Selective Protection of 2′,2′-Difluorodeoxycytidine (Gemcitabine)

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Gemcitabine (**1**) is a promising new anticancer agent used in pancreatic cancer. Improvement in the selective targeting of compound **1** and other cytotoxic agents to solid tumors may be enhanced by conjugation to ligands that target peripheral benzodiazepine receptors (PBRs) located on mitochondria and known to be overexpressed in human brain tumors. Development of such chemical conjugates requires selective protection on 4-NH_2, 3′-OH, and 5′-OH of compound **1.** All three monoprotected and three diprotected gemcitabine derivatives (**2** to **7**) were synthesized in good yield by employing a single commonly used protecting reagent, di-*tert*-butyl dicarbonate, under different conditions. Consequently, the three mono-ligand-gemcitabine conjugates coupled at 4-NH_2, 3′-OH, and 5′-OH respectively (**14** to **16**) were synthesized in high yield using the PBR ligand PK11195. This selective protection/deprotection strategy offers a relatively straightforward means to modify other nucleosides.

Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) (**1**) is a promising antineoplastic drug approved for use in pancreatic cancer. In comprehensive preclinical and clinical studies,[1] it has shown activity against a wide spectrum of human solid tumors including nonsmall cell lung, pancreatic, colon, breast, bladder, ovarian, head and neck, cervical and hepatocellular cancers. The hydrochloride of compound **1** (GEMZAR) (**1a**) is now marketed in many countries.[1]

Selective drug delivery to solid tumors continues to be a problem that can be addressed by the development of chemical conjugates that bind receptors overexpressed in tumors. Peripheral benzodiazepine receptors (PBRs) located on the outer membrane of mitochondria may serve as such a target as they are overexpressed in brain tumors relative to normal brain.[2] A host of PBR ligands are known that can bind to human PBRs (i.e., PK11195) and rat PBR (i.e., Ro 5 4864 and PK11195).[2] Compound **1** is an attractive candidate for development as a PBR ligand drug conjugate since its uptake into brain is expected to be limited because of its hydrophilicity. An immunoglobulin conjugate of compound **1** has been reported.[3] The PBR ligand−drug conjugates are unique in that they are of low molecular weight and target an intracellular receptor as opposed to the more common cell surface receptor sought in other drug delivery systems.

PBR ligands may be covalently coupled to compound **1** at 4-NH_2, 3′-OH, and 5′-OH positions directly or via a linker. All conjugates linked at these different positions are potentially interesting because they may possess different PBR binding affinities and cytotoxicity. Many different protection/deprotection strategies of nucleoside monomers have been established.[4] In those strategies, however, multiple protecting reagents were employed to reach the goal. We disclose here that the *tert*-butoxycarbonyl (Boc) group serves as an useful selective protecting group in gemcitabine derivative synthesis. All three monoprotected and three diprotected gemcitabine derivatives (**2**−**7**) were prepared, respectively, in good yield by employing one single commonly used protecting reagent di-*tert*-butyl dicarbonate (DBDC) under different conditions. The usefulness of this strategy was demonstrated by the synthesis of three PBR ligand−gemcitabine conjugates coupled at 4-NH_2, 3′-OH, and 5′-OH, respectively (**14** to **16**), in high yield and in a relatively simple manner.

Results and Discussion

When DBDC was added to a solution of **1a** in dioxane−aqueous NaOH, three products formed in 10 min, and the product profile changed over time. The structures of the three products were determined in separate experiments as 3′-*O*-Boc-gemcitabine (**2**), 3′-*O*-5′-*O*-di-Boc-gemcitabine (**3**), and 5′-*O*-Boc-gemcitabine (**4**). Thus, various conditions and procedures were studied to achieve selective protection of **1** in good yield. The results are shown in Scheme 1. Under condition a with 1 equiv of DBDC, **2** was obtained in 85% isolated yield, and the same product

