PREPARATION OF OPTICALLY ACTIVE 15-EPOXY-α- LINOLENIC ACIDS AND THEIR ANTI-RICE BLAST FUNGUS ACTIVITIES#

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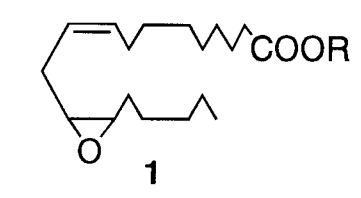
Abstract-By the action of NBS in aq. DME, α-linolenic acid was oxidized to 15-bromo-16-hydroxy-α-linolenic acid in 35% conversion yield. The bromohydrin was treated with lipase-PS and vinyl acetate to give the resolved acetate and bromohydrin. (15*S*,16*R*)- and (15*R*,16*S*)-Epoxy-α-linolenic acids were prepared from the corresponding resolved products. A comparison of anti-rice blast fungus activities showed that an unnatural epoxide is stronger than the natural epoxide.

In our previous paper, we have demonstrated that the epoxy fatty acids from rice plants as exemplified by epoxides (**1**) and (**2**) possess anti-rice blast fungus activity.[1,2] They showed the specific rotations ($[\alpha]_D$) of -0.30° for **1** and +0.27° for **2**, respectively. The (12*R*,13*S*) configuration of the former epoxide was determined by observation of a positive Cotton effect in the CD spectrum of the corresponding allyl benzoate (**3**) derived from **1** by ring-opening with LDA, followed by esterification with (*p*)Br-C_6H_4COCl. The absolute configuration of **2** has remained undetermined due to the inapplicability of the ring-opening conditions to the epoxide (**2**).



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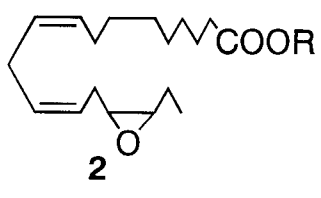

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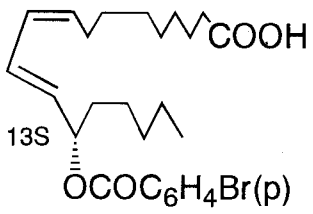

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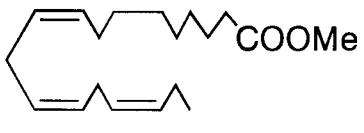
λ_{ext}^{CD} nm (Δε) =249 (+1.43)



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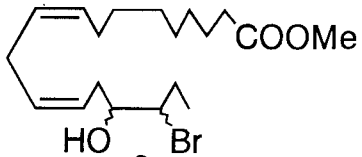

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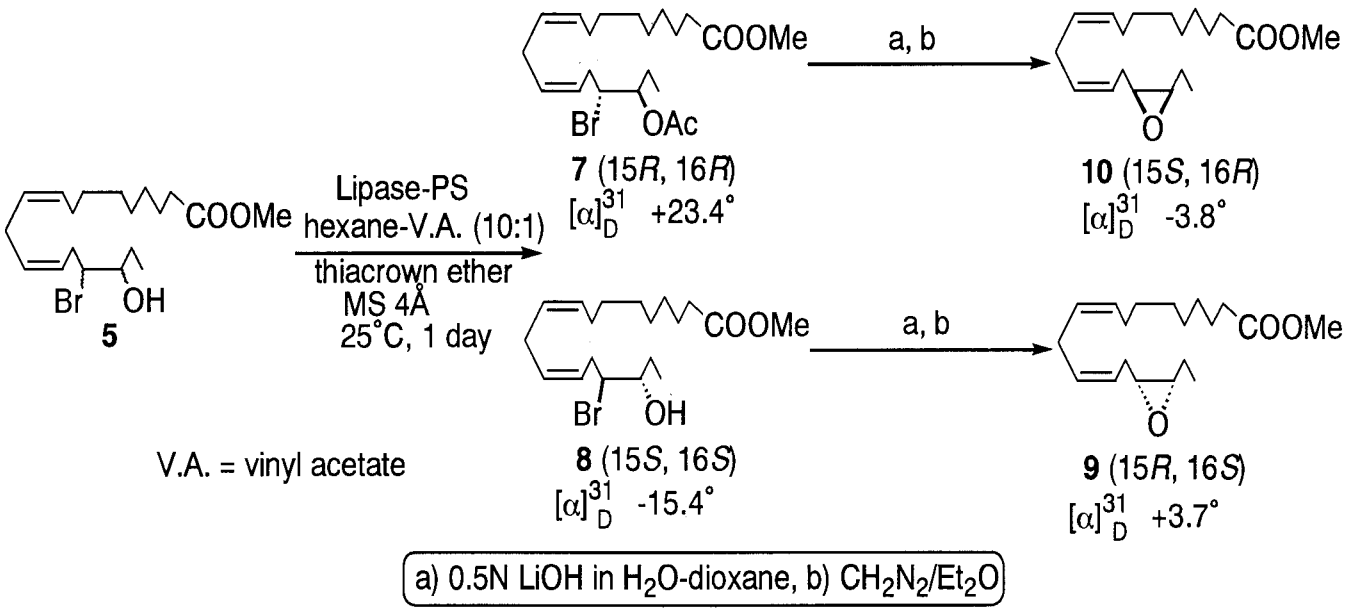
Dedicated to Professor Teruaki Mukaiyama for the memory of his seventythird birthday



