*Tetrahedron* Vol. 49, No. 30, pp. 6613-6618, 1993
Printed in Great Britain

0040-4020/93 $6.00 + .00
Pergamon Press Ltd

# Synthesis of Deuterium Labelled (11*S*,12*R*)- and (11*R*,12*S*)-[$^2H_{14}$]-Palmitic Acids; a Facile Route to Highly Labelled Fatty Acids

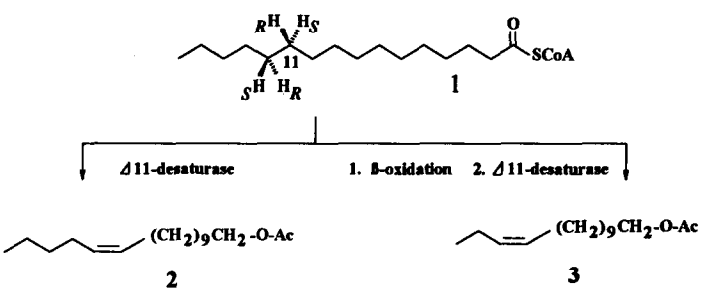
**Christian Frößl and Wilhelm Boland***

Institut für Organische Chemie der Universität, Richard-Willstätter-Allee 2, D-76131 Karlsruhe 1, Germany

(*Received in Germany* 21 *April* 1993)

**Abstract:** Chiral, highly deuterated palmitic acids are synthesised from 2,2'-bithienyl in three steps by two alkylations and reductive desulfurisation for the introduction of twelve deuterium atoms. The chiral building blocks are obtained from a,ß-unsaturated acids by enantiospecific reduction with broken cells of the microorganism *Clostridium tyrobutyricum.*

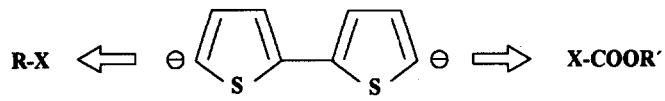
The chain length and the position of the double bond(s) of the majority of the hitherto known lepidopteran pheromones is the result of two major enzymatic activities. There exist different desaturases which in combination with ß-oxidation(s) at different stages of the pheromone biosynthesis are responsible for the structural diversity of this class of compounds which is known today.




The most common type of desaturation is Δ11, but Δ9 and Δ10 are also known.[1,2] While the mechanism(s) of the iron containing Δ9-, Δ6-, Δ5- and Δ4-desaturases from mammalian, bacterial or algal sources are well established,[3] only little is known about the Δ11-desaturases from lepidopterans.[1,2] One of the most promising strategies to screen for (mechanistic) similarities between the enzymes of different biological origins is the stereochemical approach. The data can be obtained from living insects relying on well established feeding techniques[2] using chiral precursors. As shown, this approach requires for chiral palmitic acids which are labelled at the carbon atoms C(11) and C(12), respectively. Enantiospecific labelling at these positions with deuterium atoms in conjunction with mass spectroscopy of the resulting metabolites can give all the information which is needed to deduce the stereochemical course of the particular fatty acid desaturase from insects. Since highly deuterated compounds exhibit lower boiling points than their hydrogen isotopomers, additional hydrogen at-

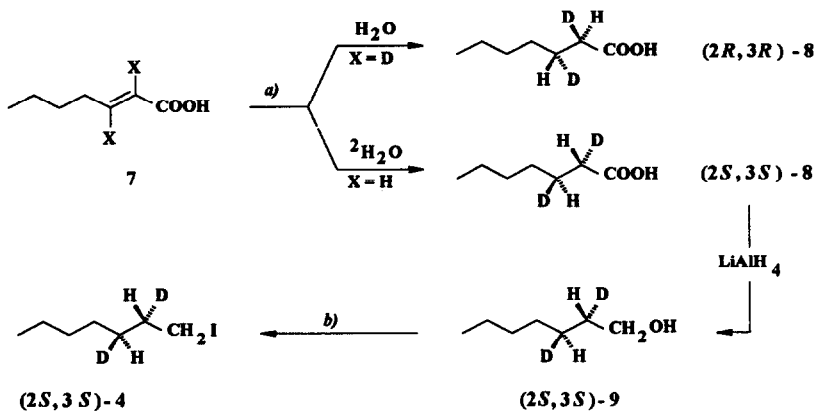
oms have to be incorporated into the precursor acid **1** to warrant gas chromatographic separation between the natural [$^1$H]- and the artificial [$^2$H]-pheromone.

