Inhibitors of lipid phosphatidate receptors: *N*-palmitoyl-serine and *N*-palmitoyl-tyrosine phosphoric acids

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Abstract An improved synthesis of two lipid phosphoric acids, *N*-palmitoyl-L-serine phosphoric acid (NP-Ser-PA) and *N*-palmitoyl-L-tyrosine phosphoric acid (NP-Tyr-PA), from the benzyl esters of L-serine and L-tyrosine is described. The sequence of *N*-acylation, followed by phosphitylation with *N,N*-diisopropyl dibenzyl phosphoramidite, oxidation to the corresponding phosphate triesters, and simultaneous debenzylation of the dibenzyl phosphate and benzyl carboxylic esters gave NP-Ser-PA and NP-Tyr-PA in high overall yields. NP-Ser-PA and NP-Tyr-PA and their D stereoisomers were potent reversible inhibitors of the lysophosphatidic acid receptors expressed in *Xenopus* oocytes, thus providing prototypic structures for the development of inhibitors of the lysophosphatidate family of phospholipid growth factors.—**Bittman, R., B. Swords, K. Liliom, and G. Tigyi.** Inhibitors of lipid phosphatidate receptors: *N*-palmitoyl-serine and *N*-palmitoyl-tyrosine phosphoric acids. *J. Lipid Res.* 1996. **37:** 391–398.

Supplementary key words chemical synthesis of lipids • oocytes • oscillatory chloride currents • PAF receptors • phospholipid growth factors

Lysophosphatidic acids (LPAs) have been shown to elicit a variety of biological responses. LPAs affect cell proliferation by growth factor-like stimulation of mitogenesis in fibroblasts (1) and are also capable of inhibiting tumor cell proliferation (2). Ester- and ether-linked LPAs cause activation of platelets isolated from feline (3) and human blood (4). The presence of a receptor for LPAs, distinct from platelet-activating factor (PAF) receptors, has been suggested and appears evident from a structural requirement for a long hydrocarbon chain covalently bonded to a phosphoric acid residue and lack of a requirement for stereospecificity (i.e., a glycerol backbone that is present in PAF) (5, 6). *N*-Palmitoyl-L-serine phosphoric acid (NP-Ser-PA) and *N*-palmitoyl-L-tyrosine phosphoric acid (NP-Tyr-PA) have been reported to inhibit human platelet aggregation elicited by 1-hexadecyl-2-lyso-*sn*-glycero-3-phosphate but not by PAF (7).

Oocytes from the frog *Xenopus laevis* are widely used as a highly sensitive bioassay for LPA analogs (5, 8, 9). In voltage-clamped oocytes, agonist binding to the LPA receptors activates phosphoinositide turnover and leads to a rise in free intracellular Ca^{2+}, which in turn leads to the opening of Ca^{2+}-activated Cl^- channels in the plasma membrane (5, 8). The size of the current and the concentration of LPA show a characteristic dose–response relationship, which makes this bioassay ideal for the quantitation of agonists or antagonists of the LPA receptor. Moreover, oocytes are more suitable than platelets for studies of responses to LPAs as oocytes do not have PAF receptors (5).

We report here an improved synthesis of NP-Ser-PA (**1**) and NP-Tyr-PA (**2**). The original synthetic method of Sugiura et al. (7) is outlined in equations 1 and 2. This method, which does not make use of standard protection-deprotection steps, has the disadvantage that the product LPAs are obtained in low yields (reported in the range of 35–40%, and lower in our hands), probably because of difficulty in purification and isolation of the LPAs from by-products present in the reaction mixture. If by-products were present in the final preparation, unequivocal interpretation of the biological activity of these compounds would be precluded.

Abbreviations: LPA, lysophosphatidic acid; NP-Ser-PA, *N*-palmitoyl-serine phosphoric acid; NP-Tyr-PA, *N*-palmitoyl-tyrosine phosphatidic acid; oleoyl-PA, 1-oleoyl-2-lyso-*sn*-glycero-3-phosphate; PAF, platelet-activating factor; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography.