Scheme1. Preparation of both enantiomers of terminal epoxides (**9**) and (**10**).
(The specific rotations were measured with *c* 1.0, MeOH.)

This paper deals with the determination of absolute configuration and estimation of enantiomeric excess of naturally occurring epoxide (**2**) by preparing both enantiomers. In addition, interest in examining the anti-rice blast fungus activity of natural and unnatural forms prompted our present study.

The preparation of both enantiomers of **2** commenced from our recent findings, in which we revealed the terminal double bonds of ω-3-unsaturated fatty acids such as α-linolenic acid (**4**), DHA and EPA can be oxidized by the application of NBS in aqueous organic solvent with high regioselectivity.[3] When α-linolenic acid methyl ester was submitted to the reaction with NBS (0.7 eq.) in aqueous DME followed by purification with HPLC, 15-bromo-16-hydroxy-α-linolenic acid methyl ester (**5**) and its positional isomer, 16-bromo-15-hydroxy-α-linolenic acid methyl ester (**6**) were obtained in 35 and 22% conversion yields, respectively, accompanying 50% recovery of the starting material (**4**).

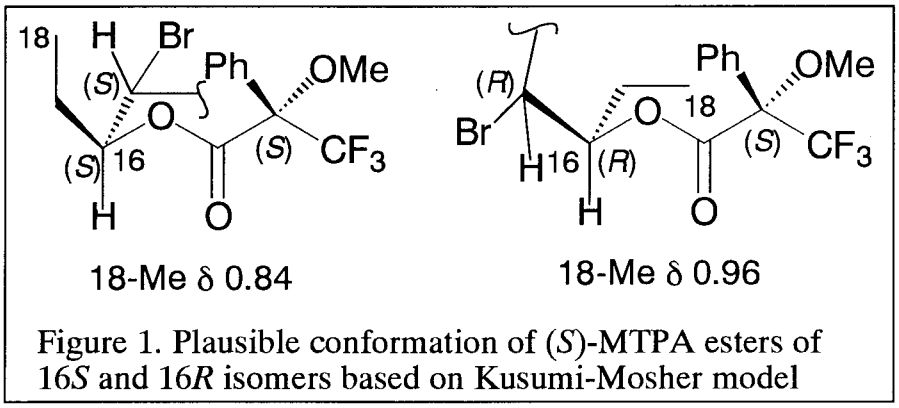


Figure 1. Plausible conformation of (*S*)-MTPA esters of 16*S* and 16*R* isomers based on Kusumi-Mosher model

