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A General and Practical Synthesis of Linear Conjugated Pentaenoic Acids

André A. Souto[a,b], A. Ulises Acuña[b] and Francisco Amat-Guerri*[a]

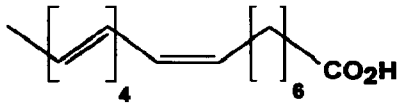
[a]Instituto de Química Orgánica, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, Spain and
[b]Instituto de Química Física *Rocasolano*, C.S.I.C., Serrano 119, 28006 Madrid, Spain

Abstract: An easy and practical synthesis of *all(E)*-2,4,6,8,10-dodecapentaenoic acid (**1**), *all(E)*-5,7,9,11,13-pentadecapentaenoic acid (**2**), *all(E)*-8,10,12,14,16-octadecapentaenoic acid (**3**) and its (8*Z*)-isomer (**4**) by application of the Wittig olefination reaction is described.

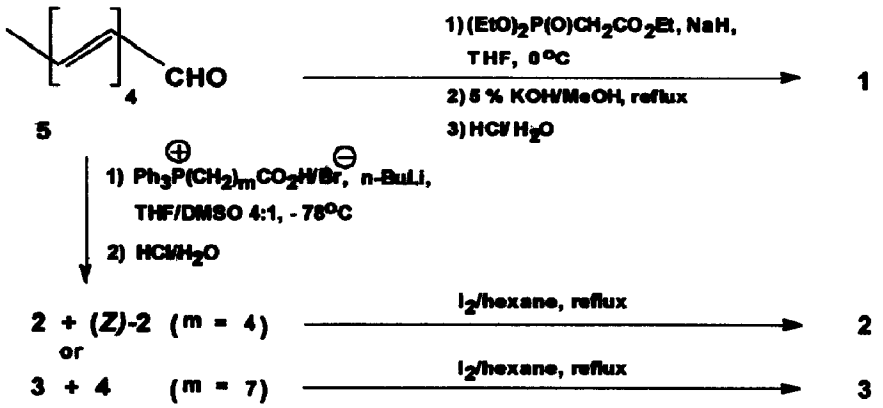
Linear fluorescent membrane probes such as β-parinaric acid (*all(E)*-9,11,13,15-octadecatetraenoic acid) are long-chain rigid polyenes that mimic the shape and size of phospholipid acyl chains. These compounds have been applied to the study of the structure and dynamics of lipid bilayers and natural membranes with considerable success, as well as to the characterization and assay of lipid-protein complexes.[1] Thus, the sensitivity of the polyene spectral parameters to its immediate environment[2,3] can be used to detect heterogeneous domains in model and natural lipid membranes.[4] On the other hand, the absorption spectrum of β-parinaric acid is centered at about 314 nm.[2] This proximity to that of tryptophan leads to some complications in its use as, for example, an electronic energy transfer (EET) acceptor for tryptophan emission in lipid-protein systems.

Extending the polyene chain by a fifth conjugated double bond will shift the absorption spectrum to the red, thus converting the probe into an almost perfect acceptor of the protein tryptophan fluorescence. In addition, excitation with the third harmonic of a Nd:YAG laser (355 nm) would be feasible. We report here the synthesis and preliminary properties of four new[5] conjugated pentaenoic acids with the structures **1**, **2**, **3** and **4**, all of them appropriate for the application indicated above. Moreover, the different aliphatic chain lengths and the location of the five double bonds at the same position –close to the terminal methyl group– make these four compounds useful for the study of structural and dynamic properties of the hydrophobic interior of the bilayer as a function of their transverse location.