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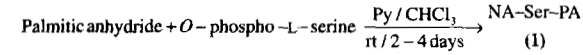
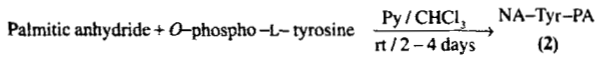


Eq. 1)



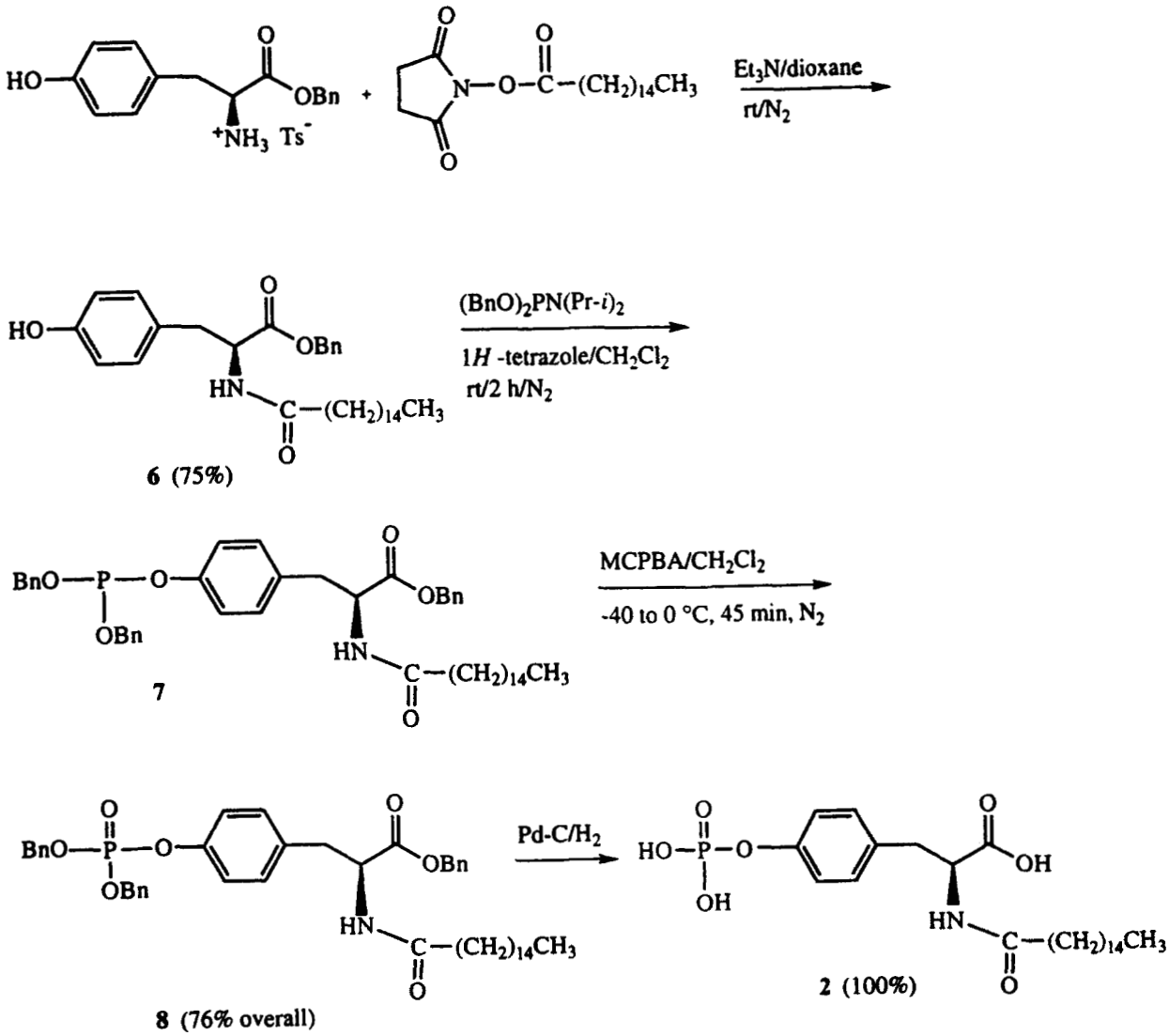
Eq. 2)

In order to obtain the desired LPA analogues in better yields and to characterize the intermediates and final products spectroscopically as well as to investigate their biological activities, we developed a new synthetic strategy based on the use of the commercially available benzyl esters of L-serine and L-tyrosine as the starting materials. **Scheme 1** shows the conversion of L-tyrosine benzyl ester into NP-Tyr-PA (**2**) in 57% overall yield; the analogous steps were used to convert L-serine benzyl ester into NP-Ser-PA (**1**) (see **Scheme 2**) in 56% overall yield. Similarly, the benzyl esters of D-serine and D-tyrosine were used to prepare NP-D-Ser-PA and NP-D-Tyr-PA.

