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Synthesis and Fungicidal Activity of O-Alkyl O-Aryl O-2-(Stearamido)ethyl Phosphates

Liang Han,[1] Jian-Rong Gao,[1] and Zheng-Ming Li[2]

[1]*State Key Laboratory Breeding Base of Green Chemistry-Synthesis Technology, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China*

[2]*National Key Laboratory of Elemento-Organic Chemistry, Tianjin 300071, People's Republic of China*

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ABSTRACT: *N-stearoylethanolamine (NAE18:0) was reacted with O-alkyl O-aryl chlorophosphate and a series of O-alkyl O-aryl O-2-(stearamido)ethyl phosphates were synthesized to explore their antifungal activity. Compared with parent NAE18:0, title compounds without substitution or with methyl substitution on a benzene ring exhibited improved antifungal activity.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:602–608, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20485

INTRODUCTION

N-acylethanolamines (NAEs), a kind of minor membrane lipid constituent, exhibit important biological and pharmacological activities in organisms [1]. More investigation supported the fact that NAEs play a lipid mediator role in plants. NAEs were implicated as transducers in plant defense signaling, identified as inhibitors of phospholipase D (α isoform) activity [2,3], and at elevated levels, interfered with normal seedling root development [4–6]. The application of exogenous NAEs further confirmed the recognition of their important role in plant [2f,7,8]. Although the above reports are exciting, NAEs have very low solubility in water and limited solubility in most common organic solvents, which discourages their further study and application. In our previous work, we found that NAE derivatives exhibit improved bioactivity and solubility. For example, after introduction of *N*-stearoylethanolamine into nitrogen mustard phosphorate, a series of O-substituted phenyl O-2-stearamidoethyl *N′N*-bis(2-chloroethy1)phosphoramidate with improved solubility and antifungal activity were obtained [9]. Moreover, the compounds, synthesized through the acylation of NAEs with aryloxyacetic acids exhibited better activity than 2,4-dichlorophenyloxyacetic acid (a traditional plant growth regulator) in stimulating hypocotyls elongation of rape [10].

In organisms, NAEs are formed from N-acylated phosphatidylethanolamine (PE) molecular species by the action of a phosphodiesterase, phospholipase D (PLD). This reaction produces a corresponding NAE and a molecule of phosphatidic acid (PA). NAEs are further hydrolyzed by an enzyme designated fatty acid amide hydrolase (FAAH), producing free fatty acid (FFA) and ethanolamine. Free fatty acids are utilized by the enzyme designated NAPE (*N*-acylphosphatidylethanolamine) synthase to acylate the amino head group of PE and produce NAPE, the precursor of NAEs, thus realizing the metabolism

Correspondence to: Liang Han; e-mail: hanliang814@163.com.
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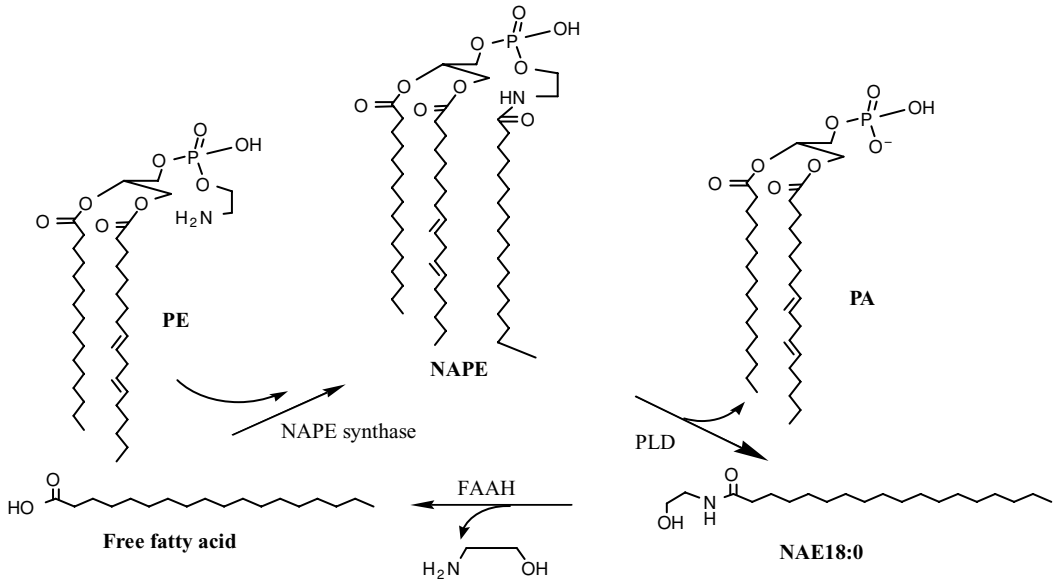
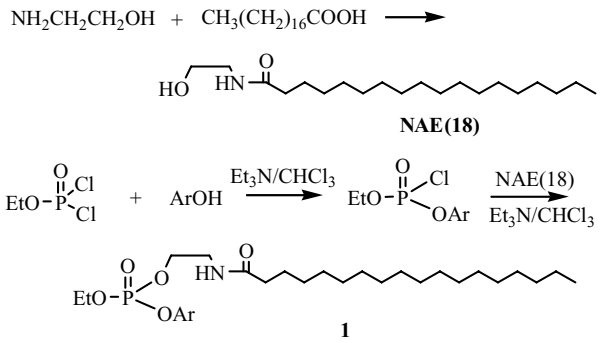