Bromohydrin (**5**) and (**6**) each provided the same *dl*-epoxide (**2**) by treatment with LiOH in aq. dioxane while the position of the hydroxyl group of **5** was confirmed by detailed H-H COSY spectrum of the corresponding acetate (**7**). The optical resolution of *dl*-bromohydrin (**5**) was effectively performed when **5** was

stirred with vinyl acetate and lipase-PS[4] in the presence of 1, 4, 8, 11-tetrathiacyclotetradecane (thiacrown ether) recently developed by Takagi's group.[5] In the absence of the thiacrown ether, the reaction proceeded quite slowly and needed more than 7 days during which period some decomposition of **5** occurred. In the presence of the thiacrown ether, however, the reaction completed after 24 h, affording the acetate (**7**) and the resolved bromohydrin (**8**). The absolute configuration of the resolved product (**8**) was elucidated by application of the Kusumi-Mosher method.[6] When *dl*-bromohydrin (**5**) was treated with (*S*)-MTPA and DCC in the presence of DMAP for 65 h at room temperature, an inseparable mixture of (*S*)-MTPA esters was formed in 98% yield in a 1 : 1 ratio, which showed the terminal methyl signals at 0.84 and 0.96 ppm in its ^{1}H NMR spectrum. As depicted in Figure 1, the 16*S* and 16*R* configurations were assignable on the basis of the Kusumi-Mosher model to the isomer showing an upfield shift (0.84 ppm) and non-shifted isomer (0.96 ppm), respectively. The resolved bromohydrin (**8**) was similarly treated with (*S*)-MTPA to furnish only an isomer of the (*S*)-MTPA ester in 85 % yield, showing the methyl signal at 0.84 ppm. This evidence suggests that the resolved bromohydrin possesses the 16*S* configuration. In order to confirm this deduction, *dl*- and resolved bromohydrins were, respectively, converted to the corresponding (*R*)-MTPA esters. The chemical shift of the methyl group from *dl*-form (**5**) was measured at 0.84 and 0.96 ppm while the methyl signal from the resolved bromohydrin (**8**) appeared only at 0.96 ppm, indicating no influence of the phenyl group of (*R*)-MTPA group on the terminal methyl group of the16*S* configuration of **8**.[7]

By treatment with LiOH in aq. dioxane, the resolved bromohydrin (**8**) and its MTPA ester provided the (15*R*,16*S*) epoxide (**9**) possessing the same values of $[\alpha]_D^{31}$+ 3.7° ± 0.1° (*c* 1.0, MeOH), demonstrating the enantiomeric excess of the resolved bromohydrin (**8**) is more than 98%.[8] The bromo acetate (**7**) was transformed into the epoxide (**10**) having $[\alpha]_D^{31}$- 3.8° (*c* 1.0, MeOH). The comparison of the specific rotation of the natural epoxide isolated from rice plants with that of the synthesized epoxides clearly indicated that the natural epoxide has (15*R*, 16*S*) configuration with less than 10% enantiomeric excess.

Our preliminary bioassay of the synthesized epoxides (**9**) and (**10**) toward the inhibition of spore germination of the rice blast fungus revealed that ID_{50} of **10** is 23 ppm while that of **9** is 37 ppm, respectively.[9] It is quite interesting to note that the unnatural form possesses stronger activity than the natural form. Further experiments seem necessary to generalize the phenomenum of stronger activities of the unnatural form.

EXPERIMENTALS

General: Unless otherwise noted, ^{1}H NMR and ^{13}C NMR spectra were recorded on solutions in $CDCl_3$ with $SiMe_4$ as an internal standard with JNM-EX 270L (270 MHz), and GSX-500 (500 MHz) spectrometers. Chemical shifts are reported in δ_H and δ_C, and *J* values are in Hz. The following multiplicities were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q; quartet; m, multiplet; br; broad. IR spectra were recorded on a Hitachi 270-30 spectrophotometer. The MS spectra were measured with Hitachi M-80B spectrometer including EIMS (electron ionization, 70 eV) and HRMS mass spectrometries. Optical rotations were measured on a JASCO DIP-370 polarimeter with a path length of 1 dm. Concentrations are given in g/100 mL. Column chromatographic purification was carried out using Kiesel gel 60, Art 7734 (70-230 mesh). HPLC were performed with Waters Associates equipment and Guard-Pak Cartridge Prep Novapak HR (Waters Associates) and μ-porasil columns, respectively. Thinlayer chromatography was carried out on aluminium sheets coated with $60F_{254}$ silica. Plates were developed using a spray of 0.5% anisaldehyde in

