STRUCTURAL DETERMINANTS OF THE TRANSIENT RECEPTOR POTENTIAL 1 (TRPV1) CHANNEL ACTIVATION BY PHOSPHOLIPID ANALOGS*

Sara L. Morales-Lázaro[1&], Barbara Serrano-Flores[1&], Itzel Llorente[1], Enrique Hernández-García[1], Ricardo González-Ramírez[2], Souvik Banerjee[3], Duane Miller[3], Veeresh Gududuru[4], James Fells[5], Derek Norman[5], Gabor Tigyi[5], Diana Escalante-Alcalde[1], and Tamara Rosenbaum[1]

[1]*Departamento de Neurodesarrollo y Fisiología, División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México; D.F., México*
[2]*Departmento de Biología Molecular e Histocompatibilidad, Hospital General "Dr. Manuel Gea González", México; D.F., México*
[3]*Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, Tennessee, USA*
[4]*RxBio Inc., Memphis, Tennessee USA*
[5]*Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA*

[&]These authors contributed equally to this work.

*Running title: ***Lipid structure-specificity and TRPV1 activation***

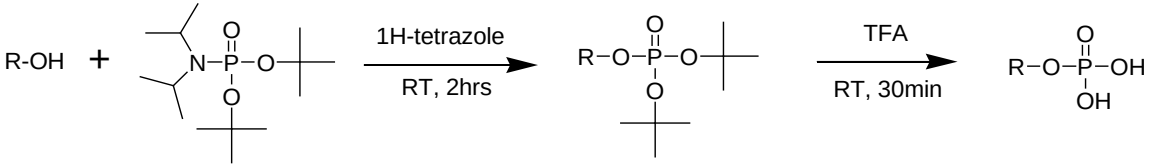
To whom correspondence should be addressed: Tamara Rosenbaum, Departamento de Neurodesarrollo y Fisiología, División Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México; D.F. México.Tel.: +5255 5622-5624; Fax: +5255 5622- 5607; Email trosenba@ifc.unam.mx