SCHEME 1 The metabolism mechanism of *N*-stearoylethanolamine.

cycle of NAEs [3a]. The metabolism mechanism of *N*-stearoylethanolamine (NAE18:0) is shown in Scheme 1. This metabolism was stimulated for several hours following treatment with a pathogen elicitor and believed to be involved in signaling pathogen perception. New bioactive compounds with good solubility may be obtained through the preparation of compounds similar with the precursor of NAEs. Therefore, a series of O-alkyl O-aryl O-2-(stearamido)ethyl phosphates were synthesized and their antifungal activity were evaluated.



SCHEME 2

RESULTS AND DISCUSSION

Synthesis of O-Ethyl O-Aryl O-2-(Stearamido)ethyl Phosphates

Several NAE types have been identified in a variety of plant species. These NAE types identified contain acyl chains of 12–18 C in length and up to three double bonds, reflecting the typical acyl moieties prevalent in higher plants [3a]. In terms of bioactivity, plants are particularly responsive to low concentration of medium chain saturated NAEs, whereas animal physiology is largely regulated by low concentration of long chain polyunsaturated NAEs (e.g., NAE 20:4) [5]. Therefore, in our primary work NAE18:0 was selected to be modified and a series of O-alkyl O-aryl O-2-(stearamido)ethyl phosphates were prepared as shown in Scheme 2.

NAE18:0 was prepared by refluxing 1 mol stearic acid with 1.5 mol ethanolamine for 6 h in 98% yield [11]. The product was purified by recrystallization from the solution of trichloromethane and acetone instead of 95% ethanol as mentioned in Ref. [8].

First, NAE18:0 was reacted with O-ethyl O-aryl chlorophosphate in the presence of Et_3N, but a poor yield was obtained. The well-known acylating catalyst 4-dimethylaminopyridine (DMAP) was used to improve the reaction yield, and the preparative reaction of compound **1o** was carried out under the optimum conditions. The reaction yield improved to 38% from 26% with 0.1 equiv DMAP as the catalyst, whereas the yield increased to 48% with 1 equiv DMAP as both the catalyst and the acid receptor. Therefore, NAE18:0 and 1 equiv DMAP were added, respectively, into the $CHCl_3$ solution of O-ethyl O-aryl chlorophosphate and the suspension was obtained owing to the poor solubility of NAE18:0. With the progress of reaction, NAE18:0 dissolved

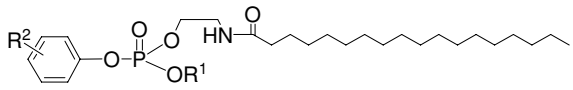
gradually and the reactant solution turned clear, which indicated the reaction happened and the products had better solubility than NAE18:0 in $CHCl_3$. After the usual workup, title compounds **1** were purified by column chromatography. Most products were solids with low melting points and the yields were still somewhat lower due to the existence of long fatty chain in NAEs.