1 n = 0
2 n = 3
3 n = 6



4

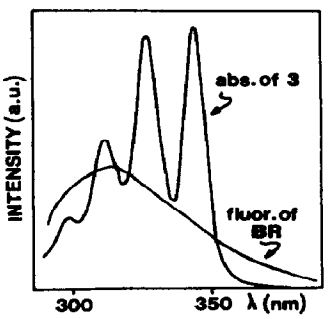


The four pentaenoic acids were obtained by Wittig olefination reaction[6] of the aldehyde *all(E)*-2,4,6,8-decatetraenal (**5**) with the appropriate phosphonium ylide. Geometrically pure **5** was readily obtained from the commercially-available aldehyde (2*E*,4*E*)-hexa-2,4-dienal, as previously described.[7]

Acid **1**, having the five double bonds conjugated with the carboxylic group, was obtained by Wittig-Horner olefination of aldehyde **5** with the ylide from triethyl phosphonoacetate, followed by hydrolysis with methanolic KOH.[8] This method had previously been successfully used in the synthesis of the diethyl ester of the related diacid *all(E)*-2,4,6,8,10-dodecapentaendioic acid.[9]

Acids **2** and **3** were synthesized[10] by reaction of aldehyde **5** with the ylide resulting from the treatment with *n*-butyllithium of (4-carboxybutyl)triphenyphosphonium bromide or (7-carboxy-heptyl)triphenylphosphonium bromide respectively, both salts being prepared by an established procedure.[11] Isomeric *E/Z* mixtures were produced in both cases, the *E/Z* ratio decreasing with the number of methylenes: 2:8 and 1:9, respectively,[12] in accordance with the known effect on the stereoselectivity of the distance of the carboxylic group from the phosphorus atom.[13] The almost quantitative conversion of these mixtures into the corresponding *all(E)*-pentaenoic acids **2**[14] and **3**[15] was attained by treatment with a trace of iodine in hexane.[16] The pure (8*Z*)-acid **4**[17] was readily isolated from its mixture with **3** by recrystallization from hexane.

When this Wittig olefination was carried out with the methyl esters of the former phosphonium salts, under the same experimental conditions, complex mixtures of products were obtained containing traces (< 5 %) of the corresponding methyl esters of acids **2**, **3** and **4**.



Preliminary spectral data of acid **3** in ethanol show an absorption spectrum with maxima at 327 and 344 nm and a fluorescence emission with maximum at 468 nm and a quantum yield six times greater than that of β-parinaric acid in this solvent. The figure shows the good overlap between the absorption spectrum of acid **3** and the tryptophan fluorescence of the membrane protein bacteriorhodopsin (BR) from *H. halobium*. These properties, together with a higher molar extinction coefficient and better thermal and

photochemical stability should render this compound especially useful as a probe for both EET and structural and dynamic properties in membrane systems. The results of current experiments directed towards demonstrating the usefulness of these applications in synthetic and natural lipid membranes will be published elsewhere.[18]

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References and notes

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2. Sklar, L.A.; Hudson, B.S.; Petersen, M; Diamond, J. *Biochemistry*, **16**, 813 (1977).

3. Ruggiero, A.; Hudson, B. *Biophys. J.*, **55**, 1111 (1989).

4. See for example: Mateo, C.R.; Brochon, J.C.; Lillo, M.P.; Acuña, A.U. *Biophys. J.*, **65**, 2237 (1993), and Mateo, C.R.; Lillo, M.P.; González-Rodríguez, J.; Acuña, A.U. *Eur. Biophys. J.*, **20**, 53 (1991).

5. A 2,4,6,8,10-dodecapentaenoic acid melting at 244-246°C and with unknown stereochemistry has been obtained previously (Kucherov, V.F.; Kovalev, B.G.; Nazarova, I.I.; Yanovskaya, L.A. *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 1512 (1960)).

6. Maryanoff, B.E.; Reitz, A.B. *Chem. Rev.*, **89**, 863 (1989).

7. The reaction of (2*E*,4*E*)-hexa-2,4-dienal with the ylide from triethyl phosphonoacetate, followed by treatment with diisobuthylaluminum hydride and oxidation with MnO_2 of the resulting allylic alcohol, yielded the corresponding aldehyde with four *all(E)*-double bonds, which after the same sequence of reactions gave aldehyde **5** with 49 % overall yield (Ley, S.V.; Smith, S.C.; Woodward, P.R. *Tetrahedron Lett.*, **29**, 5829 (1988)).

