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Asymmetric synthesis and reactivity of potent sialyltransferase inhibitors based on transition-state analogues: Supplementary data

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Protocol for the synthesis of α-hydroxyphosphonates

As described for previous syntheses of sialyltransferase inhibitors [6], the requisite α-hydroxyphosphonates were obtained by dissolving the appropriate aldehyde (1 eq.) in a small amount of CH_2Cl_2 and reacting with either dibenzyl or diallyl protected phosphonic acid diester (2 eq.) in the presence of a few drops of NEt_3. After stirring the solution for 18 h, the reaction mixture was concentrated and purified by silica gel flash chromatography, to afford the desired α-hydroxyphosphonates in high yield.

Diallyl (*S/R*)-1-hydroxy-2-phenylethylphosphonate (12a)

^{1}H NMR (250 MHz, $CDCl_3$): δ 1.89–1.97 (d, 1H, OH), 3.12–3.31 (m, 2H, ArCH_2), 4.55–4.65 (m, 5H, CH_2CH=CH_2, 1-H), 5.22–5.47 (m, 4H, CH_2CH=CH_2), 5.84–6.03 (m, 2H, CH_2C*H*=CH_2), 7.29 (s, 5H, ArH).

Tetraallyl (*S/R*)-1-hydroxy-2-phenylethylbisphosphonate (12b)

^{1}H-NMR (250 MHz, $CDCl_3$): δ 3.39 (d, ^3J(2,P) = 13.8 Hz, 2H, 2-H), 4.47–4.61 (m, 8H, CH_2CH=CH_2), 5.16–5.41 (m, 8H, CH_2CH=CH_2), 5.77–5.90 (m, 4H, CH_2C*H*=CH_2), 7.22–7.27 (m, 3H, ArH), 7.33–7.37 (m, 2H, ArH).

Diallyl (*S/R*)-1-hydroxy-2,2-diphenylethylphosphonate (12c)

^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.72 (bs, 1H, OH), 4.03–4.18 (m, 1H, 2″-H), 4.21–4.52 (m, 4H, CH_2CH=CH_2), 4.71 (dd, 1H, CHP), 5.10–5.23 (m, 4H, CH_2CH=CH_2), 5.62–5.84 (m, 2H, CH_2C*H*=CH_2), 7.13–7.39 (m, 10H, ArH).

Dibenzyl (*S/R*)-1-(3-trifluoromethyl)phenyl-1-hydroxymethylphosphonate (14c)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.87–5.10 (m, 5H, $ArCH_2$, 1-H), 7.17–7.68 (m, 14H, $ArCH_2$, and ArH); Calc. for $C_{22}H_{20}O_4F_3P$, C: 60.55, H: 4.62. Found C: 60.51, H: 4.67.

Dibenzyl (*S/R*)-1-(4-acetamido)phenyl-1-hydroxymethylphosphonate (14d)

^{1}H NMR (250 MHz, $CDCl_3$): δ 2.15 (s, 3H, NAc), 4.78–4.99 (m, 5H, $ArCH_2$, 1-H), 7.16–7.44 (m, 14H, $ArCH_2$, ArH), 7.7 (bs, 1H, NH).

Diallyl (*S/R*)-1-hydroxy-1-(4-nitro) phenylmethylphosphonate (14e)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.46–4.58 (m, 4H, CH_2CH=CH_2), 5.17–5.31 (m, 5H, CH_2CH=CH_2, 1-H), 5.75–5.92 (m, 2H, CH_2C*H*=CH_2), 7.63–7.67 (m, 2H, ArH), 8.17–8.20 (m, 2H, ArH).

Diallyl (*S/R*)-1-(4-amyl)phenyl-1-hydroxymethylphosphonate (14f)

^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, ^3J(5″, 4″) = 6.8 Hz, 3H, 5″-H), 1.25–1.41 (m, 4H, 3″-H, 4″-H), 1.52–1.64 (m, 2H, 2″-H), 2.58 (t, ^3J(1″,2″) = 6.8 Hz, 2H, 1″-H), 4.34–4.49 (m, 4H, CH_2CH=CH_2), 5.00 (m, ^2J(H,P) = 10.3 Hz, 1H, 1-H), 5.75–5.92 (m, 2H, CH_2CH=CH_2), 7.63–7.67 (m, 2H, ArH), 8.17–8.20 (m, 2H, ArH).

