



## The first total synthesis of (±)-4-methoxydecanoic acid: a novel antifungal fatty acid

Néstor M. Carballeira<sup>a,\*</sup>, Carlos Miranda<sup>a</sup>, Keykavous Parang<sup>b</sup>

<sup>a</sup> Department of Chemistry, University of Puerto Rico, Rio Piedras campus, P.O. Box 23346, San Juan, PR 00931, USA

<sup>b</sup> Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI, USA

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### ABSTRACT

The hitherto unknown (±)-4-methoxydecanoic acid was synthesized in six steps and in 25% overall yield starting from commercially available 4-penten-1-ol. The title compound demonstrated 17-fold higher antifungal activity (MIC = 1.5 mM) against *Candida albicans* ATCC 60193 and *Cryptococcus neoformans* ATCC 66031 when compared to unsubstituted *n*-decanoic acid. Our results demonstrate that mid-chain methoxylation appears to be a viable strategy for increasing the fungitoxicity of fatty acids.

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The (±)-4-hydroxydecanoic acid is an elusive fatty acid to isolate from either a natural or synthetic source because of its tendency to easily cyclize to the well-known  $\gamma$ -decalactone.<sup>1</sup> In fact, the spectral data for 4-hydroxydecanoic acid were first reported in 1996 by G. Feron and collaborators, attesting to the difficulty in isolating the pure hydroxylated fatty acid from the  $\gamma$ -decalactone.<sup>1</sup> The  $\gamma$ -decalactone is an important compound for the aroma and food industries since the compound imparts a peach-apricot flavor to foods<sup>2,3</sup> and is also responsible for the fruity flower odor of gardenia perfumes.<sup>1</sup> The  $\gamma$ -decalactone has also been used in <5 ppm concentrations in a selected number of cigarette brands.<sup>4</sup>

There are just a few scattered reports on the toxicity of (±)-4-hydroxydecanoic acid but it has been reported that the presence of a hydroxyl group in the acyl chain greatly decreases toxicity. However, the  $\gamma$ -decalactone has been reported to have higher antibacterial activity than (±)-4-hydroxydecanoic acid since it inhibits the growth of some bacteria such as *Bacillus subtilis* and some fungi such as *Fusarium oxysporum* and *Trichothecium roseum* at concentrations between 0.1 and 0.7 mM.<sup>1</sup> It should also be noted here that the 3-(*R*)-hydroxydecanoic acid has been isolated from *Lactobacillus plantarum* and displayed considerable antifungal activity (MIC = 10–100  $\mu$ g/mL) against different molds and yeasts.<sup>5</sup>

We have previously shown that  $\alpha$ -methoxylation increases the antifungal activity of fatty acids,<sup>6,7</sup> but nothing is known as to the effect of mid-chain methoxylation on the antifungal activity of

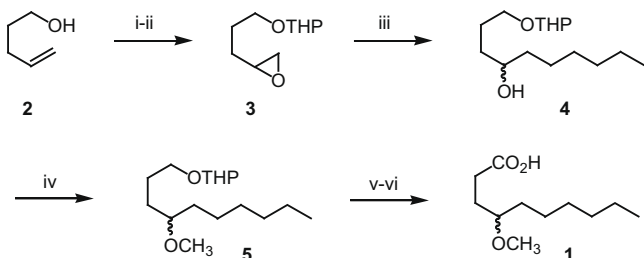
these compounds. We envisioned that a compound such as the (±)-4-methoxydecanoic acid (**1**) could be a good starting template to study such an effect. In addition, we were expecting this methoxylated fatty acid to be more fungitoxic than the corresponding compounds, unsubstituted *n*-decanoic acid (capric acid) or isolated (±)-4-hydroxydecanoic acid. The choice of a 10-carbon chain length for this study is justified by the fact that capric acid kills *Candida albicans* at 10 mM by the postulated mechanism of the fungal plasma membrane disintegration while other saturated fatty acids are not effective.<sup>8</sup>

