Synthesis of New Paramagnetic Fatty Acids and Lipophilic Spin Labels

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Abstract: Starting from readily available five-membered cyclic nitrones, paramagnetic analogues of palmitic and hexadec-2E-enoic acids are described with a range of pyrrolidine ring orientations. Herein we report the synthesis of 3,4-disubstituted lipophilic pyrroline nitroxides through a palladium-catalyzed cross-coupling reaction. Lipophilic phosphonium salt and SH-specific labels (methanethiosulfonates and isoselenuronium salts) with allylic and propargylic terminal groups are also described.

Key words: fatty acids, free radicals, Grignard reaction, lipids, palladium-catalyzed reactions

It was the widespread occurrence of lipids as fuel molecules, signal molecules and membrane components that directed the researchers’ attention to this field. Synthetic lipids with a nitroxide or with a fluorescent probe have been extensively used in the past decades as useful molecular tools in ESR and fluorescence spectroscopy for studying the structure and function of complex systems such as phospholipid bilayers and membranes. Until now, various spin-labeled analogues of naturally occurring lipids have been synthesized with a nitroxide radical either on the polar head-group or on the acyl chains. The interaction of fatty acid spin labels with membrane components reflects the structure and function of the local environment with a high degree of sensitivity to motion and molecular rotation within the membrane organization. Paramagnetic fatty acids have been used to study bilirubin-phospholipid interactions, binding properties of bovine β-lactoglobulin and oxidative membrane damage. Lipophilic nitroxides can prevent peroxide-induced oxidative stress and apoptosis as shown with mitochondria-targeted nitroxides. These potential applications have inspired researchers to synthesize a range of paramagnetically modified fatty acids for more than 40 years. The most simple and widely used approach is the synthesis of DOXYL fatty acids [e.g. 2-(3-carboxyethyl-4,4-dimethyl-2-dodecyl-3-oxyl)oxazolidine radical] using fatty acid ketosteres and 2-amino-2-methylpropanol as starting materials. However, these compounds have limited stability toward reduction and protonation on the ring. To eliminate this problem we have reported the synthesis of more stable pyrrolidine (proxyl and azetoxyl) based fatty acids, starting from lipophilic nitrones, through a Grignard reaction of an unsaturated α-bromo alkene, followed by oxidation of the terminal double bond. Synthesis of 3,4-disubstituted pyrroline nitroxide based fatty acids and oxa-oleic acid was also reported.

In this paper we report the synthesis of novel 2,2- and 2,5-disubstituted pyrroline nitroxide fatty acids using a Grignard reaction, and 3,4-disubstituted pyrroline nitroxide fatty acids through a Suzuki reaction. Such compounds were also converted to their thiol-specific reagents and membrane probes. Treatment of 5,5-dimethyl-1-pyrroline N-oxide (DMPO; 1) or 2,5-dimethyl-1-pyrroline N-oxide10 (7) with dodecylmagnesium bromide in diethyl ether, yielded the trisubstituted nitrones 2 and 8, respectively, after oxidation of the resulting hydroxylamines to the nitrones by activated MnO2, analogously to the earlier procedures (Scheme 1). Further treatment of nitrones 2 and 8 with alkynylmagnesium bromide, generated from propargyl alcohol and excess ethylmagnesium bromide15,16 in tetrahydrofuran, afforded nitroxides 3 and 9 after oxidation with PbO2. In these new lipophilic paramagnetic compounds the alkyl chains are embedded in the 2,2- and 2,5-orientations. Because the orientation of the spin-labeled fatty acids in a membrane is a crucial issue, this can be varied through the extent of chain saturation in addition to the position and orientation of the nitroxide ring. Since, the synthesis of lipophilic nitroxides with different chain rigidity was a real challenge and an evident aim, we reduced the propargylic and allylic alcohols to the corresponding bromides 5a, 6a, 11a and 12a via their mesylates, as described earlier. The propargylic (5a, 11a) and allylic (6a, 12a) bromides were converted to methanethiosulfonates (5b, 6b, 11b and 12b) with NaSSO2CH3 in water–acetone, to give the reversible, SH-specific, lipophilic reagents. From the allylic bromides and selenourea, the more water soluble, SH-reactive isoselenuronium salts 6c and 12c were also synthesized.

