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METHODS

Efficient Synthesis of the Very-Long-Chain n-3 Fatty Acids, Tetracosahexaenoic Acid (C_{24}:6n-3) and Tricosahexaenoic Acid (C_{23}:6n-3)

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Abstract Tetracosahexaenoic acid (C_{24}:6n-3, THA, **3**) is an essential biosynthetic precursor in mammals of docosahexaenoic acid (C_{22}:6n-3, DHA, **1**), the end-product of the metabolism of n-3 fatty acids. THA **3** is present in commercially valuable fishes, such as flathead flounder. Tricosahexaenoic acid (C_{23}:6n-3, TrHA, **2**), an odd-numbered-chain fatty acid, has been identified from marine organisms such as the dinoflagellate, *Amphidinium carterae*. To date, few studies have examined THA **3** and TrHA **2** due to difficulties in detecting and identifying these compounds, so their chemical and biological properties remain poorly characterized. Only one methodology for the chemical synthesis of THA **3** has been presented, and no method for the synthesis of TrHA **2** has been reported. We report here the efficient synthesis of THA **3** in four steps in 56% overall yield, and the synthesis of TrHA **2** in six steps in 48% overall yield. We also present the synthesis of Δ^2-THA **4**, an intermediate of β-oxidation of THA **3** to DHA **1**, in three steps in 73% overall yield.

Keywords Tetracosahexaenoic acid · Tricosahexaenoic acid · Docosahexaenoic acid · Chemical synthesis · Alzheimer's disease · β-oxidation · Biosynthetic precursor · Odd-numbered fatty acids · Fish oil · DHA metabolism

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Abbreviations

ALA	α-Linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
THA	Tetracosahexaenoic acid
TrHA	Tricosahexaenoic acid
TAG	Triacyl-*sn*-glycerol

Introduction

The polyunsaturated fatty acid, docosahexaenoic acid (C_{22}:6n-3, DHA, **1**), is largely found in neuronal tissues such as brain and retina [1]. DHA **1** is an essential component of neuronal membranes, is a precursor of potent neuroprotective mediators, and is important in helping to prevent both cardiovascular disease and metabolic syndrome [2, 3]. Mammals obtain DHA **1** directly from dietary sources, especially fish, but can also generate DHA **1** in the liver from n-3 fatty acid precursors obtained from eating plants [4–6]. The capacity of the human liver to produce DHA **1** may become critical for maintaining normal levels of DHA **1** in the brain and retina [4, 6–8] if insufficient DHA **1** is available from the diet, as is often the case in modern societies [9].

The pathway for DHA biosynthesis in the liver is shown in Fig. 1. α-Linolenic acid (18:3n-3, ALA) is converted to eicosapentaenoic acid (20:5n-3, EPA), which is converted into docosapentaenoic acid (22:5n-3, DPA) and then into tetracosahexaenoic acid (24:6n-3, THA, **3**). Desaturase and elongase enzymes are localized in the endoplasmic reticulum of the hepatocyte and act on n-3 fatty acids. Desaturase progressively introduces double bonds, and elongase