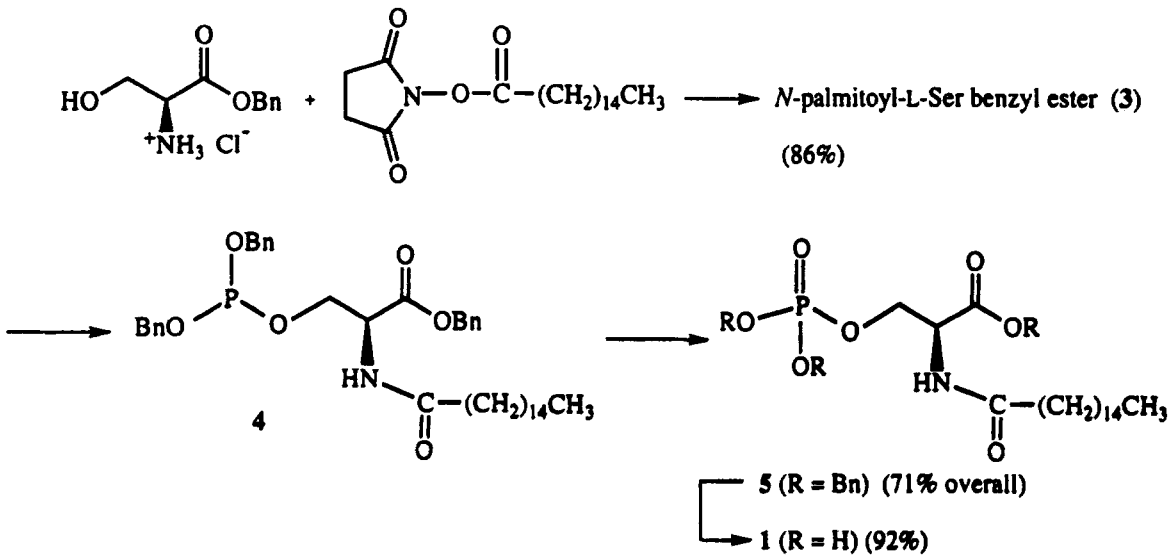
MATERIALS AND METHODS

Chemicals

L-Serine benzyl ester and L-tyrosine benzyl ester were purchased from Sigma Chemical Co. Palmitic acid *N*-hydroxysuccinimide and dibenzyl *N,N*-diisopropyl phosphoramidite were purchased from Aldrich Chemical Co. 1-Oleoyl-2-lyso-*sn*-glycero-3-phosphate (oleoyl-LPA) was obtained from Avanti Polar Lipids (Alabaster, AL).



Scheme 1. Synthesis of NP-Tyr-PA (**2**).



Scheme 2. Synthesis of NP-Ser-PA (**1**).

All reactions were carried out under dry conditions. Anhydrous solvents were obtained as follows: dichloromethane and triethylamine were refluxed over CaH_2 for several hours and distilled. Dioxane was distilled. Flash chromatography was performed on Merck Kieselgel 60 silica and TLC was carried out on Merck 60 silica gel GF_{254} plates.

Spectroscopy

^1H, ^{31}P, and ^{13}C NMR spectra were recorded on an IBM-Bruker spectrometer operating at 200, 80, and 50 MHz, respectively. Chemical shifts (δ) are reported in ppm; ^1H spectra were referenced to TMS and ^{31}P NMR to 85% H_3PO_4 as an external standard. In the carbon spectra, the carbon atoms are numbered from the terminus. HR-FAB mass spectra were recorded at the Michigan State University mass spectrometry facility.

Synthesis of *N*-palmitoyl-L-serine benzyl ester (3)

L-Serine benzyl ester HCl salt (50 mg, 2.16 mmol), palmitic acid *N*-hydroxysuccinimide (1 eq., 760 mg, 2.16 mmol) and dry triethylamine (1.1 eq., 2.37 mmol, 0.33 ml) in dry dioxane (20 ml) were stirred at ambient temperature for 6 h under an atmosphere of N_2. After the solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (150 ml) and washed with saturated aqueous sodium hydrogen carbonate (2 × 30 ml), followed by brine (2 × 30 ml). The organic phase was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude product was flash chromatographed on 30 g of silica gel, eluting with chloroform and 2% methanol in chloroform. Appropriate fractions were pooled and evaporated under reduced pressure to give compound **3** as a white solid (800 mg, 86%). ^1H NMR δ ($CDCl_3$) 0.88 (3H, t, CH_3, J = 6.0 Hz), 1.32 (24H, m, CH_2 × 12), 1.60 (2H, m, RCH_2CH_2CO), 2.24 (2H, t, RCH_2CON, J = 7.6 Hz), 3.92 (2H, dd, CH_2OH, J = 3.9 and 11.2 Hz), 4.71 (1H, m, CHNH), 5.19 (2H, s, $C_6H_5CH_2OCO$), 6.67 (1H, d, NH, J = 7.5 Hz), 7.33 (5H, s, Ar). ^{13}C NMR δ ($CDCl_3$) 14.03 (C1), 22.64 (C2), 25.54 (C4), 29.23 to 29.63 (C5 to C14), 31.87 (C3), 36.45 (C15), 54.71 (CHNH), 63.28 (COH), 67.39 ($C_6H_5CH_2OCO$), 128.07 to 128.59 (Ar, *o*, *m*, *p*), 135.17 (Ar), 170.48 (C=O), 173.90 (C=O).

