European Journal of Medicinal Chemistry 47 (2012) 485–492

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of cyclopentane-linked alkyl phosphocholines as potential anticancer agents that act by inhibiting Akt phosphorylation

Md. Maqusood Alam [a,b], Eun-Ha Joh [b], Yuri Kim [a,b], Yeon Il Oh [a,b], Jongki Hong [a,b], Baek Kim [a,c], Dong-Hyun Kim [a,b], Yong Sup Lee [a,b,*]

[a] Department of Pharmacy, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea
[b] Department of Life and Nanopharmaceutical Science, Kyung Hee University, Republic of Korea
[c] Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642, USA

ARTICLE INFO

Article history:
Received 24 September 2011
Received in revised form
9 November 2011
Accepted 9 November 2011
Available online 20 November 2011

Keywords:
Alkylphosphocholine
Antitumor
Akt phosphorylation
Miltefosine
Erufosine
Edelfosine

ABSTRACT

Three new series of novel alkylphosphocholine (APC) derivatives containing a cyclopentane ring near the phosphocholine head group were synthesized. In the first set of analogues, the phosphocholine head group was attached to the secondary alcohol of *trans*-2-(hydroxymethyl)cyclopentanol, whereas in the second and third sets of analogues, the phosphocholine head group was linked to the primary alcohol of *trans*- and *cis*-2-(hydroxymethyl)cyclopentanol, respectively. Of the compounds synthesized, compound **6d** most potently inhibited Akt phosphorylation with an IC_{50} value of 3.6 μM, its potency was greater than the reference compounds miltefosine, perifosine, and erufosine. Compounds **6b** and **6d** exhibited the most potent growth-inhibitory effects on A549, MCF-7, and KATO-III human cancer cell lines. These compounds also showed more active anti-proliferative effects than the reference compounds. Importantly, the cytotoxic effects of these compounds on A549 cell line were proportional to their abilities to inhibit Akt phosphorylation, which supports that these synthesized APC compounds are novel inhibitors of the Akt cell survival pathway.

1. Introduction

The majority of the anti-cancer drugs currently available for the treatment of human cancer have well established shortcomings, such as, poor efficiency, non-selectivity, and high toxicity. Furthermore, the identification of potent, selective, and less toxic anti-cancer agents remains an important, but challenging goal of medicinal chemistry [1–3]. Alkylphosphocholines (APCs) belong to a class of lipid molecules that include miltefosine (HePC, **1**), perifosine (OPP, **2**), erufosine ($ErPC_3$, **3**) and edelfosine (ET-18-OMe, **4**), which have known antitumor properties [4]. Unlike conventional chemotherapeutics, these compounds structurally resemble membrane lipid components, and thus, exhibit adequate drug delivery to target tumor tissues. However, they can also target primarily membrane-bound signals associated with cancer-specific cellular phenotypes, such as, cellular proliferation and survival. More specifically, the possible mechanisms underlying the actions of APCs have been reported to involve the inhibition of PI3K/Akt [5], signal transduction pathways [6], and the induction of apoptosis [7–9]. However, details of the mechanisms involved have not been elucidated [4,6]. Nevertheless, it is known that APCs do not target the DNA and they have cell selective effects, specifically by inhibiting the proliferation of cancer cells at low concentrations but do not affect normal cells [10–12].

Miltefosine (**1**, Fig. 1) shows a wide range of antitumor effect and has been approved for the topical treatment of skin metastases from breast cancer (Milterx®) [13,14], and for the oral treatment of leishmaniasis (Impavido®) [15,16]. However, the oral use of miltefosine in cancer patient has been associated with dose-limiting gastrointestinal side effects. Perifosine (**2**) is a synthetic, substituted heterocyclic APC that belongs to the second generation of APCs that exhibit significant anti-proliferative activity *in vitro* and *in vivo* in several human tumor model systems [5,17,18]. Perifosine is being currently investigated in Phase II clinical studies for cancer treatment, but is restricted to oral applications due to its hematological and biochemical toxicities. Furthermore, the gastrointestinal side effects of perifosine also prevent its effective oral dosing treatment [19]. Erufosine (**3**), another prospective APC, shows strong antitumor activity against a variety of tumor cells *in vitro* and *in vivo* [20,21]. Erufosine now represents the first intravenous APC and has been administered in a clinical Phase I

* Corresponding author. Department of Pharmacy, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea. Tel.: +82 2 961 0370; fax: +82 2 966 3885.
E-mail address: kyslee@khu.ac.kr (Y.S. Lee).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.11.018

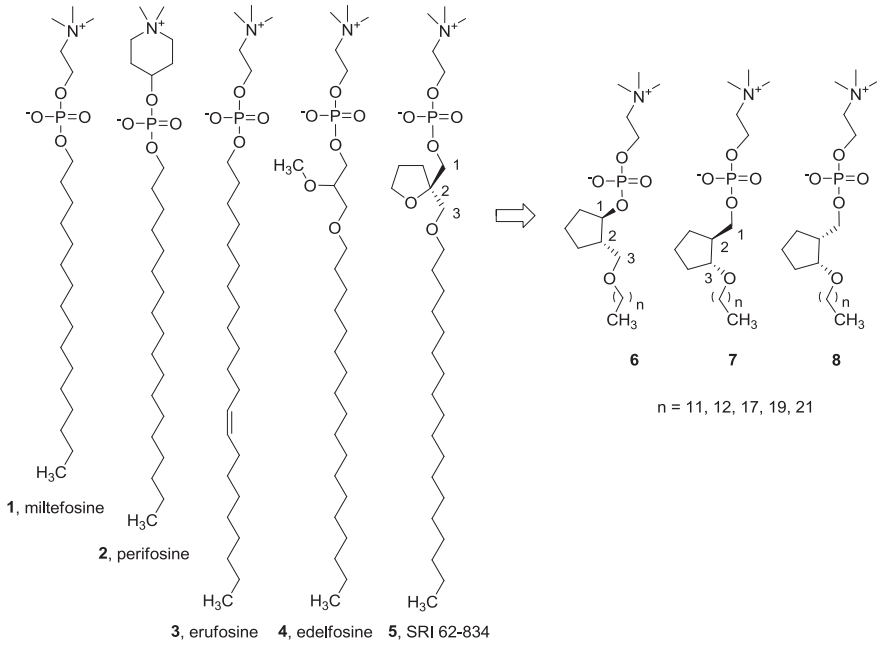


Fig. 1. Structures of reported APCs and target compounds.

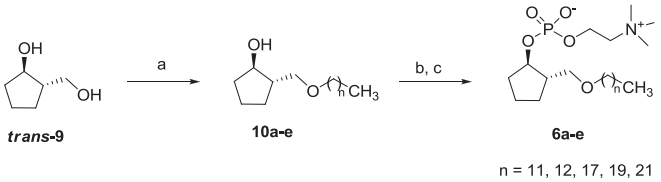
trial in tumor patients [22]. The 1-*O*-methyl-rac-glycero-3-phosphocholine (edelfosine, **4**) is also a promising antitumor compound since it exhibits potent *in vitro* and *in vivo* antitumor activity in various cancer cell lines [23]. However, its potency in Phase II clinical studies was only moderate [24].

Akt is a serine/threonine kinase and is generally activated in cancer cells. Akt regulates a wide range of downstream targets that regulate tumor associated cell processes such as cell growth, cell cycle progression, survival, migration, epithelial mesenchymal transition and angiogenesis [25]. Furthermore, the inhibition of Akt signaling leads to programmed cell death. High levels of active Akt have been found in many types of human tumors [26], and thus Akt is viewed as a promising target for cancer chemotherapy. Accordingly, several APC compounds have been synthesized as inhibitors of Akt signaling and were tested in a variety of tumor cells [27–29,34]. However, these APCs had moderate potency as compared with other antitumor agents, and they had some adverse effects, which include general problem with respect to red blood cells [30]. Thus, more extensive structure–activity relationship (SAR) studies are needed to facilitate the design of novel anticancer APC compounds. Here, we describe the chemical synthesis and biological evaluation of a series of new APC derivatives.

