Supporting information

Application of ProTide Technology to Gemcitabine: A successful approach to overcome the key cancer resistance mechanisms leads to a new agent (NUC-1031) in clinical development.

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Methods and Materials: Anhydrous solvents were obtained from Aldrich and used without further purification. Amino acid esters were purchased from Carbosynth, Carboxypeptidase Y, human serum and buffers from Sigma-Aldrich. All reactions were carried out under an argon atmosphere. Reactions were monitored with analytical TLC on Silica Gel 60-F254 precoated aluminium plates and visualised under UV (254 nm) and/or with ^{31}P NMR spectra. Column chromatography was performed on silica gel (35–70 μM). Proton (^{1}H), carbon (^{13}C), phosphorus (^{31}P) and fluorine (^{19}F) NMR spectra were recorded on a Bruker Avance 500 spectrometer at 25°C. Spectra were auto-calibrated to the deuterated solvent peak and all ^{13}C NMR and ^{31}P NMR were proton-decoupled. The purity of final compounds was verified to be >95% by HPLC analysis using Varian Polaris C18-A (10 μM) as an analytic column with a gradient elution of H_2O/MeOH from 100/0 to 0/100 in 45 min. (method 1), and with a gradient elution of H_2O/CH_3CN from 100/0 to 0/100 in 35 min. (method 2). The HPLC analysis was conducted by Varian Prostar (LC Workstation-Varian prostar 335 LC detector).

General Method for the Preparation of phosphorochloridates (3b-l).
Anhydrous triethylamine (2.0 mol eq.) was added dropwise at -78ºC to a stirred solution of the appropriate aryl dichlorophosphate (1.0 mol eq.) and an appropriate amino acid ester (1.0 mol eq.) in anhydrous DCM under argon atmosphere. Following the addition, the reaction mixture was allowed to slowly warm to RT and stirred for 1-2 h. A formation of desired compound was monitored by ^{31}P NMR. After the reaction was completed, the solvent was evaporated under reduced pressure and the resulting residue was re-dissolved in anhydrous Et_2O and filtered. The filtrate was reduced to dryness to give a crude product as an oil, which was in some cases used without further purification in the next step. Most of aryl phosphorochloridates, in particular those obtained from the amino acid tosylate salt were purified by flash column chromatography using EtOAc/Hexane (7:3) as an eluent.

General procedure A: synthesis of 3′-*O*-(*tert*-butoxycarbonyloxy)–gemcitabine ProTides 5b-l. To a stirring solution of 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine **4** (1.0 mol/eq.) dissolved in anhydrous THF, *tert*-BuMgCl (1.2 mol/eq. 1.0 M solution in THF) was added under an argon atmosphere, followed by immediate addition of an appropriate phosphorochloridate (2.0 mol/ eq.) dissolved in anhydrous THF. The resulting reaction mixture was stirred at room temperature overnight (16 - 18 h). The solvent was removed under reduced pressure and the residue was purified by column chromatography using gradient of eluent (DCM/MeOH 99:1 to 97:3 to 95:5).
General procedure B: synthesis of gemcitabine ProTides 6b-l. A mixture of 3'-*O*-(*tert*-butoxycarbonyloxy)-gemcitabine ProTides **5b-l** in TFA/DCM (1:1) was stirred at 0°C for 3 hours. The solvents were evaporated and the residues was treated with saturated $NaHCO_3$, and extracted with EtOAc. The organic layers were combined, dried ($MgSO_4$), filtered, reduced to dryness and purified on silica gel with gradient of eluent (DCM/MeOH 95:5 to 94:6 to 92:8).

Chemical syntheses:

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl (pentoxy-L-alaninyl)]phosphate (5b) was prepared according to the general procedure **A** from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.68 mmol, 0.25 g), *tert*-BuMgCl (0.82 mmol, 0.82 mL) and phenyl(pentoxy-L-alaninyl) phosphorochloridate (1.37 mmol, 0.45 g). Purification by column chromatography with gradient of CH_2Cl_2/MeOH (100% to 95:5%) afforded the title compound as a white solid; yield, 84% (0.38 g). Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.68, 3.58. ^{19}F NMR (470 MHz, MeOD): δ_F – 114.81, – 115.34 (2 x d, *J* = 247.8 Hz, *F*), – 119.41 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.60, 7.45 (2 x d, $J_{H–H}$ = 7.57 Hz, 1H, *H*-6), 7.40 – 7.35 (m, 2H, Ar*H*), 7.29 – 7.19 (m, 3H, Ar*H*), 6.38 – 6.32 (m, 1H, *H*-1'), 5.96, 5.90 (2 x d, $J_{H–H}$ = 7.57 Hz, *H*-5), 5.30 – 5.23 (m, 1H, *H*-3'), 4.56 – 4.38 (m, 3H, 2 x *H*-5', *H*-4'), 4.12 – 3.98 (m, 3H, OCH_2CH_2, C*H*CH_3), 1.64 – 1.58 (m, 2H, OCH_2CH_2), 1.51, 1.50 (2 x s, 9H, C(CH_3)$_3$), 1.41 – 1.28 (m, 7H, 2 x CH_2 ester, CHCH_3), 0.92 – 0.89 (m, 3H, CH_3 ester).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-*O*-[phenyl(pentoxy-L-alaninyl)]phosphate (6b) was obtained from the 2′-deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl(pentoxy-L-alaninyl)]phosphate **5b** (0.53 mmol, 0.35 g) as a white solid. Yield, 84% (0.24 g). (ES+) *m/z*, found: (M + Na^+) 583.17 $C_{23}H_{31}F_2N_4O_8NaP$ required: (M^+), 560.18. Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.77, 3.69. ^{19}F NMR (470 MHz, MeOD): δ_F – 117.92, – 118.10 (2 x d, *J* = 237.4 Hz, *F*), – 119.40 (d, *J* = 247.0 Hz, broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.59, 7.53 (2 x d, *J* = 7.37 Hz, 1H, *H*-6), 7.40 – 7.36 (m, 2H, Ar*H*), 7.28 – 7.19 (m, 3H, Ar*H*), 6.30 – 6.25 (m, 1H, *H*-1'), 5.93 – 5.88 (2 x d, *J* = 7.37 Hz, *H*-5), 4.56 – 4.37 (m, 2H, *H*-5'), 4.28 – 4.22 (m, 1H, *H*-3'), 4.13 – 4.05 (m, 3H, *H*-4', OCH_2CH_2), 4.02 – 3.96 (m, 1H, C*H*CH_3), 1.65 – 1.61 (m, 2H, OCH_2CH_2), 1.39 – 1.32 (m, 7H, 2 x CH_2 ester, CHCH_3), 0.92 – 0.89 (2 x t, *J* = 6.07 Hz, 3H, CH_3 ester). ^{13}C NMR (125 MHz, MeOD): δ_C 175.18, 174.92 (2 x d, $^3J_{C–P}$ = 7.75 Hz, *C*=O ester), 167.64 (*C*-NH_2, base), 157.73 (*C*=O base), 152.15 (d, $^2J_{C–P}$ = 10.7 Hz, *C*O-Ar), 142.40, 142.38 (*C*H-base), 130.2 (d, $^3J_{C–P}$ = 1.88 Hz, *C*H-Ar), 126.34 (*C*H-Ar), 124.8 (d, $^1J_{C–F}$ = 257.9 Hz, *C*F_2), 121.46, 121.42 (2 x d, $^3J_{C–P}$ = 1.91 Hz, *C*H-Ar), 96.77, 96.75 (*C*H-base), 85.96 (broad signal, *C*-1'), 80.36 (*C*-4'), 71.40, 71.32 (2 x d, $^2J_{C–F}$ = 34.4 Hz, *C*-3'), 66.54, 66.50 (O*C*H_2 ester), 65.76, 65.65 (2 x d, $^2J_{C–P}$ = 5.90 Hz, *C*-5'), 51.81 (d, $^2J_{CP}$ = 5.34 Hz, *C*HCH_3), 29.40, 29.13, 23.36 (*C*H_2 ester), 20.60, 20.43 (2 x d, $^3J_{C–P}$ = 8.42 Hz, CH*C*H_3), 14.35 (*C*H_3 ester). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 24.35 min and t_R = 24.97 min (47%, 52%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl (hexoxy-L-alaninyl)]phosphate (5c) was prepared according to the general procedure **A** from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.55 mmol, 0.20 g), *tert*-BuMgCl (0.66 mmol, 0.66 mL) and phenyl(hexoxy-L-alaninyl) phosphorochloridate (1.10 mmol, 0.38 g). Purification by column chromatography with gradient of CH_2Cl_2/MeOH (100% to 95:5%) afforded the title compound as a white solid. Yield, 58% (0.21 g). Mixture of diastereoisomers (45%, 55%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.72, 3.64. ^{19}F NMR (470 MHz, MeOD): δ_F –115.01, – 115.54 (2 x d, *J* = 242.0 Hz, *F*), – 119.40 (broad signal, *J* = 253.0 Hz, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.60, 7.47 (2 x d, *J* = 7.40 Hz, 1H, *H*-6), 7.41 – 7.36 (m, 2H, Ar*H*), 7.29 – 7.19 (m,