Synthesis of *N*-palmitoyl-L-tyrosine benzyl ester (6)

L-Tyrosine benzyl ester *p*-toluenesulfonate salt (500 mg, 1.13 mmol), palmitic acid *N*-hydroxysuccinimide (1 eq., 400 mg, 1.13 mmol), and dry triethylamine (1.1 eq., 1.24 mmol, 0.17 ml) in dry dioxane (15 ml) were stirred at ambient temperature for 6 h under an atmosphere of N_2. The solvent was evaporated under reduced pressure to give a residue that was dissolved in CH_2Cl_2 (150 ml) and washed with saturated aqueous sodium hydrogen carbonate (2 × 30 ml), followed by brine (2 × 30 ml). The organic phase was dried over $MgSO_4$, the solvent was evaporated under reduced pressure, and the crude product was chromatographed on 30 g of silica gel. Elution with chloroform and then with 2% methanol in chloroform, followed by evaporation under reduced pressure, gave compound **6** as a white solid (430 mg, 75%). ^1H NMR δ ($CDCl_3$) 0.88 (3H, t, CH_3, J = 6.2 Hz), 1.25 (24H, m, CH_2 × 12), 1.58 (2H, m, RCH_2CH_2CO),

2.17 (1H, t, RCHCON, J = 7.1 Hz), 2.34 (1H, t, RCHCON, J = 7.3 Hz), 3.02 (2H, t, $HOC_6H_4CH_2$, J = 6.0 Hz), 4.94 (1H, m, CHNH), 5.15 (2H, d, $C_6H_5CH_2OCO$, J = 5.8 Hz), 6.03 (1H, d, NH, J = 8.0 Hz), 6.66 (2H, d, tyrosine Ar, J = 8.4 Hz), 6.83 (2H, d, tyrosine Ar, J = 8.4 Hz), 7.32 (5H, m, Ar). ^{13}C NMR δ ($CDCl_3$) 14.07 (C1), 22.68 (C2), 24.76 ($HOC_6H_4CH_2$), 25.57 (C4), 29.35 to 29.66 (C5 to C14), 31.91 (C3), 36.58 (C15), 53.20 (CHNH), 67.34 ($C_6H_5CH_2OCO$), 115.52 (tyrosine Ar, *o, m*), 127.08 (tyrosine Ar, *o, m*), 128.55 to 130.34 (Ar, *o, m, p* and tyrosine Ar, *o, m, p*), 135.05 (Ar), 155.36 (tyrosine Ar), 171.82 (C=O), 173.30 (C=O).

Synthesis of dibenzylphosphoryl-*N*-palmitoyl-L-serine benzyl ester (5)

A solution of compound **3** (133 mg, 0.31 mmol) and 1*H*-tetrazole (3 eq., 65 mg, 0.921 mmol) in dry CH_2Cl_2 (6 ml) was treated with dibenzyl *N,N*-diisopropyl phosphoramidite (1.5 eq., 159 mg, 0.46 mmol, 1.09 ml of a 423.4 mM solution in CH_2Cl_2). The reaction mixture was stirred for 2 h at ambient temperature under an atmosphere of N_2. The phosphite **4** was oxidized as follows. The reaction mixture was cooled to -40°C and a solution of MCPBA (2 eq., 0.61 mmol, 106 mg) in dry CH_2Cl_2 (5 ml) was added dropwise. The resulting solution was stirred at 0°C for 45 min, then CH_2Cl_2 (100 ml) was added, and the mixture was washed with 10% sodium sulfite solution (2 × 20 ml), saturated sodium hydrogen carbonate solution (2 × 15 ml), water (15 ml), and brine (20 ml). The organic portion was dried ($MgSO_4$) and evaporated under reduced pressure to give a pale gum. The crude product was flash chromatographed on silica gel, eluting with 25% ethyl acetate in hexane. Pooling and evaporation of appropriate fractions gave compound **5** as a white gum/foam (152 mg, 71%); R_f 0.37 (ethyl acetate/hexane, 1:1). ^1H NMR δ ($CDCl_3$) 0.86 (3H, t, CH_3, J = 6.5 Hz), 1.25 (24H, m, CH_2 × 12), 1.58 (2H, m, RCH_2CH_2CO), 2.14 (2H, t, RCH_2CO, J = 7.1 Hz), 4.28 (2H, m, $C_6H_5CH_2$), 4.83 (1H, m, CHNH), 4.96 (4H, dd, $C_6H_5CH_2OPO$ × 2, J = 3.6 and 8.5 Hz), 5.14 (2H, s, $C_6H_5CH_2O$), 6.62 (1H, d, NH, J = 7.8 Hz), 7.31 (15H, m, Ar). ^{31}P NMR δ ($CDCl_3$) -1.94.