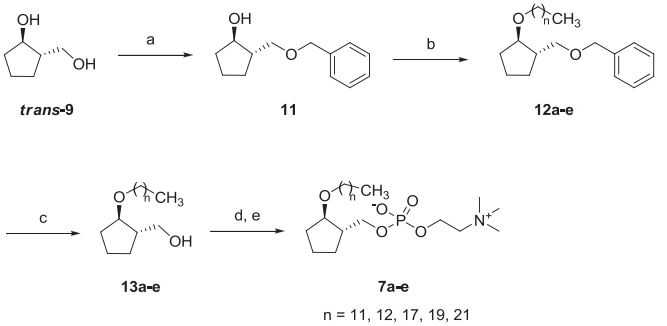
The synthesis of conformationally restricted molecules to improve the activity of the parent molecule is a common strategy in medicinal chemistry. In this line, conformationally restricted structure of **4** such as SRI 62-834 (**5**, Fig. 1), which joined C2 carbon and methyl of C2 methoxy group into a furan ring, has been described [31,32]. A view to develop potent Akt phosphorylation inhibitory compounds, we also considered creating a cyclopentane ring since it would restrict the conformations of alkyl chain in APC derivatives, and thus, produce different profiles of anti-cancer activity and side effects. In our work, we focused on the restriction of the conformational flexibility of C1–C2 or C2–C3 bond in edelfosine (**4**) by joining these bonds into the cyclopentane ring leading to compounds **6** and **7**. Here, we describe the synthesis of a new series of APCs that possess a cyclopentane ring near the position of alkylphosphocholine head group and their Akt phosphorylation inhibitory effects and cytotoxicities against human cancer cell lines. The *cis*-isomer of **7** (**8**) was also designed to explore the influence of geometry of the cyclopentane ring on the activity. Additionally, we investigated the effect of alkyl chain length variation on their biological activity (Fig. 1).

2. Chemistry

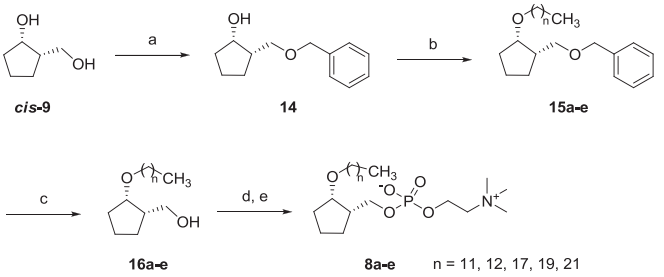
The synthetic procedures for new APC derivatives are depicted in Schemes 1–3. Syntheses of **6a–e** were started from the alkylation of *trans*-2-(hydroxymethyl)cyclopentanol (***trans*-9**) [33]. Treatment of diol **9** with several bromoalkanes in the presence of NaH in DMF afforded 2-(alkoxymethyl)cyclopentanols **10a–e**



Scheme 1. Reagents and conditions: (a) bromoalkane, NaH, DMF, 0 °C; (b) 2-chloro-1,3,2-dioxaphospholane-2-oxide, NEt_3, benzene, rt; (c) TMA, CH_3CN, 65 °C.



Scheme 2. Reagents and conditions: (a) benzyl bromide, NaH, DMF, −60 °C; (b) bromoalkane, NaH, rt (c) 10% Pd/C, MeOH or THF, rt; (d) 2-chloro-1,3,2-dioxaphospholane-2-oxide, NEt_3, benzene, rt; (e) TMA, CH_3CN, 65 °C.



Scheme 3. Reagents and conditions: (a) benzyl bromide, NaH, DMF, −60 °C; (b) bromoalkane, NaH, DMF, rt; (c) 10% Pd/C, MeOH or THF, rt; (d) 2-chloro-1,3,2-dioxaphospholane-2-oxide, NEt_3, benzene, rt; (e) TMA, CH_3CN, 65 °C.

(Scheme 1). Subsequent phosphorylations of **10a**–**e** with 2-chloro-1,3,2-dioxaphospholane-2-oxide and ring opening of the resulting phospholane intermediates with trimethylamine (TMA) afforded the target compounds **6a**–**e** [34].

In the second and third sets of reactions, the primary alcohol of the *trans*- and *cis*-diols **9** were benzylated by treating benzyl bromide and NaH in DMF to afford compounds **11** and **14**, respectively (Schemes 2 and 3) [35]. Subsequent alkylation of the secondary alcohols **11** and **14** with bromoalkane furnished **12a**–**e** and **15a**–**e**, which after treatment with 10% Pd/C under hydrogen atmosphere in methanol or THF afforded alcohols **13a**–**e** and **16a**–**e**. Finally, the coupling of phosphocholine head group on compounds **13a**–**e** and **16a**–**e** with 2-chloro-1,3,2-dioxaphospholane-2-oxide and followed by ring opening of phospholane intermediate by TMA provided the desired compounds **7a**–**e** and **8a**–**e**, respectively.

3. Results and discussion

3.1. Akt phosphorylation inhibition effects of APC compounds

Inhibition of Akt signaling leads to the apoptosis and growth inhibition of tumor cells, which commonly display elevated levels of Akt activity, and thus Akt has become a promising anti-cancer target. It has been reported that several types of anti-cancer lipids (APC) inhibit Akt phosphorylation and activate apoptosis of cancer cells in *in vivo* [5]. Hence, we examined whether our newly synthesized APC compounds inhibit Akt phosphorylation and thereby induce growth inhibition of cancer cells. For the test of the effect of APC compounds on Akt phosphorylation, we employed the A549 human epithelial lung cancer cell line [36]. Akt phosphorylation was stimulated by adding 10 μg/mL insulin to the cell line and then the effects of APC compounds on the level of Akt phosphorylation was examined. Initially, we assayed Akt phosphorylation inhibition effects of the synthesized compounds at a concentration of 10 μM, and determined the IC_{50} value of any compound that inhibited its phosphorylation by more than 50%. The activity data of new APC compounds are summarized at Table 1.

In the SAR study of compounds **6a**–**e**, **7a**–**e** and **8a**–**e**, we found that the Akt phosphorylation inhibitory effects of **6a**–**e** series compounds, which possess a phosphocholine head group at the secondary alcohol of *trans*-2-(alkyloxymethyl)cyclopentanol, were higher than those of **7a**–**e** or **8a**–**e** series compounds. However, compounds **7d**, **7e** and **8d** also showed potent inhibitory activities with IC_{50} values 9.1, 6.5 and 16.3 μM, respectively. Compound **7d**, which contains *trans* orientated cyclopentanediol and the longest alkyl chain (C-20), had a two-fold greater effect (IC_{50} = 9.1 μM) than its *cis* counterpart compound **8d** (IC_{50} = 16.3 μM). In addition, compounds **6b** and **7e** exhibited potent inhibitory effects with IC_{50} values of 6.6 and 6.5 μM, respectively, which were comparable to those of the references compounds $ErPC_3$ and HePC. However, **6d**, in which the phosphocholine moiety is linked through the secondary alcohol of *trans*-2-(alkyloxymethyl)cyclopentanol, was found to be most active with an IC_{50} of 3.6 μM. Notably, **6d** was almost three times more potent than $ErPC_3$ and HePC, and twice as potent as OPP.

Table 1
The Akt phosphorylation inhibitory activities of alkylphosphocholines **6a**–**8e**.

