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Synthesis and Cytostatic Evaluation of 4-*N*-Alkanoyl and 4-*N*-Alkyl Gemcitabine Analogues

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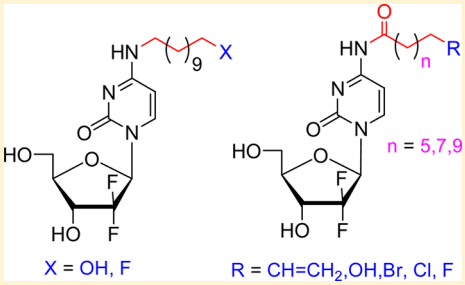
Supporting Information

ABSTRACT: The coupling of gemcitabine with functionalized carboxylic acids (C9−C13) or reactions of 4-*N*-tosylgemcitabine with the corresponding alkyl amines afforded 4-*N*-alkanoyl and 4-*N*-alkyl gemcitabine derivatives. The analogues with a terminal hydroxyl group on the alkyl chain were efficiently fluorinated under conditions that are compatible with protocols for ^{18}F labeling. The 4-*N*-alkanoylgemcitabines showed potent cytostatic activities in the low nanomolar range against a panel of tumor cell lines, whereas cytotoxicity of the 4-*N*-alkylgemcitabines were in the low micromolar range. The cytotoxicity for the 4-*N*-alkanoylgemcitabine analogues was reduced approximately by 2 orders of magnitude in the 2′-deoxycytidine kinase (dCK)-deficient CEM/dCK$^-$ cell line, whereas cytotoxicity of the 4-*N*-alkylgemcitabines was only 2−5 times lower. None of the compounds acted as efficient substrates for cytosolic dCK; therefore, the 4-*N*-alkanoyl analogues need to be converted first to gemcitabine to display a significant cytostatic potential, whereas 4-*N*-alkyl derivatives attain modest activity without measurable conversion to gemcitabine.



INTRODUCTION

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine, dFdC) is a chemotherapeutic nucleoside analogue used in the treatment of solid tumors in various cancers.[1,2] Synthesized first in 1988 by Hertel et al.,[3] gemcitabine represents first-line therapy for pancreatic and nonsmall cell lung cancers.[4−6] Gemcitabine is hydrophilic by nature, and its cellular uptake is primarily facilitated by human equilibrative nucleoside transport protein 1 (hENT1).[7] Gemcitabine is activated via phosphorylation to its 5′-monophosphate (dFdCMP) by deoxycytidine kinase (dCK).[8,9] dFdCMP then undergoes subsequent phosphorylation by intracellular kinases to its diphosphate (dFdCDP) and triphosphate (dFdCTP) forms.[10,11] dFdCTP can incorporate into DNA and inhibit DNA polymerase by chain termination during DNA replication and repair processes, invariably triggering apoptosis.[10−12] It can also participate in self-potentiation by inhibiting CTP synthetase and depleting CTP pools available to compete with dFdCTP for incorporation into RNA.[12,13] Moreover, dFdCD(T)P inhibits both R1 and R2 subunits of ribonucleotide reductase (RNR),[14−20] depleting the deoxyribonucleotide pool available to compete with dFdCTP for incorporation into DNA.[15,16] Gemcitabine is therapeutically restricted by high toxicity to normal cells and rapid intracellular deamination into inactive 2′,2′-difluorouridine (dFdU) by cytidine deaminase (CDA).[21]

Because clinical studies have indicated that prolonged infusion times with lower doses of gemcitabine can be effective while reducing toxicity to normal cells,[22,23] various prodrug strategies have been developed featuring acyl modifications on either the exocyclic 4-*N*-amine or 5′-hydroxyl group.[24] The hydrolyzable amide modifications facilitate a slow release of gemcitabine, increasing its bioavailability and uptake while also providing resistance to enzymatic deamination.[25−32] In 2004, Immordono et al. reported the increased anticancer activity of 4-*N*-stearoyl gemcitabine, which was stable in plasma and showed an improved resistance to deamination.[28] Couvreur and Cattel developed the 4-*N*-squalenoyl gemcitabine prodrug (SQgem) as a chemotherapeutic nanoassembly that accumulates in the cell membranes prior to releasing gemcitabine[26] (Figure 1). SQgem overcomes the low efficacy of gemcitabine in chemoresistant pancreatic cell lines and is currently undergoing preclinical development.[31] The orally active 4-*N*-valproylgemcitabine prodrug **1** (LY2334737), currently undergoing phase I clinical trials,[30,32] was designed to be resistant to deamination by hydrolysis under acidic conditions similar to those found in the human digestive system[25,29] while systematically releasing gemcitabine upon action by carboxylesterase 2. Recently, the gemcitabine prodrug with a Hoechst conjugate attached to the 4-amino group targeting extracellular DNA has been reported with low toxicity but high tumor efficacy.[27] In addition, the lipophilic gemcitabine prodrug CP-4126 with the 5′-OH group esterified with an elaidic fatty acid has shown antitumor activity in various xenograft models.[33] It remains active when orally administered;[33] however, it has not yet met criteria for advancement to phase II clinical trials.[34]