Synthesis of dibenzylphosphoryl-*N*-palmitoyl-L-tyrosine benzyl ester (8)

A solution of compound **6** (163 mg, 0.32 mmol) and 1*H*-tetrazole (3 eq., 0.96 mmol, 70 mg) in dry CH_2Cl_2 (8 ml) was treated with dibenzyl *N,N*-diisopropyl phosphoramidite (1.5 eq., 166 mg, 0.48 mmol, 1.14 ml of a 423.4 mM solution in CH_2Cl_2). The reaction mixture was stirred at ambient temperature under an atmosphere of N_2 for 2 h. The phosphite **7** was oxidized as follows. The reaction mixture was cooled to -40°C and a solution of MCPBA (2 eq., 0.64 mmol, 110 mg) in dry CH_2Cl_2 (5 ml) was added dropwise. The resulting solution was stirred at 0°C for 45 min, then CH_2Cl_2 (100 ml) was added, and the mixture was washed with 10% sodium sulfite solution (2 × 20 ml), saturated sodium hydrogen carbonate solution (2 × 15 ml), water (15 ml), and brine (20 ml). The organic portion was dried ($MgSO_4$) and evaporated under reduced pressure to give a pale gum. The crude product was flash chromatographed on silica gel, eluting with 25% ethyl acetate in hexane. Pooling and evaporation of appropriate fractions gave compound **8** as a colorless oil (186 mg, 76%); R_f 0.57 (ethyl acetate/hexane, 1:1). ^1H NMR δ ($CDCl_3$) 0.87 (3H, t, CH_3, J = 6.0 Hz), 1.25 (24H, m, CH_2 × 12), 1.58 (2H, m, RCH_2CH_2CO), 2.15 (2H, t, RCH_2CO, J = 7.2 Hz), 3.06 (2H, dd, $POCH_2C_6H_5$, J = 2.8 and 5.6 Hz), 4.89 (1H, d, CHNH, J = 7.6 Hz), 5.10 (6H, dd, $C_6H_5CH_2OPO$ × 2, J = 4.3 and 9.1 Hz and $C_6H_5CH_2O$), 6.11 (1H, d, NH, J = 7.7 Hz), 6.94 (4H, m, tyrosine Ar), 7.31 (15H, m, Ar). ^{31}P NMR δ ($CDCl_3$) -7.48.

Synthesis of *N*-palmitoyl-L-serine phosphoric acid (1)

Catalytic hydrogenolysis of compound **5** (152 mg, 0.22 mmol) in ethanol (8 ml) was carried out using palladium on charcoal, with stirring at ambient temperature and atmospheric pressure under an atmosphere of H_2 for 16 h. The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was evaporated under reduced pressure to give the title compound **1** as a white solid (85 mg, 92%); R_f 0.19 (chloroform–methanol–acetone–acetic acid–water, 4.5:1:2:1.3:0.5). Analytical HPLC (see below). Final purification to homogeneity was achieved by semipreparative HPLC on a normal-phase column (see below). FAB HRMS *m/z* calcd for $C_{19}H_{39}NO_7P(MH^+)$: calcd: 424.2419. Found: 424.2477. ^1H NMR δ ($CDCl_3/CD_3OD$) 0.88 (3H, unresolved t, CH_3), 1.26 (26H, m, CH_2 × 13), 2.37 (2H, m/br s, RCH_2CO), 3.59 (2H, m, $POCH_2$), 4.21 to 4.75 (4H, m, CHNH, NH, and OH), 8.08 (1H, br s, CO_2H). ^{31}P NMR δ ($CDCl_3/CD_3OD$) -1.54.