Compounds	# of alkyl chain	% inhibition of Akt phosphorylation at 10 μM	IC_{50} (μM)[a]
6a	12	36.24 ± 7.35	–
6b	13	65.37 ± 9.77	6.6 ± 2.1
6c	18	63.43 ± 21.4	10.3 ± 4.6
6d	20	74.43 ± 4.04	3.6 ± 0.6
6e	22	58.25 ± 2.75	10.0 ± 0.9
7a	12	31.71 ± 12.9	–
7b	13	40.77 ± 3.36	–
7c	18	32.36 ± 10.6	–
7d	20	58.89 ± 2.24	9.1 ± 0.4
7e	22	67.96 ± 3.36	6.5 ± 0.6
8a	12	30.42 ± 7.84	–
8b	13	44.66 ± 8.89	–
8c	18	35.18 ± 4.19	–
8d	20	54.36 ± 1.94	16.3 ± 5.3
8e	22	36.24 ± 5.93	–
1, HePC		56.31 ± 16.47	9.6 ± 3.5
2, OPP		67.96 ± 13.73	5.3 ± 0.8
3, $ErPC_3$		55.33 ± 12.35	8.4 ± 0.6

[a] IC_{50} was defined as the concentration that resulted in 50% inhibition. Data are presented as the means ± SDs of three independent experiments.

3.2. Evaluations of the in vitro anti-cancer activities of the APC derivatives

We evaluated the cytotoxic effects of APC derivates on three human cancer cell lines: A549 (lung cancer), MCF-7 (breast carcinoma), and KATO-III (gastric carcinoma) by using flow cytometry (FACS). Cytotoxicities of derivatives are expressed as half maximal inhibitory concentrations (IC_{50}), which were determined by exposing cells to the compounds for 24 h (Table 2). Miltefosine (HePC, **1**), perifosine (OPP, **2**) and erufosine ($ErPC_3$, **3**) were also used as the reference compounds. Most of the APC derivatives

Table 2
Cytotoxic effects of alkylphosphocholines **6a**-**8e** against selected human cancer cell lines.

Compounds	# of alkyl chain	IC_{50} (μM)[a]		
		A549[b]	MCF-7[c]	KATO-III[d]
6a	12	11.0 ± 0.6	11.8 ± 0.1	10.6 ± 1.7
6b	13	6.1 ± 0.1	7.7 ± 0.8	10.2 ± 0.3
6c	18	10.8 ± 0.5	13.8 ± 0.7	10.4 ± 2.4
6d	20	6.4 ± 0.1	10.8 ± 1.2	8.7 ± 1.0
6e	22	16.0 ± 3.0	16.3 ± 4.5	11.9 ± 0.8
7a	12	–	14.7 ± 1.6	10.4 ± 0.9
7b	13	9.4 ± 0.1	17.8 ± 4.9	10.1 ± 0.1
7c	18	19.3 ± 3.7	15.7 ± 1.5	8.2 ± 0.3
7d	20	9.8 ± 0.0	15.6 ± 3.4	7.7 ± 0.5
7e	22	11.1 ± 0.0	19.5 ± 9.5	17.6 ± 11.9
8a	12	26.3 ± 2.1	24.2 ± 6.7	7.4 ± 3.8
8b	13	10.2 ± 0.4	10.7 ± 0.2	12.1 ± 0.7
8c	18	17.8 ± 0.9	13.2 ± 1.1	9.2 ± 1.4
8d	20	9.7 ± 0.1	16.3 ± 0.8	8.8 ± 1.2
8e	22	23.3 ± 9.6	12.8 ± 1.0	8.6 ± 0.9
1, HePC		9.7 ± 0.2	11.4 ± 2.7	12.1 ± 3.3
2, OPP		7.0 ± 0.6	13.3 ± 0.7	12.8 ± 0.4
3, $ErPC_3$		16.9 ± 0.7	13.4 ± 0.9	9.3 ± 1.2

[a] IC_{50} was defined as the concentration resulting in 50% inhibition.
[b] Human lung cancer.
[c] Breast cancer.
[d] Gastric cancer. Data are presented as the means ± SDs of three independent experiments.

exhibited comparable or better cytotoxicity than the reference compounds (Table 2). In general, as was observed above for Akt phosphorylation inhibitory effects, **6a**–**e** series displayed more potent cytotoxicity than either **7a**–**e** or **8a**–**e** series compounds. Compound **6b**, with a C-13 alkyl chain, had the greatest cytotoxic effect on A549 and MCF-7 cell lines with IC_{50} values of 6.1 and 7.7 μM, respectively. Compound **6d**, which was the most potent Akt phosphorylation inhibitor, also exhibited a potent and well balanced cytotoxicity profile and its potencies against the three cancer lines were superior to those of HePC, OPP, or $ErPC_3$.

In the second series of compounds (**7a**–**e**), the phosphocholine head group is attached to the primary alcohol of *trans*-2-(hydroxymethyl)cyclopentanol. Compound **7d**, which has a C-20 alkyl chain, was found to have a potent cytotoxic effect on A549 and KATO-III cancer cell lines with IC_{50} values of 9.8 and 7.7 μM, respectively. On the other hand, compound **7e** exhibited moderate cytotoxicity against KATO-III, although this compound more potently inhibited the phosphorylation of Akt than any other compound in this series.

In the third series of compounds **8a**–**e**, the phosphocholine head group is attached to the primary alcohol of *cis*-2-(hydroxymethyl)cyclopentanol. In this series, compound **8d** showed potent cytotoxic activity against A549 with an IC_{50} value of 9.7 μM. Compounds **8a** and **8d**–**e** showed potent cytotoxic effects against the KATO-III cancer cell line. However, the inhibitory effects of these compounds on Akt phosphorylation were only moderate, suggesting that other possible mechanism of action on the cytotoxicity against of this cell line may be involved.

In most cases, the length of the alkyl chain had little effect on cytotoxicities, but as was observed for compounds **6d**, **7d** and **8d**, all of which possessed a C-20 alkyl chain, showed potent cytotoxic effects against A549 and KATO-III cell lines. Notably, the potent inhibitory effects of **6d**, **7d** and **8d** on Akt phosphorylation were found to be well correlated with cytotoxicities against the A549 cell line. Of the APC derivatives examined, compounds **6b** and **6d**, in which substituents were *trans*-orientated, were found to potently inhibit Akt phosphorylation and to have marked cytotoxic effects on the three human tumor cell lines. Furthermore, the potencies of these compounds were greater than those of HePC, OPP, and $ErPC_3$. Finally, the data obtained in the cytotoxicity assay showed good correlations between ability to inhibit Akt phosphorylation and anti-cancer activity, especially for the A-549 cell line.

4. Conclusion

We synthesized a new series of APCs that possess a cyclopentane ring near the position of alkylphosphocholine head group and evaluated their Akt phosphorylation inhibitory effects and cytotoxicities against human cancer cell lines. Of the APC derivatives synthesized, compounds **6b**, **6d** and **7e** exhibited greatest inhibition of Akt phosphorylation with IC_{50} values of 6.6, 3.6 and 6.5 μM respectively, and their potencies were higher than the reference compounds $ErPC_3$ and HePC. Notably, **6d** was found to be almost three times more potent than $ErPC_3$ and HePC and twice as potent as OPP. In addition, compounds **6b** and **6d** had greatest cytotoxic effects on A549, MCF-7, and KATO-III cell lines, and these effects were greater than the reference compounds. The inhibitory effects of **6b** and **6d** on Akt phosphorylation were found to be well correlated with their anti-cancer activities against the A549 cell line.

5. Experimental section

5.1. General instrumentation and chemicals

All the solvents were purified and dry condition. NMR spectra of all new compounds were recorded on Bruker AC 400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm, downfield from internal TMS standard. FABMS data were obtained on a JEOL JMS SX-102A spectrometer in the positive modes. Analytical thin layer chromatography (TLC) was carried out using precoated silica gel (E. Merck Kiesegel $60F_{254}$, layer thickness 0.25 mm), and flash column chromatography was performed using Merck Kiesegel 60 Art 9385 (230–400 mesh).

5.2. Syntheses

5.2.1. General procedure for the synthesis of ***10a***–***e***

To a stirred solution of (±)-(*trans*-2-hydroxymethyl)cyclopentanol (***trans*-9**, 1 eq.) in DMF (5 mL) was added sodium hydride (60% dispersion in mineral oil, 1.5 eq.) at 0 °C. The mixture was stirred for 30 min and then treated with several bromoalkanes (1.2 eq) at the same temperature. After stirring at 0 °C (for compounds **10a** and **10b**) or rt (for compounds **10c**–**e**) for 4 h, the reaction mixture was poured into ice water and extracted twice with diethyl ether. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated, and purified by column chromatography (ethyl acetate/*n*-hexane = 1:4) on silica gel to afford **10a**–**e**.

