Supporting Information

"Design and synthesis of potent antileishmanial cycloalkylidene-substituted ether phospholipid derivatives"

Theodora Calogeropoulou, Panagiotis Angelou, Anastasia Detsi, Irene Fragiadaki, Effie Scoulica**

Experimental Procedures and Spectroscopic Data for Compounds 3a, 3b, 4a, 4b, 5a, 5b, 7a, 7b, 8a, 8b, 10a, 10b, 11a, 11b, 13, 14, 15, 17 and 18

All reactions were carried out under scrupulously dry conditions. NMR spectra of all new compounds were recorded in $CDCl_3$, unless stated otherwise, using a Bruker AC 300 spectrometer operating at 300 MHz for ^1H, 75.43 MHz for ^{13}C, and 121.44 MHz for ^{31}P. ^1H NMR spectra are reported in units δ with $CHCl_3$ resonance at 7.24 ppm used as the chemical shift resonance. ^{13}C NMR shifts are expressed in units relative to $CDCl_3$ at 77.00 ppm, while ^{31}P NMR spectra are reported in units of δ relative to 85% H_3PO_4 used as an external standard. Silica gel plates (Merck F_{254}) were used for thin-layer chromatography. Chromatographic purification was performed with silica gel (200-400 mesh).

5-(Cyclodecylidene)pentanoic acid (3a)

To a suspension of (4-carboxybutyl)triphenylphosphonium bromide (**2**) (2.22 g, 5 mmol) in THF (15 mL) was added bis(trimethylsilyl)potassium amide (1.99 g, 10 mmol), at 0 ^oC. The mixture which acquired a red colour was stirred at room temperature for 15 min. A solution of cyclodecanone (**1a**) (386 mg, 2.5 mmol) in THF (10 mL) was then added dropwise and the mixture was stirred at room temperature for 12 h. Subsequently, the reaction was quenched with H_2O and

the aqueous layer was acidified with 10% HCl to pH 2 and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4) and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 95:5 and then petroleum ether/EtOAc 80:10) to afford acid **3a**, as a colourless oil (205 mg, 35%). ^{1}H NMR (δ) 5.13 (t, *J*= 6.72 Hz, 1H), 2.36 (t, *J*= 7.33 Hz, 2H), 2.91-2.15 (m, 6H), 1.72–1.41 (m, 16H).

Methyl 5-(cyclodecylidene)pentanoate (4a)

A solution of the acid **3a** (193 mg, 0.81 mmol) in MeOH (10 mL) containing 0.25 mL conc. H_2SO_4 was stirred at 40 ^{0}C for 3 h. The solvent was evaporated in vacuo, H_2O was added residue and the resulting solution was extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4) and the solvent was evaporated in vacuo, to afford pure ester **4a** as colourless oil (195 mg, 95%); ^{1}H NMR (δ) 5.13 (t, *J* = 6.71 Hz, 1H), 3.66 (s, 3H), 2.32 (t, *J*= 7.93 Hz, 2H), 2.16-2.08 (m, 6H), 1.72–1.40 (m, 16H).

5-(Cyclodecylidene)pentanol (5a)

To a cooled suspension of $LiAlH_4$ (58 mg, 1.754 mmol) in THF (2 mL) was added dropwise a solution of methyl 5-(cyclodecylidene)pentanoate (**4a**) (194 mg, 0.77 mmol) in THF (3 mL) and the mixture was stirred at room temperature for 2h. Subsequently, the reaction mixture was cooled, a mixture of H_2O/THF (1:1, 1 mL) was added dropwise, followed by addition of EtOAc (15 mL) and anhydrous Na_2SO_4 until the mixture became clear. The resulting suspension was filtered off, the filtrate was evaporated in vacuo and the residue was subjected to flash column chromatography (petroleum ether/Et_2O 75:25) to produce the pure alcohol **5a** as a colourless oil (140 mg, 82%). ^{1}H NMR (δ) 5.15 (t, *J*= 6.71 Hz, 1H), 3.64 (t, *J*= 6.71 Hz, 2H), 2.21-1.95 (m, 6H), 1.51–1.28 (m, 18H); ^{13}C NMR (δ) 138.4, 126.1, 62.8, 35.1, 32.5, 30.8, 27.8, 25.9, 25.7, 24.8, 24.7, 24.5, 23.1; Anal ($C_{15}H_{28}O$) C, H.