Biological Activities

Since NAEs were implicated as transducers in plant defense signaling and they could activate the expression of PAL2 (phenylalanine-ammonia lyase) [1], we primarily studied the antifungal activities of the derivatives in vitro against *Gibberella zeae*, *Alternaria solani*, *Phoma asparagi*, *Physalospora piricola*, and *Cercospora arachi dicola*. The inhibition percent in these assays over control samples without fungicides is listed in Table 1 and compared with that of NAE18:0. The data indicate that NAE18:0 only exhibits certain inhibition activity against *Alternaria solani* and *Physalospora piricola*, whereas compound **1a** show improved inhibition activity against all the tested fungi.

The bioactivities of title compounds **1b–1p** with different substitutions on a benzene ring were investigated. Compounds **1l, 1m**, and **1n** with a methyl group on the benzene ring exhibit improved activities against all the tested fungi compared with NAE18:0. For example, at the concentration of 50 mg/L, the inhibition percent of compound **1m** against *Phoma asparagi*, is 52.8%, whereas NAE18:0 shows no activity. However, the presence of a halogen atom on the benzene ring was unfavorable and compounds **1c–1e** showed no fungicidal activity. The bioactivity seemed to be associated with the electron density on the benzene ring. Compounds **1m, 1n** with high electron density on the benzene ring exhibit better antifungal activities than parent NAE18:0, whereas those with low electron density on the benzene ring such as **1b–1e** and **1i** have inferior inhibition activities to NAE18:0. Surprisedly, compound **1g**, bearing a fluorine atom on the benzene ring, exhibited greater increased fungicidal activities against *Gillerella zeae* and *Alternaria solani* compared with NAE18:0. Another exception is compound **1k**

TABLE 1 Antifungal Activity of Title Compounds and NAE(18:0) In Vitro (50 mg/L, Relative Inhibitory Ratio %)



			Ratio (%)				
Compound	R^1	R^2	*Gibberella zeae*	*Alternaria solani*	*Phoma asparagi*	*Physalospora piricola*	*Cercospora arachi dicola*
1a	Et	H	10	42.9	33.3	33.8	20.6
1b	Et	3-Cl	0	0	0	23.1	0
1c	Et	4-Cl	0	0	0	0	0
1d	Et	2,4-Cl	0	0	0	0	0
1e	Et	2-Br	0	0	0	0	0
1f	Et	4-Br	0	0	0	30.8	0
1g	Et	4-F	16.0	40.5	0	20	0
1h	Et	2-NO_2	0	0	22.2	23.1	11.8
1i	Et	4-NO_2	0	0	0	12.3	0
1j	Et	3-CH_3O	0	0	44.4	30.8	20.6
1k	Et	2-CH_3	0	0	0	27.7	0
1l	Et	3-CH_3	16	23.8	22.2	12.3	14.7
1m	Et	3,4-CH_3	40	42.9	52.8	38.5	14.7
1n	Et	2,6-CH_3	14	23.8	30.6	30.8	14.7
1o	Et	4-^{t}Bu	0	0	0	12.3	0
1p	Et	2-CH_3-4-NO_2	0	0	0	0	11.8
1q	Pr	H	0	0	0	12.3	0
1r	ClEt	H	0	0	0	12.3	0
1s	BnOEt	H	0	19.4	19.4	0	0
1t	[a]	H	0	0	0	12.3	0
NAE18	–	–	0	14.3	0	24.4	0

[a](Tetrahydrofuran-2-yl)methyl substituted.

with ortho methyl substitution on the benzene ring that only exhibits comparable fungicidal activity against *Physalospora piricola* with NAE18:0 and fails to act against other fungi.

In addition, we replaced an ethyl group with other alkyl groups and prepared compounds **1q–1t** to investigate the fungicidal activity variety along with different alkyl groups. Unfortunately, the change of the alkyl group does not contribute to the improvement of inhibition activity and the bioactivities of compounds **1q–1t** are inferior to those of **1a** and even parent NAE18:0.

CONCLUSION

In conclusion, a series of the phosphate derivatives of NAE18:0 were synthesized through reaction of NAE18:0 with O-alkyl O-aryl chlorophosphates. All compounds have better solubility than NAE18:0 and are soluble in $CHCl_3$. The bioactivity data indicate that title compounds without substitution or with methyl substitution on benzene ring exhibit improved fungicidal activities compared with NAE18:0. Certain relationship is observed between the inhibition activity and the electron density on the benzene ring and those with high electron density on the benzene ring show better bioactivities and broader antibacterial spectrum than NAE18:0. The further study for the bioactivity evaluation of these compounds and NAE18:0 is under consideration.