Heating a mixture of triphenylphosphine and allylic bromide 6a at reflux, yielded the compound 6d, which was suitable for membrane studies since the triphenylphosphonium cation probably has increased affinity toward mitochondrial membranes.
acid synthesis was achieved through aldehydes 13 and 16, which were synthesized by oxidation of alcohols 4 and 10 by MnO₂ in chloroform (Scheme 2).²³ These allylic aldehydes were then oxidized to the corresponding carboxylic acids 14 and 17 with excess Ag₂O in a THF–dioxane–NaOH (aq) system.²⁴ The resulting acids were protected as their methyl esters by treatment with iodomethane in acetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).²⁵ The saturated fatty acids were prepared by reduction of the α,β-unsaturated double bond with the safe and convenient Ehrenkaufer reduction, applying ammonium formate and Pd/C.²⁶ The hydrolysis of crude saturated esters with NaOH and acidification of the mixture afforded the paramagnetic palmitic acids 15 and 18 with different nitrooxide ring orientations.

Previously, synthesis of pyrroline fatty acids and lipophilic spin labels involved treatment of the pivalate of a paramagnetic allylic alcohol with an organocopper deriv-
ative generated from a Grignard reagent and copper cyanide. This compound was then subjected to allylic bromination which required utilization of a protecting group. This tedious procedure can now be avoided through the use of Suzuki–Miyaura coupling of commercially available long-chain boronic acids and β-bromo-α,β-unsaturated aldehyde as we reported for the synthesis of other 3,4-disubstituted pyrroline nitroxides. This procedure was a significant breakthrough since it allowed C–C bond formation in the presence of nitroxide free radical.

Treatment of vinyl halogenide with dodecyl boronic acid (1.1 equiv) in the presence of Ag₂O (3 equiv), Ph₃As (0.2 equiv) and PdCl₂(CH₃CN)₂ (0.05 equiv) in THF at reflux resulted in the formation of compound (Scheme 3). Lipophilic aldehyde could be reduced with sodium borohydride in ethanol to give alcohol, which was converted to allylic bromide via its mesylate. Nucleophilic substitution with NaSSO₂CH₃ yielded allylic methanethiosulfonate, a lipophilic, SH-specific spin-labeled molecule. Oxidation of aldehyde with NaClO₂–H₂O₂ in a water–acetonitrile mixture afforded paramagnetic pentadec-2-Z-enoic acid (22).

The methanethiosulfonates 5b, 6b, 11b, 12b and 21c were studied in aqueous phosphatidylcholine (PC) lipid vesicle solutions and the results confirmed the importance of the nitroxide ring orientation and lipid chain saturation/rigidity. Room-temperature EPR spectra of aqueous PC lipid vesicle solutions (20 mg/mL) containing 1 mole% of the methanethiosulfonate compounds 5b, 6b, 11b, 12b or 21c are shown in Figure 1. The spectra of 5b and 6b reveal incomplete averaging of the anisotropic components of the hyperfine interaction, in contrast to the behavior of the other samples. This phenomenon was further studied by EPR spectroscopy of planar supported PC multilayers containing 6b (Figure 1a). From the effective hyperfine splitting, determined for the parallel and perpendicular orientations of the membrane plane with respect to the magnetic field, an order parameter of S = 0.15 was calculated according to the method published earlier. The or-