5-(Cyclopentadecylidene)pentanoic acid (3b)

Following the procedure described for the preparation of compound **3a**, we obtained acid **3b**, using cyclopentadecanone **(1b)** (1.4 g, 6.2 mmol) as the starting ketone. The crude mixture was purified using flash column chromatography (petroleum ether/EtOAc 85:15) to afford compound **3b** as a colourless oil (1.53 g, 75%); ^{1}H NMR (δ) 11.22 (bs, 1H), 5.08 (t, *J*= 6.72 Hz, 1H), 2.33 (t, *J*= 7.33 Hz, 2H), 2.05-1.94 (m, 6H), 1.66 (q, *J*= 7.94 Hz, 2H), 1.40–1.31 (m, 24H).

Methyl 5-(Cyclopentadecylidene)pentanoate (4b)

Prepared following the procedure described for ester **4a**, starting from acid **3b** (1.53 g, 4.95 mmol). After purification by flash column chromatography (petroleum ether/EtOAc 98:2), ester **4b** was obtained as a colourless oil (1.36 g, 85%); ^{1}H NMR (δ) 5.02 (t, *J*= 6.72 Hz, 1H), 3.56 (s, 3H), 2.18 (t, *J*= 7.33 Hz, 2H), 1.96-1.88 (m, 6H), 1.57 (q, *J*= 7.32 Hz, 2H) 1.27–1.24 (m, 24H).

5-(Cyclopentadecylidene)pentanol (5b)

Prepared following the procedure described for alcohol **5a** starting from methyl 5-(cyclopentadecylidene)pentanoate (**4b**) (1.36 g, 4.21 mmol). Alcohol **5b** was obtained as a colorless oil (1.41 g, 82%); ^{1}H NMR (δ) 5.07 (t, *J*= 6.71Hz, 1H), 3.54 (t, *J*= 6.11 Hz, 2H), 2.96 (bs, 1H), 2.01-1.90 (m, 6H), 1.52 (q, *J*= 7.32 Hz, 2H), 1.32–1.28 (m, 26H); ^{13}C NMR (δ) 140.2, 124.7, 62.7, 37.5, 32.4, 29.9, 27.8, 27.6, 27.5, 27.4, 27.2, 26.8, 26.7, 26.2; Anal ($C_{20}H_{38}O$), C, H.

Methyl 11-(cyclodecylidene)undecanoate (7a)

To a suspension of (10-methoxycarbonyl)decyltriphenylphosphonium bromide **(6)** (1.08 g, 2 mmol) in THF (20 mL) was added bis(trimethylsilyl)potassium amide (399 mg, 2 mmol), at 0 ^{o}C. The mixture acquired a red colour and was stirred at room temperature for 15 min. A solution of cyclodecanone **(1a)** (77 mg, 0.5 mmol), in THF (10 mL) was then added dropwise and the mixture was refluxed (80 ^{o}C) for 12 h. Subsequently, the reaction was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. The residue was purified using flash column chromatography

(petroleum ether/Et_2O 97:3) to afford ester **7a**, as a colourless oil (60 mg, 36%); ^{1}H NMR (δ) 5.13 (t, *J*= 6.71 Hz, 1H), 3.64 (s, 3H), 2.27 (t, *J*= 7.33 Hz, 2H), 2.14-1.92 (m, 4H), 1.66–1.25 (m, 30H).

11-(Cyclodecylidene)undecanol (8a)

Prepared following the procedure described for alcohol **5a**, starting from methyl 11-(cyclodecylidene)undecanoate (**7a**) (60 mg, 0.18 mmol). The colourless oily product (50 mg, 91%) was used without further purification; ^{1}H NMR (δ) 5.14 (t, *J*= 6.71Hz, 1H), 3.62 (t, *J*= 6.71 Hz, 2H), 2.15-1.93 (m, 4H), 1.67–1.25 (m, 32H); ^{13}C NMR (δ) 137.9, 126.6, 63.0, 36.1, 32.8, 30.8, 29.8, 29.3, 28.1, 25.8, 25.7, 24.8, 24.7, 25.5, 23.1, 22.7; Anal ($C_{21}H_{40}O$) C, H.

Methyl 11-(cyclopentadecylidene)undecanoate (7b)

Following the procedure described for the preparation of compound **7a**, we obtained ester **7b**, starting from cyclopentadecanone **(1b)** (523 mg, 2.33 mmol). The crude mixture was purified by flash column chromatography (petroleum ether/Et_2O 97:3) to afford compound **7b** as a colourless oil (680 mg, 72%); ^{1}H NMR (δ) 5.08 (t, *J*= 6.71 Hz, 1H), 3.63 (s, 3H), 2.27 (t, *J*= 7.94 Hz, 2H), 1.97-1.91 (m, 6H), 1.65-1.56 (m, 2H) 1.33–1.25 (m, 36H).