3H, Ar*H*), 6.36 – 6.30 (m, 1H, *H*-1'), 5.96, 5.84 (2 x d, *J* = 7.40 Hz, *H*-5), 5.29 – 5.20 (m, 1H, *H*-3'), 4.56 – 4.37 (m, 3H, 2 x *H*-5', *H*-4'), 4.15 – 4.09 (m, 2H, OCH_2), 4.03 – 3.97 (m, 1H, C*H*CH_3), 1.66 – 1.60 (m, 2H, OCH_2CH_2), 1.52, 1.50 (2 x s, 9H, $C(CH_3)_3$), 1.41 – 1.30 (m, 9H, 3 x CH_2 ester, CHCH_3), 0.92 – 0.89 (m, 3H, CH_3 ester).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[phenyl(hexoxy-L-alaninyl)]phosphate (6c) was obtained from 2′-deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl(hexoxy-L-alaninyl)]phosphate (**5c**) (0.31 mmol, 0.21 g) as a white solid. Yield, 37% (0.066 g). (ES+) *m/z*, found: (M + Na^+) 597.2650. $C_{24}H_{33}F_2N_4O_8NaP$ required: (M^+) 574.20. Mixture of diastereoisomers (45%, 55%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.77, 3.68. ^{19}F NMR (470 MHz, MeOD): δ_F – 117.84, – 118.35 (2 x d, *J* = 238.0 Hz, *F*), – 119.96 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.60, 7.54 (2 x d, *J* = 4.09 Hz, 1H, *H*-6), 7.41 – 7.37 (m, 2H, Ar*H*), 7.29 – 7.20 (m, 3H, Ar*H*), 6.30 – 6.25 (m, 1H, *H*-1'), 5.93, 5.88 (2 x d, *J* = 4.09 Hz, *H*-5), 4.56 – 4.37 (m, 2H, 2 x *H*-5'), 4.28 – 4.21 (m, 1H, *H*-3'), 4.14 – 4.09 (m, 3H, *H*-4', OCH_2), 4.02 – 3.96 (m, 1H, C*H*CH_3), 1.65 – 1.60 (m, 2H, OCH_2CH_2), 1.40 – 1.32 (m, 9H, 3 x CH_2 ester, CHCH_3), 0.92 – 0.90 (m, 3H, CH_3 ester). ^{13}C NMR (125 MHz, MeOD): δ_C 175.16, 174.94 (2 x d, $^3J_{C-P}$ = 4.41 Hz, *C*=O ester), 167.63 (*C*-NH_2, base), 157.70 (*C*=O base), 152.16 (d, $^2J_{C-P}$ = 6.93 Hz, *C*O-Ar), 142.41, 142.38 (*C*H-base), 130.88 (d, $^3J_{C-P}$ = 3.48 Hz, *C*H-Ar), 126.32 (*C*H-Ar), 124.50 (d, $^1J_{C-F}$ = 258.0 Hz, *C*F_2), 121.45, 121.41 (2 x d, $^3J_{C-P}$ = 2.31 Hz, *C*H-Ar), 96.73, 96.70 (*C*H-base), 86.21, 85.98 (broad signal, *C*-1'), 80.37 (*C*-4'), 71.40, 70.97 (2 x d, $^2J_{C-F}$ = 22.40 Hz, *C*-3'), 66.53, 66.52 (O*C*H_2 ester), 65.75, 65.62 (2 x d, $^2J_{C-P}$ = 4.97 Hz, *C*-5'), 51.80, 51.66 (2 x d, $^2J_{C-P}$ = 5.34 Hz, *C*HCH_3), 32.58 (OCH_2CH_2), 29.97, 29.66, 26.63, 23.36 (*C*H_2 ester), 20.56, 20.39 (2 x d, $^3J_{C-P}$ = 6.90 Hz, CH*C*H_3), 14.37 (*C*H_3 ester). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 26.27 min and t_R = 26.85 min (47%, 52%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-O-[phenyl-(2,2-dimethylpropoxy-L-alaninyl)]-phosphate (5d) was prepared according to the general procedure **A** from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.23 g, 0.63 mmol), *tert*-BuMgCl (0.75 mL, 0.75 mmol) and phenyl(2,2-dimethylpropoxy-L-alaninyl) phosphorochloridate (0.42 g, 1.26 mmol). Purification by column chromatography with gradient of CH_2Cl_2/MeOH (100% to 95:5%) afforded the title compound as a white solid. Yield, 34% (0.28 g). Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.76, 3.72. ^{19}F NMR (470 MHz, MeOD): δ_F – 114.9 (d, *J* = 252 Hz), – 115.0 (d, *J* = 252 Hz, *F*), – 119.2, (– 120.5) (broad signal, *F*). ^{1}H NMR (MeOD, 500 MHz) δ_H 7.87, 7.73 (2 x d, 1H, *J* = 7.20 Hz, *H*-6), 7.39 – 7.19 (m, 5H, Ar*H*), 6.33 – 6.27 (m, 1H *H*-1'), 6.12, 6.02 (2 x d, 1H, *J* = 7.20 Hz, *H*-5), 5.30 – 5.23 (m, 1H, *H*-3'), 4.56 – 4.42 (m, 1H, *H*-4'), 4.05 – 3.98 (m, 2H, C*H*CH_3, *H*-5'), 3.88 – 3.67 (m, 3H, OC$H_2C(CH_3)_3$, *H*-5'), 1.50, 1.49 (2 x s, 9H, $C(CH_3)_3$), 1.43 – 1.38 (m, 3H, CHCH_3), 0.98, 0.95 (2 x s, 9H, $OCH_2C(CH_3)_3$).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-*O*-[phenyl-(2,2-dimethylpropoxy-L-alaninyl)]-phosphate (6d) was prepared according to the standard procedure **B** from 2′-deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl(2,2-dimethylpropoxy-L-alaninyl)]phosphate (**5d**) (0.28 g, 0.42 mmol), DCM (5 mL), TFA (5 mL). Purification of the crude compound gave the target product **6d** as a white solid. Yield, 35% (0.083 g). (ES+) *m/z*, found: (M + Na^+) 583.1786.