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\text{Scheme 3 Reagents and conditions: (a) C}_{12}\text{H}_{25}\text{B(OH)}_2 (1.1 equiv), AsPh}_3 (0.2 equiv), \text{PdCl}_2(\text{CH}_3\text{CN})_2 (0.05 equiv) in \text{THF at reflux, 18 h, 74%}; (b) \text{NaBH}_4 (2 equiv), \text{EtOH, r.t., 30 min, 85%}; (c) \text{MsCl (1.1 equiv), Et}_3\text{N (1.1 equiv), 0 °C→r.t., 1 h then LiBr (2.0 equiv), acetone, reflux, 30 min, 50%}; (d) \text{NaSSO}_2\text{CH}_3 (2.0 equiv), acetone–H₂O, 30 min, 50 °C, 56%; (e) \text{NaClO}_2 (1.1 equiv), H₂O₂ (1.0 equiv), H₂O–MeCN, KH₂PO₄, 0 °C→r.t., 1 h then Na₂S₂O₅, H⁺, 56%.} 
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\text{Figure 1 Room-temperature EPR spectra of aqueous phosphatidylcholine (PC) lipid vesicle solutions (20 mg/mL) containing 1 mole% of the methanethiosulfonate compounds 5b, 6b, 11b, 12b or 21c. (a) In addition to the vesicle spectrum (top), spectra of oriented quartz-supported planar PC multilayers containing 1 mole% of 6b are shown for membrane planes parallel and perpendicular to the external magnetic field (bottom). (b) Nearly complete averaging of the anisotropic components of the hyperfine tensor is revealed for 5b, 11b, 12b and 21c in PC vesicles.} 
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der parameter of 5b was found to be significantly smaller (oriented spectra not shown), which shows that replacement of the double bond by a triple bond leads to different reorientational behavior of the nitroxide rings. The orientation of the nitroxide with respect to the lipid axes did not allow determination of accurate order parameters for compounds 11b, 12b or 21c. Rapid reorientation around the lipid chain axis, in combination with axes wobbling, leads to nearly complete averaging of the anisotropic components of the hyperfine interaction.

In summary, new procedures have been developed for the synthesis of paramagnetic fatty acids and spin labels. The methods outlined above allow synthesis of stable pyrroline and pyrrolidine nitroxides with variable nitroxide ring orientation, ring saturation, chain length and rigidity/flexibility. Further studies and applications of paramagnetic fatty acids are in progress and the new PROXYL-palmitic acid possesses advantages over 7-DOXYL-stearic acid in the study of the fatty acid binding site of mitochondrial uncoupling protein.35

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Most of the compounds are either oils or low-melting-point solids which solidify in a freezer. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyzer. IR (Specord 85) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a Thermoquest Automass Multi and VG TRIO-2 instruments in the EI mode. 1H NMR spectra were recorded with Varian Unity Inova 400 WB spectrometer. Chemical shifts are referenced to TMS. Measurements were run at 298 K probe temperature in CDCl3 solution. ESR spectra were taken on Miniscope MS 200 in 10–4 M CHCl3 solution and all monoradicals gave triplet line a N = 14.7–15.1 G. EPR spectra of lipid vesicle solutions and hydrated planar-multilayers were measured with a Varian E-101. Flash chromatography was performed using Merck Kieselgel GF254. Chromatographic purification was performed using silica gel (0.040–0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.2 cm) coated with Merck Kieselgel GF254. n-Dodecylboronic acid was purchased from Alfa Aesar or prepared as published earlier.34 Compound 1 and all other reagents were purchased from Aldrich. Compounds 71 and 19 were prepared according to published procedures.

2,2-Dimethyl-5-dodecyl-3,4-dihydro-2H-pyrroline-1-oxide (2) and 2,5-Dimethyl-2-dodecyl-3,4-dihydro-2H-pyrroline-1-oxide (8)

To a dodecyl magnesium bromide solution in Et2O (40 mL) [made from Mg turnings (1.2 g, 50.0 mmol) and 1-bromododecane (12.45 g, 50.0 mmol)], nitrene 1 or 7 (4.52 g, 40.0 mmol) dissolved in anhyd THF (20 mL) was added dropwise under N2, and the mixture was stirred under reflux for 4 h. After cooling to 0 °C, the mixture was quenched with sat. aq NH4Cl solution (20 mL). The organic layer was separated and the aqueous layer was washed with CHCl3 (2 × 10 mL). The combined organic phases were dried (MgSO4), filtered and evaporated. The residue was redissolved in CHCl3 solution and all monoradicals gave triplet line a N = 14.7–15.1 G. EPR spectra of lipid vesicle solutions and hydrated planar-multilayers were measured with a Varian E-101. Flash chromatography was performed using Merck Kieselgel GF254. n-Dodecylboronic acid was purchased from Alfa Aesar or prepared as published earlier.34 Compound 1 and all other reagents were purchased from Aldrich. Compounds 71 and 19 were prepared according to published procedures.

