. 权利要求 1 或权利要求 3 的结晶奥贝胆酸 C 型，其特征在于包括在约 4.2、6.4、8.3、9.5、11.1、12.2、12.5、12.6、15.5、15.8、16.0、16.3、16.7、18.6 和 19.0° 2θ 处的特征峰的 X 射线衍射图。

5. 权利要求 1 或 3-4 中任一项的结晶奥贝胆酸 C 型，其特征在于包括在约 4.2、6.4、8.3、9.5、11.1、12.2、12.5、12.6、15.5、15.8、16.0、16.3、16.7、17.0、17.8、18.6、18.8、19.0、20.5 和 20.9° 2θ 处的特征峰的 X 射线衍射图。

6. 权利要求 1-5 中任一项的结晶奥贝胆酸 C 型，其中所述 X 射线衍射图使用 Cu Kα 辐射在衍射计上收集。

7. 权利要求 1-6 中任一项的结晶奥贝胆酸 C 型，其特征在于包括在约 12.0- 约 12.8 和约 15.4- 约 21.0 处的特征峰的 X 射线衍射图。

8. 一种结晶奥贝胆酸 C 型，其特征在于在约 98±2℃处具有吸热值的示差扫描量热法 (DSC) 温谱图。

9. 权利要求 1-8 中任一项的结晶奥贝胆酸 C 型，其特征还在于在约 98±2℃处具有吸热值的示差扫描量热法 (DSC) 温谱图。

10. 一种用于制备奥贝胆酸 1 型的方法，所述方法包括奥贝胆酸的晶体形式作为合成中间体。

11. 一种用于制备奥贝胆酸 1 型的方法，所述方法包括将结晶奥贝胆酸转化为奥贝胆酸 1 型。

12. 权利要求 11 的方法，所述方法包括以下步骤

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

13. 权利要求 11-12 中任一项的方法，所述方法包括以下步骤

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

14. 权利要求 11-13 中任一项的方法，所述方法包括以下步骤

使 3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 NaOH 反应形成 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

15. 权利要求 11-14 中任一项的方法，所述方法包括以下步骤

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯与 CH_3CHO 反应形成 3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 NaOH 反应形成 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

16. 权利要求 11-15 中任一项的方法，所述方法包括以下步骤

使 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯，

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯与 CH_3CHO 反应形成 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 NaOH 反应形成 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

17. 权利要求 11-16 中任一项的方法，所述方法包括以下步骤

使 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸与 CH_3OH 和 H_2SO_4 反应形成 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯，

使 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯，

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯与 CH_3CHO 反应形成 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯与 NaOH 反应形成 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 E- 或 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸，和将结晶奥贝胆酸转化为奥贝胆酸 1 型。

18. 权利要求 10-17 中任一项的方法，其中所述结晶奥贝胆酸是 C 型。

19. 权利要求 18 的方法，其中结晶奥贝胆酸 C 型的特征在于类似于图 5 所示 X 射线衍射图的 X 射线衍射图。

20. 权利要求 18 或 19 中任一项的方法，其中所述结晶奥贝胆酸 C 型从乙酸正丁酯结晶出来。

21. 权利要求 11-17 中任一项的方法，其中将结晶奥贝胆酸 C 型转化为奥贝胆酸 1 型包

括将结晶奥贝胆酸 C 型溶于 NaOH 水溶液中并加入 HCl 的步骤。

22. 权利要求 21 的方法，其中将分离的结晶奥贝胆酸 C 型在约 80℃下真空干燥。

23. 权利要求 12-17 中任一项的方法，其中使 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸与 $NaBH_4$ 反应形成结晶奥贝胆酸在约 85℃－约 110℃的温度下在碱性水溶液中进行。

24. 权利要求 23 的方法，其中所述温度为约 90℃－约 105℃。

25. 权利要求 23-24 中任一项的方法，其中所述碱性水溶液是 NaOH 水溶液。

26. 权利要求 25 中任一项的方法，其中所述碱性水溶液是 50% wt NaOH 溶液和水的混合物。

27. 权利要求 13-17 中任一项的方法，其中使 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸与 Pd/C 和氢气反应形成 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸在约 20℃－约 40℃温度下和在约 4- 约 5 巴的压力下进行。

28. 权利要求 27 的方法，其中反应混合物的有机相用活性碳处理。

29. 权利要求 27-28 中任一项的方法，其中所述温度为约 25℃－约 35℃。

30. 权利要求 27-29 中任一项的方法，其中所述压力为约 4.5- 约 5.5 巴。

31. 权利要求 27-30 中任一项的方法，其中将氢化反应混合物搅拌约 1 小时。

32. 权利要求 27-31 中任一项的方法，其中将 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 2 小时－约 5 小时。

33. 权利要求 27-32 中任一项的方法，其中将 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 3 小时。

34. 权利要求 14-17 中任一项的方法，其中使 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯与 NaOH 反应形成 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸在约 20℃－约 60℃的温度下进行。

35. 权利要求 34 的方法，其中所述温度为约 20℃－约 25℃。

36. 权利要求 34-35 中任一项的方法，其中使 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯与 NaOH 的反应在甲醇、水和 NaOH 溶液中进行。

37. 权利要求 34-36 中任一项的方法，其中所述 NaOH 溶液为 50% wt 水性溶液。

38. 权利要求 15-17 中任一项的方法，其中使 3α,7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯与 CH_3CHO 反应形成 E- 或 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯在约 -50℃－约 -70℃的温度下在 BF_3 存在下在极性非质子溶剂中进行。

39. 权利要求 38 的方法，其中所述极性非质子溶剂是二氯甲烷。

40. 权利要求 38-39 中任一项的方法，其中所述 BF_3 为乙腈中的 16% wt 溶液。

41. 权利要求 38-40 中任一项的方法，其中所述温度为约 -60℃－约 -65℃。

42. 权利要求 16-17 中任一项的方法，其中使 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α,7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯在约 -10℃－约 -30℃的温度下在极性非质子溶剂中进行。

43. 权利要求 42 的方法，其中所述极性非质子溶剂是四氢呋喃。

44. 权利要求 42-43 中任一项的方法，其中所述温度为约 -20℃ - 约 -25℃。

45. 权利要求 42-44 中任一项的方法，其中将 3α - 羟基 -7- 酮 -5β - 胆烷 -24- 酸甲酯与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 的反应搅拌约 2 小时。

46. 权利要求 17 的方法，其中将 3α - 羟基 -7- 酮 -5β - 胆烷 -24- 酸与 CH_3OH 和 H_2SO_4 反应形成 3α - 羟基 -7- 酮 -5β - 胆烷 -24- 酸甲酯加热约 3 小时，并将反应混合物的 pH 用碱性水溶液调节至约 6.5- 约 8.0 的 pH 值。

47. 权利要求 46 的方法，其中所述 3α - 羟基 -7- 酮 -5β - 胆烷 -24- 酸甲酯的分离还包括加入活性碳。

48. 权利要求 46-47 中任一项的方法，其中 3α - 羟基 -7- 酮 -5β - 胆烷 -24- 酸与 CH_3OH 和 H_2SO_4 的反应在甲醇中进行。

49. 权利要求 46-48 中任一项的方法，其中所述碱性溶液是 NaOH 水溶液。

50. 权利要求 46-49 中任一项的方法，其中所述 pH 为约 7.0- 约 7.5。

51. 一种通过权利要求 10-50 中任一项的方法产生的化合物 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸，其中所述 E/Z 异构体比率大于约 50%、大于约 60%、大于约 70%、大于约 80%、大于约 83%、大于约 85%、大于约 90%、大于约 93%、大于约 95% 或大于约 99%。

52. 权利要求 51 的化合物，其中所述 E/Z 比率通过 HPLC 测定。

53. 权利要求 51-52 中任一项的化合物，其中所述比率大于约 80%。

54. 权利要求 51-53 中任一项的化合物，其中所述比率大于约 83%。

55. 权利要求 51-54 中任一项的化合物，其中所述比率大于约 85%。

56. 权利要求 51-55 中任一项的化合物，其中所述比率大于约 90%。

57. 权利要求 51-56 中任一项的化合物，其中所述比率大于约 93%。

58. 权利要求 51-57 中任一项的化合物，其中所述比率大于约 95%。

59. 权利要求 51-58 中任一项的化合物，其中所述比率大于约 99%。

60. 奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物，其效能大于约 98%。

61. 权利要求 60 的奥贝胆酸，其中所述奥贝胆酸的效能大于约 98%。

62. 权利要求 60 的奥贝胆酸，其中所述奥贝胆酸的效能通过减去水、硫酸灰分、残余溶剂和其它有机杂质的量来确定。

63. 权利要求 61-62 中任一项的奥贝胆酸，其效能大于约 98.5%。

64. 权利要求 61-63 中任一项的奥贝胆酸，其效能大于约 99.0%。

65. 权利要求 61-64 中任一项的奥贝胆酸，其效能大于约 99.5%。

66. 权利要求 61-65 中任一项的奥贝胆酸，其中所述奥贝胆酸含有总共小于约 2% 的一种或多种选自以下的杂质 :6- 乙基乌索脱氧胆酸、3α - 羟基 -6α - 乙基 -7-cheto-5β - 胆烷 -24- 酸、6β - 乙基鹅脱氧胆酸、3α，7α - 二羟基 -6- 亚乙基 -5β - 胆烷 -24- 酸、鹅脱氧胆酸和 3α (3α，7α - 二羟基 -6α - 乙基 -5β - 胆烷 -24- 酰基氧基)-7α - 羟基 -6α - 乙基 -5β - 胆烷 -24- 酸。

67. 权利要求 66 的奥贝胆酸，其中所述奥贝胆酸含有总共小于约 1.5% 的杂质。

68. 权利要求 66-67 中任一项的奥贝胆酸，其中所述奥贝胆酸 1 型含有总共小于约 1.4% 的杂质。

69. 权利要求 66-68 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 1.2% 的水。

70. 权利要求 66-69 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 1.0% 的水。

71. 权利要求 66-70 中任一项的奥贝胆酸，其中所述奥贝胆酸含有总共小于约 0.15% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。

72. 权利要求 66-71 中任一项的奥贝胆酸，其中所述奥贝胆酸含有总共小于约 0.06% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。

73. 权利要求 66-72 中任一项的奥贝胆酸，其中所述奥贝胆酸含有总共小于约 0.05% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。

74. 权利要求 66-73 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.15% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。

75. 权利要求 66-74 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.06% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。

76. 权利要求 66-75 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.05% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。

77. 权利要求 66-76 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.15% 的 6β- 乙基鹅脱氧胆酸。

78. 权利要求 66-77 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.06% 的 6β- 乙基鹅脱氧胆酸。

79. 权利要求 66-78 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.05% 的 6β- 乙基鹅脱氧胆酸。

80. 权利要求 66-79 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 3% 的鹅脱氧胆酸。

81. 权利要求 66-80 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 1% 的鹅脱氧胆酸。

82. 权利要求 66-81 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.2% 的鹅脱氧胆酸。

83. 权利要求 66-82 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.15% 的 3α(3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。

84. 权利要求 66-83 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.06% 的 3α(3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。

85. 权利要求 66-84 中任一项的奥贝胆酸，其中所述奥贝胆酸含有小于约 0.05% 的 3α(3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。

86. 权利要求 61-85 中任一项的奥贝胆酸，其中奥贝胆酸是奥贝胆酸 1 型。

87. 结晶奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物，其中所述结晶奥贝胆酸是 C 型。

88. 权利要求 87 的结晶奥贝胆酸，其中所述结晶奥贝胆酸的效能大于约 90%。

89. 权利要求 88 的结晶奥贝胆酸 C 型，其中所述奥贝胆酸 C 型的效能通过减去水、硫酸

灰分、残余溶剂和其它有机杂质的量来确定。

90. 权利要求 87 的结晶奥贝胆酸 C 型，其中所述溶剂合物是水合物。

91. 权利要求 88-90 中任一项的结晶奥贝胆酸 C 型，其效能大于约 92%。

92. 权利要求 88-91 中任一项的结晶奥贝胆酸 C 型，其效能大于约 94%。

93. 权利要求 88-92 中任一项的结晶奥贝胆酸 C 型，其效能大于约 96%。

94. 权利要求 88-93 中任一项的结晶奥贝胆酸 C 型，其效能大于约 98%。

95. 权利要求 88-94 中任一项的结晶奥贝胆酸 C 型，其效能大于约 99%。

96. 权利要求 87-95 中任一项的结晶奥贝胆酸 C 型，其中所述结晶奥贝胆酸 C 型含有总共小于约 4% 的一种或多种选自以下的杂质 :6- 乙基乌索脱氧胆酸、3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸、6β- 乙基鹅脱氧胆酸、3α,7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸、鹅脱氧胆酸和 3α(3α,7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。

97. 权利要求 96 的结晶奥贝胆酸 C 型，其中所述总杂质小于约 3.8%。

98. 权利要求 96-97 中任一项的结晶奥贝胆酸 C 型，其中所述总杂质小于约 3.6%。

99. 一种包含通过权利要求 10-50 中任一项的方法产生的奥贝胆酸 1 型和药学上可接受的载体的药物组合物。

100. 一种包含结晶奥贝胆酸和药学上可接受的载体的药物组合物。

101. 权利要求 100 的药物组合物，其中所述结晶奥贝胆酸是 C 型。

102. 一种治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的权利要求 60-86 中任一项的或通过权利要求 10-50 中任一项的方法产生的奥贝胆酸 1 型或权利要求 99 的药物组合物。

103. 一种治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的权利要求 1-9 或 87-98 中任一项的结晶奥贝胆酸或权利要求 100-101 中任一项的药物组合物。

104. 权利要求 102-103 中任一项的方法，其中所述疾病或病况选自胆道闭锁、胆汁郁积性肝病、慢性肝病、非酒精性脂肪性肝炎 (NASH)、丙型肝炎感染、酒精性肝病、原发性胆汁性肝硬化 (PBC)、进行性纤维变性所致肝损伤、肝纤维变性和心血管疾病包括动脉粥样硬化、动脉硬化、高胆固醇血症和高脂血症。

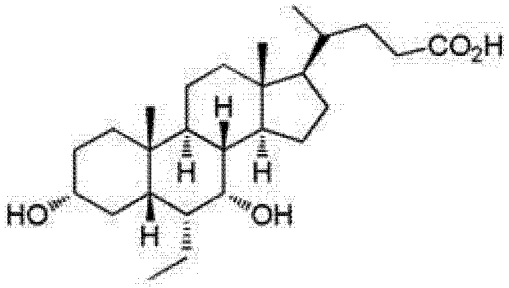
105. 一种用于降低受试者的甘油三酯的方法，所述方法包括给予有效量的权利要求 60-86 中任一项的或通过权利要求 10-50 中任一项的方法产生的奥贝胆酸 1 型或权利要求 99 的药物组合物。

106. 一种用于降低受试者的甘油三酯的方法，所述方法包括给予有效量的权利要求 1-9 或 87-98 中任一项的结晶奥贝胆酸或权利要求 100-101 中任一项的药物组合物。

奥贝胆酸的制备、用途和固体形式

发明概要

[0001] 本发明涉及奥贝胆酸(obeticholic acid)(一种FXR的激动剂)、奥贝胆酸的制备方法、包含奥贝胆酸的药物制剂及所述药物制剂的治疗用途。



奥贝胆酸

(亦称为 INT-747)

[0002] 本发明涉及特征在于包括在约4.2、6.4、9.5、12.5和16.7° 2θ处的特征峰的X射线衍射图的结晶奥贝胆酸C型。结晶奥贝胆酸C型的特征在于基本类似于图5所示X射线衍射图的X射线衍射图,且其特征还在于在约98±2℃处具有吸热值的示差扫描量热法(DSC)温谱图。

[0003] 本发明涉及用于制备奥贝胆酸1型的方法,所述方法包括将结晶奥贝胆酸转化为奥贝胆酸1型的步骤。

[0004] 本发明涉及用于制备奥贝胆酸1型的方法,所述方法包括使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0005] 本发明涉及用于制备奥贝胆酸1型的方法,所述方法包括使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸;使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸;并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0006] 本发明涉及用于制备奥贝胆酸1型的方法,所述方法包括使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯与NaOH反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸;使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸;使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸,并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0007] 本发明涉及用于制备奥贝胆酸1型的方法,所述方法包括使3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯与CH_3CHO反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯;使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯与NaOH反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸;使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成

3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸；使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸，并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0008] 本发明涉及用于制备奥贝胆酸1型的方法，所述方法包括使3α-羟基-7-酮-5β-胆烷-24-酸甲酯与$Li[N(CH(CH_3)_2)_2]$和$Si(CH_3)_3Cl$反应形成3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯；使3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯与CH_3CHO反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯；使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯与NaOH反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸；使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸；使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸，并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0009] 本发明涉及用于制备奥贝胆酸1型的方法，所述方法包括使3α-羟基-7-酮-5β-胆烷-24-酸与CH_3OH和H_2SO_4反应形成3α-羟基-7-酮-5β-胆烷-24-酸甲酯；使3α-羟基-7-酮-5β-胆烷-24-酸甲酯与$Li[N(CH(CH_3)_2)_2]$和$Si(CH_3)_3Cl$反应形成3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯；使3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯与CH_3CHO反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯；使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯与NaOH反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸；使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸；使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸，并将结晶奥贝胆酸转化成奥贝胆酸1型的步骤。

[0010] 本发明涉及用于制备奥贝胆酸1型的方法，其中将结晶奥贝胆酸C型转化为奥贝胆酸1型包括将结晶奥贝胆酸C型溶于NaOH水溶液中并加入HCl的步骤。

[0011] 本发明涉及用于制备奥贝胆酸1型的方法，其中使3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸与$NaBH_4$反应形成结晶奥贝胆酸在约85℃-约110℃的温度下在碱性水溶液中进行。

[0012] 本发明涉及用于制备奥贝胆酸1型的方法，其中使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸与Pd/C和氢气反应形成3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸在约100℃-约105℃的温度下和在约4-约5巴的压力下进行。

[0013] 本发明涉及用于制备奥贝胆酸1型的方法，其中使E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯与NaOH反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸在约20℃-约60℃的温度下进行。

[0014] 本发明涉及用于制备奥贝胆酸1型的方法，其中使3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯与CH_3CHO反应形成E-或E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯在约-50℃-约-70℃的温度下在BF_3存在下在极性非质子溶剂中进行。

[0015] 本发明涉及用于制备奥贝胆酸1型的方法，其中使3α-羟基-7-酮-5β-胆烷-24-酸甲酯与$Li[N(CH(CH_3)_2)_2]$和$Si(CH_3)_3Cl$反应形成3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯在约-10℃-约-30℃的温度下在极性非质子溶剂中进行。

[0016] 本发明涉及用于制备奥贝胆酸 1 型的方法，其中将 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸与 CH_3OH 和 H_2SO_4 反应形成 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯加热约 3 小时，并将反应混合物的 pH 用碱性水溶液调节至约 6.5- 约 8.0 的 pH 值。

[0017] 本发明涉及奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物，其效能 (potency) 大于约 98%、大于约 98.5%、大于约 99.0% 或大于约 99.5%。本发明涉及包含通过本发明的方法产生的奥贝胆酸 1 型和药学上可接受的载体的药物组合物。

[0018] 本发明涉及治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的奥贝胆酸 1 型。所述疾病或病况选自胆道闭锁、胆汁郁积性肝病、慢性肝病、非酒精性脂肪性肝炎 (NASH)、丙型肝炎感染、酒精性肝病、原发性胆汁性肝硬化 (PBC)、进行性纤维变性所致肝损伤、肝纤维变性和心血管疾病包括动脉粥样硬化、动脉硬化、高胆固醇血症和高脂血症。本发明涉及用于降低受试者的甘油三酯的方法，所述方法包括给予有效量的奥贝胆酸 1 型。

[0019] 附图简述

图 1 是实施例 1 步骤 4 的粗制化合物 5 的 HPLC-UV/MS 色谱图，以 1 mg/mL 注入，注射体积为 3μl。色谱图按照实施例 2 所述方法获得。

[0020] 图 2 是实施例 1 步骤 4 的化合物 5 的 HPLC-UV/MS 色谱图，以 1 mg/mL 注入纯化参比，注射体积为 20μL。色谱图按照实施例 2 所述方法获得。

[0021] 图 3 是采用 HPLC 方法的实施例 1 步骤 4 的粗制化合物 5 的 UV 色谱图。色谱图按照实施例 2 所述方法获得。

[0022] 图 4A 是来自用 HPLC 方法纯化分离的实施例 1 步骤 4 化合物 5 主峰级分 (RT 29.0 分钟）的 m/z 850.61914 ± 3ppm 的精确离子迹线（参见实施例 2)。

[0023] 图 4B 是来自用 HPLC 方法纯化分离的实施例 1 步骤 4 化合物 5 次峰级分 (RT 29.9 分钟）的 m/z 850.61914 ± 3ppm 的精确离子迹线（参见实施例 2)。

[0024] 图 4C 是来自实施例 1 步骤 4 粗制化合物 5 的 m/z 850.61914 ± 3ppm 的精确离子迹线（参见实施例 2)。

[0025] 图 4D 是来自实施例 1 步骤 4 化合物 5 纯化参比的 m/z 850.61914 ± 3ppm 的精确离子迹线（参见实施例 2)。

[0026] 图 5 是结晶奥贝胆酸 C 型的 XRPD 衍射图（参见实施例 3)。

[0027] 图 6 显示结晶奥贝胆酸 C 型的 TGA 和 DSC 温谱图（参见实施例 3)。

[0028] 图 7 显示在 25℃、110℃和 120℃下结晶奥贝胆酸的 VT-XRPD 衍射图（参见实施例 3)。

[0029] 图 8A 是结晶奥贝胆酸 C 型的 GVS 等温线图（参见实施例 3)。

[0030] 图 8B 是结晶奥贝胆酸 C 型的 GVS 动力曲线（参见实施例 3)。

[0031] 图 8C 显示在 GVS 分析前后结晶奥贝胆酸 C 型的 XRPD 衍射图（参见实施例 3)。

[0032] 图 9 显示在 40℃ /75% RH 下保存前后结晶奥贝胆酸 C 型的 XRPD 衍射图（参见实施例 3)。

[0033] 图 10 是第 1 批的奥贝胆酸 1 型的 XRPD 衍射图（参见实施例 5)。

[0034] 图 11 显示第 1、2、3、4、5 和 6 批的奥贝胆酸 1 型的 XRPD 衍射图（参见实施例 5)。

[0035] 图 12 是 d_6-DMSO 中第 1 批奥贝胆酸 1 型的 NMR 波谱（参见实施例 5)。

[0036] 图 13 显示第 1、2、3、4、5 和 6 批奥贝胆酸 1 型的 ^{1}H NMR 波谱（参见实施例 5）。

[0037] 图 14 是来自区域 10-75 ppm 的奥贝胆酸 1 型的 ^{13}C DEPTQ NMR 波谱的放大（参见实施例 5）。

[0038] 图 15 是来自区域 0-75 ppm 的奥贝胆酸 1 型抑制性季碳的 ^{13}C DEPT135 NMR 波谱的放大（参见实施例 5）。

[0039] 图 16 是奥贝胆酸 1 型的定量 ^{13}C NMR （参见实施例 5）。

[0040] 图 17 是图 16 的 32.3 ppm 处峰的放大图（参见实施例 5）。

[0041] 图 18 是第 1 批奥贝胆酸 1 型的 FT-IR 波谱（参见实施例 5）。

[0042] 图 19 显示第 1 批奥贝胆酸 1 型的 TGA 和 DSC 温谱图（参见实施例 5）。

[0043] 图 20 显示第 1 批奥贝胆酸 1 型的调整 DSC 温谱图（参见实施例 5）。

[0044] 图 21 显示第 1、2、3、4、5 和 6 批奥贝胆酸 1 型的 TGA 迹线（参见实施例 5）。

[0045] 图 22 显示第 1、2、3、4、5 和 6 批奥贝胆酸 1 型的 DSC 迹线（参见实施例 5）。

[0046] 图 23A 是偏振光显微术下的第 1 批奥贝胆酸 1 型的照片。图 23B 是偏振光显微术下的第 2 批奥贝胆酸 1 型的照片。图 23C 是偏振光显微术下的第 3 批奥贝胆酸 1 型的照片。图 23D 是偏振光显微术下的第 4 批奥贝胆酸 1 型的照片。图 23E 是偏振光显微术下的第 5 批奥贝胆酸 1 型的照片。图 23F 是偏振光显微术下的第 6 批奥贝胆酸 1 型的照片。

[0047] 图 24 显示第 1 批奥贝胆酸 1 型的 GVS 等温线图（参见实施例 5）。

[0048] 图 25 显示第 1 批奥贝胆酸 1 型的 GVS 动力曲线（参见实施例 5）。

[0049] 图 26 显示在 GVS 前后第 1 批奥贝胆酸 1 型的 XRPD 衍射图（参见实施例 5）。

[0050] 图 27 是在 3 种不同的甲醇 / 水比率下奥贝胆酸 1 型的 pKa 测量的曲线图（参见实施例 5）。

[0051] 图 28 是奥贝胆酸 1 型的 Yasuda-Shedlovsky 曲线（参见实施例 5）。

[0052] 图 29 是显示奥贝胆酸 1 型的依赖于 pH 的种类分布的示图（参见实施例 5）。

[0053] 图 30 是显示通过电势测定法获得的奥贝胆酸 1 型的差异曲线的示图（参见实施例 5）。

[0054] 图 31 显示奥贝胆酸 1 型的亲油性概况（参见实施例 5）。

[0055] 图 32 显示在 40℃ /75% RH 下保存后第 1 批奥贝胆酸 1 型的 XRPD 衍射图（参见实施例 5）。

[0056] 图 33 显示在 25℃ /97% RH 下保存后第 1 批奥贝胆酸 1 型的 XRPD 衍射图（参见实施例 5）。

[0057] 图 34 显示以 50% 置信水平显示非氢原子的各向异性原子位移椭圆体的晶体结构的奥贝胆酸 G 型分子的示图（参见实施例 6）。

[0058] 图 35 显示奥贝胆酸 G 型晶体结构的分子间氢键的示图，其中氢键用虚线表示（参见实施例 6）。

[0059] 图 36 显示所收集的晶体的模拟粉末图、实验图和奥贝胆酸 G 型的 XRPD 重叠（参见实施例 6）。

[0060] 图 37 显示口服给予 20 mg/kg 的奥贝胆酸 1 型和结晶 F 型后血清奥贝胆酸概况相对于时间的曲线图（参见实施例 7）。

[0061] 图 38 显示在给药后不同的时间间隔奥贝胆酸 1 型和结晶 F 型的牛磺缀合物的血

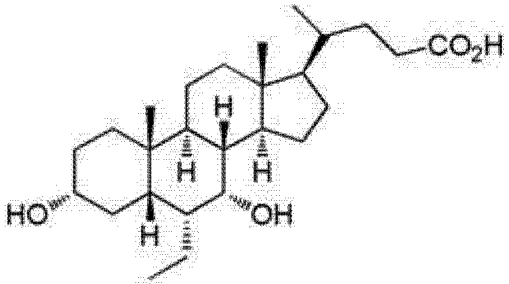
清浓度的曲线（参见实施例 7）。

[0062] 图 39 显示 1 型的 DSC 曲线（参见实施例 7）。

[0063] 图 40 显示 F 型的 DSC 曲线（参见实施例 7）。

[0064] 发明详述

本申请涉及具有以下化学结构的奥贝胆酸，一种药学活性成分（亦称为 INT-747）：



奥贝胆酸

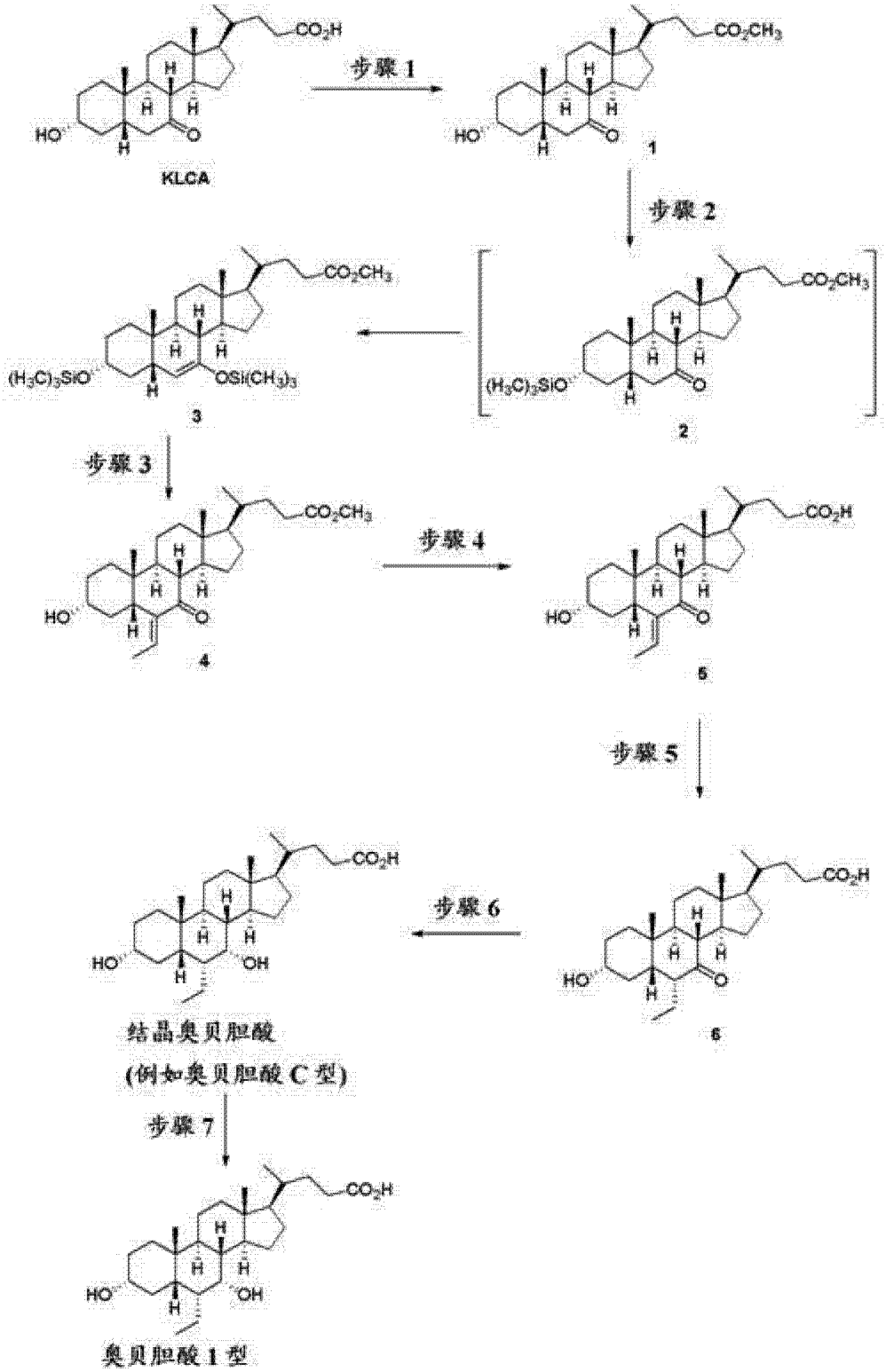
(亦称为 INT-747)

其包括基本上纯的奥贝胆酸；包含结晶奥贝胆酸作为合成中间体的奥贝胆酸的制备方法和证实在制备奥贝胆酸的方法中奥贝胆酸和合成中间体的存在和纯度的分析方法。本申请还描述了奥贝胆酸的药物组合物和制剂以及所述组合物的用途。

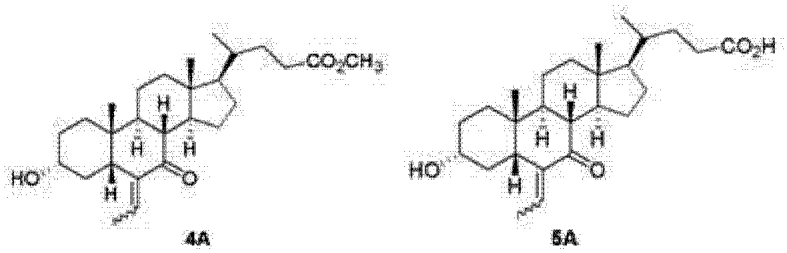
[0065] 制备奥贝胆酸的方法

本申请涉及用于制备高纯度奥贝胆酸的方法。本申请的方法见流程 1。该方法是 6 步合成接着一个纯化步骤以产生高纯度的奥贝胆酸。

[0066] 流程 1



本发明的方法还包括流程 1 的方法，其中化合物 4 和 5 各自包含通过以下化合物 4A 和 5A 的结构说明的 E 和 Z 异构体的混合物：



在一个实施方案中，E/Z-3α－羟基-6-亚乙基-7-酮-5β－胆烷-24-酸甲酯（4A）的E/Z异构体比率为约50%、大于约60%、大于约70%、大于约80%、大于约83%、大于约85%、大于约90%、大于约93%、大于约95%或大于约99%。在一个实施方案中，E/Z比率通过HPLC测定。在一个实施方案中，该比率大于约80%。在一个实施方案中，该比率大于约83%。在一个实施方案中，该比率大于约85%。在一个实施方案中，该比率大于约90%。在一个实施方案中，该比率大于约93%。在一个实施方案中，该比率大于约95%。在一个实施方案中，该比率大于约99%。

[0067] 在一个实施方案中，E/Z-3α－羟基-6-亚乙基-7-酮-5β－胆烷-24-酸（5A）的E/Z异构体比率为约50%、大于约60%、大于约70%、大于约80%、大于约83%、大于约85%、大于约90%、大于约93%、大于约95%或大于约99%。在一个实施方案中，E/Z比率通过HPLC测定。在一个实施方案中，该比率大于约80%。在一个实施方案中，该比率大于约83%。在一个实施方案中，该比率大于约85%。在一个实施方案中，该比率大于约90%。在一个实施方案中，该比率大于约93%。在一个实施方案中，该比率大于约95%。在一个实施方案中，该比率大于约99%。

[0068] 本领域未曾报告过本申请的方法。该方法为6步合成接着一个纯化步骤。步骤1是在酸性催化剂存在下并加热使用甲醇使7-酮石胆酸（KLCA）的C-24羧酸的酯化得到甲酯化合物1。步骤2是使用强碱由化合物1形成硅烯醇醚，接着用氯硅烷处理得到化合物3。步骤3是硅烯醇醚化合物3和乙醛的羟醛缩合反应形成化合物4（或化合物4A）。步骤4是酯水解，即化合物4（或化合物4A）的C-24甲酯经皂化得到羧酸化合物5（或化合物5A）。步骤5是化合物5（或化合物5A）的6-亚乙基部分的氢化，接着异构化得到化合物6。步骤6是化合物6的7-酮基选择性还原为7α－羟基得到结晶奥贝胆酸。步骤7是结晶奥贝胆酸转化为奥贝胆酸1型。

[0069] 本发明的方法涉及用于制备奥贝胆酸1型的方法，其中该方法利用奥贝胆酸的晶体形式作为合成中间体。

[0070] 在一个实施方案中，本发明涉及用于制备奥贝胆酸1型的方法，所述方法包括将结晶奥贝胆酸转化为奥贝胆酸1型的步骤。

[0071] 在一个实施方案中，本发明涉及用于制备奥贝胆酸1型的方法，所述方法包括以下步骤

使3α－羟基-6α－乙基-7-酮-5β－胆烷-24-酸（6）与$NaBH_4$反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸1型。

[0072] 在一个实施方案中，本发明涉及用于制备奥贝胆酸1型的方法，所述方法包括以下步骤

使 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5A) 与 Pd/C 和氢气反应形成 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6)，

使 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0073] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 E-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5) 与 Pd/C 和氢气反应形成 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6)，

使 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0074] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯 (4A) 与 NaOH 反应形成 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5A)，

使 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5A) 与 Pd/C 和氢气反应形成 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6)，

使 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0075] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 E-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯 (4) 与 NaOH 反应形成 E-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5)，

使 E-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5) 与 Pd/C 和氢气反应形成 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6)，

使 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0076] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α,7-二三甲基甲硅氧基-5β-胆-6-烯-24-酸甲酯 (3) 与 CH_3CHO 反应形成 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯 (4A)，

使 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯 (4A) 与 NaOH 反应形成 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5A)，

使 E/Z-3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸 (5A) 与 Pd/C 和氢气反应形成 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6)，

使 3α-羟基-6α-乙基-7-酮-5β-胆烷-24-酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆

酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0077] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯 (3) 与 CH_3CHO 反应形成 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4)，

使 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4) 与 NaOH 反应形成 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5)，

使 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5) 与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6)，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0078] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (1) 与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯 (3)，

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯 (3) 与 CH_3CHO 反应形成 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4A)，

使 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4A) 与 NaOH 反应形成 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5A)，

使 E/Z-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5A) 与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6)，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0079] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α- 羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (1) 与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯 (3)，

使 3α，7- 二三甲基甲硅氧基 -5β- 胆 -6- 烯 -24- 酸甲酯 (3) 与 CH_3CHO 反应形成 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4)，

使 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (4) 与 NaOH 反应形成 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5)，

使 E-3α- 羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸 (5) 与 Pd/C 和氢气反应形成 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6)，

使 3α- 羟基 -6α- 乙基 -7- 酮 -5β- 胆烷 -24- 酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0080] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸 (KLCA) 与 CH_3OH 和 H_2SO_4 反应形成 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1)，

使 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1) 与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯 (3)，

使 3α，7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯 (3) 与 CH_3CHO 反应形成 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (4A)，

使 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (4A) 与 NaOH 反应形成 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A)，

使 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A) 与 Pd/C 和氢气反应形成 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6)，

使 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0081] 在一个实施方案中，本发明涉及用于制备奥贝胆酸 1 型的方法，所述方法包括以下步骤

使 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸 (KLCA) 与 CH_3OH 和 H_2SO_4 反应形成 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1)，

使 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1) 与 $Li[N(CH(CH_3)_2)_2]$ 和 $Si(CH_3)_3Cl$ 反应形成 3α，7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯 (3)，

使 3α，7- 二三甲基甲硅氧基 -5β－胆 -6- 烯 -24- 酸甲酯 (3) 与 CH_3CHO 反应形成 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (4)，

使 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (4) 与 NaOH 反应形成 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5)，

使 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5) 与 Pd/C 和氢气反应形成 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6)，

使 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6) 与 $NaBH_4$ 反应形成结晶奥贝胆酸，和

将结晶奥贝胆酸转化为奥贝胆酸 1 型。

[0082] 在一个实施方案中，本发明涉及使用结晶奥贝胆酸制备奥贝胆酸 1 型的方法。在另一个实施方案中，结晶奥贝胆酸是 C 型。在一个实施方案中，结晶奥贝胆酸 C 型的特征在于类似于图 5 所示 X 射线衍射图的 X 射线衍射图。在一个实施方案中，结晶奥贝胆酸 C 型用乙酸正丁酯结晶和重结晶。

[0083] 步骤 1

步骤 1 是 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸 (KLCA) 与 CH_3OH 和 H_2SO_4 形成 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1) 的反应。在步骤 1 的一个实施方案中，将反应混合物加热约 3 小时，并用碱性水溶液调节反应混合物的 pH 至约 6.5- 约 8.0 的 pH 值。在一个实施方案中，3α－羟基 -7- 酮 -5β－胆烷 -24- 酸甲酯 (1) 的分离还包括用活性碳处理。在一

个实施方案中，3α－羟基－7－酮－5β－胆烷－24－酸甲酯（1）的分离不另包括用活性碳处理。在一个实施方案中，不用活性碳处理的3α－羟基－7－酮－5β－胆烷－24－酸甲酯（1）的分离提供较高的收率。在一个实施方案中，使3α－羟基－7－酮－5β－胆烷－24－酸（1）与CH_3OH和H_2SO_4的反应在甲醇中进行。在一个实施方案中，碱性溶液是NaOH水溶液。在一个实施方案中，pH值为约7.0－约7.5。

[0084] 在一个实施方案中，甲醇用作甲基化试剂以及反应溶剂。在一个实施方案中，含有产物的溶液用活性碳处理约30分钟并过滤除去碳固体物。在一个实施方案中，含有产物的溶液不用活性碳处理。为了沉淀产物，加入约5℃－约20℃的水和晶种材料。在另一个实施方案中，水为约10℃－约15℃。在一个实施方案中，产物以离心分离，用甲醇和水的混合物洗涤。在一个实施方案中，湿料的含水量通过Karl Fischer（KF）定量测定。在一个实施方案中，在用于下一步骤之前，将材料在转筒式干燥机中干燥。在一个实施方案中，在用于下一步骤之前，不将材料干燥。

[0085] 步骤2

步骤2是3α－羟基－7－酮－5β－胆烷－24－酸甲酯（1）与$Li[N(CH(CH_3)_2)_2]$和$Si(CH_3)_3Cl$形成3α，7－二三甲基甲硅氧基－5β－胆－6－烯－24－酸甲酯（3）的反应。在一个实施方案中，步骤2在约－10℃－约－30℃的温度下在极性非质子溶剂中进行。在一个实施方案中，极性非质子溶剂是四氢呋喃。在一个实施方案中，温度为约－20℃－约－25℃。在一个实施方案中，将3α－羟基－7－酮－5β－胆烷－24－酸甲酯（1）与$Li[N(CH(CH_3)_2)_2]$和$Si(CH_3)_3Cl$的反应搅拌约2小时。

[0086] 在一个实施方案中，将化合物1装入惰性条件下的反应器中。在另一个实施方案中，残余的水和甲醇在约65℃和常压下通过重复共沸蒸馏除去。在另一个实施方案中，需要时，将THF加入残余物中，重复蒸馏约4次。在另一个实施方案中，重复蒸馏约3次、约2次或约1次。在一个实施方案中，含有产物的剩余溶液的最终含水量为≤ 0.05%（Karl Fischer滴定）。水可以水解三甲基氯硅烷，其在稍后加入该步骤中。在一个实施方案中，使产物的溶液预先冷却至约－10℃－约－30℃，然后加入三甲基氯硅烷。在另一个实施方案中，使溶液预先冷却至约－20℃－约－25℃。在一个实施方案中，将强碱和THF装入单独的反应器中，并冷却至约－10℃－约－30℃。在一个实施方案中，强碱是二异丙基氨基锂。在另一个实施方案中，反应器是惰性的，例如在氮气氛或氩气氛下。在另一个实施方案中，使碱和THF的溶液冷却至约－20℃－约－25℃。在一个实施方案中，在约－10℃－约－30℃下，将3α－羟基－7－酮－5β－胆烷－24－酸甲酯、THF和三甲基氯硅烷的冷却的无水溶液装入碱性溶液中。在另一个实施方案中，温度为约－20℃－约－25℃。在一个实施方案中，搅拌反应混合物约2小时。在一个实施方案中，对于后处理，将反应混合物加入预冷的酸性溶液中。在另一个实施方案中，酸性溶液是柠檬酸水溶液。在一个实施方案中，在加入后，分离水相并弃去。在一个实施方案中，通过在约50℃下真空蒸馏，从有机相中除去溶剂。在一个实施方案中，分离的残余物是3α，7α－二三甲基甲硅氧基－5β－胆－6－烯－24－酸甲酯（3），在下一步骤中“照原样”使用。或者，可在步骤3前将化合物3纯化。

[0087] 步骤3

步骤3是3α，7－二三甲基甲硅氧基－5β－胆－6－烯－24－酸甲酯（3）与CH_3CHO形成3α－羟基－6－亚乙基－7－酮－5β－胆烷－24－酸甲酯（4）的反应。在一个实施方案中，步骤

3 在约 -50℃ - 约 -70℃的温度下在 BF_3存在下在极性非质子溶剂中进行。在一个实施方案中，极性非质子溶剂是二氯甲烷。在一个实施方案中，BF_3为乙腈中的 16% wt 溶液。在一个实施方案中，温度为约 -60℃ - 约 -65℃。

[0088] 在一个实施方案中，将含化合物 3 的极性非质子溶剂装入惰性反应器中。在另一个实施方案中，极性非质子溶剂是前一步骤的残余溶剂（例如 THF）。在一个实施方案中，加入 THF 以利于馏出残余的水和二异丙胺。在约 50℃的最大温度下，真空馏出残余量的极性非质子溶剂。将含化合物 3 的残余物中的含水量限于≤ 0.5% (Karl Fischer 滴定）。然后将含化合物 3 的残余物溶于极性非质子溶剂中，并预冷却至约 -50℃ - 约 -70℃。极性非质子溶剂是二氯甲烷。在另一个实施方案中，将极性非质子溶剂中的含化合物 3 的残余物预冷却至约 -60℃ - 约 -65℃。加入乙醛 (CH_3CHO)。将极性非质子溶剂和三氟化硼 (BF_3) 溶剂化络合物装入单独的反应器中，然后冷却至约 -50℃ - 约 -70℃。在另一个实施方案中，极性非质子溶剂是二氯甲烷。在另一个实施方案中，三氟化硼溶剂化络合物是三氟化硼乙腈络合物。BF_3溶液的温度为约 -60℃ - 约 -65℃。在约 -60℃ - 约 -65℃下将含有化合物 3 和乙醛的溶液加入 BF_3溶液中。在另一个实施方案中，含化合物 3 和乙醛的溶液是无水的。在一个实施方案中，在约 -60℃ - 约 -65℃下搅拌反应混合物约 2 小时，加热至 23℃ - 约 28℃，再搅拌约 2 小时，并冷却至约 2℃ - 约 10℃用于水解 / 后处理。在一个实施方案中，加入和搅拌的总时间为约 4 小时。在一个实施方案中，对于后处理，将反应器中的冷却溶液加入预冷的碱性水溶液中。在另一个实施方案中，碱性水溶液为约 50% wt 氢氧化钠 (NaOH；苛性钠）。在一个实施方案中，分离各相，将（下层）有机层转移到单独的反应器中。在一个实施方案中，尽可能地在不超过 (NMT) 50℃时通过蒸馏从有机层中除去溶剂。在一个实施方案中，残余物包含 3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4) 和一些剩余的乙腈和二氯甲烷。要了解，步骤 4 可形成 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4A)。步骤 3 的产物直接用于步骤 4。

[0089] 步骤 4

步骤 4 是 3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4) 与 NaOH 形成 E-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸 (5) 的反应。在一个实施方案中，在步骤 4 之前，将步骤 3 的残余物加热至约 45℃ - 约 60℃以除去残余量的溶剂。在一个实施方案中，温度为约 49℃ - 约 55℃。在一个实施方案中，使 3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4) 与 NaOH 反应的酯水解反应在约 20℃ - 约 25℃下在甲醇、水和 NaOH 溶液中进行。

[0090] 在一个实施方案中，将起反应的 3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4) 装入反应器中。在另一个实施方案中，反应器是惰性的，例如在氮气氛或氩气氛下。在 NMT 50℃的温度下，真空馏出残余量的溶剂。在一个实施方案中，将残余物加热至约 45℃ - 约 60℃。在另一个实施方案中，将残余物加热至约 49℃ - 约 55℃。在另一个实施方案中，将步骤 3 的残余物（化合物 4）溶于甲醇和水和碱性水溶液中。在另一个实施方案中，碱性水溶液为约 50% wt 氢氧化钠 (NaOH；苛性钠）。步骤 4 的酯水解反应在约 20℃ - 约 60℃下进行，并搅拌直到水解反应完成。在一个实施方案中，酯水解在约 20℃ - 约 25℃下进行。检查反应混合物的 pH 以证实 pH> 12。如果 pH < 12，则加入另外的 NaOH。反应混合物用水稀释，并调节温度至约 20℃ - 约 35℃。另一方面，反应混合物用水稀释，并调节温度

至约 25℃ - 约 35℃。在一个实施方案中，对于后处理，分离各相，将下层的水层转移到单独的反应器中，弃去有机层。化合物 5 在水相中。在一个实施方案中，将乙酸乙酯和酸加入含化合物 5 的水相中，以充分搅拌进入水层中。在另一个实施方案中，酸是柠檬酸水溶液。在一个实施方案中，分离各相，弃去下层的水层。化合物 5 在有机层中。在一个实施方案中，乙酸乙酯从有机层中馏出，并再加入乙酸乙酯。在一个实施方案中，重复蒸馏直到馏出液的含水量为 NMT 1% 或直到达到恒定的沸点。在一个实施方案中，使悬浮液冷却至约 10℃ - 约 30℃，分离，并用乙酸乙酯洗涤。在另一个实施方案中，使所得的含化合物 5 的悬浮液冷却至约 20℃ - 约 25℃。在一个实施方案中，所得产物的干燥在约 60℃下真空进行（例如转筒式干燥机）。

[0091] 在一个实施方案中，使用乙醇使粗制的 E-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸 (5) 结晶。在一个实施方案中，将乙醇和粗制化合物 5 装入反应器中。在另一个实施方案中，反应器是惰性的。在一个实施方案中，为了溶解粗制化合物 5，将混合物加热至回流。在一个实施方案中，以受控制的冷却变速使混合物冷却至约 15℃ - 约 20℃。在一个实施方案中，结晶化合物 5 使用离心机分离，然后用乙酸乙酯洗涤。在一个实施方案中，结晶化合物 5 的干燥在真空下（例如转筒式干燥机）和在约 60℃下进行。可取样品以测量纯化的化合物 5 的含量测定、纯度和水分含量。在一个实施方案中，纯化的化合物 5 含有 3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸的 E 和 Z 异构体两者。在一个实施方案中，E/Z 比率为约 99:1、约 98:2、约 95: 5、约 90:10、约 85:15、约 80:20、约 75:25、约 70:30、约 65:35、约 60:40、约 55:45 或约 50:50。有关纯化的化合物 5 的鉴定和表征的全部详情参见实施例 2。

[0092] 步骤 4 还可以作为 E/Z 异构体的混合物的化合物开始进行。例如，步骤 4 是 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4A) 与 NaOH 形成 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸 (5A) 的反应。在一个实施方案中，在步骤 4 之前，将步骤 3 的残余物加热约 45℃ - 约 60℃以除去残余量的溶剂。在一个实施方案中，温度为约 49℃ - 约 55℃。在一个实施方案中，涉及使 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4A) 与 NaOH 反应的酯水解反应在约 20℃ - 约 25℃下在甲醇、水和 NaOH 溶液中进行。在一个实施方案中，NaOH 溶液是 50% wt 水性溶液。

[0093] 在一个实施方案中，将起反应的 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸甲酯 (4A) 装入反应器中。在另一个实施方案中，反应器是惰性的，例如在氮气氛或氩气氛下。在 NMT 50℃的温度下，真空馏出残余量的溶剂。在一个实施方案中，将残余物加热至 45℃ - 约 60℃。在一个实施方案中，温度为约 49℃ - 约 55℃。在一个实施方案中，将步骤 3 的残余物（化合物 4A）溶于甲醇和水和碱性水溶液中。在另一个实施方案中，碱性水溶液是约 50% wt 氢氧化钠 (NaOH ；苛性钠）。步骤 4 的酯水解反应在约 20℃ - 约 60℃下进行，并搅拌直到水解反应完成。在一个实施方案中，酯水解在约 20℃ - 约 25℃下进行。检查反应混合物的 pH 以证实 pH> 12。如果 pH < 12，则加入额外的 NaOH。反应混合物用水稀释，并调节温度至约 25℃ - 约 35℃。在一个实施方案中，对于后处理，分离各相，将下层的水层转移到单独的反应器中，弃去有机层。化合物 5A 在水相中。在一个实施方案中，将乙酸乙酯和酸加入含化合物 5A 的水相中，以充分搅拌进入水层中。在另一个实施方案中，酸是柠檬酸水溶液。在一个实施方案中，分离各相，弃去下层的水层。化合物 5A 在有机层