$C_{23}H_{31}F_2N_4O_8NaP$ required: (M^+), 560.48. Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.81, 3.69. ^{19}F NMR (470 MHz, MeOD): δ_F – 117.8, – 118.0 (2 x d, *J* = 239 Hz, *F*), – 119.5 (– 120.05) (broad signal, *F*). ^1H NMR (500 MHz, MeOD): δ_H 7.58, 7.53 (2 x d, *J* = 7.54 Hz, 1H, *H*-6), 7.35 – 7.24 (m, 2H, Ar*H*), 7.28 – 7.19 (m, 3H, Ar*H*), 6.28 (apparent quartet, *J* = 7.90 Hz, 1H, *H*-1'), 5.92, 5.88 (2 x d, *J* = 7.54 Hz, 1H, *H*-5), 4.56 – 4.36 (m, 2H, *H*-5'), 4.27 – 4.21 (m, 1H, *H*-3'), 4.13 – 4.09 (m, 1H, *H*-4'), 4.06 – 3.99 (m, 1H, C*H*CH_3), 3.88 – 3.75 (m, 2H, OCH_2C$(CH_3)_3$), 1.42 – 1.38 (m, 3H, CHCH_3), 0.95, 0.94 (2 x s, 9H, $OCH_2C(CH_3)_3$). ^{13}C NMR (125 MHz, MeOD): δ_C 175.13, 174.92 (2 x d, $^3J_{C-P}$ = 4.93 Hz, *C*=O, ester), 167.66 (*C*-NH_2), 157.76, 157.72 (*C*=O, base), 152.17, 152.12 (*C*O-Ar), 142.48, 142.35 (*C*H-base), 130.93, 130.90, 126.35 (*C*H-Ar), 124.59, 124.53 (2 x d, $^1J_{C-F}$ = 259 Hz, CF_2), 121.47, 121.44 (*C*H-Ar), 96.78, 96.75 (*C*H-base), 85.99 (broad t, $^2J_{C-F}$ = 27.0 Hz, *C*-1'), 80.36 (apparent t, $^3J_{C-F}$ = 8.0 Hz, *C*-4'), 75.51, 75.49 (OCH_2C$(CH_3)_3$), 71.44, 71.06 (2 x d, $^2J_{C-F}$ = 23.0 Hz, *C*-3'), 65.85, 65.72 (2 x d, $^2J_{C-P}$ = 5.07 Hz, *C*-5'), 51.93, 51.7 (*C*HCH_3), 32.37, 32.35 ($OCH_2C(CH_3)_3$), 26.80 ($OCH_2C(CH_3)_3$), 20.75, 20.55 (2 x d, $^3J_{C-P}$ = 6.44 Hz, CH*C*H_3). Reverse HPLC, eluting with H_2O/CH_3CN from 100/0 to 0/100 in 35 min, showed one peak of diastereoisomers with t_R = 19.55 min (99%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-O-[phenyl -(cyclohexoxy-L-alaninyl)]-phosphate (5e) was prepared according to the standard procedure **A**, from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.13 g, 0.36 mmol), phenyl(cyclohexoxy-L-alaninyl) phosphorochloridate (0.24 g, 0.72 mmol), *t*BuMgCl (0.43 mmol, 0.43 mL), and anhydrous THF (8 mL). Column purification furnished the compound **5e** as a white solid. Yield, 83% (0.20 g). Mixture of diastereoisomers. ^{31}P NMR (202 MHz, MeOD): δ_P 3.86, 3.78. ^{19}F NMR (470 MHz, MeOD): δ_F – 114.9, – 115.0 (2 x d, *J* = 248 Hz, *F*), – 119.10, (– 119.03) (broad signal, *F*). ^1H NMR (500 MHz, MeOD): δ_H 7.98, 7.84 (2 x d, *J* = 7.50 Hz, *H*-6), 7.38 – 7.13 (m, 5H, Ar*H*), 6.32 – 6.14 (m, 2H, *H*-1', *H*-5), 5.36 – 5.29 (m, 1H, *H*-3'), 4.81 – 4.76 (m, 1H, OC*H*-ester), 4.54 – 4.49 (m, 3H, 2 x *H*-5', *H*-4'), 3.98 – 3.96 (m, 1H, C*H*CH_3), 1.84 – 1.72 (m, 4H, 2 x CH_2-ester), 1.76 – 1.73 (m, 2H, CH_2-ester), 1.53 – 1.35 (m, 18H, 3 x CH_2-ester, C(C$H_3)_3$, CHCH_3).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-*O*-[phenyl-(cyclohexoxy-L-alaninyl)]-phosphate (6e) was prepared according to the standard procedure **B**, from the 2′-deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl-(cyclohexoxy)-L-alaninyl)]phosphate **5e** (0.20 g, 0.29 mmol), DCM (3 mL), TFA (3 mL). Column purification gave the target product **6e** as a white solid. Yield, 18% (0.030 g). (ES+) *m/z*, found: (M + Na^+) 595.2650. $C_{24}H_{31}F_2N_4O_8NaP$ required: (M^+), 572.50. Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.79, 3.71. ^{19}F NMR (470 MHz, MeOD): δ_F – 118.09, – 118.24 (2 x d, *J* = 241 Hz, 1*F*), – 119.8 (– 119.7) (broad signal, 1*F*). ^1H NMR (500 MHz, MeOD): δ_H 7.59, 7.54 (2 x d, *J* = 7.26 Hz, *H*-6, 7.41 – 7.36 (m, 2H, Ar*H*), 7.29 – 7.20 (m, 3H, Ar*H*), 6.29 – 6.24 (m, 1H, *H*-1'), 5.91, 5.87 (2 x d, *J* = 7.26 Hz, *H*-5), 4.78 – 4.73 (m, 1H, OC*H*-ester), 4.56 – 4.36 (m, 2H, *H*-5'), 4.26 – 4.20 (m, 1H, *H*-3'), 4.13 – 4.07 (m, 1H, *H*-4'), 3.98 – 3.92 (m, 1H, C*H*CH_3), 1.84 – 1.81 (2H, CH_2-ester), 1.76 – 1.73 (m, 2H, CH_2-ester), 1.57 – 1.29 (m, 9H, 3 x CH_2-ester, CHCH_3). ^{13}C NMR (125 MHz, MeOD): δ_C 174.55, 174.34 (2 x d, $^3J_{C-P}$ = 3.77 Hz, *C*=O, ester), 167.67 (*C*-NH_2), 157.78, 157.75 (*C*=O base), 152.18, 152.13 (2 x d, $^3J_{C-P}$ = 6.88 Hz, *C*O-Ar), 142.47, 142.35 (*C*H-base), 130.89, 130.85 126.30 (*C*H-Ar), 124.53, 122.46 (2 x d, $^1J_{C-F}$ =