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Diallyl (*S/R*)-1-hydroxy-1-(pyridin-2-yl) methylphosphonate (14g)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.42–4.68 (m, 4H, CH_2CH=CH_2), 5.13–5.41 (m, 5H, CH_2CH=CH_2, CHP), 5.76–5.98 (m, 2H, CH_2C*H*=CH_2), 7.28 (m, 1H, ArH), 7.54 (m, 1H, ArH), 7.73 (m, 1H, ArH), 8.58 (m, 1H, ArH).

Diallyl (*S/R*)-1-hydroxy-1-(pyridin-4-yl) methylphosphonate (14h)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.47–4.53 (m, 4H, CH_2CH=CH_2), 5.15 (d, ^2J(H, P) = 13.3 Hz, 1H, CHP), 5.17–5.31 (m, 4H, CH_2CH=CH_2), 5.80–5.96 (m, 2H, CH_2C*H*=CH_2), 7-42–7.45 (m, 2H, ArH), 8.52–8.54 (m, 2H, ArH).

Diallyl (*S/R*)-1-hydroxy-1-(naphthalin-2-yl) methylphosphonate (14j)

^{1}H-NMR (250 MHz, $CDCl_3$): δ 4.43–4.52 (m, 4H, CH_2CH=CH_2), 4.67–4.73 (m, 1H, CHP), 5.10–5.30 (m, 4H, CH_2CH=CH_2), 5.74–5.86 (m, 2H, CH_2C*H*=CH_2), 7.44–7.49 (m, 2H, ArH), 7.57–7.61 (m, 1H, ArH), 7.78–7.83 (m, 3H, ArH), 7.95 (s, 1H, ArH).

Diallyl (*S/R*)-1-hydroxy-1-(naphthalin-1-yl) methylphosphonate (14k)

^{1}H-NMR (250 MHz, $CDCl_3$): δ 3.47 (bs, 1H, OH), 4.18–4.47 (m, 4H, CH_2CH=CH_2), 5.01–5.24 (m, 4H, CH_2CH=CH_2), 5.60–5.85 (m, 2H, CH_2C*H*=CH_2), 5.89 (d, ^2J(1′, P) = 11.3 Hz, 1H, CHP), 7.45–7.52 (m, 3H, ArH), 7.79–7.89 (m, 3H, ArH), 8.05–8.09 (m, 1H, ArH); Analysis for $C_{17}H_{19}O_4P$: Calc. C: 64.2, H: 6.0. Found C: 63.7, H: 5.9.

Diallyl (*S/R*)-1-hydroxy-1-(quinolin-2-yl) methylphosphonate (14l)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.27–4.55 (m, 2H, CH_2CH=CH_2), δ 4.56–4.80 (m, 2H, CH_2CH=CH_2), 4.95–5.23 (m, 5H, 1-H, CH_2CH=CH_2), 5.65–5.85 (m, 1H, CH_2C*H*=CH_2), 5.86–6.08 (m, 1H, CH_2C*H*=CH_2), 7.50–8.20 (m, 6H, quinolin-H); MALDI-MS (positive mode, matrix: DHB) *m/z* = 320 ([M+H]$^+$, 100%), 342 ([M + Na]$^+$, 20%), 358 ([M + K]$^+$, 10%); 319.3 for $C_{16}H_{18}NO_4P$.

Diallyl (*S/R*)-1-hydroxy-1-(6-pyrimidon-4-yl) methylphosphonate (14m)

^{1}H NMR (250 MHz, $CDCl_3$): δ 4.59–4.66 (m, 4H, CH_2CH=CH_2), 4.96 (d, ^2J(H, P) = 16.8 Hz, 1H, CHP), 5.20–5.41 (m, 4H, CH_2CH=CH_2), 5.89–6.05 (m, 2H, CH_2C*H*=CH_2), 6.66 (s, 1H, 5-H), 8.15 (s, 1H, 2-H); Analysis for $C_{11}H_{15}N_2O_5P$: Calc. C: 46.2, H: 5.3, N: 9.8. Found C: 46.2, H: 5.2, N: 9.5.

