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The use of (*S*)-(−)-1-(1-naphthyl)ethylamine as a resolving agent for α-methoxy fatty acids

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Abstract

A general method was developed for the diastereoselective resolution of α-methoxy fatty acids utilizing (*S*)-(−)-1-(1-naphthyl)ethylamine as resolving agent. The diastereomeric amides can be easily separated by silica gel column chromatography and/or capillary gas chromatography, thus allowing for a preparative and analytical method for determining the enantiomeric purity of naturally occurring and/or synthetic α-methoxy fatty acids. The first synthesis of the naturally occurring (*R*)-2-methoxyhexadecanoic acid was also accomplished in four steps starting from commercially available (±)-2-hydroxyhexadecanoic acid.

1. Introduction

α-Methoxy fatty acids are natural acids, most commonly found in the phospholipids of marine organisms, with the *R* configuration at the stereogenic center.[1] Recent examples include (*Z*)-2-methoxy-5-hexadecenoic acid and (*Z*)-2-methoxy-6-hexadecenoic acid, initially identified in Caribbean sponges, and recently shown to possess antimicrobial activity against Gram-positive bacteria.[1c,d] Recently, the more classical α-methoxyhexadecanoic acid was also identified for the first time in nature in an *Amphimedon* sponge.[1e] Other longer chain analogs include the (2*R*,21*Z*)-2-methoxy-21-octacosenoic acid, which was the first naturally occurring α-methoxy fatty acid reported from a phospholipid.[1b]

Despite the fact that there have been many methods developed for the synthesis of optically active α-hydroxy fatty acids, including asymmetric syntheses or lipase mediated resolutions,[2] not much attention has been given to either the asymmetric synthesis and/or diastereomeric resolution of α-methoxy fatty acids. One approach to an optically active α-methoxy fatty acid could be the simple methylation of an optically active α-hydroxy fatty acid, but this approach has not been explored. One potential problem during such a methylation could be the partial racemization at the stereogenic center, and thus a mild

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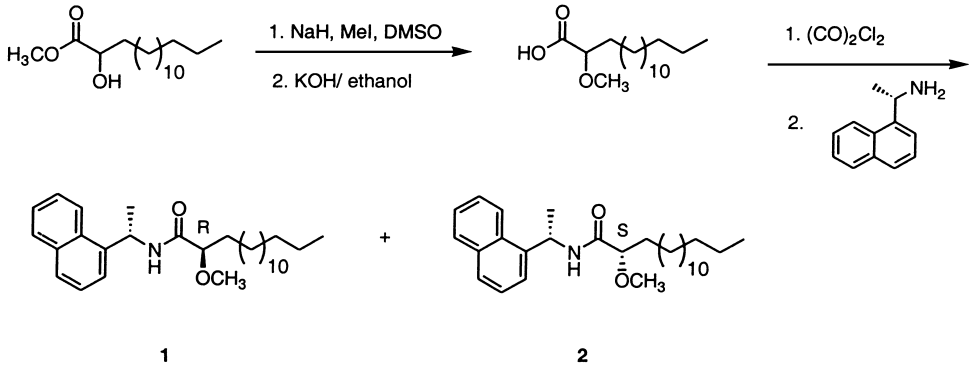
method should be employed. Moreover, a quick analytical method must also be developed for the quick assessment of the enantiomeric purity of the product.

1-(1-Naphthyl)ethylamine is an interesting reagent that has been used in the resolution of α- and β-methylated fatty acids.[3] Previous work has shown that the resulting diastereoisomers can be easily separated by flash column chromatography.[3] The naphthyl substituent helps in the chromatographic separation through its size and easy detection by ultraviolet spectroscopy, and it can be removed by acid hydrolysis, a process not reported to cause racemization at the stereogenic center. However, this methodology has not been extended to α-methoxylated fatty acids. Therefore, in this work we explore the use of 1-(1-naphthyl)ethylamine in the resolution of racemic α-methoxy fatty acids which can be developed into a general method to assess the optical purity of these compounds by either capillary gas chromatography and/or ^{1}H NMR. We also report the first synthesis of (*R*)-2-methoxyhexadecanoic acid.