中。在一个实施方案中，将乙酸乙酯从有机层中馏出，并再加入乙酸乙酯。在一个实施方案中，重复蒸馏直到馏出液的含水量为 NMT 1% 或直到达到恒定的沸点。在一个实施方案中，使悬浮液冷却至约 10℃ - 约 30℃，分离，并用乙酸乙酯洗涤。在另一个实施方案中，使所得的含化合物 5A 的悬浮液冷却至约 20℃ - 约 25℃。在一个实施方案中，所得产物的干燥在约 60℃下真空进行（例如转筒式干燥机）。化合物 5A 可无需纯化继续进行至步骤 5。

[0094] 在一个实施方案中，粗制的 E/Z-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸 (5A) 使用乙醇结晶。在一个实施方案中，将乙醇和粗制化合物 5A 装入反应器中。在另一个实施方案中，反应器是惰性的。在一个实施方案中，为了溶解粗制的化合物 5A，将混合物加热至回流。在一个实施方案中，以受控制的冷却变速使混合物冷却至约 15℃ - 约 20℃。在一个实施方案中，结晶化合物 5A 使用离心机分离，然后用乙酸乙酯洗涤。在一个实施方案中，结晶化合物 5A 的干燥在真空下（例如转筒式干燥机）和在约 60℃下进行。在一个实施方案中，步骤 4 的分离结晶产物是化合物 5。

[0095] 备选步骤 4

化合物 5 可按照备选方法制备。在一个实施方案中，将化合物 4 装入惰性反应器中。在约 50℃ （最大）下，可真空馏出残余量的溶剂（例如乙腈、二氯甲烷）。将残余物溶于甲醇中，并冷却。加入自来水和苛性钠 (50% wtNaOH)。在一个实施方案中，在约 20℃ - 约 25℃下搅拌反应混合物约 4 小时。溶液用自来水稀释，并加入甲苯。在搅拌后，分离各相，将下层的水层转到惰性反应器中。弃去有机层。在充分搅拌进入水层的情况下加入乙酸乙酯和柠檬酸溶液。分离各相，弃去下层的水层。将有机层转移到惰性反应器中。从有机层中馏出乙酸乙酯，并再加入乙酸乙酯。在一个实施方案中，重复该操作直到馏出液的含水量不超过约 1% 或直到达到恒定的沸点。使该悬浮液冷却至约 20℃ - 约 25℃，化合物 5 用惰性离心机分离，并用乙酸乙酯洗涤。干燥在转筒式干燥机中在真空和约 60℃下进行。

[0096] 该备选步骤 4 还可以作为 E/Z 异构体的混合物的化合物开始进行。在一个实施方案中，将化合物 4A 装入惰性反应器中。在约 50℃ （最大）下，可真空馏出残余量的溶剂（例如乙腈、二氯甲烷）。将残余物溶于甲醇中，并冷却。加入自来水和苛性钠 (50%wt，NaOH)。在一个实施方案中，在约 20℃ - 约 25℃下搅拌反应混合物约 4 小时。溶液用自来水稀释，并加入甲苯。在搅拌后，分离各相，将下层的水层转到到惰性反应器中。弃去有机层。在充分搅拌进入水层的情况下加入乙酸乙酯和柠檬酸溶液。分离各相，弃去下层的水层。将有机层转移到惰性反应器中。从有机层中馏出乙酸乙酯，并再加入乙酸乙酯。在一个实施方案中，重复该操作直到馏出液的含水量不超过约 1% 或直到达到恒定的沸点。使该悬浮液冷却至 20℃ -25℃，化合物 5A 用惰性离心机分离，并用乙酸乙酯洗涤。干燥在转筒式干燥机中在真空和约 60℃下进行。

[0097] 步骤 5

步骤 5 是 E-3α - 羟基 -6- 亚乙基 -7- 酮 -5β - 胆烷 -24- 酸 (5) 与 Pd/C 和氢气形成 3α - 羟基 -6α - 乙基 -7- 酮 -5β - 胆烷 -24- 酸 (6) 的反应。步骤 5 可以同步（氢化和异构化同时）或分两阶段（氢化接着异构化）进行。在一个实施方案中，步骤 5 在约 90℃ - 约 110℃的温度下和在约 4- 约 5 巴的压力下进行。在一个实施方案中，在后处理期间，反应混合物的有机相用活性碳处理。在一个实施方案中，压力为约 4.5- 约 5.5 巴。在另一个实施方案中，压力为约 5 巴。在一个实施方案中，将氢化反应混合物搅拌约 1 小时。在一个实施

方案中，将 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5) 与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 2 小时－约 5 小时。在一个实施方案中，将 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5) 与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 3 小时。

[0098] 在一个实施方案中，E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5) 与 Pd/C 和氢气的反应在碱性溶液存在下进行。在一个实施方案中，碱性溶液是 50% wt 氢氧化钠 (NaOH ；苛性钠）溶液。在氢化反应后，将反应混合物加热至 100℃ （以进行从 β 构型到 α 构型的 C-6 位的异构化），然后冷却至约 40℃－约 50℃。对于后处理，滤出 Pd/C。在一个实施方案中，向滤液中加入乙酸正丁酯和酸。在另一个实施方案中，酸是盐酸 (HCl)。在检查 pH 值以确保它是酸性的后，将水相分离并弃去。含有产物的有机相用活性碳处理。在一个实施方案中，滤出活性碳，所得的含有产物的滤液通过蒸馏浓缩，并使所得悬浮液冷却至约 10℃－约 30℃。在另一个实施方案中，使悬浮液冷却至约 15℃－约 20℃。分离含有化合物 6 的悬浮液，并用乙酸正丁酯洗涤。使用压力过滤器将化合物 6 过滤。在一个实施方案中，干燥在压力过滤器中在约 80℃下真空进行。

[0099] 在步骤 5 的一个实施方案中，将 E-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5)、水、NaOH 溶液（例如 50% wt）和 Pd/C 在约 5 巴的 H_2 气体中并在约 100℃－约 105℃的温度下混合直到 H_2 吸收停止。使反应混合物冷却至约 40℃－约 50℃，并滤出 Pd/C。然后将乙酸正丁酯和 HCl 加入含有化合物 6 的溶液中。在一个实施方案中，分离水相并弃去。含有化合物 6 的有机相用活性碳处理。滤出碳，且将滤液移到另一个反应器中，在其中通过蒸馏浓缩，然后使悬浮液冷却至约 5℃－约 20℃。在一个实施方案中，化合物 6 通过过滤分离，将滤液在压力过滤器中在约 80℃下真空干燥。

[0100] 步骤 5 还可以作为 E/Z 异构体的混合物的化合物开始进行。例如，步骤 5 是 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A) 与 Pd/C 和氢气并加热形成 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6) 的反应。步骤 5 可以同步（氢化和异构化同时）或分两阶段（氢化，接着异构化）进行。一方面，步骤 5 在约 90℃－约 110℃的温度下和在约 4- 约 5 巴的压力下进行。在一个实施方案中，在后处理期间，反应混合物的有机相用活性碳处理。在一个实施方案中，压力为约 4.5- 约 5.5 巴。在另一个实施方案中，压力为约 5 巴。在一个实施方案中，将氢化反应混合物搅拌约 1 小时。在一个实施方案中，使 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A) 与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 2 小时－约 5 小时。在一个实施方案中，使 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A) 与 Pd/C 和氢气的反应加热至约 100℃，并搅拌约 3 小时。

[0101] 在一个实施方案中，使 E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A) 与 Pd/C 和氢气的反应在碱性溶液存在下进行。在一个实施方案中，碱性溶液是 50% wt 氢氧化钠 (NaOH ；苛性钠）溶液。在氢化反应后，将反应混合物加热至 100℃ （以进行从 β 构型到 α 构型的 C-6 位的异构化），然后冷却至约 40℃－约 50℃。对于后处理，滤出 Pd/C。在一个实施方案中，向滤液中加入乙酸正丁酯和酸。在另一个实施方案中，酸是盐酸 (HCl)。在检查 pH 值以确保它是酸性的后，将水相分离并弃去。含有产物的有机相用活性碳处理。在一个实施方案中，滤出活性碳，所得的含有产物的滤液通过蒸馏浓缩，并使所得悬浮液冷却至约 10℃－约 30℃。在另一个实施方案中，使悬浮液冷却至约 15℃－约 20℃。分离含有化合物 6 的悬浮液，并用乙酸正丁酯洗涤。使用压力过滤器将化合物 6 过滤。在一个实

施方案中，干燥在压力过滤器中在约 80℃下真空进行。

[0102] 在步骤 5 的一个实施方案中，E/Z-3α－羟基 -6- 亚乙基 -7- 酮 -5β－胆烷 -24- 酸 (5A)、水、NaOH 溶液（例如 50% wt）和 Pd/C 在约在 5 巴的 H_2 气体中并在约 100℃－约 105℃的温度下混合直到 H_2 吸收停止。使反应混合物冷却至约 40℃－约 50℃，并滤出 Pd/C。然后将乙酸正丁酯和 HCl 加入含有化合物 6 的溶液中。在一个实施方案中，分离水相并弃去。含有化合物 6 的有机相用活性碳处理。滤出碳，且将滤液移到另一个反应器中，在其中通过蒸馏浓缩，然后使悬浮液冷却至约 5℃－约 20℃。在一个实施方案中，化合物 6 通过过滤分离，将滤液在压力过滤器中在约 80℃下真空干燥。

[0103] 在另一个实施方案中，上述制备化合物 6 的氢化 / 异构化反应分两阶段进行（从化合物 5 或化合物 5A 开始）。首先，氢化在约 4-5 巴下进行，然后其次，将反应混合物加热至约 20℃－约 40℃。加热反应混合物将 6- 位的乙基异构化为所需的 α 构型。将反应混合物加热直到异构化完成。

[0104] 步骤 6

步骤 6 是 3α－羟基 -6α－乙基 -7- 酮 -5β－胆烷 -24- 酸 (6) 与 $NaBH_4$ 形成结晶奥贝胆酸的反应。在一个实施方案中，步骤 6 在约 85℃－约 110℃的温度下在碱性水溶液中进行。在一个实施方案中，温度为约 90℃－约 95℃。在一个实施方案中，碱性水溶液是 NaOH 水溶液。在一个实施方案中，碱性水溶液是 50% wt NaOH 溶液和水的混合物。在一个实施方案中，搅拌化合物 6 和 $NaBH_4$ 的反应混合物约 3 小时－约 5 小时。在另一个实施方案中，搅拌反应混合物约 4 小时。

[0105] 对于后处理，在反应完成后，使混合物冷却至约 80℃，并转移到冷却的反应器中。在一个实施方案中，在约 20℃－约 60℃下，加入乙酸正丁酯和酸。在一个实施方案中，温度为约 40℃－约 45℃。在另一个实施方案中，酸是柠檬酸。在检查 pH 值以确保它是酸性的后，将水相分离并弃去。含有产物的有机相通过蒸馏浓缩。在一个实施方案中，将乙酸正丁酯加入残余物中并再次馏出。在一个实施方案中，再次将乙酸正丁酯加入残余物中，然后慢慢冷却。在另一个实施方案中，在约 50℃下向残余物中接晶种。在另一个实施方案中，在结晶出现后，将混合物加热至 52℃，然后慢慢冷却至约 15℃－约 20℃。在另一个实施方案中，使残余物冷却至约 15℃－约 20℃。在一个实施方案中，所得奥贝胆酸用乙酸正丁酯洗涤。在一个实施方案中，分离奥贝胆酸，并用乙酸正丁酯洗涤（例如在压力过滤器中）。在另一个实施方案中，压力过滤器是惰性的。将结晶产物在约 60℃下真空干燥。在一个实施方案中，将所得结晶奥贝胆酸从有机溶剂（例如庚烷）中分离。有关结晶奥贝胆酸 C 型的鉴定和表征的全部详情参见实施例 3。

[0106] 步骤 7

步骤 7 是结晶奥贝胆酸 C 型向奥贝胆酸 1 型的转化。在一个实施方案中，步骤 7 包括将结晶奥贝胆酸 C 型溶于 NaOH 水溶液中并加入 HCl 的步骤。

[0107] 在一个实施方案中，在约 20℃－约 50℃下，将结晶奥贝胆酸溶于水和苛性钠溶液 (50% wt) 中。在一个实施方案中，温度为约 30℃－约 40℃。在一个实施方案中，结晶奥贝胆酸是 C 型。在一个实施方案中，在约 20℃－约 50℃下，将所得的结晶奥贝胆酸 C 型溶液加入稀酸中。在另一个实施方案中，温度为约 30℃－约 40℃。在另一个实施方案中，酸是盐酸（例如 37%）。在一个实施方案中，37% 盐酸溶液用水稀释至以体积计小于约 1%。在一个

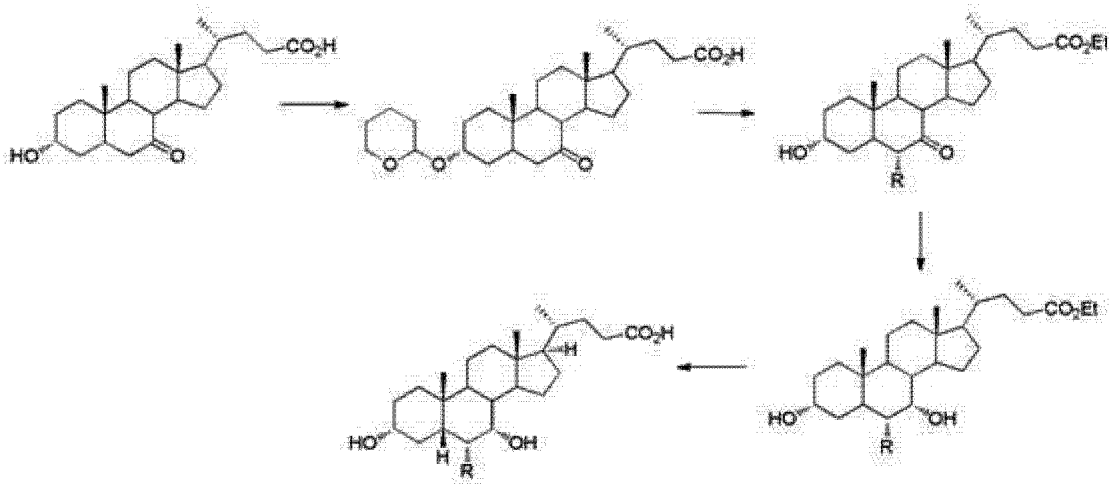
实施方案中，37% 盐酸溶液用水稀释至以体积计约 0.7%。在一个实施方案中，在约 20℃ - 约 50℃下搅拌产物在稀酸中的悬浮液约 30 分钟。在另一个实施方案中，温度为约 30℃ - 约 40℃。在一个实施方案中，在 NMT 约 20℃下分离奥贝胆酸 1 型并用水洗涤（例如在压力过滤器中）。在一个实施方案中，在 NMT 约 20℃下分离奥贝胆酸 1 型并用水洗涤（例如在压力过滤器中）。在另一个实施方案中，压力过滤器是惰性的。将产物在压力过滤器中在 NMT 约 50℃的温度下真空干燥。

[0108] 本申请的方法在奥贝胆酸 1 型制备中利用结晶中间体，其出乎意料地导致总体制备和最终产物的纯度显著改进。具体地讲，合成的步骤 6 产生奥贝胆酸的新晶体形式。这种晶体形式的产生导致基本上纯的奥贝胆酸 1 型。

[0109] 本申请的方法相对于先有技术公开的方法是一种改进。奥贝胆酸的制备公开于美国公开号 2009/0062526 A1（本文称为“‘526 公开文本”）、美国专利号 7,138,390（本文称为“‘390 专利”）和 WO 2006/122977（本文称为“‘977 申请”）。

[0110] 以下流程 3 中描述了‘390 专利中制备奥贝胆酸的方法（本文称为“‘390 方法”）(R 为乙基）：

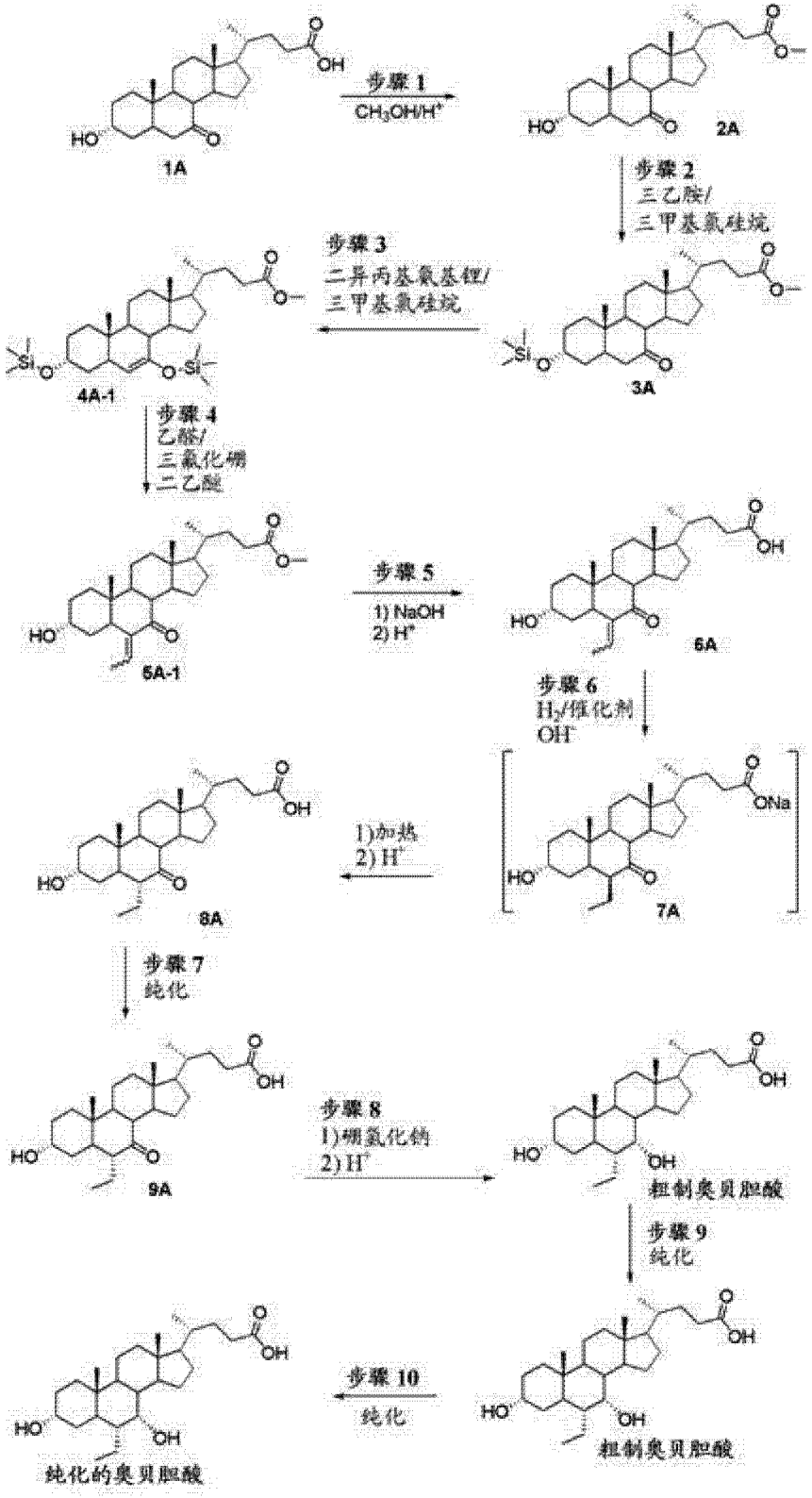
流程 3



即使该方法包括很少的步骤，但它存在一系列缺点。在所有步骤中，反应产物在色谱柱上纯化，即一种非常昂贵的分离方法，不可用于工业规模。此外，步骤 2 的反应收率极低 (12-13%)，随之总收率大大降低，低于 3.5%。该方法还使用六亚甲基磷酰胺作为反应物，这是一种已知的致癌物。

[0111] ‘977 申请中制备奥贝胆酸的方法描述于流程 4。

[0112] 流程 4



制备奥贝胆酸的‘977 方法是 8 步合成方法,其包括一个纯化步骤（步骤 7）接着 2 个另外的纯化步骤。在‘977 方法和本申请的方法之间有许多差异。下表 A 描述了两种方法间的至少一些差异：

表 A:‘977方法和本申请的方法之间的差异

合成步骤	变化		变化的优势
	'977 方法	本申请的方法	
步骤 1	甲磺酸	硫酸	规模和安全性(甲磺酸盐)
	30%氨(水溶液)	NaOH (水溶液)	按比例扩大
	无纯化/处理	使用活性碳处理	改进纯度/颜色
步骤 2 (申请步骤 2 的方法组合了'977 方法步骤 2 和 3)	三乙胺	二异丙基氨基锂 (LDA)	LDA 是该步骤合适的替代试剂
	甲苯	四氢呋喃(THF)	THF 是该步骤合适的替代试剂
	无酸性猝灭	在柠檬酸溶液中猝灭	按比例扩大
步骤 3 (申请步骤 3 的方法同'977 步骤 4)	三氟化硼二乙醚	三氟化硼乙腈络合物	处理醚合物的安全顾虑(用乙醚的暴露危险)
步骤 4 (申请步骤 4 的方法同'977 步骤 5)	甲苯	甲醇	安全性(甲苯); 规模
	磷酸(水溶液)猝灭	柠檬酸(水溶液)猝灭	按比例扩大
	无纯化/处理	结晶步骤是后处理的一部分	改进纯度
步骤 5 (申请步骤 5 的方法组合了'977 方法步骤 6 和 7)	磷酸(水溶液)猝灭	盐酸(水溶液)猝灭	按比例扩大
	无纯化/处理	使用活性碳处理	改进纯度/颜色
	纯化按步骤 7 进行	结晶步骤是后处理的一部分	按比例扩大
步骤 6 (申请步骤 6 的方法组合了'977 方法的步骤 8 和 9)	二氯甲烷	乙酸正丁酯	安全性(二氯甲烷)
	磷酸(水溶液)猝灭	柠檬酸(水溶液)猝灭	按比例扩大
	纯化按步骤 9 进行 – 使用二氯甲烷/乙酸乙酯	结晶步骤是后处理的一部分 – 使用乙酸正丁酯	规模和安全性(二氯甲烷)
步骤 7 (申请步骤 7 的方法同'977 步骤 10)	氨溶液	NaOH 溶液	按比例扩大
	磷酸(水溶液)猝灭	盐酸(水溶液)猝灭	按比例扩大

本申请的方法与'977 方法相比的差异导致该方法的重大改进,包括涉及规模优化、安全性以及纯度的改进和整体方法的改进。通过本申请的方法产生的奥贝胆酸的纯度是基本纯的。具体地讲,通过本申请的方法产生的奥贝胆酸基本上比通过先有技术的方法(包括'390 方法和'977 方法)产生的奥贝胆酸更纯。例如,通过本申请的方法生产的奥贝胆酸和通过'977 方法生产的奥贝胆酸的化验证书提供的结果比较见下表 B。杂质的百分比采用 HPLC 方法测定。

[0113] 表 B:由本申请的方法和'977方法产生的奥贝胆酸的杂质的比较

参数	规格界限	本申请的方法	'977 方法
水(KF)	NMT 4.5%	1.0%	2.1%
杂质 1 和杂质 4	NMT 0.15%	<0.05%	<0.05%
杂质 2	NMT 0.15%	<0.05%	<0.1%
杂质 3	NMT 0.15%	<0.05%	<0.1%
杂质 5	NMT 3.0%	0.2%	1.0%
杂质 6	NMT 0.15%	<0.05%	<0.05%

杂质 1 是 6-乙基乌索脱氧胆酸。

杂质 2 是 3α-羟基-6α-乙基-7-cheto-5β-胆烷-24-酸。

杂质 3 是 6β-乙基鹅脱氧胆酸。

杂质 4 是 3α,7α-二羟基-6-亚乙基-5β-胆烷-24-酸。

杂质 5 是鹅脱氧胆酸。

杂质 6 是 3α(3α,7α-二羟基-6α-乙基-5β-胆烷-24-酰基氧基)-7α-羟基-6α-乙基-5β-胆烷-24-酸(6ECDCA 二聚体)。

NMT 是指"不超过"。

结晶奥贝胆酸作为合成中间体

目前正在开发奥贝胆酸作为呈非晶体固体的活性药物成分。为了促进奥贝胆酸的开发,进行了初步的结晶和多晶现象研究以确定晶体形式是否容易获得,并且如果容易获得,则它们是否适于开发。在所设计的更好理解该材料在不同溶剂中的行为的初步溶解度筛选之后,似乎该材料具有形成凝胶的趋势,并且可能结晶。然后进行了广泛的多晶型物筛选,将该材料暴露于大范围的溶剂和结晶条件中以鉴定和表征尽可能多的相关多晶型物。在该筛选期间,发现了 5 种不同的固体形式。

[0114] 奥贝胆酸的 3 种形式 (A、C 和 D) 是含有 0.25 mol eq 水和不同量的各种有机溶剂的混合水合物 / 溶剂合物。在加热时,这些固体同时失去结晶性和溶剂,并且遗憾地是,由于其低的熔解温度和高的溶剂含量所致,这些溶剂化形式不适于进一步开发作为药物成分。还注意到,存在这种类型的类似的"不适宜"形式。例如,在稍后的实验中发现低熔点溶剂化形式,以及另一种形式的单晶,其通过 SCXRD (单晶 X 射线衍射) 显示为一水合物 / 茴香醚溶剂合物。

[0115] 其余两种形式是较高熔点的,且可能更有前景,但是它们中的一种 (G 型) 尽管许多尝试,但都无法按比例扩大再生产,也无法重复。仅生产这种形式的困难便使之不适于开发。可再现地制备剩余的非溶剂化 F 型,但它需要大量的重结晶程序并使用硝基甲烷,这是一种有毒溶剂,并且如果被胺、碱金属、强酸或高温或绝热压缩激活则可能爆炸。有关硝基甲烷的残留水平的顾虑注定 F 型也不适用于开发。

[0116] 初步结晶和多晶型物研究的整体结果显示,该材料可形成不同形式的结晶材料,但结晶材料或形式无一视为适于开发。

[0117] 没多久发现产生结晶奥贝胆酸在本申请方法倒数第二步中作为中间体的重要性。结晶奥贝胆酸可采用本申请的方法容易地大规模分离。从初步结晶和多晶型物研究来看,确定该结晶奥贝胆酸与 C 型一致。在本申请方法步骤 7 中作为合成中间体产生的结晶奥贝胆酸的形成、易于分离和高纯度的确对制备基本上纯的奥贝胆酸至关重要。

[0118] 在一个实施方案中,本发明涉及特征在于包括在约 4.2、6.4、9.5、12.5 和 16.7° 2θ 处的特征峰的 X 射线衍射图的结晶奥贝胆酸 C 型。在一个实施方案中,X 射线衍射图包

括在约 4.2、6.4、9.5、12.5、12.6、15.5、15.8、16.0、16.7 和 19.0° 2θ 处的特征峰。在一个实施方案中，X 射线衍射图包括在约 4.2、6.4、8.3、9.5、11.1、12.2、12.5、12.6、15.5、15.8、16.0、16.3、16.7、18.6 和 19.0° 2θ 处的特征峰。在一个实施方案中，X 射线衍射图包括在 约 4.2、6.4、8.3、9.5、11.1、12.2、12.5、12.6、15.5、15.8、16.0、16.3、16.7、17.0、17.8、18.6、18.8、19.0、20.5 和 20.9° 2θ 处的特征峰。在一个实施方案中，本发明涉及特征在于基本类似于图 5 所示 X 射线衍射图的 X 射线衍射图的结晶奥贝胆酸 C 型。在一个实施方案中，X 射线衍射图在使用 Cu Kα 辐射 (40 kV，40 mA) 的衍射计上收集。在一个实施方案中，X 射线衍射图包括在约 12.0- 约 12.8 和约 15.4- 约 21.0 处的特征峰。

[0119] 在一个实施方案中，本发明涉及特征在于在约 98±2℃处具有吸热值的示差扫描量热法 (DSC) 温谱图的结晶奥贝胆酸 C 型，如通过 Mettler DSC 823e 仪器测量。在一个实施方案中，示差扫描量热法 (DSC) 温谱图具有在约 98±2℃处的吸热值，如通过 Mettler DSC 823e 仪器测量。

[0120] 在一个实施方案中，本发明涉及结晶奥贝胆酸，其中所述结晶奥贝胆酸是 C 型，且具有大于约 90% 的纯度。在一个实施方案中，所述结晶奥贝胆酸 C 型的纯度通过 HPLC 测定。在一个实施方案中，本发明涉及结晶奥贝胆酸 C 型或其药学上可接受的盐、溶剂合物或氨基酸缀合物。在一个实施方案中，溶剂合物是水合物。在一个实施方案中，纯度大于约 92%。在一个实施方案中，纯度大于约 94%。在一个实施方案中，纯度大于约 96%。在一个实施方案中，纯度大于约 98%。在一个实施方案中，纯度大于约 99%。

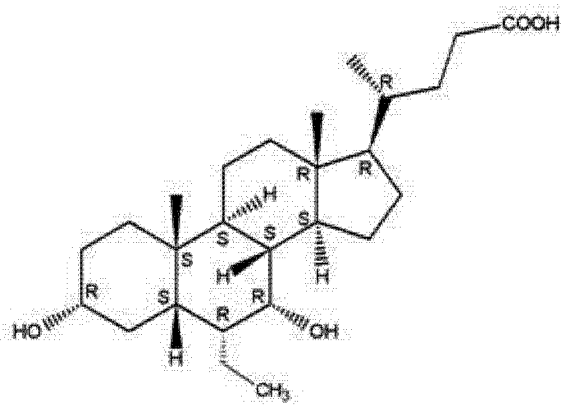
[0121] 在一个实施方案中，本发明涉及结晶奥贝胆酸，其中所述结晶奥贝胆酸是 C 型，并具有大于约 90% 的效能。在一个实施方案中，所述结晶奥贝胆酸 C 型的纯度通过 HPLC 和 / 或本领域已知的其它分析程序测定。在一个实施方案中，本发明涉及结晶奥贝胆酸 C 型或其药学上可接受的盐、溶剂合物或氨基酸缀合物。在一个实施方案中，溶剂合物是水合物。在一个实施方案中，效能大于约 92%。在一个实施方案中，效能大于约 94%。在一个实施方案中，效能大于约 96%。在一个实施方案中，效能大于约 98%。在一个实施方案中，效能大于约 99%。

[0122] 在一个实施方案中，本发明涉及含有总共小于约 4% 的一种或多种选自以下杂质的结晶奥贝胆酸 C 型 :6- 乙基乌索脱氧胆酸、3α - 羟基 -6α - 乙基 -7-cheto-5β - 胆烷 -24- 酸、6β - 乙基鹅脱氧胆酸、3α，7α - 二羟基 -6- 亚乙基 -5β - 胆烷 -24- 酸、鹅脱氧胆酸和 3α (3α，7α - 二羟基 -6α - 乙基 -5β - 胆烷 -24- 酰基氧基)-7α - 羟基 -6α - 乙基 -5β - 胆烷 -24- 酸。在一个实施方案中，总杂质小于约 3.8%。在一个实施方案中，总杂质小于约 3.6%。

[0123] 本申请的实施例 3 提供奥贝胆酸的这种新晶体形式的全面表征。

[0124] 获得奥贝胆酸的单晶 X 射线结构，并指定绝对立体化学。例如，在以 0.1℃ / 分钟冷却至 5℃接着在室温 /50℃ 8 小时周期成熟 1 周后，由奥贝胆酸从乙腈溶液中重结晶获得的晶体，测定结晶奥贝胆酸 G 型的单晶 X 射线结构。

[0125] 结构是正交晶系的，空间群 $P2_12_12_1$，在不对称单元中含有 1 分子的奥贝胆酸。最终的 R1 [I>2 σ(I)] = 3.22%。分子的绝对立体化学如下所示用 Flack 参数 = -0.01 (13) 测定。该结构有序 (no disorder)。

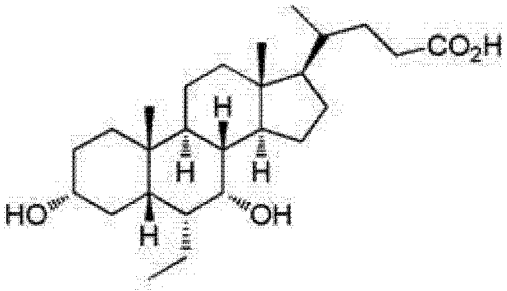


[0126] 进行了奥贝胆酸 1 型（非晶体）与结晶奥贝胆酸 F 型的生物利用度研究（实施例 7）。研究结果显示固体奥贝胆酸的物理状态在口服给予受试者时可在分子的生物利用度中起作用。口服给药的血浆动力学和肠吸收的效率和固体奥贝胆酸 1 型（非晶体）和结晶 F 型的药代动力学按照本领域已知方法评价。本发明的实施例 8 显示在给予奥贝胆酸的 1 型或 F 型后的奥贝胆酸血浆浓度相对于时间的概况、t_{max}、C_{max}和 AUC（参见图 37-38）。结晶 F 型具有比奥贝胆酸 1 型（非晶体）高的生物利用度。血浆概况表明 F 型更有效地吸收（较高的 AUC），甚至动力学更有规律，反映了药物在肠内容物中的最佳分布。

[0127] 奥贝胆酸 1 型（非晶体）的水溶性比 F 型略高。F 型显得是稳定的，因为热解重量分析 (TGA) 在所研究的温度范围内未显示任何重量减轻。

[0128] 基本上纯的奥贝胆酸

本申请提供基本上纯的奥贝胆酸及其药学上可接受的盐、溶剂合物或氨基酸缀合物：



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奥贝胆酸

(亦称为 INT-747)

[0129] 药学活性成分奥贝胆酸的其它名称为 INT-747、3α，7α－二羟基 -6α－乙基 -5β－胆烷 -24- 酸、6α－乙基－鹅脱氧胆酸、6- 乙基 -CDCA、6ECDCA 和胆烷 -24- 酸，6- 乙基 -3,7- 二羟基 -，(3α，5β，6α，7α)-。

[0130] 本申请提供包含奥贝胆酸 1 型的组合物和用于合成高纯度奥贝胆酸 1 型的方法，其是安全的且大规模生产奥贝胆酸。一方面，奥贝胆酸 1 型以商业规模方法生产。术语“商业规模方法”是指以至少约 100 克的单批次运行的方法。一方面，本申请的方法以高收率 (>80%) 和有限的杂质生产奥贝胆酸 1 型。

[0131] 本文所用术语“纯度”是指基于 HPLC 的奥贝胆酸的量。纯度以化合物的“有机”纯度为基础。纯度不包括水、溶剂、金属、无机盐等的任何量的度量。一方面，通过比较峰下面积，将奥贝胆酸的纯度与参比标准的纯度进行比较。另一方面，已知的纯度标准是奥贝胆

酸参比标准。一方面，奥贝胆酸具有大于约 96% 的纯度。一方面，奥贝胆酸具有大于约 98% 的纯度。例如，奥贝胆酸 1 型的纯度为 96.0%、96.1%、96.2%、96.3%、96.4%、96.5%、96.6%、96.7%、96.8%、96.9%、97.0%、97.1%、97.2%、97.3%、97.4%、97.5%、97.6%、97.7%、97.8%、97.9%、98.0%、98.1%、98.2%、98.3%、98.4%、98.5%、98.6%、98.7%、98.8%、98.9%、99.0%、99.1%、99.2%、99.3%、99.4%、99.5%、99.6%、99.7%、99.8% 或 99.9%。例如，奥贝胆酸 1 型的纯度 为 98.0%、98.1%、98.2%、98.3%、98.4%、98.5%、98.6%、98.7%、98.8%、98.9%、99.0%、99.1%、99.2%、99.3%、99.4%、99.5%、99.6%、99.7%、99.8% 或 99.9%。例如，奥贝胆酸的纯度为 98.0%、98.5%、99.0%、99.5%、99.6%、99.7%、99.8% 或 99.9%。例如，奥贝胆酸的纯度是 98.5%、99.0% 或 99.5%。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0132] 在一个实施方案中，本发明涉及纯度大于约 98% 的奥贝胆酸。在一个实施方案中，纯度通过 HPLC 测定。在另一个实施方案中，本发明涉及奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物。在一个实施方案中，纯度大于约 98.5%。在一个实施方案中，纯度大于约 99.0%。在一个实施方案中，纯度大于约 99.5%。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0133] 本文所用术语“效能”是基于已知标准（例如约 95%- 约 102% 的接收标准）的量的奥贝胆酸的量的度量。效能考虑所有可能的杂质包括水、溶剂、有机和无机杂质。一方面，已知标准是奥贝胆酸。一方面，奥贝胆酸具有大于约 96% 的效能。一方面，奥贝胆酸具有大于约 98% 的效能。一方面，已知标准是奥贝胆酸。另一方面，效能是 100% 减水、硫酸灰分、残余溶剂和其它杂质内容物的量，其它杂质内容物例如 6- 乙基乌索脱氧胆酸、3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸、6β- 乙基鹅脱氧胆酸、3α,7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸、鹅脱氧胆酸和 3α (3α,7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在另一个实施方案中，效能说明了因水、溶剂、金属、无机盐和其它无机或有机杂质所引起的杂质。例如，奥贝胆酸 1 型的效能 为 96.0%、96.1%、96.2%、96.3%、96.4%、96.5%、96.6%、96.7%、96.8%、96.9%、97.0%、97.1%、97.2%、97.3%、97.4%、97.5%、97.6%、97.7%、97.8%、97.9%、98.0%、98.1%、98.2%、98.3%、98.4%、98.5%、98.6%、98.7%、98.8%、98.9%、99.0%、99.1%、99.2%、99.3%、99.4%、99.5%、99.6%、99.7%、99.8% 或 99.9%。一方面，奥贝胆酸 1 型的效能为 98.0%、98.1%、98.2%、98.3%、98.4%、98.5%、98.6%、98.7%、98.8%、98.9%、99.0%、99.1%、99.2%、99.3%、99.4%、99.5%、99.6%、99.7%、99.8% 或 99.9%。例如，奥贝胆酸的效能为 98.0%、98.5%、99.0%、99.5%、99.6%、99.7%、99.8% 或 99.9%。例如，奥贝胆酸的效能为 98.5%、99.0% 或 99.5%。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0134] 在一个实施方案中，本发明涉及含有总共小于约 2% 的一种或多种选自以下杂质的奥贝胆酸 :6- 乙基乌索脱氧胆酸、3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸、6β- 乙基鹅脱氧胆酸、3α,7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸、鹅脱氧胆酸和 3α (3α,7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，杂质总共小于约 1.5%。在一个实施方案中，杂质总共小于约 1.4%。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0135] 在一个实施方案中，奥贝胆酸含有小于约 10% 的水、小于约 9% 的水、小于 8% 的水、小于 7% 的水、小于 6% 的水、小于 5% 的水、小于 4% 的水、小于 3% 的水、小于 2% 的水或小于

1% 的水。在一个实施方案中，奥贝胆酸含有小于约 1.2% 的水。在一个实施方案中，奥贝胆酸含有小于约 1.0% 的水。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0136] 在另一个实施方案中，奥贝胆酸含有不超过 (NMT) 0.15% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。在另一个实施方案中，奥贝胆酸含有总共小于约 0.07% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有总共小于约 0.06% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有总共小于约 0.05% 的 6- 乙基乌索脱氧胆酸和 3α，7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0137] 在一个实施方案中，奥贝胆酸含有不超过 (NMT) 0.15% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.07% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.06% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.05% 的 3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0138] 在一个实施方案中，奥贝胆酸含有不超过 (NMT) 0.15% 的 6β- 乙基鹅脱氧胆酸。在一个实施方案中，奥贝胆酸含有小于约 0.07% 的 6β- 乙基鹅脱氧胆酸。在一个实施方案中，奥贝胆酸含有小于约 0.06% 的 6β- 乙基鹅脱氧胆酸。在一个实施方案中，奥贝胆酸含有小于约 0.05% 的 6β- 乙基鹅脱氧胆酸。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0139] 在一个实施方案中，奥贝胆酸含有不超过 (NMT) 3% 的鹅脱氧胆酸 (CDCA)。在一个实施方案中，奥贝胆酸含有小于约 1% 的 CDCA。在一个实施方案中，奥贝胆酸含有小于约 0.5% 的 CDCA。在一个实施方案中，奥贝胆酸含有小于约 0.3% 的 CDCA。在一个实施方案中，奥贝胆酸含有小于约 0.2% 的 CDCA。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0140] 在一个实施方案中，奥贝胆酸含有不超过 (NMT) 4% 的 CDCA 和 6- 乙基乌索脱氧胆酸。

[0141] 在一个实施方案中，奥贝胆酸含有不超过 (NMT) 1.5% 的 3α (3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 1% 的 3α (3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.07% 的 3α (3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.06% 的 3α (3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸含有小于约 0.05% 的 3α (3α，7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。在一个实施方案中，奥贝胆酸是奥贝胆酸 1 型。

[0142] 口服制剂和给药

奥贝胆酸用于口服给药。在一个实施方案中，制剂经口服给药以预防或治疗 FXR 介导的疾病和病况。在一个实施方案中，制剂包含奥贝胆酸 1 型。在另一个实施方案中，制剂包含基本上纯的奥贝胆酸。

[0143] 适于口服给药的制剂可作为以下提供：独立单位，例如各含有预定量的奥贝胆酸的片剂、胶囊剂、扁囊剂（药剂师用于提供药物的糯米胶囊剂）、锭剂；散剂或颗粒剂；水性或非水性液体中的溶液剂或混悬剂；或水包油或油包水乳剂。

[0144] 本发明的制剂可通过任何合适的方法制备，通常通过将奥贝胆酸与液体或细微的固体载体或两者按所需要的比例均匀和精细地混合，然后必要时，使所得混合物做成所需形状。

[0145] 例如可通过压制包含奥贝胆酸的粉末或颗粒和一种或多种任选成分（例如粘合剂、润滑剂、惰性稀释剂或表面活性分散剂）的精细混合物，或通过模压活性成分粉末和惰性液体稀释剂的精细混合物，来制备片剂。

[0146] 例如，可根据受试者的体重，例如介于约 30 kg- 约 70 kg 之间的人，将一种或多种片剂给予以达到目标剂量水平。

[0147] 在一个实施方案中，受试者是儿童，制剂被用于治疗胆道闭锁。胆道闭锁，亦称为“肝外肝管缺失症 (extrahepatic ductopenia)”和“进行性闭塞性胆管病 (progressive obliterative cholangiopathy)”，是一种先天性或获得性肝病和所移植的肝同种异基因移植物慢性排斥的主要形式之一。在先天性形式中，肝和小肠间的胆总管闭塞或缺乏。获得性类型最常发生在自身免疫性疾病的情况下，是所移植的肝同种异基因移植物慢性排斥的主要形式之一。

[0148] 患胆道闭锁的婴儿和儿童患有具有所有以下常见伴随特征的进行性胆汁郁积：黄疸、瘙痒症、吸收不良伴生长迟缓、脂溶性维生素缺乏、高脂血症和最终肝硬化伴门静脉高压。如未诊断出，则该病况导致肝衰竭—但非核黄疸，因为肝仍能够结合胆红素，且结合的胆红素不能够跨越血脑屏障。病因未知。唯一有效的治疗无疑是手术，例如肝门肠吻合术或肝移植。

[0149] 在一个实施方案中，儿童进行肝门肠吻合术，其中当儿童出生时没有胆管或在出生时胆管完全闭塞时，肝门肠吻合术有效地为他们提供功能性胆管。

[0150] 除了上文明确提到的成分以外，考虑所述制剂类型，本发明的口服制剂可包括药学领域技术人员已知的其它作用剂。合适的口服制剂可包括矫味剂。

[0151] 在一个实施方案中，本发明涉及奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物的药物制剂，其中奥贝胆酸通过本发明的方法产生（奥贝胆酸 1 型）。在另一个实施方案中，制剂经口服给予。

[0152] 在一个实施方案中，制剂呈片剂形式。在另一个实施方案中，制剂包含奥贝胆酸和选自以下的一种或多种组分：微晶纤维素、羟基乙酸淀粉钠、硬脂酸镁、包衣材料或胶态二氧化硅。在一个实施方案中，包衣材料是 Opadry® 包衣材料。

[0153] 在另一个实施方案中，制剂包含约 0.1 mg- 约 1500 mg 奥贝胆酸 / 片。在另一个实施方案中，制剂包含约 1 mg- 约 100 mg。在另一个实施方案中，制剂包含约 1 mg- 约 50 mg。在另一个实施方案中，制剂包含约 1 mg- 约 30 mg。在另一个实施方案中，制剂包含约 4 mg- 约 26 mg。在另一个实施方案中，制剂包含约 5 mg- 约 25 mg。在一个实施方案中，制剂包含约 1 mg- 约 2 mg。在一个实施方案中，制剂包含约 1.2 mg- 约 1.8 mg。在一个实施方案中，制剂包含约 1.3 mg- 约 1.7 mg。在一个实施方案中，制剂包含约 1.5 mg。

[0154] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案

中，制剂包含约 1 mg 奥贝胆酸、约 180- 约 190 mg 微晶纤维素、约 10- 约 15 mg 羟基乙酸淀粉钠、约 1- 约 3 mg 硬脂酸镁和约 5 mg- 约 10 mg 包衣材料。在一个实施方案中，包衣材料是 Opadry® 包衣材料。

[0155] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 1 mg 奥贝胆酸、约 185.0 mg 微晶纤维素、约 12.0 mg 羟基乙酸淀粉钠、约 2.0 mg 硬脂酸镁和约 8.0 mg 包衣材料。在一个实施方案中，包衣材料是 Opadry® 包衣材料。

[0156] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 5 mg 奥贝胆酸、约 175- 约 190 mg 微晶纤维素、约 10- 约 15 mg 羟基乙酸淀粉钠、约 1- 约 3 mg 硬脂酸镁和约 5 mg- 约 10 mg 包衣材料。在一个实施方案中，包衣材料是 Opadry® 包衣材料。

[0157] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 5 mg 奥贝胆酸、约 181.0 mg 微晶纤维素、约 12.0 mg 羟基乙酸淀粉钠、约 2.0 mg 硬脂酸镁和约 8.0 mg 包衣材料。在一个实施方案中，包衣材料是 Opadry® 包衣材料。

[0158] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 10 mg 奥贝胆酸、约 170 mg- 约 180 mg 微晶纤维素、约 10 mg- 约 15 mg 羟基乙酸淀粉钠、约 1 mg- 约 3 mg 硬脂酸镁和约 5 mg- 约 10 mg 包衣材料。在一个实施方案中，包衣材料为 Opadry® 包衣材料。

[0159] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 10 mg 奥贝胆酸、约 176.0 mg 微晶纤维素、约 12.0 mg 羟基乙酸淀粉钠、约 2.0 mg 硬脂酸镁和约 8.0 mg 包衣材料。在一个实施方案中，包衣材料为 Opadry® 包衣材料。

[0160] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 25 mg 奥贝胆酸、约 150 mg- 约 160 mg 微晶纤维素、约 10 mg- 约 15 mg 羟基乙酸淀粉钠、约 1 mg- 约 3 mg 硬脂酸镁、约 5- 约 10 mg 包衣材料和约 1- 约 10 mg 胶态二氧化硅。在一个实施方案中，包衣材料为 Opadry® 包衣材料。

[0161] 在一个实施方案中，制剂包含约 1 mg- 约 25 mg 奥贝胆酸 / 片。在一个实施方案中，制剂包含约 25 mg 奥贝胆酸、约 157.0 mg 微晶纤维素、约 12.0 mg 羟基乙酸淀粉钠、约 2.0 mg 硬脂酸镁、约 8.0 mg 包衣材料和约 4.0 mg 胶态二氧化硅。在一个实施方案中，包衣材料为 Opadry® 包衣材料。

[0162] 除非另有说明，否则本文所用所有百分比和比率都以重量计。百分比二聚体杂质以面积百分比基础计，通常通过分析型 HPLC 定量。

[0163] 在整个说明书中，在组合物描述为具有、包括或包含具体组分时，预期组合物也基本上由或由所述组分组成。同样地，在方法或过程描述为具有、包括或包含具体的方法步骤时，所述方法也基本上由或由所述方法步骤组成。另外，应了解，步骤的顺序或执行某些操作的顺序并不重要，只要本发明保持可操作性即可。然而，可同时进行两个或更多个步骤或操作。

[0164] 片剂的配制

薄膜衣片剂			
组分	每片的量	功能	参考标准
1 mg 片剂			
奥贝胆酸	1.0 mg*	API	HSE
微晶纤维素	185.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色、白色或黄色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
5 mg 片剂			
奥贝胆酸	5.0 mg*	API	HSE
微晶纤维素	181.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色、白色或黄色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
10 mg 片剂			
奥贝胆酸	10.0 mg*	API	HSE
微晶纤维素	176.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色、白色或黄色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
25 mg 片剂			
奥贝胆酸	25.0 mg*	API	HSE
微晶纤维素	157.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
胶态二氧化硅	4.0 mg	助流剂	USP-NF/EP/JP
Opadry® II 绿色、白色或黄色	8.0 mg	包衣材料	HSE
总重	208.0 mg		

API: 活性药物成分
HSE = 内部规格
USP-NF = 美国药典国家处方集
Ph Eur = 欧洲药典
JP = 日本药典
* 所提供的奥贝胆酸量假设 API 是无水的且 100%纯的；实际量根据所用的药物批次的效能调节，并相应减少微晶纤维素的量。

在一个实施方案中，片剂包含黄色 Opadry®。在另一个实施方案中，片剂包含白色 Opadry®。在另一个实施方案中，片剂包含绿色 Opadry®。

[0165] 药物组合物

奥贝胆酸，包括奥贝胆酸 1 型、奥贝胆酸的基本纯形式和奥贝胆酸的晶体形式或其药学上可接受的盐、溶剂合物或氨基酸缀合物可用于多种医药目的。奥贝胆酸可用于预防或治疗 FXR 介导的疾病和病况的方法。在一个实施方案中，疾病或病况选自胆道闭锁、胆汁郁积性肝病、慢性肝病、非酒精性脂肪性肝炎 (NASH)、丙型肝炎感染、酒精性肝病、原发性胆汁性肝硬化 (PBC)、进行性纤维变性所致肝损伤、肝纤维变性和心血管疾病包括动脉粥样硬化、动脉硬化、高胆固醇血症和高脂血症。在一个实施方案中，奥贝胆酸 1 型可用于降低甘油三酯的方法。在一个实施方案中，结晶奥贝胆酸可用于降低甘油三酯的方法。奥贝胆酸 1 型或结晶奥贝胆酸可提高 HDL。奥贝胆酸 1 型或结晶奥贝胆酸的其它作用包括降低碱性磷酸酶 (ALP)、胆红素、ALT、AST 和 GGT。