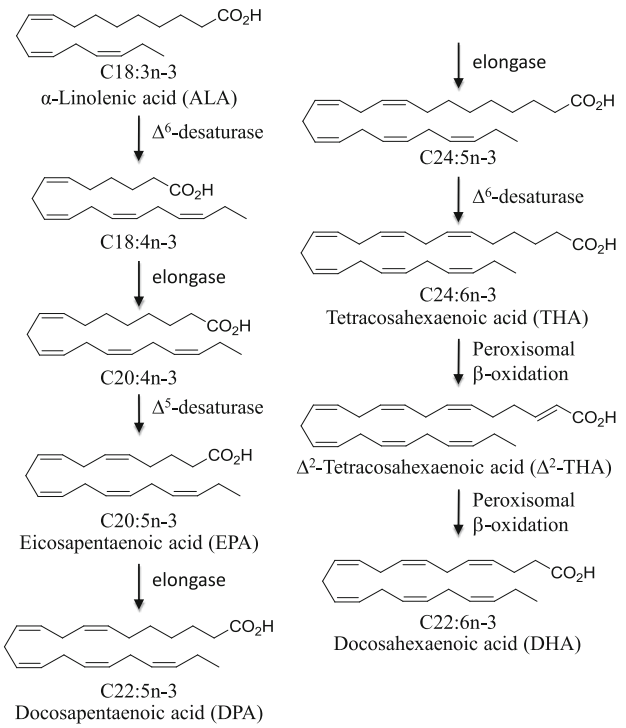


Fig. 1 Metabolic pathway of bioconversion of n-3 polyunsaturated fatty acids

extends shorter-chain n-3 fatty acids by two-carbon units, thereby generating very-long-chain THA **3** [10]. THA **3** is then transported into the peroxisomes and converted to DHA **1** by β-oxidation reactions that involve several specific enzymes: acyl-coenzyme A oxidase, D-bifunctional protein, and peroxisomal thiolases [11–14]. DHA **1**, the end-product of the metabolism of n-3 fatty acids, is delivered to the brain by the circulatory system and accumulates in the brain membranes. In contrast, THA **3** is not incorporated into the brain membranes but is an essential precursor of DHA **1**. Recently, Piomelli's group reported that levels of DHA precursors, particularly ALA and THA **3**, are elevated in the livers of patients with Alzheimer's disease, whereas expression of peroxisomal D-bifunctional protein, which catalyzes the conversion of THA **3** to DHA **1**, is reduced [15]. Piomelli et al. concluded that deficient liver biosynthesis of DHA **1** correlates with cognitive impairment in Alzheimer's patients. Thus, the critical importance of THA **3** in mammals is becoming clearer.

THA **3** is found in various marine organisms [16–18]. Nichols et al. [19] reported that THA **3** constitutes 9.3% of the total fatty acids in the jellyfish *Aurelia* sp. Recently, Tomita and Ando reported that THA **3** is found preferentially at the *sn*-2 position (1.6–23.3 mol%) of triacyl-*sn*-glycerols (TAG) from flathead flounder flesh [20]. Flathead flounder is extensively consumed by humans; therefore, we intake THA **3** in the form of TAG concentrating THA **3** at the *sn*-2 position. Odd-numbered very-long-chain

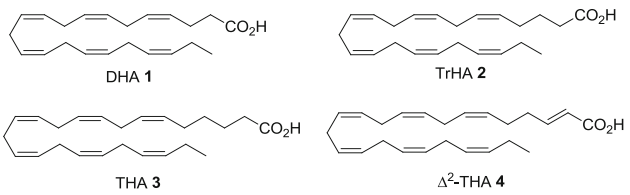


Fig. 2 Chemical structures of the fatty acids discussed in this paper. Docosahexaenoic acid (**1**), tricosahexaenoic acid (**2**), tetracosahexaenoic acid (**3**) and Δ^2-tetracosahexaenoic acid (**4**)

polyunsaturated fatty acids have also been identified from marine organisms [21]. Odd-chain fatty acids can be used in lipid metabolism [22]. The C_{23} fatty acid, tricosahexaenoic acid (C_{23}:6n-3, TrHA **2**), is found in the dinoflagellate, *Amphidinium carterae* [23]. To date, few studies have examined THA **3** and TrHA **2** due to difficulties in detecting and identifying these compounds, so their chemical and biological properties remain poorly characterized. To our knowledge, only one chemical synthesis method for THA **3** has been reported [24, 25], and none for TrHA **2**. In the course of our synthetic and structural biological studies of polyunsaturated fatty acids [26–30], we attempted to derive very-long-chain n-3 fatty acids from DHA ester. In this paper, we report the efficient chemical synthesis of THA **3**, TrHA **2**, and an intermediate of β-oxidation of THA **3** to DHA **1**, Δ^2-THA **4** (Fig. 2).