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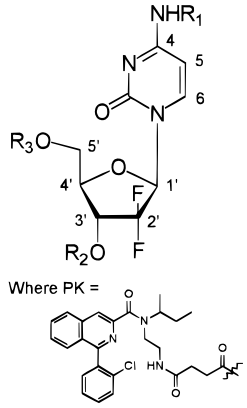
Scheme 1[a]



	R1	R2	R3
1	H	H	H
2	H	Boc	H
3	H	Boc	Boc
4	H	H	Boc
5	Boc	H	Boc
6	Boc	H	H
7	Boc	Boc	H
8	Boc	Boc	Boc
11	PK	Boc	Boc
12	Boc	PK	Boc
13	Boc	Boc	PK
14	PK	H	H
15	H	PK	H
16	H	H	PK

1a = **1** HCl

1a —a, 85%→ **2**

1a —b, c, 90%→ **3**

3 —d, 85%→ **4**

1a —e, d→ **5** + **6** (48% 41%)

2 —f, 95%→ **7**

[a] (a) Dioxane−water (4/1), Na_2CO_3, DBDC (1−5 equiv); (b) dioxane−1 M aqueous KOH (1/1), DBDC (excess); (c) dioxane, DBDC (excess), then 1 M aqueous KOH (1/1 to dioxane); (d) MeOH−1 M aqueous Na_2CO_3 (1/1); (e) dioxane, TEA, DMAP, DBDC (excess); (f) dioxane, DBDC (excess), 37 °C.

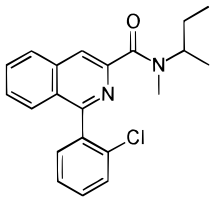
Table 1. Chemical Shift Data (ppm)[a]

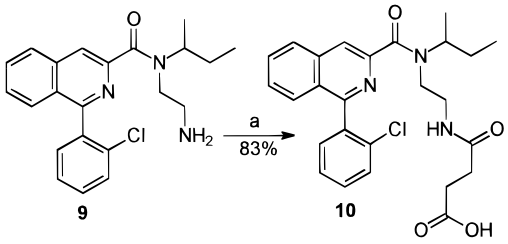
	5	6	1′	3′	4′	5′A	5′B
1	5.98	7.85	6.25	4.35	3.95	3.95	3.82
2	5.96	7.75	6.33	5.32	4.21	3.95	3.82
3	5.98	7.62	6.34	5.28	4.42	4.52	4.45
4	6.01	7.62	6.33	4.33	4.17	4.53	4.38
5	7.27	8.04	6.32	4.36	4.24	4.54	4.42
6	7.23	8.31	6.27	4.54	4.02	4.02	3.86
7	7.27	8.20	6.37	5.36	4.30	4.00	3.86
8	7.28	8.02	6.38	5.32	4.50	4.55	4.50

[a] The 600 MHz ^{1}H NMR spectra were obtained in acetone-d_6 with 2% D_2O. The position numbers are as indicated in Scheme 1.

profile was obtained with 5 equiv of DBDC based on TLC analysis. Excess DBDC was used for all the following experiments. A 1:1 mixture of **2** and **3** was obtained under condition b with excess DBDC, and the crude product mixture was converted into **3** under condition c to give 90% overall yield. Compound **3** was partially deprotected under condition d to give **4** in 85% isolated yield and recovered **1** in 10%. When **1a** was treated under forced condition e with excess DBDC, a mixture of two nonpolar products formed based on TLC analysis. The structure of the major product was determined in a separate experiment as 4-*N*-3′-*O*-5′-*O*-tri-Boc-gemcitabine (**8**). The minor product was less polar than **8** by TLC, and its structure was not determined. If pyridine was used instead of dioxane, a similar product profile was obtained based on TLC analysis. The product mixture was partially deprotected under condition d to give 4-*N*-5′-*O*-di-Boc-gemcitabine (**5**) in 48% isolated yield plus 4-*N*-Boc-gemcitabine (**6**) in 41% isolated yield. Finally, 4-*N*-3′-*O*-Boc-gemcitabine (**7**) was obtained in 95% isolated yield by using **2** as the starting material under condition f. Interestingly, compound **8** was not detected by TLC analysis under this condition. If triethylamine or diisopropylethylamine was added, a mixture of **7** and **8** formed. When **1a** was used instead of **2** under condition f, no reaction occurred after 48 h based on TLC analysis, most likely because **1a** is practically insoluble in dry dioxane. The structure determination of compounds **2** thru **8** was based on ^{1}H NMR and ESI MS. The chemical shift data are listed in Table 1.