The current work summarises a versatile approach to chiral, highly deuterated fatty acids requiring only three steps starting from 2,2´-bithienyl **5** and chirally labelled [$^2H_2$]-heptyl iodides **4**.




**4** **5** **6**

The bithienyl **5** represents a bifunctional starting material which can be successively alkylated at both ends using different electrophiles. The product can be desulfurised with Ni-catalysts[5] yielding an unbranched hydrocarbon segment. If the reaction is carried out with $NaB^2H_4$ as the reducing agent, a total of twelve deuterium atoms can be introduced which is sufficient for a base-line separation of natural and labelled products by gas chromatography. If X = Hal, the two chiral carbons atoms C(11) and C(12) of **1** correspond to C(3) and C(4) of the electrophile **4** (R = $C_7H_{15}$). As shown below, the two chiral centres are easily generated from the α,ß-unsaturated acids **7** by an enantiospecific reduction using a biocatalyst.

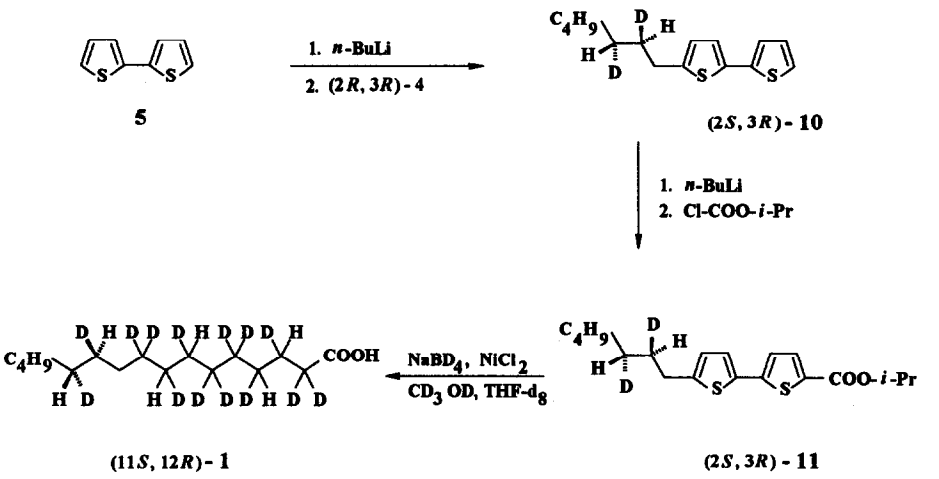



*a)* broken cells of *Clostridium tyrobutyricum*, phosphate buffer (pH = 7.0), $H_2$ gas *b)* $I_2$, $P(Ph)_3$, imidazole

The enoate reductase from the bacterium *Clostridium tyrobutyricum* (*C. La.* 1) catalyses the enantiospecific transfer of two hydrogen atoms across the double bond of a broad range of α,ß-unsaturated acids.[6,7] The process is well suited for preparative scale reactions. As outlined, the reduction can be carried out in $^1$H-buffers as well as in buffered $^2H_2O$-solutions. The hydrogen or deuterium atoms are delivered to the two trigonal centres at C(2) and C(3) of **7** in an exclusive *anti-Si-Si* fashion[8] and, hence, both enantiomers of the acids (2*R*,3*R*)-**8** and (2*S*,3*S*)-**8** are available by proper combination of the precursor **7** (X = H or $^2$H) with the suitable buffer system ($H_2O$ or $^2H_2O$). As proven by their mandelate-diesters and $^1$H NMR[7,9] the acids (2*S*,3*S*)-**8** and (2*R*,-3*R*)-**8** are virtually optically pure. Reduction and iodination[10] provides the chiral heptyl iodides (2*R*,3*R*)-**4** and

