Synthesis and biological evaluation of substituted phosphate triester alkyl lyso phospholipids (ALPs) as novel potential anti-neoplastic agents

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Abstract Phosphate triester derivatives of the anti-neoplastic alkyl lyso phospholipid (ALP) have been prepared as novel potential therapeutic agents. In particular, symmetrical phosphate triesters have been prepared, using phosphorochloridate chemistry. The compounds have been fully characterised by a range of techniques, and assayed for their inhibition of DNA synthesis by mammalian cells in culture. The compounds are generally inhibitory towards DNA synthesis in the μM range. However, the magnitude of the effect varies greatly with the phosphate structure; alkynyl and glycol substituted phosphates being especially potent.

Key words: Lysophospholipid; ALP; DNA synthesis

1. Introduction

It is known that certain phospholipid analogues may have an anti-cancer action; the alkyl lysophospholipids (ALPs; **1** in Fig. 1) are synthetic molecules with biological activity [1,2,3]. Whilst the details of the mechanisms of action of these compounds remain to be elucidated, the cytotoxic effects of lysophospholipids are thought to involve several processes [4]. In high concentrations lysophospholipids have a cytolytic detergent action, and may form extracellular micelles which act as sinks for cholesterol and other essential lipids. Below the critical micelle concentration (CMC), lysophospholipids may be incorporated into cell membranes and modify their structure and function [5]. Some of these compounds may reach the cell interior and interfere with some of the processes involved in membrane signal transduction [6] and this last process may be of particular importance with regard to anti-proliferative effects. Vogler and co-workers [7] have examined the effect on proliferation inhibition, estimated by thymidine incorporation and clonogenic assays, of a series of glycerol phosphocholine analogues with varying substitutions in the sn-1 and sn-2 positions.

In the work reported in this paper, we have investigated the effect of a series of phosphate substituted ALPs on cell proliferation, using a radio-thymidine incorporation assay to measure DNA synthesis. Cells were exposed to ALP analogues in concentrations below the CMC and DNA synthesis monitored after a 24-h exposure.

Given our experience in the improvement of the therapeutic performance of bio-active nucleosides by suitable phosphate modification [8,9,10,11,12] we wondered if a similar strategy might lead to enhancement of the properties of the parent ALP. In a recent paper [13] we described our initial results regarding the synthesis of some novel ALPs, modified in the phosphate region. It was a notable and surprising observation that simple dialkyl blocked phosphate derivatives are active in vitro and that simple structure activity trends appear to operate. In this paper we describe the synthesis of some novel ALPs, further modified in the phosphate region in the hope of improving activity at lower concentration, and we discuss their evaluation in vitro. In particular we study the effect on anti-cancer activity of substitution within the alkyl-phosphate region.

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2. Materials and methods

All experiments involving water-sensitive reagents were carried out under scrupulously dry conditions. Where needed, anhydrous solvents and reagents were obtained in the following ways: triethylamine and pyridine were refluxed over CaH_2 for several hours and distilled. Pyridine was further stored over KOH pellets. Diethyl ether was refluxed over calcium hydride for several hours and further dried over activated 4A molecular sieves. Phosphoryl chloride was distilled at atmospheric pressure. Commercially available Merck kieselgel was used for the preparative TLC and the components were visualised by iodine. Column chromatography was carried out using Woelm silica (32–63 mesh) as the stationary phase. The ratio of silica-compound varied between 50:1 and 100:1 (w/w). Phosphorus-31 NMR spectra were recorded on a Jeol FX90Q instrument operating at 36.2 MHz and are reported in units of δ relative to 85% phosphoric acid as external standard, positive shifts are downfield. Carbon-13 NMR spectra were recorded on a JEOL FX270 spectrometer operating at 67.9 MHz, or a Bruker AM360 instrument operating at 90.6 MHz. Shifts are expressed in units of δ relative to $CDCl_3$ at 77.20 ppm. Both phosphorus-31 and carbon-13 NMR spectra were proton noise decoupled and all signals were singlets unless otherwise stated. H-1 NMR spectra were recorded on a JEOL FX270 spectrometer operating at 270 MHz, or a Bruker AM360 machine operating at 360 MHz and are reported in units of δ relative to internal $CHCl_3$ at 7270 ppm unless otherwise stated. All NMR spectra were recorded in $CDCl_3$. Mass spectra were recorded in Fast Atom Bombardment (FAB) mode on a VG 70–250 spectrometer.