2-Dodecyl-2-(3-hydroxyprop-1-ynyl)-5,5-dimethylpyrrolidin-1-oxyl Radical (3) and 2-Dodecyl-5-(3-hydroxyprop-1-ynyl)-2,5-dimethylpyrrolidin-1-oxyl Radical (9)

To a stirred solution of alkynylmagnesium bromide [made from propargyl alcohol (1.68 g, 30.0 mmol) and ethylmagnesium bromide (60.0 mmol, 20 mL, 3.0 M Et2O stock solution)] in THF (20 mL) was added, dropwise, a solution of compound 2 or 8 (5.62 g, 20.0 mmol) dissolved in THF (30 mL) at −5 °C under N2. After stirring the mixture at r.t. for 2 h, aq NH4Cl solution (5.0 M, 30 mL) was added. The organic phase was separated, dried (MgSO4) and evaporated. The residue was redissolved in CHCl3 (20 mL). Ph2O (480 mg, 2.0 mmol) was added and O2 was bubbled through for 30 min. The mixture was filtered, evaporated and purified by flash chromatography (hexane–EtOAc, 4:1) to give 3 or 9.

3

Yield: 4.5 g (67%); yellowish-brown oil; Rf = 0.38 (hexane–EtOAc, 2:1).

IR (neat): 3460 (OH) cm−1.

MS (EI, 70 eV): m/z (%) = 336 (2) [M]+, 291 (1), 168 (100).

Anal. Calcd for C21H38NO: C, 73.95; H, 11.39; N, 4.16. Found: C, 73.01; H, 11.25; N, 4.22.

2-Dodecyl-2-(3-hydroxyprop-1-enyl)-5,5-dimethylpyrrolidin-1-oxyl Radical (4) and 2-Dodecyl-5-(3-hydroxyprop-1-enyl)-2,5-dimethylpyrrolidin-1-oxyl Radical (10)

To a solution of compound 3 or 10 (3.36 g, 10.0 mmol) in anhyd THF (15 mL) was added, dropwise with stirring, a 70% solution of sodium bis(2-methoxy)aluminum hydride (SMEAH, 8 mL, 3.5 M in toluene, 28.0 mmol) diluted with THF (10 mL). The resulting mixture was stirred under N2 for 5 h at r.t. and then cautiously decomposed by the dropwise addition of aq NaOH (10%, 10 mL) at 0 °C. The organic phase was separated and the aqueous phase was

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extracted with CH₂Cl₂ (2 × 15 mL). The combined organic phase was dried (MgSO₄), filtered, and evaporated. The residue was dissolved in CHCl₃ (30 mL). PbO₂ (2.39 g, 10.0 mmol) was added and O₂ was bubbled through for 30 min. The PbO₂ was filtered off and the solvent was evaporated to give the crude product that was purified by flash column chromatography (hexane–EtOAc, 2:1) to give compound 4 or 10.

4
Yield: 1.62 g (48%); brown oil; Rₛ = 0.28 (hexane–EtOAc, 2:1).
IR (neat): 3440 (OH), 1650 (C=C) cm⁻¹.
Analysis Calcd for C₂₁H₃₇BrNO: C, 63.15; H, 9.34; N, 3.51. Found: C, 62.74; H, 9.80; N, 3.35.

2-(3-Bromoprop-1-yl)-5-dodecyl-2,5-dimethylpyrrolidin-1-yloxyl Radical (12a)
Yield: 861 mg (42%); red oil; Rₛ = 0.67 (hexane–Et₂O, 2:1).
IR (neat): 1650 (C=C) cm⁻¹.
MS (EI, 70 eV): m/z (%) = 304/302 (1/1) [M⁺], 289 (40), 232/230 (55/55), 81 (100).

3-Bromomethyl-4-dodecyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxyl Radical (21b)
Yield: 1.0 g (50%); yellow oil; Rₛ = 0.50 (hexane–Et₂O, 2:1).
IR (neat): 1670 (C=C) cm⁻¹.
MS (EI, 70 eV): m/z (%) = 402/400 (2/2) [M⁺], 372/370 (5/5), 278 (79), 43 (100).
Analysis Calcd for C₂₁H₂₉BrNO: C, 62.83; H, 9.79; N, 3.49. Found: C, 62.77; H, 9.80; N, 3.44.