(2*S*,3*S*)-**4** in 53% overall yield from the achiral precursor(s) **7**. [2,3-$^2H_2$]-**7** is available according to standard procedures.[7]

The use of three eq. of bithienyl **5** and 1.5 eq. of *n*-BuLi minimises *bis*-metallation of **5** (< 5%) and warrants an optimal exploitation of the chiral iodides (2*S*,3*S*)-**4** or (2*R*,3*R*)-**4**, respectively. Remetallation and subsequent treatment of the lithium salt of **10** with the electrophile **6** (X = Cl) furnishes the esters (2*R*,3*S*)- and (2*S*,3*R*)-**11** in good overall yield ( 44%) from **5**.




The desulfurisation[5] is readily accomplished with nickel boride prepared from anhydrous $NiCl_2$ and $NaB^2H_4$ in MeOH-$d_4$/THF-$d_8$. The deuterated solvents are essential to warrant a high degree of isotopic labelling. Final saponification with $K_2CO_3$ in aq. MeOH provides the labelled palmitic acids (11*S*,12*R*)- or (11*R*,12*S*)-[$^2H_{14}$]-**1** without loss of deuterium from C(2). According to $^1H$ NMR (deuteration rate of the C(2) at $\delta$ = 2.25) and mass spectrometry an average of ≥ 95% isotope incorporation per methylene group atom is achieved by the above method.

First experiments with females of *Mamestra brassicae* indicate an excellent incorporation of labelled **1** yielding the highly deuterated pheromone **2**. As anticipated, the deuterated metabolite and the natural [$^1H$]-pheromone exhibit base-line separation upon gas chromatographic separation. Experimental details and the stereochemical course of the desaturase from *M. brassicae* will be reported in due course.[11]

## EXPERIMENTAL

General remarks. Reactions were performed under argon. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying. Boiling points are not corrected. The following spectroscopic and analytical instruments were used: $^1H$- and $^{13}C$ NMR: Bruker Cryospec WM 250 and Bruker WM 400; $CDCl_3$, TMS as internal standard. IR: Perkin-Elmer-882 IR spectrophotometer. MS: Finnigan MAT 90 GLC/-MS system and Finnigan ITD 800 combined with a Carlo-Erba gas chromatograph, model Vega, equipped with a fused-silica capillary SE 30, (10m x 0.32 mm); carrier gas, He at 30cm/s; scan range: 35-350 Dalton/s. Analytical GLC: Carlo-Erba gas chromatograph, HRGC 5300, Mega series, equipped with fused silica capillaries,

SE 30 (10m x 0.32mm); $H_2$ at 30 cm/s as carrier. Silica gel, Si 60, (0.040-0.063 mm, E. Merck, Darmstadt, FRG) was used for column chromatography.

**Microbial Reduction of Heptenoic Acids (7); General Procedure:** *Clostridium tyrobutyricum* (Strain: *C. La 1*, DSM 1460) was grown, stored, and manipulated as described.[6] For the experiment in $^2H_2O$-buffer, wet packed cells were freeze dried (under exclusion of oxygen) and resuspended in buffered $^2H_2O$. Reduction of 7: A total volume of 260 ml containing the sodium salt of [$^1$H]- or [2,3-$^2H_2$]-7 (4.56 g, 30.0 mmol), 40.0 g of wet packed cells, 11 mg tetracyclin·HCl, methylviologen (93 mg, 0.03 mmol) and 0.1 M potassium-phosphate buffer (from $H_2O$ or $^2H_2O$) at p(D)H 7.0 is shaken at 35° under an atmosphere of $H_2$ gas. The progress of the reaction is monitored by the consumption of the hydrogen gas using a *Warburg* manometer or by GLC. The reduction is complete within two days. The mixture is acidified (pH = 1.5) by addition of dil. $H_2SO_4$ and the product is extracted with ether (3 x 100 ml). Drying, evaporation of the solvents and rapid filtration over a small column of silica gel yields the crude acids **8** (hexane/ether, 70:30) which are used for the next step without further purification . Yield: 2.93 g (74%).