Furthermore, unlike (±)-4-hydroxydecanoic acid, (±)-4-methoxydecanoic acid (**1**) cannot be cyclized to the corresponding  $\gamma$ -lactone thus allowing the facile study of its fungitoxicity. This investigation was also designed to determine whether mid-chain methoxylation is a viable substitution to  $\alpha$ -methoxylation for enhancing the antifungal activity of fatty acids such as capric acid. Previous studies by our group with the (±)-2-methoxydecanoic acid have shown that the 2-OMe-10:0 acid is only 1.5-fold more fungitoxic than capric acid (10:0) against *C. albicans* (ATCC 14053). However, against *Cryptococcus neoformans* (ATCC 66031) the 2-OMe-10:0 acid was not more antifungal than capric acid.<sup>6</sup>

Our six-step synthesis started with the protection of the primary alcohol of commercially available 4-penten-1-ol (**2**) with dihydropyran (DHP) and catalytic amounts of *p*-toluenesulfonic acid (PTSA) in  $\text{CHCl}_3$  at rt for 5 h to afford the 1-[(tetrahydropyran-2-yl)oxy]-2-pentene in an 88% yield (Scheme 1). The double bond was effectively epoxidized in the presence of magnesium monoperoxyphthalate (MMPP) in EtOH as solvent for 48 h, to yield

\* Corresponding author. Tel.: +1 787 764 0000x4791; fax: +1 787 756 8242.

E-mail address: [nmcarballeira@uprrp.edu](mailto:nmcarballeira@uprrp.edu) (N.M. Carballeira).



**Scheme 1.** Reagents and conditions: (i) DHP/PTSA,  $\text{CHCl}_3$ , rt, 5 h, 88%; (ii) MMPP/EtOH, 48 h, 89%; (iii)  $\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{MgBr}$ ,  $\text{Cu(I)/THF}$ ,  $-78^\circ\text{C}$  to  $-30^\circ\text{C}$ , 81%; (iv)  $\text{NaH/CH}_3\text{I}$ , THF,  $0^\circ\text{C}$  to rt, 2 h, 88%; (v) PTSA,  $\text{CHCl}_3$ ,  $45^\circ\text{C}$ , 2 h, 73%; (vi) PDC/DMF, 24 h, 63%.

the 4,5-epoxy-1-[(tetrahydropyran-2-yl)oxy]pentane (**2**) in 89% yield after purification of the crude product using silica gel column chromatography (60–200 mesh) and eluting with hexane/diethyl ether (8:2). MMPP turned out to be more efficient than the classical *m*-chloroperoxybenzoic acid (*m*-CPBA) in epoxidizing these alkenes, since the latter reagent only afforded moderate to low yields even after long reaction times. The THP-protected epoxide **3** was then opened with 1-pentylmagnesium bromide assisted by catalytic amounts of copper(I) chloride in THF at a reaction temperature range of  $-78^\circ\text{C}$  to  $-30^\circ\text{C}$ , which afforded the desired 4-hydroxy-1-[(tetrahydropyran-2-yl)oxy]decane (**4**) in an 81% yield after silica gel (60–200 mesh) column chromatographic purification. The free hydroxyl group in **4** was then readily methylated with methyl iodide in the presence of sodium hydride in THF, which afforded the 4-methoxy-1-[(tetrahydropyran-2-yl)oxy]decane (**5**) in an 88% yield. Deprotection of the primary alcohol was effectively accomplished with PTSA in  $\text{CHCl}_3$  at  $45^\circ\text{C}$  for 2 h, which afforded the (±)-4-methoxydecan-1-ol in a 73% yield (Scheme 1). Final oxidation to the acid was accomplished by reaction of the alcohol with pyridinium dichromate (PDC) in DMF for 24 h, which resulted in a 63% yield of **1**.<sup>9</sup> The overall yield for this six-step synthesis was 25%.

The most significant absorption in the NMR spectrum of **1** was observed for the carbons and hydrogens bearing the methoxy functionality. For example, the methoxy protons resonated at  $\delta$  3.32 ppm and the methoxy carbon was observed at  $\delta$  56.5 ppm, while the methine hydrogen ( $\text{CHOCH}_3$ ) resonated at  $\delta$  3.20 ppm and the methine carbon ( $\text{CHOCH}_3$ ) at  $\delta$  79.9 ppm. These  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR displacements seem to be characteristic for saturated mid-chain methoxylated fatty acids and useful as a future reference for other similar analogs. It is also interesting to mention that in the 70 eV electron impact (EI) mass spectrum of **1** the typical McLafferty rearrangement of fatty acids at  $m/z = 60$  was greatly reduced (1% relative abundance) by the presence of the methoxy functionality at C-4. In the mass spectrum of **1** the  $\alpha$ -fragmentation at both sides of the methoxylated carbon predominated, but the fragments containing the carboxyl end (at  $m/z = 117$  corresponding to  $\text{C}_5\text{H}_9\text{O}_3^+$  and at  $m/z = 85$  corresponding to  $\text{C}_4\text{H}_5\text{O}_2^+$ ) were the most abundant.