Synthesis of *N*-palmitoyl-L-tyrosine phosphoric acid (2)

Compound **8** (186 mg, 0.24 mmol) in ethanol (8 mL) was treated with palladium on charcoal and stirred at ambient temperature and atmospheric pressure under an atmosphere of H_2 for 12 h. The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was evaporated under reduced pressure to give the title compound **2** as a white solid (120 mg, 100%); R_f 0.27 chloroform–methanol–acetone–acetic acid–water (4.5:1:2:1.3:0.5). Analytical HPLC (see be-

low). Final purification to homogeneity was achieved by semipreparative HPLC on a normal-phase column (see below). FAB HRMS *m/z* calcd for $C_{25}H_{43}NO_7P$ (MH^+): calcd: 500.2732. Found: 500.2769. ^1H NMR δ ($CDCl_3/CD_3OD$) 0.88 (3H, t, CH_3, J = 5.6 Hz), 1.26 (24H, m, CH_2 × 12), 1.57 (2H, m/br s, RCH_2CH_2CO), 2.18 (2H, m/br s, RCH_2CO), 3.08 (2H, m, $C_6H_4CH_2$), 4.74 (1H, m/br s, CHNH), 5.37 (3H, m, NH and OH × 2), 7.13 (4H, m, tyrosine Ar). ^{31}P NMR δ ($CDCl_3/CD_3OD$) -1.90.

HPLC characterization of NP-Tyr-PA and NP-Ser-PA

NP-Tyr-PA and NP-Ser-PA reaction products were characterized by normal-phase HPLC using a Microsorb column (4.6 mm × 250 mm, Rainin Instruments Co., Woburn, MA) packed with 5 -μm silica operated in a two-pump gradient system (Waters) at a flow rate of 1 ml/min. The effluent was monitored by using an evaporative light scattering detector (ELSD IIA, Varex Corp., Burtonsville, MD). Solvent A was composed of chloroform–methanol–ammonium hydroxide 80:19:5:0.5, whereas solvent B consisted of chloroform–methanol–water–ammonium hydroxide 41:50:8.5:0.5. HPLC grade solvents were obtained from J. T. Baker (Phillipsburg, NJ). For analytical HPLC, 1.3 mg of NP-Tyr-PA or NP-Ser-PA was dissolved in 100 μl of solvent B and injected with a U6K injector (Waters). A linear gradient of eluent B was developed from 50 to 100% over 20 min, followed by isocratic application of 100% of solvent B for 55 min. To monitor the chromatogram, a flow splitter was used, diverting 0.2 ml/min of eluent to the detector. t_R of NP-Tyr-PA, 23.8 min; t_R of NP-Ser-PA, 27.1 min. Semipreparative HPLC was carried out using a 5-μm silica gel column (1 cm × 25 cm) at a flow rate of 2 ml/min. Fractions of NP-Tyr-PA and NP-Ser-PA were collected, and dried under nitrogen and used in the oocyte bioassays.

Electrophysiological recording of LPA-elicited membrane currents in *Xenopus* oocytes

Details of oocyte isolation and electrophysiological recording using a standard two electrode voltage clamp amplifier (Gene Clamp 500, Axon Instruments, Foster City, CA) have been described previously (8). Briefly, stage V–VI oocytes were denuded of the follicular cell layers with type A collagenase treatment (Boehringer, Indianapolis, IN; 1.3 mg/ml in Barth's solution). Electrophysiological recording was carried out within 7 days after isolation by holding the membrane potential at -60 mV. The different lipid phosphatidic acids were dissolved in DMSO at a concentration of 1 mM and diluted further in normal frog Ringer's solution (5 mM HEPES, 120 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, pH 7.0) and applied through superfusion at a flow rate of ~4 ml/min. Oscillatory membrane currents were recorded in a NIC-