**(2*R*,3*R*)-[2,3-$^2H_2$]-Heptanol (2*R*,3*R*-(9)).**
A soln. of the above acid (2.9 g, 22 mmol) in THF (50 ml) is added to a chilled suspension of $LiAlH_4$ (0.92 g, 24 mmol) in the same solvent (100 ml). The mixture is refluxed for 14 h, cooled and hydrolysed with water and dil. HCl (2 N). Usual workup and distillation affords the alcohol (2*R*,3*R*)-**9**. Yield: 2.15 g (83%). B.p.: 68°C/ 20 Torr. $^1$H NMR (250 MHz, $CDCl_3$): δ 3.63 (d, 2H), 1.55 (s,br., 2H), 1.29 (s, 8H), 0.89 (t, 3H); IR (neat): 3332, 2958, 2924, 2858, 2154, 1464, 1410, 1376, 1033 cm$^{-1}$. MS (%): 117($M^{+\bullet}$-1, 0.05), 100(9), 85(7), 84(3), 72(17), 71(100), 70(41), 69(10); HR-MS *m/z* calcd. for $C_7H_{13}{}^2H_2O$ ($M^{+\bullet}$-H): 117.1248, found 117.1245.

**(2*S*,3*S*)-[2,3-$^2H_2$]-Heptanol (2*S*,3*S*-(9)).** From (2*S*,3*S*)-**9** (2.5 g, 21 mmol) as described above. Yield: 1.93 g (86%). Spectroscopic data identical with (2*R*,3*R*)-**9**.

**(2*R*,3*R*)-1-Iodo-[2,3-$^2H_2$]-heptane (2*R*,3*R*-(4)).**
A chilled soln. of triphenylphosphane (5.14 g, 19.6 mmol) and imidazole (1.33 g, 19.6 mmol) in 70 ml ether/$CH_3CN$ (3:1, v/v) is treated within 20 min with iodine (4.97 g, 19.6 mmol), and the soln. is allowed to come to rt. The mixture is chilled, and the alcohol (2*R*,3*R*)-9 (2.1 g, 17.8 mmol) is slowly added. Stirring is continued for 2 h at rt. Extractive workup with pentane (3 x 50 ml) and chromatography (silica gel, pentane) yields (2*R*,3*R*)-**4** as a colourless liquid. Yield: 3.51 g (86%). $^1$H NMR (250 MHz, $CDCl_3$): δ 3.17 (d, 2H), 1.81 (s,br., 1H), 1.29 (s,br., 9H), 0.89 (t, 3H); IR (neat): 2956, 2921, 2855, 2158, 1463, 1428, 1376, 1187 cm$^{-1}$. MS (%): 228 ($M^{+\bullet}$, 100), 156(39), 154(10), 141(10), 128(16), 127(41), 101(63); HR-MS *m/z* calcd. for $C_7H_{13}{}^2H_2I$: 228.0343, found 228.0358.

**(2*S*,3*S*)-1-Iodo-[2,3-$^2H_2$]-heptane ( 2*S*,3*S*-(4)).**
From (2*S*,3*S*)-**9** (1.90 g, 16.1 mmol) as described before. Yield: 3.43 g (93%). Spectroscopic data identical with (2*R*,3*R*)-**4**.

**5-((2*S*,3*R*)-[2,3-$^2H_2$]-Heptyl)-2,2′-bithienyl (2*S*,3*R*-(10)).**
A cold soln. of (-78°C) 2,2′-bithienyl **5** (3.27 g, 19.7 mmol) in THF (70 ml) is gradually treated with *n*-BuLi (3.93 ml, 9.8 mmol; 2.5 M soln. in hexane). The coolant is removed for 10 min, followed by recooling (-78°C) and addition of a soln. of the iodide (2*R*,3*R*)-**4** (1.50 g, 6.6 mmol) in 10 ml of 1,3-dimethyl-2-imidazolidinone (DMEU). The temperature is maintained for 30 min and then the mixture is allowed to come to rt within two h. Water (20 ml) is added, and the product is extracted with pentane. Usual workup and chromatography (silica gel, pentane) affords moderately pure **10** which is further purified by chromatography on reversed-phase (C18) using methanol for elution. Yield: 1.13 g (65%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 7.16 (d, J = 1.1 Hz, 1H), 7.14 (d, J = 1.1 Hz, 1H), 6.97-7.09 (m, 2H), 6.66 (d, J = 3.5, 1H), 2.77 (d, J = 7.5 Hz, 2H), 1.64 (m,br., 1H), 1.30 (m,br., 7H), 0.88 (t, 3H); IR (neat): 3108, 3069, 2955, 2921, 2853, 2152, 1515, 1464, 1425, 1202, 1115, 1045, 910, 837, 816, 794 $cm^{-1}$. MS (%): 266($M^{+\bullet}$, 39), 181(10), 180(10), 179(100), 69(39); HR-MS *m/z* calcd. for $C_{15}H_{18}{}^2H_2S_2$: 266.1132, found 266.1097.