[0166] 在一个实施方案中，本发明涉及包含奥贝胆酸和药学上可接受的载体的药物组合物，其中奥贝胆酸通过本发明的方法产生，例如，奥贝胆酸 1 型。在一个实施方案中，药物组合物包含基本纯的奥贝胆酸和药学上可接受的载体。在另一个实施方案中，药物组合物包含结晶奥贝胆酸和药学上可接受的载体。在另一个实施方案中，结晶奥贝胆酸是 C 型。

[0167] 在一个实施方案中，本发明涉及治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的通过本发明的方法产生的奥贝胆酸 1 型或其药物组合物。在一个实施方案中，本发明涉及治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的通过本发明方法产生的基本纯的奥贝胆酸或其药物组合物。在一个实施方案中，本发明涉及治疗或预防受试者的 FXR 介导的疾病或病况的方法，所述方法包括给予有效量的结晶奥贝胆酸或其药物组合物。在另一个实施方案中，结晶奥贝胆酸是 C 型。在一个实施方案中，结晶奥贝胆酸是 A 型。在一个实施方案中，结晶奥贝胆酸是 C 型。在一个实施方案中，结晶奥贝胆酸是 D 型。在一个实施方案中，结晶奥贝胆酸是 F 型。在一个实施方案中，结晶奥贝胆酸是 G 型。

[0168] 在另一个实施方案中，疾病或病况是心血管疾病或胆汁郁积性肝病和用于降低甘油三酯。在另一个实施方案中，心血管疾病是动脉粥样硬化或高胆固醇血症。在另一个实施方案中，受试者是哺乳动物。在另一个实施方案中，哺乳动物是人。

[0169] 在另一个实施方案中，口服、胃肠外或局部给予化合物或药物组合物。在另一个实施方案中，口服给予化合物或药物组合物。

[0170] 在一个实施方案中，本发明涉及抑制患有胆汁郁积病况的受试者的纤维变性的方法，所述方法包括给予受试者有效量的奥贝胆酸或其药物组合物的步骤，其中奥贝胆酸通过本发明的方法产生。在一个实施方案中，本发明涉及抑制未患胆汁郁积病况的受试者的纤维变性的方法，所述方法包括给予受试者有效量的奥贝胆酸或其药物组合物的步骤，其中奥贝胆酸通过本发明的方法产生。在实施方案中，待受抑制的纤维变性存在于其中表达 FXR 的器官中。

[0171] 在一个实施方案中，胆汁郁积病况定义为具有异常升高的碱性磷酸酶、7- 谷氨酰转肽酶 (GGT) 和 5’ 核苷酸酶的血清水平。在另一个实施方案中，胆汁郁积病况进一步定义为呈现至少一个临床症状。在另一个实施方案中，症状为搔痒（瘙痒症）。在另一个实施方案中，纤维变性选自肝纤维变性、肾纤维变性和肠纤维变性。在另一个实施方案中，胆汁郁积病况选自原发性胆汁性肝硬化、原发性硬化性胆管炎、药物诱发性胆汁郁积、遗传性胆汁郁积和妊娠肝内胆汁淤积症。在另一个实施方案中，受试者未患与选自以下疾病或病况有

关的胆汁郁积病况：原发性肝癌和胆道癌、转移性癌、脓毒症、长期胃肠道外全面营养、囊性纤维化和肉芽肿肝病。

[0172] 在另一个实施方案中，受试者患有与选自以下疾病有关的肝纤维变性：乙型肝炎；丙型肝炎；寄生虫性肝病；移植后细菌、病毒和真菌感染；酒精性肝病（ALD）；非酒精性脂肪肝病（NAFLD）；非酒精性脂肪性肝炎（NASH）；由甲氨蝶呤、异烟肼、酚丁、甲基多巴、氯丙嗪、甲苯磺丁脲或胺碘达隆诱发的肝病；自身免疫性肝炎；结节病；肝豆状核变性（Wilson's disease）；血色素沉积症；戈谢病（Gaucher's disease）；III、IV、VI、IX 和 X 型糖原贮积病；α_1- 抗胰蛋白酶缺乏；泽尔韦格综合征（Zellweger syndrome）；酪氨酸血症；果糖血症；半乳糖血症；与巴德 - 基亚里综合征（Budd-Chiari syndrome）、静脉闭塞性病或门静脉血栓形成有关的血管紊乱；和先天性肝纤维变性。

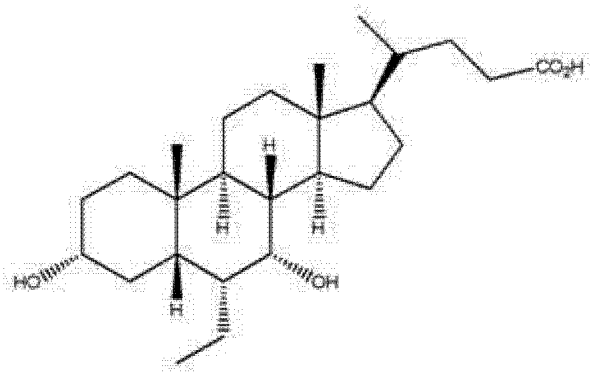
[0173] 在另一个实施方案中，受试者患有与选自以下疾病有关的肠纤维变性：克罗恩病（Crohn's disease）、溃疡性结肠炎、放射后结肠炎和显微镜下结肠炎。

[0174] 在另一个实施方案中，受试者患有与选自以下疾病有关的肾纤维变性：糖尿病性肾病、高血压肾硬化、慢性肾小球性肾炎、慢性移植物肾小球病、慢性间质性肾炎和多囊肾病。

[0175] 定义

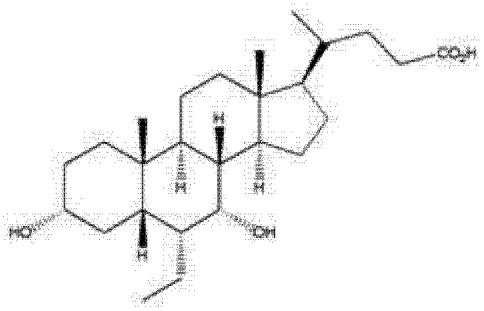
为方便起见，在此汇集了用于本说明书、实施例和随附权利要求书中的某些术语。

[0176] 本文所用术语“奥贝胆酸”或“OCA”是指具有以下化学结构的化合物：



。奥贝胆酸的其它化学名称包括：3α，7α - 二羟基 -6α - 乙基 -5β - 胆烷 -24- 酸、6α - 乙基 - 鹅脱氧胆酸、6- 乙基 -CDCA、6ECDCA、胆烷 -24- 酸，6- 乙基 -3，7- 二羟基 -，(3α，5β，6α，7α)- 和 INT-747。奥贝胆酸的 CAS 登记号是 459789-99-2。该术语是指奥贝胆酸的所有形式，例如非晶体、晶体和基本纯的。

[0177] 本文所用术语“结晶奥贝胆酸”是指具有以下化学结构的化合物的任何晶体形式：



。结晶奥贝胆酸意指化合物在三维空间中结晶成为

特定的晶体堆积排列或具有外面平面的化合物。奥贝胆酸（或其药学上可接受的盐、氨基酸缀合物、溶剂合物）的晶体形式可结晶成不同的晶体堆积排列，其全部都具有相同的奥贝胆酸的元素组成。不同的晶体形式通常具有不同的 X 射线衍射图、红外线谱、熔点、密度硬度、晶形、光学性质和电性质、稳定性和溶解度。重结晶溶剂、结晶速率、保存温度和其它因素可使一种晶体形式占优势。奥贝胆酸的晶体可通过在不同条件下（例如不同溶剂、温度等）结晶来制备。

[0178] 本文所用术语“结晶奥贝胆酸 C 型”是指具有基本类似于图 5 所示 X 射线衍射图的 X 射线衍射图的奥贝胆酸的晶体形式，例如实施例 3 中表征的晶体形式。

[0179] 本文所用术语“基本上纯的奥贝胆酸”是指具有大于约 95% 的效能的奥贝胆酸。奥贝胆酸的效能考虑奥贝胆酸样品中的杂质，包括例如水、溶剂和其它有机和无机杂质。在另一个实施方案中，效能的已知标准为 100% 奥贝胆酸，通过从 100% 的已知标准减去杂质（例如溶剂、水和其它有机和无机杂质）的百分比，求出效能。一方面，无机杂质包括例如无机盐和硫酸灰分。一方面，有机杂质包括 6- 乙基乌索脱氧胆酸、3α- 羟基 -6α- 乙基 -7-cheto-5β- 胆烷 -24- 酸、6β- 乙基鹅脱氧胆酸、3α,7α- 二羟基 -6- 亚乙基 -5β- 胆烷 -24- 酸、鹅脱氧胆酸和 3α (3α,7α- 二羟基 -6α- 乙基 -5β- 胆烷 -24- 酰基氧基)-7α- 羟基 -6α- 乙基 -5β- 胆烷 -24- 酸。杂质的量可通过本领域已知方法测定，例如 HPLC、NMR 或出自美国药典或欧洲药典的方法或这些方法中两种或更多种的组合。

[0180] 本文所用术语“纯度”是指获自例如 HPLC 的化合物的化学分析。在一个实施方案中，通过用于比较的各个峰的峰下面积，对化合物的纯度与参比标准（例如奥贝胆酸）的纯度进行比较。在一个实施方案中，纯度说明样品中的有机杂质。

[0181] 本文所用术语“反应混合物”是指一种或多种物质混合在一起的混合物。在一个实施方案中，物质的混合或组合引起一种或多种原始物质的化学转化或改变。

[0182] 本文所用术语“奥贝胆酸 1 型”是指非晶奥贝胆酸。在一个实施方案中，奥贝胆酸的这种形式通过结晶奥贝胆酸作为合成中间体来产生。例如，奥贝胆酸的这种形式通过结晶奥贝胆酸 C 型作为合成中间体，通过本申请的方法产生。在一个实施方案中，奥贝胆酸 1 型是用作药学活性成分的形式。有关更多详情参见实施例 5。

[0183] “治疗”包括导致病况、疾病、病症等改善的任何作用，例如减轻、降低、调节或消除。疾病状态的“治疗”或“医治”包括 :抑制疾病状态，即阻止疾病状态或其临床症状的发展 ;或缓解疾病状态，即引起疾病状态或其临床症状的暂时或永久消退。

[0184] “预防”疾病状态包括在可能暴露于疾病状态或有疾病状态倾向，但尚未遭受或显示疾病状态的症状的受试者中使疾病状态的临床症状不发生。

[0185] “疾病状态”意指任何疾病、病症、病况、症状或适应症。

[0186] 本文所用术语“有效量”是指在给予适当剂量后产生急性或慢性治疗作用的奥贝胆酸（例如 FXR 激活性配体）的量。作用包括预防、纠正、抑制或逆转疾病 / 病况（例如肝、肾或肠的纤维变性）的症状、病征和基础病理和相关并发症至任何可检测的程度。

[0187] “治疗有效量”意指当给予哺乳动物用于治疗疾病时，足以对疾病实现所述治疗的奥贝胆酸的量。“治疗有效量”将随奥贝胆酸、疾病及其严重程度和待治疗的哺乳动物的年龄、体重等而变化。

[0188] 奥贝胆酸的治疗有效量可与用于给予人或动物的药学上可接受的载体一起配制。

因此，可通过例如口服、胃肠外或局部途径给予奥贝胆酸或其制剂，以提供有效量的化合物。在备选实施方案中，按照本发明制备的奥贝胆酸可用来包覆或浸渍医疗装置，例如支架。

[0189] 本文所用“药理作用”包括在达到预期治疗目的的受试者中产生的作用。在一个实施方案中，药理作用意指接受治疗的受试者的主要适应症受到防止、缓解或减轻。例如，药理作用是在受治疗的受试者中导致主要适应症受到防止、缓解或减轻的药理作用。在另一个实施方案中，药理作用意指接受治疗的受试者的主要适应症的病症或症状受到防止、缓解或减轻。例如，药理作用是在受治疗的受试者中导致主要适应症受到防止或减轻的药理作用。

[0190] 本发明还包括同位素标记的奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物，其与本发明化学式和下文所述的那些相同，只是一个或多个原子被原子质量或质量数不同于天然最常见的原子质量或质量数的原子置换。可掺入奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物的同位素的实例包括氢、碳、氮、氟的同位素，例如 ^{3}H、^{11}C、^{14}C 和 ^{18}F。

[0191] 含有上述同位素和 / 或其它原子的其它同位素的奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物在本发明的范围内。同位素标记的奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物，例如放射性同位素例如 ^{3}H、^{14}C 掺入其中的那些，可用于药物和 / 或底物组织分布测定法。因其容易制备和可检测所致，氚化（即 ^{3}H）和碳 -14（即 ^{14}C）同位素是特别优选的。另外，被较重同位素（例如氘即 ^{2}H）取代，可提供产生自较大代谢稳定性的某些治疗优势，例如体内半寿期延长或剂量需要量降低，因此，在某些情况下可能是优先的，一般可用容易获得的同位素标记的试剂取代非同位素标记的试剂，通过进行本发明的流程和 / 或实施例中公开的程序，来制备同位素标记的奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物。在一个实施方案中，奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物不是同位素标记的。在一个实施方案中，氚化奥贝胆酸可用于生物分析法。在另一个实施方案中，奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物是放射性标记的。

[0192] “几何异构体”意指其存在归因于双键周围的位阻旋转的非对映异构体。这些构型在其名称中通过前缀顺式和反式或 Z 和 E 区分，它表明了按照 Cahn-Ingold-Prelog 原则，基团位于分子双键的同侧或相对侧。

[0193] “溶剂合物”意指含有化学计算量或非化学计算量的溶剂的溶剂加成形式。奥贝胆酸可具有在结晶固体状态中截留固定摩尔比率的溶剂分子的趋势，因此形成溶剂合物。如果溶剂为水，则所形成的溶剂合物是水合物，在溶剂是醇时，所形成的溶剂合物是醇化物。水合物通过水的一个或多个分子与水在其中保持其分子状态为 H_2O 的物质之一结合而形成，所述结合能够形成一种或多种水合物。另外，本发明的化合物，例如化合物的盐，可以水合或未水合的（无水）形式或作为与其它溶剂分子的溶剂合物存在。水合物的非限制性实例包括一水合物、二水合物等。溶剂合物的非限制性实例包括乙醇溶剂合物、丙酮溶剂合物等。

[0194] “互变异构体”是指其结构在原子排列中显著不同，但以容易和快速平衡存在的化合物。要理解，奥贝胆酸可描述为不同的互变异构体。还应理解，当本发明的奥贝胆酸和合

成中间体具有互变异构形式时，所有互变异构形式预期落入本发明的范围内，奥贝胆酸的命名不排除任何互变异构体形式。本发明的奥贝胆酸和合成中间体可以几种互变异构形式存在，包括酮－烯醇。例如，在酮－烯醇互变异构中，发生电子和氢原子的同时位移。互变异构体作为在溶液中互变异构体组的混合物存在。在固体形式中，通常一种互变异构体占优势。虽然可能描述了一种互变异构体，但本发明包括本发明化合物的全部互变异构体。

[0195] 因此要理解的是，产生于不对称碳原子的异构体（例如所有对映异构体和非对映异构体）包括在本发明的范围内，除非另有说明。可通过经典分离技术并通过立体化学上控制的合成，以基本上纯的形式获得所述异构体。此外，本申请书中论述的结构和其它化合物和部分还包括其全部互变异构体。适当时，烯烃可包括 E- 或 Z- 几何结构。奥贝胆酸和合成中间体可以立体异构体形式存在，因此可作为各个立体异构体或作为混合物产生。

[0196] “药物组合物”是含有奥贝胆酸的呈适于给予受试者的形式的制剂。在一个实施方案中，药物组合物是散装形式或单位剂型。以剂量单位形式配制组合物以易于给药和剂量均匀性，将是有利的。本文所用剂量单位形式是对于待治疗的受试者适于作为单位剂量的物理独立单位；每个单位含有经计算以产生所需治疗作用的预定量的活性试剂以及所需的药用载体。本发明的剂量单位形式的规格受制于或直接取决于活性试剂的特殊性质和待实现的特定治疗作用，以及调节用于治疗个体的所述活性剂领域的内在限制。

[0197] 单位剂型是多种形式的任一种，包括例如胶囊剂、IV 袋、片剂、气雾剂吸入器上的单流向泵或小瓶。组合物的单位剂量中的奥贝胆酸（例如奥贝胆酸或其药学上可接受的盐、溶剂合物或氨基酸缀合物的制剂）的量是有效量，并随所涉及的具体治疗而变化。本领域的技术人员应认识到，有时需要根据患者的年龄和病况对剂量作例行变化。剂量还将取决于给药途径。考虑多种途径，包括口服、经肺、直肠、胃肠外、经皮、皮下、静脉内、肌内、腹膜内、吸入、含服、舌下、胸膜内、鞘内、鼻内等。用于本发明化合物的局部或经皮给药的剂型包括散剂、喷雾剂、软膏剂、糊剂、乳膏剂、洗剂、凝胶、溶液剂、贴剂和吸入剂。在一个实施方案中，奥贝胆酸在无菌情况下与药学上可接受的载体混合，并与需要的任何防腐剂、缓冲剂或抛射剂混合。

[0198] 术语“闪释剂量 (flash dose)”是指是快速分散性剂型的奥贝胆酸制剂。

[0199] 术语“即释”定义为奥贝胆酸以相对短的时间（一般至多约 60 分钟）从剂型中释放。术语“改良释放”的定义包括延缓释放、延长释放和脉冲释放。术语“脉冲释放”定义为药物从剂型中的一系列释放。术语“延时释放”或“延长释放”定义为奥贝胆酸在一段长的时间内从剂型中持续释放。

[0200] “受试者”包括哺乳动物，例如人、伴侣动物（例如狗、猫、鸟等）、农场动物（例如牛、绵羊、猪、马、禽等）和实验动物（例如大鼠、小鼠、豚鼠、鸟等）。在一个实施方案中，受试者是人。在一个实施方案中，受试者是儿童（例如约 30 kg- 约 70 kg）。在一个实施方案中，儿童进行肝门肠吻合术，其中当儿童出生时没有胆管或在出生时胆管完全闭塞时，肝门肠吻合术有效地为他们提供功能性胆管。

[0201] 本文所用短语“药学上可接受的”是指在合理医学判断的范围内，适用于与人类和动物的组织接触而无过度毒性、刺激性、变态反应或其它问题或并发症、与合理的受益风险比相当的那些化合物、材料、组合物、载体和 / 或剂型。

[0202] “药学上可接受的赋形剂”意指可用于制备药物组合物的基本上安全、无毒且生物

学或其它方面都无不良的赋形剂，包括对于兽药用以及人药物使用是可接受的赋形剂。用于本说明书和权利要求书的“药学上可接受的赋形剂”包括一种和一种以上所述赋形剂。

[0203] 虽然可在没有任何配制的情况下直接给予奥贝胆酸，但奥贝胆酸通常以包含药学上可接受的赋形剂和奥贝胆酸的药物制剂的形式给予。这些制剂可通过多种途径给予，包括口服、含服、直肠、鼻内、经皮、皮下、静脉内、肌内和鼻内。本文在题为“口服制剂和给药”的部分中进一步描述了奥贝胆酸的口服制剂。

[0204] 在一个实施方案中，可经皮给予奥贝胆酸。为了经皮给予，需要经皮递送装置(“贴剂”)。所述经皮贴剂可用来提供本发明化合物的受控量的连续或不连续的输注。用于递送药剂的经皮贴剂的制备和使用是本领域众所周知的。参见例如美国专利号 5,023,252。可制备所述贴剂用于药剂的连续、脉动或按需递送。

[0205] 在本发明的一个实施方案中，提供制剂中包含适于含服和 / 或舌下或经鼻给药的至少上述奥贝胆酸的药物制剂。该实施方案提供以避免胃并发症的方式给予奥贝胆酸，例如通过胃系统和 / 或经过肝的首过代谢。该给药途径还可减少吸收时间，提供更快速起效的治疗益处。本发明的化合物可提供特别有利的溶解特征以利于舌下 / 含服制剂。所述制剂通常需要相对高浓度的活性成分以将足量的活性成分递送到舌下 / 口腔黏膜的有限表面积上达制剂与所述表面积接触的相对短的持续时间，以供吸收活性成分。因此，非常高的奥贝胆酸活性，结合其高溶解度，有利于其舌下 / 含服制剂的适合性。

[0206] 奥贝胆酸优选以单位剂型配制，各个剂量含有约 0.1 mg- 约 1500 mg。在另一个实施方案中，制剂包含约 1 mg- 约 100 mg。在另一个实施方案中，制剂包含约 1 mg- 约 50 mg。在另一个实施方案中，制剂包含约 1 mg- 约 30 mg。在另一个实施方案中，制剂包含约 4 mg- 约 26 mg。在另一个实施方案中，制剂包含约 5 mg- 约 25 mg。在一个实施方案中，制剂包含约 1 mg- 约 2 mg。在一个实施方案中，制剂包含约 1.2 mg- 约 1.8 mg。在一个实施方案中，制剂包含约 1.3 mg- 约 1.7 mg。在一个实施方案中，制剂包含约 1.5 mg。术语“单位剂型”是指适于作为单位剂量用于人类受试者和其它哺乳动物的物理独立单位，每个单位含有经计算产生所需治疗作用的预定量的活性物质以及合适的上述药用赋形剂。

[0207] 奥贝胆酸通常在广泛的剂量范围内有效。例如，每日剂量通常落入约 0.0001- 约 30 mg/kg 体重的范围内。在治疗成人时，呈单剂量或分剂量的约 0.1- 约 15 mg/kg/ 天的范围是特别优选的。在实施方案中，制剂包含约 0.1 mg- 约 1500 mg。在另一个实施方案中，制剂包含约 1 mg- 约 100 mg。在另一个实施方案中，制剂包含约 1 mg- 约 50 mg。在另一个实施方案中，制剂包含约 1 mg- 约 30 mg。在另一个实施方案中，制剂包含约 4 mg- 约 26 mg。在另一个实施方案中，制剂包含约 5 mg- 约 25 mg。在一个实施方案中，制剂包含约 1 mg- 约 2 mg。在一个实施方案中，制剂包含约 1.2 mg- 约 1.8 mg。在一个实施方案中，制剂包含约 1.3 mg- 约 1.7 mg。在一个实施方案中，制剂包含约 1.5 mg。然而，应理解，实际给予的奥贝胆酸的量可通过医生根据相关情况，包括待治疗的病况、所选的给药途径、所给予的奥贝胆酸的形式、各个患者的年龄、体重和反应及患者症状的严重程度来决定，因此上述剂量范围无意以任何方式限制本发明的范围。在某些情况下，低于上述范围下限的剂量水平可能就足够，而在其它情况下，可应用还更大的剂量而不引起任何有害副作用，前提是首先将所述较大的剂量分成几个较小的剂量以在一整天内给予。

[0208] “本发明的方法”是指如本文所述制备奥贝胆酸的方法，其中所述方法包括结晶奥

贝胆酸。

[0209] “纤维变性”是指涉及组织或器官中发生的过量纤维结缔组织（例如疤痕组织）的病况。这种疤痕组织的产生可在响应因疾病、创伤、化学毒性等造成的感染、炎症或器官损伤而发生。纤维变性可发生在多个不同的组织和器官中，包括肝、肾、肠、肺、心脏等。

[0210] 本文所用术语“抑制”或“阻止”是指对疾病或病况的发生或发展的任何可检测的积极作用。所述积极作用可包括延缓或防止疾病或病况的至少一个症状或病征的发作，缓解或逆转症状或病征，且减慢或防止症状或病征进一步恶化。

[0211] 本文所用“胆汁郁积病况”是指其中发生在肝或胆管中的胆汁从肝中的分泌受损或受阻的任何疾病或病况。肝内胆汁郁积和肝外胆汁郁积是胆汁郁积病况的两种类型。肝内胆汁郁积（发生在肝内）最常见于原发性胆汁性肝硬化、原发性硬化性胆管炎、脓毒症（全身性感染）、急性酒精性肝炎、药物毒性、胃肠道外全面营养（静脉内提供营养）、恶性肿瘤、囊性纤维化和妊娠。肝外胆汁郁积（发生在肝外）可由胆管肿瘤、狭窄、囊肿、憩室、胆总管中结石形成、胰腺炎、胰腺肿瘤或假囊肿和由附近器官的疱块或肿瘤所致压迫引起。

[0212] 胆汁郁积病况的临床症状和病征包括：搔痒（瘙痒症）、疲劳、黄疸性皮肤或眼、无法消化某些食物、恶心、呕吐、灰白色大便、赤尿和右上象限腹痛。在临床上可根据一套标准临床实验室试验诊断和跟踪患有胆汁郁积病况的患者，包括测量患者血清中的碱性磷酸酶、γ－谷氨酰转肽酶 (GGT)、5’核苷酸酶、胆红素、胆汁酸和胆固醇水平。总的来说，如果诊断标志物碱性磷酸酶、GGT 和 5’核苷酸酶所有 3 种的血清水平被视为异常升高，则患者被诊断为患有胆汁郁积病况。这些标志物的正常血清水平可从实验室到实验室和从方法到方法改变至某种程度，这取决于试验方案。因此，医生可根据具体实验室和试验方法，确定什么是各种标志物异常升高的血液水平。例如，患有胆汁郁积病况的患者血液中一般有大于约 125 IU/L 碱性磷酸酶、大于约 65 IU/L GGT 和大于约 17 NIL 5’核苷酸酶。由于血清标志物水平的变化性，除至少一种上述症状（例如搔痒（瘙痒症））以外，可根据这 3 种标志物的水平异常来诊断胆汁郁积病况。

[0213] 术语“器官”是指由细胞和组织组成并在生物体中进行某些特定功能的分化结构（如在心脏、肺、肾、肝等中）。该术语还包括执行功能或在活动中协调合作的身体部件（例如构成视器的眼和相关结构）。术语“器官”还包括可能发育成为完整结构的分化细胞和组织的任何部分结构（例如肝叶或肝瓣）。

[0214] 本文引用的所有公开文本和专利文件通过引用结合到本文中，就像所述公开文本或文件具体而单独指明通过引用结合到本文中一样。公开文本和专利文件的引用无意承认任一个是有关的现有技术，也不构成对所述文件的内容或日期的任何承认。虽然现已通过书面描述对本发明进行了描述，但本领域技术人员应认识到，本发明可在各种实施方案中予以实践，上面的描述和下面的实施例用于说明目的而非对随附权利要求书进行限制。

[0215] 在本说明书中，单数形式还包括复数，除非文中另有明确说明。除非另有定义，否则本文所用的所有技术和科学术语具有本发明所属技术领域的普通技术人员通常所理解的相同含义。在有冲突的情况下，以本说明书为准。

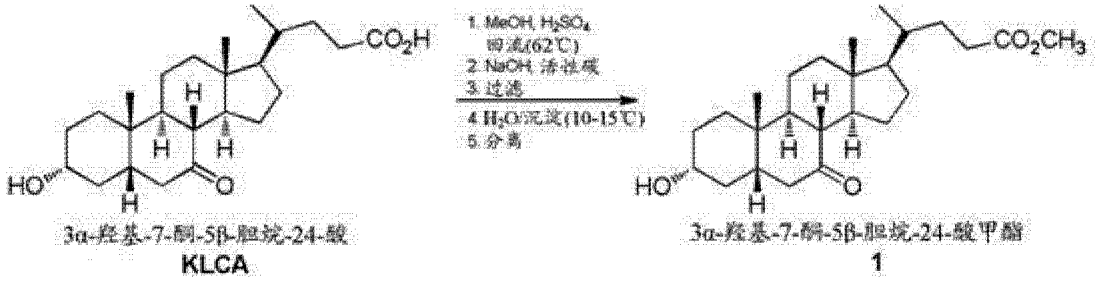
[0216] 除非另有说明，否则本文所用的所有百分比和比率以重量计。

实施例

[0217] 实施例 1:奥贝胆酸的合成

本合成程序中所提及的化合物编号是指流程 1 中和对应于各步骤的反应中的化合物编号。

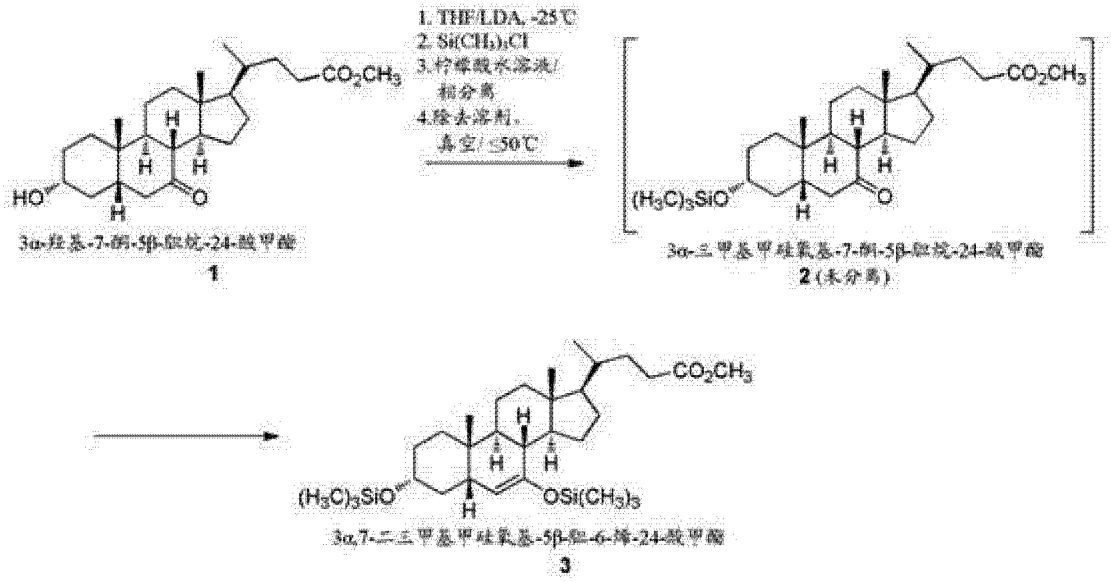
[0218] 步骤 1—3α-羟基 -7-酮 -5β-胆烷 -24-酸甲酯 (1)的制备:



反应 1:7- 酮石胆酸 (KLCA) 的 C-24 羧酸的酯化

在酸性催化剂(硫酸,1.0 mL)存在下,使用甲醇(2500 mL)使 3α-羟基-7-酮-5β-胆烷-24- 酸 (KLCA;500.0 g,1.28 mol)酯化,并加热直到 62℃ -64℃约 3 小时,得到 3α-羟基 -7- 酮 -5β- 胆烷 -24- 酸甲酯 (1)。在该反应中,甲醇起甲基化试剂以及反应溶剂的作用。对于后处理,用氢氧化钠溶液 (2N) 调节 pH 值至 pH 7.0-7.5。溶液用活性碳 (25 g) 处理约 30 分钟,过滤除去碳固体物。或者,溶液不用活性碳处理。为了沉淀产物,在 15 分钟内加入 10℃ -15℃的水 (625 mL),并加入晶种材料。在 10℃ -15℃下搅拌反应混合物 1 小时。在约 20-25 分钟内加入另一部分水 (1875 mL)。在 10℃ -15℃下搅拌产物悬浮液 30 分钟。产物用离心机分离,用甲醇和水的混合物 (1:1,350 mL) 洗涤。通过 Karl Fischer (KF) 定量测定湿料的含水量。将材料在转筒式干燥机中在 NMT 70℃下真空干燥。材料也可用于下一步骤而无需干燥。收率(以干燥产物计算)为 501.4 g (1.24 mol,96.8%)。

[0219] 步骤 2 - 3α,7α-二三甲基甲硅氧基 -5β-胆 -6-烯 -24-酸甲酯 (3)的制备:

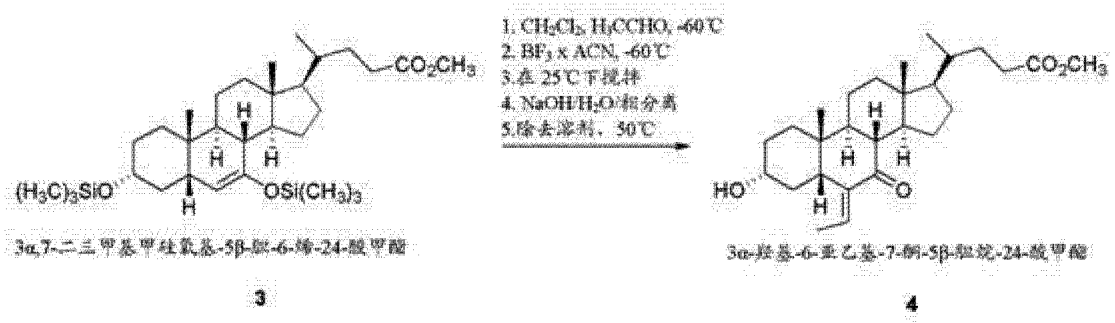


反应 2:从 7- 酮石胆酸甲酯形成硅烯醇醚

将含有残余的水和甲醇的化合物 1 (60.69 g,150 mmol,以干物质计算)装入惰性条件下的反应器中,并溶于四氢呋喃 (THF,363 mL) 中。通过在约 65℃和常压下重复共沸蒸馏除去水和甲醇。需要时将 THF 加入残余物中,重复蒸馏约 4 次。剩余的溶液必须具有≤ 0.05%

的最终含水量(Karl Fischer滴定)。将该溶液预冷至-20℃至-25℃,然后在约30-45分钟中加入三甲基氯硅烷(73.33 g,675 mmol,4.5当量)。在氮气氛下,将二异丙基氨基锂(28% LDA溶液,900 mmol)和THF (504 mL)装入单独的惰性反应器中,并冷却至-20℃至-25℃。将化合物1的无水冷却溶液、THF (84 mL)和三甲基氯硅烷装入-20℃至-25℃下的LDA溶液中。然后,搅拌反应混合物约2小时。对于后处理,将反应混合物加入2℃-8℃的预冷的柠檬酸水溶液(34.6 g,在300 mL中)中。在加入后,分离水相并弃去。在最大50℃下通过真空蒸馏从有机相中除去液体。分离的残余物含有化合物3和一些残余溶剂,并"照原样"用于下一步骤。

[0220] 步骤3 - 3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸甲酯(4)的制备:

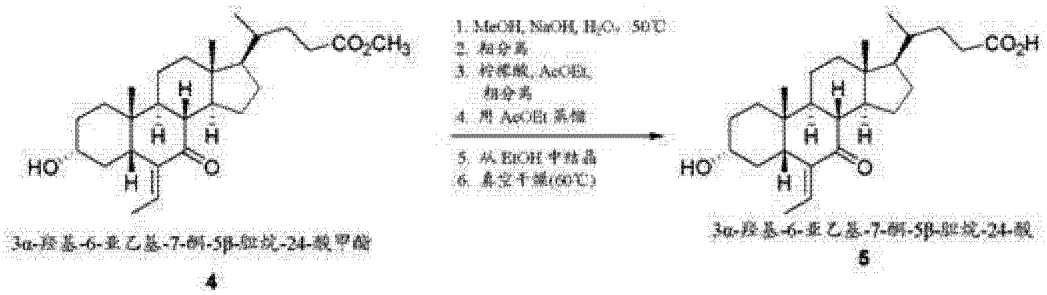


3 4

反应3:硅烯醇醚和乙醛的羟醛缩合

将THF中的化合物3 (164.68 g,300 mmol,以干燥物质计算)溶液装入惰性反应器中。在50℃的最大温度下,真空馏出残余量的THF。将残余物的含水量限于≤ 0.5% (Karl Fischer滴定)以继续进行。然后将残余物溶于二氯甲烷(200 mL),并预冷至-60℃至-65℃。然后加入乙醛(33.8 mL,600 mmol)。在氮气氛下,将二氯甲烷(700 mL)和三氟化硼(乙腈中的16 wt%溶液,318 g,750 mmol)乙腈络合物装入单独的反应器中,然后冷却至-60℃至-65℃。在-60℃至-65℃下,加入无水化合物3溶液。在-60℃至-65℃下搅拌反应混合物约2小时,加热至23℃-28℃,再搅拌约3小时,并冷却至约2℃-10℃用于水解/后处理。对于后处理,将反应器中的冷却溶液加入50% wt苛性钠(40 mL)和660 mL的水的预冷水性溶液中。在充分搅拌约10分钟后,分离各相,将(下层)有机层转移到单独的反应器中。在NMT 50℃下通过蒸馏尽可能地从有机层中除去溶剂。将由化合物4和一些剩余的乙腈和二氯甲烷组成的残余物装入滚筒中。化合物4A (E/Z-异构体的混合物)也可通过上述用于步骤3的方法制备。

[0221] 步骤4 - 3α-羟基-6-亚乙基-7-酮-5β-胆烷-24-酸(5)的制备:



4 5

反应4:C-24酯的皂化

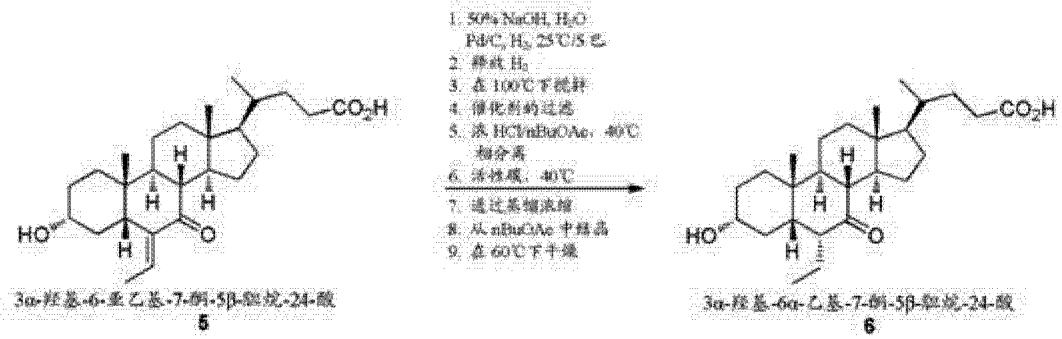
将化合物4 (258.37 g,600 mmol,以干燥物质计算)装入惰性反应器中。在NMT 50℃

的温度下，真空馏出残余量的溶剂。将残余物溶于甲醇 (360 mL) 和水 (54 mL) 中，加入苛性钠 50% wt (54 mL)。将反应混合物加热至 49℃ -53℃，在该温度下搅拌至少 2 小时。检查反应混合物的 pH 以证实 pH 为 > 12。如果 pH < 12，则再加入 NaOH，重复 2 小时反应时间。溶液用水 (1000 mL) 稀释，并调节温度至 25℃ -35℃。对于后处理，使反应混合物静置至少 30 分钟。分离各相，将下层的水层转移到单独的反应器，弃去有机层。加入乙酸乙酯 (1400 mL) 和柠檬酸水溶液 (244 g，在 480 mL 中）以充分搅拌进入水层中。在 25℃ -35℃下搅拌反应混合物 10 分钟。分离各相，弃去下层的水层。从有机层中馏出乙酸乙酯，并再加入乙酸乙酯 (800 mL)。重复该操作直到馏出液的含水量为 NMT 1% 或直到达到恒定沸点。使悬浮液冷却至 20℃ -25℃，搅拌 30 分钟，然后分离产物，用乙酸乙酯 (100 mL，3-4 次）洗涤。在转筒式干燥机中在约 60℃下进行真空干燥。产量为 118.71 g (47.5%，来自 KLCA) 的粗制化合物 5。化合物 4A (E/Z 异构体的混合物）也可用作产生化合物 5A (E/Z 异构体的混合物）的起始原料。

[0222] 然后使用乙醇使粗制化合物 5 结晶。用于结晶的粗制化合物也可为 E/Z 异构体的混合物化合物 5A。将乙醇 (390-520 mL) 和粗制化合物 5 (130 g) 装入惰性反应器中。为了溶解粗制化合物 5，将反应混合物加热至回流。然后，在 3-5 小时内以受控制的冷却变速按线性分布使反应混合物冷却至 15℃ -20℃。结晶化合物 5A 用离心机分离，然后用乙酸乙酯 (50-100 mL，2 次）洗涤。干燥在转筒式干燥机中在真空和约 60℃下进行。这产生 85.8 g (66%) 产量。取样品以测量纯化的化合物 5 的含量测定、纯度和水分含量。纯化的化合物 5 是 3α-羟基 -6- 亚乙基 -7- 酮 -5β- 胆烷 -24- 酸的 E 异构体。有关纯化的化合物 5 的鉴定和表征的全部详情参见实施例 2。纯化的化合物 5 (E 异构体）的分离可为任选的。E 异构体和 Z 异构体具有不同的溶解度。E 异构体不太溶解和结晶，使得 Z 异构体可被洗掉。

[0223] 制备化合物 5 的备选方法如下。将化合物 4 (111.96 g) 装入惰性反应器中。在最大 50℃下，真空馏出残余量的溶剂（例如乙腈、二氯甲烷）。将残余物溶于甲醇 (156 mL) 中，冷却至约 10℃。加入自来水 (23.4 mL) 和苛性钠 50% (23.4 mL)。在约 20℃ - 约 25℃下搅拌反应混合物约 4 小时。溶液用自来水 (433 mL) 稀释，加入甲苯 (144 mL)。在搅拌后，分离各相，将下层水层转移到惰性反应器中。弃去有机层。在充分搅拌到水层的同时，加入乙酸乙酯 (607 mL) 和柠檬酸溶液 (105.7 g，在 208 mL 的水中）。分离各相，弃去下层水层。将有机层转移到惰性反应器中。从有机层馏出乙酸乙酯，并再加入乙酸乙酯 (347 mL)。在一个实施方案中，用乙酸乙酯 (173 mL) 重复该操作直到馏出液的含水量不超过约 1% 或直到达到恒定沸点。使该悬浮液冷却至 20℃ -25℃。用惰性离心机将化合物 5 分离，并用乙酸乙酯 (3-4 次各 43 mL) 洗涤。干燥在转筒式干燥机中在真空和约 60℃下进行 (64.8% 收率，基于化合物 1)。化合物 4A (E/Z 异构体的混合物）也可用作步骤 4 的起始原料以产生化合物 5A (E/Z 异构体的混合物）。

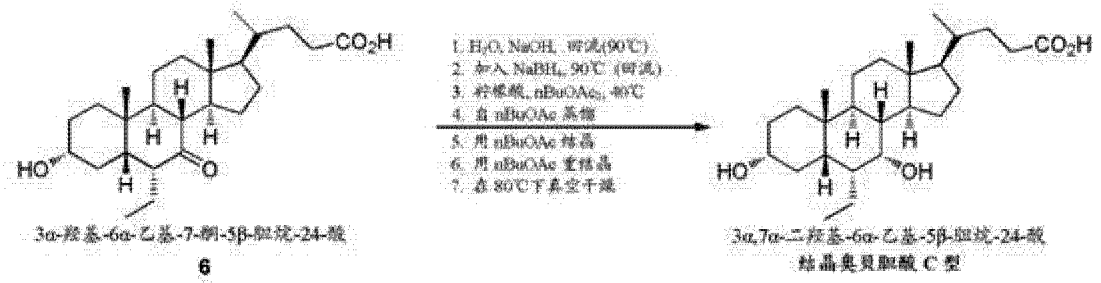
[0224] 步骤 5 - 3α-羟基 -6α-乙基 -7-酮 -5β-胆烷 -24-酸 (6) 的制备：



反应 5 :6- 亚乙基部分的氢化

将纯化的化合物 5 (110 g,264 mmol)、水 (1100 mL)、50% 的苛性钠溶液 (35.8 mL,682 mmol) 和钯催化剂 (Pd/C,11 g) 的混合物装入氢化反应器中。调节温度至 25℃ -35℃,反应器用氮气 (2 巴) 吹扫 3 次,用氢气 (1 巴) 吹扫 3 次。这些压力值相对于环境压力 (= 0 巴) 给出。施加 5 巴的氢压,并在 1.5 小时的时间内将反应混合物加热至 100℃ (用于 α 位的异构化),然后在保持 4.5-5 巴氢压的同时搅拌 3 小时。然后使反应混合物冷却至 40℃ -50℃。对于后处理,滤出 Pd/C。向滤液中加入乙酸正丁酯 (1320 mL) 和盐酸 (67.8 mL,815 mmol,37%)。分离水相并弃去。有机相在 40-50℃下用活性碳 (5.5 g) 处理约 10 分钟。滤出活性碳,滤液通过蒸馏浓缩,在 2-3 小时内使使所得悬浮液冷却至 15℃ -20℃。沉淀的化合物 6 经分离,并用乙酸正丁酯 (160 mL) 洗涤。产物使用压力过滤器过滤。干燥在压力过滤器中在约 60℃下真空进行。这产生 89.8 g (81.2%) 的化合物 6。化合物 5A (E/Z 异构体的混合物) 可用于步骤 5 以制备化合物 6。

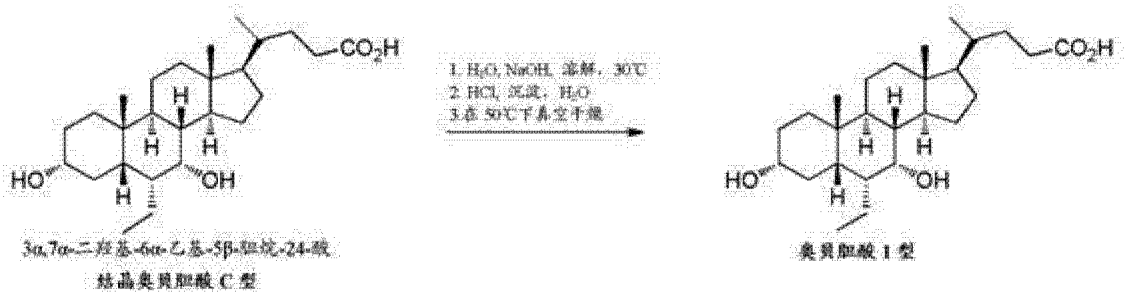
[0225] 步骤 6 - 3α,7α-二羟基 -6α-乙基 -5β-胆烷 -24-酸 (奥贝胆酸) 的制备 :



反应 6 :7- 酮至 7α- 羟基的选择性还原

在 50% wt 氢氧化钠溶液 (1.5 mL) 和水 (20 mL) 的混合物中,在 90℃ -105℃下使化合物 6 (86 g,205.4 mmol)、水 (688 mL) 和 50% 氢氧化钠溶液 (56.4 mL) 的混合物与硼氢化钠 (7.77 g,205.4 mmol) 反应。将反应混合物加热至回流,并搅拌至少 3 小时。对于后处理,在反应完成后,使反应混合物冷却至约 80℃,并转移到冷却的反应器中。在 30℃ -50℃下,加入水 (491 mL) 中的乙酸正丁酯 (860 mL) 和柠檬酸 (320.2 g,无水)。在检查 pH 值以确保是酸性后,分离水相并弃去。有机相经转移并蒸馏。残余物用乙酸正丁酯稀释,并慢慢冷却至 15℃ -20℃ , 粗制奥贝胆酸使用离心机过滤。湿产物从乙酸正丁酯中结晶出来。产物奥贝胆酸经分离,并在惰性压力过滤器中用乙酸正丁酯 (43 mL,4 次) 洗涤。干燥在压力过滤器中在约 80℃下真空进行。这产生 67.34 g (77.9%) 的结晶奥贝胆酸。有关结晶奥贝胆酸的鉴定和表征的全部详情参见实施例 3。

[0226] 步骤 7 - 奥贝胆酸 1 型的制备 :



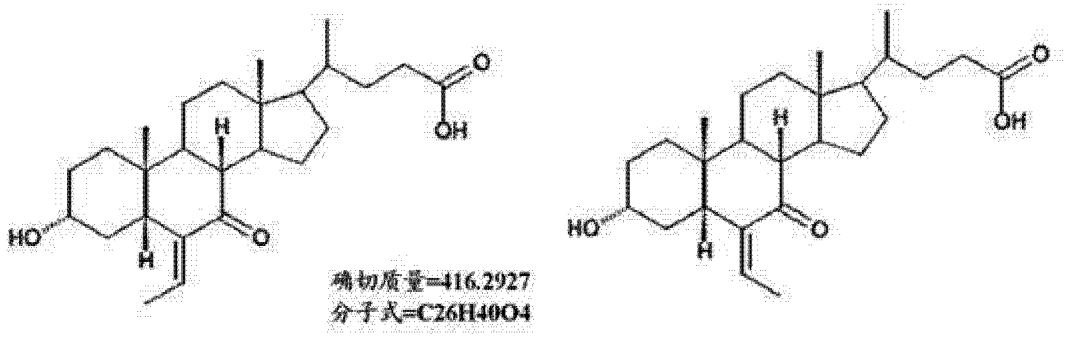
反应 7：自结晶奥贝胆酸 C 型制备奥贝胆酸 1 型

将结晶奥贝胆酸 C 型 (58 g) 溶于 30℃ -40℃的水 (870 mL) 和苛性钠溶液 (50%,8.7 mL,166 mmol) 中。搅拌混合物直到全部固体溶解。产物使用下列后处理沉淀。通过过滤器将奥贝胆酸溶液慢慢加入 30℃ -40℃的含稀盐酸 (37%,16.05 mL,193 mmol) 的水 (870 mL) 中。在下 30℃ -40℃搅拌悬浮液约 30 分钟，然后冷却至不超过 (NMT) 20℃。产物经分离，在惰性压力过滤器中用水 (465 mL,6 次）洗涤。干燥在真空压力过滤器中在 NMT 50℃的温度下进行。这产生 53.2 g (91.7%) 的奥贝胆酸 1 型。

[0227] 实施例 2：E-3α-羟基 -6-亚乙基 -7-酮 -5β-胆烷 -24-酸 (5)的表征

化合物 5 是本申请方法的关键中间体。化合物自乙酸乙酯分离，然后用乙醇结晶出来。高纯度化合物 5 可供化合物 6 及随后结晶奥贝胆酸 C 型和奥贝胆酸 1 型（包括基本上纯的奥贝胆酸）的有效而高产量的生产。

[0228] 使用 ^{1}H NMR、^{13}C NMR 和质谱法证实了来自实施例 1 步骤 4 的化合物 5 的结构。在通过 LC/MS 联用经质量控制方法 1 产生的 UV 色谱图中，来自步骤 4 的粗产物产生保留时间 (RT) 为 27.457 分钟的主产物和 RT 为 28.078 分钟的副产物。两种产物为化合物 5 的 E/Z 异构体：