Synthesis of Allylic and Propargylic Methanethiosulfonates; General Procedure
A solution of allylic/propargylic bromide (1.0 mmol) and Na₂SSCH₃ (268 mg, 2.0 mmol) in an acetone–H₂O mixture (4:1, 10 mL) was warmed to 50 °C, until consumption of the starting material was observed (monitored by TLC, ~30 min). After evaporation of the acetone, the oily residue was partitioned between CHCl₃ (15 mL) and H₂O (5 mL). The organic phase was separated, dried (MgSO₄), and evaporated. The residue was purified by flash column chromatography to give the methanethiosulfonates in 38–59% yield.

2-(3-Methanethiosulfonylprop-1-ynyl)-2-dodecyl-5,5-dimethylpyrrolidin-1-yloxyl Radical (5a)
Yield: 0.98 g (49%); yellowish-red oil; Rₛ = 0.69 (hexane–Et₂O, 2:1).
Analysis Calcd for C₂₁H₂₉BrNO: C, 63.15; H, 9.34; N, 3.44. Found: C, 62.83; H, 9.79; N, 3.49. Found: C, 62.74; H, 9.80; N, 3.35.

2-(3-Methanethiosulfonylprop-1-ynyl)-2-dodecyl-5,5-dimethylpyrrolidin-1-yloxyl Radical (12b)
Yield: 229 mg (53%); beige solid; Rₛ = 0.39 (hexane–EtOAc, 2:1).
IR (nujol): 1650 (C=C) cm⁻¹.
MS (El, 70 eV): m/z (%) = 432 (4) [M⁺], 323 (3), 290 (5), 264 (100).
Anal. Calcd for C₃₉H₅₄BrNOP: C, 70.57; H, 8.20; N, 2.11. Found: C, 61.10; H, 9.72; N, 3.10.

3-Methanethiosulfonylethyl-4-dodecyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yl oxyl Radical (21c)

Yield: 242 mg (56%); orange oil; Rf = 0.43 (hexane–EtOAc, 2:1).
IR (neat): 1650 (C=C) cm⁻¹.
MS (El, 70 eV): m/z (%) = 432 (7) [M⁺], 430 (7), 306 (11), 278 (38), 152 (100).
Anal. Calcd for C₂₂H₄₃BrN₃OSe: C, 50.38; H, 8.26; N, 8.01. Found: C, 50.43; H, 8.22; N, 7.88.

Synthesis of Isoselenuronium Salts; General Procedure

A solution of compound 6a or 12a (401 mg, 1.0 mmol) and sele­nourea (123 mg, 1.0 mmol) was refluxed in anhyd acetone (10 mL) for 30 min. After cooling, the precipitated isoseluronium salt was filtered off to give the title compounds as water-soluble SH-reagents.

2-(3-Isoselenuroniumprop-1-yl)-2-dodecyl-5,5-dimethyl­pyrrolidin-1-yl oxyl Radical Bromide Salt (6d)

Yield: 424 mg (81%); yellow solid; mp 138–140 °C; Rf = 0.5 (CHCl₃–MeOH, 4:1).
IR (nujol): 3250, 3100 (NH), 1660 (C=N) cm⁻¹.
MS (El, 70 eV): m/z (%) = 352 (2), 322 (1), 307 (3), 184 (76), 43 (100).

(E)-3-(2-Dodecyl-1-oxyl-5,5-dimethylpyrrolidin-2-yl)acrylic Acid Radical (14) and (E)-3-(5-Dodecyl-1-oxyl-2,5-dimethylpyrrolidin-2-yl)acrylic Acid (17)

To a stirred suspension of freshly precipitated Ag₂O (924 mg, 4.0 mmol) and aq NaOH (10%, 20 mL), aldehyde 13 or 16 (672 mg, 2.0 mmol) dissolved in a mixture of THF (5 mL) and dioxane (2 mL), was added at 50 °C and the mixture was stirred for 2 h at this temperature. The reaction mixture was filtered through Celite and the filtrate was acidified cautiously with aq H₂SO₄ (5%). The solvent was evaporated off and the residue was purified by flash column chromatography (CHCl₃–Et₂O, 2:1) to give the title compounds in 35–41% yields.

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crude ester, which was dissolved in MeOH (10 mL) and aq NaOH (10%, 5 mL) was added. The solution was refluxed for 1 h and allowed to stand overnight at r.t. After the MeOH was removed under vacuum, the aqueous phase was acidified with aq H$_2$SO$_4$ (5%), and the mixture was extracted with CHCl$_3$ (2 × 15 mL), the combined organic phase was dried (MgSO$_4$), filtered and the mixture was concentrated in vacuo. The residue was purified by flash column chromatography to yield the acids as pale-yellow semisolids 15 and 18 in 25–31% yield.