2. Results and discussion

After reviewing several chiral reagents we chose (*S*)-(−)-1-(1-naphthyl)ethylamine as the resolving agent to develop a methodology for the rapid gas chromatographic determination of the absolute stereochemistry of α-methoxy fatty acids. Towards these ends a synthesis was developed that started with commercially available methyl 2-hydroxyhexadecanoate, which was conveniently methylated with NaH and MeI in dimethyl sulfoxide, the best conditions we found to carry out this transformation (Scheme 1). Further saponification with KOH in ethanol readily afforded the (±)-2-methoxyhexadecanoic acid. Reaction in ether with excess oxalyl chloride resulted in the corresponding acid chloride to which was added, at 0°C, (*S*)-(−)-1-(1-naphthyl)ethylamine. The resulting diastereomeric mixture was easily separated, for final characterization, using silica gel column chromatography with benzene:ethyl acetate (9:1) as eluent, which was the best solvent combination we found to separate these diastereoisomers. However, we noticed that these naphthyl amides readily separated into two well-resolved peaks using non-polar capillary gas chromatography with either an HP-5MS (Hewlett–Packard) or an SPB-5 (Supelco) capillary column, both of which contain poly(5% diphenyl/95% dimethylsiloxane) as the stationary phase (Fig. 1). As determined by the lipase mediated synthesis which will be described below, the (*R*)-2-methoxyamide elutes before the (*S*)-2-methoxyamide, reflecting their boiling point elution order. This means that, without any physical separation, it is possible to determine the enantiomeric composition of any mixture of 2-methoxyhexadecanoic acids by capillary gas chromatography. These results, therefore, lay the foundation for the development of a more general gas chromatographic technique to assess the enantiomeric purity of α-methoxylated fatty acids.



Scheme 1.

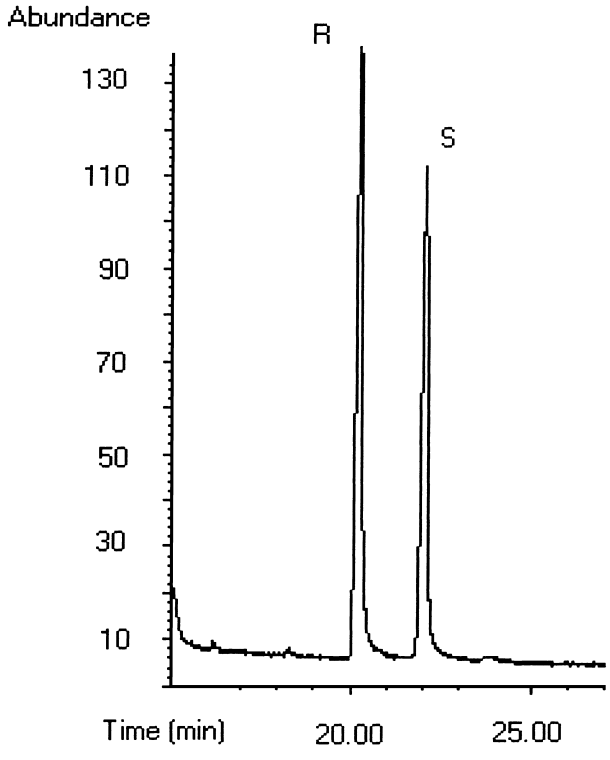
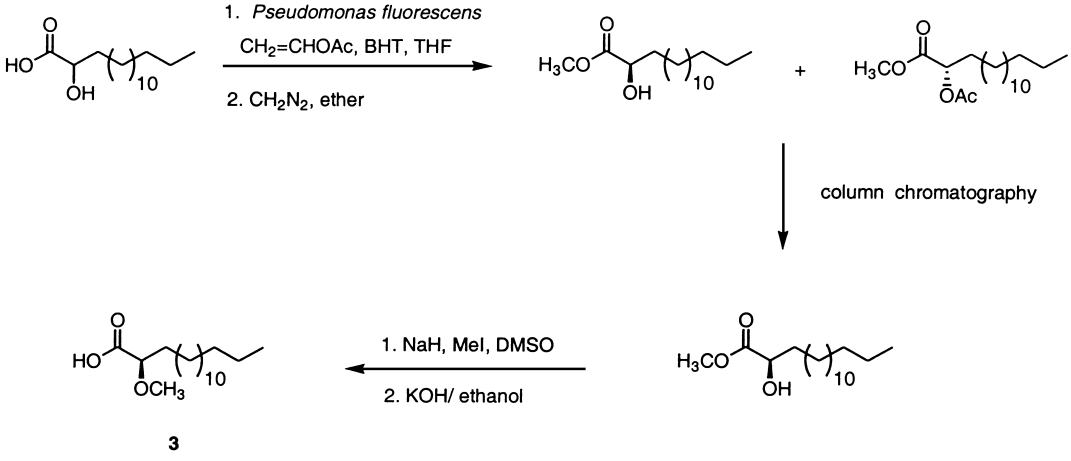


Figure 1.