**5-((2*R*,3*S*)-[2,3-$^2H_2$]-Heptyl)-2,2′-bithienyl (2*R*,3*S*-(10)).**
From (2*S*,3*S*)-**4** (3.40 g, 14.8 mmol) as described before. Yield: 2.83 g (72%). Spectroscopic data identical with (2*S*,3*R*)-**10.**

**2-Methylethyl 5-((2*S*,3*R*)-[2,3-$^2H_2$]-heptyl)-2′,5-bithienyl-2-methanoate (2*S*,3*R*-(11)).**
A cold soln (-78°C) of (2*R*,3*R*)-**10** (0.296 g, 1.11 mmol) in THF (5 ml) is gradually treated with n-BuLi (0.47 ml, 1.17 mmol; 2.5 M in hexane). The temperature is maintained with stirring for 20 min. Then the mixture is recooled (-78°C) and transferred by a syringe into a cold (-78°C) soln. of the chloro formate **6** (0.205 g, 1.67 mmol) in toluene (1.7 ml). After 30 min, the temperature is raised to -20°C and after 4 h the mixture is hydrolysed with cold water (0°C). Extractive workup (ether) and chromatography (silica gel. pentane/ether, 95:5) furnishes **11** as a colourless liquid. Yield: 0.26 g (67%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 7.64 (d, J = 3.9 Hz, 1H), 7.08 (d, J = 3.6 Hz, 1H), 7.04 (d, J = 3.9 Hz, 1H), 6.70 (d, J = 3.5 Hz, 1H), 5.20 (sept. 1H), 2.78 (d, J = 7.6 Hz, 2H), 1.70 (m,br., 1H), 1.33 (d, 6H), 1.29 (d, 2H), 1.30 (m,br., 7H), 0.89 (t, 3H); IR (neat): 3073, 2984, 2958, 2913, 2872, 2854, 2139, 1694, 1522, 1479, 1467, 1438, 1350, 1289, 1178, 1143, 1092, 1035, 797, 782, 752 $cm^{-1}$. MS (%): 352($M^{+\bullet}$,100), 310(18), 293(9), 265(20), 237(3), 223(94), 179(4), 134(4); HR-MS *m/z* calcd. for $C_{19}H_{24}{}^2H_2O_2S_2$: 352,1500, found 352.1475.

**2-Methylethyl 5-((2*R*,3*S*)-[2,3-$^2H_2$]-heptyl)-2,5-bithienyl-2-methanoate (2*R*,3*S*-(11)).**
From (2*S*,3*S*)-**10** (0.730 g, 2.74 mmol) as described before. Yield: 0.83 g (86%). Spectroscopic data identical with (2*R*,3*R*)-**11**.

**(11*S*,12*R*)-[2,2′, 3,4,5,5′,6,6′,7,8,9,9′,11,12-$^2H_{14}$]-Hexadecanoic acid (11*S*,12*R*-(1)).**
To a chilled suspension of (2*R*,3*R*)-**11** (0.10 g, 0.28 mmol) and anhydrous $NiCl_2$ (0.777 g, 6 mmol) in MeOH-$d_4$ (10 ml) and THF-$d_8$ (3 ml) is gradually added $NaB^2H_4$ (1.00 g, 23.9 mmol) within two h. Stirring is continued for 2 h. Water (20 ml) and ether (50 ml) are added, and, after extractive workup, the crude ester is purified by chromatography (silica gel, pentane/ether (95/5)). Yield: 0.055 g. The free acid is obtained by stirring the ester with $K_2CO_3$ (0.105 g) in aq. MeOH (5 ml, 90%). After 2 days another portion of $K_2CO_3$ (0.105 g) is added, and stirring is continued for two days more prior to acidification with dil. HCl (pH 4) and extraction