The antifungal activity of **1** was determined against a fluconazole-resistant strain of *C. albicans* (ATCC 60193) and against *C. neoformans* (ATCC 66031) following our previously published protocol (Table 1).<sup>6,7</sup> *n*-Decanoic acid was also tested as a control. As can be seen from the data given in Table 1 the (±)-4-methoxydecanoic acid (**1**) was approximately 17-fold more antifungal against both fungal strains (MIC = 1457  $\mu\text{M}$ ) when compared to *n*-decanoic acid (MIC = 25,478  $\mu\text{M}$ ). Therefore, the antifungal results clearly show

**Table 1**

Antifungal activity (MIC values,  $\mu\text{M}$ ) against *Candida albicans* (SDB) and *Cryptococcus neoformans* (SDB) at  $35\text{--}37^\circ\text{C}$  after 24–48 h<sup>a</sup>

Compound	<i>C. albicans</i> ATCC 60193	<i>C. neoformans</i> ATCC 66031
(±)-4-Methoxydecanoic acid ( <b>1</b> )	1457	1457
Decanoic acid	25,478	25,478
Fluconazole	>500	<0.9
Amphotericin B	<0.3	<0.3
DMSO	>5000	>5000

<sup>a</sup> The results are the average of three separate experiments. The upper limit of the standard error of the mean (SEM) was  $\pm 10\%$ .

that C-4 methoxylation increased the antifungal activity of the parent *n*-decanoic acid. In fact, for *n*-decanoic acid C-4 methoxy substitution seems to be more effective than C-2 methoxy substitution in increasing the antifungal activity of *n*-decanoic acid.<sup>6</sup>

As to the reasons for the better antifungal activity of **1** over that of *n*-decanoic acid we can only speculate at this stage and more mechanistic studies are required. The addition of the C-4 methoxy functionality possibly makes the fatty acid more soluble than unsubstituted *n*-decanoic acid thus facilitating its interaction with the target sites. In addition, based on the previously published antifungal mechanism of decanoic acid<sup>8</sup> we can also speculate that acid **1** seems to be able to more efficiently disrupt the fungal membranes due to the mid-chain methoxy substitution. Moreover, the title compound **1** may also inhibit fatty acid biosynthesis within the fungi interacting with some key enzymes. In summary, our results clearly demonstrate that mid-chain methoxylated fatty acids are valuable compounds that can be optimized for developing more potent antifungal agents and thus merit further scrutiny in the search for better antifungal analogs.

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- Spectral data for the (±)-4-methoxydecanoic acid (**1**): Transparent oil; IR (neat):  $\nu_{\text{max}}$  3500–2500, 2928, 1712, 1462, 1377, 1282, 1096, 936  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  3.32 (s, 3H,  $-\text{OCH}_3$ ), 3.20 (m, 1H, H-4), 2.43 (t,  $J = 7.5$  Hz, 2H, H-2), 1.97–1.67 (m, 2H, H-3), 1.52 (m, 2H, H-5), 1.27 (m, 8H,  $-\text{CH}_2-$ ), 0.87 (t,  $J = 6.7$  Hz, 3H,  $-\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75.5 MHz):  $\delta$  179.6, 79.9, 56.5, 33.1, 31.8, 29.9, 29.4, 28.2, 25.1, 22.6, 14.0; GC-MS (70 eV)  $m/z$  (rel. intensity) 201( $\text{M}^+ - 1$ , 0.1), 187(2), 170(1), 169(2), 129(18), 116(54), 97(16), 85(100), 71(14), 60(1), 57(7), 55(24); HRMS (APCI): calcd for  $\text{C}_{11}\text{H}_{23}\text{O}_3$  ( $\text{M} + \text{H}^+$ ) 203.1642, found: 203.1639.