2.1. 3-O-*hexadecyl*-2-O-*methyl*-*rac*-*glycerol* (**2**)

This was prepared entirely as recently described by us [13].

2.2. 3-O-*hexadecyl*-2-O-*methyl*-rac-*glycerol*-*bis* (*methyl glycolyl*)*phosphate* (**3a**)

1-*O*-Hexadecyl-2-*O*-methyl-*rac*-glycerol (**2**) (140 mg, 0.43 mmol) was dissolved in pyridine (20 ml), and di(methyl glycolyl)phosphorochloridate (352 mg, 1.35 mmol) was added dropwise with vigorous stirring, after stirring for 5 h at ambient temperature, the reaction was quenched with water (2 ml), and the solvent was removed under reduced pressure. The resulting oil was isolated by preparative thin layer chromatography using 5% methanol in chloroform as eluant to give the product as an oil (151 mg, 57.6%); δ_P-2 ; δ_H 0.89 (3H, t, ω-CH_3), 1.26 (26H, bs, $CH_2 \times 13$), 1.60 (2H, m, OCH_2CH_2R), 3.44–3.53 (8H, m, $CHCH_2OCH_2$, OCH_3), 3.80 (6H, s, CH_3OCO), 4.20–4.45 (2H, m, $CHCH_2OP$), 4.72 (4H, m, $COCH_2OP$); FAB MS 577 (MH^++Na, 34), 555.3289 (MH^+, $C_{26}H_{52}O_{10}P$ requires 555.3298, 54), 313 ($C_{20}H_{41}O_2^+$, 25).

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*2.3. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis{(s)-(−)-methyl lactyl}phosphate (**3b**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of di{(s)-(−)-methyl lactyl}phosphorochloridate (815 mg, 2.83 mmol) and (**2**) (128 mg, 0.39 mmol) the product was obtained as an oil (72 mg, 31.6%) ; δ_P −5.8; δ_H 0.85 (3H, t, ω-CH_3), 1.26 (26H, bs, $CH_2 \times 13$), 1.59–1.65 (8H, m, OCH_2CH_2R, $CH_3CH \times 2$), 3.47 (8H, m, $CHCH_2OCH_2$, OCH_3), 3.78 (6H, s, $CH_3OCO \times 2$), 4.21 (2H, m, $CHCH_2OP$), 4.93 (2H, m, COCHOP); FAB MS 605 (MH^++Na, 7), 583.3591 (MH^+, $C_{28}H_{56}O_{10}P$ requires 583.3611, 70), 497 ($C_{24}H_{50}O_8P^+$, 8), 313 ($C_{20}H_{41}O_2^+$, 10), 271 ($(CH_3OCOCHCH_3O)_2PO_2H_2^+$, 50), 185 ($CH_3OCOCHCH_3OPO_3H_3^+$, 20), 87 ($C_4H_7O_2^+$, 40).

*2.4. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (2-propyn-1-yl)phosphate (**3c**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(propargyl)phosphorochloridate (268 mg, 1.39 mmol) and (**2**) (122 mg, 0.37 mmol) the product was obtained as an oil (58 mg, 32%); δ_P −2; δ_H 0.86 (3H, t, ω-CH_3), 1.20–1.33 (26H, m, $CH_2 \times 13$), 1.51 (2H, m, OCH_2CH_2R), 2.60 (2H, m, C≡CH), 3.38–3.53 (8H, m, $CHCH_2OCH_2$, OCH_3), 4.09–4.28 (2H, m, $CHCH_2OP$), 4.65–4.78 (4H, m, $C{\equiv}CCH_2 \times 2$); FAB MS 487.3186 (MH^+, $C_{26}H_{48}O_6P$ requires 487.3189, 100), 449 ($C_{24}H_{46}O_6P^+$, 10), 313 ($C_{20}H_{41}O_2^+$, 15), 175 ($(HC{\equiv}CCH_2O)_2PO_2H^+$, 27), 137 ($HC{\equiv}CCH_2OPO_3H_3^+$, 70).