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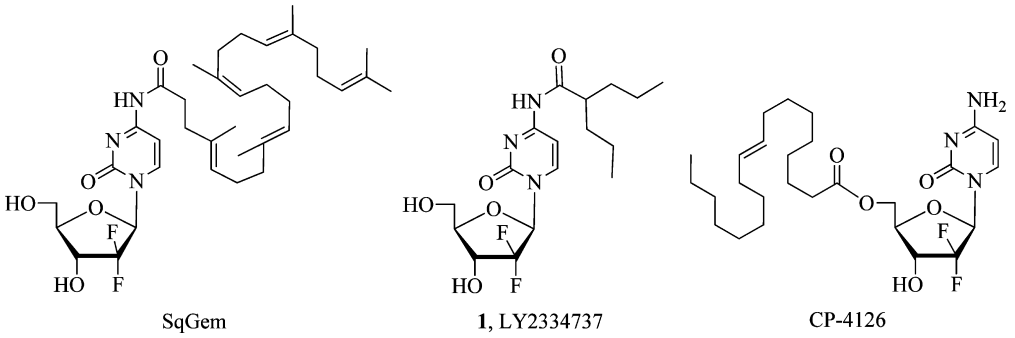
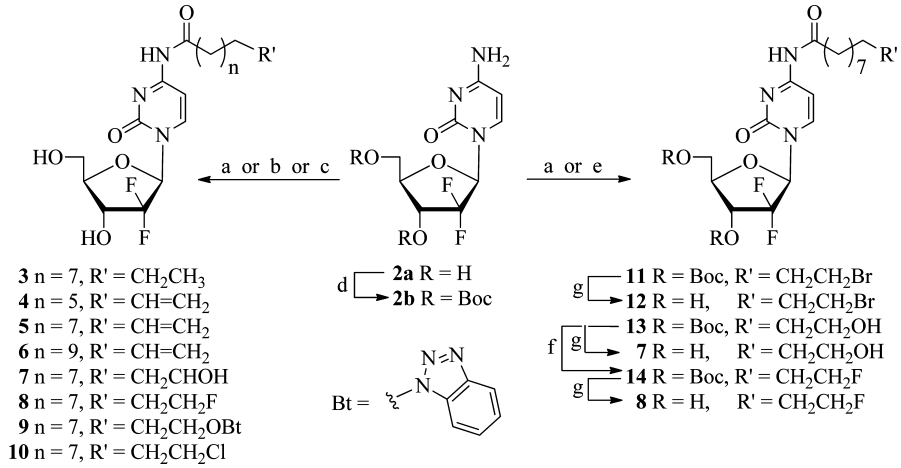


Figure 1. The acylated gemcitabine prodrugs.

Scheme 1. Synthesis of Lipophilic 4-*N*-Alkanoyl Gemcitabine Derivatives[a]



[a]Reagents and conditions: (a) R′$(CH_2)_n$COOH/NMM/HOBt/EDCI/DMF/DMSO/60 °C/overnight; (b) (i) TMSCl/Pyr/CH_3CN, 0 °C/2.5 h, (ii) $BrCH_2(CH_2)_9$COOH/CDI/CH_3CN/65 °C/overnight; (c) $BrCH_2(CH_2)_9$COOH/ClCOOEt/Et_3N/DMF (d) $(Boc)_2O$/KOH/1,4-dioxane; (e) $BrCH_2(CH_2)_9$COCl/$NaHCO_3$/CH_2Cl_2; (f) DAST/CH_2Cl_2; (g) TFA

Very recently, the 5′-acylated gemcitabine prodrugs with a coumarin or boron-dipyrromethene/biotin conjugate have been reported for monitoring drug delivery at subcellular levels by fluorescence imaging.[35,36]

1-(2′-Deoxy-2′-^{18}F-fluoro-β-D-arabinofuranosyl)cytosine ([^{18}F]-FAC) was developed by Radu et al.[37] as a PET tracer possessing a substrate affinity for dCK and CDA comparable to gemcitabine. Determination of [^{18}F]-FAC uptake and pretreatment levels of dCK serve as a noninvasive prognosticator for gemcitabine chemotherapy response.[37−40] dCK-specific PET tracers, such as 1-(2′-deoxy-2′-^{18}F-fluoro-β-L-arabinofuranosyl)-cytosine ([^{18}F]-L-FAC) and 1-(2′-deoxy-2′-^{18}F-fluoro-β-L-arabinofuranosyl)-5-methylcytosine (L-^{18}F-FMAC), which possess no substrate affinity to CDA, were also developed to study uptake of [^{18}F]-FAC and serve as additional predictive tools for gemcitabine treatment response.[41,42]

Herein, we report synthesis and cytostatic activity of a series of gemcitabine analogues with 4-*N*-alkanoyl or 4-*N*-alkyl chains modified with a terminal hydroxyl, halide, or alkene groups. The 4-*N*-alkyl analogues stable toward deamination were designed to examine their anticancer activities and also to explore their compatibility with radiofluorination protocols.

CHEMISTRY

Condensation of gemcitabine (**2a**, dFdC) with undecanoic acid under peptide coupling conditions[25,43] (*N*-dimethylaminopropyl)-*N*′-ethyl-carbodiimide (EDCI)/1-hydroxybenzotriazole (HOBt)/*N*-methylmorpholine (NMM)) in DMF/DMSO (3:1) at 60 °C afforded 4-*N*-undecanoylgemcitabine **3** (50%, Scheme 1). Analogous coupling of **2a** with 8-nonenoic acid, 10-undecenoic acid, 12-tridecenoic acid, 11-fluoroundecanoic acid (**S4**; see the Supporting Information), or 11-hydroxyundecanoic acid afforded 4-*N*-acyl analogues **4**−**8** in 40−66% yield after silica gel purification. It is noteworthy that these couplings in the presence of HOBt typically progressed to >90% completion (TLC), whereas the 1,1′-carbonyldiimidazole-(CDI)-mediated coupling[44] of **2a** with the corresponding carboxylic acids in CH_3CN and pyridine without HOBt proceeded less efficiently.