*5.2.1.1. (±)-trans-2-(Dodecyloxymethyl)cyclopentanol (**10a**).* The compound **10a** (367 mg) was obtained according to the general procedure 5.2.1 from ***trans*-9** (300 mg, 2.59 mmol) and 1-bromododecane (771 mg, 3.10 mmol). Yield 50%; ^1H NMR (400 MHz, $CDCl_3$) δ 3.94 (m, 1H), 3.68–3.53 (m, 2H), 3.49–3.27 (m, 2H), 2.10–1.51 (m, 8H), 1.25 (brs, 18H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.1.2. (±)-trans-2-(Tridecyloxymethyl)cyclopentanol (**10b**).* The compound **10b** (821 mg) was obtained according to the general procedure 5.2.1 from ***trans*-9** (700 mg, 6.03 mmol) and 1-bromotridecane (2.0 g, 7.23 mmol). Yield 46%; ^1H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.69–3.54 (m, 2H), 3.49–3.27 (m, 2H), 2.22–1.51 (m, 7H), 1.25 (brs, 22H), 0.88 (t, J = 6.7 Hz, 3H).

*5.2.1.3. (±)-trans-2-(Octadecyloxymethyl)cyclopentanol (**10c**).* The compound **10c** (287 mg) was obtained according to the general procedure 5.2.1 from ***trans*-9** (300 mg, 2.59 mmol) and 1-bromooctadecane (948 mg, 2.84 mmol). Yield 30%; ^1H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.69–3.54 (m, 2H), 3.50–3.27 (m, 2H), 2.20–1.51 (m, 8H), 1.25 (brs, 31H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.1.4. (±)-trans-2-(Icosyloxymethyl)cyclopentanol (**10d**).* The compound **10d** (390 mg) was obtained according to the general procedure 5.2.1 from ***trans*-9** (300 mg, 2.59 mmol) and 1-bromoeicosane (1.12 g, 3.11 mmol). Yield 38%; ^1H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.68–3.3.53 (m, 2H), 3.49–3.27 (m, 2H), 2.12–1.51 (m, 7H), 1.41–1.10 (brs, 36H), 0.88 (t, J = 6.7 Hz, 3H).

*5.2.1.5. (±)-trans-2-(Docosyloxymethyl)cyclopentanol (**10e**).* The compound **10e** (195 mg) was obtained according to the general procedure 5.2.1 from ***trans*-9** (300 mg, 2.59 mmol) and 1-bromodocosane (1.21 g, 3.11 mmol). Yield 18%; ^1H NMR (400 MHz, $CDCl_3$) δ 3.95 (m, 1H), 3.67–3.53 (m, 2H), 3.49–3.27 (m, 2H), 2.16–1.52 (m, 7H), 1.42–1.10 (brs, 40H), 0.88 (t, J = 6.4 Hz, 3H).

5.2.2. General procedure for the synthesis of compound ***11*** and ***14***

To a stirred solution of ***trans*-9** or ***cis*-9** (1 eq.) in DMF (5 mL) was added sodium hydride (60% dispersion in mineral oil, 1.5 eq.) at −60 °C. The mixture was stirred for 10 min and treated with benzyl bromide (1 eq.). After stirring for 2 h at the same temperature, the reaction mixture was poured into ice water and extracted twice with diethyl ether. The combined organic layer was dried with over $MgSO_4$, concentrated, and purified by column

chromatography (ethyl acetate/*n*-hexane = 1:4) on silica gel to afford **11** and **14**.

*5.2.2.1. (±)-trans-2-(Benzyloxymethyl)cyclopentanol (**11**).* The compound **11** (725 mg) was obtained according to the general procedure 5.2.2 from ***trans*-9** (600 mg, 5.17 mmol) and benzyl bromide (632 μL, 5.17 mmol). Yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.25 (m, 5H), 4.54–4.43 (m, 2H), 3.98 (m, 1H), 3.57 (m, 1H), 3.36 (t, J = 9.0 Hz, 1H), 2.22–1.18 (m, 7H).

*5.2.2.2. (±)-cis-2-(Benzyloxymethyl)cyclopentanol (**14**).* The compound **14** (300 mg) was obtained according to the general procedure 5.2.2 from ***cis*-9** (350 mg, 3.01 mmol) and benzyl bromide (370 μL, 3.01 mmol). Yield 48%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.24 (m, 5H), 4.61–4.47 (m, 2H), 4.07 (m, 1H), 3.76–3.61 (m, 2H), 2.10 (m, 1H), 1.85–1.44 (m, 6H).

*5.2.3. General procedure for the synthesis of **12a–e** and **15a–e***

To a stirred solution of **11** and **14** (1.2 eq.) in DMF (10 mL) was added NaH (60% dispersion in mineral oil, 1.5 eq.) at 0 °C and stirred for 30 min. To the mixture was added bromoalkane (1.2 eq.) and stirred at rt overnight. The reaction mixture was then poured into ice water and extracted three times with diethyl ether. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated, and purified by column chromatography (ethyl acetate/*n*-hexane = 1:9) on silica gel to afford **12a–e** and **15a–e**.