这两种异构体在 MS/MS 谱中显示相同的精确质量和相同断片。通过质谱数据无法区分它们。

[0229] 采用半制备方法分离 E/Z 异构体峰，采用两阶段法证实 E/Z 异构体的结构。HPLC 质量控制方法 1 使用不挥发的磷酸缓冲液，因此，使用不挥发的缓冲液的直接 LC/MS 联用是不可能的。用于调整该方法的初步试验显示，对于 E/Z 异构体的充分分离，仅 UPLC 方法允许非常高的板数 (plate number)。两阶段方法如下：步骤 A 是用新开发的 UPLC/MS 方法鉴定两个样品中的 E/Z 异构体，步骤 B 是用 HPLC 方法 2 分离 E/Z 异构体峰的级分，并随后用 UPLC/MS 方法 1 鉴定。该方法的实验详情如下：

表 C

1. MS 相容性 UPLC 方法(方法 1)	
仪器:	Accela UPLC与LTQ FTSpectrometer联用(ThermoScientific)
柱:	200 x 2mm Hypersil Gold 1.9 µm
洗脱液:	A: 水+ 10 mM Ammoniumformiat + 0.1%甲酸 B: 乙腈
梯度:	45% B在20分钟内至60% B (10分钟等度梯度)
流速:	0.4 ml/分钟，40℃柱温
检测:	MS: ESI 阳离子和阴离子; UV: PDA 200.600 nm
质量分辨	R=100000 ICR
样品:	1 mg/ml在水/乙腈(1:1)中，3µl/20µl注入
2. HPLC (方法2)	
仪器:	Agilent1100 HPLC (Agilent Technologies)
柱:	125 x 4mm Purospher STAR C18 5 Lm
洗脱液:	A: 含磷酸的水pH 2.6 B: 乙腈
梯度:	30% B在10分钟内至35% B在30分钟内至60% B 在1分钟内至90% B (9分钟等度梯度)
流速:	1 ml/分钟，35℃柱温
检测:	UV: DAD 200 – 400 nm (UVA 200 nm)
样品:	10 mg/ml在水/乙腈(9:1)中，25µl注入

结果见图 1 和 2。图 1 和 2 是“粗制化合物 5”（图 1）和高效 UPLC 柱上获得的化合物 5“纯化参比”（图 2）的 UPLC UV/MS 色谱图。对于图 1，将样品以 1 mg/mL 溶于 ACN/H_2O 1:1；200x2mm Hypersil GOLD R122；LMA:H20+10mM AF + 0.1%HFo；LMB:ACN；45%-20-60%(10)；0.4mL/ 分钟；40℃;UVA=200nm；3 µ L 注射体积。对于图 2，将样品以 1 mg/ml 溶于 ACN/H_2O；200x2mm Hypersil GOLD R122；A：10mM AF + 0.1%HFo；B:ACN；45%-20-60%B(10)；0.4mL/ 分钟；20 µ L 注射体积。在 2 个样品中，主要组分（RT 9.92 分钟）和次要组分（RT 10.77 分钟）的分子量与预期相同，如下所示阳离子和阴离子测量数据的表 D 和 E 所示，两种化合物的精确质量与所提供的结构一致：

表 D：阳离子测量的数据

RT (分钟)	离子 m/z	分子式	结构建议
9.98	417.30008	$C_{28}H_{41}O_4$ ΔM 0.35 ppm	M+H E 异构体
	833.59381	$C_{52}H_{81}O_8$ ΔM 1.45 ppm	2M+H E 异构体
	850.61938	$C_{52}H_{84}O_8N$ ΔM 0.28 ppm	2M+NH4 E 异构体
10.77	417.30023	$C_{28}H_{41}O_4$	M+H Z 异构体
	833.59409	$C_{52}H_{81}O_8$	2M+H Z 异构体
	850.61984	$C_{52}H_{84}O_8N$	2M+NH4 Z 异构体

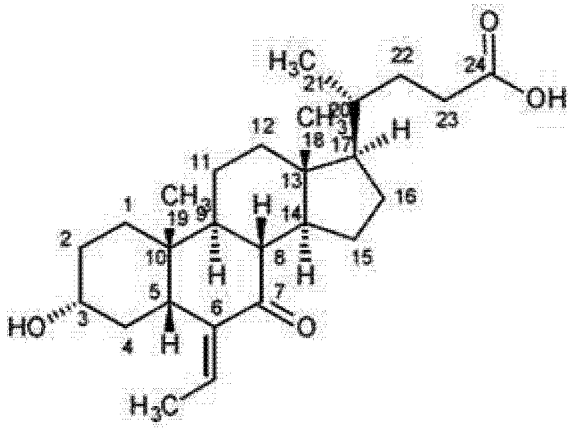
表 E：阴离子测量的数据

RT (分钟)	离子 m/z	分子式	结构建议
9.98	415.28520	$C_{26}H_{38}O_4$ ΔM -0.44 ppm	M-H Z 异构体
	461.29051	$C_{27}H_{41}O_6$ ΔM -0.76 ppm	M+Formiat Z 异构体
	831.57683	$C_{52}H_{79}O_8$ ΔM -1.46 ppm	2M-H Z 异构体
10.77	415.28545	$C_{26}H_{38}O_4$	M-H E 异构体
	461.29069	$C_{27}H_{41}O_6$	M+Formiat E 异构体
	831.57739	$C_{52}H_{79}O_8$	2M-H E 异构体

为了确保质量控制 HPLC 方法 2 的可移植性 (portability)，在所述条件下确切地重复原始分离。按半制备法分离主峰和次峰。具有捕获级分的明显位置的所得 UV 色谱图见图 3。图 3 是使用 HPLC 方法 2 的粗制化合物 5 的 UV 色谱图；125x4mm Purospher STAR C18 5 µm AG；LMA:H2O pH 2.6mit H_3PO_4;LMB:ACN；30%B-10-35%-30-60%-1-90%(9)；1 mL/ 分钟；35℃;UVA=200nm；ohne MS；25 mL。随后，用新开发的 UPLC/MS 方法分别分析分离级分。为了评价，使用 850.61914±3ppm 的准分子离子 [2M+NH4] 的精确离子迹线。所得的主峰级分、次峰级分和两个样品的色谱图见图 4 (A-D)。MS 研究表明，通过质量控制方法 2 产生的在 RT 27.457 分钟和 RT 28.078 分钟处的 2 个峰是分子式 $C_{26}H_{40}O_4$的 2 个异构体。该分子式与针对 E/Z 异构体提出的结构一致。因此，UPLC-MS 方法的开发表明，3α－羟基－亚乙基 -7- 酮 -5β－胆 -24 酸的 E/Z 异构体是以高分辨率在色谱法中可分离的。得自 FR-ICR 质谱仪的精确 MS 数据与针对 E/Z 异构体提出的结构一致。对于 2 个异构体，得到相同的分子式 $C_{26}H_{40}O_4$。

[0230] 由于用 HPLC 方法 2 的 E/Z 异构体峰的半制备分离和用 UPLC-MS 方法的后续鉴定，我们可证实通过质量控制方法 2 产生的 2 个峰 (RT 27.457 分钟和 RT 28.078 分钟，参见图 1) 是具有分子式 $C_{26}H_{40}O_4$的 2 个异构体。该分子式与提议的 E/Z- 异构体的结构一致。结合下述 NMR 结果，得到下列分配：RT 27.457 分钟属于 E- 异构体，RT 28.078 分钟属于 Z- 异构体。

[0231] 3α－羟基－亚乙基 -7- 酮 -5β－胆 -24 酸的 E 异构体的 ^1H 和 ^{13}C 位移的分配如下。位移按照以下文献估算：“L. Bettarello 等, II Farmaco 55 (2000), 51-55 (物质 3α－羟基 -7- 酮 -5β－胆烷 -24- 酸)。



[0232] 表 F：^1H 位移分配 (^1H-NMR，500 MHz，303K，CD_3OD)

化学位移[ppm]	强度[H]	峰裂数	分配
6.10	1	Q	25
3.61	1	M	3
2.69	1	DD	5
2.28	2	DT	23
1.72	3	D	26
1.05	3	S	19
0.99	3	D	21
0.70	3	S	18

表 G：^{13}C 位移分配（^{13}C-NMR，125 MHz，303K，CD_3OD）

化学位移[ppm]	峰裂数	分配
207.5	S	7
178.1	S	24
145.3	S	6
130.4	D	25
71.0	D	3
56.0	S	17
52.0 和 50.1	D 各个	8 和 14
46.9	D	5
44.7	S	13
40.7	D	9
40.3	T	12*
38.3	T	4*
36.5	D	20
35.8	S	10
35.4	T	1
32.3 和 32.0	T 各个	22 和 23
30.5	T	2*
29.4	T	16*
27.0	T	15*
23.2	Q	19
22.4	T	11
18.9	Q	21
12.7	Q	26
12.5	Q	18

S = 单峰

D = 双峰

T = 三重峰

Q = 四重峰

M = 多重峰

DD = 双双重峰

DT = 双三重峰

实施例 3：结晶奥贝胆酸 C型的表征

来自流程 1 步骤 6 和实施例 1 的产物的全固态表征显示奥贝胆酸是晶体。该晶体形式被标记为 C 型。下面是概述结晶奥贝胆酸 C 型的表征的表格：

表 G：结晶奥贝胆酸 C 型特征概述

技术	结晶奥贝胆酸C型
外观	白色粉末
NMR	与大约3.5% w/w 己烷的提供结构一致
XRPD	结晶
TGA	在室温-85℃（0.4%）和85-115℃（4.1%）之间重量减轻
DSC	97.9℃开始吸热
GVS	略微吸湿的，在90% RH下1.2%水吸收
Karl Fisher水测定	1.5% w/w
在40℃/75% RH下的稳定性	型或结晶性无改变

热分析

在配备34位自动进样器的Mettler DSC 823e中收集DSC（示差扫描量热法）数据。使用检定的铟针对能量和温度校准仪器。通常将针孔铝锅中的各样品0.5-1 mg以10℃•分钟$^{-1}$从25℃加热至350℃。在样品上方以50 ml•分钟$^{-1}$保持氮气吹扫。仪器控制和数据分析软件是STARe v 9.20。

[0233] 在配备34位自动进样器的Mettler TGA/SDTA 851e上收集TGA（热解重量分析）数据。使用检定的铟对仪器进行温度校正。通常将5-10 mg的各个样品加载到预先称重的铝坩埚中，以10℃·分钟$^{-1}$从环境温度加热至300℃。在样品上方以50 ml·分钟$^{-1}$保持氮气吹扫。仪器控制和数据分析软件是STARe v 9.20。

[0234] 通过结晶奥贝胆酸C型的TGA观察到2个重量减轻步骤。第一个发生在室温（室温）和85℃之间(0.41%)，第二个发生在85℃-115℃之间(4.10%)。第一重量减轻步骤可归因于水分丢失，而第二步骤归因于剩余水分丢失（水负责约1.2%重量减轻）和结合的庚烷丢失（约3.4%重量减轻）。结晶奥贝胆酸C型含有0.15和0.2摩尔间的溶剂（庚烷）和约1.5% w/w (0.3摩尔)。结晶奥贝胆酸C型的DSC温谱图含有一个吸热峰。这是相当陡峭的，从约98℃开始。参见图6。不同的溶剂会具有不同的沸点，因此在DSC和TGA实验中在不同温度下会蒸发。

[0235] X射线粉末衍射 (XRPD)分析

Bruker AXS C2 GADDS

使用Cu Kα辐射(40 kV,40 mA)、自动化XYZ阶段、用于自动样品定位的激光视频显微镜和HiStar二维平面检测器，在Bruker AXS C2 GADDS衍射计上收集X射线粉末衍射图。X射线光学系统由与0.3 mm针孔准直仪联用的单一Göbel多层镜(single Göbel multilayer mirror)组成。采用经检定的标准NIST 1976 Corundum（平板）进行每周性能检查。

[0236] 射束发散性，即X射线束在样品上的有效尺寸为约4 mm。采用样品—检测器距离为20 cm的θ-θ连续扫描模式，这提供3.2°-29.7°的有效2θ范围。通常将样品暴露于X射线束120秒钟。用于数据收集的软件为用于WNT 4.1.16的GADDS，并应用Diffrac *Plus* EVA v 9.0.0.2或v 13.0.0.2分析并呈现数据。

[0237] 环境条件：原样使用未研磨的粉末，制备在环境条件下运行的样品作为平板样本。将约1-2 mg的样品在玻璃载玻片上轻轻压平得到平直表面。

[0238] 非环境条件：将在非环境条件下运行的样品铺在含热传导化合物的硅片上。然后将样品以约10℃·分钟$^{-1}$加热至合适的温度，随后保持恒温约1分钟后，开始数据收集。

[0239] Bruker AXS/Siemens D5000

使用Cu Kα辐射(40 kV,40 mA)、θ-θ测角仪、V20的发散性和接收缝隙、石墨二级单色器和闪烁计数器，在Siemens D5000衍射计上收集X射线粉末衍射图。采用检定的金刚砂(Corundum)标准(NIST 1976)对仪器进行性能检查。用于数据收集的软件为Diffrac *Plus* XRD Commander v2.3.1，并应用Diffrac *Plus* EVA v 11,0.0.2或v 13.0.0.2分析并呈现数据。

[0240] 原样使用粉末，将样品在环境条件下运行作为平板样本。将约20 mg样品轻轻塞进切成磨光的零背景(510)硅片的空腔中。在分析期间使样品在自己平面上旋转。数据收集的详情为：

- 角范围：2-42 ° 2θ
- 步长：0.05 ° 2θ
- 收集时间：4 s·步骤$^{-1}$

Bruker AXS D8 Advance

使用Cu Kα辐射(40 kV,40 mA)、θ-2θ测角仪、V4的分散性和接收缝隙、Ge单色器

和Lynxeye检测器，在Bruker D8衍射计上收集X射线粉末衍射图。采用检定的金刚砂标准(NIST 1976)对仪器进行性能检查。用于数据收集的软件为Diffrac *Plus* XRD Commander v 2.5.0，并应用Diffrac *Plus* EVA v 11.0.0.2或v 13.0.0.2分析并呈现数据。

[0241] 原样使用粉末，将样品在环境条件下运行作为平板样本。将约5 mg样品轻轻塞进切成磨光的零背景(510)硅片的空腔中。在分析期间使样品在自己平面上旋转。数据收集的详情为：

- 角范围：2-42 ° 2θ
- 步长：0.05 ° 2θ
- 收集时间：0.5 s•步骤$^{-1}$

XRPD显示在Bruker AXS D8 Advance收集从本发明方法步骤6分离的粉末。参见图5。下表中提供X射线衍射图的相应数据。用于数据收集的软件为Diffrac *Plus* XRD Commander v2.6.1，应用Diffrac *Plus* EVA v13.0.0.2或v15.0.0.0分析并呈现数据。原样使用粉末，将样品在环境条件下运行作为平板样本。将样品轻轻塞进切成磨光的零背景(510)硅片的空腔中。在分析期间使样品在自己平面上旋转。数据收集的详情为：

- 角范围：2-42 ° 2θ
- 步长：0.05 ° 2θ
- 收集时间：0.5 s•步骤$^{-1}$

表H：结晶奥贝胆酸C型的X射线衍射图数据

峰	角2-θ（°）	d值（埃）
1	4.2	21.0203
2	6.35	13.90839
3	8.298	10.64718
4	9.5	9.30229
5	11.05	8.00042
6	12.246	7.22192
7	12.498	7.07692
8	12.647	6.99367
9	15.497	5.71337
10	15.843	5.5895
11	15.998	5.53561
12	16.346	5.41836
13	16.695	5.30601
14	16.996	5.21251
15	17.849	4.96547
16	18.593	4.76844
17	18.798	4.71689
18	19.047	4.65579
19	20.493	4.33041
20	20.894	4.24808

VT-XRPD（可变温度-X射线衍射）显示DSC温谱图所见吸热相当于样品的去溶剂化，因为在加热时未观察到形态变化。在DSC和VT-XRPD数据之间存在温度差异，因为VT-XRPD实验在样品所暴露的大空间中进行，而DSC实验在有限的封闭空间中进行。这种差异为约20℃，并解释了为什么在DSC实验中样品在低得多的温度下融化，而在VT-XRPD实验中样品在110℃下仍呈现晶体。VT-XRPD显示材料中溶剂的干燥导致结晶性丧失，这与材料呈溶剂

化形式一致。参见图 7。

[0242] 重量法蒸汽吸附 (GVS)

使用由 DVS 内部控制软件 v 1.0.0.30 控制的 SMS DVS 内部水分含量吸附分析仪，获得吸着等温线。通过仪器控制将样品温度保持在 25℃下。以 200 ml • 分钟 $^{-1}$的总流速，通过将干氮和湿氮气流混合，来控制湿度。通过位于样品附近的经校正的 Rotronic 探头 (1.0-100% RH 的动态范围)，测定相对湿度。通过微量天平 (精确度 ±0.005 mg) 不断监测随 % RH (相对湿度) 变化的样品的重量变化 (质量弛豫 (mass relaxation))。

[0243] 在环境条件下将 5-20 mg 样品置于去皮的网孔不锈钢篮中。在 40% RH 和 25℃ (典型的室内条件) 下加载和卸载样品。如下概述进行吸湿等温线 (2 次扫描得到 1 个完整循环)。标准等温线在 25℃下在 0.5-90% RH 范围内以 10% RH 间隔进行。应用 DVS 分析套件 v6.0.0.7，在 Microsoft Excel 中进行数据分析。SMS DVS 内部实验的方法参数如下 :

参数	值
吸附—扫描 1	40-90%
解吸 / 吸附—扫描 2	90-0,0-40%
间隔 (% RH)	10
扫描次数	2
流速 (ml • 分钟 $^{-1}$)	200
温度 (℃)	25
稳定性 (℃ • 分钟 $^{-1}$)	0.2
吸附时间 (小时)	6 小时终止

样品在完成等温线后回收，并通过 XRPD 再次分析。

[0244] 结晶奥贝胆酸 C 型的分析显示，样品是略微吸湿的，因为在 0-90% RH 间观察到 1.18% 的质量增加。这种水的吸收在整个分析中是稳定的，并且对于所有步骤都达到平衡。曲线的滞后少，表明了样品容易丢失已吸附的水。在 GVS 分析后的 XRPD 分析表明，样品无改变。参见图 8A、8B 和 8C。

[0245] 通过 Karl Fischer滴定 (KF)的水测定

使用 Hydranal Coulomat AG 试剂和氩气吹扫，用 Mettler Toledo DL39 库伦计测量了各样品的含水量。将称重的固体样品引入与 subaseal 连接以避免进水的铂 TGA 盘的管中。每次滴定使用约 10 mg 样品，并且进行一式两份测定。

[0246] Karl Fischer 分析表明结晶奥贝胆酸 C 型含有 1.5% 水，相当于约 0.3 摩尔水。

[0247] 在 40℃和 75% RH下的一周稳定性

如下测定 40℃和 75% RH (相对湿度) 下奥贝胆酸的稳定性。将奥贝胆酸样品在潮湿箱中在 40℃ /75% RH 下保存 1 周。通过 XRPD 再次分析样品，发现无变化。

[0248] 固态研究表明，需要存在相对大量的有机溶剂以使奥贝胆酸 C 型结晶。奥贝胆酸 1 型样品将自发结晶形成贮存中的结晶奥贝胆酸 C 型是极不可能的。

[0249] 实施例 4:奥贝胆酸片剂

下表显示奥贝胆酸片剂的定量组成。5 mg、10 mg 和 25 mg 制剂被用作 3 期临床试验材料。

[0250] 表 I :薄膜衣片剂

薄膜衣片剂			
组分	每片的量	功能	参考标准
1 mg 片剂			
奥贝胆酸	1.0 mg*	API	HSE
微晶纤维素	185.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色或白色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
5 mg 片剂			
奥贝胆酸	5.0 mg*	API	HSE
微晶纤维素	181.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色或白色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
10 mg 片剂			
奥贝胆酸	10.0 mg*	API	HSE
微晶纤维素	176.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
Opadry® II 绿色或白色	8.0 mg	包衣材料	HSE
总重	208.0 mg		
25 mg 片剂			
奥贝胆酸	25.0 mg*	API	HSE
微晶纤维素	157.0 mg*	填充剂/粘合剂	USP-NF/EP/JP
羟基乙酸淀粉钠	12.0 mg	崩解剂	USP-NF/EP/JP
硬脂酸镁	2.0 mg	润滑剂	USP-NF/EP/JP
胶态二氧化硅	4.0 mg	助流剂	USP-NF/EP/JP
Opadry® II 绿色或白色	8.0 mg	包衣材料	HSE
总重	208.0 mg		

API: 活性药物成分
HSE = 内部规格
USP-NF= 美国药典国家处方集
Ph Eur = 欧洲药典
JP = 日本药典
*所提供的奥贝胆酸量假设 API 是无水和 100%纯的; 实际量根据所用的药物批次的效能调节, 并相应减少微晶纤维素的量。

实施例 5:奥贝胆酸 1型的表征

奥贝胆酸 1 型是指奥贝胆酸的非晶形式。奥贝胆酸的这种形式可通过结晶奥贝胆酸作为合成中间体产生。奥贝胆酸 1 型可用作药学活性成分。如下表征和分析奥贝胆酸 1 型。

[0251] 第 1 批奥贝胆酸 1 型采用下列技术表征 :通过 X 射线粉末衍射 (XPRD) 对结晶性的评价,^{1}H 和 ^{13}C 核磁共振 (NMR)、傅里叶变换红外光谱法 (FT-IR)、光学评价 (例如粒子形态 / 粒径)、热性质 (例如示差扫描量热法 (DSC) 和热解重量分析 (TGA))、通过 Karl Fischer (KF) 的水测定、在 40℃和 75%RH 下贮存和 2 周后通过 XRPD 再次分析、通过电势测定方法的 pKa、通过电势测定法的 Log P/D (辛醇 / 水) 和采用重量法蒸汽吸附 (GVS ;例如用通过 XRPD 收集的固体分析的完全吸附 - 解吸循环) 的水分含量的稳定性。还采用下列技术表征并比较了奥贝胆酸 1 型的其它 5 个批次 (例如第 2、3、4、5 和 6 批) :通过 XRPD 评价并与主要的第 1 批图的比较、^{1}H 和 ^{13}C NMR、FT-IR、光学评价 (例如粒子形态 / 粒径)、热性质 (例如 DSC、TGA 和高温载台显微术) 和通过 KF 的水测定。

[0252] X射线粉末衍射 (XRPD)分析

使用 Cu Kα 辐射 (40 kV,40 mA)、自动化 XYZ 阶段、用于自动样品定位的激光视频显微镜和 HiStar 二维平面检测器,在 Bruker AXS C2 GADDS 衍射计中收集 X 射线粉末衍射图。X 射线光学系统由与 0.3 mm 针孔准直仪联用的单一 Göbel 多层镜组成。射束发散性,即 X 射线束在样品上的有效尺寸为约 4 mm。采用样品—检测器距离为 20 cm 的 θ - θ 连续扫描模式,这得到 3.2° - 29.7° 的有效 2θ 范围。通常将样品暴露于 X 射线束 120 秒钟。用于数据收集的软件为用于 WNT 4.1.16 的 GADDS,并应用 Diffrac *Plus* EVA v 9.0.0.2 或 v 13.0.0.2 分析并呈现数据。

[0253] 原样使用未研磨的粉末,制备在环境条件下运行的样品作为平板样本。将约 1-2 mg 样品在硅片上轻轻压平得到平直表面。衍射图显示奥贝胆酸 1 型是非晶体的 (参见图 10 和图 11)。

[0254] NMR表征

在配备自动进样器和由 DRX400 控制台控制的 Bruker 400 MHz 仪器中收集 NMR 波谱。采用标准 Bruker 加载实验,应用带有 Topspin v 1.3 (patch level 8) 运行的 ICONNMR v4.0.4 (build 1),实现自动化实验。对于非常规光谱法,通过仅使用 Topspin 获得数据。在 *d6*-DMSO 中制备样品,除非另有说明。离线分析采用 ACD SpecManager v 9.09 (build 7703) 的进行。

[0255] 图 12 显示第 1 批的 ^{1}H NMR 波谱。还记录了第 2-6 批的 ^{1}H NMR 波谱,并与第 1 批的波谱进行了比较。参见图 13。波谱全都类似,但是含不同量的水。在 0.75 ppm 和 2 ppm 之间的一大群质子的积分中注意到一些差异,其中峰重叠,无法分别积分。表 J 显示在第 1-6 批的波谱中积分的质子总数,考虑了 0.75 - 2 ppm 区域的变化。

[0256] 表 J

批号	通过积分的 H 的数目（不包括 COOH）
1	43
2	42
3	40
4	41
5	42
6	41-42

不包括羧酸质子，使得质子数应为 43，但实际上它在 6 个波谱间从 40 到 43 之间变化。然而，其中来自 (0.75-2 ppm) 范围的变化的面积是相当宽的，并且是因基线质量所致，不能依靠该积分。

[0257] 因为波谱不能完全指定，并且积分改变，因此记录了第 2 批的 ^{13}C NMR 波谱。图 14 显示 DEPTQ 波谱，其中 CH_2 和季碳峰指向上，而 CH_3 和 CH 基团指向下。有 13 个峰指向下，其对应于 9 个 CH 和 4 个 CH_3 基团。这与结构一致。在 175 ppm 处观察到羧酸碳的峰。为了目标区域清楚起见，将其从该展开图中排除。然而，仅有 11 个峰指向上，但是应有 12 个，因为分子中有 10 个 CH_2 基团和 2 个季碳（不包括羰基）。一个碳显得与另一个信号重叠。因此，收集 DEPT135 波谱，抑制季碳信号，其可显示重叠信号是否是四重的。参见图 15。DEPT135 波谱与 DEPTQ 波谱的比较显示 1 个峰（在 42.5 ppm 处）消失。在分子中有 2 个季碳，其应相当于 2 个峰消失。因此重叠碳信号是四重的。

[0258] 另外，进行了测定碳的弛豫时间的实验以测定其中丢失的季碳信号与另一个碳信号重叠。参见图 16。该 ^{13}C 波谱含有被积分的峰。这表明在 32.3 ppm 处的峰说明 2 个碳。有关在 32.3 ppm 处的峰的展开图的参见图 17。因此，目前通过积分说明了 26 个碳（包括羧酸），这与结构一致。

[0259] 通过 ATR的 FT-IR

在配备 Universal ATR 采样配件的 Perkin-Elmer Spectrum One 中收集数据。应用波谱 v5.0.1 软件，收集并分析数据。参见图 18。

[0260] 通过示差扫描量热法 (DSC) 和热解重量分析 (TGA) 的热分析

在配备 50 位自动进样器的 TA Instruments Q2000 中收集 DSC 数据。使用检定的铟，针对能量和温度校准校正仪器。通常将针孔铝锅中的 0.5-3 mg 各样品以 10℃·分钟 $^{-1}$ 从 25℃加热至 300℃。在样品上方以 50 ml·分钟 $^{-1}$ 保持氮气吹扫。仪器控制软件是 Advantage for Q Series v2.8.0.392 和 Thermal Advantage v4.8.3，应用 Universal Analysis v4.3A 分析数据。对调制 DSC，样品如前制备，将锅以 2℃·分钟 $^{-1}$ 从 25℃加热至 200℃。调制器条件为 0.20℃的幅度和 40 s 的周频。采样间隔为 1 sec/pt。

[0261] 在配备 16 位自动进样器的 TA Instruments Q500 TGA 中收集 TGA 数据。使用检定的 Alumel 对仪器进行温度校准。通常将 5-10 mg 各样品加载到预去皮的铂坩埚和铝 DSC 锅中，以 10℃·分钟 $^{-1}$ 从环境温度加热至 350℃。在样品上方以 60 ml·分钟 $^{-1}$ 保持氮气吹扫。仪器控制软件是 Advantage for Q Series v2.8.0.392 和 Thermal Advantage v4.8.3。

[0262] 通过 DSC 和 TGA 进行第 1 批的热分析。TGA 迹线（参见图 19）显示在环境温度和 121℃之间重量减轻 1.7%，这可能是水的丢失。DSC 迹线（参见图 19）显示宽泛的低温吸热（可能相当于水丢失），接着在 94℃下开始小的吸热。

[0263] 该第二个吸热可能表明玻璃转化，通过调制 DSC 作进一步研究（参见图 20）。该技

术使得能够将可逆转事件（例如玻璃转化）与不可逆转事件（例如溶剂丢失或晶体形式融溶）分开来。调制 DSC 中的可逆热流迹线显示玻璃转化为拐点 (Tg) 位于 95℃的步骤。这对于玻璃转化是高的，且表明 1 型是稳定的。在不可逆热流迹线中在 89℃处开始的小的吸热相当于在玻璃转化温度下疏松材料的分子弛豫。

[0264] DSC 迹线（参见图 19）显示分解在 220℃附近开始，这还相当于 TGA 迹线弯曲向下。

[0265] 第 1、2、3、4、5 和 6 批的 TGA 迹线具有类似形状（图 21）。在环境和 120℃间测量的重量减轻见表 K。它们与通过 NMR 观察到的不同量的水一致。进一步通过 Karl Fischer (KF) 水滴定定量测定这些量。参见通过 FK 的水测定。

[0266] 表 K：接受样品的 TGA 重量减轻概要

批号	通过 TGA 的重量减轻
1	1.7%
2	0.6%
3	1.2%
4	0.9%
5	1.5%
6	1.6%

图 22 显示用于比较的 6 个批次的 DSC 迹线。迹线相似，具有不同大小的宽的低温吸热，与不同量的水一致，接着在玻璃转化温度附近的小的吸热，如 DSC 和 TGA 节所见。结果概括于表 L。

[0267] 表 L：接受样品的 DSC 结果概要

批号	第 1 吸热，宽	第 2 吸热，小	开始分解
1	28.3J/g，Tmax = 64℃	1.2J/g，Tonset =94℃	220℃
2	7.4J/g，Tmax =48℃	1.4J/g，Tonset =94℃	220℃
3	无	2.0J/g，Tonset =89℃	175℃
4	14.5J/g，Tmax = 58℃	1.3J/g，Tonset =94℃	200℃
5	12.2J/g，Tmax = 59℃	1.2J/g，Tonset =94℃	175℃
6	28.7J/g，Tmax =59℃	1.5J/g，Tonset =94℃	200℃

偏振光显微术 (PLM)

样品用带有用于图像捕捉的数字视频摄录机的 Leica LM/DM 偏振光显微镜进行研究。将少量的各样品置于玻璃载玻片上，在硅油中固定，盖上盖玻片，尽可能地使各个颗粒分开。样品用适当的放大倍数和与 λ 假色滤镜联用的部分偏振光观察。

[0268] 图 23A-23F 显示第 1、2、3、4、5 和 6 批是由小的不规则颗粒形成的大的硬聚结物构成的材料。第 1、2、3、4、5 和 6 批看起来全都类似。在平面偏振光下未观察到双折射，这与该材料是非晶体的一致。粒径的范围为小于 1μm-3 μm。这些小的颗粒尺寸表明它们极快地析出。

[0269] 重量法蒸汽吸附 (GVS)

使用由 SMS 分析套件软件控制的 SMS DVS 内在水分含量吸附分析仪，获得吸着等温线。通过仪器控制将样品温度保持在 25℃下。以 200 ml·分钟 $^{-1}$ 的总流速，通过将干氮和湿氮气流混合来控制湿度。通过位于样品附近的经校正的 Rotronic 探头 (1.0-100% RH 的动态范围），测定相对湿度。通过微量天平（精确度 ± 0.005 mg）不断监测随 %RH 变化的样品的重量变化（质量弛豫）。

[0270] 通常在环境条件下，将 5-20 mg 样品置于去皮的网孔不锈钢篮中。在 40% RH 和 25℃（典型的室内条件）下加载和卸载样品。如下概述进行吸湿等温线 (2 次扫描得到 1

个完整循环）。标准等温线在25℃下在0.5-90%RH范围内以10% RH间隔进行。

[0271] 表M

参数	值
吸附—扫描1	40-90
解吸/吸附—扫描2	85-无水,无水-40
间隔(% RH)	10
扫描次数	2
流速(ml•分钟$^{-1}$)	200
温度(℃)	25
稳定性(℃•分钟$^{-1}$)	0.2
吸附时间(小时)	6小时终止

在25℃下获得第1批的重量法蒸汽吸附(GVS)等温线,见图24。样品显得是适度吸湿的,从0到90%相对湿度(RH)的总重量变化为3.8%。滞后(吸附和解吸曲线间的面积)小,表明了固体相当容易释放吸附的水。未观察水合物的形成。在整个实验后没有可观的重量变化(0.3%)。

[0272] GVS的动态图(图25)显示水的吸附大多发生在非常高的湿度下,解吸发生在非常低的湿度下。在吸附阶段,样品相当快地达到平衡直到80% RH,并花较长时间在90% RH下平衡。解吸时,质量在所有步骤中稳定。

[0273] 在完成GVS后,回收样品,通过XRPD再次分析,分析表明该材料仍是非晶体的(图26)。

[0274] 通过Karl Fischer (KF)的水测定

使用Hydranal Coulomat AG试剂和氩气吹扫,用Mettler Toledo DL39库伦计测量了各样品的含水量。将称重的固体样品引入与subaseal连接以避免进水的铂TGA盘上的管中。每次滴定使用约10 mg样品,并且进行一式两份测定。

[0275] 通过库仑分析的Karl Fischer的水滴定得到2.4 wt%水的结果。这略微高于通过TGA观察到重量减轻。它可能意味着一些水不是在加热时从材料中释放,而是可能因为这两种技术的不同实验程序所致。

[0276] 通过库仑分析的Karl Fischer测定各批次的含水量。表N显示这些结果,并且将其与较早获得的Karl Fischer结果和通过TGA观察到的重量减轻进行比较。数据是一致的,因为在所有3项分析中趋势是相同的。较早获得的Karl Fischer数据显示比在此获得的结果低的含水量。这与材料是吸湿的一致,虽然一些样品比其它样品吸收更多的水。TGA重量减轻一贯比通过Karl Fischer滴定获得的结果低,这可能意味着一些水被截留在材料中,在加热时不释放,但是也可能是由实验程序所致。

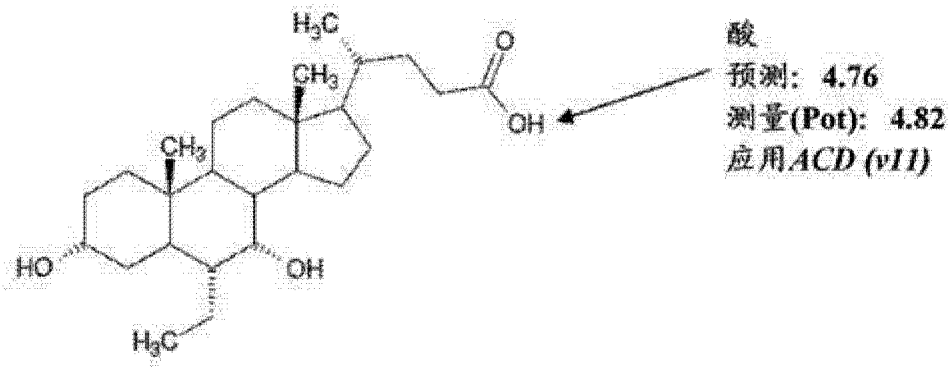
[0277] 表N:Karl Fischer (KF)结果和含水量数据概要

批号	KF含水量	较早的KF结果	TGA重量减轻
1	2.4%	2.1%	1.7%
2	1.9%	0.4%	0.6%
3	2.5%	1.4%	1.2%
4	2.2%	0.92%	0.9%
5	2.3%	0.53%	1.5%
6	2.8%	2.1%	1.6%

pKa测定和预测

在带有D-PAS附件的Sirius GlpKa仪器中收集pKa测定数据。在25℃下在水性溶液

中通过 UV 和在甲醇水混合物中通过电势测定法进行测量。用 0.15 M KCl (aq) 对滴定介质进行离子强度调节 (ISA)。通过 Yasuda-Shedlovsky 外推，将甲醇水混合物中的实测值校正至 0% 共溶剂。应用 Refinement Pro 软件 v1.0 对数据进行精修。pKa 值的预测应用 ACD pKa 预测软件 v9 进行。



[0278] 奥贝胆酸的 pKa 使用甲醇作为共溶剂通过电势测定法测量（图 27）并应用 Yasuda-Shedlovsky 外推外推至 0% 共溶剂（图 28）。pKa 使得能够测定指定 pH 下化合物的中性和电离形式的比例。图 29 显示取决于 pH 的种类的分布。

[0279] Log P测定

使用 3 种比率的辛醇 :离子强度调节 (ISA) 的水，在 Sirius GlpKa 仪器通过电势测定滴定法收集数据，以得到 Log P、Log $P_{离子}$ 和 Log D 值。应用 Refinement Pro 软件 v1.0 对数据进行精修。Log P 值的预测应用 ACD v9 和 Syracuse KOWWIN v1.67 软件进行。

[0280] 表 0 :预测和实测的 LogP

ACD (V9) 预测的 LogP	5.81
实测的 LogP	5.54
实测的 $LogP_{离子}$	1.58
实测的 LogD7.4	2.98

应用 ACD 软件预测 LogP，然后通过电势测定法测量。在 3 种不同的辛醇 /ISA 水比率下进行 3 个滴定，得到图 30 中绘制的不同曲线。黑色曲线是纯的水溶液 pKa 滴定，3 个有色曲线对应于 3 种辛醇 /ISA 水比率。pKa 中的位移使得能够测定 LogP。

[0281] 亲油性曲线 (logD 随 pH 而变化）见图 31。Log D 是分配系数，代表在特定 pH 下存在的所有物类的综合亲油性。LogP 是化合物常数，其相当于纯的中性物类的分配系数，而 $LogP_{离子}$ 是纯的电离物类的分配系数。LogP 和 $LogP_{离子}$ 可自亲油性曲线测定为 Y 轴分别与 pH 标度开始时的正切（当分子完全是中性形式时）和 pH 标度结束时的正切（当分子完全完全电离时）的交叉点。

[0282] 在 40℃和 75% RH及 25℃和 97% RH下的 2周稳定性

在固体形式的加速稳定性试验中，将第 1 批样品贮存在 40℃和 75% 相对湿度 (RH) 下。将另一个样品贮存在 25℃和 97% 相对湿度下以检查极高湿度的作用。在 5 天后和在 2 周后通过 XRPD 再次分析两个样品。2 个样品在 2 个贮存条件下保持非晶体持续高达 2 周，表明 1 型在这些条件下是稳定的。参见图 32 和图 33。

[0283] 所分析的 6 个批次全是非晶体的。用调制 DSC 实验在 95℃下测量玻璃转化温度。对于采用的所有分析技术，6 个批次显得非常相似，其间的唯一差异时其含水量，通过 Karl Fischer 滴定从 1.9% 到 2.8% 变化。热分析显示不同量的水，并表明分解在 175-220℃附近

开始。实测 pKa 为 4.82，LogP 为 5.54。显微评价显示极小的不规则颗粒形成大的硬聚结物。

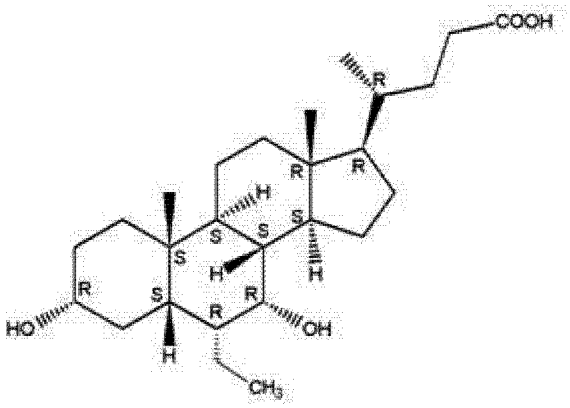
[0284] 稳定性试验表明，在加速条件 (40℃ /75% RH) 下或在高湿度 (25℃ /97% RH) 下 2 周后，该材料仍是非晶体的。重量法蒸汽吸附 (GVS) 分析表明该材料仅是适度吸湿的，从 0 至 90% 相对湿度 (RH) 总重量增加 3.8%。在 GVS 下未观察到水合物形成。在 GVS 后通过 XRPD 再次分析的样品依旧是非晶体的。高玻璃转化温度和稳定性试验结果表明该非晶体形式是稳定的。

[0285] 实施例 6：单晶 X射线结构和绝对立体化学

在以 0.1℃ / 分钟冷却至 5℃接着在室温 /50℃ 8 小时周期成熟 1 周后，由奥贝胆酸从乙腈溶液中重结晶获得的晶体，测定奥贝胆酸的单晶 X 射线结构 (参见图 34)。结构与 G 型一致，产生模拟的 XRPD 图作为该材料的参比图。可通过使在例如乙腈中的奥贝胆酸溶液冷却来制备 G 型。

[0286] 结构是正交晶系的，空间群 $P2_12_12_1$，在不对称单元中含有 1 分子的奥贝胆酸。最终 R1 [I>2 σ(I)] = 3.22%。晶体显示概略尺寸为 0.4 x 0.4 x 0.3mm 的棱晶形态。确定分子的绝对立体化学在手性中心 C5、C9、C10 和 C14 上为 *S*，在手性中心 C3、C6、C7、C8、C13、C17 和 C22 上为 *R*，Flack 参数 =-0.01 (13)。对于在 *R* 构型中具有手性中心 C5、C9、C10 和 C14，在 *S* 构型中具有手性中心 C3、C6、C7、C8、C13、C17 和 C22 的倒置结构，Flack 参数 = 1.01(13)，证实了上述指派。

[0287] 总之，该结构具有强数据集且有序。



[0288] 用于指派立体化学的软件 (PLATON) 确定手性中心 (C8) 为 *R* 立体中心，而 ACD 软件 (以及 Cahn-Ingold-Prelog) 对 (C8) 的指派为 *S*。然而，对于 B/C 环系反式环连接点的指派从晶体结构来看是绝对确定的。

[0289] 对 Bijvoet 差异使用 Bayesian 统计测定的绝对结构 (Hooft 等，*J. Appl. Cryst.*，(2008)，41，96-103) 显示，所提供的是正确的绝对结构的概率为 1.000，而绝对结构是外消旋孪晶或非真的概率分别为 0.000 和 0.000。通过该程序计算 Flack 等同物及其不确定度为 -0.019(17)。

[0290] 奥贝胆酸的结构含有稠合在一起的 1 个 5 元环和 3 个 6 元环。对 5 元环 (C13、C14、C15、C16 和 C17) 的构象分析显示对于这个环最密折起的描述符 (closest puckering descriptor) 是半椅式。对 3 个 6 元环 (C1、C2、C3、C4、C5 和 C10) ；(C5、C6、C7、C8、C9 和 C10) 和 (C8、C9、C11、C12、C13 和 C14) 的构象分析显示对于这些环最密折起的描述符是椅

式。

[0291] 在晶体结构中观察到 2 个独特的分子间氢键。每分子的奥贝胆酸与分别具有氧 O1 和 O4 （用作氧的供体）、O3 和 O1（用作接纳体）的奥贝胆酸的 2 个不同的对称相关分子形成氢键，O1— H1C---O3 [D • • • A = 2.7419(12)Å] 和 O4— H4C---O1 [D • • • A = 2.6053(13)Å （参见图 35)。这些相互作用产生复杂的三维氢键网。最终傅立叶差异图显示最大和最小电子强度分别为 0.402 和 -0.176 eÅ-3。

[0292] 该结构的计算的 XRPD 图与实验批次的重叠表明，晶体与大部分一致，且是奥贝胆酸 G 型（参见图 36)。

[0293] 表 1. 奥贝胆酸 G型的晶体数据

结晶溶剂	乙腈
结晶方法	在RT/50℃下成熟
经验式	$C_{26}H_{44}O_4$
式量	420.63
温度	100(2) K
波长	1.54178 Å
结晶粒度	0.40 x 0.40 x 0.30 mm
晶癖	无色棱晶
晶系	斜方晶
空间群	$P2_12_12_1$
晶胞大小	a = 8.72510(10) Å α = 90°
	b = 12.69860(10)Å β = 90°
	c = 22.5408(2)Å γ = 90°
体积	2497.44(4) $Å^3$
Z	4
密度(计算值)	1.119 Mg/m^3
吸收系数	0.574 mm^{-1}
F(000)	928

表 2. 奥贝胆酸 G型的数据收集和结构精修

衍射计	SuperNova，Dual，在零下的Cu，Atlas
辐射源	SuperNova (Cu) X射线源，CuKα
数据收集方法	ω扫描
用于数据收集的θ范围	9.15-74.49°
下标范围	$-10 \leq h \leq 10$，$-15 \leq k \leq 15$，$-28 \leq l \leq 26$
收集的反射	50001
独立反射	5073 [R(int) = 0.0220]
独立反射的覆盖度	99.4%
检查反射的变化	N/A
吸收校正	来自等同物的半经验
最大和最小传输	1.00000和0.78605
结构解析技术	直接
结构解析程序	SHELXTL (Sheldrick，2001)

精修技术	对F^2的全矩阵最小二乘
精修程序	SHELXTL (Sheldrick，2001)
函数最小化	$\Sigma w(F_o^2-F_c^2)^2$
数据/约束/参数	5073/0/286
对F^2的拟合优度	1.060
Δ/σ_{max}	0.001
最终R指数	
5039数据；I>2σ(I)	R1 = 0.0320，wR2 = 0.0859
全部数据	R1 = 0.0322，wR2 = 0.0861
加权方式	计算$w=1/[\sigma^2(F_o^2)+(0.0503P)^2+0.5520P]$
	其中$P=(F_o^2+2F_c^2)/3$
绝对结构参数	-0.01(13)
最大差异峰和空穴	0.402和-0.176 $eÅ^{-3}$
结构的精修概要如下：	
有序的非H原子，XYZ	随意精修
有序的非H原子，U	各向异性
H原子(在碳上)，XYZ	连接原子上的理想化位置
H原子(在碳上)，U	结合原子的U(eq)的适当倍数
H原子(在杂原子上)，XYZ	随意精修
H原子(在杂原子上)，U	各向同性
无序原子，OCC	非无序
无序原子，XYZ	非无序
无序原子，U	非无序

实施例 7:奥贝胆酸 1型 （非晶体 ）和晶体 （F型 ）形式之间的生物利用度差异

固体奥贝胆酸的物理状态在口服给予受试者（例如大鼠）时可在分子的生物利用度中起作用。进行了下述研究以评价在单次口服给药后的血清动力学和肠吸收的效率和奥贝胆酸的固体非晶体和晶体形式的药代动力学。对在给予奥贝胆酸 1 型（非晶体）或 F 型后的奥贝胆酸血浆浓度与时间的分布、t_{max}、C_{max}和 AUC 进行了比较（参见图 37-38）。

[0294] 将奥贝胆酸 1 型（非晶体）和 F 型给予大鼠，在不同的时间收集各动物血液持续至少 3 小时。针对奥贝胆酸的各个形式对 6 只动物进行了研究。

[0295] 实验方案：

所用的试验物质是奥贝胆酸 1 型（非晶体）和晶体 F 型。可通过从乙腈或硝基甲烷中成熟来制备 F 型。把制剂制备成 pH 4 的水中的混悬剂。研究模型是约 225- 约 250 g 的成年雄性 Sprague Dawley 大鼠 (Harlan Laboratories)。各剂量途径使用 6 只动物。剂量为 PO 20 mg/kg/5 mL。在用奥贝胆酸制剂治疗前，使动物禁食过夜。口服给药通过胃管饲进行。

[0296] 在第一天，给动物装上植入左颈静脉的导管 (SOP VIVO/SAF6)，通过异氟烷达到麻醉。在手术恢复一天后开始实验。通过在肝素化注射器 (Na Heparin) 中的导管采集约 500 μL 血液 (250 μL 血浆)，并立即收集在冰 / 水浴中的微管中。在 1 小时内，使样品在 4℃下以 10000xg 离心 5 分钟。将血清立即转移到微管中，并在 -20℃下贮存。给药后 30 分钟、1 小时、1.3 小时、2 小时和 3 小时收集血样。采用 HPLC-ES/MS/MS 定量方法分析血清样品。采用非区室方法进行药代动力学研究。

[0297] 结果 ：

图 37 中报告了在 2 种固体形式的 20 mg/Kg b.w 口服单剂给药后奥贝胆酸的平均血浆浓度。值是各制剂 6 套实验的均值。图中报告了标准差。

[0298] 在给予晶体形式后，1.5 小时后达到 Cmax，血浆奥贝胆酸浓度遵循具有一个最大值的正常动力学，3 小时后剂量是 Cmax 的几乎一半。

[0299] 在给予奥贝胆酸 1 型（非晶体）1 型后的动力学概况不同于晶体 F 型的动力学概况。30 分钟后获得早期血浆浓度峰，2 小时后获得第二个峰。6 只大鼠中数据的变化性非常低，这一行为在统计学上不同于所述晶体形式的特点。对于所述晶体形式，所研究的 3 小时的 AUC 较高。动力学表明，3 小时后奥贝胆酸仍存在于血浆中。之前已证实，奥贝胆酸通过肝的途径产生肝代谢物牛磺缀合物，它分泌进入胆汁，并在肠肝循环中蓄积。因此，牛磺缀合物的测量可用来确定通过肝的途径的奥贝胆酸的量。图 38 中报告了牛磺缀合物形成的速率，其显示牛磺缀合物形成较快，并且在给予所述晶体形式后达到较高的浓度。

[0300] 熔点和玻璃转化

奥贝胆酸 1 型（非晶体）1 型和晶体 F 型的熔点使用常规方法测量。测量作为参比化合物的鹅脱氧胆酸和乌索脱氧胆酸的熔点。一式三份进行测量。对于晶体形式，从固态到液态的转变定义为熔解温度 (T_m)，而对于非晶体形式定义为玻璃化温度转变 (T_g)。表中报告了测量值，以摄氏℃和开尔文° K 两者表示。

[0301] 表 3:奥贝胆酸 (1型和 F型) 和 CDCA和 UDCA的熔点

	实验数据		文献数据	
化合物	T_m(℃)	T_g(℃)	T_m(℃)	T_g(℃)
CDCA	136-140	-	119 143 163	98
UDCA	203-207	-	203	105
奥贝胆酸	120-124 235-237	108-112	-	-

	实验数据			文献数据		
化合物	T_m(°K)	T_g(°K)	T_g/T_m (°K)	T_m(°K)	T_g(°K)	T_g/T_m (°K)
CDCA	409-413	-	-	392 416 436	371	0,85
UDCA	476-480	-	-	477	378	0,79
奥贝胆酸	393-397 508-510	381-385	0,75	-	-	0,75

结果：

针对 CDCA 和 UDCA 得到的值与之前报告的值一致，其中 UDCA 的熔点高于 CDCA 的熔点。1 型的转变玻璃化温度 Tg（102-112℃）低于 F 型的熔点温度 Tm（120-124℃）。当比较 2 种固态形式时，此观察模式与之前报告的数据一致。F 型在较高温度（235-237℃）下具有额外转变。

[0302] 以开尔文度表示的最高熔点温度和玻璃转化温度间的比率与其它药物和其它胆汁酸十分类似（J. Kerc 等 Thermochim. Acta, 1995 (248) 81-95）。