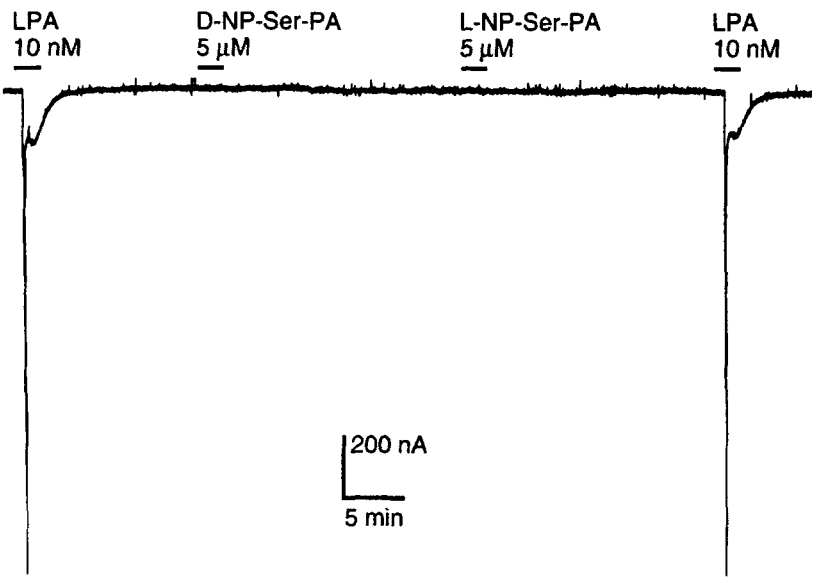


Fig. 1. Oscillatory Cl^- currents in oocytes. Application of 10 nM oleoyl-LPA to the oocyte evoked large oscillatory currents, whereas application of both the D- and L-stereoisomers of NP-Ser-PA at 5 μM failed to elicit any currents. After the compounds were washed out as described previously (2) for the time indicated, the oocyte still responded to 10 nM oleoyl-LPA with the original sensitivity. Downward deflection of the trace represents inward currents.

310 digital oscilloscope (Nicolet, Madison, WI) and analyzed by the RICO software package (version 1.01).

RESULTS

Chemical synthesis of NP-Ser-PA (1) and NP-Tyr-PA (2)

The first step in the conversion of L-serine and L-tyrosine into **1** and **2** is the *N*-acylation of the benzyl ester-protected amino acids using an activated palmitic acid, i.e., palmitic acid *N*-hydroxysuccinimide, in dry dioxane. Yields of 75–86% of *N*-acylated products **3** and **6** were obtained after purification by flash chromatography. The structures of the products were confirmed by ^{1}H and ^{13}C NMR spectroscopy.

The next step involves formation of the phosphate moiety. Attempts were made to treat the *N*-acyl amino acid benzyl esters with phosphorylating agents. For example, dimethyl phosphochloridate (10) was used, but only moderate yields of the corresponding dimethyl-phosphoryl-*N*-palmitoyl-amino acid benzyl esters were obtained. Similarly, phosphitylation (11) of the hydroxyl group with phosphorus trichloride, imidazole, and triethylamine, followed by oxidation of the *H*-phosphonate intermediate to the *N*-palmitoyl-amino acid benzyl ester phosphoric acid, gave poor yields of products. Presumably, the poor yields arose because of side reactions, and in the latter case because of difficulty in isolating the polar phosphoric acid intermediates.

O-Phosphitylation was performed using the reactive phosphitylating reagent *N,N*-diisopropyl dibenzyl phosphoramidite (12) in the presence of tetrazole as condensing agent, followed by oxidation, without isolation, of the phosphite triester intermediates **4** and **7** with *m*-chloroperbenzoic acid to give the corresponding phosphate triesters **5** and **8.** This method proved to be successful in terms of the yields (71–76%) obtained after purification by flash chromatography. As the amino group was acylated in the first step, there is no possibility of *N*-phosphitylation. The structures of the products were confirmed by ^{1}H and ^{31}P NMR spectroscopy. The chemical shifts observed in the ^{31}P NMR spectra of intermediates **5** and **8** are δ -1.94 and -7.48 ppm, respectively, which are typical of phosphates of this type (13). The ^{1}H NMR spectra revealed the phosphorus-coupled methylenes of the dibenzyl phosphate at δ 4.96 and 5.10, respectively. In both cases the signals are split into double doublets.