Unexpectedly, the HOBt-promoted coupling of **2a** with 11-bromoundecanoic acid led to the formation of 4-*N*-[11-(1*H*-benzotriazol-1-yloxy)undecanoyl derivative **9** in a 53% yield rather than the expected 4-*N*-(11-bromoundecanoyl) derivative **12**. Other attempts to synthesize the bromo derivative either by transiently protecting **2a** with a trimethylsilyl group[45] followed by treatment with 11-bromoundecanoic acid/CDI or by employing a mixed anhydride procedure[26] (11-bromoundecanoic acid/ethyl chloroformate/TEA) gave chloro derivative **10** instead. The labile nature of the terminal bromide necessitated an alternative approach for the preparation of **12**. We found that condensation of 3′,5′-di-*O*-Boc-protected gemcitabine[46] **2b** with 11-bromoundecanoyl chloride (**S5**; see the Supporting Information) provided bromo derivative **11** in a 33% isolated yield. The deprotection of **11** with TFA gave desired **12** (86%).

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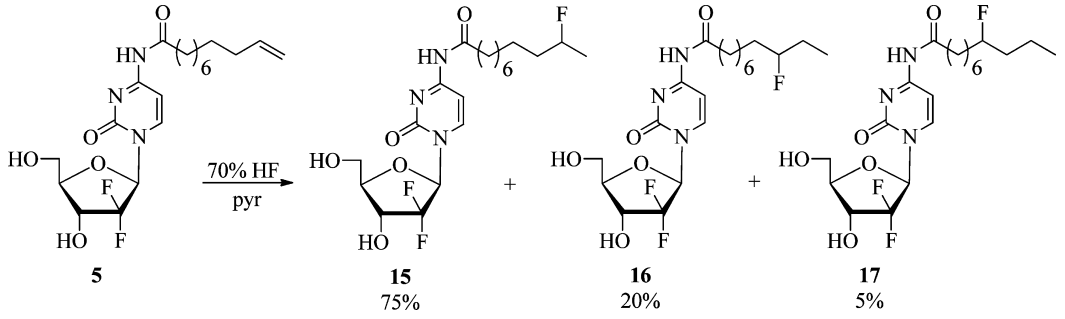
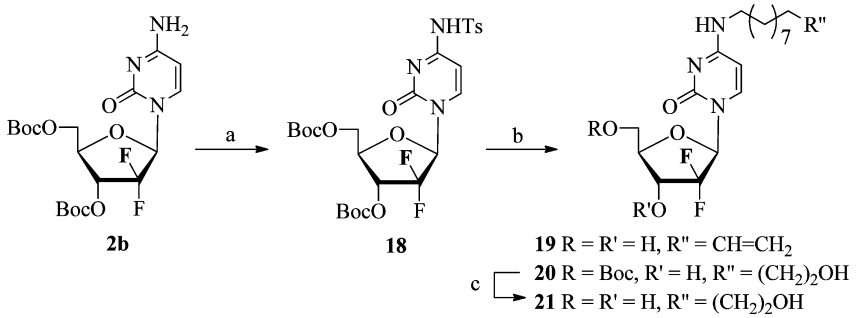
Scheme 2. Synthesis of Fluorinated 4-*N*-Alkanoyl Gemcitabine Derivatives by the Addition of HF to the Olefin

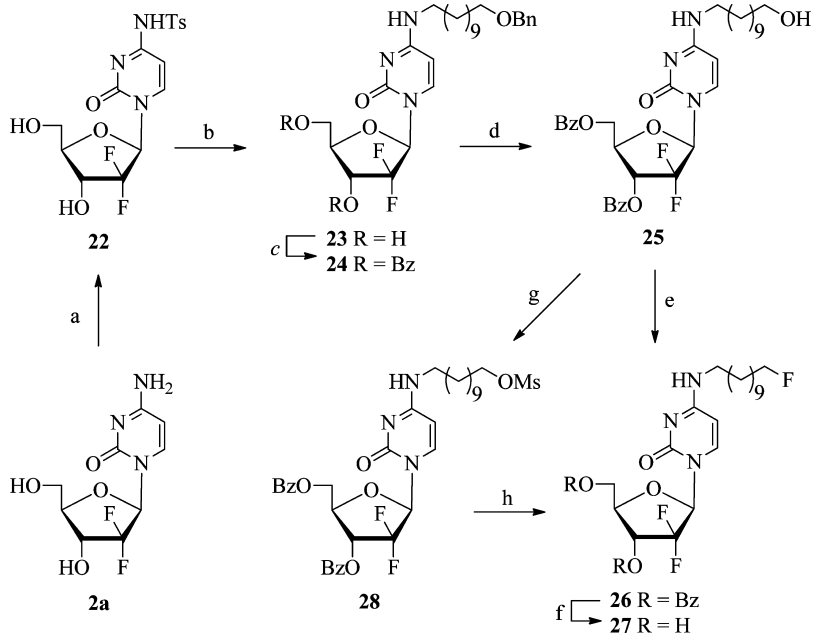


Scheme 3. Synthesis of 4-*N*-Alkyl Gemcitabine Derivatives[a]