*5.2.3.1. ((((±)-trans-2-(Dodecyloxy)cyclopentyl)methoxy)methyl) benzene (**12a**).* The compound **12a** (240 mg) was obtained according to the general procedure 5.2.3 from compound **11** (200 mg, 0.97 mmol) and 1-bromododecane (289 mg, 1.16 mmol). Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 4.53–4.47 (m, 2H), 3.75 (m, 1H), 3.47–3.26 (m, 4H), 2.21 (m, 1H), 1.93–1.49 (m, 6H), 1.25 (brs, 20H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.2. ((((±)-trans-2-(Tridecycloxy)cyclopentyl)methoxy)methyl) benzene (**12b**).* The compound **12b** (350 mg) was obtained according to the general procedure 5.2.3 from compound **11** (300 mg, 1.46 mmol) and 1-bromotridecane (459 mg, 1.76 mmol). Yield 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 4.53–4.49 (m, 2H), 3.76 (m, 1H), 3.48–3.26 (m, 4H), 2.21 (m, 1H), 1.96–1.49 (m, 6H), 1.25 (brs, 21H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.3. ((((±)-trans-2-(Octadecyloxy)cyclopentyl)methoxy)methyl) benzene (**12c**).* The compound **12c** (303 mg) was obtained according to the general procedure 5.2.3 from compound **11** (200 mg, 0.97 mmol) and 1-bromooctadecane (389 mg, 1.16 mmol). Yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 4.53–4.48 (m, 2H), 3.76 (m, 1H), 3.44–3.26 (m, 4H), 2.21 (m, 1H), 1.96–1.49 (m, 7H), 1.25 (brs, 31H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.4. ((((±)-trans-2-(Icosyloxy)cyclopentyl)methoxy)methyl) benzene (**12d**).* The compound **12d** (252 mg) was obtained according to the general procedure 5.2.3 from compound **11** (300 mg, 1.46 mmol) and 1-bromoeicosane (632 mg, 1.75 mmol). Yield 36%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.21 (m, 5H), 4.51–4.50 (m, 2H), 3.76 (m, 1H), 3.49–3.24 (m, 4H), 2.12 (m, 1H), 1.87–1.41 (m, 6H), 1.25 (brs, 36H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.5. ((((±)-trans-2-(Docosyloxy)cyclopentyl)methoxy)methyl) benzene (**12e**).* The compound **12e** (250 mg) was obtained according to the general procedure 5.2.3 from compound **11** (370 mg, 1.80 mmol) and 1-bromodocosane (840 mg, 2.11 mmol). Yield 27%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 4.53–4.49 (m, 2H), 3.75 (m, 1H), 3.47–3.26 (m, 4H), 2.21 (m, 1H), 1.92–1.49 (m, 7H), 1.25 (brs, 40H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.6. ((((±)-cis-2-(Dodecyloxy)cyclopentyl)methoxy)methyl)benzene (**15a**).* The compound **15a** (120 mg) was obtained according to the general procedure 5.2.3 from compound **14** (100 mg, 0.49 mmol) and 1-bromododecane (146 mg, 0.58 mmol). Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.24 (m, 5H), 4.56–4.41 (m, 2H), 3.82 (m, 1H), 3.65 (m, 1H), 3.45–3.41 (m, 2H), 3.28 (m, 1H), 2.12 (m, 1H), 1.87–1.41 (m, 7H), 1.25 (brs, 19 H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.7. ((((±)-cis-2-(Tridecyloxy)cyclopentyl)methoxy)methyl)benzene (**15b**).* The compound **15b** (215 mg) was obtained according to the general procedure 5.2.3 from compound **14** (200 mg, 0.97 mmol) and 1-bromotridecane (307 mg, 1.16 mmol). Yield 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.24 (m, 5H), 4.56–4.42 (m, 2H), 3.83 (m, 1H), 3.67 (m, 1H), 3.46–3.42 (m, 2H), 3.28 (m, 1H), 2.12 (m, 1H), 1.87–1.41 (m, 7H), 1.25 (brs, 21H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.8. ((((±)-cis-2-(Octadecyloxy)cyclopentyl)methoxy)methyl)benzene (**15c**).* The compound **15c** (150 mg) was obtained according to the general procedure 5.2.3 from compound **14** (200 mg, 0.97 mmol) and 1-bromooctadecane (388 mg, 1.16 mmol). Yield 34%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.26 (m, 5H), 4.57–4.42 (m, 2H), 3.83 (m, 1H), 3.67 (m, 1H), 3.49–3.24 (m, 3H), 2.13 (m, 1H), 1.88–1.41 (m, 7H), 1.25 (brs, 31H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.9. ((((±)-cis-2-(Icosyloxy)cyclopentyl)methoxy)methyl)benzene (**15d**).* The compound **15d** (252 mg) was obtained according to the general procedure 5.2.3 from compound **14** (300 mg, 1.46 mmol) and 1-bromoeicosane (632 mg, 1.75 mmol). Yield 36%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.24 (m, 5H), 4.57–4.42 (m, 2H), 3.83 (m, 1H), 3.67 (m, 1H), 3.49–3.24 (m, 3H), 2.13 (m, 1H), 1.87–1.41 (m, 6H), 1.25 (brs, 36H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.3.10. ((((±)-cis-2-(Docosyloxy)cyclopentyl)methoxy)methyl)benzene (**15e**).* The compound **15e** (282 mg) was obtained according to the general procedure 5.2.3 from compound **14** (350 mg, 1.70 mmol) and 1-bromodocosane (793 mg, 2.04 mmol). Yield 32%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.26 (m, 5H), 4.57–4.42 (m, 2H), 3.83 (m, 1H), 3.67 (m, 1H), 3.47–3.24 (m, 2H), 3.28 (m, 1H), 2.14 (m, 1H), 1.88–1.41 (m, 7H), 1.25 (brs, 39H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4. General procedure for the synthesis of **13a–e** and **16a–e***

To a stirred solution of compound **12a–e** and **15a–e** (1 eq.) in dry methanol or THF (10 mL) was added catalytic amount of 10% Pd/C and stirred under hydrogen atmosphere at rt overnight. The catalyst was filtered off and the filtrate was concentrated to afford **13a–e** and **15a–e**.

*5.2.4.1. ((±)-trans-2-(Dodecyloxy)cyclopentyl)methanol (**13a**).* The compound **13a** (70 mg) was obtained according to the general procedure for 5.2.4 from compound **12a** (130 mg, 0.34 mmol). Yield 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.69–3.27 (m, 4H), 2.25–1.53 (m, 7H), 1.25 (brs, 20H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.2. ((±)-trans-2-(Tridecyloxy)cyclopentyl)methanol (**13b**).* The compound **13b** (58 mg) was obtained according to the general procedure 5.2.4 from compound **12b** (100 mg, 0.26 mmol). Yield 73%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.68–3.27 (m, 4H), 2.13–1.51 (m, 7H), 1.25 (brs, 22H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.3. ((±)-trans-2-(Octadecyloxy)cyclopentyl)methanol (**13c**).* The compound **13c** (150 mg) was obtained according to the

general procedure 5.2.4 from compound **12c** (250 mg, 0.54 mmol). Yield 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (m, 1H), 3.64–3.30 (m, 4H), 2.11–1.52 (m, 7H), 1.25 (brs, 32H), 0.88 (t, J = 6.4 Hz, 3H)

*5.2.4.4. ((±)-trans-2-(Icosyloxy)cyclopentyl)methanol (**13d**).* The compound **13d** (182 mg) was obtained according to the above general procedure 5.2.4 from compound **12d** (300 mg, 0.67 mmol). Yield 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.68–3.27 (m, 4H), 2.12–1.51 (m, 7H), 1.25 (brs, 36H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.5. ((±)-trans-2-(Docosyloxy)cyclopentyl)methanol (**13e**).* The compound **13e** (182 mg) was obtained according to the above general procedure 5.2.4 from compound **12e** (160 mg, 0.31 mmol). Yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.69–3.27 (m, 4H), 2.10–1.53 (m, 8H), 1.25 (brs, 40H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.6. ((±)-cis-2-(Dodecyloxy)cyclopentyl)methanol (**16a**).* The compound **16a** (70 mg) was obtained according to the general procedure 5.2.4 from compound **15a** (130 mg, 0.34 mmol). Yield 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (m, 1H), 3.78–3.26 (m, 4H), 3.01 (t, J = 6.0 Hz, 1H), 2.10 (m, 1H), 1.84–1.48 (m, 8H), 1.25 (brs, 18H), 0.88 (t, J = 6.7 Hz, 3H).

*5.2.4.7. ((±)-cis-2-(Tridecyloxy)cyclopentyl)methanol (**16b**).* The compound **16b** (130 mg) was obtained according to the general procedure 5.2.4 from compound **15b** (200 mg, 0.52 mmol). Yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (m, 1H), 3.74–3.24 (m, 4H), 3.01 (t, J = 5.4 Hz, 1H), 2.09 (m, 1H), 1.84–1.47 (m, 8H), 1.25 (brs, 20H), 0.88 (t, J = 6.7 Hz, 3H).

*5.2.4.8. ((±)-cis-2-(Octadecyloxy)cyclopentyl)methanol (**16c**).* The compound **16c** (80 mg) was obtained according to the general procedure 5.2.4 from compound **15c** (200 mg, 0.44 mmol). Yield 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (m, 1H), 3.75–3.28 (m, 4H), 3.04 (t, J = 5.4 Hz, 1H), 2.12 (m, 1H), 1.81–1.54 (m, 7H), 1.25 (brs, 31H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.9. ((±)-cis-2-(Icosyloxy)cyclopentyl)methanol (**16d**).* The compound **16d** (140 mg) was obtained according to the general procedure 5.2.4 from compound **15d** (250 mg, 0.51 mmol). Yield 69%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (m, 1H), 3.79–3.26 (m, 4H), 3.04 (t, J = 5.4 Hz, 1H), 2.09 (m, 1H), 1.86–1.48 (m, 8H), 1.25 (brs, 34H), 0.88 (t, J = 6.8 Hz, 3H).

*5.2.4.10. ((±)-cis-2-(Docosyloxy)cyclopentyl)methanol (**16e**).* The compound **16e** (142 mg) was obtained according to the general procedure 5.2.4 from compound **15e** (280 mg, 0.54 mmol). Yield 63%; ^{1}H NMR data (400 MHz, $CDCl_3$) δ 3.97 (m, 1H), 3.77–3.26 (m, 4H), 3.04 (t, J = 5.8 Hz, 1H), 2.12 (m, 1H), 1.85–1.48 (m, 7H), 1.25 (brs, 39H), 0.88 (t, J = 6.7 Hz, 3H).