11-(Cyclopentadecylidene)undecanol (8b)

Prepared following the procedure described for alcohol **5a** starting from methyl 11-(cyclopentadecylidene)undecanoate (**7b**) (680 mg, 1.67 mmol). Alcohol **8b** was obtained as a colourless oil (640 mg, quantitative). ^{1}H NMR (δ) 5.11 (t, *J*= 6.72Hz, 1H), 3.63 (t, *J*= 6.10 Hz, 2H), 1.98-1.92 (m, 6H), 1.67-1.52 (m, 2H) 1.34–1.26 (m, 38H); Anal ($C_{26}H_{50}O$) C,H.

***trans*-2-(Dimethylamino)cyclopentanol (10a)**

A mixture containing 6-oxadicyclo[3.1.0]hexane **(9a)** (964 mg, 11.5 mmol) and 40% aqueous solution of dimethylamine (2.3 mL, 46 mmol) was stirred at ambient temperature for 12 h. Subsequently, H_2O was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo to

provide pure aminalcohol **10a** as an orange oil (531 mg, 36%); ^{1}H NMR (δ) 4.07 (m, 1H), 2.78 (bs, 1H), 2.45 (m, 1H), 2.28 (s, 6H), 1.94-1.42 (m, 6H); ^{13}C NMR (δ) 75.5, 75.1, 43.3, 34.2, 26.8, 21.4.

***trans*-(2-Hydroxy-cyclopentyl)- *N*,*N*,*N*-trimethylammonium toluene-4-sulfonate (11a)**

Methyl *p*-toluene sulfonate (3.07 gr, 16.48 mmol) was added to a solution of *trans*-2-(dimethylamino)cyclopentanol (**10a**) (531 mg, 4.12 mmol) in Et_2O (5 mL) and the mixture was stirred at ambient temperature. for 48h. The solvent was then evaporated in vacuo to provide a yellow solid, which was recrystallized from boiling acetone. The precipitate was triturated with Et_2O and dried over P_2O_5 to give salt **11a** as a white solid (1.21 g, 93%). mp 161-164 ^{o}C; ^{1}H NMR (δ) 7.73 (d, *J*=7.94 Hz, 2H), 7.16 (d, *J*=7.33 Hz, 2H), 4.48 (m, 1H), 3.84 (m, 1H), 3.22 (s, 9H), 2.33 (s, 3H), 2.08-1.61 (m, 6H); Anal ($C_{15}H_{25}NO_4S$) C, H, N.

***trans*-2-(Dimethylamino)cyclohexanol (10b)**

Prepared following the procedure described for the synthesis of aminoalcohol **10a**, starting from 7-oxabicyclo[4.1.0]heptane **(9b)** (2.91 g, 29.7 mmol). Compound **10b** was obtained as an orange oil (2.66 g, 63%). ^{1}H NMR (δ) 3.90 (bs, 1H*H*), 3.35-3.27 (m, 1H), 2.19 (s, 6H), 2.18-2.09 (m, 2H), 1.77-1.68 (m, 3H), 1.30-1.05 (m, 4H); ^{13}C NMR (δ) 69.6, 69.3, 40.2, 33.2, 25.3, 24.2, 20.4.

***trans*- (2-Hydroxy-cyclohexyl)- *N*,*N*,*N*-trimethylammonium toluene-4-sulfonate (11b)**

Following the procedure described for the preparation of compound **11a**, we obtained salt **11b**, starting from *trans*-2-(dimethylamino)cyclohexanol (**10b**) (2.66 g, 18.6 mmol). White solid (5.18 g, 85%), mp 200-203 ^{o}C; ^{1}H NMR (δ) 7.72 (d, *J*= 7.94 Hz, 2H), 7.14 (d, *J*= 7.33 Hz, 2H), 3.76-3.68 (ddd, *J*= 10.38, 10.06, 4.53 Hz, 1H), 3.55-3.47 (ddd, *J*= 11.6, 10.06, 3.66 Hz, 1H), 3.23 (s, 9H), 2.31 (s, 3H), 2.20-2.02 (2H), 1.82-1.66 (m, 2H), 1.61-1.18 (m, 4H); Anal ($C_{16}H_{27}NO_4S$) C, H, N.