2M sulfuric acid. The α-linolenic acid methyl ester was purified using silica gel impregnated with 5% $AgNO_3$ column chromatography eluted with mixed solvents of hexane : AcOEt by changing the ratio from 60 : 1 to 30 : 1 and finally to 10 :1. Solvents and commercially available reagents were dried and purified if necessary before use according to standard procedures. All the derivatives described in this paper are pale yellow oily substances and the purities were confirmed by ^{13}C NMR spectra. The usual work up involved dilution of the reaction mixture with water, extraction with ether and evaporation after washing the organic extracts with water and brine, followed by drying over Na_2SO_4.

15-Bromo-16-hydroxy-α-linolenic Acid Methyl Ester (5): Water (13 mL) was added to dimethoxyethane (DME) (50 mL) solution of α-linolenic acid methyl ester (**4**) (1.47 g, 5.03 mmol) to obtain the saturated solution of α-linolenic acid methyl ester (**4**). A mass of NBS (120 mg) was added to the stirred saturated solution under ice cooling in the dark at argon atmosphere. The reaction was monitored with KI-Starch paper. After complete disappearance of HOBr in the solution, another mass of NBS (120 mg) was again added; finally a total amount of 630 mg (3.52 mmol) of NBS was added. The reaction mixture was treated as usual and the crude reaction mixture was separated by SiO_2 (74 g) column chromatography eluted with hexane-AcOEt (40 :1) to obtain first the recovered α-linolenic acid methyl ester (**4**) (730 mg, 50 %) and then a mixture of monobromohydrins (590 mg). The mixture of monobromohydrins was submitted to HPLC purification with μ-porasil column eluted with hexane : AcOEt (15 :1) under the flow rate of 3.5 mL/min to obtain pure bromohydrins (**5**) (338 mg, 35% conversion yield) and **6** (215 mg, 22% conversion yield) each as a pale yellow oil. Bromohydrin (**5**) δ_H (270 MHz, $CDCl_3$) 0.98 (3H, t, J = 7.6 Hz), 1.31 (8H, br), 1.61 (4H, m), 2.04 (2H, m), 2.31 (2H, t, J = 7.6 Hz), 2.78 (4H, m), 3.40 (1H, m), 3.67 (3H, s), 4.08 (1H, m), and 5.42 (4H, m). δ_C (67.8 MHz, $CDCl_3$) 174.3 (s), 131.4 (d), 130.7 (d), 127.1 (d), 125.7 (d), 74.2 (d), 63.1 (d), 51.5 (q), 34.1 (t), 33.8 (t), 29.5 (t), 29.1 (t) x 2, 29.0 (t) x 2, 27.2 (t), 25.8 (t), 24.9 (t), and 10.0 (q). EIMS m/z (relative intensity) 388 (M^+ 1.2%), 291 (M-Br-H_2O, 75), and 277 (M-Br-OH-CH_3, 100). HRMS Calcd for $C_{19}H_{33}O_3Br$; 388.1613. Observed; 388.1610. Bromohydrin (**6**) δ_H (270 MHz, $CDCl_3$) 1.07 (3H, t, J = 7.3 Hz), 1.31 (8H, br d), 1.60-1.65 (4H, m), 2.0 (2H, m), 2.41 (2H, t, J = 6.9 Hz), 2.83 (2H, t, J = 6.6 Hz), 3.55 (1H, m), 3.67 (3H, s), 4.04 (1H, m), and 5.32-5.56 (4H, m). δ_C (67.8 MHz, $CDCl_3$) 174.3 (s), 131.7 (d), 130.6 (d), 127.2 (d), 124.3 (d), 73.2 (d), 66.0 (d), 51.5 (q), 34.1 (t), 33.9 (t), 29.5 (t), 29.1 (t) x 2, 29.0 (t), 28.8 (t), 27.2 (t), 25.9 (t), 24.9 (t), and 12.6 (q). HRMS Calcd for $C_{19}H_{33}O_3Br$; 388.1613. Observed; 388.1617.