Scheme 2[a]





10 + **3** —b, 85%→ **11** —c, d, 84%→ **14**

10 + **5** —e, 88%→ **12** —c, d, 88%→ **15**

10 + **7** —e, 86%→ **13** —c, d, 90%→ **16**

[a] (a) Succinic anhydride, DIEA; (b) DCC, 1-hydroxybenzotriazole; (c) TFA; (d) $NaHCO_3$; (e) DCC, DMAP.

PK11195 is a model human PBR ligand[5] and served as the PBR ligand to synthesize conjugates **14**, **15**, and **16** by coupling compound **10** with di-protected gemcitabine derivatives (**3**, **5**, and **7**) followed by deprotection using TFA as shown in Scheme 2. Compound **10** was synthesized from succinic anhydride and compound **9**, which was prepared as described in the literature.[5] To assign the ^{1}H NMR spectrum of compound **10**, it was compared to the spectrum of an authentic sample of PK11195 [Research Biochemical, Natick, MA 01760]. Both spectra indicated slow rotation about the amide C−N bond. The structure determination of conjugates **14**, **15**, and **16** was based on ^{1}H 1D and 2D NMR, and FAB

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HRMS. All NMR spectra of compounds **11**−**16** also indicated slow rotation about the amide bond and are available as Supporting Information with detailed peak assignment. The three mono-PBR ligand−gemcitabine conjugates were obtained in high yield, demonstrating the usefulness of the selective protection/deprotection strategy disclosed here. This strategy may be applied to other PBR ligand−nucleoside conjugates, and generally to other types of chemical conjugates involving nucleosides in which a selective linkage is required.

Experimental Section

Gemcitabine hydrochloride (**1a**) was obtained from Eli Lilly and Co. All other reagents were commercially available. ACROS silica gel (35−70 μm) was used for flash chromatography. 600 MHz 1D ^{1}H, 2D ^{1}H−^{1}H COSY NMR spectra were obtained in acetone-d_6 with 2% D_2O. Coupling constants are reported in hertz.

3′-*O*-(*tert*-Butoxycarbonyl)gemcitabine (2). To a stirred mixture of **1a** (60 mg, 0.2 mmol) and Na_2CO_3 (106 mg), in 4 mL of dioxane and 1 mL of water was added DBDC (44 mg, 0.2 mmol), and the resulting mixture stirred at 24 °C for 48 h. After 2 mL of water was added, the mixture was extracted with 2 × 30 mL of EtOAc. The organic extracts were washed with water (5 mL) and brine (5 mL), dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (CH_2Cl_2−acetone−EtOH 1:1:0.02) to give **2** (62 mg, 85%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except a singlet (δ 1.49, 9H). ESI MS *m*/*z* 364 [positive, (M + H)], 362 [negative, (M − H)].

3′,5′-*O*-Bis(*tert*-Butoxycarbonyl)gemcitabine (3). To a stirred solution of **1a** (600 mg, 2 mmol) in 40 mL of 1 M aqueous KOH was added DBDC (4.36 g, 20 mmol) dropwise to 40 mL of dioxane over 20 min. The reaction mixture was then stirred at 24 °C for an additional 40 min and extracted with EtOAc (3 × 80 mL). The organic extracts were washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was a mixture of **2** and **3** (about 1:1 by TLC). To a stirred clear solution of the above residue and DBDC (4.36 g) in 40 mL of dioxane at 24 °C was added 40 mL of 1 M aqueous KOH. The reaction progress was followed by TLC. After 30 min, the reaction was nearly complete to give only one major product **3** that was extracted by a similar procedure as described above. Flash chromatography (CH_2Cl_2−acetone 1:1) gave **3** (833 mg, 90%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except two singlets (δ 1.50, 9H; 1.47, 9H). ESI MS *m*/*z* 464 [positive, (M + H)], 462 [negative, (M − H)].