8. Synthesis and data for **1**: Triethyl phosphonoacetate (168 mg, 0.75 mmol) and aldehyde **5** (100 mg, 0.68 mmol) were successively added with stirring under argon to a suspension of sodium hydride (0.80 mmol, 38 mg of a 1:1 w/w dispersion in mineral oil) in THF (10 mL) at 0°C. After 15 min the mixture was poured into ice-water and the yellow precipitate which appeared was filtered, washed with water and suspended in 5 % KOH in methanol (10 mL), the suspension then being briefly heated at reflux. After cooling, the precipitate was filtered and washed with acetone and cold 5 % aqueous HCl. Acid **1** was recrystallized from ethanol. Yield 60 %, mp 248-250°C. MS, m/z (%): 190 (M^+, 100), 145 (45), 129 (33), 117 (27). UV (ethanol), λ_{max} (nm) (ε, $M^{-1}cm^{-1}$): 328 (42200), 342 (62300), 358 (60600). IR (KBr), ν_{max} (cm^{-1}): 3050b, 1680s, 1610m, 1001vs. ^1H NMR (300 MHz, DMSO-d_6): δ 1.77 (dd, J = 1.5 and 7.0 Hz, CH_3), 5.84 (dq, J = 14.9 and 7.0 Hz, H-11), 5.87 (d, J = 15.1 Hz, H-2), 6.17 (ddd, J = 1.5, 10.5 and 14.9 Hz, H-10), 6.45 (m, H-4), 6.24-6.55 (m, H-6 to H-9), 7.24 (dd, J = 11.3 and 15.1 Hz, H-3), 12.10 (broad, CO_2H). ^{13}C NMR (DMSO-d_6): δ 18.6 (C-12), 121.4, 129.9, 130.5, 131.7, 132.1, 132.2, 135.9, 137.6, 141.0, 144.5 (C-2 to C-11), 167.9 (C-1).

9. Boschelli, D.; Takemasa, T.; Nishitani, Y.; Masamune, S. *Tetrahedron Lett.*, **26**, 5239 (1985).

10. Synthesis of **2**, **3** and **4**: A 2.5 M solution of butyllithium in hexane (1.05 mL, 2.9 mmol) was added, at room temperature with stirring under argon, to a suspension of the appropriate carboxyalkyltriphenylphosphonium bromide (0.75 mmol) in THF (8 mL) and DMSO (2 mL). After stirring for 10 min, the mixture was cooled to -78°C and aldehyde **5** (100 mg, 0.68 mmol) was added. The stirring was continued at the same temperature for 30 min, while a precipitate appeared. The mixture was allowed to warm to room temperature and was then poured into ethyl acetate (15 mL) and 5 % aqueous HCl (15 mL). The aqueous layer was separated and extracted with more ethyl acetate (3x15 ml), the combined organic extracts were washed with water and brine, and then dried. After solvent elimination, the product was purified by flash column chromatography (silicagel, hexane-ethyl acetate 4:1 v/v as eluent) yielding the *E/Z* mixture of **2** and its *5(Z)*-isomer (2:8, 50 % yield) or of **3** and **4** (1:9, 54% yield). Each mixture (100 mg) was refluxed for 10 min in hexane (250 mL) under argon with a trace of iodine-saturated hexane (10 µL). On cooling, crystals of *all(E)*-pentaenoic acid **2** or **3** separated. Overall yields were 45 % and 49 %, respectively. White needles of **4** were obtained by recrystallization in hexane of the mixture **3** plus **4**.

11. The phosphonium salts were obtained by the reaction between triphenylphosphine and 5-bromopentanoic acid or 8-bromooctanoic acid in acetonitrile (Dawson, M.I; Vasser, M. *J. Org. Chem.*, **42**, 2783 (1977)).