Materials and Methods

General Experimental Procedures

DHA ethyl ester **5** was a kind gift from the Maruha Nichiro holdings (Tsukuba, Japan). All the other reagents were purchased from commercial sources and used without further purification. Organic solvents used were dried by standard methods. All reactions were performed under a nitrogen atmosphere. Silica gel (wako gel C200) was used for column chromatography, and pre-coated silica gel $60F_{254}$ plates (0.25 mm, Merck) were used for TLC. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded using a Bruker AV300 instrument in $CDCl_3$ solution with TMS as an internal standard and the chemical shifts are given in δ values. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet. Mass spectra were recorded on a JEOL MS700 spectrometer using NBA as positive-ion FAB matrix.

(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-Docosa-4,7,10,13,16,19-Hexaenal (**6**)

To a stirred solution of DHA ethyl ester **5** (157 mg, 0.440 mmol) in CH_2Cl_2 (1 mL) was added 1.0 M

diisobutylaluminium hydride (DIBAL-H) in toluene (523 μL, 0.523 mmol) at −78 °C and the mixture was stirred for 1 h. The reaction was quenched with 1 N HCl aq. and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (9 g, 5% EtOAc–hexane) to give aldehyde **6** (117 mg, 85%). A large scale preparation of **6** was performed using 10.1 g of DHA ester **5** under the similar conditions and 7.45 g of pure compound **6** was obtained (84% yield).

^{1}H NMR ($CDCl_3$) δ: 9.78 (1 H, m, H-1), 5.33–5.44 (12 H, m, H-4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 2.78–2.92 (10 H, m, H-6, 9, 12, 15, 18), 2.51 (2 H, m, H-2), 2.40 (2 H, m, H-3), 2.08 (2 H, quint, $J = 7.5$ Hz, H-21), 0.97 (3 H, t, $J = 7.5$ Hz, H-22); ^{13}C NMR ($CDCl_3$) δ: 201.9, 132.0, 129.4, 128.6, 128.4, 128.29, 128.27, 128.1 (2 carbons), 127.90, 127.86, 127.7, 127.0, 43.7, 25.64 (3 carbons), 25.59, 25.5, 20.6, 20.1, 14.3; HRMS (FAB): Calcd for $C_{22}H_{33}O$ $[M + H]^+$: 313.2531; found: 313.2537.

Methyl (2*E*,6*Z*,9*Z*,12*Z*,15*Z*,18*Z*,21*Z*)-Tetracosa-2,6,9,12,15,18,21-Heptaenoate (**7**)

To a stirred suspension of sodium hydride (0.71 g, 29.6 mmol) in THF (6 mL) was added trimethyl phosphonoacetate (4.25 mL, 29.6 mmol) at 0 °C and the mixture was stirred for 10 min. A solution of aldehyde **6** (4.63 g, 14.8 mmol) in THF (45 mL) was added to the mixture. The reaction mixture was stirred at 0 °C for 1 h, then quenched with water at 0 °C and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (60 g, 0.5% EtOAc–hexane) to give 2*Z*-**7** (0.39 g, 7%) and 2*E*-**7** (4.71 g, 87%) in this order.

2*E*-**7** $R_f = 0.51$ (10% EtOAc-hexane). ^{1}H NMR ($CDCl_3$) δ: 6.96 (1 H, dt, $J = 15.6$, 6.6 Hz, H-3), 5.84 (1 H, d, $J = 15.6$ Hz, H-2), 5.28–5.46 (12 H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 3.73 (3 H, s, H-CO_2Me), 2.78–2.91 (10 H, m, H-8, 11, 14, 17, 20), 2.25 (4 H, m, H-4, 5), 2.08 (2 H, quint, $J = 7.5$ Hz, H-23), 0.97 (3 H, t, $J = 7.5$ Hz, H-24); ^{13}C NMR ($CDCl_3$) δ: 167.0, 148.6, 132.0, 129.1, 128.6, 128.29, 128.26, 128.22 (2 carbons), 128.08, 128.06, 128.02, 127.9, 127.0, 121.3, 51.4, 32.1, 25.7, 25.6 (3 carbons), 25.5, 20.6, 14.3; HRMS (FAB): Calcd for $C_{25}H_{37}O_2$ $[M + H]^+$: 369.2794; found: 369.2780. 2*Z*-**7** $R_f = 0.65$ (10% EtOAc-hexane). ^{1}H NMR ($CDCl_3$) δ: 6.22 (1 H, dt, $J = 11.5$, 7.5 Hz, H-3), 5.79 (1 H, dt, $J = 11.5$, 1.7 Hz, H-2), 5.26–5.45 (12 H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 3.70 (3 H, s, H-CO_2Me), 2.68–2.91 (12 H, m, H-4, 8, 11, 14, 17, 20), 2.22 (2 H, m, H-5), 2.07 (2 H, quint, $J = 7.5$ Hz, H-23), 0.97 (3 H, t, $J = 7.5$ Hz, H-24); ^{13}C NMR ($CDCl_3$) δ: 166.7, 149.8, 132.0, 128.8 (2 carbons), 128.6, 128.25, 128.23, 128.18, 128.16, 128.09 (2 carbons), 127.9, 127.0, 119.7, 51.0, 28.8, 26.6, 25.6 (4 carbons), 25.5, 20.6, 14.3; HRMS (FAB): Calcd for $C_{25}H_{37}O_2$ $[M + H]^+$: 369.2794; found: 369.2792.