*2.5. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (3-butyn-1-yl)phosphate (**3d**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(3-butyn-1-yl)phosphorochloridate (165 mg, 0.75 mmol) and (**2**) (123 mg, 0.38 mmol) the product was obtained as an oil (85 mg, 44%) ; δ_P −3 ; δ_H 0.85 (3H, t, ω-CH_3), 1.22–1.35 (26H, m, $CH_2 \times 13$), 1.51 (2H, m, OCH_2CH_2R), 2.02 (2H, m, HC≡C , J = 2.6 Hz), 2.52 (4H, m, $C{\equiv}CCH_2$, J = 2.7 Hz), 3.38–3.56 (8H, m, $CHCH_2OCH_2$, OCH_3), 4.05–4.25 (6H, m, CH_2OP, $CHCH_2OP$) ; FAB MS 537 (MH^++Na, 4), 515.3549 (MH^+, $C_{28}H_{52}O_6P$ requires 515.3502, 100), 463 ($C_{24}H_{48}O_6P^+$, 3), 313 ($C_{20}H_{41}O_2^+$, 18), 203 ($(HC{\equiv}CCH_2CH_2O)_2PO_2^+$, 21), 151 ($HC{\equiv}CCH_2CH_2OPO_3H_3P^+$, 30).

*2.6. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (glycidol)phosphate (**3e**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(glycidol)phosphorochloridate (290 mg, 1.30 mmol) and (**2**) (136 mg, 0.42 mmol) the product was obtained as an oil (33 mg, 15.2%); δ_P 0; δ_H 0.83 (3H, t, ω-CH_3), 1.20–1.35 (26H, m, $CH_2 \times 13$), 1.50 (2H, m, OCH_2CH_2R), 2.63 (4H, bs, oxiryl-$CH_2OP \times 2$), 3.21–3.46 (8H, m, $CHCH_2OCH_2$, OCH_3), 4.17 (2H, m, CH_2OP); EI MS 545 (MH^++Na, 70), 523 (MH^+, 36), 467 ($C_{23}H_{48}O_7P^+$, 3), 313 ($C_{20}H_{41}O_2^+$, 7), 211 ($(OCH_2CHCH_2O)_2PO_2H_2^+$, 13), 155 ($OCH_2CHCH_2OPO_3H_3^+$, 15), 57 ($OCH_2CHCH_2^+$, 100).

*2.7. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis(1,2-O-isopropylidene-rac-glycerol-3-yl)phosphate (**3f**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(1,2-*O*-isopropylidene-*rac*-glycerol-3-yl)phosphorochloridate (175 mg, 0.75 mmol) and (**2**) (123 mg, 0.38 mmol) the product was obtained as an oil (79 mg, 28.9%); δ_P −1.8; δ_H 0.88 (3H, t, ω-CH_3), 1.19–1.35 (26H, m, $CH_2 \times 13$) 1.37 (6H, s, ipr-CH_3), 1.42 (6H, s, ipr-CH_3), 1.55 (2H, m, RCH_2CH_2O), 3.35–3.55 (8H, m, $CHCH_2OCH_2$, OCH_3), 3.82 (2H, m, $CHCH_2OP \times 2$), 4.00–4.35 (10H, m, $OCH_2CHO \times 2$, $CH_2OP \times 3$) ; FAB MS 661 (MH^++Na, 2), 639.4133 (MH^+, $C_{32}H_{64}O_{10}P$ requires 639.4237, 15), 525 ($C_{26}H_{54}O_8P^+$, 10), 328 ($(C_6H_{11}O_3)_2PO_2H_2^+$, 3), 313 ($C_{20}H_{41}O2^+$, 15), 211 ($C_6H_{12}O_6P^+$, 47), 115 ($C_6H_{11}O_2^+$, 100).