15
Yield: 177 mg (25%); mp 43–45 °C; $R_f$ = 0.37 (CHCl$_3$-Et$_2$O, 2:1).
IR (nujol): 3000 (OH), 1690 (C=O).
MS (EI, 70 eV): $m/z$ (%) = 354 (5) [M$^+$], 282 (20), 186 (100).
Anal. Calcd for C$_{21}$H$_{38}$NO$_2$: C, 74.95; H, 11.38; N, 4.16. Found: C, 74.53; H, 11.91; N, 4.14. Found: C, 74.53; H, 11.88; N, 4.10.

18
Yield: 219 mg (31%); mp 45–47 °C; $R_f$ = 0.22 (CHCl$_3$-Et$_2$O, 2:1).
IR (nujol): 3100 (OH), 1680 (C=O), 1620 (C=C) cm$^{-1}$.
MS (EI, 70 eV): $m/z$ (%) = 352 (33) [M$^+$], 337 (28), 324 (35), 168 (51), 43 (100).
Anal. Calcd for C$_{21}$H$_{40}$NO$_3$: C, 74.50; H, 11.91; N, 4.14. Found: C, 74.53; H, 11.88; N, 4.10.

3-Formyl-4-dodecyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxyl Radical (20)
A stirred solution of aldehyde 19 (1.24 g, 5.0 mmol), PdCl$_2$(MeCN)$_2$ (65 mg, 0.25 mmol), Ph$_3$As (305 mg, 1.0 mmol) and H$_2$O$_2$ (30%, 0.1 mL), was added, dropwise over 30 min, to a well-stirred solution of aldehyde 20 (1.00 g, 3.0 mmol) in EtOH (20 mL), and the mixture was stirred at reflux for 18 h under N$_2$. After cooling, the mixture was filtered through Celite, the filtrate was evaporated and the residue was partitioned between brine (10 mL) andEt$_2$O (15 mL). The aqueous layer was washed with Et$_2$O (15 mL) and the combined ethereal solution was dried, filtered, evaporated and the residue was purified by flash column chromatography (hexane–Et$_2$O, 97:3) to yield aldehyde 20 as a yellow oil.

Yield: 1.24 g (74%); $R_f$ = 0.46 (hexane–Et$_2$O, 2:1).
IR (neat): 1670 (C=O), 1600 (C=C) cm$^{-1}$.
MS (EI, 70 eV): $m/z$ (%) = 336 (2) [M$^+$], 308 (17), 306 (12), 293 (51), 43 (100).

3-Carboxy-4-dodecyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxyl Radical (22)
To a well-stirred solution of aldehyde 20 (336 mg, 1.0 mmol), KH$_2$PO$_4$ (70 mg, 0.51 mmol) in MeCN–H$_2$O (5:3, 8 mL) and aq H$_2$O$_2$ (30%, 0.1 mL), was added, dropwise over 30 min, NaClO$_2$ (200 mg, 2.2 mmol) dissolved in H$_2$O (5 mL) at 0 °C. The solution was stirred at r.t. for 1 h then Na$_2$S$_2$O$_5$ (100 mg) was added and the solution was cautiously acidified with aq HCl (1.0 M). The solution was extracted with Et$_2$O (2 × 10 mL) and the combined organic phase was dried (MgSO$_4$), filtered and evaporated to give a residue that was purified by flash column chromatography (hexane–EtOAc, 2:1) to give the title acid.

Yield: 200 mg (56%); yellow solid; mp 53–54 °C; $R_f$ = 0.67 (CHCl$_3$-Et$_2$O, 2:1).
IR (nujol): 3100 (OH), 1680 (C=O), 1620 (C=C) cm$^{-1}$.
MS (EI, 70 eV): $m/z$ (%) = 352 (33) [M$^+$], 337 (28), 324 (35), 168 (51), 43 (100).
Anal. Calcd for C$_{21}$H$_{40}$NO$_3$: C, 71.50; H, 10.81; N, 3.86. Found: C, 71.50; H, 10.81; N, 3.86.

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