12. E/Z ratio analyzed by HPLC, reverse-phase column, acetonitrile-acetic acid 95:5 v/v as eluent.

13. Maryanoff, B.E.; Reitz, A.B.; Duhl-Emswiler, B. A. *J. Am. Chem. Soc.* **107**, 217 (1985).

14. **2**: mp 127-130°C. MS, m/z (%): 232 (M^+, 100), 159 (11), 145 (52), 131 (49), 117 (56). UV (ethanol), λ_{max} (nm) (ε, $M^{-1}cm^{-1}$): 312 (57000), 327 (95000), 344 (98600). IR (KBr), ν_{max} (cm^{-1}): 3050b, 1700s, 1001vs. ^1H NMR (200 MHz, DMSO-d_6): δ 1.60 (m, H-3), 1.76 (d, J = 7.0 Hz, CH_3), 2.10 (m, H-4), 2.22 (t, J = 7.2 Hz, H-2), 5.75 (m, H-5 and H-14), 6.10-6.30 (m, H-6 to H-13), 12.10 (broad, $CO_2\underline{H}$). ^{13}C NMR (DMSO-d_6): δ 18.5 (C-15), 24.4 (C-3), 31.9 (C-2), 33.3 (C-4), 130.2, 130.9, 131.4, 131.5, 132.3, 132.7, 132.9, 133.0, 133.6, 134.6 (C-5 to C-14), 174.6 (C-1).

15. **3**: mp 138-140°C. MS, *m/z* (%): 274 (M^+, 42), 159 (21), 145 (88), 131 (60), 117 (52). UV (ethanol), λ_{max} (nm) (ε, $M^{-1}cm^{-1}$): 312 (60000), 327 (98000), 344 (103000). IR (KBr), ν_{max} (cm^{-1}): 3050b, 1700s, 1001vs. ^1H NMR (200 MHz, DMSO-d_6): δ 1.30 (m, H-4 to H-6), 1.50 (m, H-3), 1.76 (d, J = 7.0 Hz, CH_3), 2.08 (m, H-7), 2.18 (m, H-2), 5.73 (m, H-8 and H-17), 6.10-7.25 (m, H-9 to H-16) and 11.80 (broad, $CO_2\underline{H}$). ^{13}C NMR (DMSO-d_6): δ 18.5 (C-18), 24.7 (C-3), 28.5, 28.6 and 28.9 (C-4 to C-6), 32.5 and 33.9 (C-2 and C-7), 130.1, 131.0, 131.2, 132.3, 132.7, 133.1, 133.2, 135.5 (C-8 to C-17), 174.8 (C-1).

16. Sklar, L.A.; Hudson, B.S.; Simoni, R.D. *Biochemistry*, **16**, 819 (1977).

17. **4**: mp 131-133°C. MS, *m/z* (%): 274 (M^+, 100), 159 (19), 145 (77), 131 (56), 117 (58). UV (ethanol), λ_{max} (nm) (ε, $M^{-1}cm^{-1}$): 314 (64000), 329 (107000), 347 (111000). IR (KBr), ν_{max} (cm^{-1}): 3050b, 1700s, 1001vs. ^1H NMR (200 MHz, DMSO-d_6): δ 1.30 (m, H-4 to H-6), 1.48 (m, H-3), 1.76 (d, J = 6.6 Hz, CH_3), 2.16 and 2.19 (two t, J = 7 Hz, H-2 and H-7), 5.47 (m, H-8), 5.77 (m, H-17), 6.05-6.62 (m, H-9 to H-16). ^{13}C NMR (DMSO-d_6): δ 18.5 (C-18), 24.7 (C-3), 27.5, 28.5, 28.6 (C-4 to C-6), 29.2 (C-7), 33.9 (C-2), 128.3, 129.2, 130.4, 130.9, 132.2, 132.7, 132.8, 133.2, 133.3, 133.5 (C-8 to C-17), 174.8 (C-1).

18. Souto, A.A.; Mateo, C.R.; Amat-Guerri, F.; Acuña, A.U., in preparation.

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