*2.8. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis [2-(hydroxymethyl)tetrahydropyranyl]phosphate (**3g**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis[2-(hydroxymethyl)tetrahydropyranyl]phosphorochloridate (337 mg, 1.08 mmol) and (**2**) (142 mg, 0.43 mmol) the product was obtained as an oil (137 mg, 52.1%); δ_P 0; δ_H 0.88 (3H, t, ω-CH_3), 1.21–1.36 (30H, m, $CH_2 \times 13$, pyran-$CH_2 \times 2$), 1.55 (8H, m, pyran-$CH_2 \times 2$), 1.82 (2H, m, RCH_2CH_2O), 3.37–3.58 (12H, m, $CHCH_2OCH_2$, OCH_3, pyran-$CH_2O \times 2$), 3.92–4.18 (8H, m, pyran-CH $\times 2$, $CH_2OP \times 3$) ; FAB MS 607.4287 (MH^+, $C_{32}H_{64}O_8P$ requires 607.4339, 40), 509 ($C_{26}H_{54}O_7P^+$, 6), 295 ($(C_6H_{11}O)_2PO_2H_2^+$, 11), 197 ($C_6H_{11}OPO_3H_3^+$, 14), 99 ($C_6H_{11}O_6^+$, 100).

*2.9. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (monomethyl ethylene glycolyl)phosphate (**3h**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(monomethyl ethylene glycolyl)phosphorochloridate (784 mg, 3.51 mmol) and ([**2**) (164 mg, 0.50 mmol) the product was obtained as an oil (43 mg, 22%); δ_P −2 ; δ_H 0.87 (3H, t, ω-CH_3), 1.18–1.34 (26H, m, $CH_2 \times 13$), 1.54 (2H, m, OCH_2CH_2R), 3.37–3.62 (18H, m, $OCH_2CH_2OP \times 2$, CH, RCH_2OCH_2, $OCH_3 \times 3$), 4.05–4.25 (6H, m, $OCH_2CH_2OP \times 2$, $CHCH_2OP$); FAB MS 527.3702 (MH^+, $C_{26}H_{56}O_8P$ requires 527.3713, 40), 469 ($C_{23}H_{50}O_7P^+$, 3), 313 ($C_{20}H_{41}O2^+$, 4), 215 ($(CH_3OCH_2CH_2O)_2PO_2H_2^+$, 26), 157 ($CH_3OCH_2CH_2OPO_3H_3^+$, 23), 59 ($CH_3OCH_2CH_2^+$, 100).

*2.10. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (monomethyl diethylene glycolyl)phosphate (**3i**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(monomethyl diethylene glycolyl)phosphorochloridate (784 mg, 3.51 mmol) and (**2**) (164 mg, 0.50 mmol) the product was obtained as an oil (107 mg, 34.7%); δ_P −4; δ_H 0.86 (3H, t, ω-CH_3), 1.18–1.35 (26H, m, CH_2 ×13), 1.54 (2H, m, OCH_2CH_2R), 3.36 (6H, s, glycol-$OCH_3 \times 2$), 3.38–3.73 (22H, m, $OCH_2CH_2OP \times 2$, $CH_3OCH_2CHO \times 2$, CH, RCH_2OCH_2, $OCH_3 \times 3$), 4.04–4.23 (6H, m, $OCH_2CH_2OP \times 2$, $CHCH_2OP$); FAB MS 637 (MH^++Na, 35), 615.4200 (MH^+, $C_{30}H_{64}O_{10}P$ requires 615.4237, 60), 583 (M^+-OCH_3, 2), 513 ($C_{25}H_{54}O_8P^+$, 3), 313 ($C_{20}H_{41}O_2^+$, 4), 303 ($(CH_3OCH_2CH_2OCH_2CH_2O)_2PO_2H_2^+$, 15), 201 ($CH_3OCH_2CH_2OCH_2CH_2OPO_3H_3^+$, 10), 103 ($CH_3OCH_2CH_2OCH_2CH_2^+$, 100), 59 ($CH_3OCH_2CH_2^+$, 88).