^{1}H NMR also proved to be a good technique to differentiate between the two diastereoisomers. Even though there were some minor differences between diastereoisomers with respect to the hydrogen signals around the chiral methoxylated carbon, the methoxy resonance turned out to be the best signal to differentiate between the amides. In this particular case, the methoxy group of the (*R*)-2-methoxyamide resonated at 3.37 ppm, while the methoxy group of the (*S*)-2-methoxyamide resonated more upfield at 3.16 ppm, almost a 0.20 ppm difference. Therefore, with ^{1}H NMR it is also possible to assess the enantiomeric purity of an α-methoxylated fatty acid through its naphthylethylamide derivative.

As mentioned before, it was possible to assign the absolute stereochemistry to each diastereoisomer using lipase mediated chemistry, but the synthesis of only one of the two diastereoisomers was sufficient to assign their absolute stereochemistry. A key step in the synthesis was the methylation of (*R*)-2-hydroxyhexadecanoic acid, as the method of choice to obtain the corresponding optically active α-methoxy acid. Therefore, the synthesis of (*R*)-2-methoxyhexadecanoic acid, the natural acid, was undertaken and this also becomes the first synthesis for this optically active methoxy acid.[1e] Our synthesis started with commercially available (±)-2-hydroxyhexadecanoic acid, for which the *S* enantiomer was selectively acetylated (47% yield out of a maximum 50%, >95% ee) with vinyl acetate in THF using the lipase *Pseudomonas fluorescens* from Aldrich (Scheme 2). In order to facilitate the separation of the acetylated acid from the non-acetylated acid, both compounds were methylated with diazomethane, mild conditions that avoided cleavage of the acetate functionality. The methyl (*R*)-2-hydroxyhexadecanoate and the methyl (*S*)-2-acetoxyhexadecanoate were then separated by silica gel column chromatography using benzene:hexane (1:1) as eluent. The purified methyl (*R*)-2-hydroxyhexadecanoate was further methylated with NaH/MeI in DMSO, and finally saponified with KOH in ethanol, which afforded the (*R*)-2-methoxyhexadecanoic acid. The enantiomeric purity (>95% ee) of the (*R*)-2-methoxyhexadecanoic

acid was verified by making the corresponding naphthylethylamide, and it corresponded to the shortest retention time (20.3 min) peak in the gas chromatogram of the two possible diastereoisomers. The naphthylethylamide of (*S*)-2-methoxyhexadecanoic acid elutes second with a longer retention time (22.1 min) in non-polar capillary gas chromatography (Fig. 1). Therefore, little racemization at the stereogenic center was observed under these conditions. This represents the first synthesis of (*R*)-2-methoxyhexadecanoic acid.



Scheme 2.

We have presented here a facile and efficient method that discriminates between enantiomeric α-methoxyhexadecanoic acids. Preliminary studies in our laboratory have revealed that this methodology can also be extended to resolve β-methoxy fatty acids.

3. Experimental

The NMR spectra were recorded in $CDCl_3$ using either a General Electric QE-300 MHz spectrometer or a Bruker DPX 300 MHz spectrometer. FT-IR spectra were recorded neat in a Magna-IR 750 Nicolet spectrometer. Mass spectra were obtained at 70 eV using GC/MS mode in a Hewlett–Packard 5972A MS ChemStation (Palo Alto, CA) equipped with a 30 m×0.25 mm special performance capillary column (HP-5MS) cross-linked with 5% phenyl methylpolysiloxane or a 30 m×0.32 mm SPB-5 (Supelco) capillary column. The temperature program was as follows: 150°C for 0.5 min, then increased at a rate of 10°C/min to 270°C and maintained for 30 min at 270°C. The carrier gas was helium at a pressure of 5 psi. Optical rotations were measured in a Perkin–Elmer 243 B polarimeter. High resolution mass spectral data were recorded on a VG Autospec high resolution mass spectrometer.