Protocol for condensation with cytidine phosphitamide 10

As described for previous syntheses of sialyltransferase inhibitors,[6] the condensation with cytidine phosphitamide (**10**) was performed as follows: racemic α-hydroxyarylphosphonates (1 eq.) and **10** (1.5 eq.) were dissolved in CH_2Cl_2 and evaporated to dryness. The remaining foam was dissolved under nitrogen in dry CH_2Cl_2, and 1-*H* tetrazole (2 eq.) was added. After the reaction mixture had been stirred for 3 h, an anhydrous solution of *t*-butyl hydroperoxide (1.5 eq.) was added, and after an additional hour, NEt_3 (50 eq.) was added. After 18h of stirring, the reaction mixture was concentrated at 20°C and purified by silica gel flash chromatography.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-2-phenylethyl]-phosphate (13a)

From (*S/R*)-**12a** (81.0 mg, 0.25 mmol): purification by FC (silica gel, 5:1 EtOAc-MeOH + 1% Et_3N) gave (*S/R*)-**13a** (130 mg, 63% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.23 (t, ^3J = 7.3 Hz, 9H, N(CH_2CH_3)$_3$), 2.02/2.03/2.09/2.10 (4s, 6H, C(O)CH_3), 2.14 (s, 3H, NH(O)CH_3), 3.21 (q, ^3J = 7.3 Hz, 6H, N(C$H_2$$CH_3$)$_3$), 3.53–4.87 (5m, 9H, 2′-H, 3′-H, 4′-H, 5′a,b-H, CH_2CH=CH_2), 4.75–4.95 (m, 1H, CHP), 5.13–5.29 (dd, ^2J(H,P) = 12.7 Hz, ^3J(H,P) = 11.4 Hz, 2H, ArCH_2), 5.29–5.43 (m, 4H, CH_2CH=CH_2), 5.78–5.94 (m, 2H, CH_2C*H*=CH_2), 6.11–6.15 (m, 1H, 1′-H), 7.19–7.49 (m, 6H, 5-H, ArH), 8.23–8.43 (m, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 712 ([M-Et_3N + H]$^-$) 672 ([M-Et_3N-Allyl]$^-$); 814.75 for $C_{35}H_{52}N_4O_{14}P_2$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1, 1- tetraallylbisphosphonato-2-phenylethyl] -phosphate (13b)

From (*S/R*)-**12b** (132 mg, 0.30 mmol): purification by FC (silica gel, 7:1 EtOAc-MeOH + 1% Et_3N) gave (*S/R*)-**13b** (140 mg, 48% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.28–1.34 (t, ^3J = 7.3 Hz, N(CH_2CH_3)$_3$),[1] 2.06/2.12 (4s, 6H, C(O)CH_3), 2.17 (s, 3H, NH(O)CH_3), 3.20 (q, ^3J = 7.3 Hz, N(C$H_2$$CH_3$)$_3$),[1] 3.62 (t, ^3J(H,P) = 11.9 Hz, 2H, ArCH_2), 4.05–4.45 (2m, 3H, 2′-H, 3′-H, 4′-H), 4.57–4.78 (m, 8H, CH_2CH=CH_2), 5.11–5.39 (m, 8H, CH_2CH=CH_2), 5.40–5.43 (m, 2H, 5′a,b-H), 5.76–6.09 (m, 4H, CH_2C*H*=CH_2), 6.19 (m, 1H, 1′-H), 7.18–7.23 (m, 3H, ArH), 7.42–7.48 (m, 2H, ArH), 7.44 (d, ^2J(5,6) = 7.6 Hz, 1H, 5-H), 8.31 (d, ^2J(6,5) = 7.6 Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin -5‘-yl)-[(*S/R*)-1-diallylphosphonato-2,2-diphenylethyl]-phosphate (13c)

From (*S*/*R*)-**12c** (90.0 mg, 0.25 mmol): purification by FC (silica gel, 5:1 EtOAc-MeOH + 1% Et_3N) gave (*S*/*R*)-**13c** (165 mg, 74% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.23 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.02/2.03/2.09/2.10 (4s, 6H, $C(O)CH_3$), 2.14/2.15 (2s, 3H, $NH(O)CH_3$), 3.21 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.93–4.54 (3m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.02–5.15 (m, 5H, CHP, $CH_2CH{=}CH_2$), 5.16–5.53 (m, 4H, $CH_2CH{=}CH_2$), 5.54–5.91 (m, 2H, $CH_2CH{=}CH_2$), 6.02 (m, 1H, 1′-H), 7.08–7.29 (m, 6H, ArH), 7.40 (d, $^3J(5,6) = 7.6$ Hz, 1H, 5-H), 7.44–7.50 (m, 4H, ArH), 8.15–8.24 (2d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) *m/z* = 748 ([M-Et_3N + H-Allyl]$^-$) 788 ([M-Et_3N + H]$^-$; 890.85 for $C_{41}H_{56}N_4O_{14}P_2$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[1,1-dibenzyl biscarboxylato-2-phenylethyl]-phosphate (11b)