5′-*O*-(*tert*-Butoxycarbonyl)gemcitabine (4). To a stirred solution of **3** (833 mg) in 40 mL of MeOH was added 40 mL of 1 M aqueous Na_2CO_3 at 24 °C. The reaction progress was followed by TLC. After 4 h, the reaction was complete to give one major product **4** and a small amount of **1**. A similar workup procedure as stated above for **2** gave **4** (555 mg, 85%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except a singlet (δ 1.47, 9H). ESI MS *m*/*z* 364 [positive, (M + H)], 362 [negative, (M − H)]. The aqueous portions during the extraction were combined and dried under reduced pressure at 50 °C. The residue was treated with acetone (2 × 20 mL). The acetone filtrate was then concentrated. Flash chromatography on a short column (CH_2Cl_2−acetone−EtOH 1:2:0.1) gave **1** (48 mg, 10%), and the ^{1}H NMR data are shown in Table 1.

4-*N*-5′-*O*-Bis(*tert*-Butoxycarbonyl)gemcitabine (5) and 4-*N*-(*tert*-Butoxycarbonyl)gemcitabine (6). To a stirred solution of **1a** (90 mg, 0.3 mmol) and DMAP (5 mg) in 5 mL of dioxane and 5 mL of TEA was added DBDC (655 mg, 3 mmol). The reaction mixture was stirred at 24 °C for 18 h. Solvents were removed under reduced pressure. The residue was treated with 100 mL of EtOAc, washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated to dryness. Flash chromatography on a short column (CH_2Cl_2−acetone 4:1) gave a nonpolar product mixture. To a stirred solution of the above mixture in 20 mL of MeOH was added 20 mL of aqueous 1 M Na_2CO_3 at 24 °C. The reaction progress was followed by TLC, and after 4 h was completed. A similar extraction procedure as stated above gave a mixture of two products **5** and **6** that were subjected to flash chromatography (CH_2Cl_2−acetone−EtOH 2:1:0 to 1:1:0.02) to give compounds **5** [(66 mg, 48%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except two singlets (δ 1.51, 9H; 1.49, 9H). ESI MS *m*/*z* 486 [positive, (M + Na)], 462 [negative, (M − H)]] and **6** [(45 mg, 41%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except a singlet (δ 1.51, 9H). ESI MS *m*/*z* 386 [positive, (M + Na)], 362 [negative, (M − H)]].

4-*N*-3′-*O*-Bis(*tert*-Butoxycarbonyl)gemcitabine (7). To a stirred solution of **2** (73 mg, 0.2 mmol) in 8 mL of dioxane was added DBDC (436 mg, 2 mmol). The reaction mixture was maintained at 37 °C, 250 rpm, in a rotary shaker for 70 h at which time the solvent was removed under reduced pressure. The residue was washed with 2 mL of water. The solids were dried and subjected to flash chromatography (CH_2Cl_2−acetone 9:1 to 4:1) to give **7** (88 mg, 95%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR data are shown in Table 1 except two singlets (δ 1.51, 9H; 1.50, 9H). ESI MS *m*/*z* 464 [positive, (M + H)], 462 [negative, (M − H)].

4-*N*-3′-*O*-5′-*O*-Tris(*tert*-Butoxycarbonyl)gemcitabine (8). In a separate experiment under similar condition as the first step for the preparation of **5** and **6**, **8** was isolated from the product mixture by flash chromatography (CH_2Cl_2−acetone 9:1), homogeneous by TLC (CH_2Cl_2−acetone 4:1). ^{1}H NMR data are shown in Table 1 except three singlets (δ 1.51, 9H; 1.50, 9H; 1.49, 9H). ESI MS *m*/*z* 564 [positive, (M + H)], 562 [negative, (M − H)].