This file contains:
Supplemental methods and references

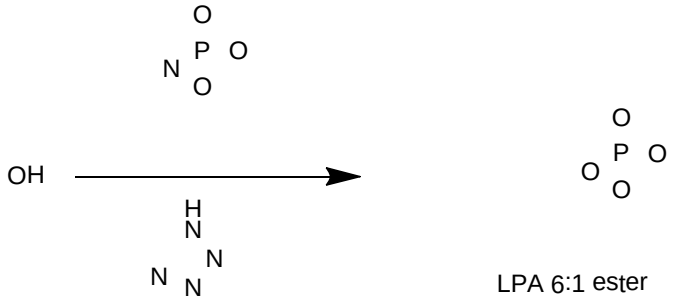
Supplemental Methods

Lipid synthesis

General Schemes

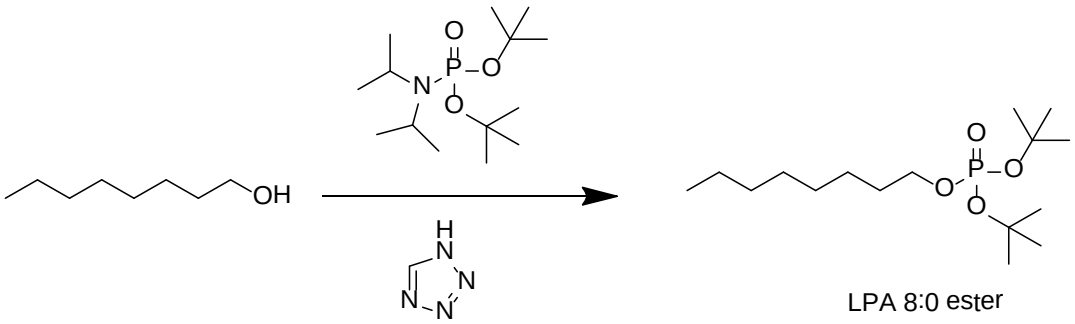


Synthesis of (E)-di-tert-butyl hex-3-en-1-yl phosphate- A volume of 1.7 mL N,N-diisopropylphosphoramidite (5.2 mmol) was added to a solution of 0.31 mL trans-3-hexenol (2.5 mmol) in 60 mL methylenechloride at ambient temperature under argon atmosphere. An amount of .58g 1H-tetrazol (8.2 mmol) was added to the reaction mixture. The reaction was continued to stir over 60 minutes. A volume of 0.6 mL 50% H_2O_2 was added to the reaction mixture at 0 ^{0}C. The reaction was continued to stir over 60 minutes. The reaction was diluted with another 60 mL methylenechloride. The methylenechloride layer was washed with 10% sodium metabisulfite, saturated sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated under vacuum, and purified by silicagel chromatography (20% EtOAc/hexanes) giving 0.64g desired product (2.2 mmol, 88%) as colorless oil. R_f = 0.23 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.6 (m, 1H), 5.4 (m, 1H), 3.9 (m, 2H), 2.3 (m, 2H), 2 (m, 2H), 1.5 (s, 18H), 0.9 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ =135.3, 124.2, 82.0, 66.6, 33.5, 29.8, 25.8, 13.7. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = -9.67. HRMS $[C_{14}H_{29}O_4PNa]^+$ calculated = 315.1701, found = 315.1705.



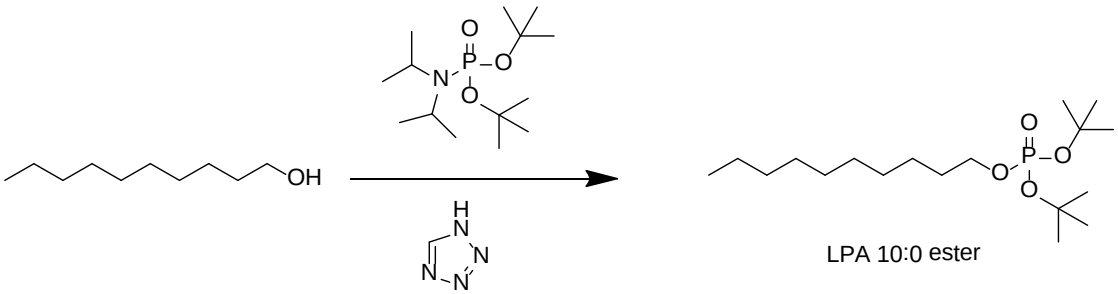
Synthesis of di-tert-butyl octyl phosphate- A volume of 1.74 mL N,N-diisopropylphosphoramidite (5.5 mmol) was added to a solution of 0.36 mL 1-octanol (2.3 mmol) in 60 mL methylenechloride at ambient temperature under argon atmosphere. An amount of .53g 1H-tetrazol (7.6 mmol) was added to the reaction mixture. The reaction was continued to stir over 60 minutes. A volume of 0.6 mL 50% H_2O_2 was added to the reaction mixture at 0 ^{0}C. The reaction was continued to stir over 60 minutes. The reaction was diluted with another 60 mL methylenechloride. The methylenechloride layer was washed with 10% sodium metabisulfite, saturated sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated under vacuum, and purified by silicagel chromatography (20% EtOAc/hexanes) giving 0.68g desired product (2.1 mmol, 91%) as colorless oil. R_f = 0.24 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 3.9 (m, 2H), 1.6 (m, 2H), 1.45 (s, 18H), 1.25 (m, 10H), 0.85 (t, 3H, *J*

= 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 81.9, 67.1, 31.8, 30.3, 30.2, 29.8, 29.2, 25.6, 22.6, 14.3. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = -9.52. HRMS $[C_{16}H_{35}O_4PNa]^+$ calculated = 345.2171, found = 345.2175.