Methyl (6*Z*,9*Z*,12*Z*,15*Z*,18*Z*,21*Z*)-Tetracosa-6,9,12,15,18,21-Hexaenoate (**8**)

To a solution of 2*E*/2*Z* mixture **7** (32 mg, 87 μmol) in MeOH (0.5 mL) was added Mg turnings (20 mg, 0.84 mmol) at 0 °C and the mixture was stirred for 3.5 h at room temperature. After addition of H_2O, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (10 g, 1% EtOAc–hexane) to give **8** (26 mg, 81%). A large scale preparation of **8** was performed using 1.07 g of 2*E*/2*Z* mixture **7** under the similar conditions and 820 mg of pure compound **8** was obtained (76% yield). ^{1}H NMR ($CDCl_3$) δ: 5.28–5.56 (12 H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 3.67 (3 H, s, H-CO_2Me), 2.73–2.92 (10 H, m, H-8, 11, 14, 17, 20), 2.32 (2 H, m, H-2), 2.08 (4 H, m, H-5, 23), 1.65 (2 H, quint, $J = 7.7$ Hz, H-3), 1.40 (2 H, quint, $J = 7.7$ Hz H-4), 0.98 (3 H, t, $J = 7.5$ Hz, H-24); ^{13}C NMR ($CDCl_3$) δ: 174.1, 132.0, 129.7, 128.6, 128.4, 128.3, 128.19, 128.16, 128.12, 128.10, 128.0, 127.9, 127.0, 51.5, 34.0, 29.0, 26.9, 25.6 (4 carbons), 25.5, 24.6, 20.6, 14.3; HRMS (FAB): Calcd for $C_{25}H_{39}O_2$ $[M + H]^+$: 371.2950; found: 371.2954.

(6*Z*,9*Z*,12*Z*,15*Z*,18*Z*,21*Z*)-Tetracosa-6,9,12,15,18,21-Hexaenoic Acid (THA **3**)

A solution of **8** (1.19 g, 3.23 mmol) in 5% KOH/MeOH–H_2O (19:1, 16 mL) was stirred at 50 °C for 1 h. The reaction mixture was neutralized with 10% HCl aq. and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (110 g, 50% EtOAc–hexane) to give THA **3** (1.01 g, 87 %). ^{1}H NMR ($CDCl_3$) δ: 5.27–5.55 (12 H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 2.74–2.91 (10 H, m, H-8, 11, 14, 17, 20), 2.36 (2 H, t, $J = 7.4$ Hz, H-2), 2.08 (4 H, m, H-5, 23), 1.66 (2 H, m, H-3), 1.42 (2 H, m, H-4), 0.98 (3 H, t, $J = 7.5$ Hz, H-24); ^{13}C NMR ($CDCl_3$) δ: 179.9, 132.0, 129.6, 128.6, 128.4, 128.3, 128.22, 128.18, 128.1, 128.03, 127.96, 128.9, 127.0, 33.9, 29.0, 26.8, 25.6 (4 carbons), 25.5, 24.3, 20.6, 14.3; HRMS (FAB): Calcd for $C_{24}H_{37}O_2$ $[M + H]^+$: 357.2794; found: 357.2790.