From (*S*/*R*)-**9b** (132 mg, 0.30 mmol): purification by FC (silica gel, 3:1 EtOAc-MeOH) gave (*S*/*R*)-**11b** (140 mg, 61% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 600 MHz): δ 1.30 (t, $^3J = 7.4$ Hz, 9H, $N(CH_2CH_3)_3$), 2.04/2.07 (2s, 6H, $C(O)CH_3$), 2.15 (s, 3H, $NH(O)CH_3$), 3.15 (q, $^3J = 7.4$ Hz, $N(CH_2CH_3)_3$), 3.62 (b, 2H, $ArCH_2$), 4.11 (bd, $^2J(5'a,5'b) = 8$ Hz, 1H, 5′a-H), 4.23 (bd, $^2J(5'a,5'b) = 8$ Hz, 1H, 5′b-H), 4.28 (b, 1H, 4′-H), 5.00 (d, $^2J = 12$ Hz, 1H, Bn), 5.04 (d, $^2J = 12$ Hz, 1H, Bn), 5.11–5.14 (m, 2H, Bn), 5.44 (m, 2H, 2′-H, 3′-H), 6.12 (d, $^3J(1',2') = 3.9$ Hz, 1H, 1′-H), 7.14–7.36 (m, 16H, ArH, 5-H), 7.42–7.48 (m, 2H, ArH), 8.35 (d, $^2J(5,6) = 7.5$ Hz, 1H, 6-H); ^{13}C NMR (D_4-MeOH, 151 MHz): δ 42.1 (C2″), 65.1 (C5′), 68.6/68,7 (2Bn), 71.8 (C3′), 75.4 (C2′), 83.0 (C4′), 89.1 (C1′), 99.2 (C5), 127–137 (ArCH), 146.6 (C6), 140.6 (ArC), 158.1 (C4), 164.4 (C2), 169/170/171/172 (5 C=O); ^{31}P NMR (D_4-MeOH, 243 MHz): δ − 2.78 (bs, $P(O)O_3$); MALDI-MS (positive mode, matrix: CHCA) *m/z* = 844 ([M-NEt_3 + Na]$^+$), 866 ([M-NEt_3-H + 2Na]$^+$), 922.3 for $C_{45}H_{55}N_4O_{15}P$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-1-(3-phenoxy) phenylmethyl]-phosphate (15b)

From (*S*/*R*)-**14b** (72.0 mg, 0.20 mmol): purification by FC (silica gel, 7:1 to 5:1 EtOAc-MeOH + 1% Et_3N) gave (*S*/*R*)-**15b** (88 mg, 50% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.29 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.03/2.06/2.07/2.08 (4s, 6H, $C(O)CH_3$), 2.16/2.17 (2s, 3H, $NH(O)CH_3$), 3.19 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.55–3.99 (3m, 2H, 5′a,b-H), 4.20–4.23 (m, 1H, 4′-H), 4.54–4.58 (m, 4H, $CH_2CH{=}CH_2$), 5.15–5.35 (m, 6H, 2′-H, 3′-H and $CH_2CH{=}CH_2$), 5.59/5.60 (2dd, $^2J(H,P) = 15.8$ Hz, $^3J(H,P) = 11.7$ Hz, 1H, CHP), 5.84–5.94 (m, 2H, $CH_2CH{=}CH_2$), 6.07/6.12 (d, $^3J(1,2) = 4.6$ Hz, 1′-H), 6.89–7.45 (m, 10H, 5-H, ArH), 8.15/8.27 (d, $^3J(6,5) = 7.5$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2‘,3‘-di-*O*-acetylcytidin-5‘-yl)-[(*S/R*)-1-dibenzylphosphonato-1-(3-trifluoromethyl) phenylmethyl]-phosphate (15c)