(±)-1-(2-Chlorphenyl)-*N*-(1-methylpropyl)-*N*-[2-[*N*-(2-hydroxycarbonyl-ethylcarbonyl)aminoethyl]]-3-isoquinolinecarboxyamide (10). To a solution of dihydrochloride of **9** (1.36 g, 3 mmol) in CH_2Cl_2 (60 mL) and DIEA (2.09 mL), was added succinic anhydride (600 mg, 6 mmol), and the reaction mixture was stirred at 24 °C for 1 h. An additional 60 mL of CH_2Cl_2 was added to the mixture that was then washed with 1 M HCl (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, and concentrated to dryness. Flash chromatography (CH_2Cl_2−acetone−HOAc 9:1:0 to 3:1:0.04) produced compound **10** (1.20 g, 83%), homogeneous by TLC (CH_2Cl_2−acetone−HOAc 3:1:0.1). ^{1}H NMR δ 8.20−8.10 (m, 2H), 7.90−7.86 (m, 1H), 7.73−7.55 (m, 6H), 4.1−3.9 (m, 1H), 3.7−3.2 (m, 4H), 2.6−2.3 (m, 4H), 2.0−1.4 (m, 2H), 1.37−1.21 (m, 3H), 0.97−0.73 (m, 3H). 2D NMR was in agreement with the structure. ESI MS *m*/*z* (relative intensity) 482/484 [3/1, positive, (M + H)], 480/482 [3/1, negative, (M − H)].

3′-*O*-5′-*O*-Bis(*tert*-Butoxycarbonyl)-4-*N*-[2-[2-[*N*-(1-methylpropyl),*N*-[1-(2-chlorophenyl)-isoquinoline-3-carbonyl]amino]ethylaminocarbonyl]ethylcarbonyl]-gemcitabine (11). A reaction mixture of **3** (93 mg, 0.2 mmol), **10** (96 mg, 0.2 mmol), DCC (83 mg, 0.4 mmol), and 1-hydroxybenzotriazole hydrate (27 mg, 0.2 mmol) in CH_2Cl_2 (10 mL) was stirred at 24 °C for 20 h. After an additional 50 mL of CH_2Cl_2 was added, it was washed with water (10 mL) and brine (2 × 10 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was stirred in 20 mL acetone for 1 h. The white solids were removed by filtration after cooled in an ice-bath. The filtrate was concentrated and subjected to flash chromatography (CH_2Cl_2−acetone 4:1 to 2:1) to give **11** (158 mg, 85%), homogeneous by TLC (EtOAc). ^{1}H NMR δ 8.23−8.11 (m, 2H), 8.03 (d, 7.6, 1H), 7.86 (m, 1H), 7.73−7.54 (m, 6H), 7.42 (m, 1H), 6.38 (m, 1H), 5.33 (m, 1H), 4.6−4.4 (m, 3H), 4.2−3.9 (m, 1H), 3.8−3.2 (m, 4H), 2.9−2.5 (m, 4H), 2.0−1.4 (m, 2H, overlap with the following two singlets), 1.50 (s, 9H), 1.47 (s, 9H), 1.37−1.21 (m, 3H), 0.96−0.73 (m, 3H). ESI MS *m*/*z* (relative intensity) 927/929 [3/1, positive, (M + H)], 925/927 [3/1, negative, (M − H)].

4-*N*-5′-*O*-Bis(*tert*-Butoxycarbonyl)-3′-*O*-[2-[2-[*N*-(1-methylpropyl),*N*-[1-(2-chlorophenyl)isoquinoline-3-carbo-