258 Hz, *C*F_2), 121.42, 121.39 (2 x d, $^4J_{C-P}$ = 1.1 Hz, *C*H-Ar), 96.68, 96.65 (*C*H-base), 86.12, 85.84 (2 x d, $^2J_{C-F}$ = 27.0 Hz *C*-1'), 80.34 (d, $^3J_{C-F}$ = 7.5 Hz, *C*-4'), 74.98, 74.76 (C*H*-ester), 71.43, 71.05 (2 x d, $^2J_{C-F}$ = 23.0 Hz, *C*-3'), 65.80, 65.65 (2 x d, $^2J_{C-P}$ = 4.40 Hz, *C*-5'), 51.80 (*C*HCH$_3$), 32.48, 32.40 (2 x *C*H_2-ester), 26.40, 24.61 (*C*H_2-ester), 20.57, 20.42 (2 x d, $^3J_{C-P}$ = 7.33 Hz, CH*C*H$_3$). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 24.11 min and t_R = 24.79 min (40%, 56%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl (benzoxy-L-alaninyl)] phosphate (5f) was prepared according to the standard procedure **A**, from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.40 g, 1.10 mmol), phenyl(benzoxy-L-alaninyl) phosphorochloridate (0.78 g, 2.20 mmol), *t*BuMgCl (1.32 mmol, 1.32 mL) and anhydrous THF (30 mL). Column purification gave the compound **5f** as a white solid. Yield, 85% (0.64 g). Mixture of diastereoisomers. ^{31}P NMR (202 MHz, MeOD): δ_P 3.74, 3.55. ^{19}F NMR (470 MHz, MeOD): δ_F – 118.07, – 118.29 (2 x d, *J* = 239 Hz, 1*F*), – 119.52 (–120.57) (broad signal, 1*F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.74, 7.61 (2 x d, *J* = 7.34 Hz, *H*-6), 7.37 – 7.18 (m, 10H, Ar*H*), 6.32 – 6.25 (m, 1H, *H*-1'), 6.04, 5.95 (2 x d, *J* = 7.34 Hz, *H*-5), 5.32 – 5.28 (m, 1H, *H*-3'), 5.13 – 5.07 (m, 2H, OCH_2Ph), 4.50 – 4.37 (m, 3H, 2 x *H*-5', *H*-4'), 4.01 – 3.96 (m, 1H, C*H*CH$_3$), 1.51, 1.50 (2 x s, 9H, C(CH_3)$_3$), 1.38 (d, *J* = 7.23 Hz, 3H, CHCH_3).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-*O*-[phenyl(benzoxy-L-alaninyl)] phosphate (6f) was prepared according to the standard procedure **B**, from 2′-deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[phenyl(benzoxy-L-alaninyl)] phosphate **5f** (0.60 g, 0.89 mmol), DCM (10 mL), TFA (10 mL). Purification on silica gel afforded the target product as a white solid. Yield, 70% (0.36 g). (ES+) *m/z,* found: (M + Na^+) 603.14. $C_{25}H_{27}F_2N_4O_8NaP$ required: (M^+) 580.47. Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 3.81, 3.64. ^{19}F NMR (470 MHz, MeOD): δ_F – 118.07, – 118.29 (2 x d, *J* = 239 Hz, 1*F*), – 119.52 (–120.57) (broad signal, 1*F*). ^{1}H NMR (500 MHz, MeOD): δ_H 7.56, 7.52 (2 x d, *J* = 7.5 Hz, 1H, *H*-6), 7.38 – 7.33 (m, 7H, Ar*H*), 7.26 – 7.19 (m, 3H, Ar*H*), 6.25 (apparent q, *J* = 7.5 Hz, 1H, *H*-1'), 5.88, 5.84 (2 x d, *J* = 7.5 Hz, 1H, *H*-5), 5.18 – 5.12 (m, 2H, OCH_2Ph), 4.49 – 4.42 (m, 1H, *H*-5'), 4.38 – 4.31 (m, 1H, *H*-5'), 4.25 – 4.18 (m, 1H, *H*-3'), 4.07 – 4.01 (m, 2H, *H*-4', C*H*CH$_3$), 1.38 (apparent t, *J* = 8.5 Hz, 3H, CHCH_3). ^{13}C NMR (125 MHz, MeOD): δ_C 174.86, 174.62 (2 x d, $^3J_{C-P}$ = 4.6 Hz, *C*=O, ester), 167.53 (*C*-NH_2), 158.03 (*C*=O, base), 152.10, 152.05 (*C*-O, Ph), 142.37, 142.28 (*C*H-base), 137.19, 136.88 (*C*-Ar), 130.95, 130.92, 130.23, 130.15, 129.78, 129.70, 129.67, 129.59, 129.55, 129.46, 129.36, 129.31, 129.22, 126.39 (*C*H-Ar), 123.56 (apparent t, $^1J_{C-F}$ = 259 Hz, *C*F_2), 124.21, 124.14, 121.55, 121.46, 121.43 (*C*H-Ar), 97.27, 97.25 (*C*H-base), 86.04, 85.67 (broad signal, *C*-1'), 80.43 (apparent t, $^3J_{C-F}$ = 8.0 Hz, *C*-4'), 71.33, 70.94 (2 x d, $^2J_{C-F}$ = 23.0 Hz, *C*-3'), 68.76, 68.08 (O*C*H_2Ph), 65.69, 65.63 (2 x d, $^2J_{C-P}$ = 5.30 Hz, *C*-5'), 53.73, 53.19 (*C*HCH$_3$), 20.48, 20.33 (2 x d, $^3J_{C-P}$ = 6.44 Hz, CH*C*H$_3$). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 24.19 min and t_R = 24.88 min (47%, 52%).

2′-Deoxy-2′,2′-difluoro-3′-(*tert*-butoxycarbonyloxy)-D-cytidine-5'-O-[1-naphthyl -(pentoxy-dimethylglycinyl)]-phosphate (5g) was prepared according to the standard Procedure **A**, from 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.3 g, 0.83