*2.11. 3-O-Hexadecyl-2-O-methyl-rac-glycerol-bis (monomethyl triethylene glycolyl)phosphate (**3j**)*

This was prepared by an analogous method to (**3a**). Thus, from the condensation of bis(monomethyl triethylene glycolyl)phosphorochloridate (650 mg, 1.59 mmol) and (**2**) (140 mg, 0.43 mmol) the product was obtained as an oil (134 mg, 47.5%); δ_P −2 ; δ_H 0.86 (3H, t, ω-CH_3), 1.21–1.35 (26H, m, $CH_2 \times 13$), 1.55 (2H, m, OCH_2CH_2R), 3.37 (6H, s, glycl-OCH_3), 3.38–3.73 (28H, m, $OCH_2CH_2OP \times 2$, $CH_2CH_2O \times 2$, $OCH_2CH_2O \times 2$, CH, RCH_2OCH_2, OCH_3), 4.08–4.22 (6H, m, $OCH_2CH_2OP \times 2$, $CHCH_2OP$); FAB MS 703.4662 (MH^+, $C_{34}H_{72}O_{12}P$ requires 703.4761,14), 671 (M^+-OCH_3, 1), 557 ($C_{27}H_{58}O_9P^+$, 2), 391 ($(CH_3OCH_2CH_2OCH_2CH_2OCH_2CH_2O)_2PO_2H_2^+$, 5), 313 ($C_{20}H_{41}O_2^+$, 3), 245 ($CH_3OCH_2CH_2OCH_2CH_2OCH_2CH_2OPO_3H_3^+$, 7), 147 ($CH_3OCH_2CH_2OCH_2CH_2OCH_2CH_2^+$, 32), 103 ($CH_3OCH_2CH_2OCH_2CH_2^+$, 30), 89 ($CH_3OCH_2CH_2OCH_2^+$, 17), 59 ($CH_3OCH_2CH_2^+$, 100).

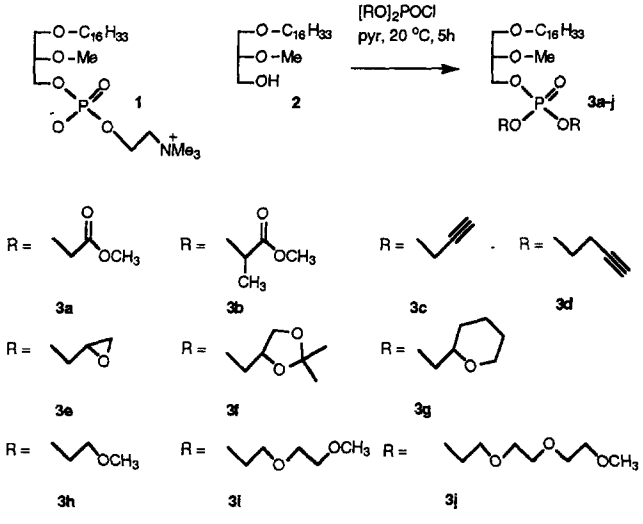


Fig. 1. Synthetic route to lipid analogues.

3.12. Tritiated thymidine incorporation assay

This assay was conducted entirely as we have recently described [13], using an exposure period of 24 h, and a labelling time of 30 min. Cells between passages 24 and 34 were used, and the reference compound hexadecyl phosphatidyl choline (Sigma) was included in the series of experiments as a source for comparison of the effectiveness of the agents tested.

3. Results

The synthetic route adopted to the lipid analogues was entirely as recently described by us for simple dialkyl phosphate triester ALPs [13]. Thus, 1-*O*-hexadecyl-2-*O*-methyl-*rac*-glycerol (**2**) was prepared from 2,3-*O*-isopropylidene-*rac*-glycerol in 4 steps in an overall yield of 31%. This material was used in a series of parallel reactions with a wide variety of dialkyl phosphorochloridates (Fig. 1), which were prepared in one step by the reaction of phosphoryl chloride with 2 equivalents of the appropriate alcohol, in the presence of triethylamine. The final products (**3a–j**) were isolated by preparative thin layer chromatography in yields ranging from 15% to 58%. All materials were pure by chromatographic and spectroscopic methods, the latter including P-31, H-1 and C-13 NMR and high resolution mass spectrometry. Carbon- and phosphorus-NMR data for all final products (**3a–j**) are given in Tables 1 and 2.