*5.2.5. General procedure for the synthesis of **6a–e**, **7a–e** and **8a–e***

To a stirred solution of compound **10a–e**, **13a–e** and **16a–e** (1eq.) in benzene (5 mL) containing triethylamine (2 eq.) was added 2-chloro-1,3,2-dioxaphospholane-2-oxide (2 eq.) at 0 °C and warmed up slowly to rt, and further stirred overnight. The precipitated triethylamine hydrochloride was filtered and washed with benzene. The combined filtrate was concentrated, diluted with CH_3CN (10 mL) and transferred into pressure bottle. The mixture was cooled to −78 °C and treated with TMA (1.5 mL) at the same temperature under nitrogen. The reactor was closed and heated at 65 °C for 18 h. After cooling back to rt, the reactor was opened. The solvent and excess TMA were evaporated. The residual solid was purified by column chromatography on silica gel, first eluting with $CHCl_3$-MeOH (9:1) for separation of nonpolar compounds, and then with $CHCl_3$-MeOH-H_2O (65:20:4). The isolated semisolid was finally precipitated from chloroform and *n*-pentane to afford **6a–e**, **7a–e** and **8a–e**.

*5.2.5.1. (±)-trans-2-(Dodecyloxymethyl)cyclopentyl 2-(trimethylammonio)ethyl phosphate (**6a**).* The compound **6a** (60 mg) was obtained according to the general procedure 5.2.5 from compound **10a** (77 mg, 0.27 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (55 μL, 0.54 mmol) and TMA (ca. 1.5 mL). Yield 49%; TLC R_f = 0.22 ($MeOH/H_2O$ = 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ 4.42 (m, 1H), 4.25 (brs, 2H), 3.88–3.70 (m, 2H), 3.67–3.65 (m, 2H), 3.48–3.38 (m, 2H), 3.22 (s, 9H), 2.19 (m, 1H), 1.94–1.51 (m, 6H), 1.41–1.29 (m, 19H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 82.9, 79.8, 72.9, 71.3, 66.6, 58.9, 54.4 (3C), 47.1, 33.5, 31.9, 29.6, 29.5, 29.3, 27.5, 27.4, 26.2, 22.9, 22.5, 14.1; FABMS *m/z* 450.5 $(M + H)^+$.

*5.2.5.2. (±)-trans-2-(Tridecyloxymethyl)cyclopentyl 2-(trimethylammonio)ethyl phosphate (**6b**).* The compound **6b** (230 mg) was obtained according to the general procedure 5.2.5 from compound **10b** (210 mg, 0.70 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (120 μL, 1.40 mmol) and TMA (1.5 mL). Yield 71%; TLC R_f = 0.22 ($MeOH/H_2O$ = 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ 4.45 (m, 1H), 4.27 (brs, 2H), 3.84–3.62 (m, 3H), 3.50–3.38 (m, 3H), 3.22 (s, 9H), 2.22 (m, 1H), 1.95–1.51 (m, 6H), 1.41–1.22 (m, 21H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 82.8, 79.2, 73.0, 71.24, 66.3, 59.0, 54.3 (3C), 47.1, 33.6, 31.8, 31.6, 30.1, 29.6, 29.5, 29.3, 27.6, 26.2, 22.8, 22.5, 14.07; FABMS *m/z* 464.5 $(M + H)^+$.

*5.2.5.3. (±)-trans-2-(Octadecyloxymethyl)cyclopentyl 2-(trimethylammonio)ethyl phosphate (**6c**).* The compound **6c** (150 mg) was obtained according to the general procedure 5.2.5 from compound **10c** (200 mg, 0.54 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (110 μL, 1.08 mmol) and TMA (1.5 mL). Yield 52%; TLC R_f = 0.22 ($MeOH/H_2O$ = 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ 4.43 (m, 1H), 4.27 (brs, 2H), 3.84–3.62 (m, 3H), 3.50–3.42 (m, 3H), 3.24 (s, 9H), 2.25 (m, 1H), 1.97–1.51 (m, 7H), 1.44–1.21 (m, 31H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 84.2, 81.1, 72.9, 71.3, 68.9, 67.5, 60.8, 54.7 (3C), 47.1 34.5, 33.2, 32.7, 31.8, 29.5 (5C), 29.38, 27.57, 27.4, 27.2, 26.1, 22.9, 22.5, 14.1; FABMS *m/z* 534.4 $(M + H)^+$.

*5.2.5.4. (±)-trans-2-(Icosyloxymethyl)cyclopentyl 2-(trimethylammonio)ethyl phosphate (**6d**).* The compound **6d** (430 mg) was obtained according to the general procedure 5.2.5 from compound **10d** (360 mg, 0.91 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (185 μL, 1.82 mmol) and TMA (1.5 mL). Yield 85%; TLC R_f = 0.22 ($MeOH/H_2O$ = 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ 4.43 (m, 1H), 4.26 (brs, 2H), 3.89–3.62 (m, 3H), 3.50–3.42 (m, 3H), 3.24 (s, 9H), 2.24 (m, 1H), 1.97–1.53 (m, 6H), 1.44–1.21 (m, 36H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 82.2, 79.3, 72.3, 70.2, 68.1, 66.0, 53.1 (3C), 45.7, 33.1, 32.5, 31.5, 31.3, 30.7, 29.5, 29.2, 28.9, 28.6, 28.4, 26.9, 26.3, 25.9, 25.3, 22.7, 22.2, 22.1, 21.8, 12.9; FABMS *m/z* 562.4 $(M + H)^+$.

*5.2.5.5. (±)-trans-2-(Docosyloxymethyl)cyclopentyl 2-(trimethylammonio)ethyl phosphate (**6e**).* The compound **6e** (180 mg) was obtained according to the general procedure 5.2.5 from compound **10e** (305 mg, 0.72 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (146 μL, 1.44 mmol) and TMA (1.5 mL). Yield (180 mg, 43%); TLC R_f = 0.22 ($MeOH/H_2O$ = 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ 4.42 (m, 1H), 4.25 (brs, 2H), 3.89–3.62 (m, 3H), 3.48–3.40 (m, 3H), 3.22 (s, 9H), 2.25 (m, 1H), 1.95–1.51 (m, 6H), 1.42–1.23 (m, 31H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 84.2, 81.4, 73.8, 70.2, 67.5, 60.2, 54.7 (3C), 47.8, 34.4, 33.0, 32.8, 31.8, 31.1, 30.7, 30.6 (6C), 30.4, 28.4, 28.3, 27.4, 27.3, 26.9, 23.9, 23.6, 14.4; FABMS *m/z* 590.4 $(M + H)^+$.

*5.2.5.6. ((±)-trans-2-(Dodecyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**7a**).* The compound **7a** (200 mg) was obtained according to the general procedure 5.2.5 from compound **13a** (150 mg, 0.52 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (105.4 μL, 1.04 mmol) and TMA (1.5 mL). Yield 86%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.44 (m, 1H), 4.26 (brs, 2H), 3.87–3.62 (m, 4H), 3.46–3.37 (m, 2H), 3.22 (s, 9H), 2.16 (m, 1H), 1.94–1.49 (m, 7H), 1.41–1.29 (m, 19H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 82.2, 73.0, 71.2, 69.2, 66.2, 59.1, 54.3 (3C), 46.5, 33.5, 31.9, 31.6, 30.1, 29.6, 29.3, 27.6, 26.2, 22.8, 22.5, 14.15; FABMS *m/z* 450.5 $(M + H)^+$.