(8-Methyl-8-aza-bicyclo[3.2.1]oct-3-ylidene)acetic acid ethyl ester (13)

Triethyl phosphonoacetate (1.79 g, 8.0 mmol) was added dropwise to a suspension of bis(trimethylsilyl)potassium amide (1.66 g, 8.32 mmol) in THF (15ml) at 0 ^{o}C. Tropinone **(12)** (1g,

7.18 mmol) in THF (15 mL) was added dropwise and the mixture was stirred at ambient temperature. for 2 h and at 80 ^{o}C for 35 h. The reaction was quenched with H_2O and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, the solvent was evaporated in vacuo and a saturated ethanolic HCl solution was added to the residue. Evaporation of the solvent gave a yellowish solid which was recrystallized from boiling acetone. After trituration with Et_2O, and drying over P_2O_5, the hydrochloride salt of ester **13** was obtained as a white solid. After treating the salt with K_2CO_3 in a mixture of CH_3COCH_3/H_2O 20:1, compound **13** was obtained as an orange oil (935 mg, 62%); ^{1}H NMR (δ) 5.30 (s, 1H), 3.74 (q, *J*= 7.2 Hz, 2H), 3.16 (d, *J*= 15.26 Hz, 1H), 2.86 (bs, 2H), 2.31 (d, *J*= 14.65 Hz, 1H), 1.99-1.97 (m, 4H), 1.64-1.56 (m, 3H), 1.12 (d, *J*= 8.55 Hz, 2H), 0.88 (t, *J*= 7.33 Hz, 3H); ^{13}C NMR (δ) 165.7, 157.2, 117.1, 61.1, 60.8, 58.9, 47.1, 41.3, 38.5, 34.6, 26.6, 26.4, 13.9.

2-(8-Methyl-8-aza-bicyclo[3.2.1]oct-3-ylidene)ethanol (14)

Prepared following the procedure described for alcohol **5a** starting from (8-methyl-8-aza-bicyclo[3.2.1]oct-3-ylidene)acetic acid ethyl ester (**13**) (500 mg, 2.39 mmol). Alcohol **14** was obtained in pure form as a yellow oil (431 mg, 100%). ^{1}H NMR (δ) 5.46 (t, *J*= 6.72 Hz, 1H), 4.11 (d, *J*= 6.11 Hz, 2H), 3.17 (bs, 2H), 2.54 (m, 1H), 2.29 (s, 3H), 2.26 (m, 1H), 1.88 (m, 3H), 1.53-1.36 (m, 3H).

3-(2-Hydroxy-ethylidene)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane, *p*-toluene sulfonate (15)

Prepared following the procedure described for salt **11a**, starting from aminalcohol **14** (431 mg, 2.58 mmol). Compound **15** was obtained as a white solid (683 mg, 75%), mp 69-73 ^{o}C; ^{1}H NMR (δ) 7.70 (d, *J*= 7.93 Hz, 2H), 7.14 (d, *J*= 7.93 Hz, 2H), 5.72 (bs, 1H), 4.06-3.91 (m, 4H), 3.29 (s, 3H), 3.11 (s, 3H), 2.99 (bs, *J*= 17.49 Hz, 1H), 2.78-2.55 (m, 2H), 2.32 (s, 3H), 2.25-2.13 (m, 3H), 1.89 (m, 2H); Anal ($C_{18}H_{27}NO_4S$) C, H, N.

4-Dimethylamino-but-2-yn-1-ol (17)

N,N-dimethyl-prop-2-ynyl-amine (**16**) (1.01 g, 12.2 mmol) was added dropwise at -55 ^{o}C to a solution of *n*-BuLi (1.6 M in THF, 14.64 mmol, 9.15 mL) in 61 mL THF and the resulting mixture was stirred for 1 h. Subsequently, a solution of paraformaldehyde (24.4 mmol, 732 mg) in 29 mL THF was added and the reaction was left to warm up to room temperature until completion of the reaction. The mixture was treated with brine at 0 ^{o}C and then extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo to afford the desired product **17** in 60% yield (828 mg) which was used for the next step without purification; ^{1}H NMR (δ) 4.28 (bs, 2H), 3.27 (bs, 2H), 2.29 (s, 6H).

Toluene-4-sulphonate(4-hydroxy-but-2-ynyl)- *N,N,N*-trimethylammonium (18)

Prepared following the procedure described for the salt **11a**, starting from 4-dimethylamino-but-2-yn-1-ol (**17**) (431 mg, 2.58 mmol). Compound **18** was obtained as a white solid (683 mg, 75%), mp 69-73 ^{o}C; ^{1}H NMR (δ) 7.61 (d, *J*= 8.1 Hz, 2H), 7.08 (d, *J*= 7.8 Hz, 2H), 4.21 (s, 2H), 4.16 (s, 2H), 3.11 (s, 9H), 2.32 (s, 3H); ^{13}C NMR (δ) 141.9, 140.1, 128.7, 125.5, 91.8, 71.8, 56.5, 52.5, 47.9, 20.9; Anal ($C_{14}H_{21}NO_4S$) C, H, N.