LPA 8:0 ester

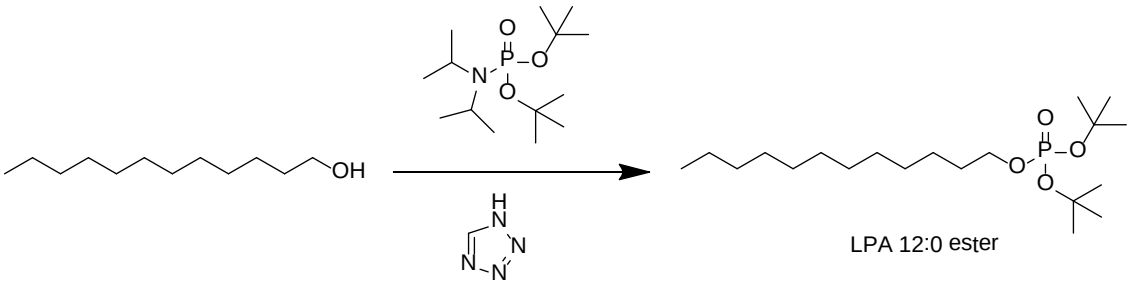
Synthesis of di-tert-butyl decyl phosphate- A volume of 1.73 mL N,N-diisopropylphosphoramidite (5.5 mmol) was added to a solution of 0.48 mL 1-decanol (2.5 mmol) in 60 mL methylenechloride at ambient temperature under argon atmosphere. An amount of .58g 1H-tetrazol (8.2 mmol) was added to the reaction mixture. The reaction was continued to stir over 60 minutes. A volume of 0.6 mL 50% H_2O_2 was added to the reaction mixture at 0 ^{0}C. The reaction was continued to stir over 60 minutes. The reaction was diluted with another 60 mL methylenechloride. The methylenechloride layer was washed with 10% sodium metabisulfite, saturated sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated under vacuum, and purified by silicagel chromatography (20% EtOAc/hexanes) giving 0.81g desired product (2.3 mmol, 92%) as colorless oil. R_f = 0.25 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 3.95 (m, 2H), 1.65 (m, 2H), 1.5 (s, 18H), 1.3 (m, 14H), 0.85 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 82.1, 66.9, 31.9, 30.3, 30.2, 29.8, 29.5, 29.3, 29.2, 25.7, 22.7, 14.2. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = -9.33. HRMS $[C_{18}H_{39}O_4PNa]^+$ calculated = 373.2484, found = 373.2485.



LPA 10:0 ester

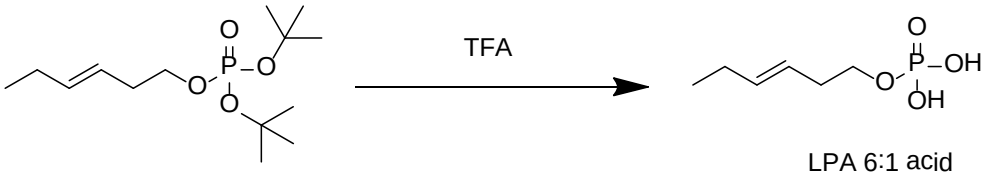
Synthesis of di-tert-butyl dodecyl phosphate- A volume of 1.72 mL N,N-diisopropylphosphoramidite (5.3 mmol) was added to a solution of 0.52 mL 1-dodecanol (2.3 mmol) in 60 mL methylenechloride at ambient temperature under argon atmosphere. An amount of .53g 1H-tetrazol (7.6 mmol) was added to the reaction mixture. The reaction was continued to stir over 60 minutes. A volume of 0.6 mL 50% H_2O_2 was added to the reaction mixture at 0 ^{0}C. The reaction was continued to stir over 60 minutes. The reaction was diluted with another 60 mL methylenechloride. The methylenechloride layer was washed with 10% sodium metabisulfite, saturated sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated under vacuum, and purified by silicagel chromatography (20% EtOAc/hexanes) giving 0.79g desired product (2.3 mmol, 91%) as colorless oil. R_f = 0.25 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 3.95 (m, 2H), 1.55 (m, 2H), 1.5 (s, 18H), 1.3 (m, 18H), 0.86 (t, 3H, *J*

= 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 81.9, 67.1, 31.9, 30.3, 30.2, 29.64, 29.62, 29.57, 29.53, 29.3, 29.2, 25.6, 22.7, 14.1. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = -9.53. HRMS $[C_{20}H_{43}O_4PNa]^+$ calculated = 401.2797, found = 401.2791.



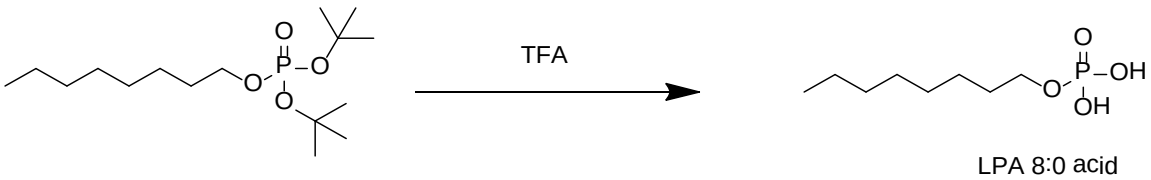
General Experimental procedure for the synthesis of alkyl phosphoric acid analogues- A volume of 0.4 mL trifluoroacetic acid was added to a solution of 100 mg di-*tert*-butyl-alkylphosphate analogues in 10 mL anhydrous methylenechloride. The solution was allowed to stir over 30 minutes at RT. At which point, the reaction was found to be completed as evident by TLC. The methylenechloride layer was evaporated out under reduced pressure. The residue was dissolved in acetonitrile. The acetonitrile layer was extracted with pentane. The acetonitrile layer was evaporated under reduced pressure giving the pure product.