EXPERIMENTAL

General

Melting points were determined on a Yanaco melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 300 M NMR spectrometer in $CDCl_3$ with TMS as internal standard, and coupling constants (J) are expressed in hertz. Elemental analyses were done on a Yanaco CHN CORDER MT-3. The reaction progress is followed with TLC plates run in PE-EtOAc solvent systems. Spots were visualized by exposure to UV light (254 nm) followed by I_2 vapor. Flash column chromatographies were carried out on silica gel with PE-EtOAc mixtures under positive pressure.

Biological Assay

The fungicidal activities of title compounds were evaluated using mycelium growth rate test. One-milliliter solution of tested compound in acetone was added into a culture plate, and then 9 mL PDA culture medium was added to obtain the flat containing 50 ppm tested compound. A bacterium tray of 4-mm diameter cut along the external edge of the hypase was removed to the flat containing tested compound and put in equilateral triangular style. Later, the culture plate was cultured at 24 ± 1°C and the expanded diameter of bacterium tray was measured after 48 h and compared with that treated with sterilization water to estimate the activity. Two replicates were included in the evaluation.

General Synthetic Procedure for Title Compounds

Ethyl or phenyl phosphorodichloride (3 mmol) was dissolved in $CHCl_3$ (5 mL), and the solution was cooled to −10°C. Phenol or alcohol (3 mmol) and Et_3N (3 mmol) in $CHCl_3$ (5 mL) were dripped into the stirred solution. The reaction mixture was continued to be stirred in ice bath for 1 h. The solution of DMAP (2.1 mmol) in $CHCl_3$ (5 mL) was dripped into the reaction solution after NAE18:0 (0.7 g) was added. Then the reaction solution was allowed to rise to room temperature, and during this period NAE18:0 dissolved gradually and the solution turned clear and light yellow. After stirring for 12 h, the reaction solution was washed with water and then dried over anhydrous magnesium sulfate. The solvent was removed, and column chromatography of the residue with PE/EtOAc (2:1, v/v) gave the product as a solid.

*O-Ethyl O-phenyl O-2-(stearamido)ethyl phosphate (***1a***).* Yellowy solid, mp 44–46°C, yield 44%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.38–7.33 (t, 2H, Ar$\underline{H}$), 7.22–7.20 (d, 3H, Ar$\underline{H}$), 6.13 (bs, 1H, N$\underline{H}$), 4.29–4.19 (m, 4H, NHCH$_2$C$\underline{H}_2$O, CH$_3$C$\underline{H}_2$OP), 3.58–3.53 (dd, 2H, NHC$\underline{H}_2$CH$_2$O), 2.15–2.10 (t, 2H, C$\underline{H}_2$CONH), 1.60–1.56 (t, 2H, C$\underline{H}_2$CH$_2$CONH), 1.38–1.34 (t, 3H, C$\underline{H}_3$CH$_2$OP), 1.25 (s, 28H, (C$\underline{H}_2$)$_{14}$), 0.90–0.86 (t, 3H, C$\underline{H}_3$(CH$_2$)$_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.84. Anal Calcd for $C_{28}H_{50}NO_5P$: C 65.73, H 9.85, N 2.74; found: C 65.60, H 10.01, N 2.86.

*O-Ethyl O-(3-chlorophenyl) O-2-(stearamido)-ethyl phosphate (***1b***).* Yellowy solid, mp <30°C, yield 40%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.28–7.24 (d, 1H, Ar$\underline{H}$), 7.22–7.19 (t, 1H, Ar$\underline{H}$), 7.17–7.09 (dd, 2H, Ar$\underline{H}$), 6.20 (bs, 1H, N$\underline{H}$), 4.26–4.18 (t, 4H, NHCH$_2$C$\underline{H}_2$O, CH$_3$C$\underline{H}_2$OP), 3.55–3.51 (dd, 2H, NHC$\underline{H}_2$-CH$_2$O), 2.15–2.11 (t, 2H, C$\underline{H}_2$CONH), 1.58–1.55 (t, 2H, C$\underline{H}_2$CH$_2$CONH), 1.36–1.33 (t, 3H, C$\underline{H}_3$CH$_2$OP), 1.23 (s, 28H, (C$\underline{H}_2$)$_{14}$), 0.87–0.84 (t, 3H, C$\underline{H}_3$(CH$_2$)$_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.21. Anal Calcd for $C_{28}H_{49}ClNO_5P$: C 61.58, H 9.04, N 2.56; found: C 61.29, H 9.43, N 2.39.