[0303] 示差扫描量热法分析

进行了示差扫描量热法（DSC）分析以更好地界定奥贝胆酸晶体和非晶体形式的熔点和物理状态。所用仪器为 Mettler Toledo DSC 821e 型。对 1 型和 F 型各约 4-5 mg 进行了分析。以 10℃ / 分钟加热速率将化合物暴露于 30-300℃的温度范围内。

[0304] 图 39 显示针对奥贝胆酸晶体 F 型获得的 DSC 曲线。检测到相当于化合物熔点的 120.04℃处的一个吸热转变。该结果还得到热台显微术（HSM）的证实；在 30℃ -240℃的范围内，所观察到的固体 - 液体转变在 122-124℃下。在 DSC 迹线中，针对 F 型获得的峰形和强度与晶体形式所显示的典型表现一致。然而，峰宽相当宽；这可能是由于不均匀的晶体所致。热解重量分析（TGA）在 30-300℃温度范围内不显示任何重量减轻。

[0305] 图 40 显示针对奥贝胆酸非晶体 1 型获得的 DSC 曲线。观察到在 79.95℃处的一个吸热转变。峰形和强度与非晶化合物所预期的表现一致。对于这些物质，固体 - 液体转变（玻璃转化）所需能量小于结晶化合物。温谱图在 30-300℃温度范围内不显示任何重量减轻。

[0306] 水溶性

奥贝胆酸 1 型（非晶体）和晶体 F 型的水溶性按照本领域已知方法测量。简单地说，

将固体悬浮于低 pH (HCl 0.1 mol/L) 的水中，并在稍稍混合下置于 25℃下一周以使之平衡。将饱和溶液过滤，通过 HPLC-ES-MS/MS 测量溶液中化合物的浓度。

[0307] 结果：

	水溶性 (μmol/L)
1 型	17.9
F 型	9.1

相对于 F 型的 9.1 μmol/L，1 型呈现较高的溶解度 17.9 μmol/L。

[0308] 按照奥贝胆酸的生物利用度数据，晶体 F 型高于奥贝胆酸 1 型（非晶体）。尽管给予 1 型后血浆浓度峰较早，但是血浆概况显示 F 型更有效地吸收（较高的 AUC），甚至动力学更有规律，反映了药物在肠内容物中的最佳分布。1 型显示这个早期峰，之后第二个峰，其 Cmax 低于 F 型的 Cmax。

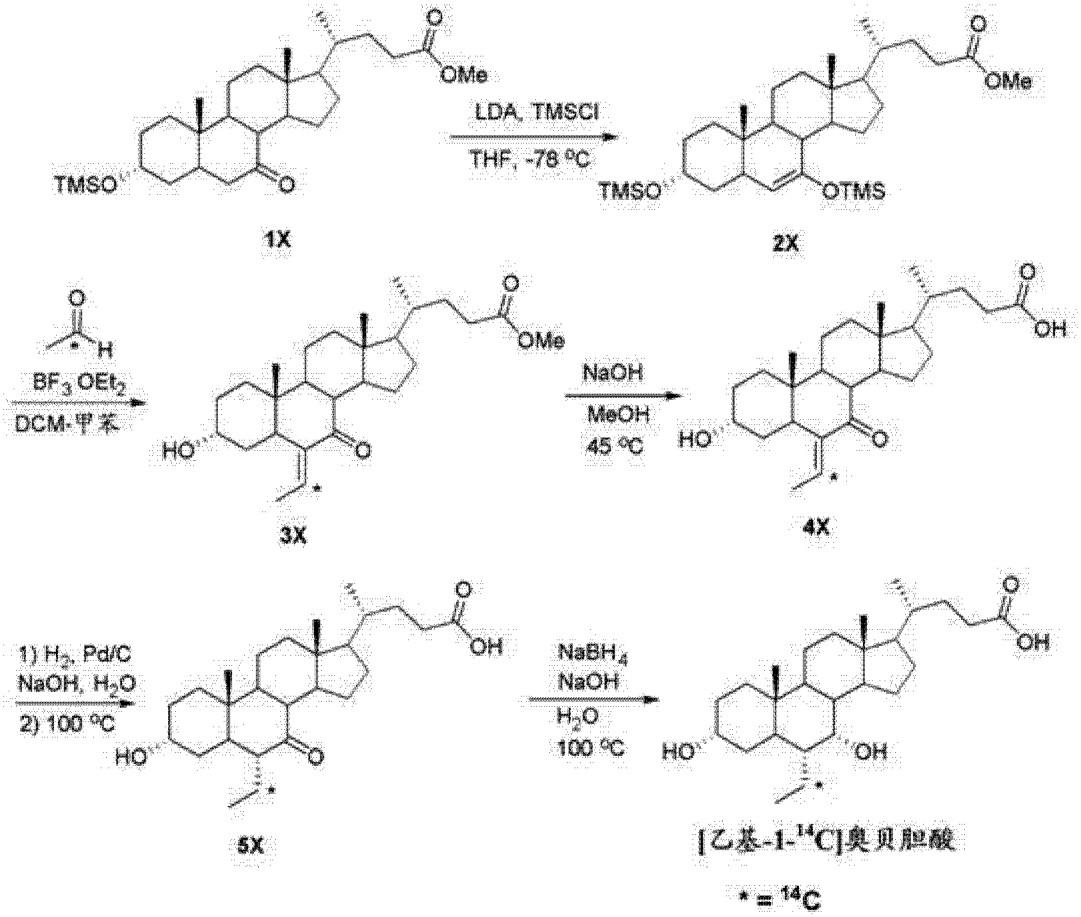
[0309] 1 型的水溶性高于 F 型的水溶性。F 型显得是稳定的，因为热解重量分析 (TGA) 在所研究的温度范围内未显示任何重量减轻。

[0310] 按照这些结果，F 型当口服给予时显得更有效地被肠吸收，并被肝吸收。F 型的主要肝代谢物牛磺缀合物的形成速率几乎是与 1 型相比的两倍，表明了更有效的转运和在肠肝循环中蓄积和 3 小时后的血清浓度。

[0311] 实施例 8：放射性标记的奥贝胆酸的制备

放射性标记的奥贝胆酸按照以下流程制备。

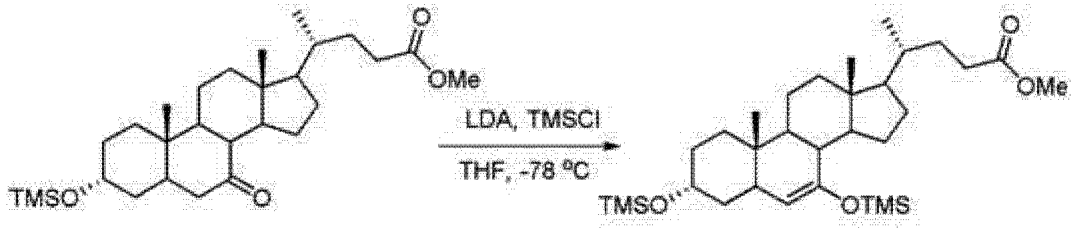
[0312] 流程 5



在 30℃下在 5-mm 外径管 (Norell, Inc. 507-HP) 中记录 $CDCl_3$ 和 MeOD-d_4 溶液中的 NMR

波谱，并在 Varian VNMRS-400 中以 ^{1}H 的 400 MHz 收集。化学位移（δ）以四甲基硅烷 (TMS = 0.00 ppm) 为基准，并用 ppm 表示。LC-MS/MS 以 Accela-Thermo Finnigan LCQ Fleet 操作 EST (-) 电离模式在离子阱质谱仪中取得。HPLC 在 Agilent 1200 系列（柱 :Xterra MS C8,250 x 4.6 mm,5 μm,40℃）连线 β-Ram 中取得。比活在 LSA (Liquid Scintillation Analyzer,Perkin Elmer,Tri-Carb 2900TR) 中取得。

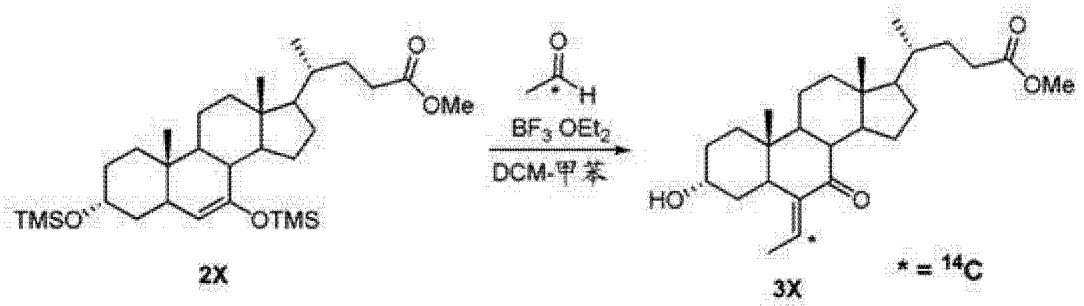
[0313] 化合物 2X 的制备



1X 2X

在 -20℃下，向二异丙胺 (1.59 g,15.8 mmol) 的无水 THF (6.0 mL) 溶液中加入 n-BuLi (6.30 mL,2.5 M,15.8 mmol)。在 -20℃下搅拌反应混合物 1 小时后，冷却至 -78℃，加入 TMSCl (1.72 g,15.8 mmol)，接着加入含化合物 1X (3.00 g,6.29 mmol) 的无水 THF (6.0 mL)。在 -78℃下搅拌反应混合物 1 小时，通过加入 $NaHCO_3$ 猝灭，并在室温下搅拌 30 分钟。有机层经分离，并真空浓缩，得到化合物 2X (3.29 g,95%)，无需进一步纯化用于下一步骤。

[0314] 化合物 3X 的制备

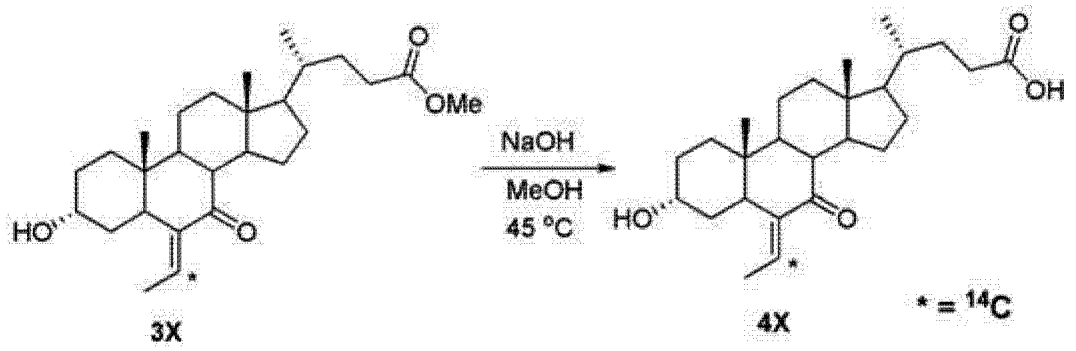


2X 3X * = ^{14}C

将含 [1-^{14}C] 乙醛 (330 mCi,5.63 mmol)（由 [^{14}C]$BaCO_3$ 制备，SA = 58.6 mCi/mmol) 的甲苯 (1.0 mL) 和含乙醛 (130 mg,2.95 mmol) 的 DCM (2.0 mL) 在 -78℃下混合，然后在 -78℃下转移到化合物 2X (3.29 g,6.00 mmol) 的 DCM (13.0 mL) 溶液中，接着加入 $BF_3 \cdot OEt_2$ (1.05 g,7.40 mmol)。在 -78℃下搅拌 1 小时后，使反应混合物升温直到 35℃，并在上述温度下搅拌 1 小时。通过加入水 (10 mL) 猝灭反应，水层用 DCM 萃取，合并的有机层经无水 Na_2SO_4 干燥，过滤后，真空浓缩。残余物用 SiO_2 柱色谱法（己烷 :EtOAc = 5:1-3:1) 纯化，得到化合物 3X (102 mCi,31%,SAW 37.0 mCi/mmol)，为白色固体。

[0315] ^{1}H-NMR ($CDCl_3$, Varian, 400 MHz) :δ 0.65 (3H, s) ;0.93 (3H, d, *J*= 6.0 Hz), 1.01 (3H, s), 1.06-1.49 (12H, m), 1.62-2.04 (7H, m), 1.69 (3H, d, J= 6.8 Hz), 2.18-2.28 (2H, m), 2.32-2.43 (2H, m), 2.58 (1H, dd, *J*= 12.8, 4.0 Hz), 3.62-3.70 (1H, m), 3.67 (3H, s), 6.18 (1H, q, *J*= 6.8 Hz)。

[0316] 化合物 4X 的制备

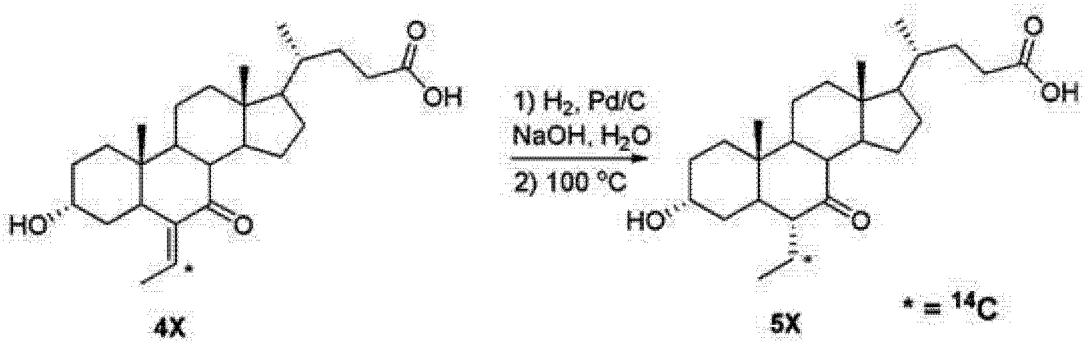


3X 4X

在室温下，向化合物 3X (102 mCi，2.75 mmol) 的 MeOH (6.0 mL) 溶液中加入含 NaOH (220 mg，5.50 mmol) 的 H_2O (3.0 mL)。在 45℃下搅拌反应混合物 1 小时后，冷却至室温，减压除去 MeOH，用 H_2O (12 mL) 稀释。水层用 H_3PO_4 酸化，用 DCM 萃取，并将有机层真空浓缩。将残余物悬浮于 Et_2O 中，经过滤收集沉淀，得到化合物 4X (86.3 mCi，85%)，为白色固体。

[0317] ^{1}H-NMR ($CDCl_3$, Varian, 400 MHz)：δ 0.63 (3H, s), 0.92 (3H, d, *J*= 6.0 Hz), 0.99 (3H, s), 1.04-1.50 (13H, m), 1.61-2.01 (7H, m), 1.67 (3H, d, *J*= 7.2 Hz), 2.21-2.28 (2H, m), 2.35-2.41 (2H, m), 2.56 (1H, dd, *J*= 12.8, 4.0 Hz), 3.58-3.69 (1H, m), 6.16 (1H, q, J= 7.2 Hz)。

[0318] 化合物 5X 的制备

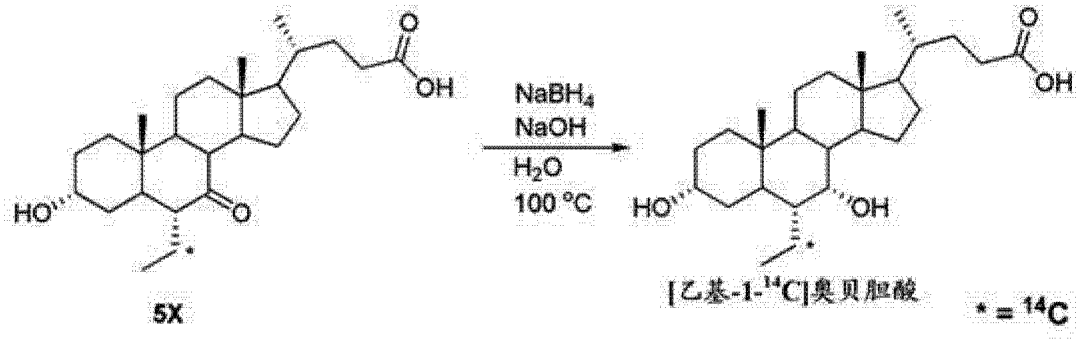


4X 5X

将化合物 4X (86.3 mCi，2.35 mmol) 和 5%- Pd/C (100 mg) 在 aq. 0.5 M NaOH (10 mL，5.0 mmol) 中的混合物在室温、H_2 气氛（气囊）下搅拌 10 小时，然后在 100℃下搅拌 14 小时。催化剂经过滤除去，用水洗涤，滤液用 H_3PO_4 酸化。经过滤收集沉淀物，将固体溶于 EtOAc 中，用盐水洗涤，通过 SiO_2 短垫过滤，并真空浓缩 。残余固体用 EtOAc 重结晶，得到化合物 5X (67.7 mCi，78%)，为白色固体。

[0319] ^{1}H-NMR (MeOD-d_4, Varian, 400 MHz)：δ 0.71 (311, s), 0.75-0.84 (1H, m), 0.81 (3H, t, *J* = 7.4 Hz), 0.921.01 (1H, m), 0.96 (3H, d, *J*= 6.4 Hz), 1.06-1.38 (7H, m), 1.25 (3H, s), 1.41-1.96 (1211, m), 2.01-2.05 (1H, m), 2.11-2.24 (2H, m), 2.30-2.37 (1H, m), 2.50 (1H, t, *J*= 11.4 Hz), 2.80-2.85 (1H, m), 3.42-3.49 (1H, m)。

[0320] [乙基-1-^{14}C] 奥贝胆酸的制备



在80℃下向化合物5X (67.7 mCi,1.83 mmol)的aq. 2 M NaOH(4.50 mL,9.00 mmol)的溶液中加入$NaBH_4$ (416 mg,11.0 mmol)的H2O (2.0 ml)溶液。在100℃下搅拌反应混合物2小时后,在室温下加入水(6.0 mL),并用H_3PO_4酸化。水层用DCM萃取,经无水Na_2SO_4干燥,通过SiO_2短垫过滤,并真空浓缩,残余物用SiO_2柱色谱法(己烷:EtOAc = 1:1-1:3)纯化,得到产物(44.0 mCi,65%),为白色固体。将产物(44.0 mCi,1.19 mmol)和奥贝胆酸(120 mg,0.285 mmol)溶于EtOAc (4 mL)中,将溶液在50℃下搅拌2小时,然后真空浓缩。将残余的油状物悬浮于Et_2O中,经过滤收集沉淀物,得到[乙基-1-^{14}C]奥贝胆酸(560 mg,38.5 mCi,SA = 29 mCi/ mmol),为白色固体。

[0321] ^{1}H-NMR ($CDCl_3$, Varian, 400 MHz):8 0.66 (3H, s), 0.88 (311, s), 0.93 (3H, t, J = 7.2 Hz), 0.93 (3H, d, I = 6.4 Hz), 0.96-1.04 (1H, m), 1.08-1.52 (14H, m), 1.51-1.60 (1011, m), 2.22-2.30 (111, m), 2.36-2.44 (1H, m), 3.38-3.45 (111, m), 3.71 (IH, s)。

[0322] LC-MS/MS (MS:LCQ Fleet):MS计算值:421.56;MS实测值:421.07 $[M-H]^-$。

[0323] Radio TLC:二氧化硅60 F_{254}的TLC板,流动相为EtOAc。放射化学纯度为98.90%,Rf = 0.675。

[0324] HPLC (Agilent 1200系列):流动相;乙腈:5 mM磷酸盐缓冲液(pH = 3):MeOH = 450:450:100。放射化学纯度为98.19% (β-ram),Rt = 20.00分钟。

[0325] [乙基-1-^{14}C]奥贝胆酸的分子式为$^{14}C_1C_{25}H_{44}O_4$,在通过LSC的29 mCi/mmol比活下的分子量为421.46。

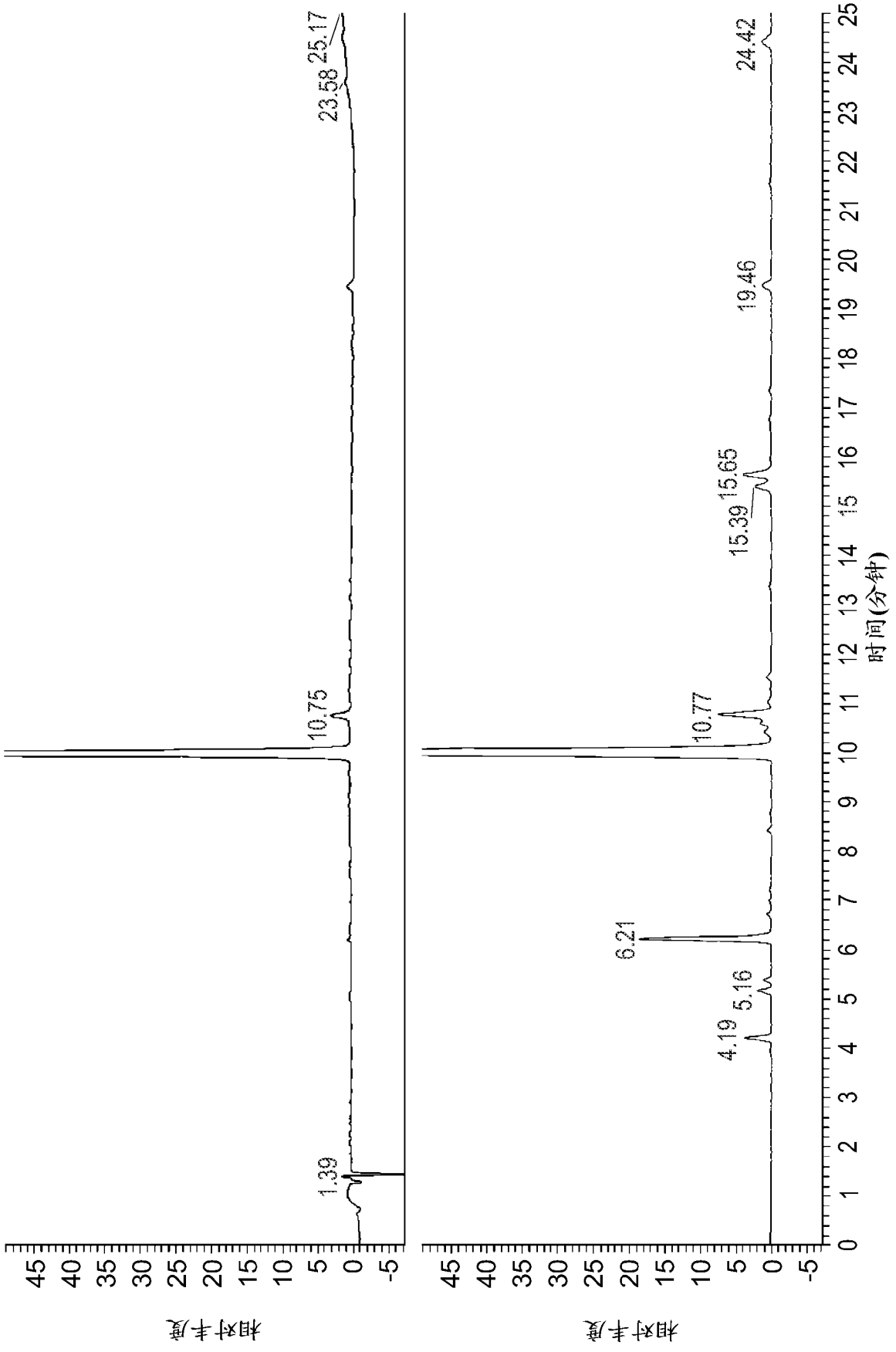

1.39
10.75
23.58
25.17
4.19
5.16
6.21
10.77
15.39
15.65
19.46
24.42
相对丰度
相对丰度
时间(分钟)

图 1

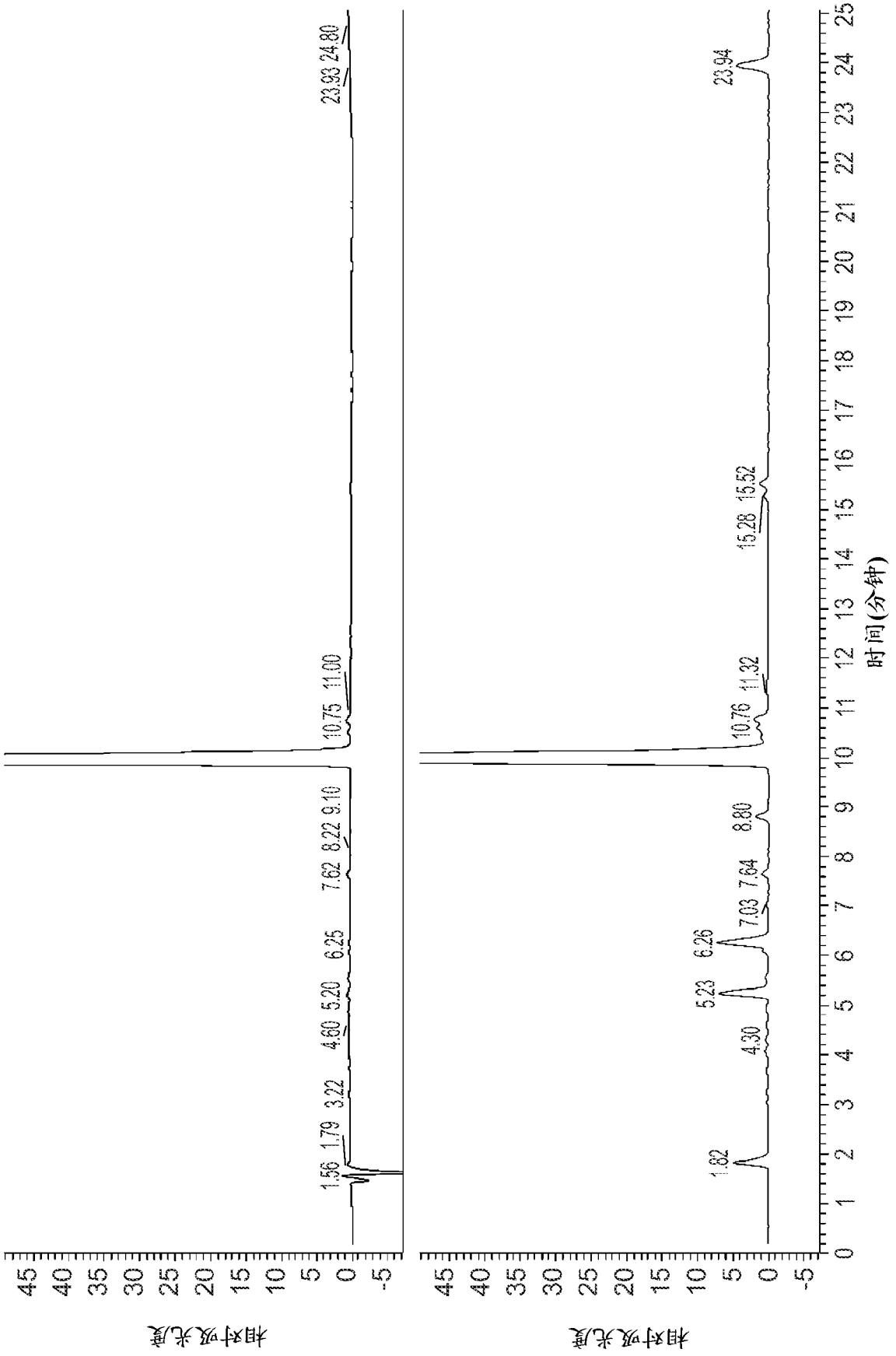

相对吸光度
1.56
1.79
3.22
4.60
5.20
6.25
7.62
8.22
9.10
10.75
11.00
23.93
24.80
相对吸光度
1.82
4.30
5.23
6.26
7.03
7.64
8.80
10.76
11.32
15.28
15.52
23.94
时间(分钟)

图 2

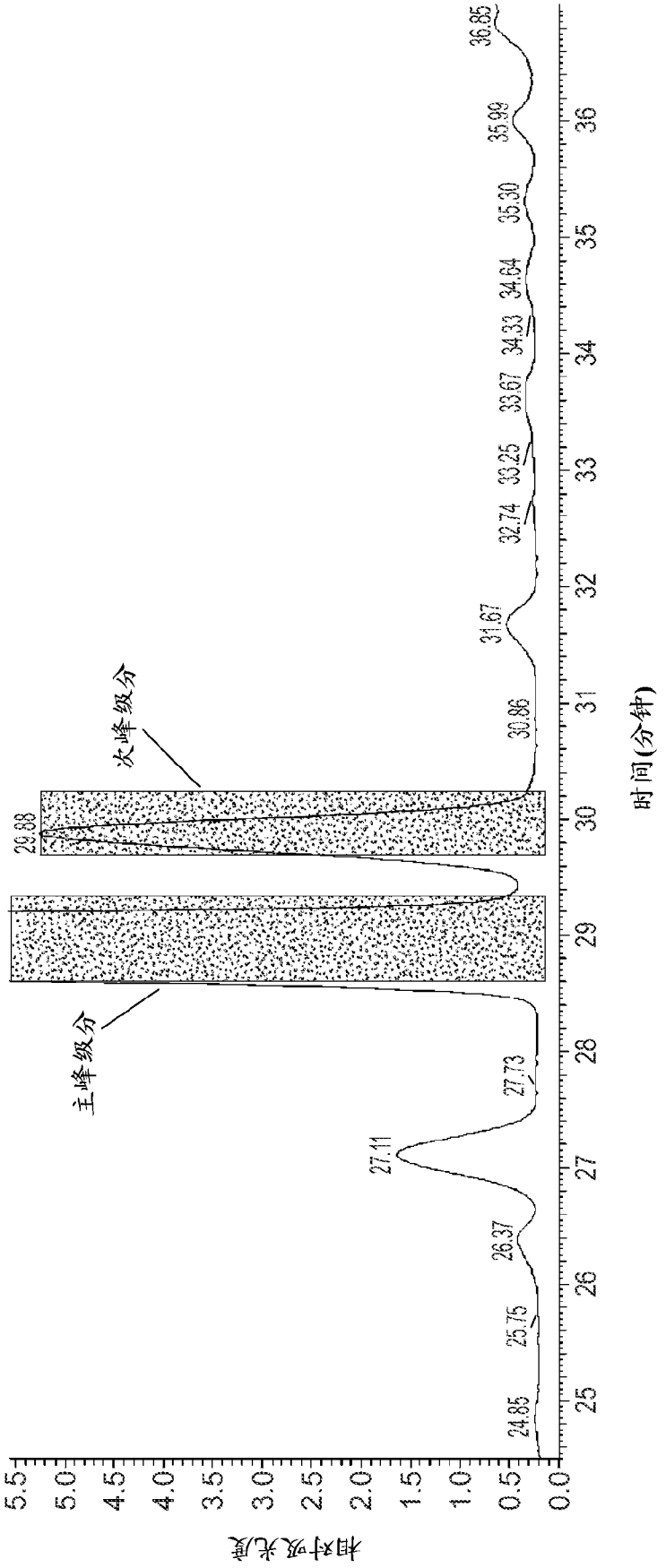

相对吸光度
时间(分钟)
主峰级分
次峰级分
24.85
25.75
26.37
27.11
27.73
29.88
30.86
31.67
32.74
33.25
33.67
34.33
34.64
35.30
35.99
36.85

图 3

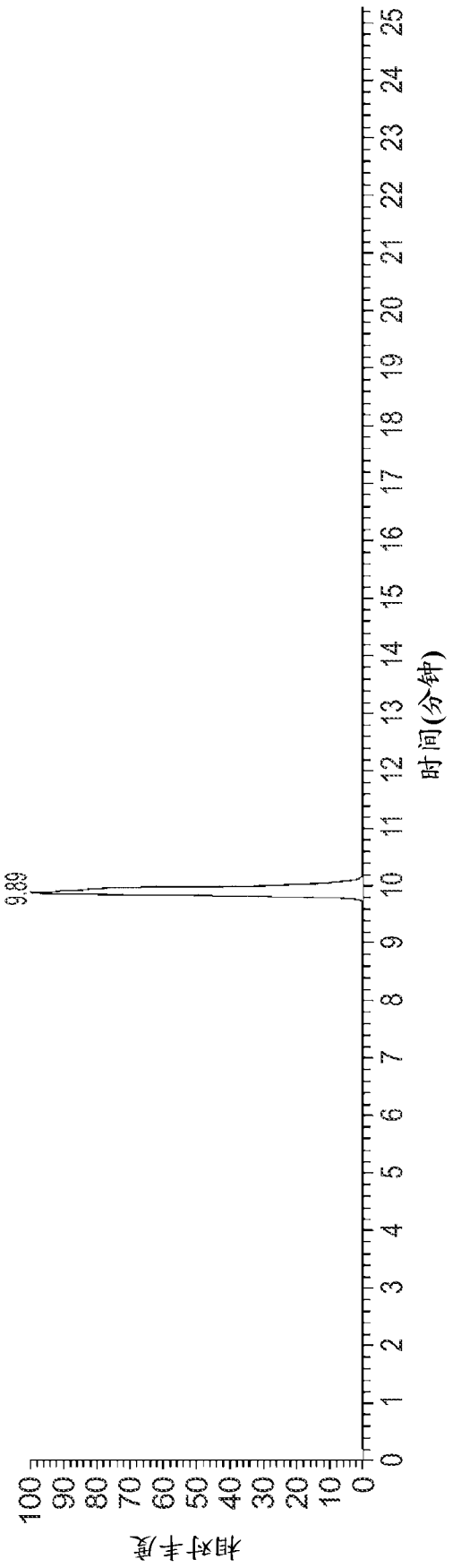


图 4A

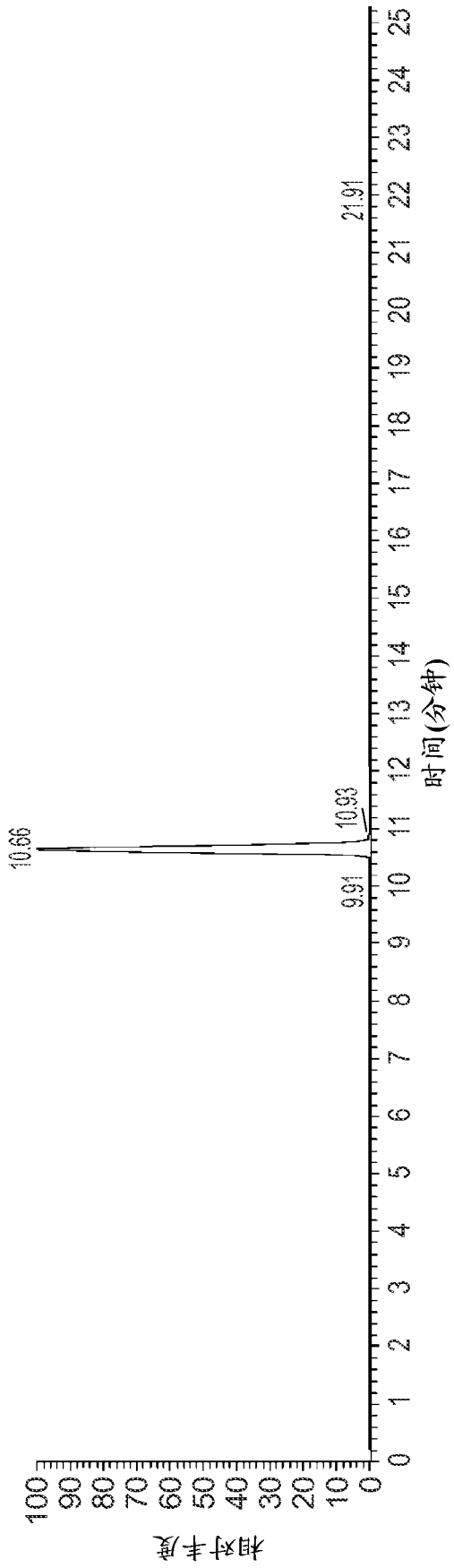


图 4B

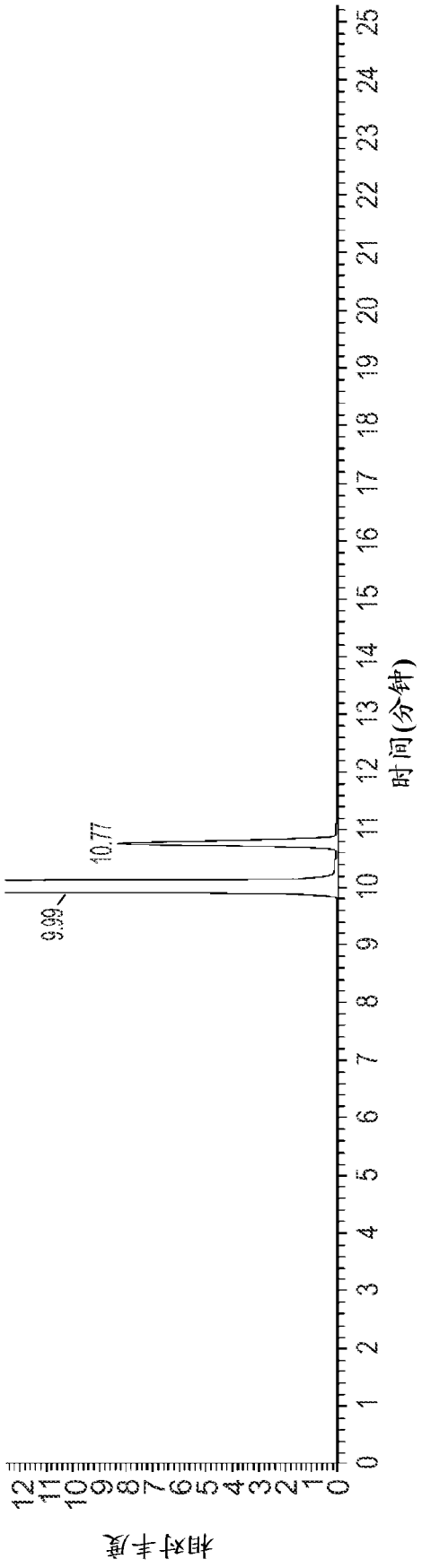


图 4C

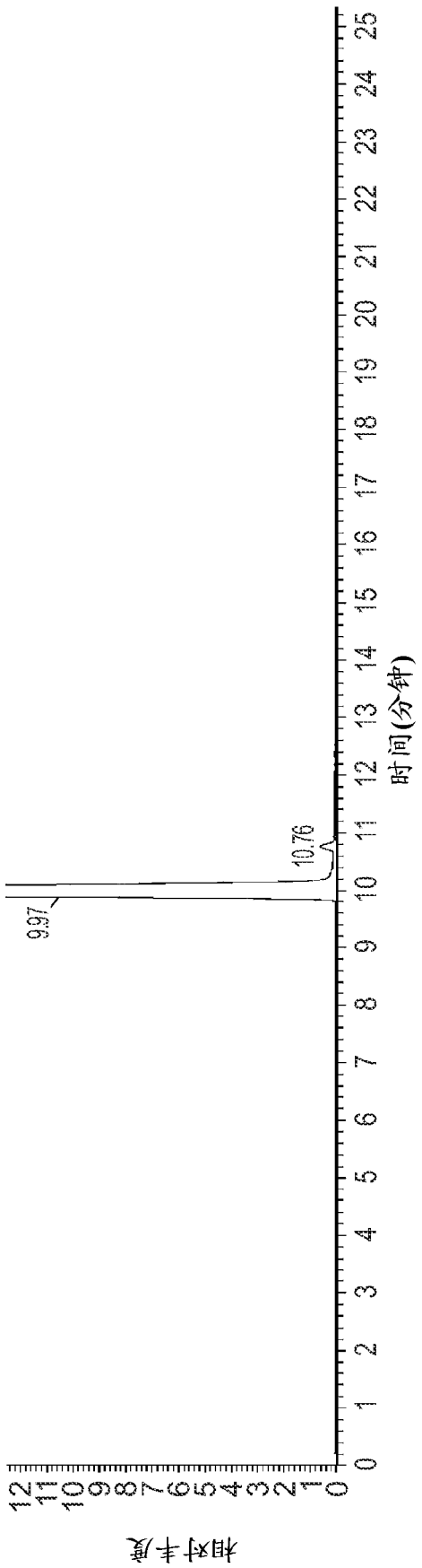


图 4D

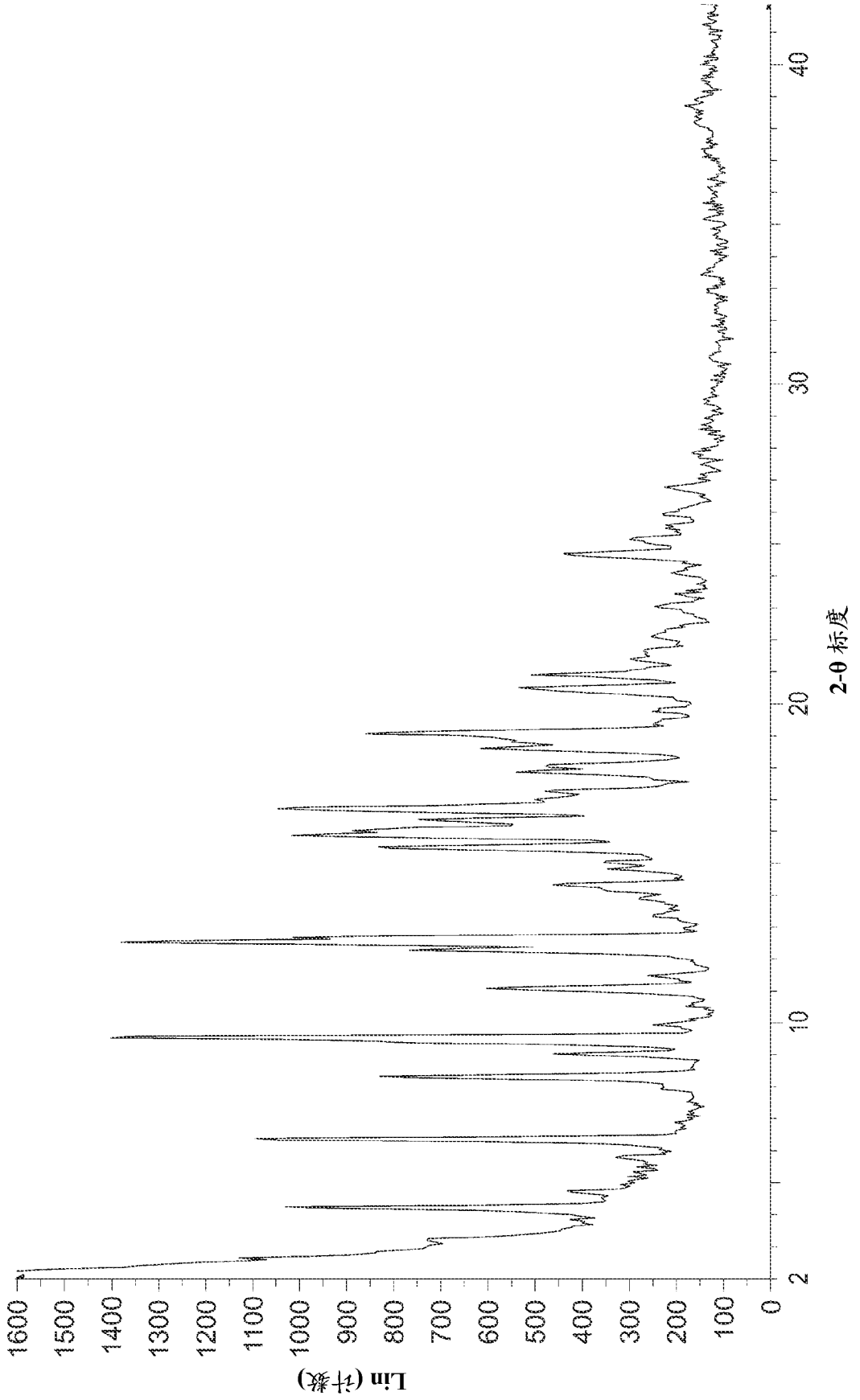

Lin (计数)
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2
10
20
30
40
2-θ 标度

图 5

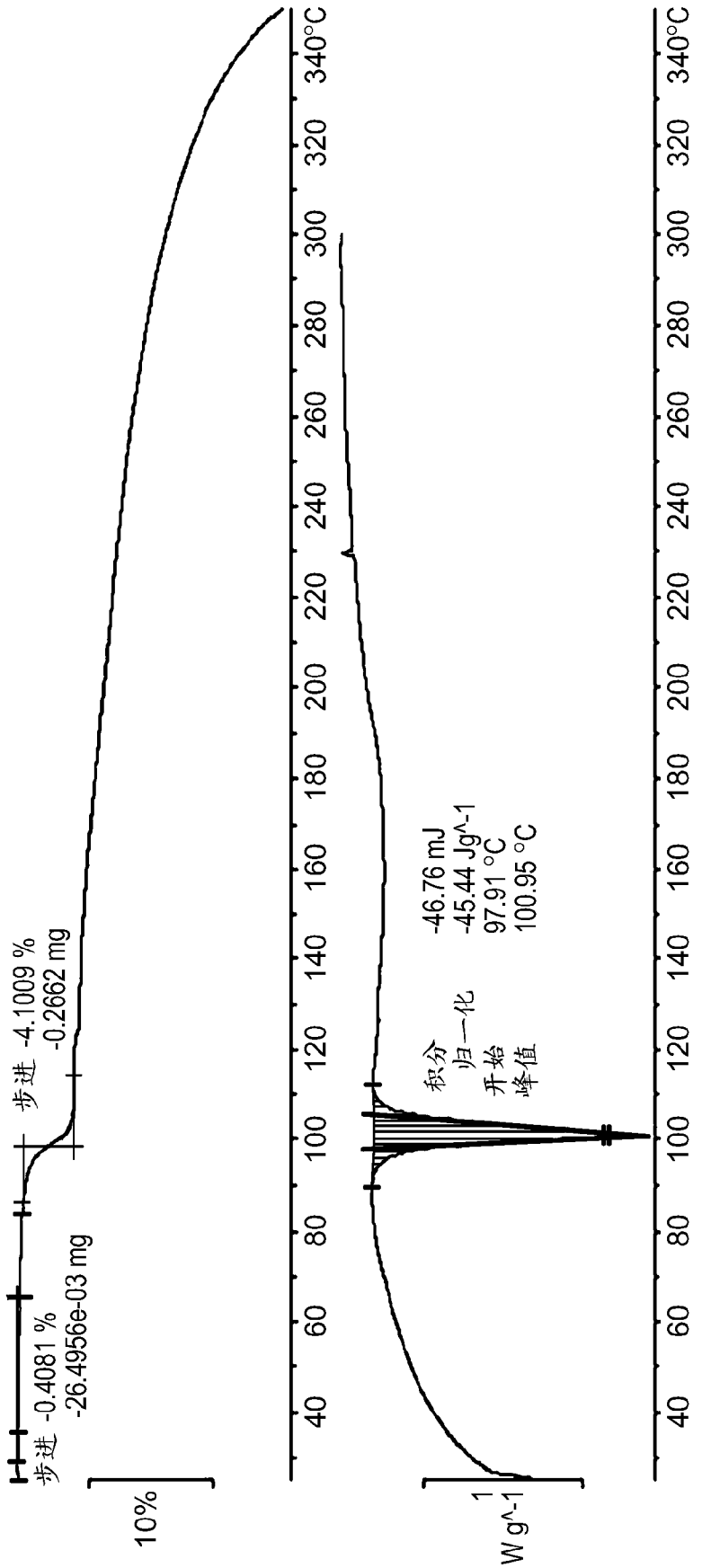

步进 -4.1009 %
-0.2662 mg
步进 -0.4081 %
-26.4956e-03 mg
10%
积分 -46.76 mJ
归一化 -45.44 Jg^-1
开始 97.91 °C
峰值 100.95 °C
1
W g^-1
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340°C

图 6

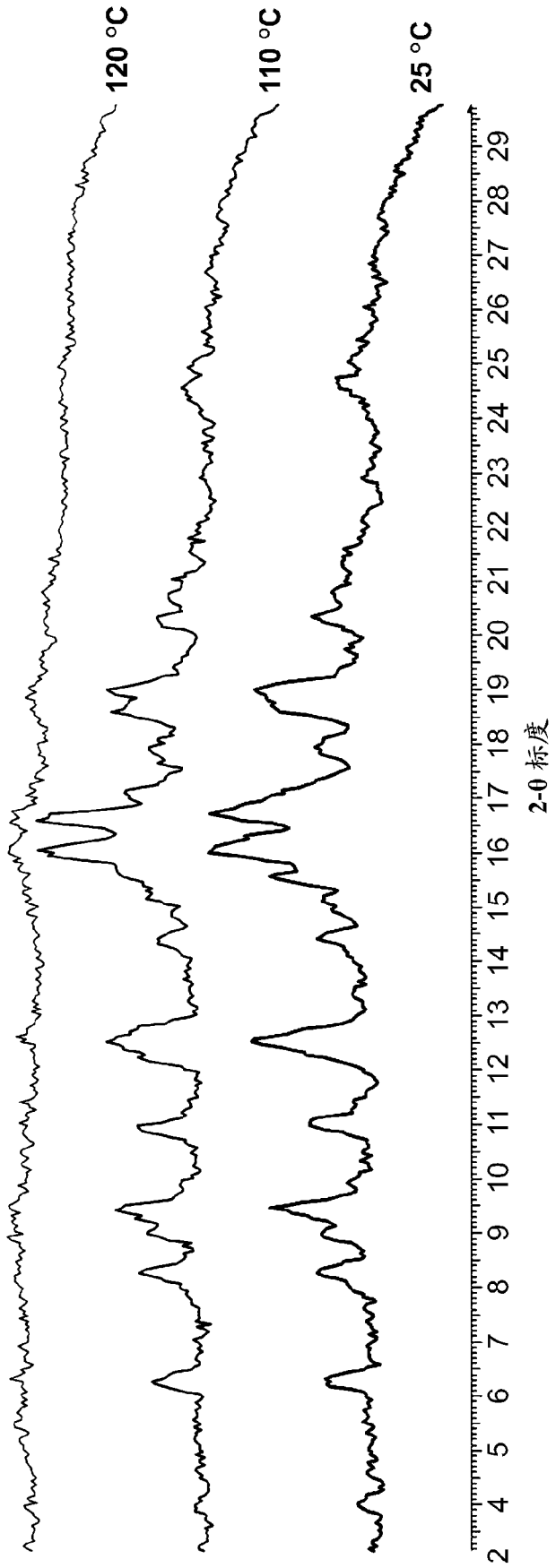

120 °C
110 °C
25 °C
2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
2-θ 标度