mmol), 1-naphthyl(pentoxy-dimethylglycinyl) phosphorochloridate (0.66 g, 1.65 mmol), *t*BuMgCl (0.99 mmol, 0.99 mL) and anhydrous THF (25 mL). Column purification gave the compound **5g** as a white solid. Yield 82% (0.49 g). Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 2.58, 2.10. ^{19}F NMR (470 MHz, MeOD): δ_F –114.71 (d, J = 237.4 Hz), –115.23 (d, J = 237.4 Hz) (1F), –118.81 (–119.29) (broad signal, 1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.25 – 8.22 (m, 1H, Ar*H*), 7.92 – 7.90 (m, 1H, Ar*H*), 7.75 – 7.70 (m, 1H, Ar*H*), 7.57 – 7.53 (m, 3H, 2 x Ar*H*, *H*-6), 7.47 – 7.24 (m, 2H, Ar*H*), 6.26 – 6.23 (m, 1H, *H*-1'), 5.74, 5.70 (2 x d, J = 7.5 Hz, 1H, *H*-5), 5.26 – 5.17 (m, 1H, *H*-3'), 4.58 – 4.46 (m, 2H, *H*-5'), 4.38 – 4.32 (m, 1H, *H*-4'), 4.24 – 4.14 (m, 2H, OCH_2CH$_2$), 1.66 – 1.61 (m, 2H, OCH$_2$CH_2), 1.55 – 1.54 (m, 6H, C(CH_3)$_2$), 1.51, 1.49 (2 x s, C(CH_3)$_3$), 1.35 – 1.30 (m, 4H, 2 x CH_2 ester), 0.87 (t, 3H, J = 3.38 Hz, CH_3 ester).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[1-naphthyl-(pentoxy-dimethylglycinyl)] -phosphate (6g) was prepared according to the Standard Procedure **B**, from the compound **5g** (0.49 g, 0.67 mmol), DCM (10 mL), TFA (10 mL). Purification on silica gel afforded the target product as a white solid. Yield 48% (0.20 g). (ES+) *m/z,* found: (M + Na$^+$) 647.19. $C_{28}H_{35}F_2N_4O_8Na$ P required: (M$^+$), 624.57. Mixture of diastereoisomers (48%, 52%). ^{31}P NMR (202 MHz, MeOD): δ_P 2.70, 2.54. ^{19}F NMR (470 MHz, MeOD): δ_F –117.57 (d, J = 237.0 Hz), –117.85 (d, J = 237.0 Hz) (1F), –119.47 (–119.97) (broad signal, 1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.25 – 8.23 (m, 1H, Ar*H*), 7.92 – 7.88 (m, 1H, Ar*H*), 7.74 – 7.71 (m, 1H, Ar*H*), 7.57 – 7.54 (m, 3H, Ar*H*), 7.47 – 7.39 (m, 2H, 1 x Ar*H*, *H*-6), 6.25 – 6.18 (m, 1H, *H*-1'), 5.72, 5.69 (2 x d, J = 7.5 Hz, 1H, *H*-5), 4.58 – 4.54 (m, 1H, *H*-5'), 4.49 – 4.45 (m, 1H, *H*-5'), 4.29 – 4.14 (m, 1H, *H*-3'), 4.12 – 4.08 (m, 3H, *H*-4', OCH_2CH$_2$), 1.63 – 1.60 (m, 2H, OCH$_2$CH_2), 1.55 – 1.54 (m, 6H, C(CH_3)$_2$), 1.34 – 1.30 (m, 4H, 2 x CH_2 ester), 0.87 (t, 3H, J = 3.38 Hz, CH_3 ester). ^{13}C NMR (125 MHz, MeOD): δ_C 176.81 (d, $^3J_{C\text{-}P}$ = 3.73 Hz, *C*=O, ester), 167.53 (*C*-NH$_2$ base), 157.63 (*C*=O, base), 148.13, 148.15 (2 x d, $^2J_{C\text{-}P}$ = 8.80 Hz, *C*O-Ar), 142.43, 142.37 (*C*H-base), 136.34 (*C*-Ar), 128.92, 127.94, 127.86, 127.84, 127.50, 127.44, 126.54, 126.51, 126.04, 125.97, 125.52 (*C*H-Ar), 123.46 (apparent t, $^1J_{C\text{-}F}$ = 261.0 Hz, *C*F$_2$), 122.93, 121.40 (*C*H-Ar), 116.52, 116.33 (2 x d, $^3J_{C\text{-}P}$ = 4.20 Hz, *C*H-Ar), 96.58 (*C*H-base), 85.71 (broad signal, *C*-1'), 80.28 (*C*-4'), 71.30, 71.07 (2 x d, $^2J_{C\text{-}F}$ = 22.5 Hz, *C*-3'), 66.77 (O*C*H$_2$ ester), 65.82, 65.74 (2 x d, $^2J_{C\text{-}P}$ = 5.2 Hz, *C*-5'), 58.29 (*C*(CH$_3$)$_2$), 29.34, 29.18 (*C*H$_2$ ester), 27.93, 27.78, 27.68 (3 x d, $^3J_{C\text{-}P}$ = 5.5 Hz, (C(*C*H$_3$)$_2$), 23.33 (*C*H$_2$ ester), 14.32 (*C*H$_3$ ester). Reverse HPLC, eluting with H$_2$O/MeOH from 100/0 to 0/100 in 35 min, showed one peaks of diastereoisomers with t_R = 28.04 min (99%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphate (5h) was prepared according to the general procedure **A** using 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.20 g, 0.55 mmol), 1-naphthyl(hexoxy-L-alaninyl) phosphorochloridate (0.44 g, 1.10 mmol) and *t*BuMgCl (0.66 mmol, 0.66 mL). Purification by column chromatography with gradient of CH$_2$Cl$_2$/MeOH (100% to 95:5%) afforded the title compound **5h** as a white solid. Yield, 45% (0.18 g). Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.13, 4.06. ^{19}F NMR (470 MHz, MeOD): δ_F – 114.81, –115.33 (2 x d, J = 251 Hz, *F*), – 119.21 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 8.22 – 8.19 (m, 1H, Ar*H*), 7.91 – 7.88 (m, 1H, Ar*H*), 7.75 – 7.70 (m, 1H, Ar*H*), 7.58 – 7.53 (m, 3H, Ar*H*), 7.46 – 7.24 (m, 2H, 1 x Ar*H*, *H*-6), 6.34 – 6.23 (m, 1H, *H*-1'), 5.83, 5.70 (2