*5.2.5.7. ((±)-trans-2-(Tridecyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**7b**).* The compound **7b** (90 mg) was obtained according to the general procedure 5.2.5 from compound **13b** (100 mg, 0.33 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (67 μL, 0.67 mmol) and TMA (1.5 mL). Yield 59%; TLC $R_f = 0.21$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.42 (m, 1H), 4.24 (brs, 2H), 3.84–3.56 (m, 4H), 3.48–3.41 (m, 2H), 3.22 (s, 9H), 2.23 (m, 1H), 1.98–1.53 (m, 7H), 1.29 (brs, 21H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 82.8, 79.2, 73.0, 71.2, 66.3, 59.0, 54.3 (3C), 46.5, 33.6, 31.8, 31.6, 30.1, 29.8, 29.7, 29.5, 27.6, 26.2, 22.8, 22.5, 14.0; FABMS *m/z* 464.5 $(M + H)^+$.

*5.2.5.8. ((±)-trans-2-(Octadecyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**7c**).* The compound **7c** (150 mg) was obtained according to the general procedure 5.2.5 from compound **13c** (130 mg, 0.35 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (71 μL, 0.70 mmol) and TMA (1.5 mL). Yield 80%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.42 (m, 1H), 4.25 (brs, 2H), 3.89–3.65 (m, 4H), 3.49–3.38 (m, 2H), 3.22 (s, 9H), 2.21 (m, 1H), 1.97–1.51 (m, 7H), 1.29 (brs, 31H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 84.2, 81.4, 73.8, 70.2, 67.5, 60.2, 54.7 (3C), 47.7, 34.6, 33.0, 32.8, 31.1, 30.7, 30.6 (3C), 30.4, 28.4, 28.3, 27.4, 27.3, 24.2, 23.9, 23.9, 23.7, 23.6, 14.4; FABMS *m/z* 534.5 $(M + H)^+$.

*5.2.5.9. ((±)-trans-2-(Icosyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**7d**).* The compound **7d** (115 mg) was obtained according to the general procedure 5.2.5 from compound **13d** (165 mg, 0.42 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (85 μL, 0.84 mmol) and TMA (1.5 mL). Yield 48%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.42 (m, 1H), 4.25 (brs, 2H), 3.89–3.61 (m, 4H), 3.49–3.38 (m, 2H), 3.22 (s, 9H), 2.18 (m, 1H), 1.97–1.51 (m, 7H), 1.29 (brs, 31H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 84.2, 81.4, 73.8, 70.2, 67.5, 60.2, 54.7 (3C), 47.7, 34.6, 33.0, 32.8, 31.1, 30.7, 30.6 (3C), 30.4, 28.4, 28.3, 27.4, 27.3, 24.2, 23.9, 23.9, 23.7, 23.6, 14.4; FABMS *m/z* 562.4 $(M + H)^+$.

*5.2.5.10. ((±)-trans-2-(Docosyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**7e**).* The compound **7e** (90 mg) was obtained according to the general procedure 5.2.5 from compound **13e** (130 mg, 0.31 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (63 μL, 0.62 mmol) and TMA (1.5 mL). Yield 42%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.41 (m, 1H), 4.24 (brs, 2H), 3.87–3.62 (m, 4H), 3.48–3.40 (m, 2H), 3.22 (s, 9H), 2.18 (m, 1H), 1.98–1.51 (m, 6H), 1.28 (brs, 40H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 84.3, 81.3, 73.8, 70.2, 67.5, 60.2, 54.7 (3C), 47.8, 34.6, 34.5, 33.1, 32.8, 31.1, 30.8, 30.7, 30.6 (5C), 30.5, 28.5, 28.3, 27.4, 27.3, 26.9, 23.7, 23.6, 14.5; FABMS *m/z* 590.4 $(M + H)^+$.

*5.2.5.11. ((±)-cis-2-(Dodecyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**8a**).* The compound **8a** (36 mg) was obtained according to the general procedure 5.2.5 from compound **16a** (35 mg, 0.12 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (24 μL, 0.24 mmol) and TMA (1.5 mL). Yield 66%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.25 (brs, 2H), 4.08 (m, 1H), 3.89–3.83 (m, 2H), 3.64–3.61 (m, 2H), 3.50–3.38 (m, 2H), 3.22 (s, 9H), 2.19 (m, 1H), 1.81–1.48 (m, 8H), 1.29 (brs, 18H), 0.89 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.9, 72.2, 69.1, 66.2, 59.5, 54.3, 45.3 (3C), 45.3, 31.9, 30.1, 29.9, 29.7, 29.6, 29.3, 27.0, 26.2, 22.7, 21.7, 14.1; FABMS *m/z* 450.5 $(M + H)^+$.

*5.2.5.12. ((±)-cis-2-(Tridecycloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**8b**).* The compound **8b** (132 mg) was obtained according to the general procedure 5.2.5 from compound **16b** (110 mg, 0.37 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (75 μL, 0.74 mmol) and TMA (1.5 mL). Yield 76%; TLC $R_f = 0.23$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.25 (brs, 2H), 4.07 (m, 1H), 3.89–3.63 (m, 4H), 3.49–3.37 (m, 2H), 3.22 (s, 9H), 2.09 (m, 1H), 1.81–1.44 (m, 8H), 1.28 (brs, 20H), 0.89 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 81.0, 71.1, 69.1, 65.8, 59.2, 54.3 (3C), 45.3, 31.9, 30.9, 30.1, 29.9, 29.7, 29.6, 29.3, 27.0, 26.2, 22.7, 21.7, 14.1; FABMS *m/z* 464.5 $(M + H)^+$.

*5.2.5.13. ((±)-cis-2-(Octadecyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**8c**).* The compound **8c** (51 mg) was obtained according to the general procedure 5.2.5 from compound **16c** (100 mg, 0.27 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (55 μL, 0.54 mmol) and TMA (1.5 mL). Yield 37%; TLC $R_f = 0.22$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.25 (brs, 2H), 4.06 (m, 1H), 3.90–3.85 (m, 2H), 3.75–3.62 (m, 2H), 3.48–3.38 (m, 2H), 3.22 (s, 9H), 2.14 (m, 1H), 1.87–1.49 (m, 8H), 1.28 (brs, 30H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 82.2, 80.1, 72.2, 70.1, 67.5, 60.3, 54.7 (3C), 46.7, 34.5, 33.0, 31.9, 31.1, 30.8, 30.7, 30.6 (5C), 30.4, 27.9, 27.7, 27.4, 27.3, 14.4; FABMS *m/z* 534.5 $(M + H)^+$.

*5.2.5.14. ((±)-cis-2-(Icosyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**8d**).* The compound **8d** (74 mg) was obtained according to the general procedure 5.2.5 from compound **16d** (120 mg, 0.30 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (61 μL, 0.60 mmol) and TMA (1.5 mL). Yield 43%; TLC $R_f = 0.23$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.28 (brs, 2H), 4.10 (m, 1H), 3.92–3.86 (m, 2H), 3.73–3.63 (m, 2H), 3.48–3.38 (m, 2H), 3.22 (s, 9H), 2.19 (m, 1H), 1.84–1.49 (m, 7H), 1.28 (brs, 35H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 82.2, 80.5, 72.2, 70.1, 67.2, 60.4, 54.7 (3C), 46.7, 34.5, 33.0, 31.9, 31.1, 30.8, 30.7 (3C), 30.6, 30.4, 27.8, 27.7, 27.4, 27.3, 24.2, 23.7, 22.6, 22.4, 14.1; FABMS *m/z* 562.4 $(M + H)^+$.

*5.2.5.15. ((±)-cis-2-(Docosyloxy)cyclopentyl)methyl 2-(trimethylammonio)ethyl phosphate (**8e**).* The compound **8e** (40 mg) was obtained according to the general procedure 5.2.5 from compound **16e** (100 mg, 0.24 mmol), 2-chloro-1,3,2-dioxaphospholane-2-oxide (48 μL, 0.48 mmol) and TMA (1.5 mL). Yield 29%; TLC $R_f = 0.23$ ($MeOH/H_2O = 9:1$); ^1H NMR (400 MHz, CD_3OD) δ 4.27 (brs, 2H), 4.10 (m, 1H), 3.91–3.63 (m, 4H), 3.48–3.38 (m, 2H), 3.22 (s, 9H), 2.19 (m, 1H), 1.79–1.51 (m, 7H), 1.28 (brs, 39H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 82.2, 80.1, 73.3, 70.1, 67.1, 60.3, 54.7 (3C), 46.7, 33.0, 31.9, 31.1, 30.8, 30.7, 30.6 (8C), 30.4, 27.8, 27.7, 27.4, 23.7, 22.6, 22.4, 14.1; FABMS *m/z* 590.4 $(M + H)^+$.