图 7

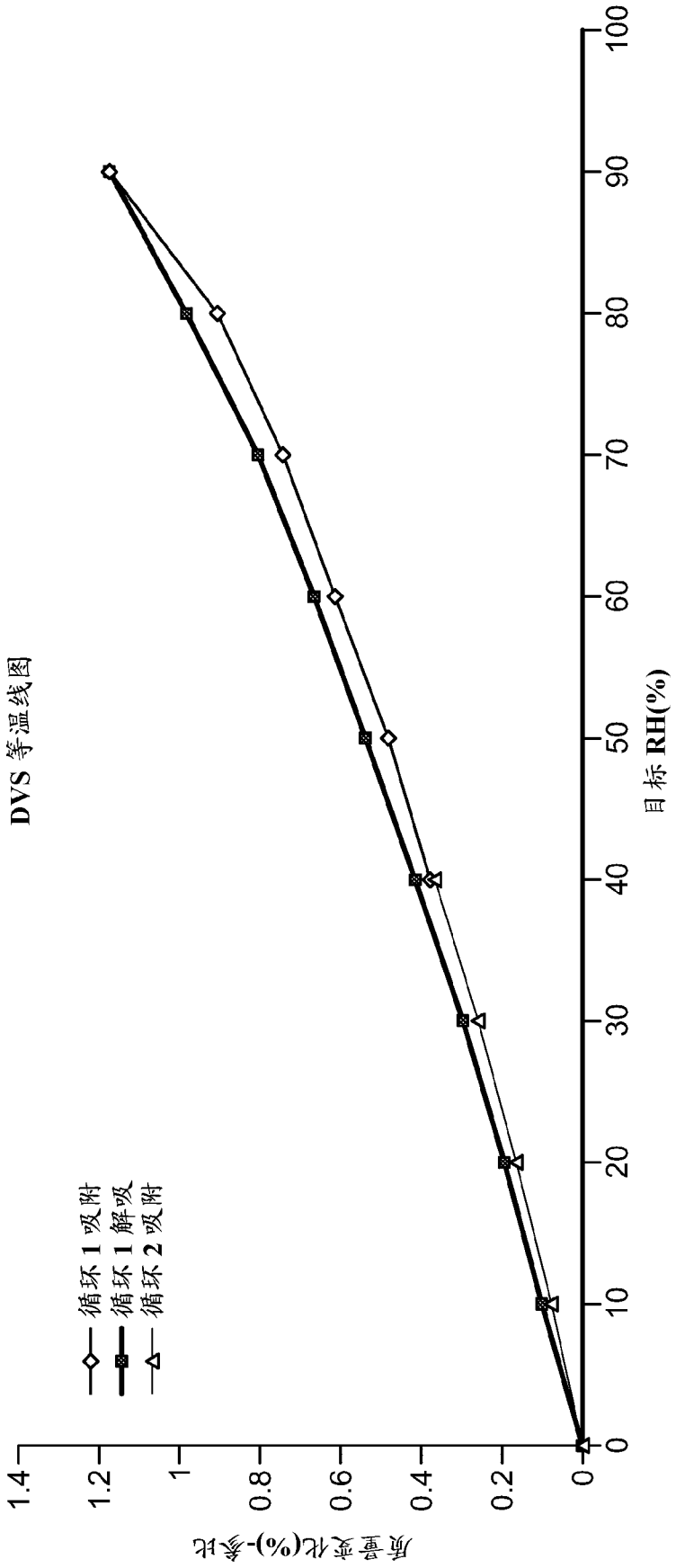

DVS 等温线图
循环 1 吸附
循环 1 解吸
循环 2 吸附
质量变化(%)-参比
目标 RH(%)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
10
20
30
40
50
60
70
80
90
100

图 8A

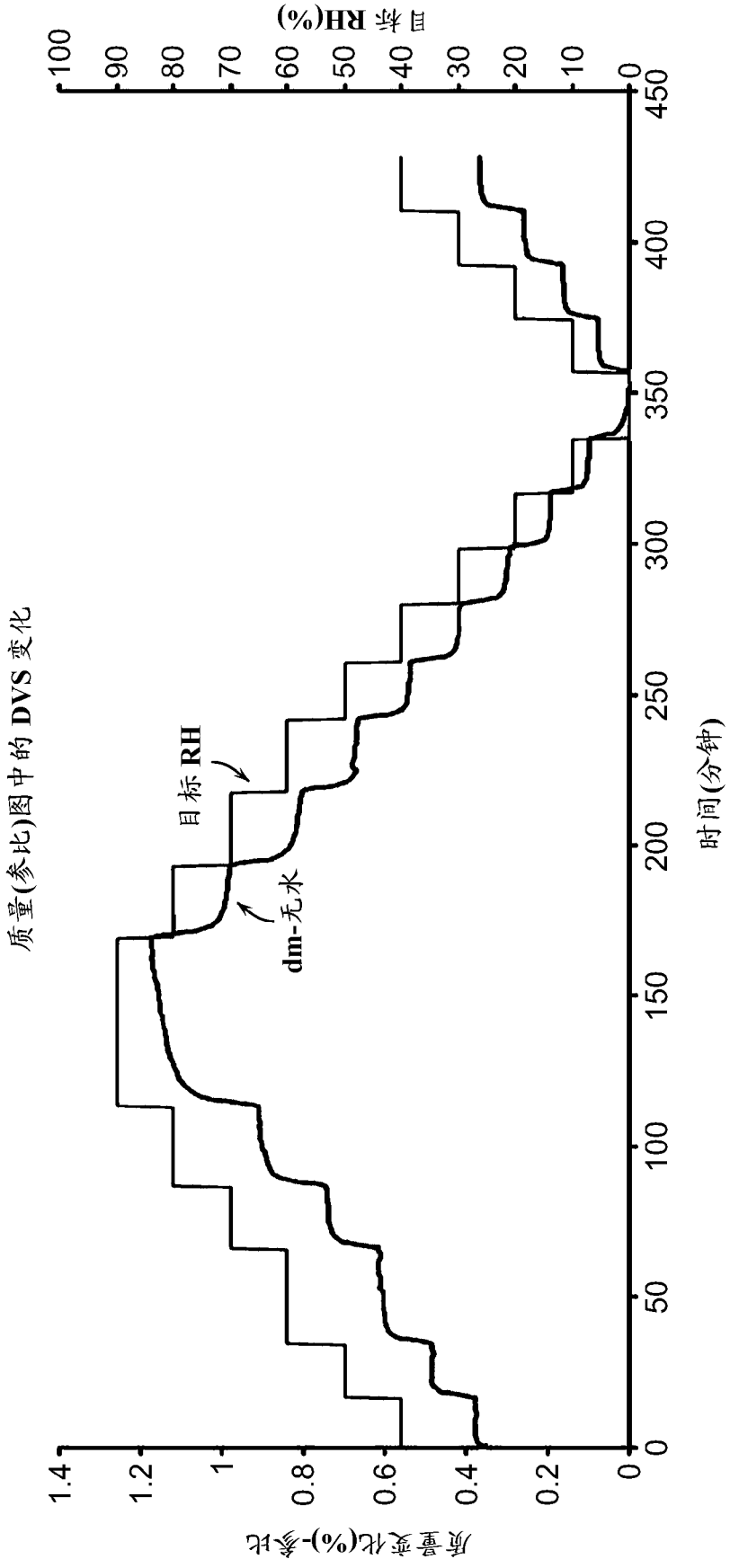

质量(参比)图中的 DVS 变化
质量变化(%)-参比
目标 RH(%)
时间(分钟)
目标 RH
dm-无水
0
0.2
0.4
0.6
0.8
1
1.2
1.4
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450

图 8B

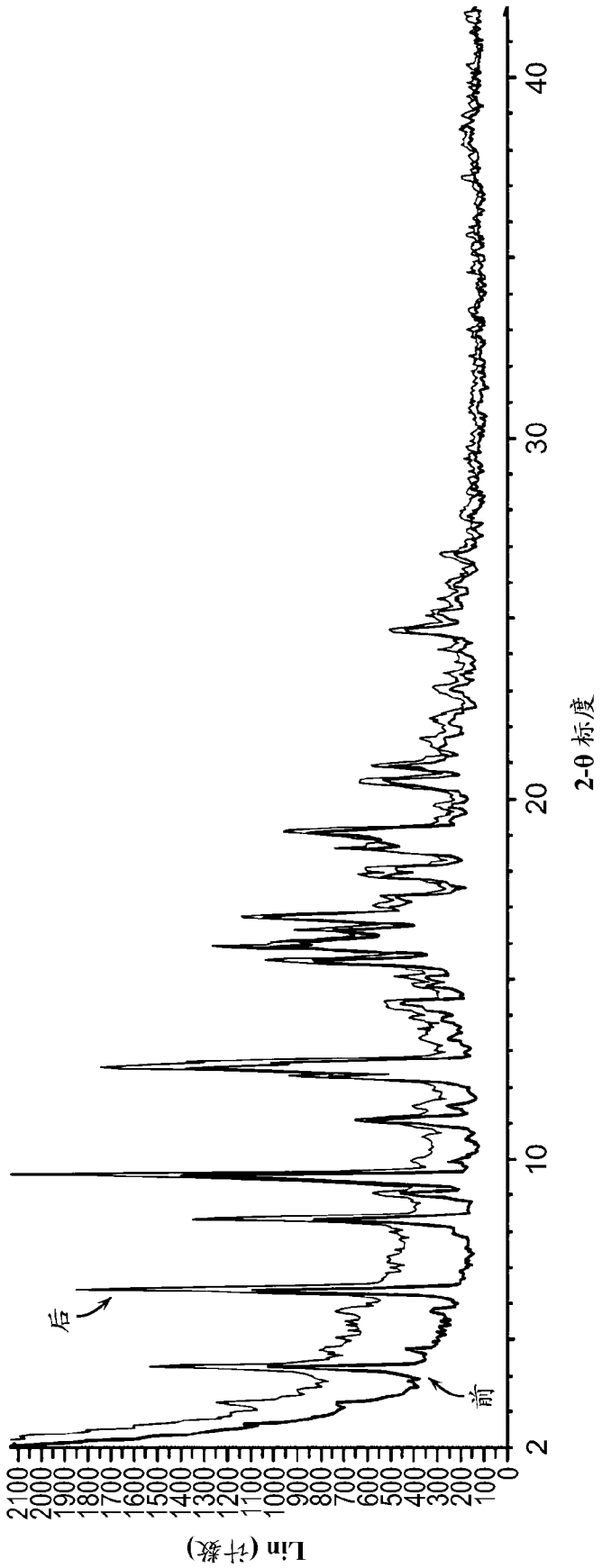

Lin (计数)
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2
10
20
30
40
2-θ 标度
后
前

图 8C

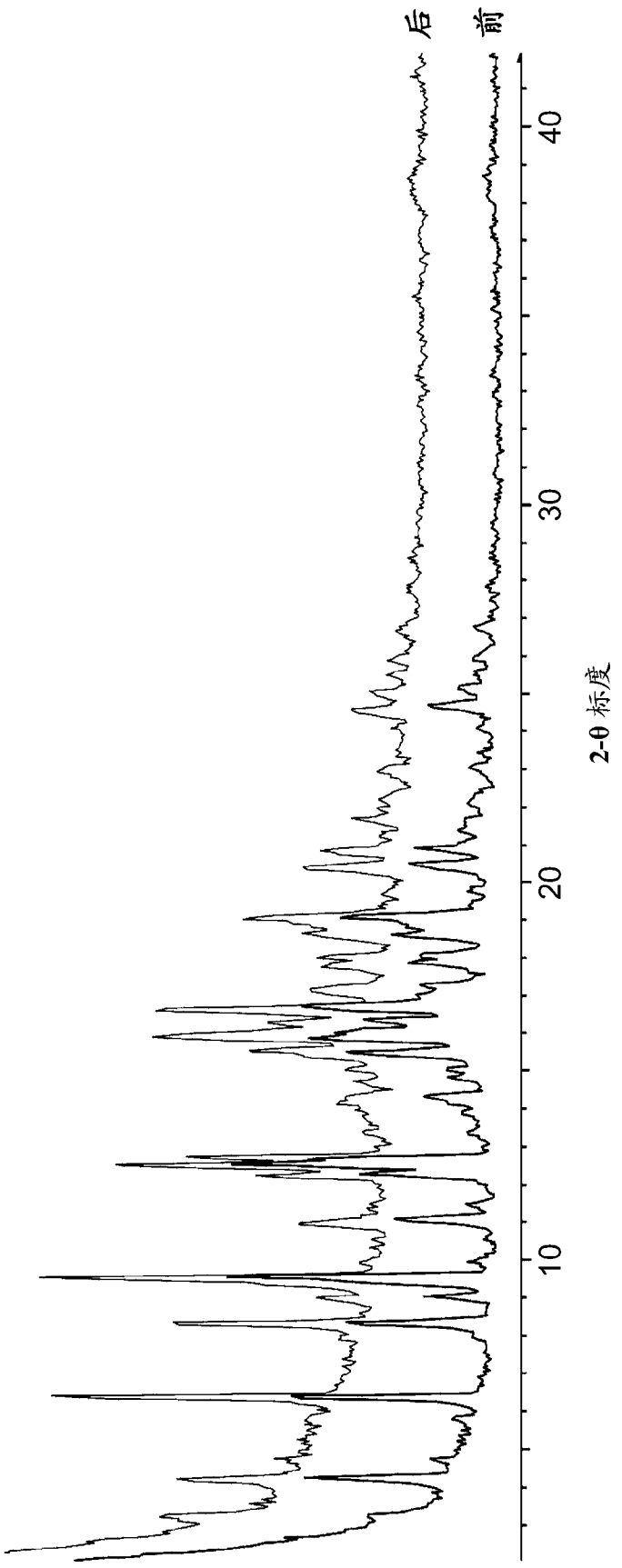


图 9

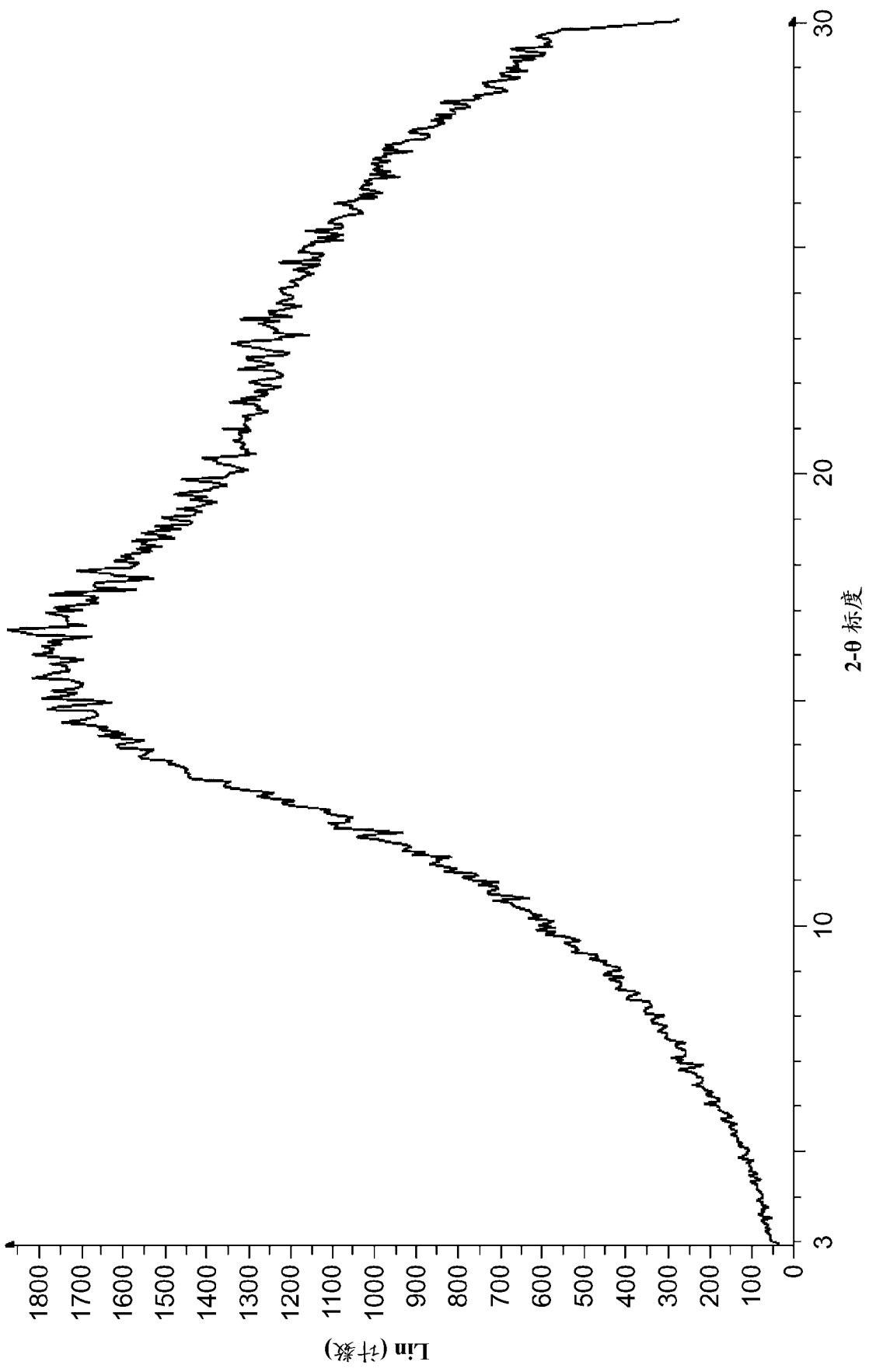

Lin (计数)
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
3
10
20
30
2-θ 标度

图 10

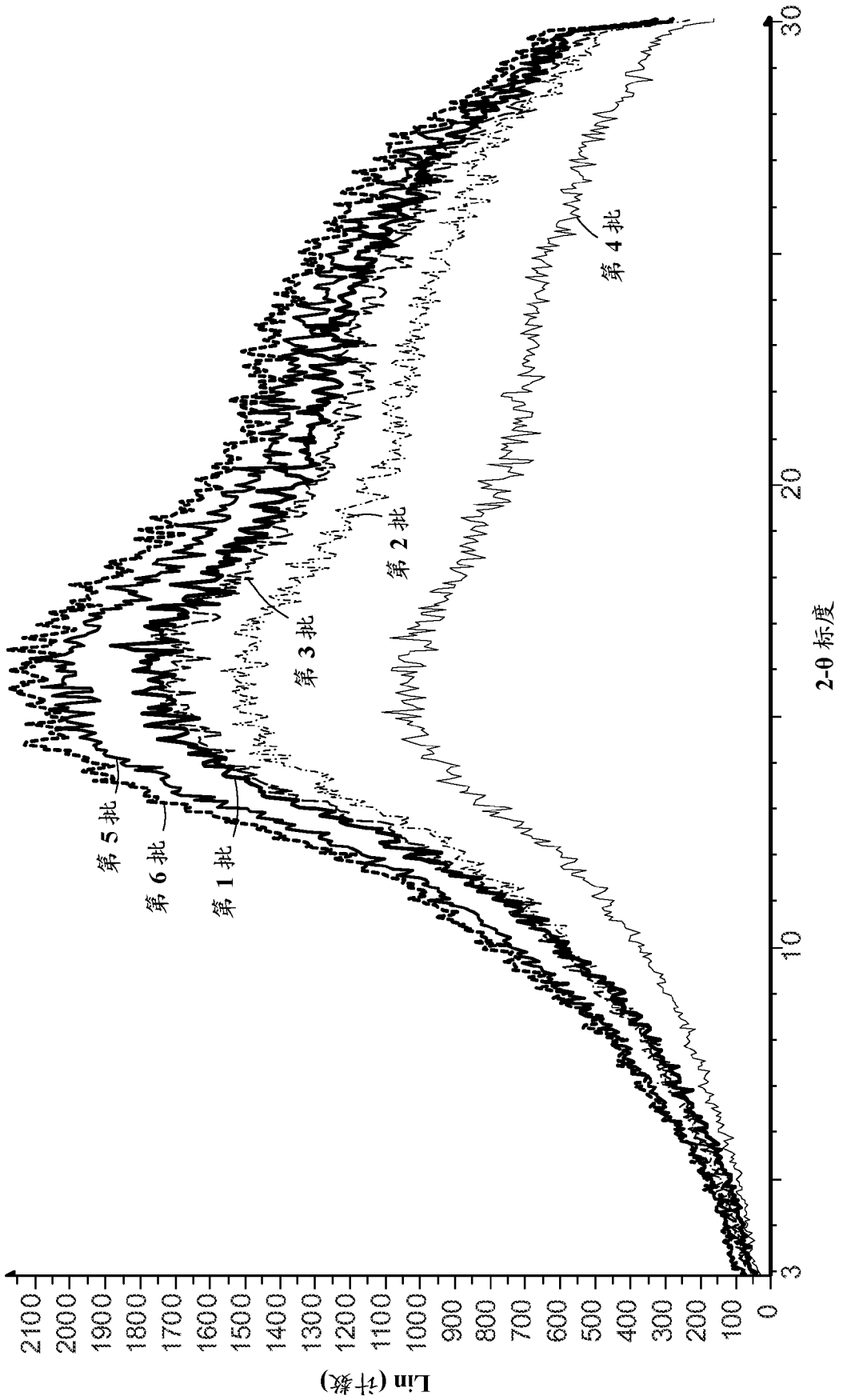


图 11

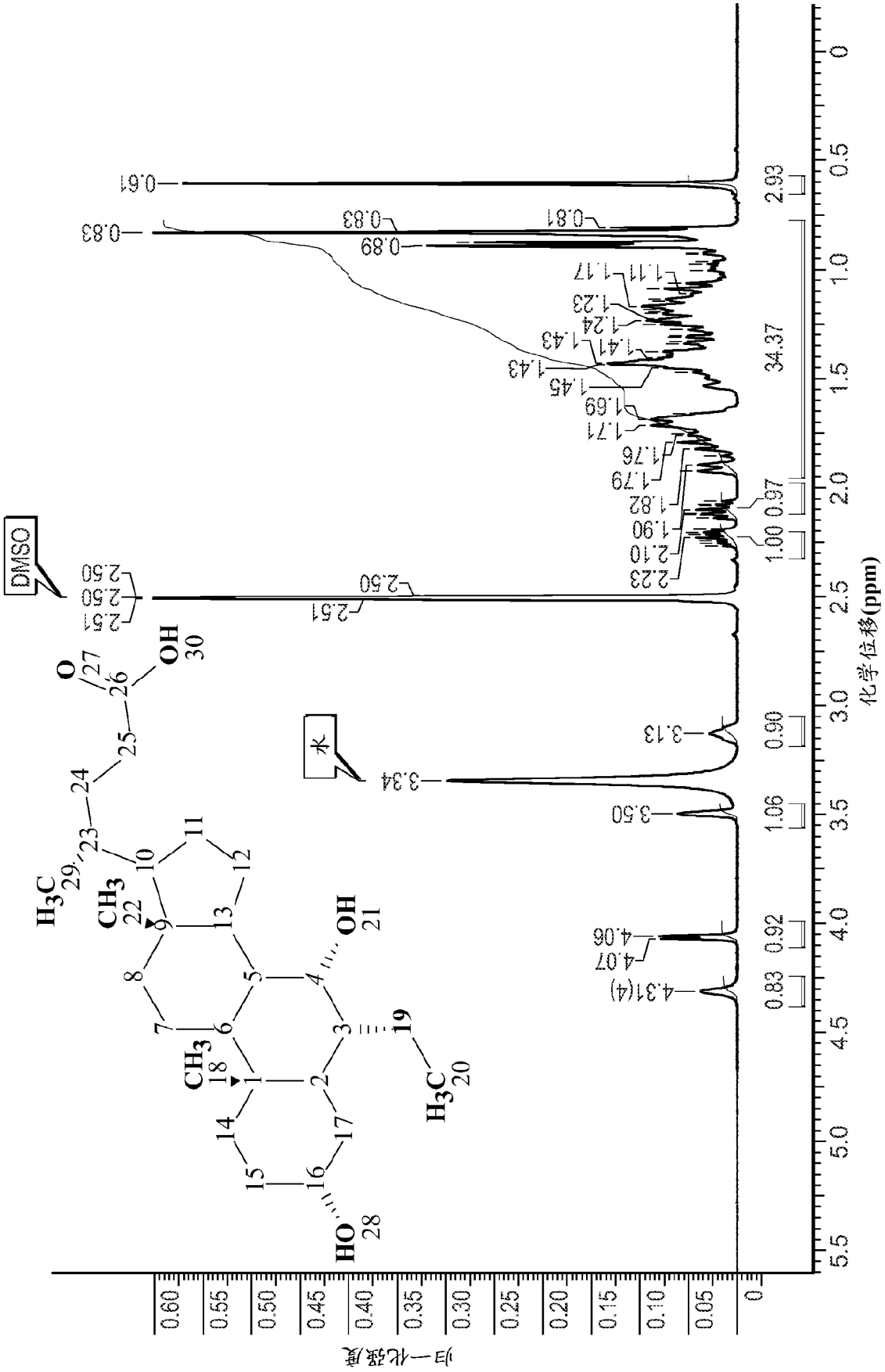


图 12

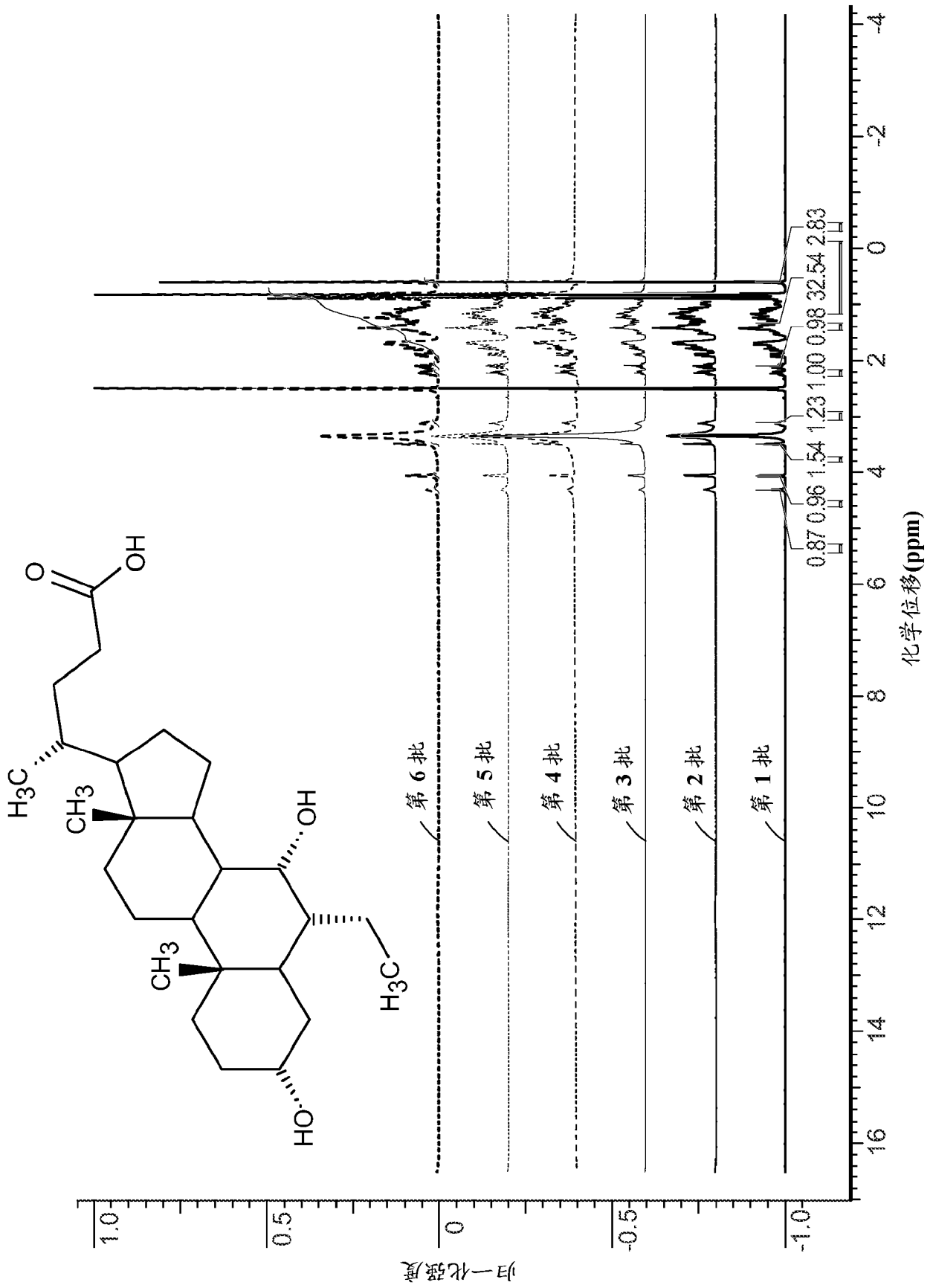

归一化强度
化学位移(ppm)
第 6 批
第 5 批
第 4 批
第 3 批
第 2 批
第 1 批
0.87 0.96 1.54 1.23 1.00 0.98 32.54 2.83
H3C
CH3
CH3
OH
HO
H3C
O
OH

图 13

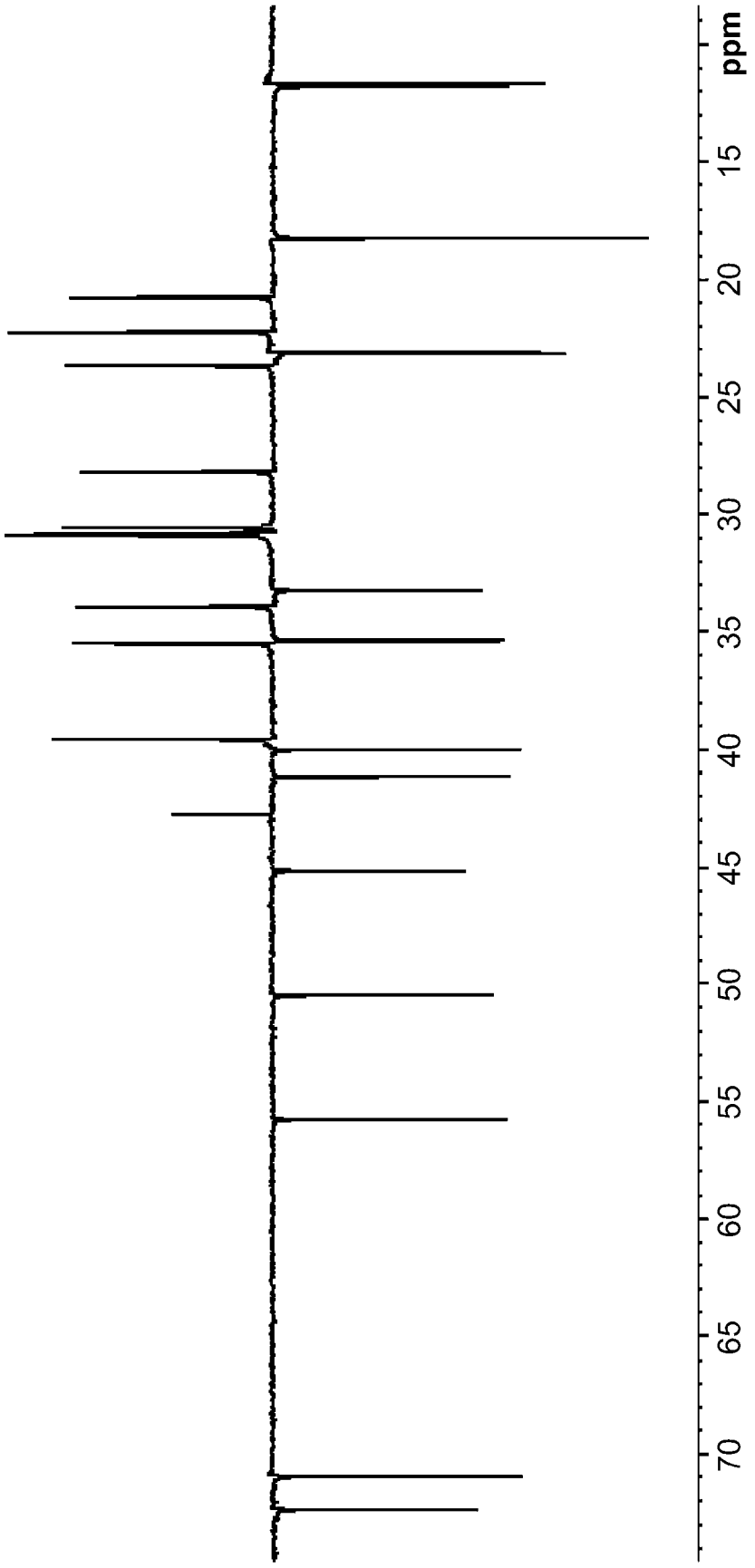


图 14

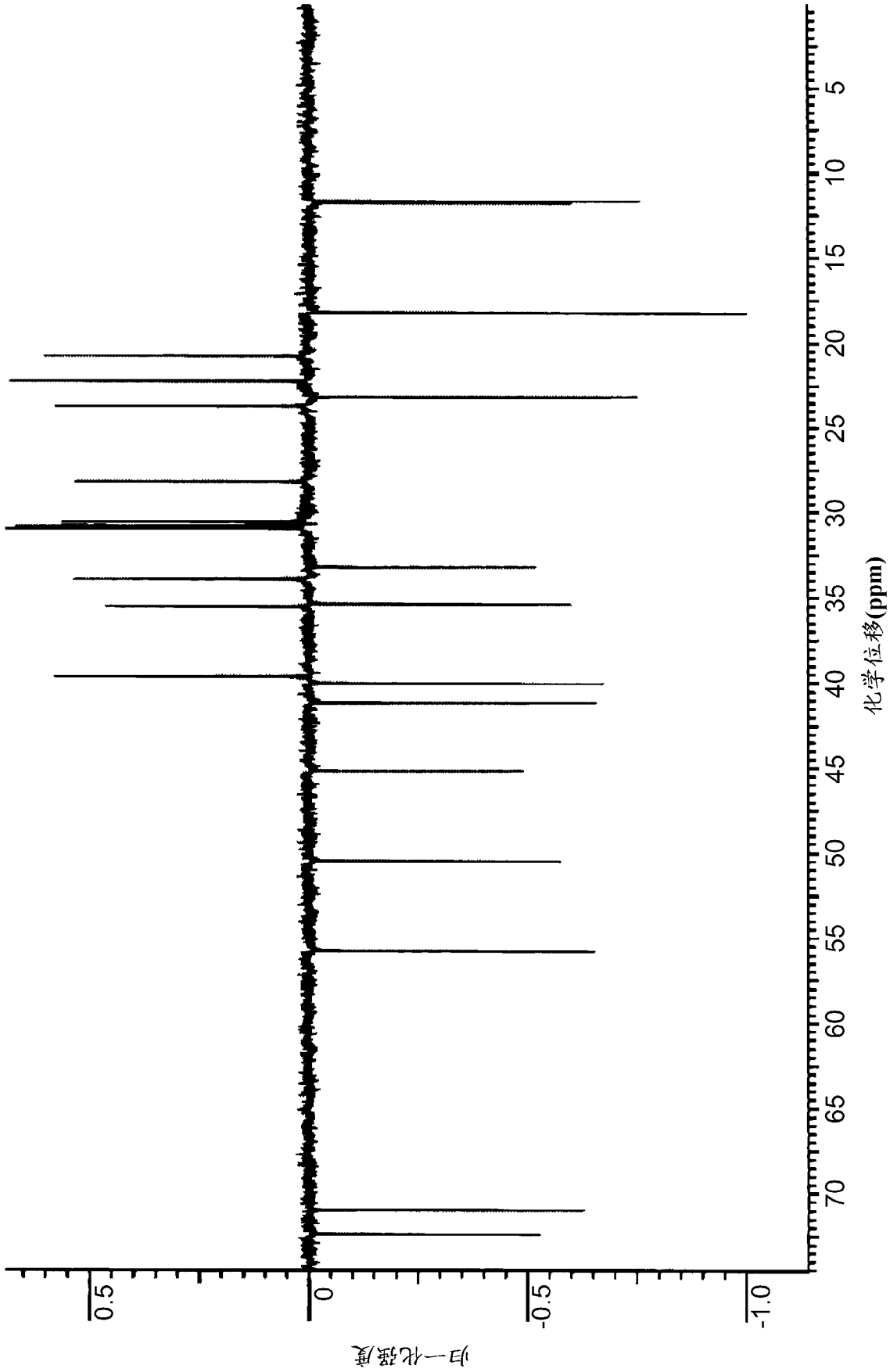


图 15

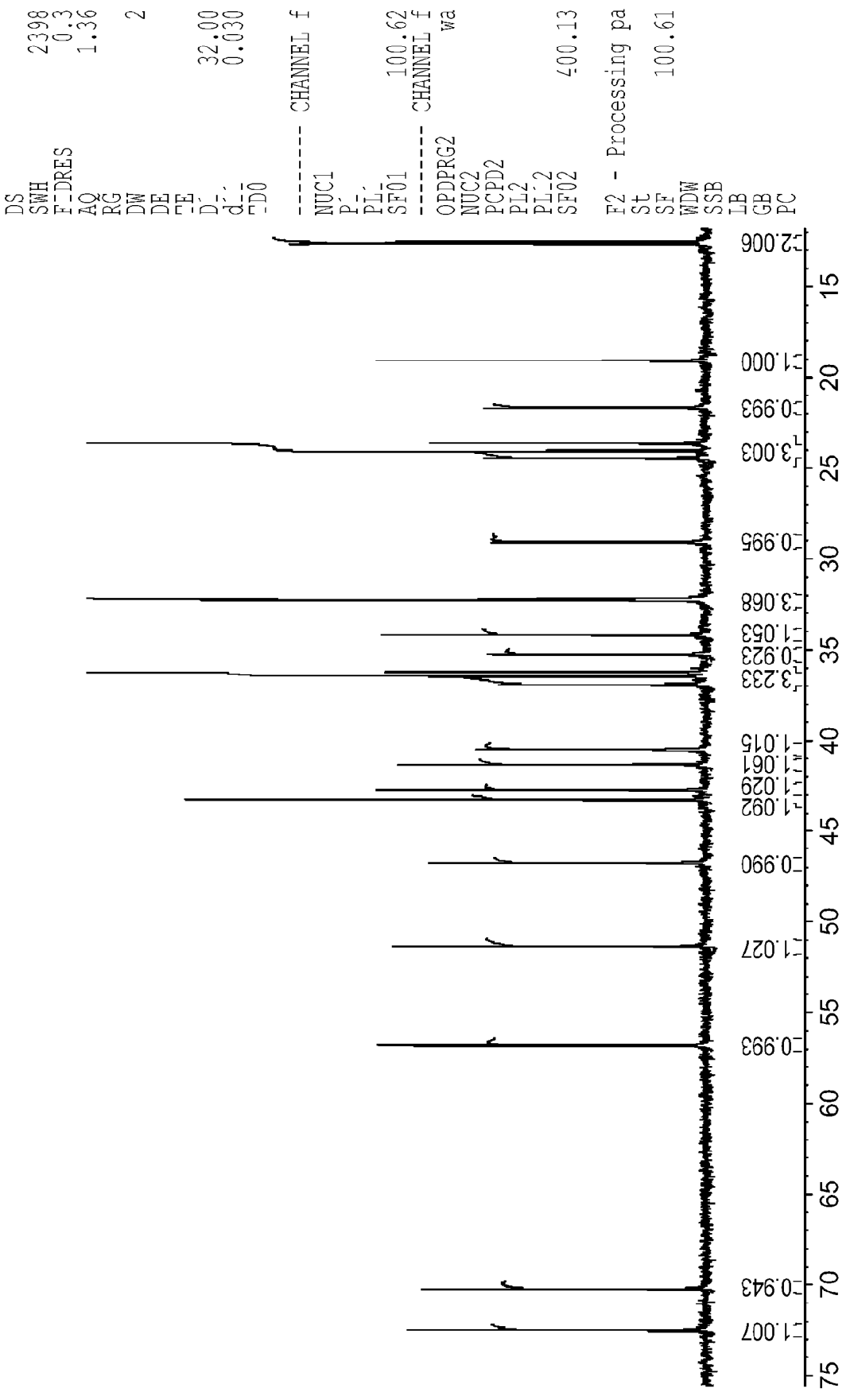

DS 2398
SWH 0.3
FIDRES 1.36
AQ
RG 2
DW
DE
TE 32.00
D1 0.030
TD0
-------- CHANNEL f
NUC1
P1
PL1 100.62
SF01 -------- CHANNEL f
OPDPRG2 wa
NUC2
PCPD2
PL2
PL12 400.13
SF02
F2 - Processing pa
St
SF 100.61
WDW
SSB
LB
GB
PC
2.006
1.000
0.993
3.003
0.995
3.068
1.053
0.923
3.233
1.015
1.061
1.029
1.092
0.990
1.027
0.993
0.943
1.007
15
20
25
30
35
40
45
50
55
60
65
70
75

图 16

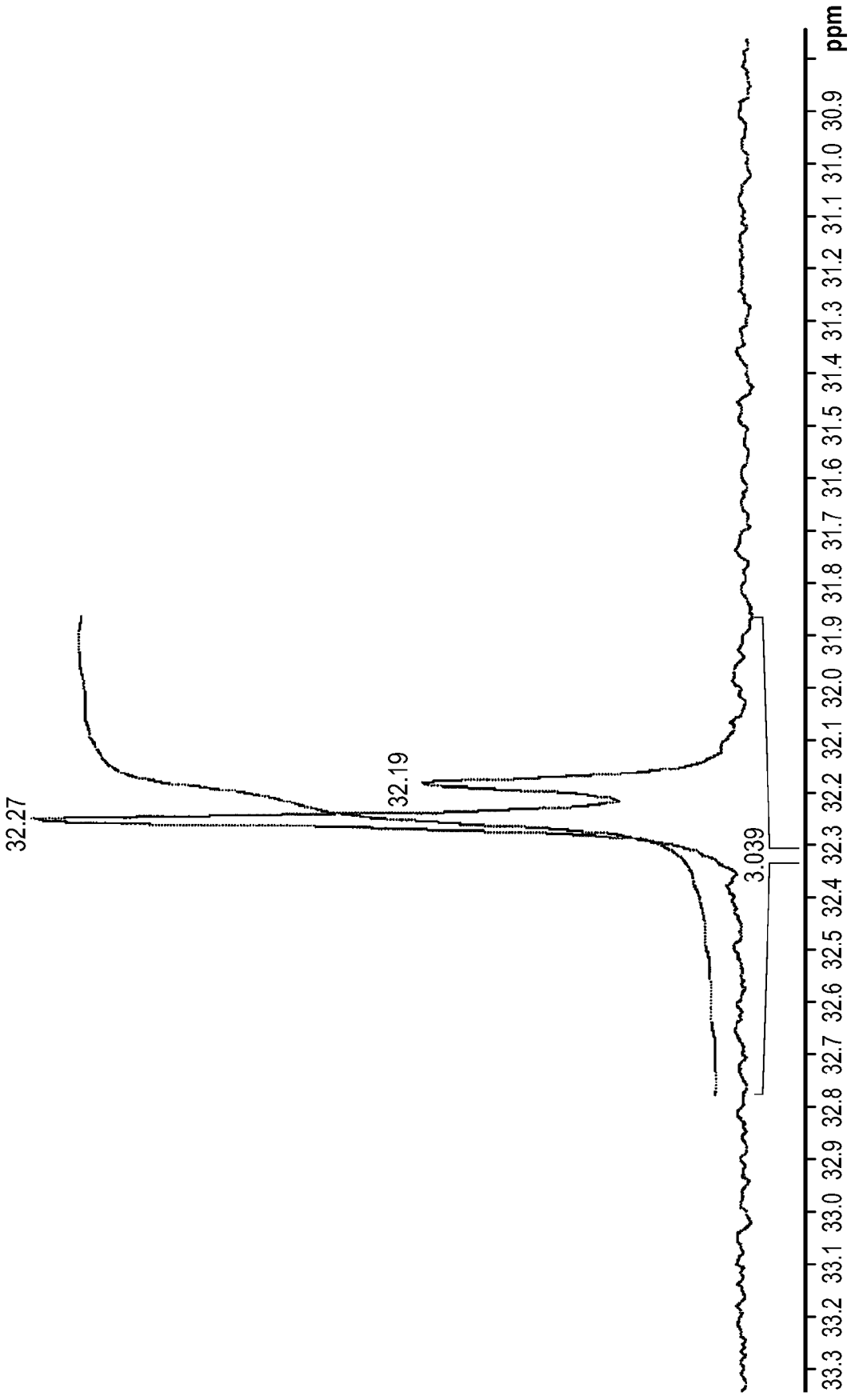

32.27
32.19
3.039
ppm
30.9
31.0
31.1
31.2
31.3
31.4
31.5
31.6
31.7
31.8
31.9
32.0
32.1
32.2
32.3
32.4
32.5
32.6
32.7
32.8
32.9
33.0
33.1
33.2
33.3

图 17

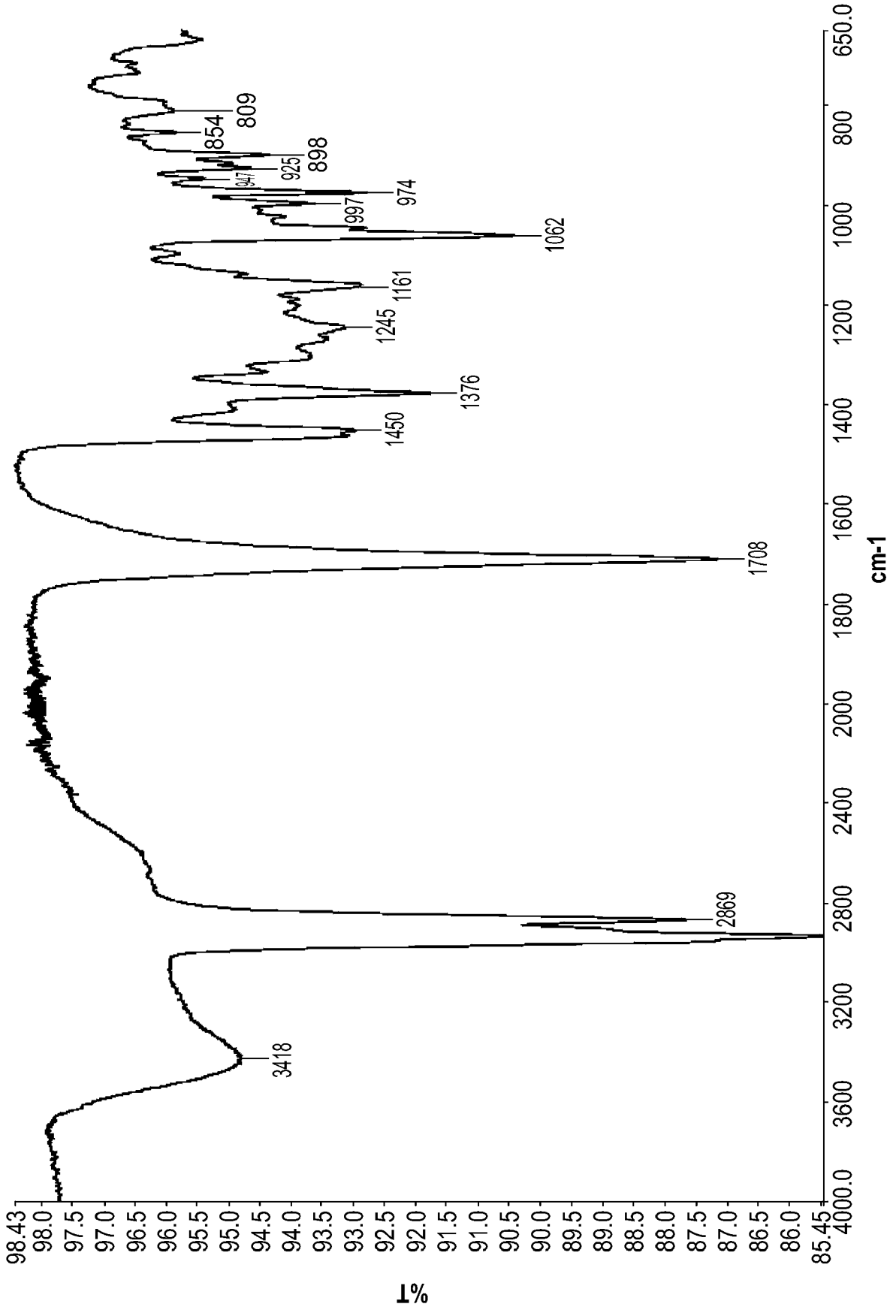

%T
cm-1
98.43
98.0
97.5
97.0
96.5
96.0
95.5
95.0
94.5
94.0
93.5
93.0
92.5
92.0
91.5
91.0
90.5
90.0
89.5
89.0
88.5
88.0
87.5
87.0
86.5
86.0
85.45
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650.0
3418
2869
1708
1450
1376
1245
1161
1062
997
974
947
925
898
854
809

图 18

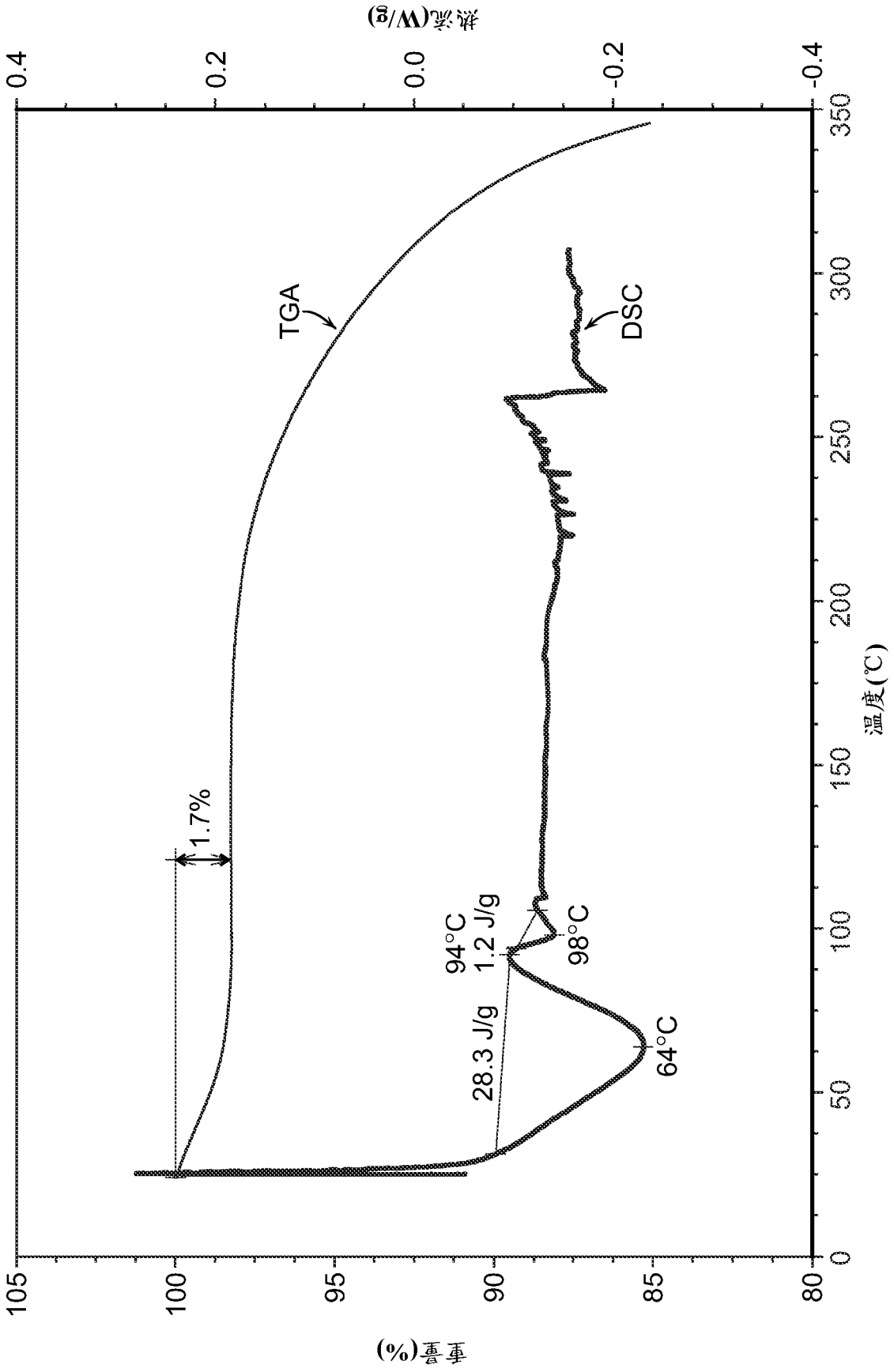

热流(W/g)
0.4
0.2
0.0
-0.2
-0.4
TGA
DSC
1.7%
94°C
1.2 J/g
98°C
28.3 J/g
64°C
105
100
95
90
85
80
重量(%)
0
50
100
150
200
250
300
350
温度(℃)