x d, J = 7.40 Hz, *H*-5), 5.26 – 5.19 (m, 1H, *H*-3'), 4.61 – 4.35 (m, 3H, 2 x *H*-5', *H*-4'), 4.11 – 4.01 (m, 3H, OCH_2CH$_2$, C*H*CH$_3$), 1.59 – 1.47 (m, 11H, OCH$_2$CH_2, C(CH_3)$_3$), 1.39 (d, J = 3.51 Hz, 3H, CHCH_3), 1.33 – 1.21 (m, 6H, 3 x CH_2 ester), 0.89 – 0.84 (m, 3H, CH_3 ester).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5'-O-[1-naphthyl(hexoxy-L-alaninyl)] phosphate (6h) was prepared according to the standard procedure **B** from **5h** (0.16 g, 0.22 mmol), DCM (2 mL), TFA (2 mL). Purification on silica gel afforded the target product as a white solid. Yield, 22% (0.030 g). (ES+) *m/z,* found: (M + Na^+) 647.19. $C_{28}H_{35}F_2N_4O_8NaP$ required: (M^+) 624.22. Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.16, 4.12. ^{19}F NMR (470 MHz, MeOD): δ_F –117.58, –118.08 (2 x d, J = 245 Hz, *F*), – 119.92 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 8.22 – 8.19 (m, 1H, Ar*H*), 7.93 – 7.90 (m, 1H, Ar*H*), 7.76 – 7.72 (m, 1H, Ar*H*), 7.58 – 7.39 (m, 5H, 4 x Ar*H*, *H*-6), 6.26 – 6.22 (m, 1H, *H*-1'), 5.78, 5.72 (2 x d, J = 7.20 Hz, *H*-5), 4.60 – 4.41 (m, 2H, *H*-5'), 4.26 – 4.17 (m, 1H, *H*-3'), 4.12 – 4.01 (m, 4H, 1 x *H*-4', OCH_2CH$_2$, C*H*CH$_3$), 1.60 – 1.57 (m, 2H, OCH$_2$CH_2), 1.37 – 1.34 (m, 4H, 2 x CH_2 ester), 1.32 – 1.29 (m, 5H, 1 x CH_2 ester, CHCH_3), 0.89 – 0.84 (m, 3H, CH_3 ester). ^{13}C NMR (125 MHz, MeOD): δ_C 175.15 (*C*=O, ester), 167.54 (*C*-NH$_2$), 157.63 (*C*=O, base), 147.97 (*C*-OAr), 142.38, 142.28 (*C*H-base), 136.35 (*C*-Ar), 128.97, 128.95, 127.92, 127.88, 127.62, 127.54, 126.56 (*C*H-Ar), 123.50 (broad signal, CF_2), 116.34, 116.32, 116.26, 116.24 (*C*H-Ar), 96.63, 96.58 (*C*H-base), 86.24 (broad signal, *C*-1'), 80.33 (broad d, $^3J_{C-F}$ = 8.0 Hz, *C*-4'), 71.15, 71.03 (2 x d, $^2J_{C-F}$ = 23.0 Hz, *C*-3'), 66.53, 66.49 (O*C*H$_2$), 65.85, 65.81 (2 x d, $^2J_{C-P}$ = 5.20 Hz, *C*-5'), 51.89, 51.80 (*C*HCH$_3$), 32.55, 32.53, 29.62, 26.60, 23.56 (*C*H$_2$ ester), 20.50, 20.37 (2 x d, $^3J_{C-P}$ = 7.50 Hz, CH*C*H$_3$), 14.38 (*C*H$_3$ ester). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 29.08 min and t_R = 29.17 min (44%, 52%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-O-[1-naphthyl(cyclopentoxy-L-alaninyl)]phosphate (5i) was prepared according to the general procedure **A** using 3′-O-(*tert*-butoxycarbonyl)-gemcitabine (0.25 g, 0.68 mmol), *tert*-BuMgCl (0.82 mL, 0.82 mmol) and 1-naphthyl(cyclopentoxy-L-alaninyl) phosphorochloridate (0.52 g, 1.37 mmol). Purification by column chromatography with gradient of CH_2Cl_2/MeOH (100% to 95:5%) afforded the title compound as a white solid. Yield 60% (0.29 g). Mixture of diastereoisomers (52%, 48%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.16, 4.08. ^{19}F NMR (470 MHz, MeOD): δ_F –114.75 (d, J = 245.0 Hz), –115.42 (d, J = 245.0 Hz, 1F), –119.22 (–120.57, broad signal, 1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.30 – 8.16 (m, 1H, Ar*H*), 7.91 – 7.87 (m, 1H, Ar*H*), 7.80 – 7.70 (m, 1H, Ar*H*), 7.58 – 7.50 (m, 2H, Ar*H*), 7.46 – 7.31 (m, 3H, 2 x Ar*H*, 1 x *H*-6), 6.33 – 6.26 (m, 1H, *H*-1'), 5.83, 5.75 (2 x d, J = 7.34 Hz, *H*-5), 5.25 – 5.08 (m, 1H, *H*-3'), 4.60 – 4.43 (m, 2H, *H*-5'), 4.39 – 4.32 (m, 1H, *H*-4'), 4.06 – 3.94 (m, 1H, OC*H* ester), 3.93 – 3.89 (m, 1H, C*H*CH$_3$), 1.87 – 1.53 (m, 8H, 4 x CH_2 ester), 1.50 – 1.47 (m, 9H, C(CH_3)$_3$), 1.37 – 1.32 (m, 3H, CHCH_3).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[1-naphthyl(cyclopentoxy-L-alaninyl)] phosphate (6i) was prepared according to the Standard Procedure **B**, from the compound **5i** (0.29 g, 0.41 mmol), DCM (10 mL), TFA (10 mL). Purification on silica gel afforded the target product as a white solid. Yield (0.12 g, 50%). (ES+) *m/z*, found: (M + Na^+) 631.18 $C_{27}H_{31}F_2N_4O_8NaP$ required: (M^+) 608.53. Mixture of diastereoisomers (52%, 48%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.21, 4.15. ^{19}F NMR

(470 MHz, MeOD): δ_F –117.55 (d, J = 242.4 Hz), –118.09 (d, J = 242.0 Hz, 1F), –119.90 (broad signal, 1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.21 – 8.18 (m, 1H, Ar*H*), 7.93 – 7.90 (m, 1H, Ar*H*), 7.77 – 7.72 (m, 1H, Ar*H*), 7.58 – 7.50 (m, 3H, Ar*H*), 7.47 – 7.38 (m, 2H, 1 x Ar*H*, 1 x *H*-6), 6.25 – 6.22 (m, 1H, *H*-1'), 5.77, 5.71 (2 x d, J = 7.20 Hz, *H*-5), 5.17 – 5.07 (m, 1H, OC*H* ester), 4.60 – 4.41 (m, 2H, *H*-5'), 4.25 – 4.17 (m, 1H, *H*-3'), 4.12 – 4.07 (m, 1H, *H*-4'), 4.03 – 3.97 (m, 1H, C*H*CH$_3$), 1.85 – 1.81 (m, 2H, CH_2 ester), 1.69 – 1.57 (m, 6H, 3 x CH_2 ester), 1.34 (d, J = 4.57 Hz, 3H, CHCH_3). ^{13}C NMR (125 MHz, MeOD): δ_C 174.93, 174.66 (2 x d, $^3J_{C\text{-}P}$ = 4.6 Hz, *C*=O ester), 167.61 (*C*-NH$_2$), 157.70 (*C*=O base), 147.94 (d, $^2J_{C\text{-}P}$ = 8.8 Hz, *C*O-Ar), 142.39, 142.27 (*C*H-base), 136.35 (*C*-Ar), 128.98, 127.93, 127.90 (*C*H-Ar), 127.86, 127.81 (*C*-Ar), 127.63, 127.54, 126.57, 126.54, 126.17, 126.13 (*C*H-Ar), 123.56 (apparent t, $^1J_{C\text{-}F}$ = 259 Hz, *C*F$_2$), 116.35, 116.33, 116.28, 116.25 (*C*H-Ar), 96.63, 96.59 (*C*H-base), 86.50 (broad signal, *C*-1'), 80.43 (broad signal, *C*-4'), 79.60, 79.57 (O*C*H ester), 71.43, 71.23 (2 x d, $^2J_{C\text{-}F}$ = 16.5 Hz, *C*-3'), 65.86, 65.65 (2 x d, $^2J_{C\text{-}P}$ = 5.30 Hz, *C*-5'), 51.91 (d, $^2J_{C\text{-}P}$ = 10.43 Hz, *C*HCH$_3$), 33.57, 33.49, 33.47 (*C*H$_2$ ester), 24.63, 24.62 (*C*H$_2$ ester), 20.46, 20.32 (2 x d, $^3J_{C\text{-}P}$ = 7.5 Hz, CH*C*H$_3$). Reverse HPLC, eluting with H$_2$O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 24.21 min and t_R = 24.76 min (45%, 52%).