*3.1. Preparation of diastereomeric naphthyl amides **1** and **2***

To a solution of a racemic mixture of 2-methoxyhexadecanoic acid (0.11 g, 0.38 mmol) in dry ether (6 ml), containing catalytic amounts of *N*,*N*-dimethylformamide (1 drop) at 0°C, was added a twofold excess of oxalyl chloride. After stirring for 4 h at rt, the mixture was concentrated to remove excess oxalyl chloride. Ether (5 ml) was added and the resulting solution cooled to 0°C. Then, a solution of (*S*)-(−)-1-(1-napthyl)ethylamine (0.06 ml, 0.39 mmol) in dry ether (10 ml) at 0°C was added dropwise to the acyl chloride. A precipitate was formed, and the reaction mixture was allowed to warm to rt and

stirred for 12 h. Then, ice-cold 1N HCl (10 mL) and water (5 mL) were added and the product extracted with ether:hexane (1:1), dried over Na_2SO_4, and concentrated affording the diastereomeric amides (0.11 g, 68%). The diastereomeric mixture was separated by silica gel column chromatography using benzene:ethyl acetate (9:1). The most mobile diastereoisomer corresponded to the (*R*)-2-methoxyamide (R_f=0.8), while the (*S*)-2-methoxyamide (R_f=0.66) eluted next.

*3.1.1. (*R*)-*N*-[(*S*)-1-(Naphthyl)ethyl]-2-methoxyhexadecanamide **1***

$[\alpha]_D^{25}$=−5.72 (*c* 0.035, $CHCl_3$); FT-IR (neat) ν_{max}: 3250 (NH), 3060, 2915, 2852, 1646 (C=O), 1546, 1472, 1378, 1126, 940, 781 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ=0.88 (3H, t, *J*=6.5 Hz, CH_3), 1.25–1.30 (26H, br s, CH_2), 1.70 (3H, d, *J*=6.8 Hz, N-CHC*H*$_3$), 3.37 (3H, s, -OCH_3), 3.68 (1H, dd, *J*=4.7 and 6.3 Hz, H-2), 5.94 (1H, q, N-C*H*CH_3), 6.76 (1H, br d, NH), 7.43–8.08 (7H, m, naphthyl); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=171.4 (s, C-1), 138.1 (s), 133.9 (s), 131.1 (s), 128.7 (d), 128.4 (d), 126.4 (d), 125.8 (d), 125.1 (d), 123.5 (d), 122.4 (d), 82.4 (d, C-2), 58.0 (q, -OCH_3), 43.9 (d, N-*C*HCH_3), 32.3 (t), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.45 (t), 29.42 (t), 29.3 (t), 24.6 (t), 22.7 (t), 20.8 (q, N-CH*C*H_3), 14.1 (q, -CH_3); GC–MS (70 eV) *m/z* (relative intensity): 439 $[M]^+$ (6), 244 (3), 243 (14), 241 (3), 211 (4), 198 (4), 182 (6), 171 (5), 170 (19), 169 (14), 156 (19), 155 (100), 154 (23), 153 (19), 152 (6), 129 (7), 128 (9), 115 (6), 111 (7), 97 (20), 95 (5), 89 (5), 85 (7), 83 (26), 81 (9), 71 (37), 69 (30), 67 (10), 58 (10), 57 (36), 55 (41); HREIMS *m/z*: 439.3447 (calcd for $C_{29}H_{45}NO_2$: 439.3450).