(2*E*,6*Z*,9*Z*,12*Z*,15*Z*,18*Z*,21*Z*)-Tetracosa-2,6,9,12,15,18,21-Heptaenoic Acid (Δ^2-THA **4**)

A solution of 2*E*-**7** (100 mg, 272 μmol) in 5% KOH/iPrOH–H_2O (19:1, 10 mL) was stirred at 50 °C for 3 h.

The reaction mixture was neutralized with 10% HCl aq. and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was passed through a short silica gel column (1 g, 70% EtOAc–hexane) to give Δ^2-THA **4** (95 mg, 99 %). ^{1}H NMR ($CDCl_3$) δ: 7.07 (1 H, dt, $J = 15.6$, 6.6 Hz, H-3), 5.84 (1 H, d, $J = 15.6$ Hz, H-2), 5.28–5.46 (12 H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 2.77–2.91 (10 H, m, H-8, 11, 14, 17, 20), 2.20–2.36 (4 H, m, H-4, 5), 2.08 (2 H, quint, $J = 7.5$ Hz, H-23), 0.97 (3 H, t, $J = 7.5$ Hz, H-24); ^{13}C NMR ($CDCl_3$) δ: 171.9, 151.3, 132.0, 129.2, 128.6, 128.3 (2 carbons), 128.2, 128.13, 128.08 (2 carbons), 128.0, 127.9, 127.0, 121.1, 32.2, 25.6 (4 carbons), 25.5, 20.5, 14.3; HRMS (FAB): Calcd for $C_{24}H_{35}O_2$ $[M + H]^+$: 355.2637; found: 355.2624.

(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-Docosa-4,7,10,13,16,19-Hexaen-1-ol (**9**)

To a stirred solution of DHA ethyl ester **5** (6.17 g, 17.3 mmol) in CH_2Cl_2 (40 mL) was added 1.0 M DIBAL-H in toluene (51.5 mL, 51.5 mmol) at 0 °C and the mixture was stirred for 1.5 h. The mixture was quenched with 1 N HCl aq. at 0 °C and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (100 g, 10% EtOAc–hexane) to give alcohol **9** (5.06 g, 93%). ^{1}H NMR ($CDCl_3$) δ: 5.28–5.47 (12 H, m, H-4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 3.66 (2 H, t, $J = 6.4$ Hz, H-1), 2.85 (10 H, m, H-6, 9, 12, 15, 18), 2.16 (2 H, quint, $J = 6.4$ Hz, H-3), 2.08 (2 H, quint, $J = 7.5$ Hz, H-21), 1.65 (2 H, quint, $J = 6.4$ Hz, H-2), 0.98 (3 H, t, $J = 7.5$ Hz, H-22); ^{13}C NMR ($CDCl_3$) δ: 132.1, 129.4, 128.6, 128.5, 128.30, 128.27, 128.20, 128.16, 128.1 (2 carbons), 127.9, 127.0, 62.5, 32.5, 25.65 (2 carbons), 25.63, 25.60, 25.5, 23.6, 20.6, 14.3; HRMS (FAB): Calcd for $C_{22}H_{35}O$ $[M + H]^+$: 315.2688; found: 315.2686.

(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-Docosa-4,7,10,13,16,19-Hexaen-1-yl 4-Methylbenzenesulfonate (**10**)