*O-Ethyl O-(4-chlorophenyl) O-2-(stearamido)-ethyl phosphate (***1c***).* ivory solid, mp 33–34°C, yield 51%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.33–7.30 (d, 2H, ArH), 7.17–7.15 (d, 2H, ArH), 6.08 (bs, 1H, NH), 4.28–4.18 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.58–3.53 (dd, 2H, $NHCH_2CH_2O$), 2.16–2.11 (t, 2H, CH_2CONH), 1.61–1.57 (t, 2H, CH_2CH_2CONH), 1.38–1.33 (t, 3H, CH_3-CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.86 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.03. Anal Calcd for $C_{28}H_{49}ClNO_5P$: C 61.58, H 9.04, N 2.56; found: C 61.59, H 9.03, N 2.68.

*O-Ethyl O-(2,4-dichlorophenyl) O-2-(stearamido)-ethyl phosphate (***1d***).* yellowy solid, mp <30°C, yield 35%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.44–7.43 (d, 1H, ArH), 7.39–7.36 (d, 1H, ArH), 7.25–7.21 (dd, 1H, ArH), 6.25 (bs, 1H, NH), 4.32–4.23 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.60–3.55 (dd, 2H, $NHCH_2CH_2O$), 2.18–2.13 (t, 2H, CH_2CONH), 1.62–1.57 (t, 2H, CH_2CH_2CONH), 1.40–1.35 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.86 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.47. Anal Calcd for $C_{28}H_{48}Cl_2$ N O_5P: C 57.93, H 8.33, N 2.41; found: C 57.82, H 8.25, N 2.41.

*O-Ethyl O-(2-bromophenyl) O-2-(stearamido)-ethyl phosphate (***1e***).* Yellowy solid, mp <30°C, yield 45%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.59–7.57 (d, 1H, ArH), 7.44–7.42 (d, 1H, ArH), 7.31–7.26 (dd, 1H, ArH), 7.08–7.04 (t, 1H, ArH), 6.16 (bs, 1H, NH), 4.32–4.24 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.58–3.54 (dd, 2H, $NHCH_2CH_2O$), 2.16–2.12 (t, 2H, CH_2CONH), 1.59–1.56 (t, 2H, CH_2CH_2CONH), 1.38–1.35 (t, 3H, CH_3CH_2OP), 1.24 (s, 28H, $(CH_2)_{14}$), 0.88–0.85 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.46. Anal Calcd for $C_{28}H_{49}BrNO_5P$: C 56.94, H 8.36, N 2.37; found: C 56.98, H 8.44, N 2.42.

*O-Ethyl O-(4-bromophenyl) O-2-(stearamido)-ethyl phosphate (***1f***).* Off-white solid, mp 38–40°C, yield 49%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.45–7.43 (d, 2H, ArH), 7.10–7.07 (d, 2H, ArH), 6.19 (bs, 1H, NH), 4.24–4.14 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.55–3.51 (dd, 2H, $NHCH_2CH_2O$), 2.13–2.09 (t, 2H, CH_2CONH), 1.58–1.55 (t, 2H, CH_2CH_2CONH), 1.35–1.32 (t, 3H, CH_3-CH_2OP), 1.23 (s, 28H, $(CH_2)_{14}$), 0.87–0.84 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.11. Anal Calcd for $C_{28}H_{49}BrNO_5P$: C 56.94, H 8.36, N 2.37; found: C 57.10, H 8.38, N 2.30.

*O-Ethyl O-(4-fluorophenyl) O-2-(stearamido)-ethyl phosphate (***1g***).* Yellowy solid, mp 38–39°C, yield 40%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.20–7.16 (dd, 2H, ArH), 7.06–7.01 (t, 2H, ArH), 6.10 (bs, 1H, NH), 4.28–4.18 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.59–3.54 (dd, 2H, $NHCH_2CH_2O$), 2.17–2.12 (t, 2H, CH_2CONH), 1.62–1.57 (t, 2H, CH_2CH_2CONH), 1.38–1.33 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.86 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.64. Anal Calcd for $C_{28}H_{49}FN$ O_5P: C 63.49, H 9.32, N 2.64; found: C 63.58, H 9.43, N 2.37.