[a]Reagents and conditions: (a) TsCl/Et_3N/1,4-dioxane; (b) $CH_2{=}CH(CH_2)_9NH_2$ or $HOCH_2CH_2(CH_2)_9NH_2$; (c) TFA

Scheme 4. Synthesis of 4-*N*-Fluoroalkyl Gemcitabine Derivatives[a]



[a]Reagents and conditions: (a) (i) TMSCl/Pyr, (ii) TsCl, (iii) MeOH/NH_3; (b) $BnO(CH_2)_{11}NH_2/Et_3N$/1,4-dioxane; (c) 2,6-lutidine/DMAP/$BzCl/CH_2Cl_2$; (d) CAN/CH_3CN; (e) $DAST/CH_2Cl_2$; (f) $MeOH/NH_3$/rt; (g) $MsCl/Et_3N/CH_2Cl_2$/0 °C; (h) $KF/K_2CO_3/K_{222}/CH_3CN$/110 °C, (ii) MeONa/MeOH/100 °C

The coupling of **2b** with 11-hydroxyundecanoic acid yielded protected 4-*N*-(11-hydroxyundecanoyl) derivative **13** (37%), bearing a hydroxyl group at the alkyl chain suitable for further chemical modifications. Thus, fluorination of **13** with DAST afforded 4-*N*-(11-fluoroundecanoyl) derivative **14** (40%). Deprotection of **13** or **14** with TFA gave **7** (87%) or **8** (82%), respectively.

Recently, Haufe et al. established the methodology for radiofluorination of terminal olefins employing the [^{18}F]-HF/pyridine reagent.[47] We performed the model fluorination under this condition by employing regular, nonradioactive HF/pyridine with olefin **5**. Thus, treatment of **5** with Olah's reagent (70% HF in pyridine) in an HDPE vessel at 0 °C for 2 h gave a regioisomeric mixture of 10-fluoro, 9-fluoro, and 8-

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Table 1. In Vitro Cytostatic Activity of Representative 4-*N*-Modified Analogues on the Tumor Cell Lines L1210, CEM/0, CEM/dCK$^-$, HeLa, and MCF-7

	IC_{50} (μM)				
compound	L1210	CEM/0	CEM/dCK$^-$	HeLa	MCF-7
1[a]	1.1 ± 0.7	5.2 ± 2.3	161 ± 8	0.76 ± 0.30	0.55 ± 0.49
2a	0.013 ± 0.001	0.069 ± 0.002	7.6 ± 0.5	0.0099 ± 0.0041	0.0072 ± 0.0002
3	0.014 ± 0.002	0.060 ± 0.012	5.8 ± 0.5	0.0089 ± 0.0024	0.0053 ± 0.0023
4	0.024 ± 0.017	0.14 ± 0.00	20 ± 2	0.042 ± 0.005	0.0079 ± 0.0002
5	0.018 ± 0.016	0.071 ± 0.015	12 ± 9	0.012 ± 0.007	0.0062 ± 0.0029
6	0.021 ± 0.018	0.069 ± 0.002	6.8 ± 1.8	0.013 ± 0.007	0.0079 ± 0.0012
7	0.023 ± 0.003	0.24 ± 0.19	19 ± 6	0.049 ± 0.030	0.0081 ± 0.0005
8	0.053 ± 0.040	0.059 ± 0.009	7.2 ± 0.8	0.011 ± 0.004	0.0077 ± 0.0006
19	7.0 ± 3.0	13 ± 6	60 ± 15	3.4 ± 0.0	28 ± 14
21	29 ± 11	86 ± 10	140 ± 28	22 ± 4	27 ± 3
27	28 ± 2	28 ± 4	134 ± 18	17 ± 4	26 ± 7

[a]In free-base form.

fluoro derivatives **15**−**17** with an isomeric ratio of 75:20:5 (91%, Scheme 2). The ^{1}H and ^{19}F NMR spectra were diagnostic for the regioisomeric composition [^{19}F δ −179.79 (m, 0.05 F), −178.83 (m, 0.2 F), −170.27 (m, 0.75 F)]. Fluorination involved Markovnikov addition of HF to the double bond, whereas generation of minor isomers **16** and **17** is attributed to migration of the carbocation along the chain during the addition reaction, as has been observed before.[48] Because addition of HF to **5** gave multiple products and radiolabeling with [^{18}F]-HF was reported to proceed with low radiochemical efficiencies,[47] whereas other commonly used fluorination protocols required conditions[49] under which the 4-*N*-alkanoyl linkage might be cleaved, we turned our attention to developing 4-*N*-alkyl gemicitabine analogues.

Synthesis of 4-*N*-alkyl gemcitabine derivatives, which, to the best of our knowledge, are limited to short 4-*N*-alkyl modifications and their anticancer activities have not been studied in depth.[50] The 4-*N*-alkyl analogues are expected to be resistant to chemical hydrolysis as well as CDA-catalyzed deamination.[51] From the available methods for the *N*-alkylation of cytosine nucleosides,[52−55] we found that the alkylation of the 4-exocyclic amine group in gemcitabine could be achieved efficiently by displacement of a 4-*N*-tosylamine group[54] with an aliphatic alkyl amine. Thus, reaction of **2b** with TsCl in the presence of Et_3N in 1,4-dioxane afforded protected 4-*N*-tosylgemcitabine **18** (45%, Scheme 3). Treatment of **18** with 10-undecenyl amine effected simultaneous displacement of the *p*-toluenesulfonamido group from the C4 position of the cytosine ring and deprotection to give 4-*N*-(10-undecenyl) derivative **19**. Analogous reaction of **18** with 11-aminoundecanol (**S7**; see the Supporting Information) gave 5′-monoprotected 11-hydroxyundecanyl analogue **20** (47%) as the major product in addition to fully deprotected **21** (24%). Deprotection of **20** with TFA provided **21** with 38% overall yield from **18**.