*3.1.2. (*S*)-*N*-[(*S*)-1-(Naphthyl)ethyl]-2-methoxyhexadecanamide **2***

$[\alpha]_D^{25}$=−25.72 (*c* 0.035, $CHCl_3$); FT-IR (neat) ν_{max}: 3308 (NH), 2956, 2918, 2853, 1651 (C=O), 1527, 1470, 1454, 1110, 809, 779 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ=0.88 (3H, t, *J*=6.7 Hz, CH_3), 1.20–1.38 (26 H, br s, CH_2), 1.67 (3H, d, *J*=6.7 Hz, N-CHC*H*$_3$), 3.16 (3H, s, -OCH_3), 3.63 (1H, dd, *J*=4.3 and 6.7 Hz, H-2), 5.94 (1H, q, N-C*H*CH_3), 6.76 (1H, br d, NH), 7.44–8.13 (7H, m, naphthyl); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=171.6 (s, C-1), 138.1 (s), 133.9 (s), 131.1 (s), 128.8 (d), 128.4 (d), 126.5 (d), 125.9 (d), 125.2 (d), 123.5 (d), 122.6 (d), 82.4 (d, C-2), 58.2 (q, -OCH_3), 43.9 (d, N-*C*HCH_3), 32.6 (t), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.45 (t), 29.36 (t), 24.7 (t), 22.7 (t), 20.7 (q, N-CH*C*H_3), 14.1 (q, CH_3); GC–MS (70 eV) *m/z* (relative intensity): 439 $[M]^+$ (3), 243 (12), 198 (4), 182 (6), 171 (4), 170 (15), 169 (12), 168 (11), 156 (19), 155 (100), 154 (23), 153 (18), 152 (6), 129 (8), 128 (10), 127 (11), 115 (7), 111 (8), 98 (3), 97 (22), 96 (3), 95 (7), 89 (6), 85 (9), 83 (32), 81 (10), 77 (5), 71 (51), 69 (40), 67 (14), 58 (15), 57 (54), 56 (10), 55 (61); HREIMS *m/z*: 439.3459 (calcd for $C_{29}H_{45}NO_2$: 439.3450).

*3.2. Methyl (*R*)-2-hydroxyhexadecanoate*

To a solution of (±)-2-hydroxyhexadecanoic acid (0.086 g, 0.31 mmol) and butylated hydroxy toluene (1 mg, BHT) in a mixture of vinyl acetate (1.0 mL) and THF (1.5 mL) (dried over molecular sieves 4 Å and passed through an alumina column) was added the lipase *Pseudomonas fluorescens* (0.101 g, Aldrich), and the mixture was stirred with heating at 65°C for 24 h. After cooling to rt, the mixture was filtered and the filtrate was concentrated in vacuo. The resulting mixture of (*R*)-2-hydroxyhexadecanoic acid and (*S*)-2-acetoxyhexadecanoic acid was methylated with an ethereal solution of diazomethane to give the corresponding fatty acid methyl esters in quantitative yields. This product was then chromatographed using silica gel as the stationary phase and benzene:hexane (1:1) as the mobile phase, affording 0.027 g (>95% ee) of unreacted methyl (*R*)-2-hydroxyhexadecanoate and 0.049 g (47% of the maximum expected of 50%) of methyl (*S*)-2-acetoxyhexadecanoate. The unreacted methyl (*R*)-2-hydroxyhexadecanoate was then used for the next step.

3.3. Methyl (R)-2-methoxyhexadecanoate

Into a two-necked 20 mL round-bottom flask, provided with a magnetic stirrer and under argon, were placed methyl 2-hydroxyhexadecanoate (0.010 g, 0.035 mmol) in DMSO (2 mL). Separately, NaH (2 equiv.) was dissolved in DMSO (1 mL) (also under a nitrogen atmosphere) and added dropwise, after which the reaction mixture was stirred at rt for 10 min. An excess of MeI was then added, and the reaction mixture was further stirred for 20 min. After this time the reaction mixture was diluted with hexane:ether (1:1) and washed twice with H_2O to remove the DMSO. The organic phase was dried over $MgSO_4$, filtered and evaporated in vacuo, affording 0.008 g (0.026 mmol, 73%) of methyl (*R*)-2-methoxyhexadecanoate.[1e]

3.4. (R)-2-Methoxyhexadecanoic acid **3**

Into a 25 mL round-bottom flask were placed methyl (*R*)-2-methoxyhexadecanoate (8 mg, 0.026 mmol) in 10 mL of 1.0 M KOH in ethanol. The reaction mixture was refluxed for 1 h and then allowed to cool to room temperature. The ethanol was evaporated in vacuo and the resulting salt washed with hexane (1×5 mL) to extract the non-saponifiable matter, which was discarded. The salt was then dissolved in water (15 mL) water and acidified with 6.0 M HCl followed by extraction with ethyl ether (3×10 mL). The organic extracts were added together and the solvent evaporated in vacuo, obtaining 6.1 mg of the acid,[1e] $[\alpha]_D^{27}$=+3.75 (*c* 0.08, $CHCl_3$), for an 80% yield.

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