*O-Ethyl O-(2-nitrophenyl) O-2-(stearamido)ethyl phosphate (***1h***).* Yellow solid, mp 34–36°C, yield 33%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.96–7.93 (d, 1H, ArH), 7.66–7.48 (m, 2H, ArH), 7.37–7.32 (t, 1H, ArH), 6.16 (bs, 1H, NH), 4.36–4.24 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.59–3.54 (dd, 2H, $NHCH_2CH_2O$), 2.20–2.15 (t, 2H, CH_2CONH), 1.63–1.58 (t, 2H, CH_2CH_2CONH), 1.40–1.35 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.86 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.94. Anal Calcd for $C_{28}H_{49}N_2O_7P$: C 60.41, H 8.87, N 5.03; found: C 60.11, H 8.36, N 4.87.

*O-Ethyl O-(4-nitrophenyl) O-2-(stearamido)ethyl phosphate (***1i***).* Yellow solid, mp 75–77°C, yield 41%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.27–8.24 (d, 2H, ArH), 7.40–7.37 (d, 2H, ArH), 6.24 (bs, 1H, NH), 4.32–4.23 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.61–3.56 (dd, 2H, $NHCH_2CH_2O$), 2.19–2.14 (t, 2H, CH_2CONH), 1.62–1.58 (t, 2H, CH_2CH_2CONH), 1.41–1.36 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.85 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.85. Anal Calcd for $C_{28}H_{49}N_2O_7P$: C 60.41, H 8.87, N 5.03; found: C60.39, H 8.85, N 5.03.

*O-Ethyl O-(3-methyloxyphenyl) O-2-(stearamido)-ethyl phosphate (***1j***).* Ivory solid, mp 41–43°C, yield 53%.^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.27–7.24 (d, 1H, ArH), 7.18–7.13 (t, 1H, ArH), 6.98–6.89 (dd, 2H, ArH), 6.42 (bs, 1H, NH), 4.29–4.24 (t, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.87 (s, 3H, CH_3– OAr), 3.59–3.54 (dd, 2H, $NHCH_2CH_2O$), 2.16–2.11 (t, 2H, CH_2CONH), 1.61–1.57 (t, 2H, CH_2CH_2CONH), 1.39–1.35 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.86 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.57. Anal Calcd for $C_{29}H_{52}N$ O_6P: C 64.30, H 9.68, N 2.59; found: C 64.18, H 9.66, N 2.59.

*O-Ethyl O-(2-methylphenyl) O-2-(stearamido)-ethyl phosphate (***1k***).* Off-white solid, mp <30°C, yield 43%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.27–7.06 (m, 4H, ArH), 6.19 (bs, 1H, NH), 4.28–4.18 (m, 4H, $NHCH_2CH_2O$, CH_3CH_2OP), 3.56–3.52 (dd, 2H, $NHCH_2CH_2O$), 2.31 (s, 3H, $ArCH_3$), 2.14–2.09 (t, 2H, CH_2CONH), 1.60–1.56 (t, 2H, CH_2CH_2CONH), 1.38–1.33 (t, 3H, CH_3CH_2OP), 1.25 (s, 28H, $(CH_2)_{14}$), 0.90–0.85 (t, 3H, $CH_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.57. Anal Calcd for $C_{29}H_{52}NO_5P$: C

66.26, H 9.97, N 2.66; found: C 66.37, H 10.02, N 2.68.

*O-Ethyl O-(3-methylphenyl) O-2-(stearamido)-ethyl phosphate (***1l***).* White solid, mp 35–36°C, yield 30%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.25–7.20 (t, 1H, ArH), 7.02–6.99 (d, 3H, Ar<u>H</u>), 6.14 (bs, 1H, N<u>H</u>), 4.26–4.18 (m, 4H, $NHCH_2C\underline{H}_2O$, $CH_3C\underline{H}_2OP$), 3.57–3.53 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.35 (s, 3H, $ArC\underline{H}_3$), 2.15–2.10 (t, 2H, $C\underline{H}_2CONH$), 1.60–1.56 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.39–1.34 (t, 3H, $C\underline{H}_3CH_2OP$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.83. Anal Calcd for $C_{29}H_{52}NO_5P$: C 66.26, H 9.97, N 2.66; found: C 66.28, H 9.92, N 2.70.