nyl]amino]ethylaminocarbonyl]ethylcarbonyl]-gemcitabine (12). To a stirred solution of **5** (46 mg, 0.1 mmol), **10** (48 mg, 0.1 mmol), and DCC (42 mg, 0.2 mmol) in 5 mL of CH_2Cl_2 was added 2 mg of DMAP, and the resulting mixture was stirred at 24 °C for 2 h. After an additional 55 mL of CH_2Cl_2 was added, it was washed with 20 mM, pH 7 phosphate buffer (2 × 20 mL) and brine (2 × 20 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was stirred in 12 mL acetone for 1 h. The white solids were removed by filtration after being cooled in an ice-bath. The filtrate was concentrated and subjected to flash chromatography (EtOAc−acetone 1:0 to 9:1) to give **12** (82 mg, 88%), homogeneous by TLC (EtOAc). ^{1}H NMR δ 8.2−8.02 (m, 3H), 7.88 (m, 1H), 7.80−7.54 (m, 6H), 7.28 (d, 7.6, 1H), 6.40 (m, 1H), 5.48 (m, 1H), 4.6−4.4 (m, 3H), 4.1−3.9 (m, 1H), 3.8−3.2 (m, 4H), 2.8−2.4 (m, 4H), 2.0−1.4 (m, 2H, overlap with the following two singlets), 1.51 (s, 9H), 1.47 (s, 9H), 1.37−1.21 (m, 3H), 0.96−0.73 (m, 3H). ESI MS *m*/*z* (relative intensity) 949/951 [3/1, positive, (M + Na)], 925/927 [3/1, negative, (M − H)].

4-*N*-3′-*O*-Bis(*tert*-Butoxycarbonyl)-5′-*O*-[2-[2-[*N*-(1-methylpropyl),*N*-[1-(2-chlorophenyl)isoquinoline-3-carbonyl]amino]ethylaminocarbonyl]ethylcarbonyl]-gemcitabine (13). To a stirred solution of **7** (46 mg, 0.1 mmol), **10** (48 mg, 0.1 mmol), and DCC (42 mg, 0.2 mmol) in 5 mL of CH_2Cl_2 was added 2 mg of DMAP. The resulting mixture was stirred at 24 °C for 2 h. After an additional 55 mL of CH_2Cl_2 was added, it was washed with 20 mM, pH 7 phosphate buffer (2 × 20 mL) and brine (2 × 20 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was stirred in 12 mL of acetone for 1 h. The white solids were removed by filtration after cooled in an ice-bath. The filtrate was concentrated and subjected to flash chromatography (CH_2Cl_2−acetone 9:1 to 4:1) to give **13** (80 mg, 86%), homogeneous by TLC (CH_2Cl_2−acetone 4:1). ^{1}H NMR δ 8.22−8.10 (m, 3H), 7.88−7.56 (m, 7H), 7.36−7.28 (m, 1H), 6.39 (m, 1H), 5.38 (m, 1H), 4.61−4.36 (m, 3H), 4.1−3.9 (m, 1H), 3.7−3.2 (m, 4H), 2.8−2.3 (m, 4H), 2.0−1.4 (m, 2H, overlap with the following two singlets), 1.49 (s, 9H), 1.48 (s, 9H), 1.36−1.20 (m, 3H), 0.95−0.72 (m, 3H). ESI MS *m*/*z* (relative intensity) 949/951 [3/1, positive, (M + Na)], 925/927 [3/1, negative, (M − H)].

4-*N*-[2-[2-[*N*-(1-Methylpropyl),*N*-[1-(2-chlorophenyl)-isoquinoline-3-carbonyl]amino]ethylaminocarbonyl]-ethylcarbonyl]-gemcitabine (14). To a stirred solution of **11** (130 mg) in 10 mL of CH_2Cl_2 at 0 °C was added 10 mL of TFA. The reaction reached completion in 1 h at the same temperature. The solvents were evaporated, and the residue was treated with EtOAc (2 × 30 mL) and 10 mL of 5% aqueous $NaHCO_3$. The organic extract was washed with 10 mL of $NaHCO_3$ and brine (2 × 10 mL), dried over Na_2SO_4, and concentrated. Crystallization from EtOAc gave **14** (85 mg, 84%) in two portions, homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR δ 8.31 (d, 7.6, 1H), 8.23−8.12 (m, 2H), 7.87 (m, 1H), 7.75−7.55 (m, 6H), 7.48 (m, 1H), 6.27 (t, 7.3, 1H), 4.43 (m, 1H), 4.15−3.70 (m, 4H), 3.6−3.2 (m, 4H), 2.9−2.5 (m, 4H), 2.1−1.4 (m, 2H), 1.36−1.20 (m, 3H), 0.96−0.72 (m, 3H). 2D NMR was in agreement with the structure. ESI MS *m*/*z* (relative intensity) 727/729 [3/1, positive, (M + H)], 725/727 [3/1, negative, (M − H)]. FAB HRMS [M + H] calcd for $C_{35}H_{38}ClF_2N_6O_7$ 727.2459, found 727.2447.