A mixture of alcohol **9** (5.06 g, 16.1 mmol) and *p*-toluenesulfonyl chloride (4.61 g, 24.2 mmol) in pyridine (25 mL) was stirred at 0 °C for 13 h. After addition of water, the mixture was extracted with EtOAc. The organic layer was washed with 1 N HCl aq., saturated $NaHCO_3$ aq. and brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (110 g, 5% EtOAc–hexane) to give tosylate **10** (6.71 g, 89%). ^{1}H NMR ($CDCl_3$) δ: 7.79 (2 H, d, $J = 8.3$ Hz, H-Ar), 7.34 (2 H, d, $J = 8.3$ Hz, H-Ar), 5.29–5.42 (12 H, m, H-4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 4.03 (2 H, t, $J = 6.5$ Hz, H-1), 2.75–2.87 (10 H, m, H-6, 9, 12, 15, 18), 2.44 (3 H, s, H-Ph-Me), 2.04–2.12 (4 H, m, H-3, 21), 1.71 (2 H, quint, $J = 6.5$ Hz, H-2), 0.97 (3 H, t, $J = 7.5$ Hz, H-22); ^{13}C NMR ($CDCl_3$) δ: 144.7, 133.2, 132.0, 129.8 (2 carbons), 129.4, 128.6, 128.29, 128.26 (2 carbons), 128.1 (2 carbons), 128.0, 127.9 (4 carbons), 127.0, 69.9, 28.8, 25.6 (3 carbons), 25.5 (2 carbons), 23.0, 21.6, 20.6, 14.3; HRMS (FAB): Calcd for $C_{29}H_{41}O_3S$ $[M + H]^+$: 469.2776; found: 469.2777.

(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*,20*Z*)-Tricosa-5,8,11,14,17,20-Hexaenenitrile (**11**)

A mixture of tosylate **10** (6.71 g, 14.3 mmol) and KCN (1.31 g, 20.1 mmol) in DMSO (40 mL) was stirred at 70 °C for 2.5 h. After addition of H_2O at 0 °C, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (100 g, 5% EtOAc–hexane) to give cyanide **11** (4.51 g, 97%). ^{1}H NMR ($CDCl_3$) δ: 5.32–5.40 (12 H, m, H-5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21), 2.78–2.87 (10 H, m, H-7, 10, 13, 16, 19), 2.34 (2 H, t, $J = 7.2$ Hz, H-2), 2.01–2.14 (4 H, m, H-4, 22), 1.66 (2 H, m, H-3), 1.42 (2 H, m, H-3), 0.98 (3 H, t, $J = 7.5$ Hz, H-23); ^{13}C NMR ($CDCl_3$) δ: 132.0, 130.3, 128.6, 128.4, 128.3 (2 carbons), 128.1, 128.0, 127.9, 127.8, 127.3, 127.0, 119.6, 26.0, 25.7 (4 carbons), 25.5, 25.3, 20.6, 16.5, 14.3; HRMS (FAB): Calcd for $C_{23}H_{34}N$ $[M + H]^+$: 324.2691; found: 324.2696.

(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*,20*Z*)-Tricosa-5,8,11,14,17,20-Hexaenal (**12**)

To a stirred solution of cyanide **11** (2.53 g, 7.86 mmol) in CH_2Cl_2 (1 mL) was added 1.0 M DIBAL-H in toluene (10.1 mL, 10.1 mmol) at −20 °C and the mixture was stirred for 1 h. The mixture was quenched with 10% aqueous potassium sodium tartrate at 0 °C and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (55 g, 3% EtOAc–hexane) to give aldehyde **12** (1.93 g, 75%). ^{1}H NMR ($CDCl_3$) δ: 9.87 (1 H, t, $J = 1.6$ Hz, H-1), 5.23–5.48 (12 H, m, H-5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21), 2.78–2.91 (10 H, m, H-7, 10, 13, 16, 19), 2.45 (2 H, td, $J = 7.3$ Hz, 1.6 Hz, H-2), 2.02–2.18 (4 H, m, H-4, 22), 1.71 (2 H, quint, $J = 7.3$ Hz, H-3), 0.97 (3 H, t, $J = 7.5$ Hz, H-23); ^{13}C NMR ($CDCl_3$) δ: 202.4, 132.0, 129.1, 128.8, 128.6, 128.3, 128.23, 128.19, 128.12 (2 carbons), 128.08, 127.9, 127.0, 43.8, 26.9, 25.7 (2 carbons), 25.6 (2 carbons), 25.5, 21.9, 20.6, 14.3; HRMS (FAB): Calcd for $C_{23}H_{35}O$ $[M + H]^+$: 327.2688; found: 327.2696.

Methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*,20*Z*)-Tricosa-5,8,11,14,17,20-Hexaenoate (**13**)

A mixture of aldehyde **12** (1.93 g, 5.92 mmol), KOH (864 mg, 15.4 mmol) and I_2 (1.95 g, 7.70 mmol) in MeOH (75 mL) was stirred at 0 °C for 1 h. After addition of 10% solution of aqueous $Na_2S_2O_3$, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (40 g, 2% EtOAc–hexane) to give methyl ester **13** (1.91 g, 91%). ^{1}H NMR ($CDCl_3$) δ: 5.28–5.44 (12 H, m, H-5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21), 3.67 (3 H, s, H-CO_2Me), 2.76–2.90 (10 H, m, H-7, 10, 13, 16, 19), 2.32 (2 H, t, $J = 7.4$ Hz, H-2), 2.04–2.12 (4 H, m, H-4, 22), 1.70 (2 H, quint, $J = 7.4$ Hz, H-3), 0.98 (3 H, t, $J = 7.5$ Hz, H-23); ^{13}C NMR ($CDCl_3$) δ: 174.0, 132.0, 128.9, 128.8, 128.6, 128.25, 128.24, 128.19, 128.15, 128.09 (2 carbons), 127.9, 127.0, 51.5, 33.4, 26.5, 26.63 (4 carbons), 25.5, 24.8, 20.6, 14.3; HRMS (FAB): Calcd for $C_{24}H_{37}O_2$ $[M + H]^+$: 357.2794; found: 357.2790.

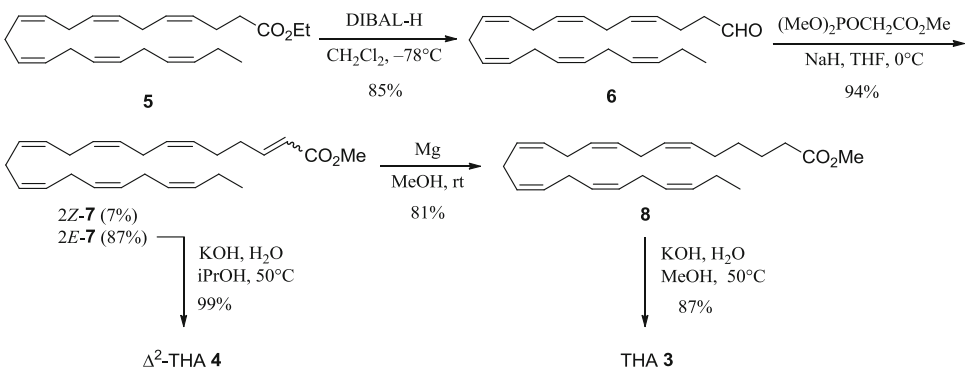
(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*,20*Z*)-Tricosa-5,8,11,14,17,20-Hexaenoic Acid (TrHA **2**)

A solution of ester **13** (1.19 g, 3.34 mmol) in 5% KOH/MeOH–H_2O (19:1, 16 mL) was stirred at 50 °C for 1 h. The reaction mixture was neutralized with 10% HCl aq. and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (110 g, 50% EtOAc–hexane) to give TrHA **2** (1.01 g, 88 %). ^{1}H NMR ($CDCl_3$) δ: 5.27–5.44 (12 H, m, H-5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21), 2.76–2.91 (10 H, m, H-7, 10, 13, 16, 19), 2.37 (2 H, t, $J = 7.4$ Hz, H-2), 2.02–2.17 (4 H, m, H-4, 22), 1.70 (2 H, quint, $J = 7.4$ Hz, H-3), 0.97 (3 H, t, $J = 7.5$ Hz, H-23); ^{13}C NMR ($CDCl_3$) δ: 179.9, 132.0, 129.0, 128.8, 128.6, 128.27, 128.20, 128.19, 128.15 (2 carbons), 128.09, 127.9, 127.0, 33.4, 26.5, 25.6 (4 carbons), 25.5, 24.5, 20.6, 14.3; HRMS (FAB): Calcd for $C_{23}H_{35}O_2$ $[M + H]^+$: 343.2637; found: 343.2643.