2′-Deoxy-2′,2′-difluoro-3′-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-O-[1-naphthyl -(cyclohexoxy-L-alaninyl)]-phosphate (5j) was prepared according to the Standard Procedure **A**, from 3′-O-(*tert*-butoxycarbonyl)-gemcitabine (0.25 g, 0.68 mmol), 1-naphthyl(cyclohexoxy-L-alaninyl) phosphorochloridate (0.54 g, 1.37 mmol), *t*BuMgCl (0.83 mmol, 0.83 mL), and anhydrous THF (20 mL). Column purification furnished the compound **5j** as a white solid. Yield 94% (0.46 g). Mixture of diastereoisomers (52%, 48%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.24, 4.13.^{19}F NMR (470 MHz, MeOD): δ_F –115.23 (d, J = 245.0 Hz), –115.65 (d, J = 242.0 Hz), (1F), –119.52 (– 119.48, broad signal), (1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.28 – 8.18 (m, 2H, Ar*H*), 7.94 – 7.89 (m, 1H, Ar*H*), 7.83 – 7.80 (m, 1H, Ar*H*), 7.76, 7.74 (2 x d, J = 7.63 Hz, *H*-6), 7.59 – 7.51 (m, 3H, Ar*H*), 6.25 (apparent quartet, J = 9.22 Hz, *H*-1'), 5.87, 5.73 (2 x d, J = 7.63 Hz, *H*-5), 5.23 – 5.19 (m, 1H, *H*-3'), 4.77 – 4.69 (m, 1H, OC*H*), 4.61 – 4.38 (m, 3H, 2 x *H*-5', *H*-4'), 4.08 – 3.94 (m, 1H, C*H*CH$_3$), 1.80 – 1.63 (m, 4H, 2 x CH_2-ester), 1.51, 1.50 (2 x s, 9H, C(CH_3)$_3$), 1.39 – 1.25 (m, 9H, 3 x CH_2 ester, 3H, CHCH_3).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[1-naphthyl-(cyclohexoxy-L-alaninyl)]-phosphate (6j) was prepared according to the Standard Procedure **B**, from **5j** (0.46 g, 0.64 mmol), DCM (4 mL), TFA (4 mL). Column purification gave the target product as a white solid. Yield 17% (0.068 g). (ES+) *m/z*, found: (M + Na$^+$) 645.20 $C_{28}H_{33}F_2N_4O_8NaP$ required: 622.55 (M$^+$). Mixture of diastereoisomers (52%, 48%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.17, 4.15. ^{19}F NMR (470 MHz, MeOD): δ_F –117.70 (d, J = 237 Hz), –117.97 (d, J = 237 Hz), (1F), –119.47 (– 119.46, broad signal), (1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.21 – 8.19 (m, 1H, Ar*H*), 7.92 – 7.90 (m, 1H, Ar*H*), 7.76 – 7.72 (m, 1H, Ar*H*), 7.58 – 7.51 (m, 3H, Ar*H*), 7.47 – 7.39 (m, 2H, *H*-6, Ar*H*), 6.25 (apparent t, J = 7.80 Hz, *H*-1'), 5.75, 5.73 (2 x d, J = 7.60 Hz, *H*-5), 4.74 – 4.69 (m, 1H, OC*H*-ester), 4.60 – 4.41 (m, 2H, *H*-5'), 4.25 – 4.16 (m, 1H, *H*-3'), 4.11 – 4.08 (m, 1H, *H*-4'), 4.04 – 3.99 (m, 1H, C*H*CH$_3$), 1.81 – 1.69 (m, 4H, 2 x CH_2-ester), 1.55 – 1.50 (m, 1H, CH_2-ester), 1.42 – 1.26 (m, 8H, 5 x CH_2-ester, 3 x H, CHCH_3). ^{13}C NMR (125 MHz, MeOD): δ_C 174.50, 174.30 (2 x d, $^3J_{C\text{-}P}$ = 3.80 Hz, *C*=O, ester), 167.59 (*C*=O, base), 157.70, 157.68 (*C*-NH$_2$, base), 152.18, 152.14 (*C*-Ar), 142.37,

142.25 (*C*H-base), 136.34, 135.91 (*C*-Ar), 128.95, 127.89, 127.62, 127.51, 126.56, 126.14, 122.71, 122.63 (*C*H-Ar), 123.30, 123.21 (broad signal, 2 x d, $^{1}J_{C\text{-}F}$ = 256 Hz, *C*F_2), 121.27, 121.16 (broad signal, 2 x d, $^{1}J_{C\text{-}F}$ = 256.0 Hz, *C*F_2), 116.32, 116.24 (*C*H-Ar), 96.60, (*C*H-base), 86.10 (broad signal *C*-1'), 80.26 (d, $^{3}J_{C\text{-}F}$ = 8.75 Hz, *C*-4'), 75.01 (C*H*-ester), 71.40, 71.30 (broad signal, *C*-3'), 65.85 (*C*-5'), 51.97 (d, $^{2}J_{C\text{-}P}$ = 10.94 Hz, *C*HCH_3), 32.42, 32.36 (*C*H_2-ester), 26.38, 26.37 (*C*H_2-ester), 24.60 (*C*H_2-ester), 20.58, 20.40 (2 x d, $^{3}J_{C\text{-}P}$ = 7.66 Hz, CH*C*H_3). Reverse HPLC, eluting with H_2O/CH_3CN from 100/0 to 0/100 in 35 min, showed one peak of diastereoisomers with t_R = 18.04 min (99.9%).

2′-Deoxy-2′,2′-difluoro-3′-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-O-[1-naphthyl (benzoxy-L-alaninyl)] phosphate (5k) was prepared according to the standard Procedure **A**, from 3′-O-(*tert*-butoxycarbonyl)-gemcitabine (0.3 g, 0.82 mmol), 1-naphthyl(benzoxy-L-alaninyl) phosphorochloridate (0.67 g, 1.65 mmol), *t*BuMgCl (0.99 mL, 0.99 mmol) and anhydrous THF (25 mL). Column purification gave the compound **5k** as a white solid. Yield 81% (0.49 g). Mixture of diastereoisomers (52%, 48%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.18. ^{1}H NMR (500 MHz, MeOD): δ_H 7.72 – 7.56 (m, 13H, Ar*H*, *H*-6), 6.23 – 6.19 (m, 1H, *H*-1'), 5.94, 5.80 (2 x d, *J* = 7.20 Hz, 1H, *H*-5), 5.23 – 5.10 (m, 3H, *H*-3', CH_2Ph), 4.51 – 4.48 (m, 3H, *H*-5', *H*-4'), 4.15 – 4.06 (m, 1H, C*H*CH_3), 1.51, 1.50 (2 x s, 9H, C(CH_3)$_3$), 1.38 (d, *J* = 7.0 Hz, CHCH_3).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[1-naphthyl(benzoxy-L-alaninyl)] phosphate (6k) was prepared according to the Standard Procedure **B**, from the compound **5k** (0.49 g, 0.67 mmol), DCM (10 mL), TFA (10 mL). Purification on silica gel afforded the target product as a white solid. Yield 47% (0.20 g). (ES+) *m/z*, found: (M + Na^+) 653.17 $C_{29}H_{29}F_2N_4O_8NaP$ required: 630.53 (M^+). Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.20, 4.10. ^{19}F NMR (470 MHz, MeOD): δ_F –117.7 (d, *J* = 240 Hz), –117.9 (d, *J* = 240 Hz) (1F), –119.3 (–120.5) (broad signal, 1F). ^{1}H NMR (500 MHz, MeOD): δ_H 8.20 – 8.17 (m, 1H, Ar*H*), 7.93 – 7.90 (m, 1H, Ar*H*), 7.73 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.58 – 7.48 (m, 3H, 2 x Ar*H*, *H*-6), 7.44 – 7.29 (m, 7H, Ar*H*), 6.22 (apparent t, *J* = 7.5 Hz, 1H, *H*-1'), 5.79, 5.73 (2 x d, *J* = 7.5 Hz, 1H, *H*-5), 5.15 – 5.10 (m, 2H, CH_2Ph), 4.53 – 4.49 (m, 1H, *H*-5'), 4.44 – 4.36 (m, 1H, *H*-5'), 4.24 – 4.04 (m, 3H, *H*-4', *H*-3', C*H*CH_3), 1.37 (d, 3H, *J* = 7.0 Hz, CH*C*H_3). ^{13}C NMR (125 MHz, MeOD): δ_C 174.88 (d, $^{3}J_{C\text{-}P}$ = 3.6 Hz, *C*=O, ester), 174.58 (d, $^{3}J_{C\text{-}P}$ = 4.5 Hz, *C*=O, ester), 167.57 (*C*-NH_2), 157.73, 157.71 (*C*=O, base), 147.96 (2 x d, $^{2}J_{C\text{-}P}$ = 7.3 Hz, *C*O-Ar), 142.32, 142.21 (*C*H-base), 137.17 (*C*-Ar), 136.33 (*C*-Ar), 129.60, 129.37, 129.33, 129.30, 128.98, 128.96, 127.92, 127.90 (*C*H-Ar), 127.85, 127.80 (*C*-Ar), 127.66, 127.58, 126.59, 126.54, 126.17 (*C*H-Ar), 123.45 (apparent t, $^{1}J_{C\text{-}F}$ = 259 Hz, *C*F_2), 122.72, 122.63, 116.40, 116.33 (2 x d, $^{3}J_{C\text{-}P}$ = 3.6 Hz, *C*H-Ar), 96.65, 96.61 (*C*H-base), 85.94 (broad signal, *C*-1'), 80.26 (*C*-4'), 71.33 (*C*-3'), 68.04 (O*C*H_2Ph), 65.82, 65.75 (2 x d, $^{2}J_{C\text{-}P}$ = 5.0 Hz, *C*-5'), 51.94, 51.82 (*C*HCH_3), 20.40, 20.26 (2 x d, $^{3}J_{C\text{-}P}$ = 7.3 Hz, CH*C*H_3). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 27.35 min and t_R = 27.85 min (42%, 57%).