3′-*O*-[2-[2-[*N*-(1-Methylpropyl),*N*-[1-(2-chlorophenyl)-isoquinoline-3-carbonyl]amino]ethylaminocarbonyl]-ethylcarbonyl]-gemcitabine (15). To a stirred solution of **12** (80 mg) in 5 mL of CH_2Cl_2 at 0 °C, 5 mL TFA was added. The reaction reached completion in 1.5 h at the same temperature. The solvents were evaporated, and the residue was treated with EtOAc (50 mL) and 5 mL of 5% aqueous $NaHCO_3$. The organic extract was washed with 5 mL of $NaHCO_3$ and brine (2 × 5 mL), dried over Na_2SO_4, and concentrated. Crystallization from EtOAc gave **15** (55 mg, 88%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR δ 8.24−8.11 (m, 2H), 7.90−7.80 (m, 2H), 7.75−7.56 (m, 6H), 6.35 (m, 1H), 6.03 (d, 7.4, 1H), 5.49 (m, 1H), 4.26−3.75 (m, 4H), 3.7−3.2 (m, 4H), 2.8−2.3 (m, 4H), 2.0−1.42 (m, 2H), 1.37−1.19 (m, 3H), 0.96−0.73 (m, 3H). 2D NMR was in agreement with the structure. ESI MS *m*/*z* (relative intensity) 727/729 [3/1, positive, (M + H)], 725/727 [3/1, negative, (M − H)]. FAB HRMS [M + H] calcd for $C_{35}H_{38}ClF_2N_6O_7$ 727.2459, found 727.2443.

5′-*O*-[2-[2-[*N*-(1-Methylpropyl),*N*-[1-(2-chlorophenyl)-isoquinoline-3-carbonyl]amino]ethylaminocarbonyl]-ethylcarbonyl]gemcitabine (16). To a stirred solution of **13** (68 mg) in 5 mL of CH_2Cl_2 at 0 °C was added 5 mL of TFA. The reaction reached completion in 12 h at the same temperature. The solvents were evaporated, and the residue was treated with EtOAc (50 mL) and 5 mL of 5% aqueous $NaHCO_3$. The organic extract was washed with 5 mL of $NaHCO_3$ and brine (2 × 5 mL), dried over Na_2SO_4, and concentrated. Crystallization from EtOAc gave **16** (48 mg, 90%), homogeneous by TLC (CH_2Cl_2−acetone−EtOH 5:4:1). ^{1}H NMR δ 8.24−8.11 (m, 2H), 7.91−7.56 (m, 8H), 6.30 (brs, 1H), 6.11−5.90 (m, 1H), 4.55−4.3 (m, 3H), 4.14 (m, 1H), 4.08−3.92 (m, 1H), 3.6−3.3 (m, 4H), 2.7−2.4 (m, 4H), 1.96−1.42 (m, 2H), 1.37−1.19 (m, 3H), 0.97−0.72 (m, 3H). 2D NMR was in agreement with the structure. ESI MS *m*/*z* (relative intensity) 727/729 [3/1, positive, (M + H)], 725/727 [3/1, negative, (M − H)]. FAB HRMS [M + H] calcd for $C_{35}H_{38}ClF_2N_6O_7$ 727.2459, found 727.2461.

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Supporting Information Available: 1D ^{1}H NMR spectra of compounds **2**−**8** and **10**−**16**, and 2D ^{1}H−^{1}H NMR spectra of compounds **10** and **14**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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