Synthesis of (E)-hex-3-en-1-yl dihydrogen phosphate- An amount of 45 mg (0.25 mmol, 83%) pure product was obtained as light yellowish oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 8.4 (bs, 2H), 5.6 (m, 1H), 5.4 (m, 1H), 4.0 (m, 2H), 2.4 (m, 2H), 2.0 (m, 2H), 0.9 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 135.8, 123.3, 67.9, 33.5, 25.6, 13.7. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = 1.3. HRMS $[C_6H_{13}O_4PNa]^+$ calculated = 203.0449, observed = 203.0458.



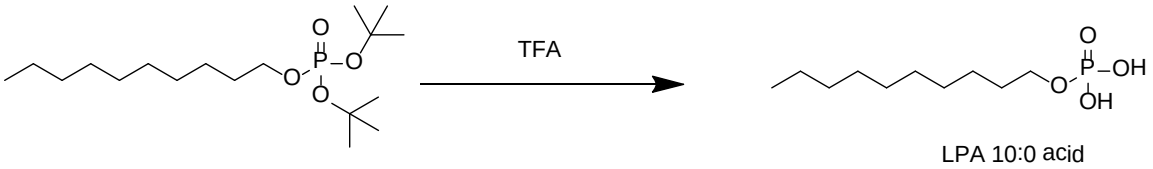
(FAP-14)

Synthesis of octyl dihydrogen phosphate- An amount of 54 mg (0.26 mmol, 84%) pure product was obtained as light yellowish oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 9.0 (bs, 2H), 4.0 (m, 2H), 1.7 (m, 2H), 1.3 (m, 10H), 0.8 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 68.3, 31.9, 30.1, 29.1, 29.08, 25.3, 22.6, 14.1. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = 1.7. HRMS $[C_{18}H_{19}O_4PNa]^+$ calculated = 233.0919, found = 233.0918.



(FAP-15)

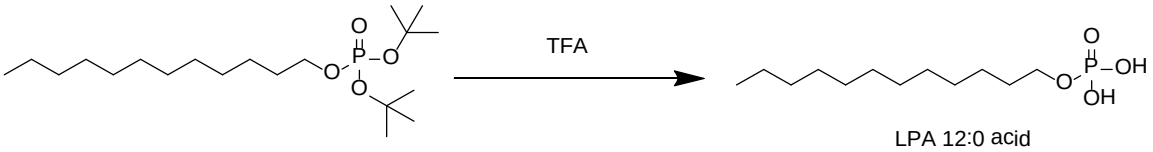
Synthesis of decyl dihydrogen phosphate- An amount of 57 mg (0.24 mmol, 85%) pure product was obtained as white wax. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 9.3 (bs, 2H), 4.0 (m, 2H), 1.7 (m, 2H), 1.3 (m, 14H), 0.8 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 68.3, 31.9, 30.1, 29.6, 29.5, 29.3, 29.2, 25.4, 22.7, 14.1. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = 2.7. HRMS $[C_{10}H_{23}O_4PNa]^+$ calculated = 261.1232, found = 261.1238.



LPA 10:0 acid

(FAP-16)

Synthesis of dodecyl dihydrogen phosphate- An amount of 58 mg (0.22 mmol, 85%) pure product was obtained as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.8 (bs, 2H), 4.05 (m, 2H), 1.7 (m, 2H), 1.3 (m, 14H), 0.85 (t, 3H, *J* = 7Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ = 68.6, 31.9, 30.1, 30.0, 29.7, 29.6, 29.5, 29.4, 29.1, 25.3, 22.7, 14.2. ^{31}P NMR ($CDCl_3$, 400 MHz) δ = 2.3. HRMS $[C_{12}H_{27}O_4PNa]^+$ calculated = 289.1545, observed = 289.1548.



LPA 12:0 acid

(FAP-17)

Supplemental References

Gududuru, V. LPA Receptor Agonists and Antagonists. U.S. Patent 8,686,177 B2, April 1, 2014.