with ether. Recrystallisation from pentane (ca. 5 ml) affords the labelled palmitic acid (11*S*,12*R*)-**1**. Overall yield from **11**: 0.047 g (59%). $^1$H NMR (250 MHz, $CDCl_3$, *i*-propyl ester): δ 5.00 (quint., 1H), 1.64 (m, 2H), 1.24 (d, 6H), 1.10-1.40 (m, 14H), 0.88 (t, 3H); IR (neat, *i*-propyl ester): 2960, 2919, 2854, 2189, 2147, 2100, 1731, 1464, 1372 1260, 1178, 1109, 867, 795 cm$^{-1}$. MS (%, methyl ester)): M+•{287(2.8), 286(5), 285(12), 284(13), 283(8), 282(3), 254(5), 253(6), 252(5), 237(8), 236(5), 235(8), 152(10), 151(12), 105(8), 91(16), 90(97), 89(59), 88(11), 78(13), 77(89), 76(100), 75(24), 61(12), 60(17); HR-MS *m/z* calcd. for $C_{17}H_{20}{}^2H_{14}O_2$ (methyl ester): 284.3438, found 284.3410.

**(11*R*,12*S*)-[2,2′, 3,4,5,5′,6,6′,7,8,9,9′,11,12-$^2H_{14}$]-Hexadecanoic acid (11*R*,12*S*-(1)).**
From (2*S*,3*S*)-**11** (0.10 g, 0.28 mmol) as described. Spectroscopic data identical with (11*R*,12*R*)-**1**.

**Acknowledgement:** Financial support by the Deutsche Forschungsgemeinschaft, Bonn, and the Fonds der Chemischen Industrie, Frankfurt, is gratefully acknowledged. We also thank the BASF, Ludwigshafen, and the Bayer AG, Leverkusen, for generous supply with chemicals and solvents. Special thanks are due to Prof. Dr. H. Simon, Technical University, Garching, for a generous supply with wet packed cells of *Clostridium tyrobutyricum* and the preparative deuteration of heptenoic acids.

## References

1. Bjostad, L.B., Wolf, W.A., Roelofs, W.L., in: *Pheromone Biosynthesis* (Prestwich, G.D., Blomquist, G.J., Eds.), Academic Press, Orlando, Florida, **1987**, 77-120.

2. Foster, S.P., Roelofs, W.L., *Experientia* **1990**, *46*, 269-273, and references cited therein.

3. Jeffcoat, R., *Essays Biochem.* **1979**, *15*, 1-36; Morris, L.J. Harris, R.V., Kelly, W., James, A.T., *Biochem. Biophys. Res. Commun.* **1967**, *28*, 904-908.

5. Back, T.G., Yang, K., *J. Chem. Soc. Chem. Commun.* **1990**, 819-8206.

6. Thanos, I., Bader, J., Günther, H., Neumann, S., Krauss, F., Simon, H., *Methods Enzymol.* **1987**, *136*, 302-317, and references cited therein.

7. Görgen, G., Boland, W., Preiss, U., Simon, H., *Helv. Chim. Acta* **1989**, *72*, 917-928.

8. Bartl, K., Calvalar, C., Krebs, T., Ripp, E., Rétey, J., Hull, W.E., Günther, H., Simon, H., *Eur. J. Biochem.* **1977**, 72, 247-250.

9. Parker, D., *J. Chem. Soc. Perkin Trans. II*, **1983**, 83-88.

10. Millar, J.G., Underhill, E.W., *J. Org. Chem.*, **1986**, *51*, 4726-4728.

11. Boland, W., Frößl, C., Schöttler, M., Tóth, M., *J. Chem. Soc. Chem. Commun.* **1993**, in press.