*O-Ethyl O-(3,4-dimethylphenyl) O-2-(stearamido)-ethyl phosphate (***1m***).* Yellowy solid, mp 34–35°C, yield 51%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.09–7.06 (d, 1H, Ar<u>H</u>), 6.98 (s, 1H, Ar<u>H</u>), 6.94–6.92 (d, 1H, Ar<u>H</u>), 6.20 (bs, 1H, N<u>H</u>), 4.27–4.17 (m, 4H, $NHCH_2C\underline{H}_2O$, $CH_3C\underline{H}_2OP$), 3.56–3.51 (dd, 2H, NH-$C\underline{H}_2CH_2O$), 2.24 (s, 3H, $ArC\underline{H}_3$), 2.22 (s, 3H, $ArC\underline{H}_3$), 2.14–2.09 (t, 2H, $C\underline{H}_2CONH$), 1.60–1.55 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.38–1.33 (t, 3H, $C\underline{H}_3CH_2OP$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.61. Anal Calcd for $C_{30}H_{54}NO_5P$: C 66.76, H 10.08, N 2.60; found: C 66.67, H 9.98, N 2.68.

*O-Ethyl O-(2,6-dimethylphenyl) O-2-(stearamido)-ethyl phosphate (***1n***).* Ivory solid, mp 50–52°C, yield 38%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.06–6.97 (m, 3H, Ar<u>H</u>), 6.07 (bs, 1H, N<u>H</u>), 4.25–4.18 (m, 4H, $NHCH_2$-$C\underline{H}_2O$, $CH_3C\underline{H}_2OP$), 3.54–3.50 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.36 (s, 6H, $ArCH_3$), 2.14–2.09 (t, 2H, $C\underline{H}_2CONH$), 1.60–1.55 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.37–1.32 (t, 3H, $C\underline{H}_3$-CH_2OP), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.19. Anal Calcd for $C_{30}H_{54}NO_5P$: C 66.76, H 10.08, N 2.60; found: C 66.61, H 10.08, N 2.64.

*O-Ethyl O-(4-tertbutylphenyl) O-2-(stearamido)-ethyl phosphate (***1o***).* Yellowy solid, mp 54–55°C, yield 48%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.37–7.34 (d, 2H, Ar<u>H</u>), 7.13–7.10 (d, 2H, Ar<u>H</u>), 6.13 (bs, 1H, N<u>H</u>), 4.28–4.19 (m, 4H, $NHCH_2C\underline{H}_2O$, $CH_3C\underline{H}_2OP$), 3.58–3.53 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.16–2.11 (t, 2H, $C\underline{H}_2CONH$), 1.62–1.57 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.39–1.34 (t, 3H, $C\underline{H}_3$- CH_2OP), 1.30 (s, 9H, ^{t}Bu<u>H</u>), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.85 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.53. Anal Calcd for $C_{32}H_{58}NO_5P$: C 67.69, H 10.30, N 2.47; found: C 67.77, H 10.38, N 2.34.

*O-Ethyl O-(2-nitro-4-methylphenyl) O-2-(stearamido)ethyl phosphate (***1p***).* Yellow solid, mp 40–42°C, yield 42%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.74 (s, 1H, Ar<u>H</u>), 7.44–7.37 (dd, 2H, Ar<u>H</u>), 6.12 (bs, 1H, N<u>H</u>), 4.32–4.22 (m, 4H, $NHCH_2C\underline{H}_2O$, $CH_3C\underline{H}_2OP$), 3.59–3.53 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.42 (s, 3H, $ArC\underline{H}_3$), 2.20–2.15 (t, 2H, $C\underline{H}_2CONH$), 1.63–1.58 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.39–1.34 (t, 3H, $C\underline{H}_3CH_2OP$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.61. Anal Calcd for $C_{29}H_{51}N_2O_7P$: C 61.03, H 9.01, N 4.91; found: C 61.19, H 8.99, N 5.01.