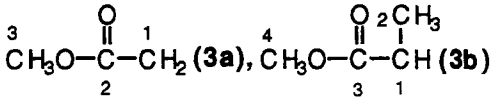
4. Discussion

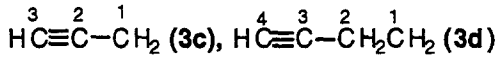
Each of the ALP derivatives (**3a–j**) and the reference compound hexadecyl phosphatidyl choline (C16PC) were tested for their ability to inhibit the incorporation of tritiated thymidine into the DNA of mammalian cells in tissue culture, using methods we have described [13]. The data are summarised in Table 3 and indicate a dose-dependent inhibition of thymidine incorporation for many of the ALP analogues studied. Especially potent compounds include those containing alkynyl (**3c** and **3d**) and glycol (**3h–3j**) structures. In these cases it is instructive to compare the data to that reported by us for simple alkyl phosphate analogues [13] where we found a sharp cut-off in activity

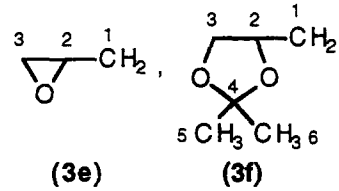
Table 1
Carbon-13 and phosphorus-31 NMR data for compounds (**3a–3f**)

	3a	3b	3c	3d	3e	3f
Alkyl						
C1	14.3	14.2	14.2	14.2	14.2	14.2
C2	22.9	23.8	22.8	22.8	22.8	22.8
C3–13	29.8–29.9	29.7–30.0	29.4–29.8	29.4–29.8	29.4–29.8	29.5–29.8
C14	26.5	26.4	26.1	26.2	26.1	26.2
C15	32.4	32.5	32.0	32.0	32.0	32.0
C16	71.1	67.8	69.2	69.3	68.4	69.3
Glycerol						
C1	72.4	72.7	72.0	72.0	71.9	72.01
C2	78.4[7]	79.4	78.97[7]	79.0[7]	78.8[7]	78.9[7]
C3	66.5[6]	67.0	67.3[6]	67.1[6]	67.2[6]	67.2[6]
OMe	58.2	58.0	58.2	58.1	58.1	58.2
P-OR						
C1″	66.3	72.9	55.4[4]	65.5[3]	50.1	67.8
C2″	174.2	19.6	79.02	20.7[7]	50.0	74.1[8]
C3″	60.9	174.2	76.5	79.4	44.6	66.3
C4″		53.4	–	70.5	–	110.0
C5″		–	–	–	–	25.4
C6″		–	–	–	–	26.8
δ_P	−2	−5.8	−2	−3	0	−1.8

All spectra are recorded in $CDCl_3$. Data are presented as δ in ppm, with 1-*O*-alkyl chains numbered from the terminus. All spectra were recorded using proton decoupling. In the case of carbon data, phosphorus coupling constants in Hz are superscripted.







(**3e**) (**3f**)

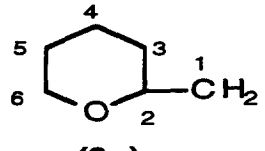
The side-chain numbers correspond to their assignments in the ^{13}C NMR Table 1.

for compounds with alkyl-phosphate chains longer than 2 carbon atoms. Thus, the dipropyl compound [13] displayed 17% (± 2) inhibition of thymidine incorporation at 100 μM, whilst we now note the somewhat similar propargyl analogue (**3c**) to show 90% (± 2%) inhibition. Similarly, the previous dibutyl compound displayed 0% (± 15) whilst the butynyl analogue (**3d**) shows 96% (± 1%) inhibition. It is very clear that, whilst a strict size limit operates for the simple dialkyl analogues, this appears not to be the case for the alkynyl analogues, at least within the limits herein described. Of course, the alkynyl systems are far more constrained in a conformational sense, being unable to adopt much of the vast conformational space available to the simple alkyl systems of analogous length — this may be one explanation for the apparent difference in steric constraints operational in the two series. Furthermore, the alkynyl terminus is inherently far more polar in nature than the corresponding methyl(ene) terminus of the simple alkyl systems, which may again lead to the apparently different steric constraints. To some extent this latter explanation is more consistent with the even more striking data on the glycol (**3h**), digol (**3i**) and trigol (**3j**) series herein described. Thus, the first of these may be rather similar in a steric sense to the simple butyl compound previously described [13]. However, as noted above, whilst the butyl compound is essentially inactive in this assay, the glycol analogue (**3h**) is potent, with >99% inhibition at 100 μM. Moreover, the trigol (**3j**), which might have been expected to be *inactive* from our previous (steric) structure–activity relationships is very potent, with >99% inhibition at 100 μM and 35% at 10μM. This material is thus perhaps 5–10-fold more potent than the reference compound C16PC.