From (*S*/*R*)-**14c** (110 mg, 0.25 mmol): purification by FC (silica gel, 9:1 to 7:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15c** (130 mg, 60% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.25 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.03/2.04/2.06/2.07 (4s, 6H, $C(O)CH_3$), 2.16/2.17 (2s, 3H, $NH(O)CH_3$), 3.13 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.80–4.22 (m, 3H, 4′-H, 5′a,b-H), 4.98–5.09 (m, 5H, $ArCH_2$, 3′-H), 5.31–5.38 (m, 1H, 2′-H), 5.54 (dd, $^3J(1,2) = 12.7$ Hz, 1H, CHP), 6.06/6.11 (2d, $^3J(1',2') = 5.8$ Hz, 1′-H), 7.19–7.49 (m, 13H, 5-H, ArH), 7.79–7.89 (m, 2H, ArH), 8.26/8.36 (2d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-(4-acetamido)phenyl-1-dibenzylphosphonatomethyl]-phosphate (15d)

From (*S*/*R*)-**14d** (213 mg, 0.50 mmol): purification by FC (silica gel, 7:1 to 5:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15d** (340 mg, 70% yield) as a pale brown lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.26 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 1.97/2.00/2.03/2.05 (4s, 6H, $C(O)CH_3$), 2.10/2.15/2.16/2.17 (4s, 6H, $NH(O)CH_3$), 3.11 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.94–4.19 (m, 3H, 4′-H, 5′a,b-H), 4.91–5.35 (m, 6H, 2′-H, 3′-H, $ArCH_2$), 5.65 (dd, $^2J(H,P) = 13.2$ Hz, $^3J(H,P) = 10.3$ Hz, 1H, CHP), 6.05/6.09 (2d, $^3J(1,2) \approx 4$ Hz, 1′-H), 7.09–7.56 (m, 15H, 5-H, ArH), 8.16/8.26 (2d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin- 5′-yl)-[(*S/R*)-1-diallylphosphonato-1-(4-nitro)phenylmethyl] -phosphate (15e)

From (*S*/*R*)-**14e** (78.0 mg, 0.25 mmol): purification by FC (silica gel, 6:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15e** (112 mg, 54% yield) as a yellow-brown lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.29 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.04/2.05/2.06/2.08 (4s, 6H, $C(O)CH_3$), 2.17/2.18 (2s, 3H, $NH(O)CH_3$), 3.19 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.85–4.26 (m, 2H, 5′a,b-H), 4.32–4.38 (m, 1H, 4′-H), 4.56–5.64 (m, 4H, $CH_2CH{=}CH_2$), 5.07–5.18 (m, 1H, 3′-H), 5.22–5.35 (m, 5H, 2′-H, $CH_2CH{=}CH_2$), 5.72/5.78 (2dd, $^2J(H,P) = 15.5$ Hz, $^3J(H,P) = 11.1$ Hz, 1H, CHP), 5.86/5.97 (m, 2H, $CH_2CH{=}CH_2$), 6.09/6.14 (2d, $^3J(1',2') = 4.7$ Hz,

1′-H), 7.34/7.37 (2d, $^3J(5,6) = 7.6$ Hz, 5-H), 8.16–8.21 (m, 2H, ArH), 8.26 (d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-(4-amyl)phenyl-1-diallylphosphonatomethyl]-phosphate (15f)

From (*S/R*)-**14f** (68.0 mg, 0.20 mmol): purification by FC (silica gel, 6:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15f** (92 mg, 53% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 0.96/0.97 (t, $^3J(5'',4'') = 6.8$ Hz, 3H, 5″-H), 1.29 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 1.23–1.47 (m, 4H, 3″-H, 4″-H), 1.54–1.65 (m, 2H, 2″-H), 2.04/2.05/2.07/2.08 (4s, 6H, $C(O)CH_3$), 2.18/2.19 (2s, 3H, $NH(O)CH_3$), 2.56–2.61 (t, $^3J(1'',2'') = 6.8$ Hz, 2H, 1″-H), 3.17 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.57–4.59 (m, 7H, 4′-H, 5′a,b-H, $CH_2CH{=}CH_2$), 5.08–5.35 (m, 6H, 2′-H, 3′-H, $CH_2CH{=}CH_2$), 5.58 (dd, $^2J(H,P) = 15.1$ Hz, $^3J(H,P) = 11.9$ Hz, 1H, CHP), 5.81–6.02 (m, 2H, $CH_2CH{=}CH_2$), 6.09/6.14 (2d, $^3J(1',2') = 5.1$ Hz, 1′-H), 7.14–7.19 (m, 2H, ArH), 7.42–7.48 (m, 3H, 5-H, ArH), 8.14/8.29 (2d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-1-(pyridin-2-yl)methyl]-phosphate (15g)