5.3. Biological activity testing

5.3.1. Akt inhibitory activity assay

The A549 human lung cancer cell line was grown to 70% confluency and serum starved for 18 h. Compounds were added at the

indicated concentrations (2, 5, 10, and 20 μM) for 2 h, and then Akt phosphorylation was stimulated by adding insulin at 10 μg/mL for 30 min. As negative control, insulin was not added and positive control insulin was added for 30 min. After 30 min of stimulation with insulin, cells were washed in ice-cold PBS and lysed using RIPA lysis buffer (Sigma–Aldrich, MO, USA). Quantification of phosphorylated Akt was performed using ELISA-based phosphor-Akt assay kits (R & D systems, MN, USA). Data are presented as the means ±SDs of three independent experiments.

5.3.2. Cell culture and measurements of cytotoxicity

The A549 human lung cancer cell line, the MCF-7 breast carcinoma cell line and the KATO-III human gastric carcinoma cell line were purchased from the Korea cell line bank (Seoul, Korea). All cell lines were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 1 mM sodium pyruvate in a humidified 5% CO_2 atmosphere at 37 °C. Cells were plated in 96-well plates at a density of 1×10^5 cells per well, 24 h prior to addition of APCs compounds. These compounds solubilized in 100% ethanol were added at the indicated concentrations (final ethanol concentration, 0.5%). After 24 h of incubation, cell death was assessed by FACS cytometry (Accuri C6 flow cytometer, Accuri, MI, U.S.A.). All studies were performed in triplicate. Data are presented as the means ±SDs of three independent experiments.

Acknowledgments

This work was supported from the World Class University Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (#R33-2008-000-10018-0). BK is a grant awardees of US NIH, NIAID (#AI107701). We also appreciate Dr. Joseph Hollenbaugh for reading the manuscript.

References

[1] A.A. Adjei, E.K. Rowinsky, Cancer Biol. Ther. 2 (2003) S5–15.
[2] J.D. Bagnato, A.L. Eilers, R.A. Horton, C.B. Grissiom, J. Org. Chem. 69 (2004) 8987–8996.
[3] A. Kamb, S. Wee, C. Lengaur, Nat. Rev. Drug Discov. 6 (2007) 115–120.
[4] S.R. Vink, W.J. van Blitterswijk, Jan H.M. Schellns, M. Verheij, Cancer Treat. Rev. 33 (2007) 191–202.
[5] G.A. Ruiter, S.F. Zerp, H. Bartelink, W.J. van Blitterswijk, M. Verheij, Anticancer Drugs 14 (2003) 167–173.
[6] H.H. Grunicke, K. Maly, F. Uberall, C. Schubert, E. Kindler, J. Stekar, H. Brachwitz, Adv. Enzym. Regul. 36 (1996) 385–407.
[7] F. Mollinedo, C. Gajate, S. Martin–Santamaria, F. Gago, Curr. Med. Chem. 11 (2004) 3163–3184.
[8] B. Ogretmen, Y.A. Hannun, Nat. Rev. Cancer 4 (2004) 604–616.
[9] M. Rahmani, E. Reese, Y. Dai, C. Bauer, S.G. Payne, P. Dent, S. Spiegel, S. Grant, Cancer Res. 65 (2005) 2422–2431.
[10] W.E. Berdel, Br. J. Cancer 64 (1991) 208–211.
[11] P. Samadder, G. Arthur, Cancer Res. 59 (1999) 4808–4815.
[12] W.J. van Blitterwijk, M. Verheij, Curr. Pharm. Des 14 (2008) 2061–2074.
[13] C. Unger, M. Peukert, H. Sindermann, P. Hilgard, G. Nagel, H. Eibl, Cancer Treat. Rev. 17 (1990) 243–246.
[14] R. Leonard, J. Hardy, G. van Tienhoven, S. Houston, P. Simmonds, M. David, J. Mansi, J. Clin. Oncol. 19 (2001) 4150–4159.
[15] A. Kuhlencord, T. Maniera, H. Eibl, C. Unger, Antimicrob. Agents Chemother. 36 (1992) 1630–1634.
[16] S. Sunder, T.K. Jha, C.P. Thakur, J. Engel, H. Sindermann, C. Fischer, K. Junge, A. Bryceson, J. Berman, New Engl. J. Med. 347 (2002) 1739–1746.
[17] S.B. Kondapaka, S.S. Singh, G.P. Dasmahapatra, E.A. Sausville, K.K. Roy, Mol. Cancer Ther. 2 (2003) 1093–1103.
[18] A.M. Senderowicz, Oncogene 22 (2003) 6609–6620.
[19] D.S. Ernst, E. Eisenhauer, N. Wainman, M. Davis, R. Lohmann, T. Baetz, K. Belanger, M. Smyle, Invest. New Drugs 23 (2005) 569–575.
[20] B. Erdlenbruch, V. Jendrossek, M. Marks, A. Hunold, H. Eibl, M. Lakomek, Anticancer Res. 18 (1998) 2551–2557.
[21] V. Jendrossek, B. Erdlenbruch, A. Hunold, W. Kugler, H. Eibl, M. Lakmek, Int. J. Oncol. 14 (1999) 15–22.
[22] L.H. Lindner, H. Eibl, M. Hossann, M. Vogeser, J. Chromatogr. B. 869 (2008) 16–19.
[23] D. Berkovic, Gen. Pharmacol. 31 (1998) 511–517.
[24] W.R. Vogler, W.E. Berdel, R.B. Geller, J.A. Brochstein, R.A. Beveridge, W.S. Dalton, K.B. Miller, H.M. Lazarus, Adv. Exp. Med. Biol. 416 (1996) 389–396.
[25] J.Q. Cheng, C.W. Lindsley, G.Z. Cheng, H. Yang, S.V. Nicosia, Oncogene 24 (2005) 7482–7492.
[26] I. Vivanco, C.L. Sawyers, Nat. Rev. Cancer 2 (2002) 489–501.
[27] W.J. Houlihan, M. Lohmeyer, P. Workman, S.H. Cheon, Med. Res. Rev. 15 (1995) 157–223.
[28] T.L. Andresen, S.S. Jensen, R. Madsen, K. Jorgensen, J. Med. Chem. 48 (2005) 7305–7314.
[29] J. Maravljak, R. Zeisig, S. Pecar, J. Med. Chem. 48 (2005) 6393–6399.
[30] I. Ahmed, J.J. Filep, J.C. Franklin, A.S. Janoff, G.R. Masters, J. Pattassery, A. Peters, J.J. Schupsky, Y. Zha, E. Mayhew, Cancer Res. 57 (1997) 1915–1921.
[31] C.M. Lazenby, M.G. Thomopson, J.A. Hickman, Cancer Res. 50 (1990) 3327–3330.
[32] W.J. Houlihan, K. Parsad, R. Underwood, O. Repic, J. Med. Chem. 39 (1996) 605–608.
[33] F. Theil, S. Ballschuh, Tetrahedron-Asymmetry 7 (1996) 3565–3572.
[34] A.N. Pinchuk, M.A. Rampy, M.C. Longino, R.W. Scott Skinner, M.D. Gross, J.P. Weichert, R.E. Counsell, J. Med. Chem. 49 (2006) 2155–2165.
[35] A. Fukuzawa, H. Sato, T. Masamune, Tetrahedron Lett. 28 (1987) 4303–4306.
[36] E. Roudier, O. Mistafa, U. Stenius, Mol. Cancer Ther. 5 (2006) 2706–2715.