*O-Propyl O-phenyl O-2-(stearamido)ethyl phosphate (***1q***).* White solid, mp 37–38°C, yield 43%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.38–7.33 (t, 2H, Ar<u>H</u>), 7.22–7.20 (d, 3H, Ar<u>H</u>), 6.18 (bs, 1H, N<u>H</u>), 4.25–4.19 (m, 2H, $NHCH_2C\underline{H}_2O$), 4.16–4.09 (dd, 2H, $CH_3CH_2C\underline{H}_2OP$), 3.57–3.52 (dd, 2H, $NHC\underline{H}_2$-CH_2O), 2.15–2.09 (t, 2H, $C\underline{H}_2CONH$), 1.78–1.67 (m, 2H, $CH_3C\underline{H}_2CH_2OP$), 1.61–1.56 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.98–0.93 (t, 3H, $C\underline{H}_3CH_2CH_2OP$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.75. Anal Calcd for $C_{29}H_{52}NO_5P$: C 66.26, H 9.97, N 2.66; found: C 66.28, H 10.01, N 2.71.

*O-(2-Chloroethyl) O-phenyl O-2-(stearamido)-ethyl phosphate (***1r***).* Yellowy solid, mp 53–55°C, yield 42%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.39–7.34 (t, 2H, Ar<u>H</u>), 7.24–7.21 (d, 3H, Ar<u>H</u>), 6.08 (bs, 1H, N<u>H</u>), 4.41–4.33 (m, 2H, $NHCH_2C\underline{H}_2O$), 4.29–4.23 (m, 2H, $ClCH_2C\underline{H}_2OP$), 3.72–3.68 (t, 2H, $ClC\underline{H}_2CH_2OP$), 3.59–3.54 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.16–2.11 (t, 2H, $C\underline{H}_2CONH$), 1.61–1.56 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3$-$(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −5.29. Anal Calcd for $C_{28}H_{49}ClNO_5P$: C 61.58, H 9.04, N 2.56; found: C 61.59, H 9.09, N 2.58.

*O-(2-Benzyloxyethyl) O-phenyl O-2-(stearamido)-ethyl phosphate (***1s***).* Off-white solid, mp <30°C, yield 51%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.34–7.30 (m, 7H, Ar<u>H</u>), 7.22–7.16 (m, 3H, Ar<u>H</u>), 6.12 (bs, 1H, N<u>H</u>), 4.55 (s, 2H, $PhC\underline{H}_2$), 4.41–4.25 (m, 2H, $NHCH_2C\underline{H}_2O$), 4.23–4.13 (m, 2H, $BnOCH_2$-$C\underline{H}_2OP$), 3.72–3.69 (t, 2H, $BnOC\underline{H}_2CH_2OP$), 3.52–3.47 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.09–2.04 (t, 2H, $C\underline{H}_2CONH$), 1.58–1.53 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.88. Anal Calcd for $C_{35}H_{56}NO_6P$: C 68.04, H 9.14, N 2.27; found: C 67.96, H 9.11, N 2.27.

*O-((Tetrahydrofuran-2-yl)methyl) O-phenyl O-2-(stearamido)ethyl phosphate (***1t***).* Yellowy solid, mp

70–72°C, yield 60%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.38–7.33 (t, 2H, Ar$\underline{H}$), 7.23–7.21 (d, 3H, Ar$\underline{H}$), 5.98 (bs, 1H, N$\underline{H}$), 4.34–4.04 (m, 3H, $NHCH_2C\underline{H}_2O$, $THFOC\underline{H}_aH_b$), 3.93–3.79 (m, 1H,$THFOCH_a\underline{H}_b$), 3.75–3.72 (t, 2H, $-CH_2CH_2C\underline{H}_2OCH-$), 3.57–3.53 (t, 1H, $-CH_2CH_2CH_2OC\underline{H}-$), 3.46–3.41 (dd, 2H, $NHC\underline{H}_2CH_2O$), 2.24–2.18 (t, 2H, $C\underline{H}_2CONH$), 2.16–2.11 (t, 2H, $-C\underline{H}_2CH_2CH_2OCH-$), 2.05−1.81 (t, 2H, $-CH_2C\underline{H}_2CH_2OCH-$), 1.66–1.61 (t, 2H, $C\underline{H}_2CH_2CONH$), 1.25 (s, 28H, $(C\underline{H}_2)_{14}$), 0.90–0.86 (t, 3H, $C\underline{H}_3(CH_2)_{14}$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): −4.92. Anal Calcd for $C_{31}H_{54}NO_5P$: C 67.48, H 9.86, N 2.54; found: C 67.45, H 9.56, N 2.89.

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