Owing to instability of the Boc protection group during displacement of the *p*-toluenesulfonamido group, we explored other protection strategies that would lead to gemcitabine derivatives suitable for the selective modification of the primary hydroxyl group on the 4-*N*-alkyl chain. Thus, transient protection of **2a** with TMSCl and subsequent treatment with TsCl followed by methanolic ammonia afforded 4-*N*-tosylgemcitabine **22** in 96% yield (Scheme 4). Displacement of the *p*-toluenesulfonamido group from **22** with *O*-benzyl-protected 11-aminoundecanol (**S11**; see the Supporting Information) proceeded efficiently to give 4-*N*-(11-benzyloxyundecanyl)gemcitabine **23** in 61% isolated yield. Subsequent treatment of **23** with BzCl yielded fully protected analogue **24** (60%). The lengthy treatment of **24** with ceric ammonium nitrate (CAN) effected the selective removal of the benzyl group to give 3′,5′-di-*O*-benzoyl-protected 11-hydroxyundecanyl analogue **25** (70%). It is noteworthy that attempted hydrogenolysis of **24** (H_2/Pd−C/EtOH/24 h) produced **25** with inconsistent yields of 5−50% in addition to substantial quantities of other byproducts including the 5,6-dihydro reduced derivative.

Fluorination of **25** with DAST afforded 4-*N*-(11-fluoroundecanyl) derivative **26**, and subsequent deprotection with methanolic ammonia at room temperature gave 4-*N*-fluoroalkyl analogue **27** (43% overall from **25**). We also examined fluorination of **25** under conditions that are compatible with general radiosynthetic protocols for ^{18}F labeling.[46,56] Thus, reaction of **25** with MsCl/Et_3N gave mesylate precursor **28** (90%). Fluorination of the latter with KF/K_2CO_3/Kryptofix 2.2.2 in CH_3CN at 110 °C for 18 min yielded protected fluoro analogue **26**, and subsequent debenzoylation with 0.5 M MeONa/MeOH at 100 °C for 8 min and purification by HPLC afforded desired 4-*N*-fluoroalkyl gemcitabine **27** (overall 62% from **28**, in a total of 50 min). This fluorination protocol meets the criteria for working with 18-fluorine isotope, which has limited availability and a short half-life (110 min) and as such is applicable for labeling studies.

CYTOSTATIC ACTIVITY

The growth-inhibitory activities of the 4-*N*-acyl (**3**−**8**) and 4-*N*-alkyl (**19**, **21**, and **27**) gemcitabine analogues were assessed on a panel of murine and human tumor cell lines (Table 1). All 4-*N*-alkanoyl **3**−**8** analogues demonstrated potent antiproliferative activities with IC_{50} values in the low nanomolar range, similar to gemcitabine **2a**, probably acting as prodrugs as established before.[25] However, 4-*N*-alkylgemcitabine derivatives **19**, **21**, and **27** showed cytostatic activities at IC_{50} values in the low to modest micromolar range. It appears that the cytostatic activity only varies slightly between compounds with different chain lengths or functional groups. The activity for the 4-*N*-acyl gemcitabine derivatives was drastically diminished (by almost 2 orders of magnitude) in the dCK-deficient CEM/dCK$^-$ cell line, again implying the role for dCK in the metabolism of these compounds.[24] Interestingly, cytotoxicity of 4-*N*-alkylgemcitabines **19**, **21**, and **27** in dCK-deficient CEM/dCK$^-$ cells was