Resolution of *dl*-Bromohydrin (5): To a hexane (4 mL) solution of *dl*-bromohydrin (**5**) (85 mg, 0.22 mmol) were added lipase PS (85 mg), 4 Å molecular sieves (*ca.* 20 mg), 1, 4, 8, 11-tetrathiacyclotetradecane (*ca.* 2 mg) and vinyl acetate (0.4 mL) and the mixture was stirred for 20 h at rt under argon atmosphere. The insoluble materials were removed through a short silica gel column and the silica gel was washed with ether. After the combined organic layers were evaporated, the residue was passed through a SiO_2 (5 g) column eluted with hexane : AcOEt (30 : 1) to isolate the bromo acetate (**7**) (41 mg, 43%) and resolved bromohydrin (**8**) (42 mg, 49%) as a pale yellow oil, respectively. Bromo acetate (**7**) $[\alpha]_D^{31}$ +23.4° (*c* 1.0, MeOH) and +29.7° (*c* 1.0, $CHCl_3$). δ_H (500 MHz, $CDCl_3$) 0.91 (18-H_3, t, J = 7.3 Hz), 1.74 (17-H_2, m), 2.04 (8-H_2, dt, J = 6.5 Hz), 2.12 (3H, s, acetate), 2.30 (2-H_2, t, J = 7.0 Hz), 2.63 (14-H_2, m), 2.76 (11-H_2, br s), 3.67 (3H, s, ester Me), 4.04 (15-H, m), 4.92 (16-H, m), 5.30 (10-H, m), 5.40 (9-H, m), 5.44 (13-H, m), and 5.52 (12-H, m). (The assignment was based on H-H COSY experiments). δ_C (125 MHz, $CDCl_3$) 9.7 (q, 18-C), 25.9 (t, 17-C), 75.8 (d, 16-C), 56.0 (d, 15-C), 33.0 (t, 14-C), 125.5 (d, 13-C), 131.4 (d, 12-C), 25.9 (t, 11-C), 127.9

(d, 10-C),130.7 (d, 9-C), 27.3 (t, 8-C), 25.0 (t, 3-C), 34.1 (t, 2-C), 174.3 (s, 1-C), 51.4 (q, Me ester), 170.5 (s) and 21.0 (q) (acetate) in addition to unassigned triplets at 29.1, 29.2, 29.3, and 29.5. HRMS Calcd for $C_{21}H_{35}O_4Br$. 430.1718. Observed 430.1701. Resolved Bromohydrin (**8**) $[\alpha]_D^{31}$ – 15.4° (*c* 1.0, MeOH) and -10.0° (*c* 1.0, $CHCl_3$). The NMR spectra (δ_H and δ_C) were identical with *dl*-bromohydrin (**5**).

MTPA Ester of Bromohydrins : A mixture of *dl*-bromohydrin (**5**) (43 mg, 0.11 mmol), DCC (43 mg, 0.22 mmol), DMAP (*ca.* 2 mg), and (*S*)-MTPA (52 mg, 0.22 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred at rt for 68 h. The reaction mixture was diluted with ether and the organic layer was successively washed with aq. $NaHCO_3$ solution and then brine and dried over Na_2SO_4. After evaporation of volatile materials, the residue was passed through a SiO_2 (2 g) column to remove urea. The (*S*)-MTPA ester of *dl*-bromohydrin (**5**) (59 mg) was purified by HPLC using μ-porasil column with hexane : AcOEt (20 :1) as an elution solvent under the flow rate of 3 mL/min to obtain an inseparable mixture of (*S*)-MTPA esters (59 mg, 98%). δ_H 0.84 (t) and 0.96 (t) in a 1 : 1 ratio (18-H_3). Similarly, the resolved bromohydrin (**8**) was, respectively, treated with (*S*)- and (*R*)-MTPA to furnish the corresponding MTPA esters. (*S*)-MTPA ester in 85 % yield after 46 h. δ_H (270 MHz, $CDCl_3$) 0.84 (18-H_3, t, J = 7.3 Hz), 2.30 (2-H_2, t, J = 7.6 Hz), 3.59 (OMe of MTPA, s), 3.66 (1-OMe, s), 4.06 (15-H, m), 5.16 (16-H, m), 5.40 (4H, m), 7.42 (3H, m), and 7.60 (2H, m). HRMS Calcd for $C_{29}H_{40}O_5BrF_3$; 604.2011. Observed; 604.1988. (*R*)-MTPA ester in 85 % yield after 46 h. δ_H (270 MHz, $CDCl_3$) 0.96 (18-H_3, t, J = 7.6 Hz), 2.30 (2-H_2, t, J = 7.3 Hz), 3.58 (OMe of MTPA, s), 3.67 (1-OMe, s), 4.04 (15-H, m), 5.11 (16-H, m), 5.40 (4H, m), 7.42 (3H, m), and 7.59 (2H, m).