2′-Deoxy-2′,2′-difluoro-3′-*O*-(*tert*-butoxycarbonyloxy)-D-cytidine-5′-*O*-[1-naphthyl(pentoxy-L-alaninyl)]phosphate (5l) was prepared according to the general procedure **A** using 3′-*O*-(*tert*-butoxycarbonyl)-gemcitabine (0.18 g, 0.49 mmol), 1-

naphthyl(pentoxy-L-alaninyl) phosphorochloridate (0.38 g, 0.99 mmol) and *t*BuMgCl (0.59 mL, 0.59 mmol). Purification by column chromatography with gradient of CH_2Cl_2/MeOH (100% to 95:5%) afforded the title compound **5l** as a white solid. Yield, 43% (0.15 g). Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.13, 4.07. ^{19}F NMR (470 MHz, MeOD): δ_F – 114.92, –115.43 (2 x d, J = 251.1 Hz, *F*), – 118.73 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 8.22 – 8.19 (m, 1H, Ar*H*), 7.93 – 7.89 (m, 1H, Ar*H*), 7.76 – 7.71 (m, 1H, Ar*H*), 7.58 – 7.52 (m, 3H, Ar*H*), 7.47 – 7.26 (m, 2H, Ar*H*, *H*-6), 6.31 – 6.25 (m, 1H, *H*-1'), 5.82, 5.78 (2 x d, J = 7.50 Hz, *H*-5), 5.25 – 5.18 (m, 1H, *H*-3'), 4.61 – 4.34 (m, 3H, 2 x *H*-5', *H*-4'), 4.09 – 4.02 (m, 3H, OCH_2CH$_2$, CHCH$_3$), 1.61 – 1.55 (m, 2H, OCH$_2$CH_2), 1.51, 1.49 (2 x s, 9H, C(CH_3)$_3$), 1.40 – 1.36 (m, 3H, CHCH_3), 1.32 – 1.28 (m, 4H, 2 x CH_2 ester), 0.89 – 0.84 (m, 3H, CH_3 ester).

2′-Deoxy-2′,2′-difluoro-D-cytidine-5′-O-[1-naphthyl(pentoxy-L-alaninyl)] phosphate (6l) was prepared according to the standard procedure **B** from **5l** (0.15 g, 0.21 mmol), DCM (2 mL), TFA (2 mL). Purification on silica gel afforded the target product as a white solid. Yield, 25% (0.032 g). (ES+) *m/z*, found: (M + Na$^+$) 633.20 $C_{27}H_{33}F_2N_4O_8NaP$ required: (M$^+$) 610.54. Mixture of diastereoisomers (49%, 51%). ^{31}P NMR (202 MHz, MeOD): δ_P 4.16, 4.12. ^{19}F NMR (470 MHz, MeOD): δ_F – 117.58, –118.08 (2 x d, J = 245 Hz, *F*), – 119.92 (broad signal, *F*). ^{1}H NMR (500 MHz, MeOD): δ_H 8.22 – 8.19 (m, 1H, Ar*H*), 7.93 – 7.90 (m, 1H, Ar*H*), 7.76 – 7.72 (m, 1H, Ar*H*), 7.58 – 7.39 (m, 5H, 4 x Ar*H*, *H*-6), 6.26 – 6.22 (m, 1H, *H*-1'), 5.78, 5.72 (2 x d, J = 7.20 Hz, *H*-5), 4.60 – 4.41 (m, 2H, *H*-5'), 4.26 – 4.17 (m, 1H, *H*-3'), 4.12 – 4.01 (m, 4H, *H*-4', OCH_2CH$_2$, CHCH$_3$), 1.60 – 1.57 (m, 2H, OCH$_2$CH_2), 1.37 – 1.34 (m, 4H, 2 x CH_2 ester), 1.32 – 1.29 (m, 3H CHCH_3), 0.89 – 0.84 (m, 3H, CH_3 ester). ^{13}C NMR (125 MHz, MeOD): δ_C 175.15 (*C*=O, ester), 167.54 (*C*-NH$_2$), 157.63 (*C*=O, base), 147.97 (*C*-OAr), 142.38, 142.28 (*C*H-base), 136.35 (*C*-Ar), 128.97, 128.95, 127.92, 127.88, 127.62, 127.54, 126.56 (*C*H-Ar), 123.50 (broad signal, CF_2), 116.34, 116.32, 116.26, 116.24 (*C*H-Ar), 96.63, 96.58 (*C*H-base), 86.24 (broad signal, *C*-1'), 80.33 (broad d, $^3J_{C-F}$ = 8.0 Hz, *C*-4'), 71.15, 71.03 (2 x d, $^2J_{C-F}$ = 23.0 Hz, *C*-3'), 66.53, 66.49 (O*C*H$_2$), 65.85, 65.81 (2 x d, $^2J_{C-P}$ = 5.20 Hz, *C*-5'), 51.89, 51.80 (*C*HCH$_3$), 29.35, 29.10 (*C*H$_2$ ester), 23.33 (*C*H$_2$ ester), 20.50, 20.37 (2 x d, $^3J_{C-P}$ = 7.50 Hz, CH*C*H$_3$), 14.25 (*C*H$_3$ ester). Reverse HPLC, eluting with H_2O/MeOH from 100/0 to 0/100 in 35 min, showed two peaks of diastereoisomers with t_R = 26.28 min and t_R = 26.76 min (45%, 52%).