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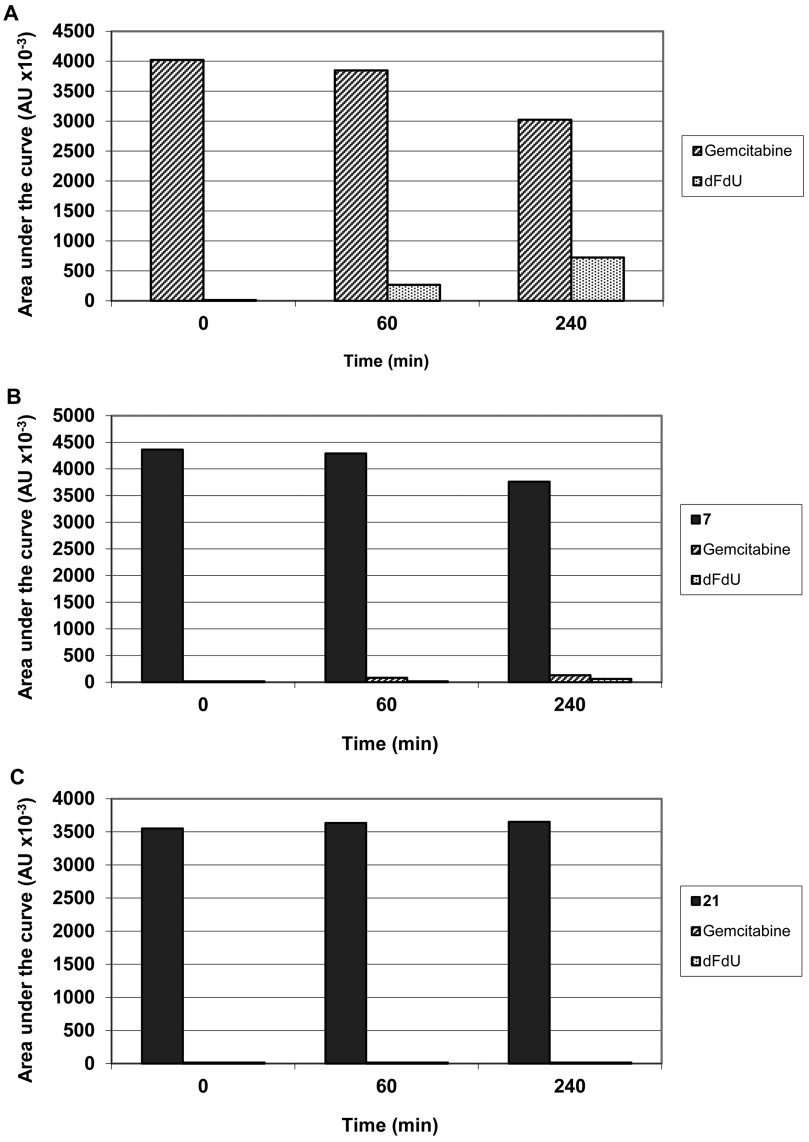

Figure 2. Time-point evaluation of the stability and resistance to deamination for gemcitabine (A), 4-*N*-alkanoylgemcitabine **7** (B), and 4-*N*-alkylgemcitabine **21** (C) in 50% human serum in PBS.

only 2−5 times lower. It is noteworthy that 4-*N*-valproylgemcitabine **1** in its free-base form showed cytostatic activity in the low micromolar range, which is more comparable to our 4-*N*-alkyl analogues than the 4-*N*-acyl counterparts. The inhibitory activities for **1** are in agreement with the cytotoxicity described by Pratt et al. on the NCI-60 panel, who reported IC_{50} values of **1** that were 80-fold greater than the value for gemcitabine on most cell lines.[30]

The selected compounds were also investigated for their interaction with dCK or mitochondrial thymidine kinase (TK-2), which also has 2′-deoxycytidine kinase activity. None of the prodrug derivatives showed significant inhibition of the phosphorylation of dCyd or dThd by dCK and TK-2, respectively. When directly evaluated as a potential substrate for dCK, 4-*N*-alkanoyl derivatives **6** and **8** displayed very poor substrate activity (<1%), whereas 4-*N*-alkyl analogues **11** and **12** displayed no measurable substrate activity under experimental conditions that converted gemcitabine to its 5′-monophosphate by at least 15%. Taken together, these findings suggest that the 4-*N*-alkanoyl analogues first need to be converted to gemcitabine before acting as an efficient substrate for dCK. This release of gemcitabine from the 4-*N*-alkanoyl prodrugs seems to occur quite efficiently given their pronounced cytostatic potential in the cell cultures and the marked loss of cytostatic activity in the dCK-deficient CEM tumor cell cultures. Instead, the rather modest antiproliferative activities of the 4-*N*-alkyl analogues may be attributed, at least to a certain extent, to a poor cellular uptake and/or to an inefficient conversion to the parental gemcitabine that may fall outside the assay's detection limits (<1%). Moreover, the fact that the cytostatic activity of the 4-*N*-alkyl analogues are only moderately decreased in dCK-deficient CEM tumor cell cultures may not only confirm a poor, if any, intracellular conversion to gemcitabine but also point to a potentially different mechanism of cytostatic activity of these prodrugs. To gain insight into the metabolism of the 4-*N*-alkylgemcitabine derivatives, the stabilities of representative 4-*N*-alkanoyl **7** and 4-*N*-alkyl **21** analogues toward hydrolysis and resistance to enzymatic deamination were evaluated in parallel with gemcitabine in human serum and in murine liver extract. Figure 2 shows that gemcitabine was deaminated to its inactive uracil derivative, dFdU, as a function of time (panel A), whereas

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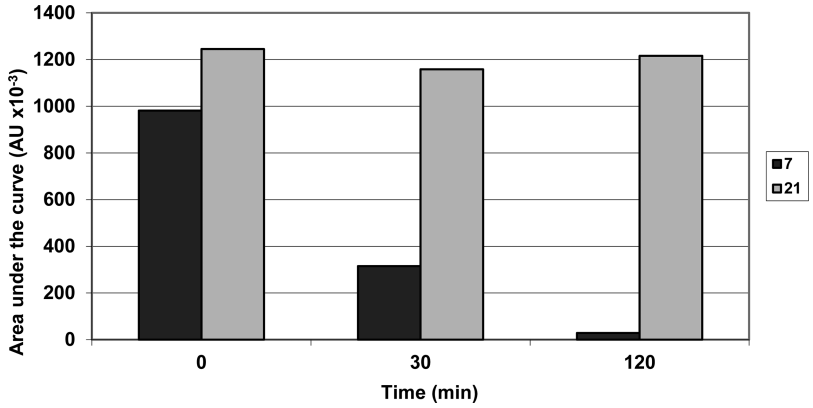

Figure 3. Time-point evaluation of the stability of 4-*N*-alkanoylgemcitabine **7** and 4-*N*-alkylgemcitabine **21** in murine liver extract in PBS.