Results and Discussion

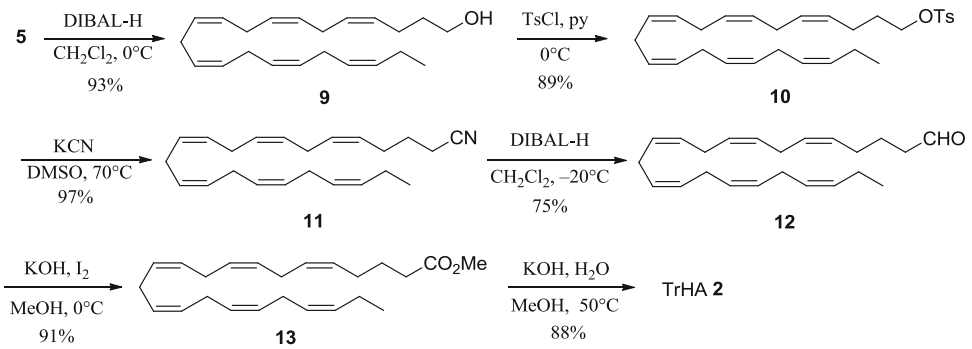
Two fatty acids, THA **3** and Δ^2-THA **4**, were synthesized using DHA ethyl ester **5** as the starting material as shown in Scheme 1. Ester **5** was reduced with 1.2 equivalents of diisobutylaluminium hydride (DIBAL-H) at −78 °C to afford aldehyde **6** in 85% yield. Aldehyde **6** was subjected to the Horner–Wadsworth–Emmons reaction using trimethyl phosphonoacetate [31] to give two-carbon elongated α,β-unsaturated ester **7** in 94% yield as a mixture of 2*E*-isomer (87%) and 2*Z*-isomer (7%). These two isomers were separated easily by silica gel column chromatography. Regioselective hydrogenation of α,β-unsaturated ester **7** was achieved by reduction using Mg turnings in MeOH to provide **8** in good yield (81%) [31–33]. Ester **8** was treated with 5% KOH/MeOH–H_2O (19:1) to provide desired compound THA **3** in 87% yield. On the other hand, Δ^2-THA **4** was obtained in 99% yield upon treatment of 2*E*-**7** with 5% KOH/iPrOH–H_2O (19:1). Thus, THA **3** was synthesized in four steps in 56% overall yield and Δ^2-THA **4** was synthesized in three steps in 73% overall yield. Kuklev et al. [24] reported the synthesis of THA **3** from DHA methyl ester, via a malonic ester derivative as a precursor of two-carbon-elongated fatty acid; he reported a 21% overall yield achieved in five steps. Thus, our synthetic route is more facile and efficient than the previously reported route.

TrHA **2** was synthesized from DHA ethyl ester **5** as shown in Scheme 2. Ester **5** was reduced with DIBAL-H at 0 °C to afford alcohol **9** in 93% yield. Alcohol **9** was then treated with TsCl to give tosylate **10** in 89% yield. Tosylate **10** was treated with KCN at 70 °C to afford one-carbon-elongated cyanide **11** in 97% yield. Cyanide **11** was reduced with DIBAL-H to afford aldehyde **12** in 75% yield. Aldehyde **12** was converted to ester **13** in 91% yield by alkaline-iodine oxidation, a reaction previously developed by our group [34]. Ester **13** was treated with 5% KOH/MeOH–H_2O (19:1) to provide desired compound TrHA **2** in 88% yield. Thus, TrHA **2** was synthesized in six steps in 48% overall yield. To our knowledge, this is the first report of the chemical synthesis of TrHA **2**.



Scheme 1 Synthetic route from DHA ester to tetracosahexaenoic acid (**3**) and Δ^2-tetracosahexaenoic acid (**4**)

Scheme 2 Synthetic route from DHA ester to tricosahexaenoic acid (**2**)



In conclusion, we have developed efficient chemical methods for the synthesis of the very-long-chain n-3 polyunsaturated fatty acids, THA **3**, Δ^2-THA **4** and TrHA **2**. Using these synthetic compounds, we are now investigating their biological activities and metabolism.

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