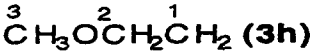
Table 2
Carbon-13 and phosphorus-31 NMR data for compounds (**3g–3j**)

	3g	**3h**	**3i**	**3j**
Alkyl				
C1	14.3	14.2	14.2	14.2
C2	22.9	22.8	22.8	22.8
C3–13	29.5–29.9	29.4–29.8	29.4–29.8	29.4–29.8
C14	26.2	26.2	26.2	26.1
C15	32.1	32.0	32.0	32.0
C16	69.7	69.5	69.6	69.5
Glycerol				
C1	72.0	72.0	72.0	72.0
C2	79.0[8]	78.9[8]	78.9[7]	78.9[7]
C3	66.7[6]	66.8	66.78	66.77
OMe	58.3	58.1	58.1	58.2
P-OR				
C1″	70.5[5]	71.4[7]	66.84	66.83
C2″	76.2[6]	66.8	70.2	71.9[5]
C3″	27.7	59.0	70.6	70.68
C4″	23.0	–	72.0	70.67
C5″	25.9	–	59.1	70.1
C6″	68.4	–	–	70.0
C7″	–	–	–	59.1
δ_P	0	−2	−4	−2

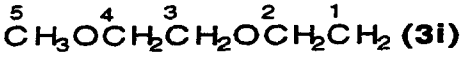
All spectra are recorded in $CDCl_3$. Data are presented as δ in ppm, with 1-*O*-alkyl chains numbered from the terminus. All spectra were recorded using proton decoupling. In the case of carbon data, phosphorus coupling constants in Hz are superscripted.



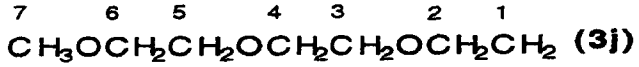
(3g)



(3h)



(3i)



(3j)

The side-chain numbers correspond to their assignments in the ^{13}C NMR Table 2.

Table 3
Inhibition by ALP derivatives of tritiated thymidine incorporation by cells in vitro

Compound	%1 @ 100 μM	%1 @ 10 μM
3a	26 (1.5)	11 (6.1)
3b	6 (2.2)	13 (2.3)
3c	90 (1.8)	3 (10.3)
3d	96 (0.8)	3 (8.5)
3e	25 (5.6)	10 (2.8)
3f	40 (2.9)	0 (1.8)
3g	48 (10.4)	8 (3.4)
3h	99.7 (0.03)	12.1 (4.8)
3i	79 (7.6)	0 (5.4)
3j	99.6 (0.04)	22 (2.9)
C16PC*	60 (6)	5.4 (5.5)

The data shown are calculated from the means of quadruplicate measurements of tritiated thymidine incorporation into trichloroacetic acid-insoluble material by sub-confluent CNCM-I222 cells, compared to appropriate solvent controls, with standard deviations in brackets. *indicates hexadecyl phosphatidyl choline was included as internal control.

The great steric demands, and most likely high flexibility of these glycol systems indicates that the presence of heteroatoms within such alkyl chains is able to entirely overcome the severe steric constrains operational for the simple alkyl phosphate analogues. As noted above for the alkynyl analogues, the polarity conferred by the heteroatoms may be instrumental in this phenomena, although the precise mechanism in operation remains unclear.

In conclusion, we note that substituted alkyl phosphate triester ALP derivatives inhibit thymidine incorporation by mammalian cells in tissue culture. The potency of inhibition varies greatly with the nature of the phosphate substituents, with alkynyl and glycol groups being especially efficacious. In some cases, particularly for a trigol derivative, the in vitro activity appears to exceed that of the reference compound by as much as an order of magnitude. Whilst the precise mechanism of action of these materials remains unclear, it is apparent that activity is tunable by changing the phosphate structure, and that the apparent severe steric constraints on the phosphate region for simple alkyl phosphates do not apply for substituted analogues. This suggests good possibilities for further optimisation of the leads we describe, and the prospect of agents emerging with improved therapeutic efficacy over existing lipid drugs.

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