4-*N*-alkanoylgemcitabine prodrug **7** was slowly converted to gemcitabine, which was then gradually deaminated to dFdU (panel B). However, 4-*N*-alkylgemcitabine derivative **21** was neither deaminated nor was there any measurable conversion to gemcitabine observed (panel C). When **7** and **21** were exposed to the murine liver extract, **7** was rapidly converted to gemcitabine (and dFdU), whereas **21** was fully stable for at least 2 h (Figure 3). These findings again support the assumption that **7** is enzymatically efficiently converted to gemcitabine, whereas **21** is not, explaining the differences in the cytostatic activity of both compounds. Although the cellular target for the antiproliferative activity of the 4-*N*-alkyl analogues is currently unclear, it may be different from the inhibition of DNA synthesis.

In conclusion, we have demonstrated that the coupling of gemcitabine with various carboxylic acids or reaction of 3′,5′-di-*O*-Boc-protected gemcitabine with acyl halides gives 4-*N*-alkanoylgemcitabine analogues with a hydroxyl, fluoro, chloro, bromo, or alkene functional group on the alkyl chain. Displacement of the *p*-toluenesulfonamido group from 4-*N*-tosylgemcitabine with alkyl amines provided 4-*N*-alkylgemcitabine analogues suitable for further chemical modifications, including fluorination compatible with synthetic protocols for ^{18}F labeling. The 4-*N*-alkanoylgemcitabine analogues showed potent antiproliferative activities against the L1210, CEM, HeLa, and MCF-7 cell lines, with IC_{50} values in the low nanomolar range, whereas the cytostatic activity of the 4-*N*-alkylgemcitabine derivatives was in the low to modest micromolar range. The 4-*N*-alkanoyl derivatives display significant cytostatic activity, acting as efficient prodrugs, whereas the 4-*N*-alkyl analogues appear to attain their modest activity without measurable conversion to gemcitabine. The cytostatic activity appears to be independent of the length of the alkyl chain and varies slightly for the different functional groups present on the molecule.

EXPERIMENTAL SECTION

The ^1H (400 MHz), ^{13}C (100 MHz), or ^{19}F (376 MHz) NMR spectra were recorded at ambient temperature in solutions of $CDCl_3$, MeOH-d_4, or DMSO-d_6, as noted. The reactions were followed by TLC with Merck Kieselgel 60-F_{254} sheets, and products were detected with a 254 nm light or with Hanessian's stain. Column chromatography was performed using Merck Kieselgel 60 (230−400 mesh). Reagent-grade chemicals were used, and solvents were dried by reflux distillation over CaH_2 under nitrogen gas unless otherwise specified, and reactions were carried out under an Ar atmosphere. The carboxylic acid and amine derivatives used for the coupling with gemcitabine were commercially available except for 11-fluoroundecanoic acid (**S4**), 11-bromoundecanoyl chloride (**S5**), 11-aminoundecanol (**S7**), and 11-benzyloxyundecan-1-amine (**S11**), which were synthesized as described in the Supporting Information. The purity of the synthesized compounds was determined to be ≥95% by elemental analysis (C, H, N) and/or HPLC on Phenomenex Gemini RP-C18 with an isocratic mobile phase (50% CH_3CN/H_2O) and a flow rate of 5 mL/min. Representative HPLC chromatograms are included in the Supporting Information.

Tumor Cell and Enzyme Sources. Murine leukemia L1210, human lymphocyte CEM, and human cervical carcinoma HeLa cell lines were obtained from ATCC (Rockville, MD). Human breast carcinoma MCF-7 cells were a kind gift from G. Peters (Amsterdam, The Netherlands). The dCK-deficient CEM cell line was obtained upon selection in the presence of araC and was found to be deficient in cytosolic dCK activity.

General Synthetic Procedure for Preparation of the 4-*N*-Acyl Gemcitabine Derivatives (3−9). Procedure A. *N*-Methylmorpholine (1.1 equiv), 1-hydroxybenzotriazole (1.1 equiv), the appropriate carboxylic acid (1.1 equiv), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (1.3 equiv) were sequentially added to a stirred solution of gemcitabine hydrochloride (**2a**, 1.0 equiv) in DMF/DMSO (3:1, 2 mL) at ambient temperature under Argon. The reaction mixture was then gradually heated to 65 °C (oil-bath) and was kept stirring overnight. After the reaction was completed (TLC), the reaction mixture was cooled to 15 °C and partitioned between a small amount of brine and EtOAc. The organic phase was separated, and the aqueous layer was extracted with fresh portions of EtOAc (3 × 30 mL). The combined organic layers were then sequentially washed with 20% $LiCl/H_2O$, saturated $NaHCO_3/H_2O$, and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give crude products **3**−**9**.