From (*S/R*)-**14g** (78.0 mg, 0.29 mmol): purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15g** (138 mg, 60% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.24–1.42 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.02/2.06/2.07 (3s, 6H, $C(O)CH_3$), 2.15 (s, 3H, $NH(O)CH_3$), 3.12–3.18 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.91–4.28 (m, 3H, 2′-H, 3′-H, 4′-H), 4.59–4.63 (m, 4H, $CH_2CH{=}CH_2$), 5.13–5.34 (m, 6H, 5′a,b-H, $CH_2CH{=}CH_2$), 5.64–5.78 (m, 1H, CHP), 5.86–5.99 (m, 2H, $CH_2CH{=}CH_2$), 6.10–6.13 (m, 1H, 1′-H), 7.28–7.32 (m, 1H, ArH), 7.49–7.51 (m, 1H, 5-H), 7.71–7.84 (2m, 2H, ArH), 8.22 (d, $^3J(5,6) = 7.3$ Hz, 0.5H, 6-H), 8.29 (d, $^3J(5,6) = 7.3$ Hz, 0.5H, 6-H), 8.43–8.49 (m, 1H, ArH); MALDI-MS (negative mode, matrix: ATT) $m/z = 660$ ([M-Et_3N^+H-Allyl]$^-$), 700 ([M-Et_3N^+H]$^-$); 801.71 for $C_{33}H_{49}N_5O_{14}P_2$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*) -1-diallylphosphonato-1-(pyridin-4-yl) methyl]-phosphate (15h)

From (*S/R*)-**14h** (78.0 mg, 0.29 mmol): purification by FC (silica gel, 5:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15h** (86 mg, 67% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.24–1.42 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.04/2.05/2.06/2.07 (4s, 6H, $C(O)CH_3$), 2.16 (s, 3H, $NH(O)CH_3$), 3.13–3.27 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 4.08–4.32 (m, 3H, 2′-H, 3′-H, 4′-H), 4.59–4.64 (m, 4H, $CH_2CH{=}CH_2$), 5.18–5.43 (m, 6H, 5′a,b-H, $CH_2CH{=}CH_2$), 5.61–5.72 (dd, $^2J(H,P) = 14.9$ Hz, $^3J(H,P) = 9.5$ Hz, 1H, CHP), 5.82–5.98 (m, 2H, $CH_2CH{=}CH_2$), 6.12–6.27 (m, 1H, 1′-H), 7.42–7.48 (2d, $^3J(5,6) = 7.6$ Hz, 1H, 5-H), 7.55–7.59 (m, 2H, ArH), 8.23–8.32 (2d, $^3J(5,6) = 7.6$ Hz, 1H, 6-H), 8.49–8.53 (m, 2H, ArH).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-1-(*N*-methylpyridinium-4-yl)methyl]-phosphate (15i)

From (*S/R*)-**15h** (100 mg, 0.12 mmol): purification by FC (silica gel, 5:1 to 1:50 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15i** (80.0 mg, 81% yield) as a colourless lyophilisate from CH_2Cl_2. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.24–1.32 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.05/2.07/2.12/2.16 (4s, 6H, $C(O)CH_3$), 2.18/2.19 (2s, 3H, $NH(O)CH_3$), 3.11–3.20 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 4.10–4.45 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.34/4.40 (2s, 3H, CH_3N^+), 4.62–4.73 (m, 4H, $CH_2CH{=}CH_2$), 5.26–5.43 (m, 5H, $CH_2CH{=}CH_2$, CHP), 5.90–6.09 (m, 2H, $CH_2CH{=}CH_2$), 6.13–6.27 (m, 1H, 1′-H), 7.48–7.51 (m, 1H, 5-H), 8.12–8.19 (m, 2H, ArH), 8.33–8.40 (m, 1H, 6-H), 8.73 (m, 1H, ArH), 8.90 (m, 1H, ArH).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*) -1-diallylphosphonato-1-(naphthalin-2-yl)methyl]-phosphate (15j)