图 19

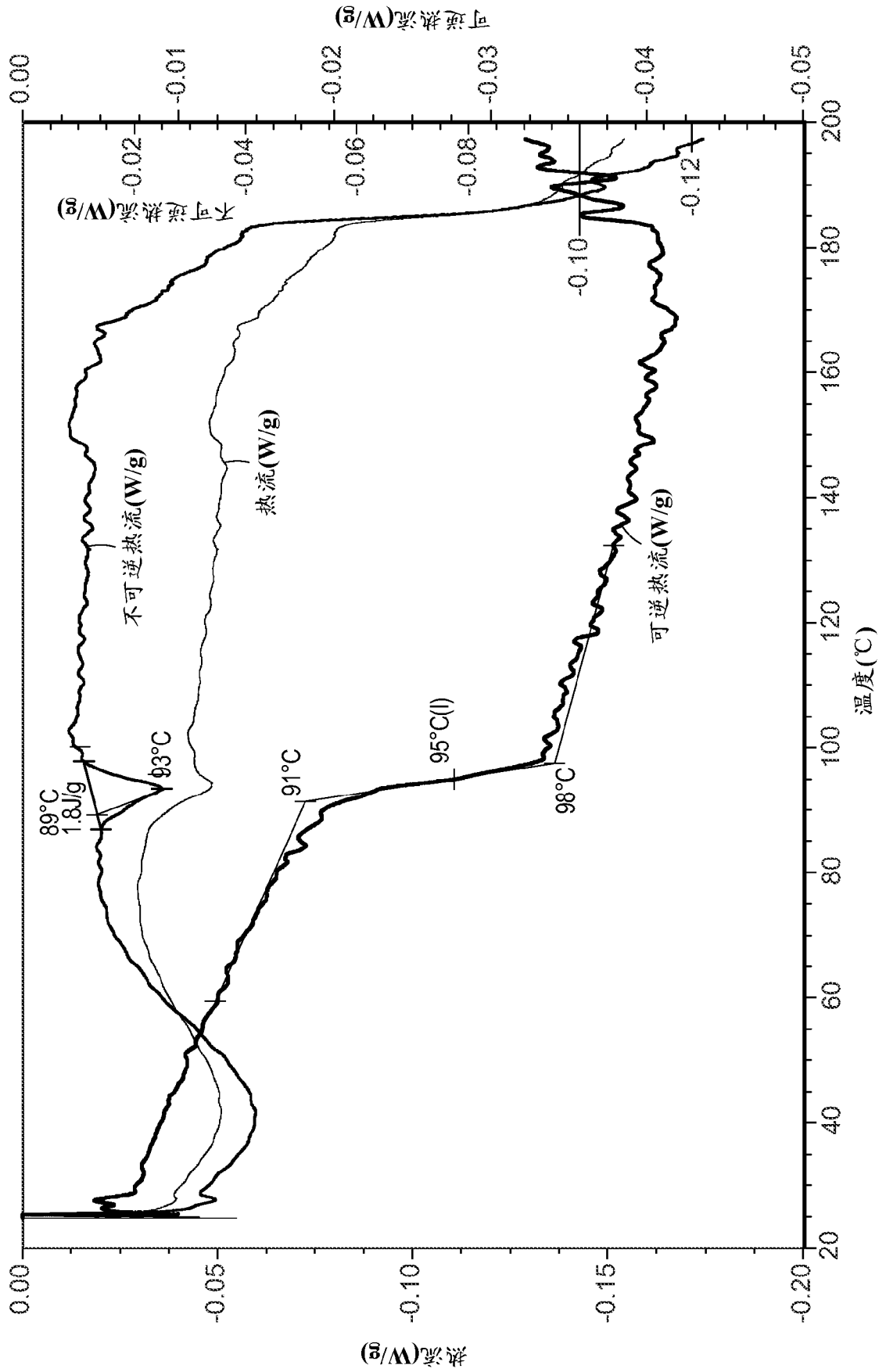

可逆热流(W/g)
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
不可逆热流(W/g)
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
不可逆热流(W/g)
热流(W/g)
可逆热流(W/g)
89°C
1.8J/g
93°C
91°C
95°C(l)
98°C
温度(℃)
20
40
60
80
100
120
140
160
180
200
热流(W/g)
0.00
-0.05
-0.10
-0.15
-0.20

图 20

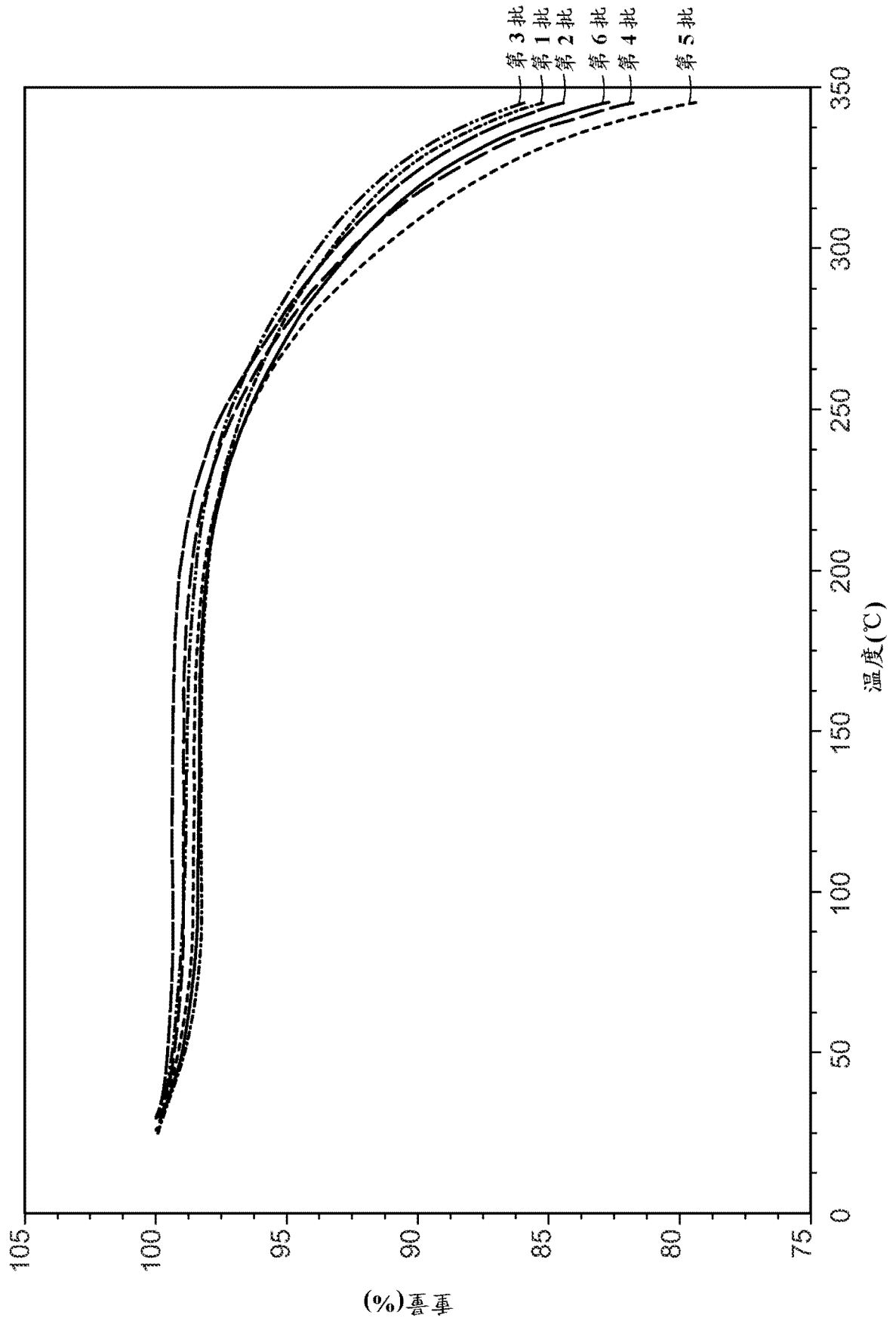

第3批
第1批
第2批
第6批
第4批
第5批
105
100
95
90
85
80
75
0
50
100
150
200
250
300
350
温度(℃)
重量(%)

图 21

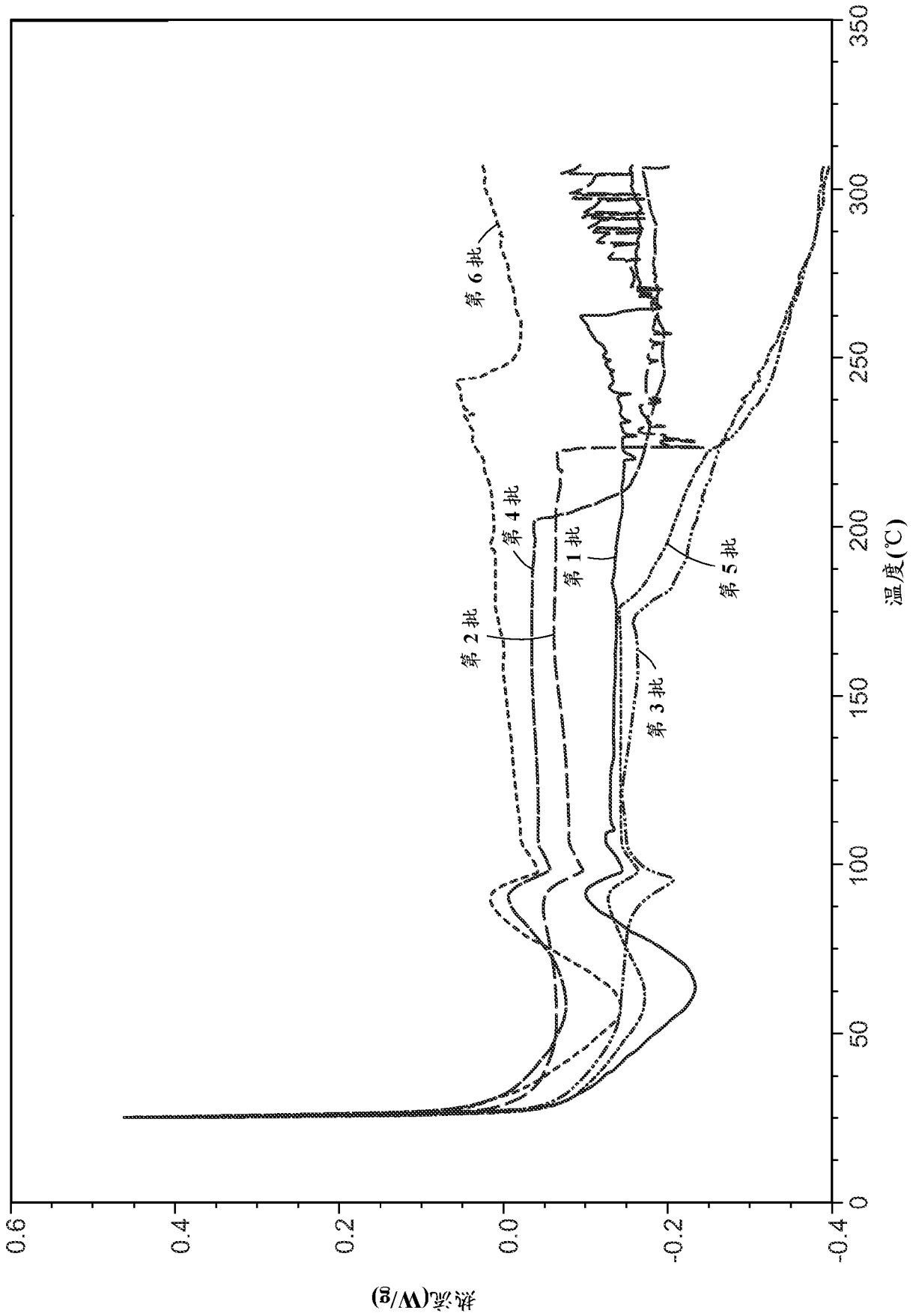

0.6
0.4
0.2
0.0
-0.2
-0.4
热流(W/g)
0
50
100
150
200
250
300
350
温度(℃)
第 6 批
第 4 批
第 1 批
第 2 批
第 5 批
第 3 批

图 22

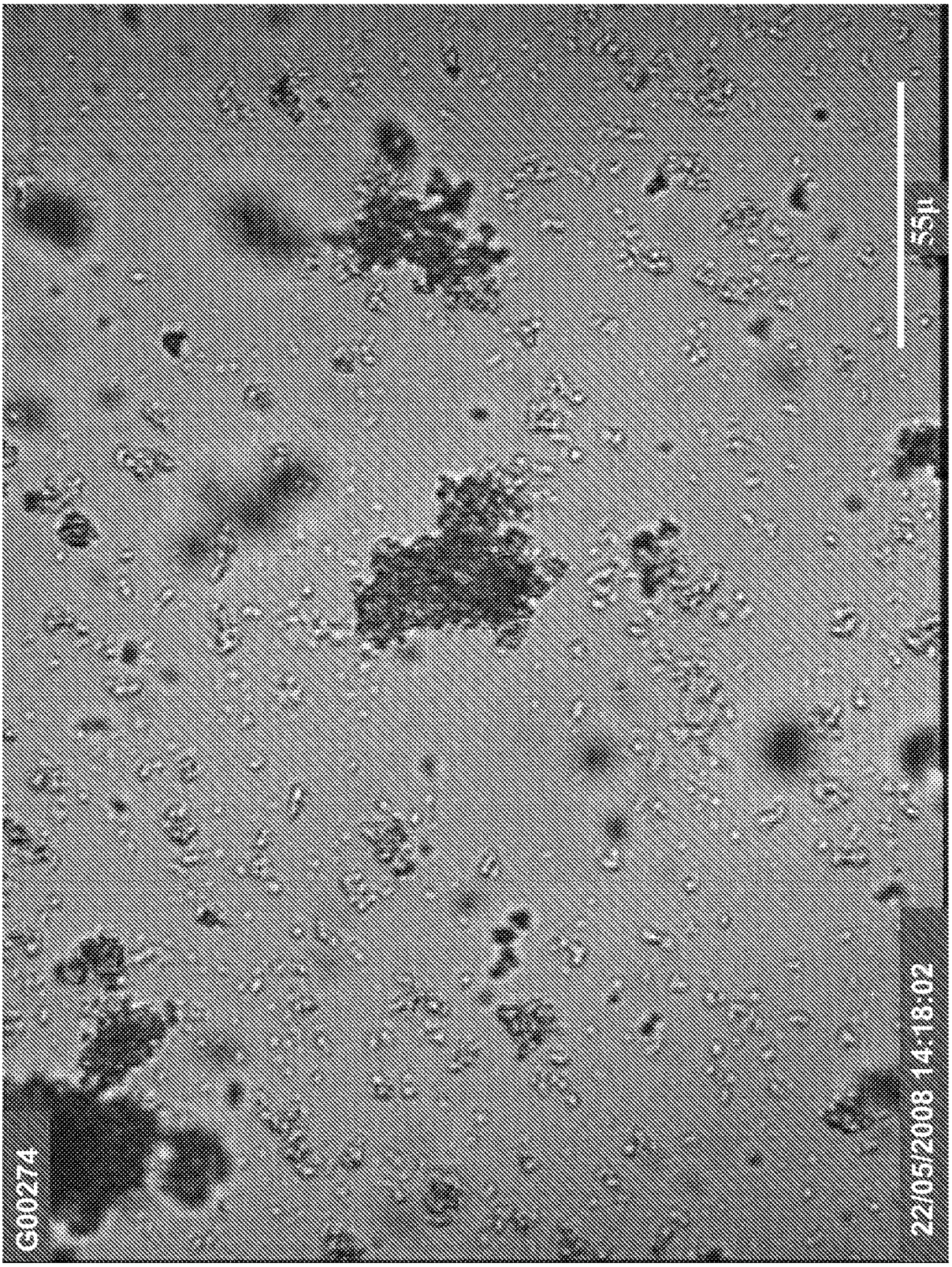


图 23A

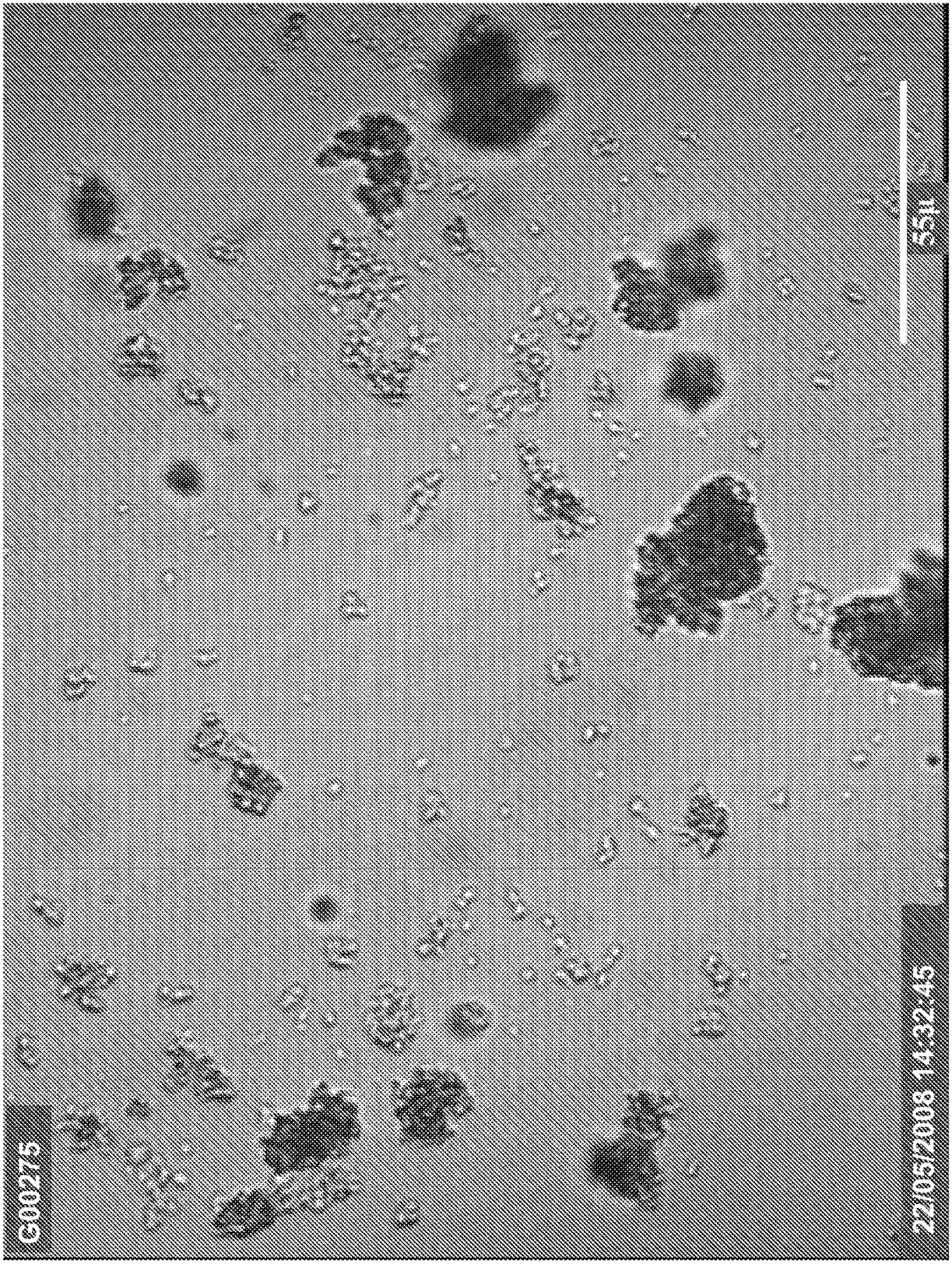


图 23B

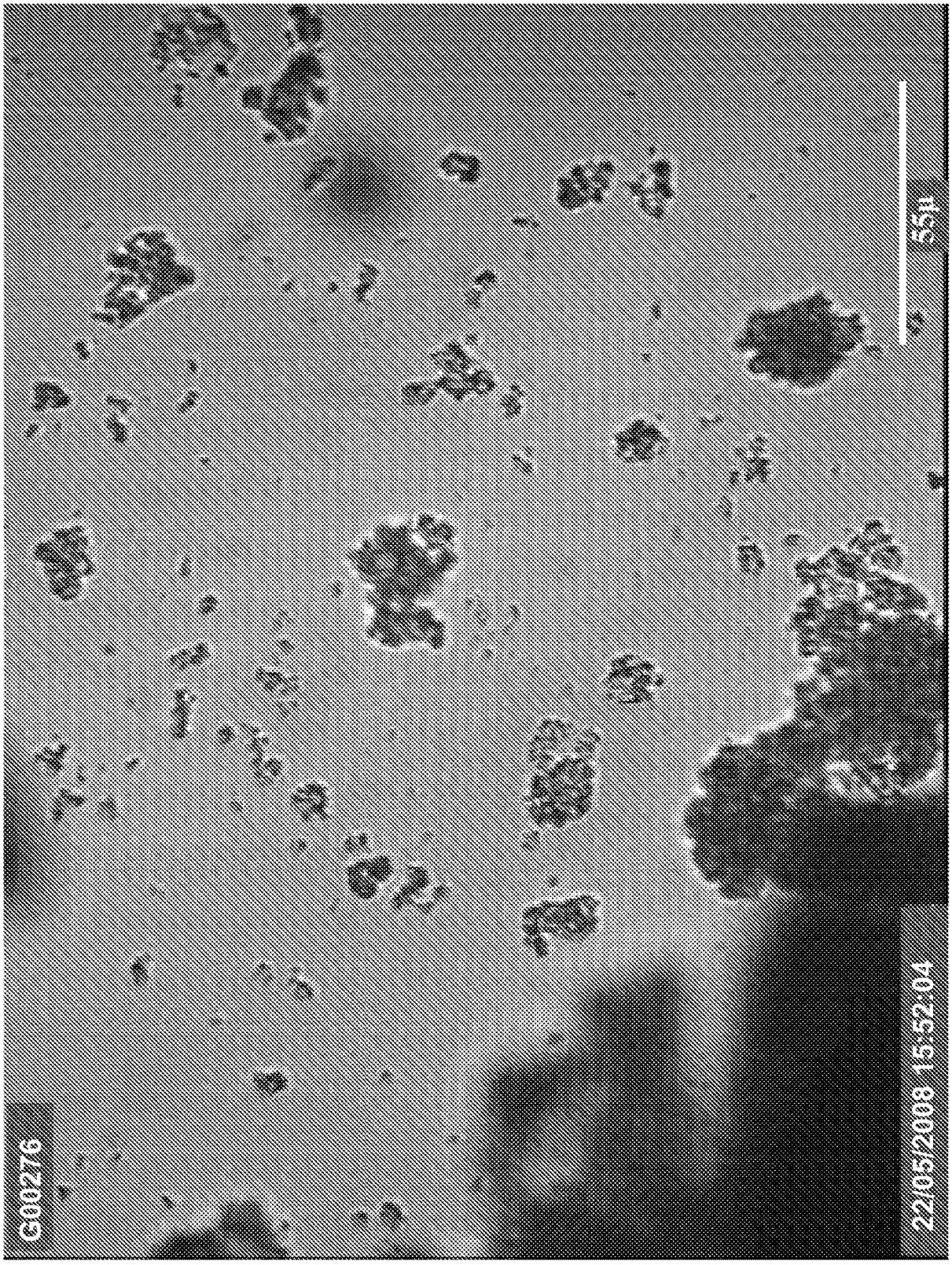


图 23C

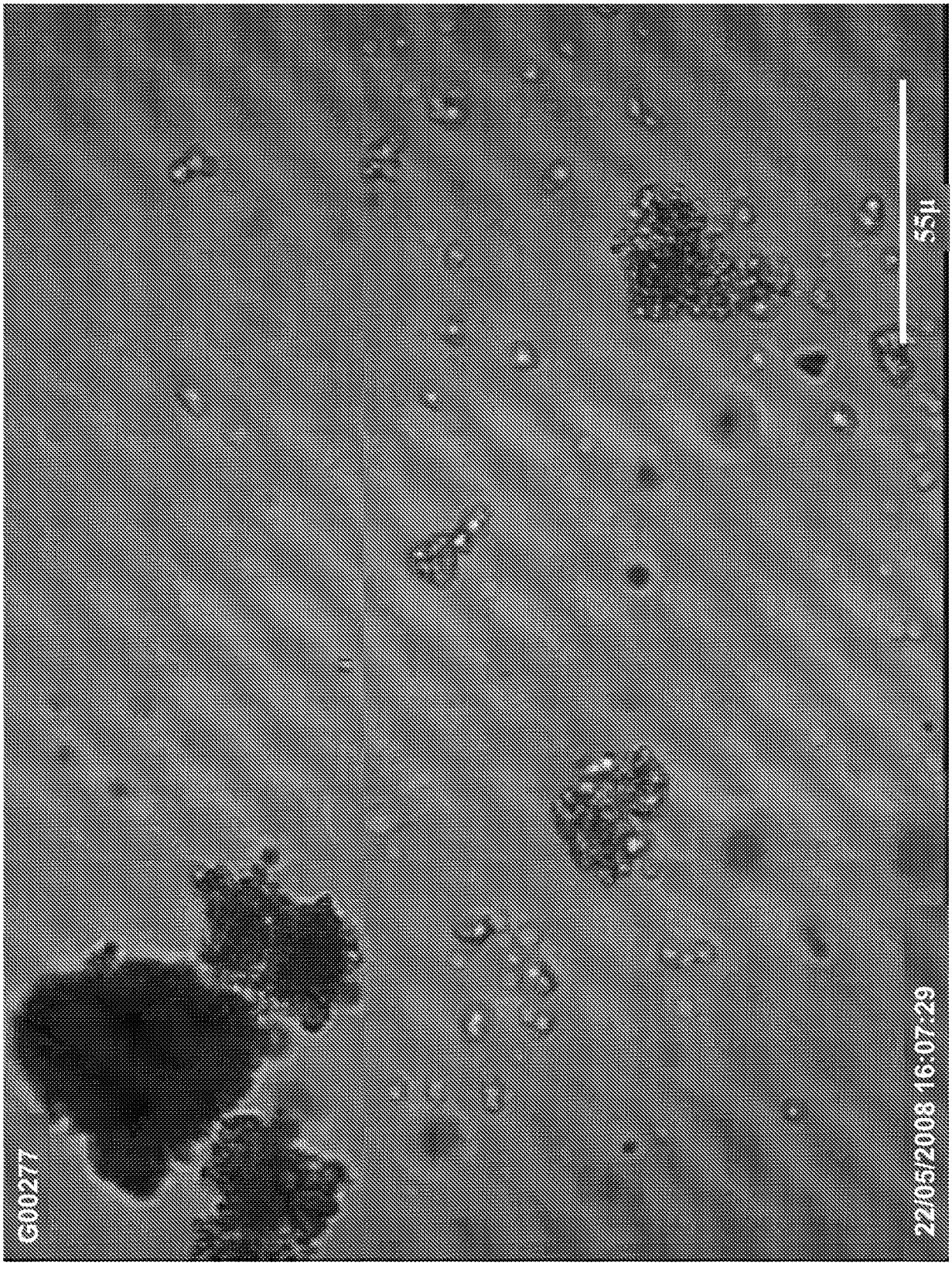


图 23D

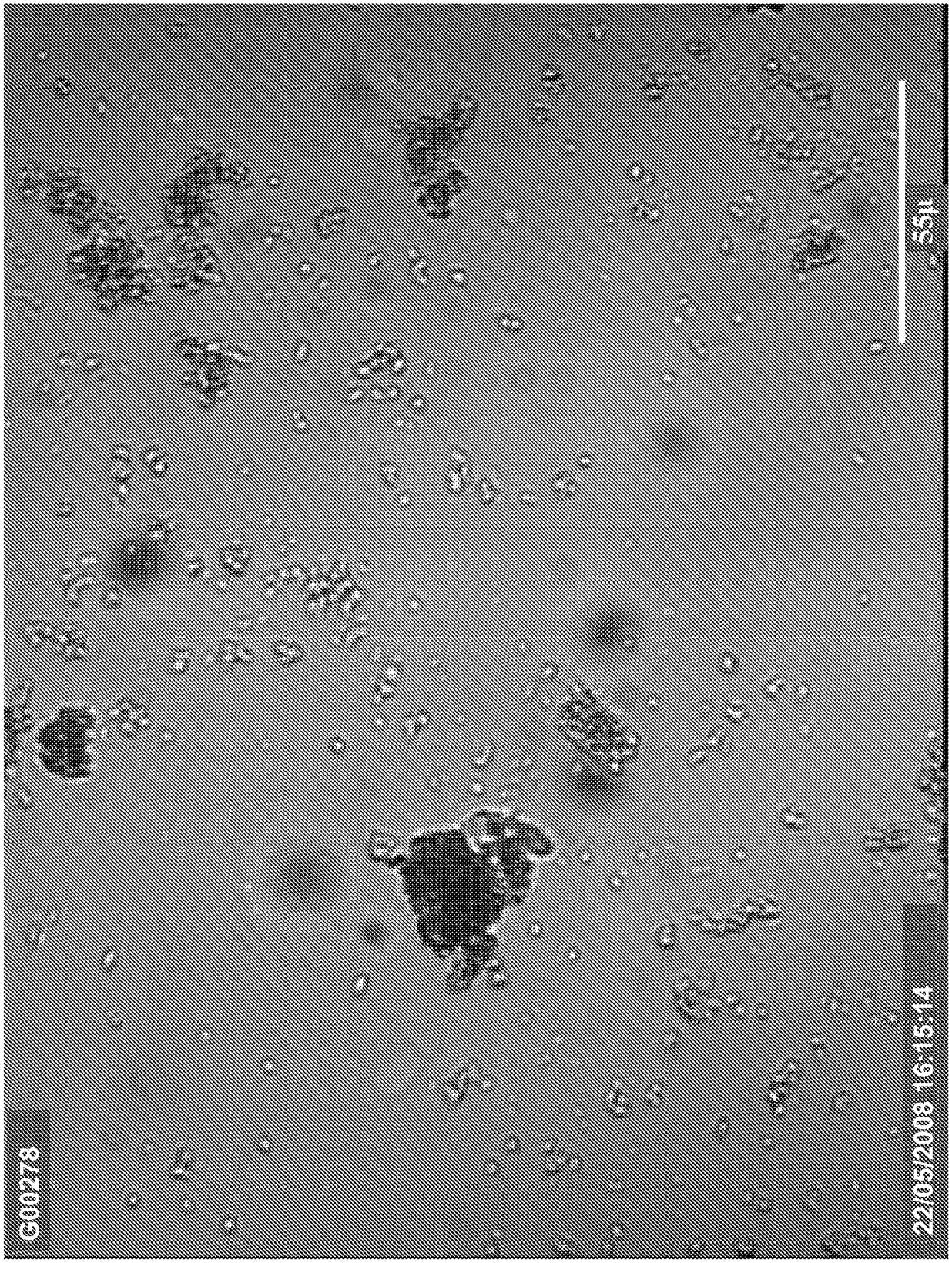


图 23E

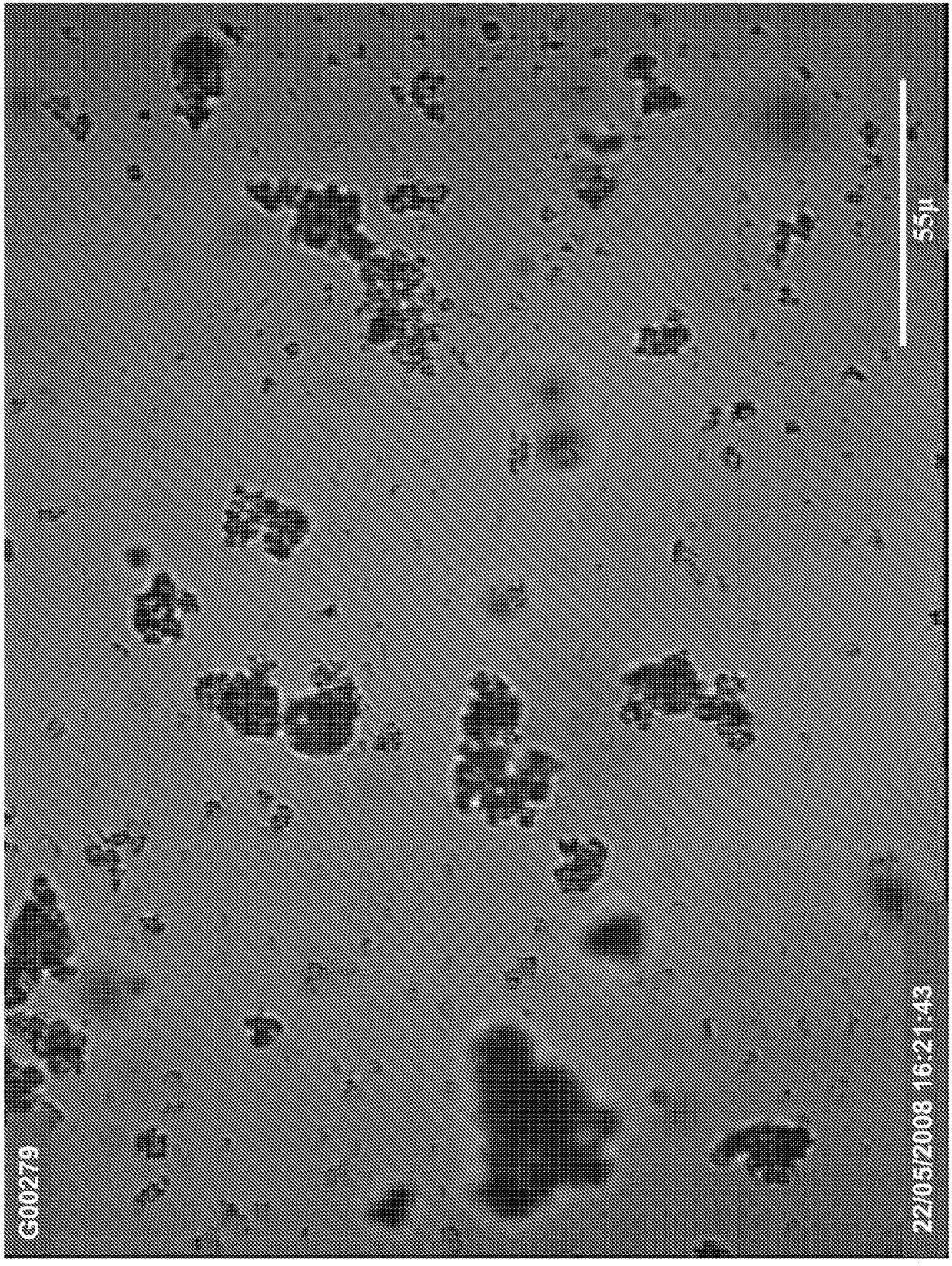


图 23F

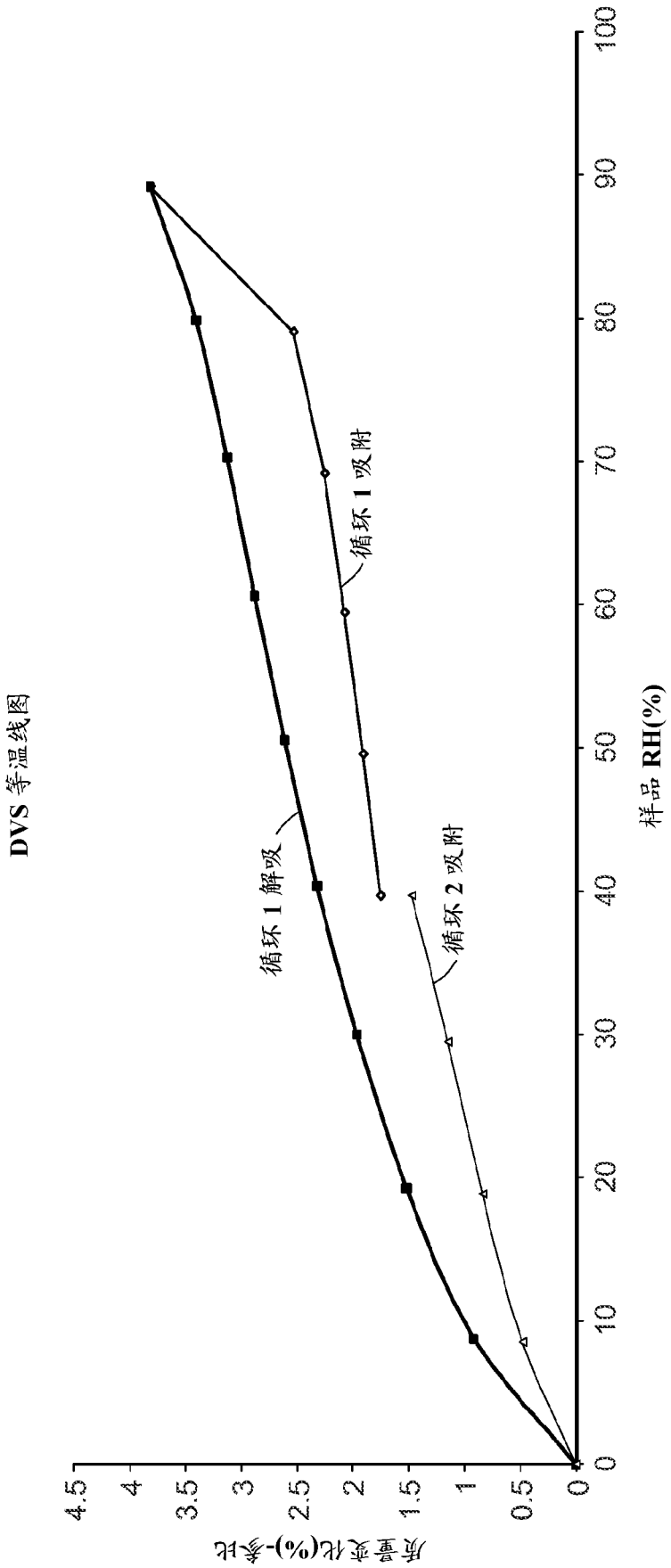

DVS 等温线图
质量变化(%)-参比
样品 RH(%)
循环 1 吸附
循环 1 解吸
循环 2 吸附
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
10
20
30
40
50
60
70
80
90
100

图 24

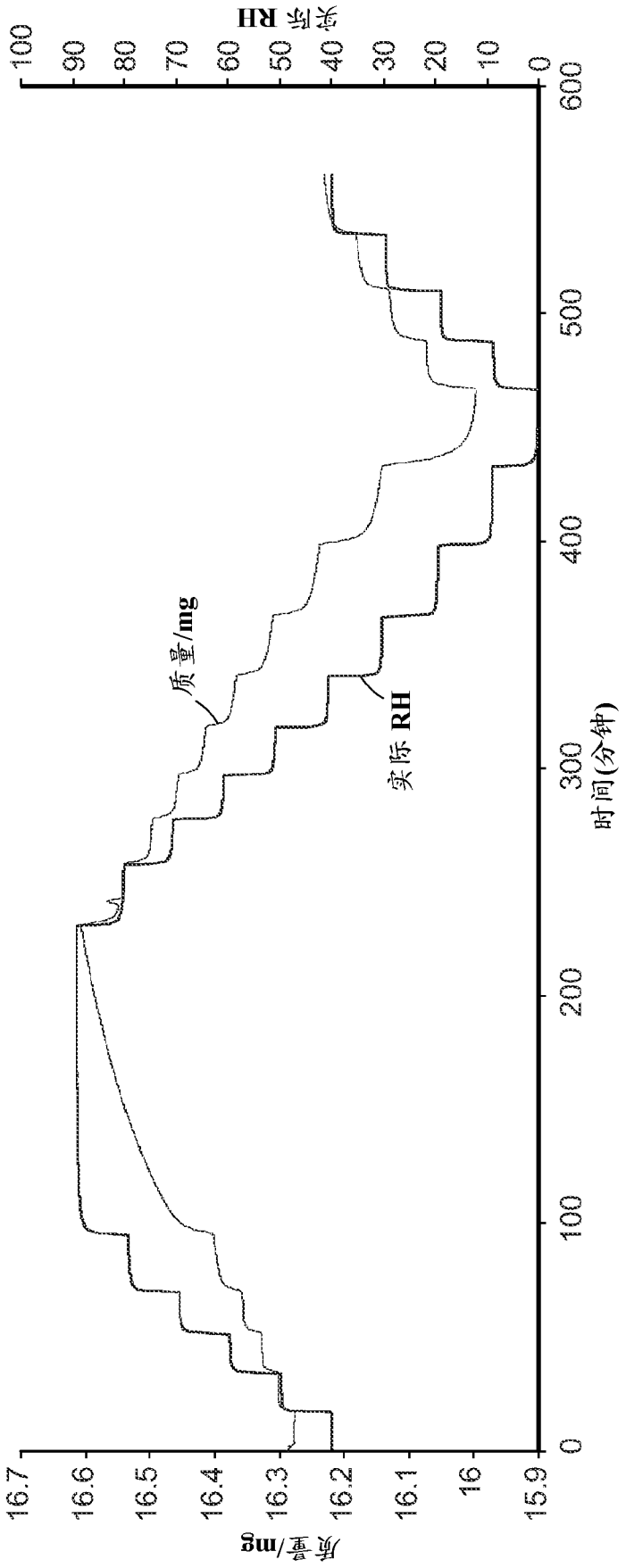

质量/mg
实际 RH
时间(分钟)
质量/mg
实际 RH
16.7
16.6
16.5
16.4
16.3
16.2
16.1
16
15.9
0
100
200
300
400
500
600
100
90
80
70
60
50
40
30
20
10
0

图 25

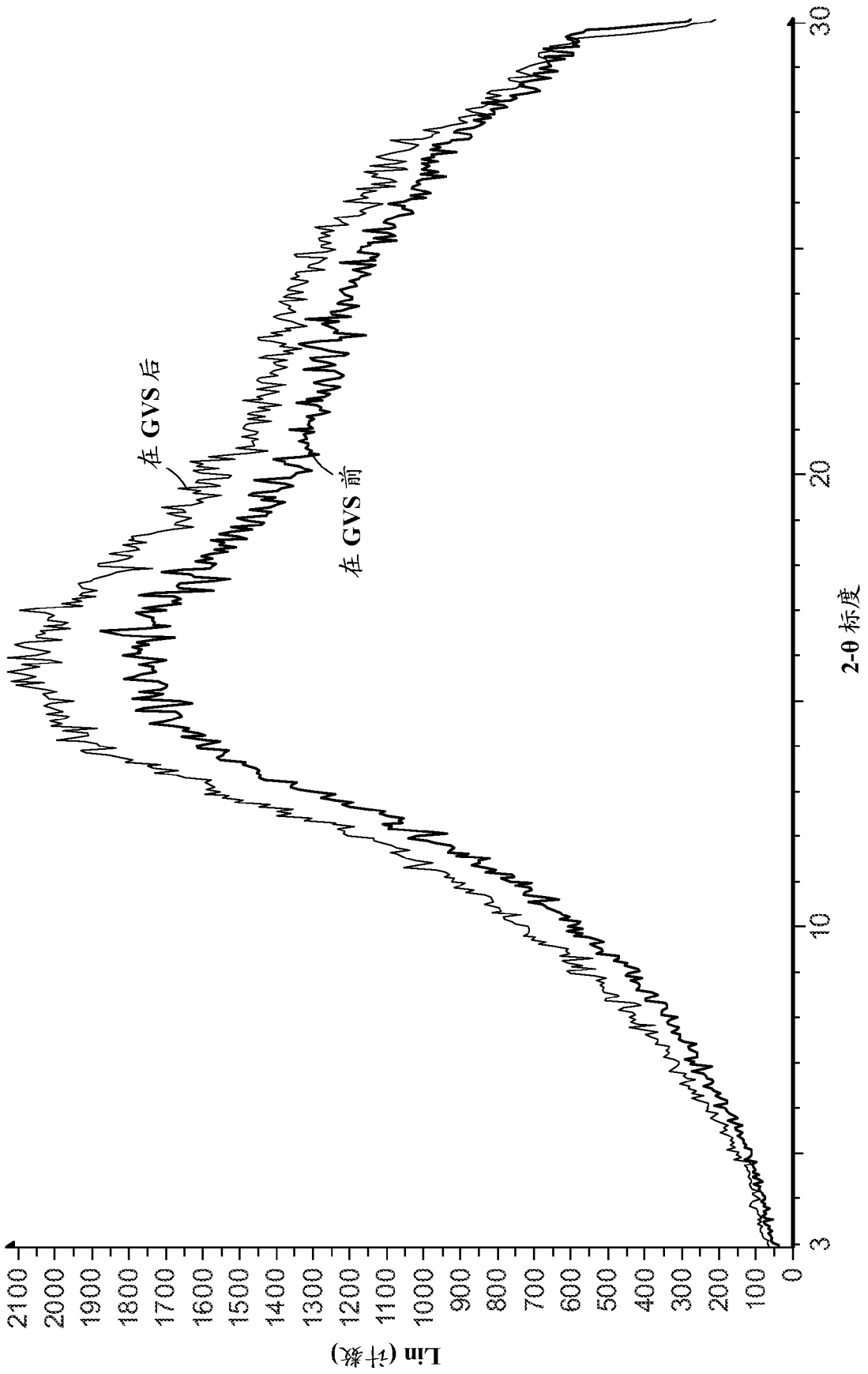

在 GVS 后
在 GVS 前
Lin (计数)
2-θ 标度
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
3
10
20
30

图 26

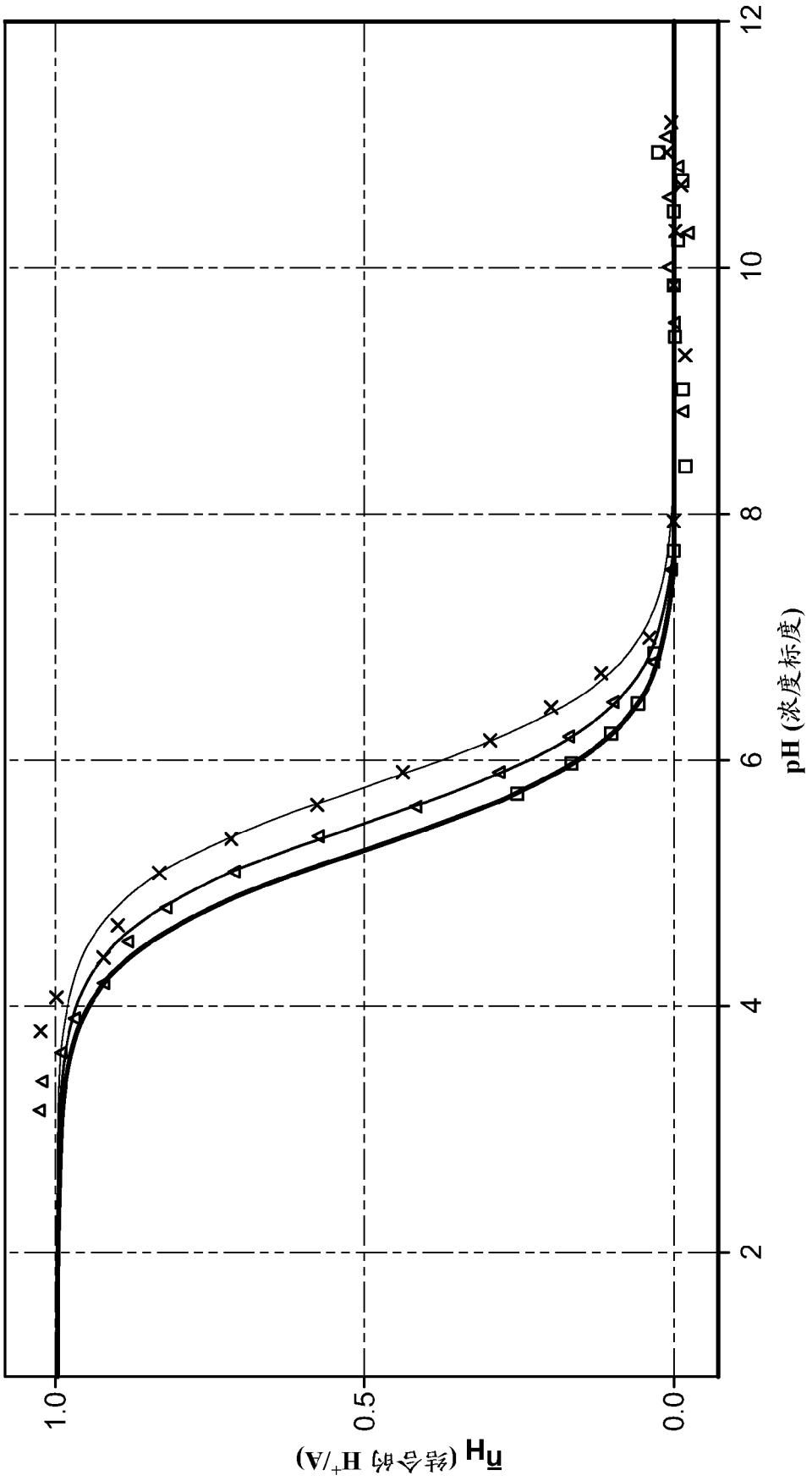


图 27

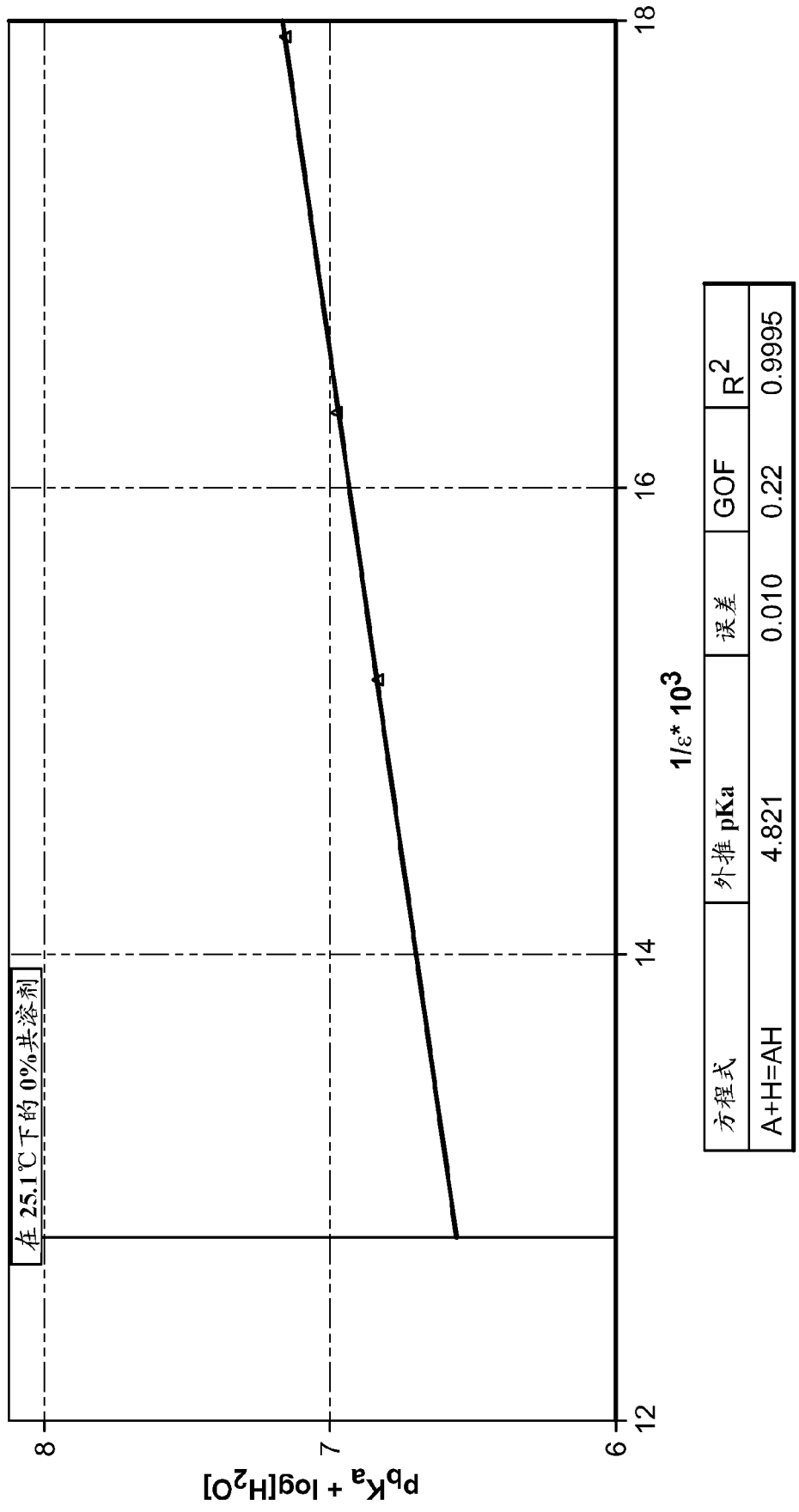

在 25.1℃下的 0%共溶剂
$pK_a + \log[H_2O]$
$1/\varepsilon^* 10^3$
8
7
6
12
14
16
18
方程式
外推 pKa
误差
GOF
R^2
A+H=AH
4.821
0.010
0.22
0.9995