Epoxy-α-linolenic Acids from Bromoacetate (7) and Resolved Bromohydrin (8): After the bromo acetate (**7**) (41 mg, 0.09 mmol) in 0.5 N LiOH / dioxane : H_2O (1 :1) (1.8 mL, 10 eq.) was stirred at rt for 20 h under argon atmosphere, 0.5 N aq. oxalic acid solution was added to make pH of the solution acidic (3 ~ 4). After usual workup, the excess CH_2N_2 in ether solution was added to the crude reaction mixture and then the solvent was removed *in vacuo*. The crude methyl ester was purified by first SiO_2 (2.5 g) column chromatography eluted with hexane : AcOEt (40 :1) and then HPLC of μ-porasil column with hexane : AcOEt (20 : 1) to obtain the β-epoxide (**10**) (21 mg, 74%) as a pale yellow oil. The resolved bromohydrin (**8**) (42 mg, 0.11 mmol) was similarly treated with 0.5 N LiOH / dioxane : H_2O (1 : 1) (2.2 mL, 10 eq.) for 20 h at rt under argon atmosphere and purified by SiO_2 column chromatography and then HPLC after esterification with diazomethane to isolate α-epoxide (**9**) (31 mg, 93%) as a pale yellow oil. β-Epoxide (**10**) $[\alpha]_D^{31}$ – 3.8° (*c* 1.0, MeOH). δ_H (270 MHz, $CDCl_3$) 1.06 (3H, t, J = 7.3 Hz), 1.31 (8H, br d), 1.59 (4H, m), 2.03 (2H, m), 2.31 (4H, m), 2.90 (4H, m), 3.67 (3H, s), and 5.41 (4H, m). δ_C (67.8 MHz, $CDCl_3$) 174.3 (s), 130.7 (d), 130.5 (d), 127.2 (d), 124.2 (d), 58.3 (d), 56.5 (d), 51.4 (q), 34.0 (t), 29.5 (t), 29.3 (t), 29.1 (t) x2, 27.2 (t), 26.1 (t), 25.8 (t), 24.9 (t), 21.0 (t), and 10.6 (q). HRMS Calcd for $C_{19}H_{32}O_3$; 308.2351. Observed; 308.2364. α-Epoxide (**9**) $[\alpha]_D^{31}$ + 3.7° (*c* 1.0, MeOH). δ_H (270 MHz, $CDCl_3$) and δ_C (67.8 MHz, $CDCl_3$) were identical with those of β-Epoxide (**10**). HRMS Calcd for $C_{19}H_{32}O_3$; 308.2351. Observed; 308.2367.

α-Epoxide (9) from MTPA Ester of 8: After the purified MTPA ester of **8** (17 mg, 0.029 mmol) in 0.5 N LiOH / H_2O : dioxane (1:1) (1.7 mL) was stirred for 36 h at rt under argon atmosphere, the reaction mixture was diluted with water and acidified with aq oxalic acid to pH *ca.* 3. The mixture was worked up as usual and the residue was taken into ether, CH_2N_2 in ether was added and then the volatile materials were removed. The residue was purified with SiO_2 (1 g) column chromatography eluted with hexane : AcOEt (40

: 1) and then with HPLC [m-porasil column, solvent; hexane : AcOEt (20 : 1)] to obtain α-epoxide (**9**) (8 mg,, 87 %). $[\alpha]_D^{26}$ + 3.6°(*c* 1.0, MeOH).

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7 The present method is also applicable to DHA, the results being published elsewhere.

8 The ee of 98 % was estimated from the detection of the minute component (less than 2 %) in the NMR spectrum.

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