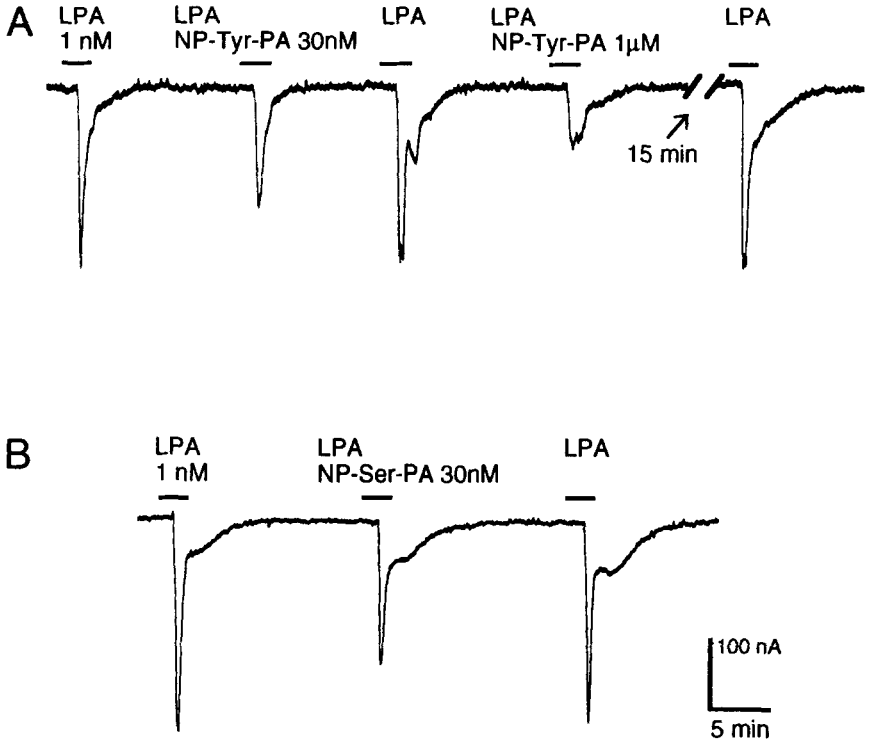


Fig. 2. Effects of NP-Tyr-PA (panel A) and NP-Ser-PA (panel B) on LPA-elicited oscillatory chloride currents in voltage-clamped *Xenopus* oocytes. The membrane potential was held at -60 mV, and oleoyl-LPA was applied by perfusion at a final concentration of 1 nM alone and mixed with the inhibitor at the concentration indicated.

The final step is the simultaneous debenzylation of both the phosphate and carboxylic acid esters. Compounds **5** and **8** were debenzylated in nearly quantitative yields by catalytic hydrogenolysis using palladium on charcoal under an atmosphere of hydrogen at atmospheric pressure. The structures of the LPA products **1** and **2** were supported by ^{1}H and ^{31}P NMR spectroscopy, although resolution of the broad signals was poor owing to the compounds' poor solubility and tendency to aggregate. Use of a mixture of $CDCl_3$ and CD_3OD (1:1) enabled sufficient solubility to obtain the spectra. The signals observed in the ^{31}P NMR spectra of LPAs **1** and **2** were very broad, but there were clear singlets at δ -1.54 and -1.90 ppm, which were shifted downfield from that of the dibenzyl phosphoryl intermediates **5** and **8** at δ -1.94 and -7.48 ppm, respectively. Fast atom bombardment high-resolution mass spectrometry showed the expected MH^+ ions for NP-Ser-PA and NP-Tyr-PA.

Effects of NP-Ser-PA and NP-Tyr-PA on oscillatory chloride currents in oocyte membranes

Figure 1 illustrates the inability of both D- and L-NP-Ser-PA at 5 μM each to activate oscillatory Cl^- currents in voltage-clamped oocytes. NP-Ser-PA was removed by washing (2), giving oocytes that still responded to 10 nM oleoyl-LPA in a manner identical to that observed prior to the application of NP-Ser-PA. Identical results were obtained with D- and L-NP-Tyr-PA at 5 μM (not shown). **Figure 2A** shows the ability of NP-Tyr-PA at 30 nM and 1 μM to inhibit oscillatory Cl^- currents in voltage-clamped oocyte membranes in the presence of 1 nM oleoyl-PA. The inhibition was dose-dependent and reversible, as indicated by the complete recovery of the response to oleoyl-LPA. As shown in Fig. 2B, the behavior of NP-Ser-PA was very similar.

DISCUSSION

Our aim was to synthesize LPA analogs in high yields in order to assay them as inhibitors/activators of the putative LPA receptors. A convenient synthesis was developed to provide lipid phosphoric acid analogues **1** and **2** in high yields in four steps from commercially available amino acid benzyl esters. The phosphitylation methodology with $(BnO)_2PN(Pr\text{-}i)_2$ proved advantageous over phosphorylation, and in the final step, simultaneous debenzylation by catalytic hydrogenolysis was a particularly useful method for obtaining high yields of pure LPA analogues as the reactions proceeded cleanly, negating purification by chromatographic techniques, and gave a volatile by-product (toluene).

While a detailed pharmacological analysis of the competitive antagonist properties of NP-Ser-PA and NP-Tyr-PA will be presented elsewhere (K. Liliom, R. Bittman, and G. Tigyi, unpublished results), we show here that both L- and D-NP-Ser-PA and NP-Tyr-PA at 5 μM, when applied to highly sensitive *Xenopus* oocytes, failed to evoke oscillatory currents characteristic for the activation of the LPA receptors (Fig. 1). However, both compounds reversibly inhibited the oscillatory Cl^- currents evoked by oleoyl-LPA in voltage-clamped *Xenopus* oocytes (Fig. 2). The present data obtained in *Xenopus* oocytes, together with those reported by Sugiura et al. (7) from studies in human platelets, indicate that NP-Ser-PA and NP-Tyr-PA inhibit physiological responses to oleoyl-LPA. The synthetic approach reported here to NP-Ser-PA and NP-Tyr-PA, via a series of protection/deprotection reactions, can be applied to the synthesis of new analogs that are receptor subtype selective antagonists of the LPA receptor family.

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