图 28

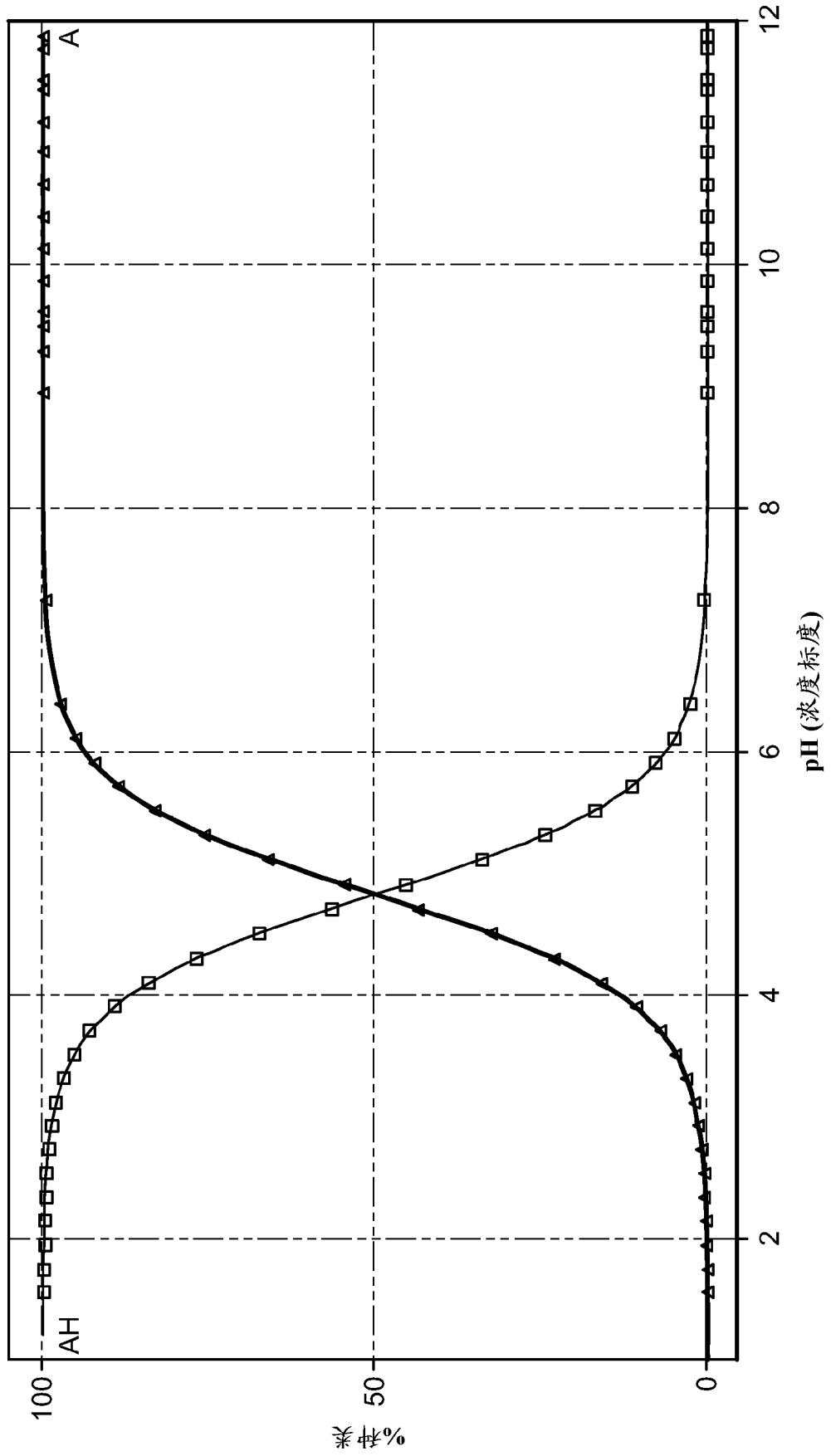


图 29

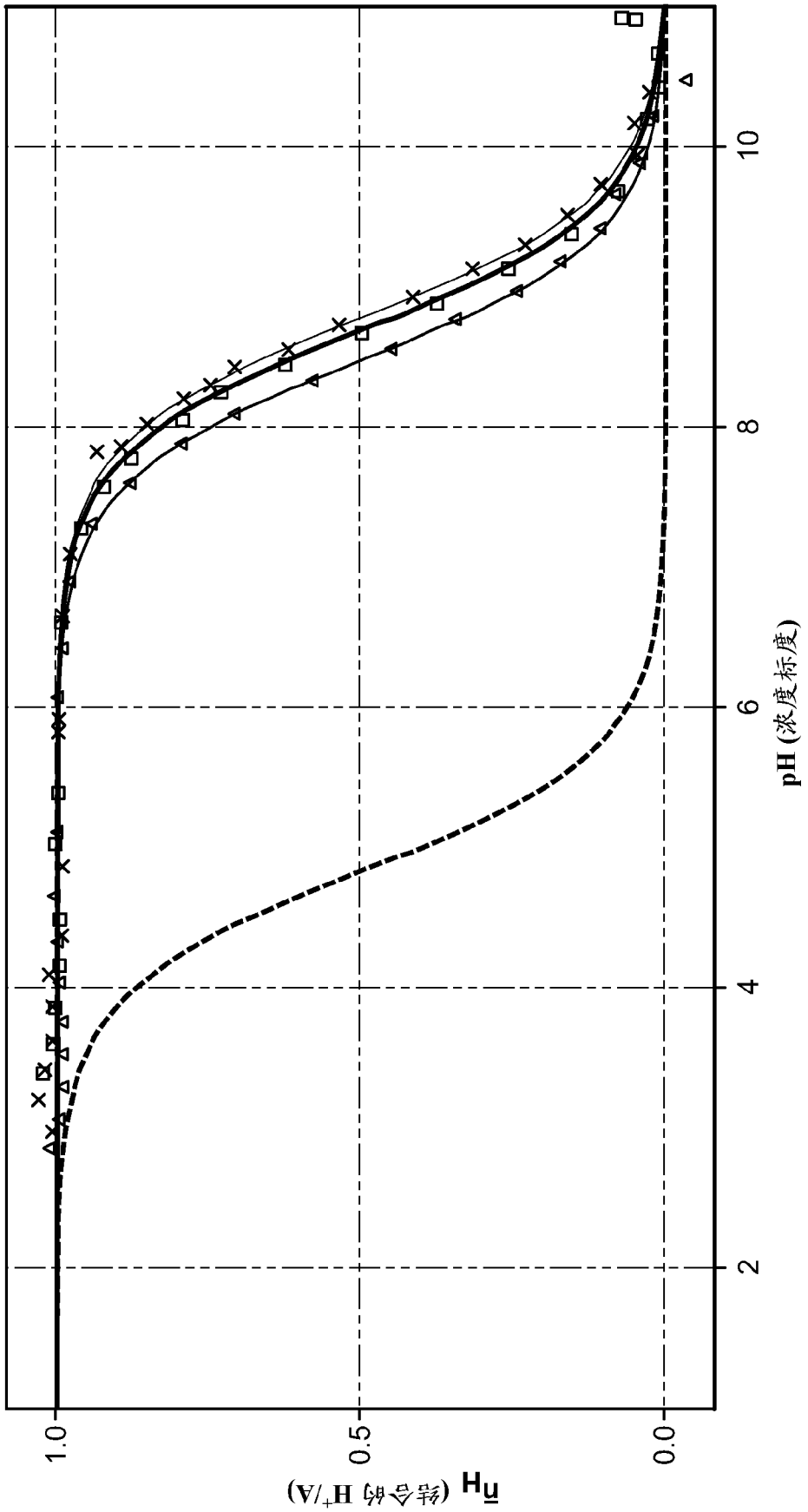


图 30

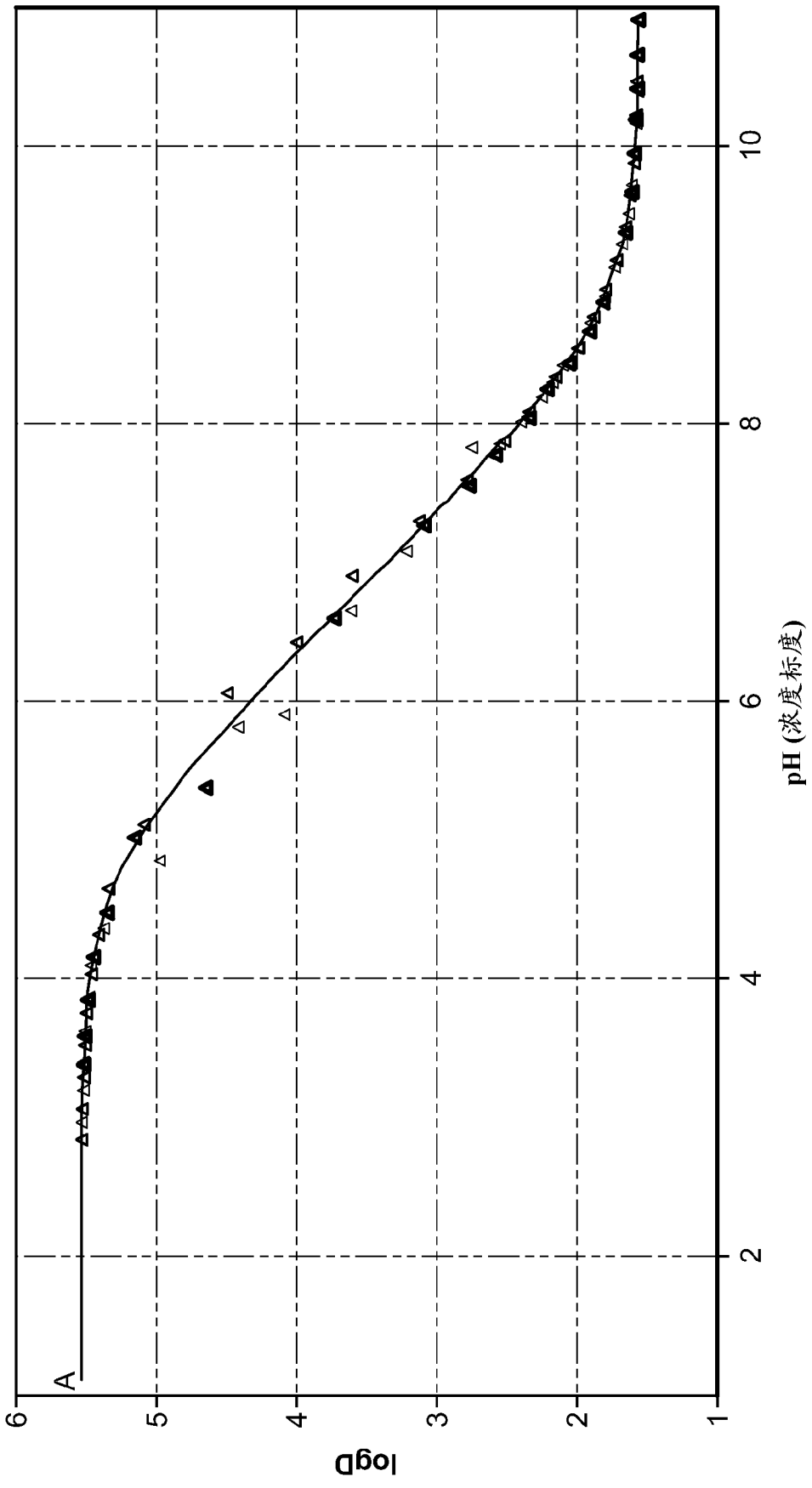


图 31

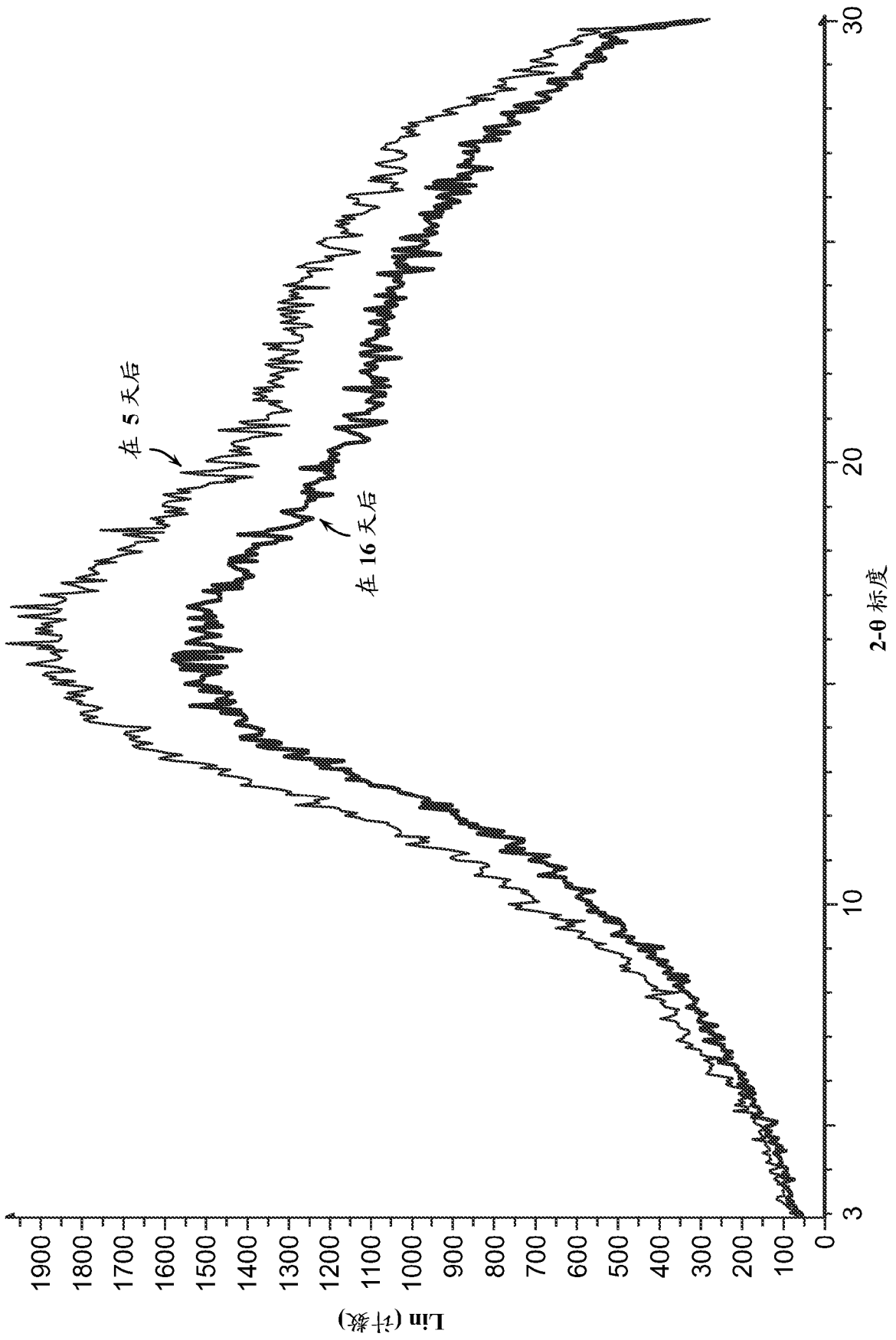

在 5 天后
在 16 天后
Lin (计数)
2-θ 标度
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
0
3
10
20
30

图 32

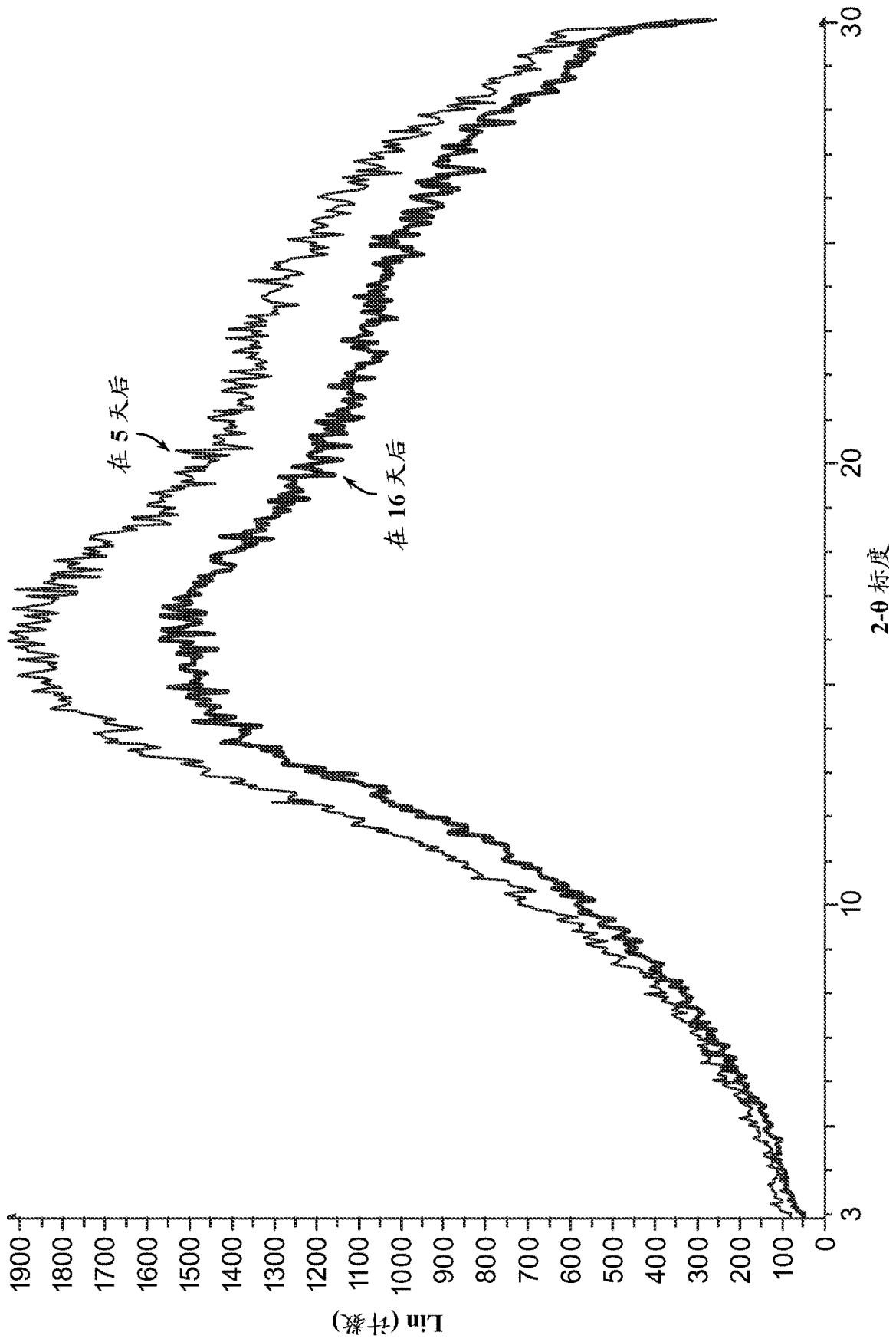

在 5 天后
在 16 天后
Lin (计数)
2-θ 标度
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
3
10
20
30

图 33

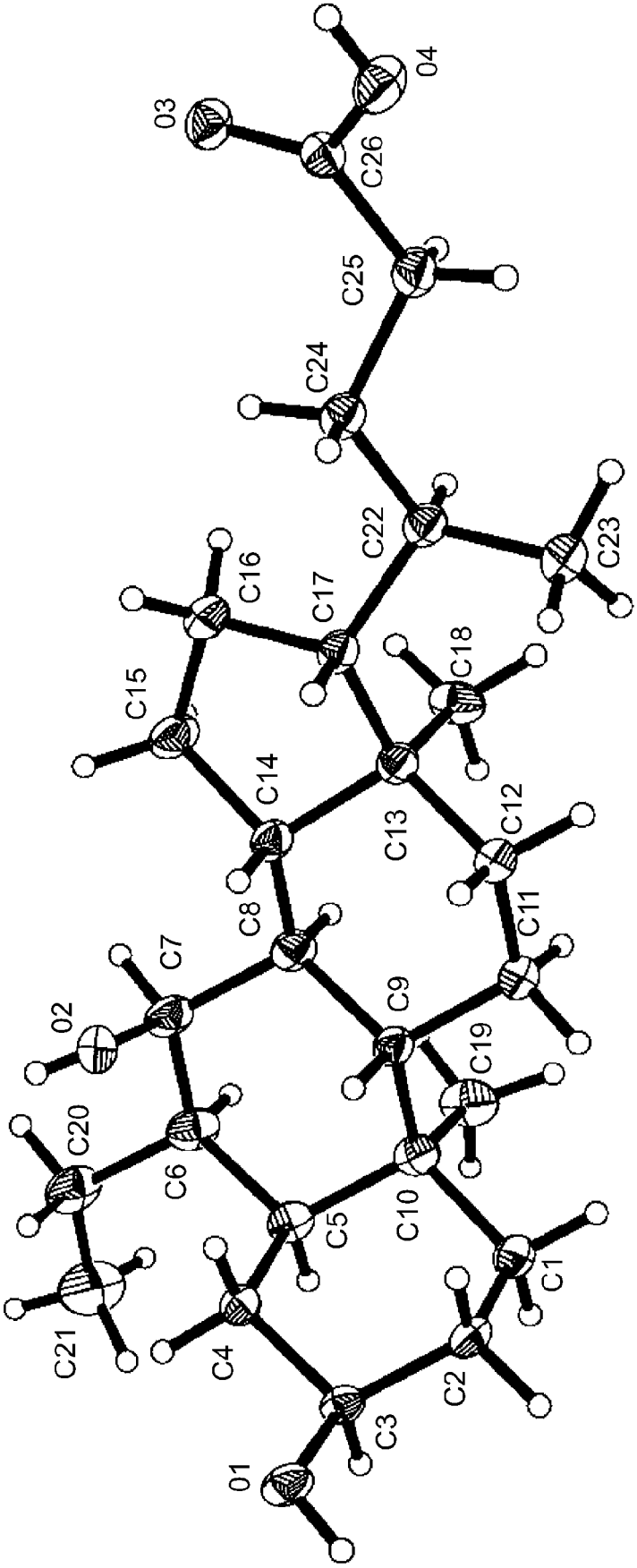

04
03
C26
C25
C24
C22
C23
C16
C17
C18
C15
C14
C13
C12
C8
C11
C7
C9
02
C19
C20
C6
C5
C10
C1
C21
C4
C2
C3
01

图 34

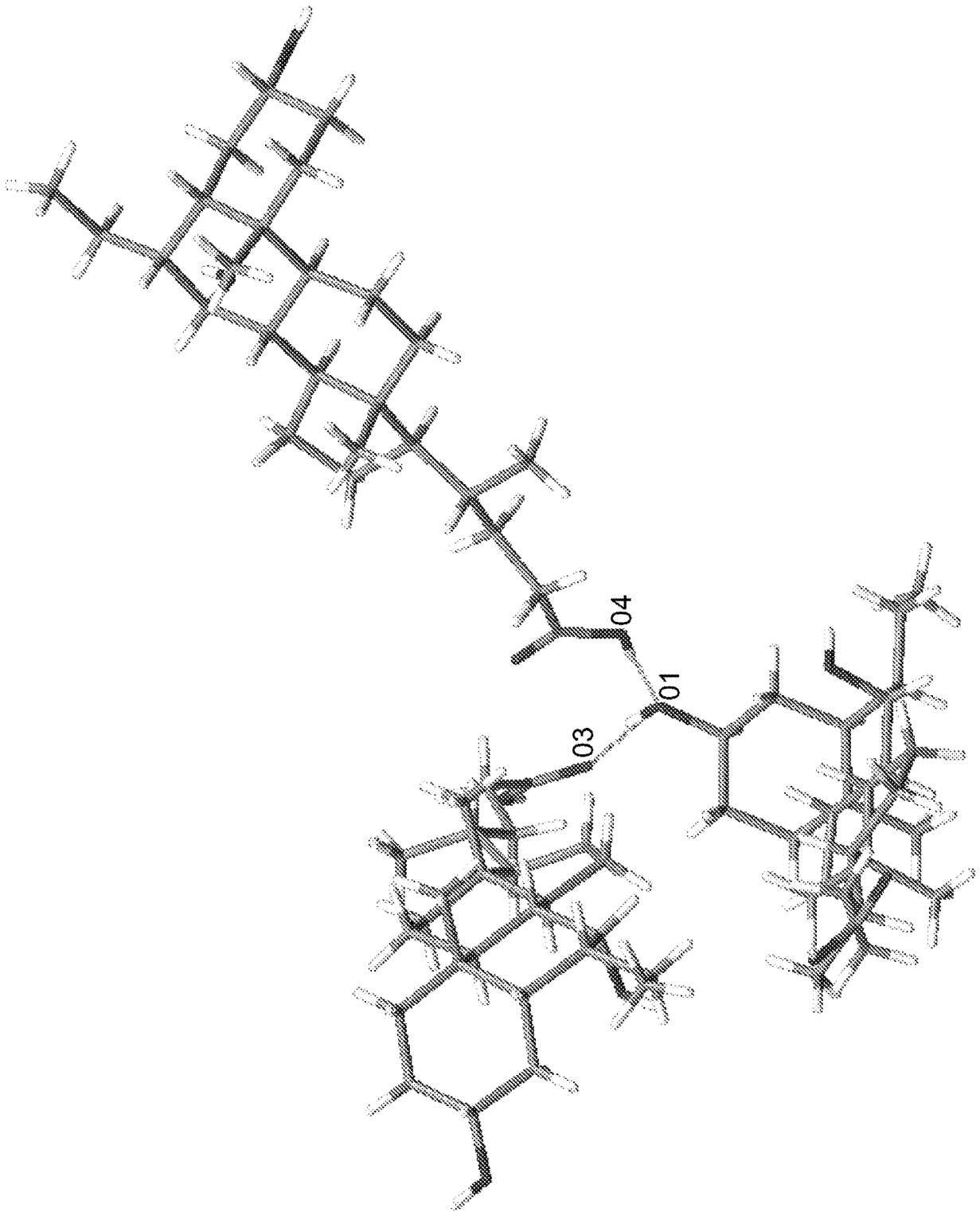


图 35

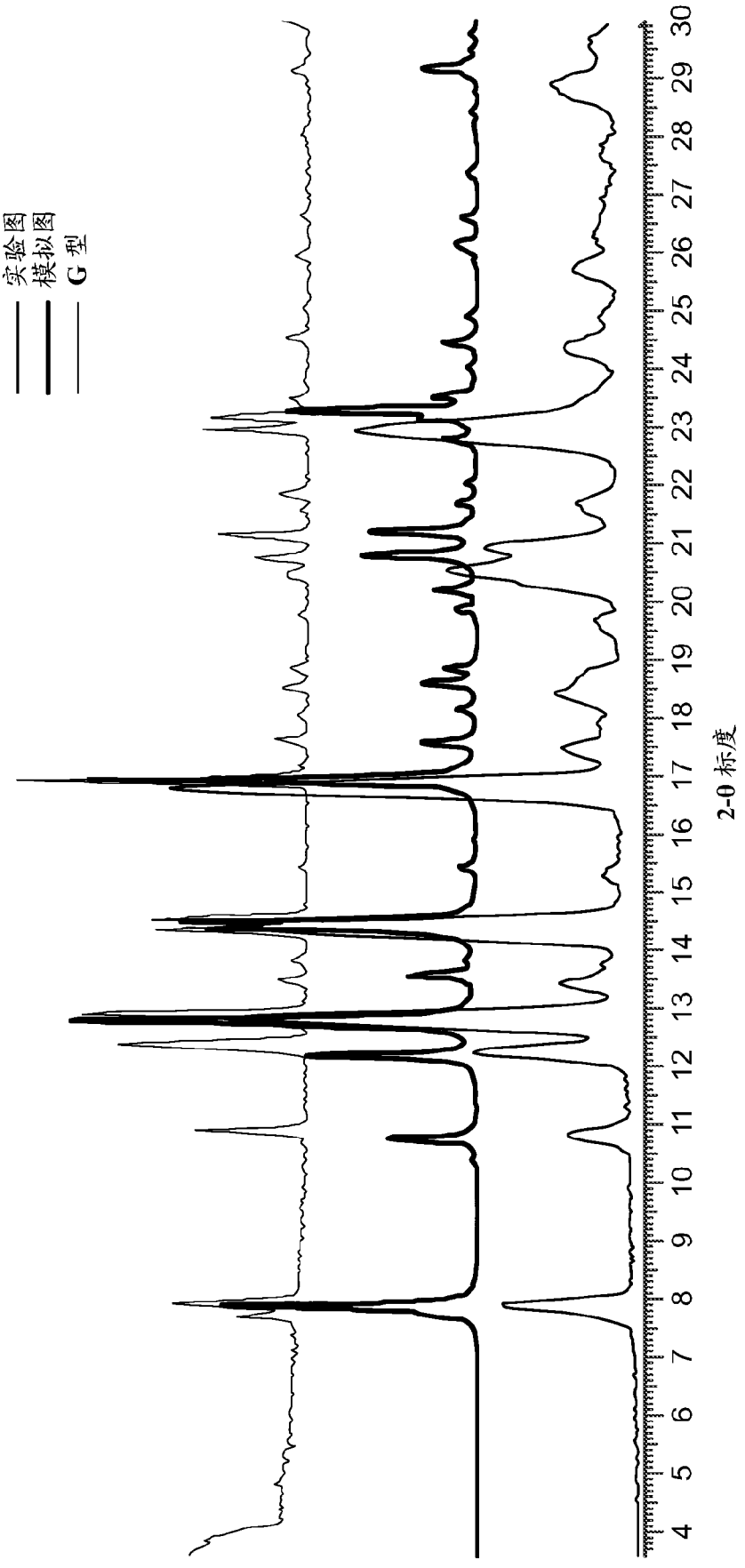

实验图
模拟图
G 型
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2-θ 标度

图 36

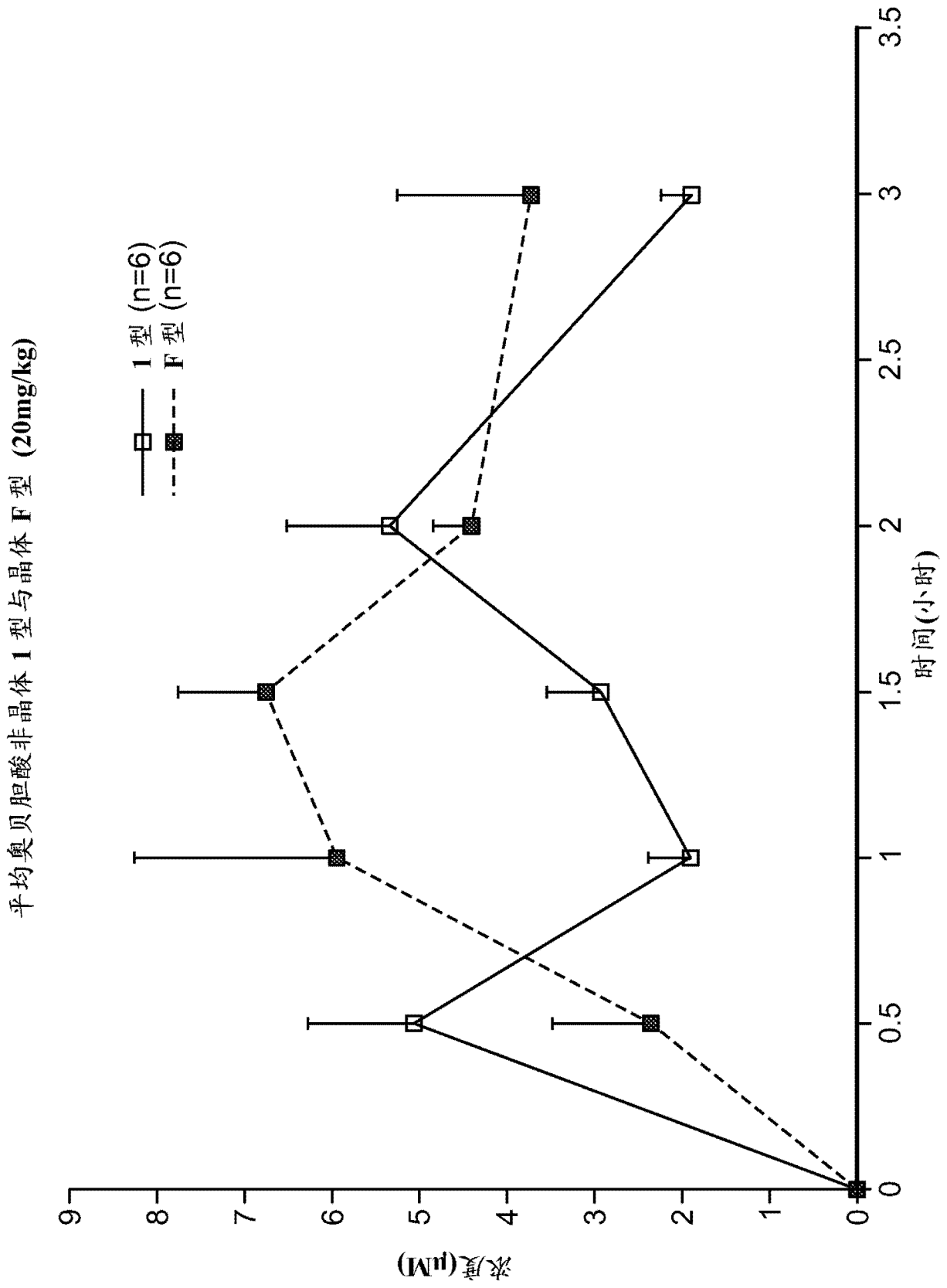

平均奥贝胆酸非晶体 1 型与晶体 F 型 (20mg/kg)
1 型 (n=6)
F 型 (n=6)
浓度(μM)
时间(小时)
0
1
2
3
4
5
6
7
8
9
0
0.5
1
1.5
2
2.5
3
3.5

图 37

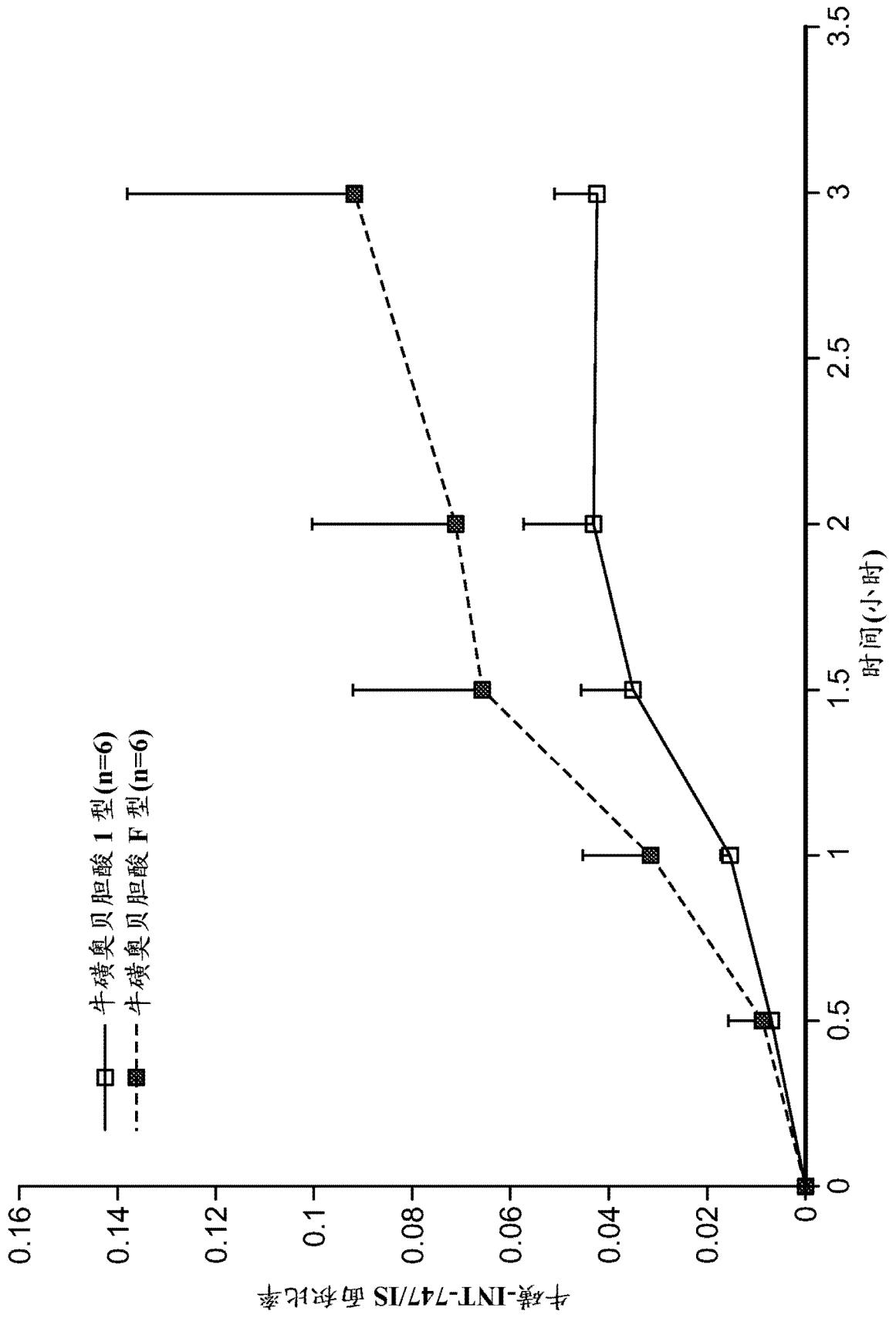

非晶体 1 型与晶体 F 型(20mg/kg)中的奥贝胆酸的平均牛磺缀合物
牛磺奥贝胆酸 1 型(n=6)
牛磺奥贝胆酸 F 型(n=6)
牛磺-INT-747/IS 面积比率
时间(小时)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.5
1
1.5
2
2.5
3
3.5

图 38

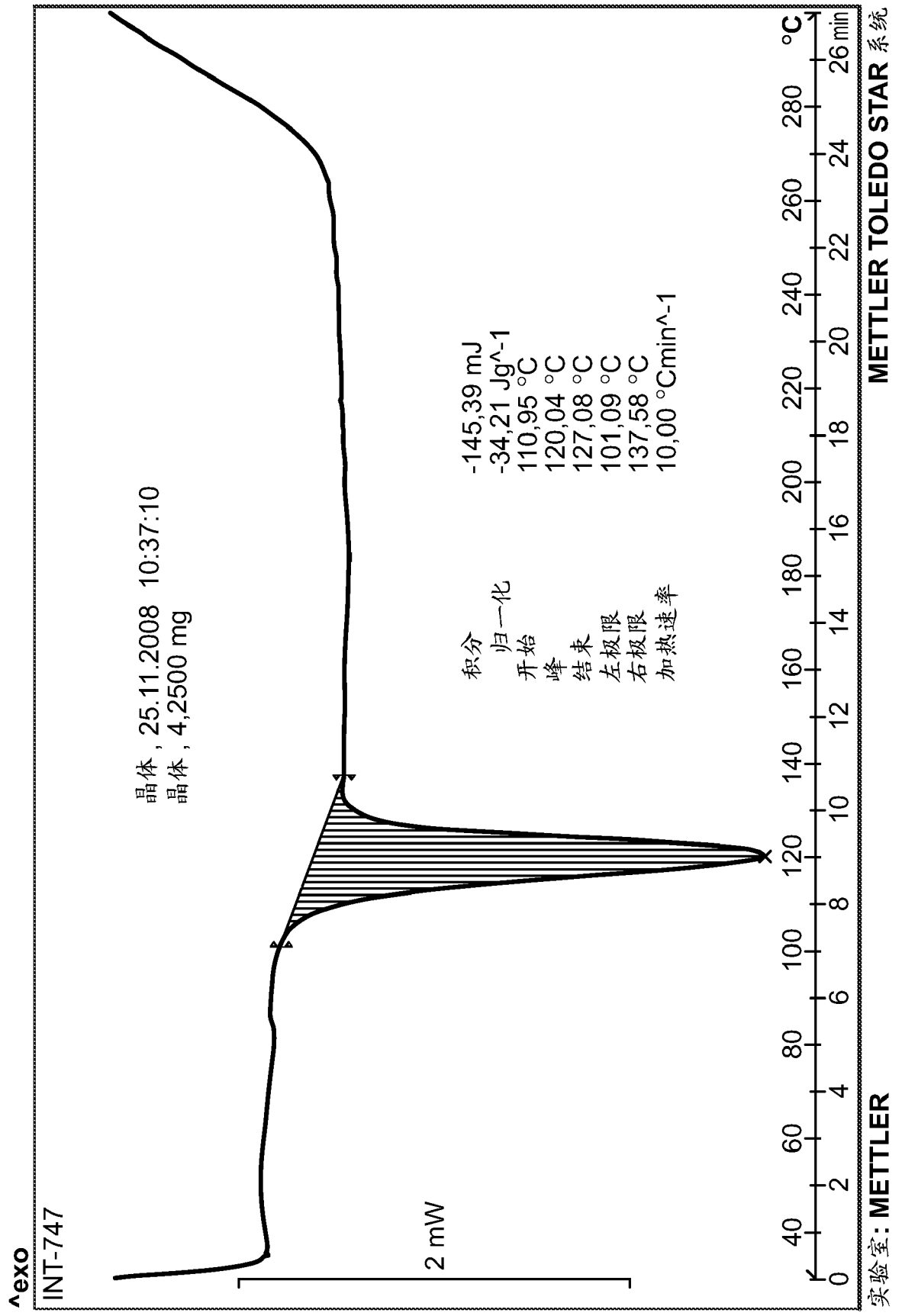

^exo
INT-747
晶体 , 25.11.2008 10:37:10
晶体 , 4,2500 mg
积分 -145,39 mJ
归一化 -34,21 Jg^-1
开始 110,95 °C
峰 120,04 °C
结束 127,08 °C
左极限 101,09 °C
右极限 137,58 °C
加热速率 10,00 °Cmin^-1
2 mW
°C
min
实验室: METTLER
METTLER TOLEDO STAR 系统

图 39

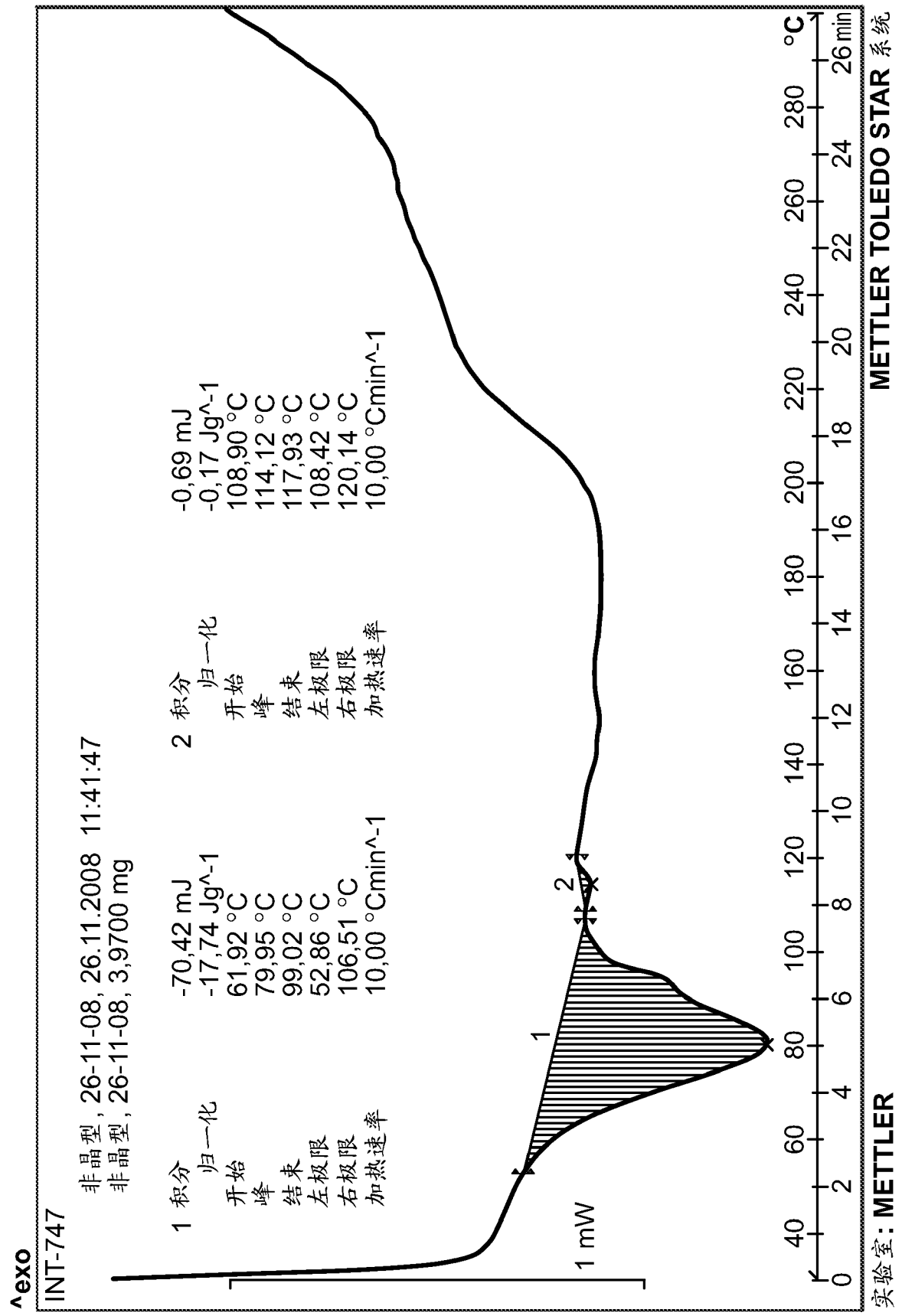
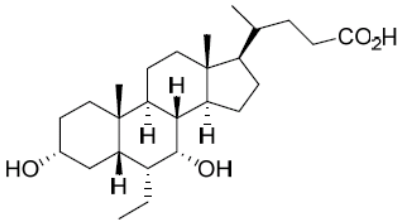

^exo
INT-747
非晶型, 26-11-08, 26.11.2008 11:41:47
非晶型, 26-11-08, 3,9700 mg
1 积分 -70,42 mJ
归一化 -17,74 Jg^-1
开始 61,92 °C
峰 79,95 °C
结束 99,02 °C
左极限 52,86 °C
右极限 106,51 °C
加热速率 10,00 °Cmin^-1
2 积分 -0,69 mJ
归一化 -0,17 Jg^-1
开始 108,90 °C
峰 114,12 °C
结束 117,93 °C
左极限 108,42 °C
右极限 120,14 °C
加热速率 10,00 °Cmin^-1
1 mW
1
2
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
实验室: METTLER
METTLER TOLEDO STAR 系统

图 40

Abstract

The present invention relates to obeticholic acid:



or a pharmaceutically acceptable salt, solvate or amino acid conjugate thereof. Obeticholic acid is useful for the treatment or prevention of a FXR mediated disease or condition, cardiovascular disease or cholestatic liver disease, and for reducing HDL cholesterol, for lowering triglycerides in a mammal, or for inhibition of fibrosis. The present invention also relates to processes for the synthesis of obeticholic acid.