From (*S/R*)-**14j** (55.6 mg, 0.18 mmol): purification by FC (silica gel, 5:1 to 3:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15j** (86.0 mg, 67% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.23–1.31 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 1.93/2.03 (2s, 6H, $C(O)CH_3$), 2.19 (s, 3H, $NH(O)CH_3$), 3.12–3.18 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.81–4.63 (m, 9H, 2′-H, 3′-H, 4′-H, 5′a,b-H, $CH_2CH{=}CH_2$), 5.12–5.32 (m, 4H, $CH_2CH{=}CH_2$), 5.72–5.83 (dd, $^2J(H,P) = 12.7$ Hz, $^3J(H,P) = 11.3$ Hz, 1H, CHP), 5.82–5.95 (m, 2H, $CH_2CH{=}CH_2$), 5.91/6.05 (2d, $^3J(1',2') = 3.6$ Hz, 1H, 1′-H), 7.28/7.39 (2d, $^3J(5,6) = 7.3$ Hz, 1H, 5-H), 7.40–7.51 (m, 2H, ArH), 7.63–7.70 (m, 1H, ArH), 7.74–7.88 (m, 3H, ArH), 8.01 (s, 1H, ArH), 8.03/8.24 (2d, $^3J(6,5) = 7.3$ Hz, 1H, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)- [(*S/R*)-1-diallylphosphoryl-1-(naphthalin-1-yl) methyl]-phosphate (15k)

From (*S/R*)-**14k** (95 mg, 0.30 mmol): purification by FC (silica gel, 5:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15k** (140 mg, 55% yield) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.23 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.01/2.02/2.04/2.05 (4s, 6H, $C(O)CH_3$), 2.17/2.18 (2s, 3H, $NH(O)CH_3$), 3.10 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.71–4.57 (m, 7H, 4′-H, 5′a,b-H, $CH_2CH{=}CH_2$), 4.90–5.31 (m, 6H, 2′-H, 3′-H,

$CH_2CH{=}CH_2$), 5.70–5.91 (m, 2H, $CH_2CH{=}CH_2$), 5.95/6.04 (2d, $^3J(1',2') = 4.8$ Hz, 1H, 1′-H), 6.48 (dd, $^2J(H,P) = 11.5$ Hz, $^3J(H,P) = 12.6$ Hz, 1H, CHP), 7.26/7.59 (2d, $^3J(5,6) = 7.6$ Hz, 1H, 5-H), 7.45–7.56 (m, 3H, ArH), 7.84–7.88 (m, 3H, ArH), 8.05 (2d, $^3J(6,5) = 7.6$ Hz, 1H, 6-H), 8.23–8.27 (m, 1.5H, ArH, 6-H).

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-1-(quinolin-2-yl) methyl]-phosphate (15l)

From (*S*/*R*)-**14l** (370 mg, 1.15 mmol): purification by FC (silica gel, 5:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15l** (390 mg, 40% yield) as a light yellow lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.30 (2t, $^3J = 7.5$ Hz, 9H, $N(CH_2CH_3)_3$), 2.01/2.02/2.06/2.09 (4s, 6H, $C(O)CH_3$), 2.17/2.18 (2s, 3H, $NH(O)CH_3$), 3.18 (2q, $^3J = 7.5$ Hz, 6H, $N(CH_2CH_3)_3$), 4.0–4.7 (m, 7H, 4′-H, 5′a,b-H, $CH_2CH{=}CH_2$), 5.02–5.35 (m, 4H, $CH_2CH{=}CH_2$), 5.4–5.5 (m, 3H, 1′-H, 2′-H, 3′-H), 5.8–6.1 (m, 2H, $CH_2CH{=}CH_2$), 6.1–6.2 (m, 1H, CHP), 7.26/7.59 (2d, $^3J(5,6) = 7.6$ Hz, 1H, 5-H), 7.4–8.5 (m, 8H, 5-H, 6-H, ArH) ; MALDI-MS (positive mode, matrix: DHB) *m/z* = 751 ($[M\text{-}Et_3N + H]^+$) 773 ($[M\text{-}Et_3N + Na]^+$), 851.3 for $C_{37}H_{51}N_5O_{14}P_2$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(*S/R*)-1-diallylphosphonato-1- (6-pyrimidon-4-yl) methyl]-phosphate (15m)

From (*S*/*R*)-**14m** (100 mg, 0.35 mmol): purification by FC (silica gel, 5:1 to 1:1 EtOAc-MeOH + 1% Et_3N) gave (S/*R*)-**15m** (78 mg, 27% yield) as a brown syrup.

Note

1. From integration it appears that one Et_3NH moiety may be associated with two phosphate moieties.