APPENDIX

Elemental Analyses

Compound	*Formula*	*% Calculated*	*% Found*
5a	$C_{15}H_{28}O$	C 80.29 H 12.58	C 80.42 H 12.34
5b	$C_{20}H_{38}O$	C 81.56 H 13.01	C 81.52 H 13.23

8a	$C_{21}H_{40}O$	C 81.75 H 13.07	C 81.89 H 13.19
8b	$C_{26}H_{50}O$	C 82.47 H 13.31	C 82.22 H 13.07
11a	$C_{15}H_{25}NO_4S$	C 57.12 H 7.99 N 4.44	C 57.35 H 8.15 N 4.29
11b	$C_{16}H_{27}NO_4S$	C 58.33 H 8.26 N 4.25	C 58.41 H 8.47 N 4.38
15	$C_{18}H_{27}NO_4S$	C 61.16 H 7.70 N 3.96	C 61.04 H 7.82 N 3.88
18	$C_{14}H_{21}NO_4S$	C 56.16 H 7.07 N 4.68	C 56.21 H 6.94 N 4.49
19	$C_{20}H_{40}NO_4P$	C 61.67 H 10.35 N 3.60	C 61.85 H 10.31 N 3.45
20	$C_{26}H_{52}NO_4P$	C 65.93 H 11.07 N 2.96	C 66.08 H 11.25 N 3.08
21	$C_{25}H_{50}NO_4P.\ 3H_2O$	C 58.45 H 10.99 N 2.73	C 58.21 H 10.67 N 2.96
22	$C_{28}H_{54}NO_4P$	C 67.30 H 10.89 N 2.80	C 67.22 H 10.57 N 2.68
23	$C_{27}H_{52}NO_5P.\ 2.5H_2O$	C 59.32 H 10.51 N 2.56	C 59.25 H 10.13 N 2.82
24	$C_{31}H_{62}NO_4P\ .3H_2O$	C 62.28 H 11.46 N 2.34	C 62.04 H 11.21 N 2.51
25	$C_{34}H_{66}NO_4P.2H_2O$	C 65.88 H 11.38 N 2.26	C 65.91 H 11.76 N 2.25
26	$C_{33}H_{64}NO_5P$	C 67.66 H 11.01 N 2.39	C 67.45 H 11.30 N 2.45
27	$C_{28}H_{54}NO_4P$	C 67.30 H 10.89 N 2.80	C 67.58 H 10.87 N 2.72

28	$C_{29}H_{56}NO_4P$	C 67.80 H 10.99 N 2.73	C 67.55 H 10.78 N 2.65
29	$C_{25}H_{48}NO_4P$.$2H_2O$	C 60.83 H 10.62 N 2.84	C 60.97 H 10.47 N 2.85
30	$C_{26}H_{50}NO_4P$	C 66.21 H 10.69 N 2.97	C 66.37 H 10.55 N 2.85
31	$C_{29}H_{52}NO_4P$	C 68.34 H 10.28 N 2.75	C 68.52 H 10.22 N 2.67
32	$C_{30}H_{54}NO_4P$	C 68.80 H 10.39 N 2.67	C 69.02 H 10.24 N 2.85
33	$C_{32}H_{54}NO_4P$	C 70.17 H 9.94 N 2.56	C 69.91 H 10.08 N 2.69
34	$C_{28}H_{48}NO_4P$ .H_2O	C 65.73 H 9.85 N 2.74	C 65.55 H 10.07 N 3.04
35	$C_{24}H_{50}NO_4P$	C 64.40 H 11.26 N 3.13	C 64.75 H 11.03 N 3.42
36	$C_{25}H_{52}NO_4P$. H_2O	C 62.60 H 11.35 N 2.92	C 62.53 H 11.51 N 3.08
37	$C_{20}H_{42}NO_4P$	C 61.35 H 10.81 N 3.58	C 61.62 H 10.63 N 3.28
38	$C_{25}H_{52}NO_4P$	C 65.04 H 11.35 N 3.03	C 65.31 H 11.05 N 3.19
39	$C_{28}H_{56}NO_4P$	C 67.03 H 11.25 N 2.79	C 67.33 H 11.15 N 2.75
40	$C_{27}H_{54}NO_5P$	C 64.38 H 10.81 N 2.78	C 64.13 H 10.84 N 2.43