4-*N*-(Undecanoyl)-2′-deoxy-2′,2′-difluorocytidine (3). Treatment of **2a** (34 mg, 0.11 mmol) with commercially available undecanoic acid (23.3 mg, 0.120 mmol) by procedure A gave 45.7 mg of the crude product, which was then column chromatographed (5% MeOH/EtOAc) to give **3** (23.8 mg, 50%) as a white solid. ^1H NMR (CD_3OD) δ 0.90 (t, J = 6.9 Hz, 3H, CH_3), 1.27−1.39 (m, 14H, 7× × CH_2), 1.63−1.70 (m, 2H, CH_2), 2.45 (t, J = 7.4 Hz, 2H, CH_2), 3.79−3.83 (m, 1H, H5′), 3.94−3.99 (m, 2H, H5′, H4′), 4.31 (dt, J = 20.8, 10.5 Hz, 1H, H3′), 6.24−6.28 (m, 1H, H1′), 7.50 (d, J = 7.6 Hz, 1H, H5), 8.34 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 14.41, 23.69, 25.93, 30.15, 30.40, 30.40, 30.56, 30.64, 33.03, 38.16, 60.29 (C5′), 70.21 (t, J = 23.1 Hz, C3′), 82.86 (d, J = 8.6 Hz, C4′), 86.44 (dd, J = 26.6, 38.3 Hz, C1′), 98.26 (C5′), 123.90 (t, J = 259.3 Hz, C2′), 145.94 (C6), 157.65 (C2), 164.80 (C4), 175.97. ^{19}F NMR (CD_3OD) δ −120.09 (br d, J = 240.9 Hz, 1F), −119.14 (dd, J = 11.3, 240.9 Hz, 1F). HRMS (ESI^+) m/z calcd for $C_{20}H_{31}F_2N_3NaO_5$ $[M + Na]^+$, 454.2124; found, 454.2136.

4-*N*-(8-Nonenoyl)-2′-deoxy-2′,2′-difluorocytidine (4). Treatment of **2a** (34 mg, 0.110 mmol) with commercially available 8-nonenoic acid (21 μL, 19.5 mg, 0.120 mmol) by procedure A gave

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29.0 mg of the crude product, which was then column chromatographed (70 → 100% EtOAc/hexane) to give **4** (20 mg, 45%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.32−1.46 (br s, 6H, 3 × CH_2), 1.65−1.69 (m, 2H, CH_2), 2.03−2.07 (m, 2H, CH_2), 2.45 (t, J = 7.4 Hz, 2H, CH_2), 3.81 (dd, J = 12.3, 2.8 Hz, 1H, H5′), 3.96−3.99 (m, 2H, H5″, H4′), 4.30 (td, J = 12.2, 8.6 Hz, 1H, H3′), 4.90−5.01 (m, 2H, CH_2), 5.81 (ddt, J = 16.9, 10.0, 3.4 Hz, 1H, CH), 6.24−6.28 (m, 1H, H1′), 7.50 (d, J = 7.6 Hz, 1H, H5), 8.34 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.90, 29.87, 29.90, 30.00, 34.79, 38.15, 60.31 (C5′), 70.23 (dd, J = 21.9, 23.4 Hz, C3′), 82.86 (C4′), 86.14 (d, J = 20.1 Hz, C1′), 98.28 (C5), 114.83, 123.94 (t, J = 259.2 Hz, C2′), 140.03, 145.97 (C6), 157.37 (C2), 164.84 (C4), 175.97. ^{19}F NMR (CD_3OD) δ −120.13 (br d, J = 242.5 Hz, 1F), −119.21 (dd, J = 11.4, 240.0 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{18}H_{25}F_2N_3NaO_5$ [M + Na]$^+$, 424.1654; found, 424.1656.

4-*N*-(10-Undecenoyl)-2′-deoxy-2′,2′-difluorocytidine (5). Treatment of **2a** (40 mg, 0.134 mmol) with commercially available undecylenic acid (31 μL, 28 mg, 0.148 mmol) by procedure A gave 114 mg of the crude product, which was then column chromatographed (80 → 100% EtOAc/hexane) to give **5** (38 mg, 66%) as a white solid. UV (CH_3OH) λ_{max} 252 nm (ε 15 150), 286 nm (ε 8950), λ_{min} 228 nm (ε 5900), 275 nm (ε 8650). ^{1}H NMR (DMSO-d_6) δ 1.23−1.29 (br s, 8H, 4 × CH_2), 1.30−1.39 (m, 2H, CH_2), 1.50−1.57 (m, 2H, CH_2), 2.01 (q, J = 7.0 Hz, 2H, CH_2), 2.40 (t, J = 7.3 Hz, 2H, CH_2), 3.66 (br d, J = 12.4 Hz, 1H, H5″), 3.81 (br d, J = 12.4 Hz, 1H, H5′), 3.89 (dt, J = 8.5, 2.7 Hz, 1H, H4′), 4.19 (q, J = 10.6 Hz, 1H, H3′), 4.93 (d quin, J = 10.1, 1.0 Hz, 1H, CH), 4.99 (d quin, J = 17.2, 1.7 Hz, 1H, CH), 5.33 (br t, J = 5.0 Hz, 1H, OH), 5.79 (tdd, J = 6.6, 10.3, 17.1 Hz, 1H, CH), 6.17 (t, J = 7.5 Hz, 1H, H1′), 6.35 (br s, 1H, OH), 7.29 (d, J = 7.6 Hz, 1H, H5), 8.24 (d, J = 7.6 Hz, 1H, H6), 10.98 (br s, 1, NH). ^{13}C NMR (CD_3OD) δ 25.95, 30.08, 30.15 (2 × CH_2), 30.37, 30.39, 34.88, 38.18, 60.32 (C5′), 70.24 (dd, J = 21.9, 23.4 Hz, C3′), 82.89 (dd, J = 2.7, 5.2 Hz, C4′), 86.48 (dd, J = 25.8, 38.2 Hz, C1′), 98.28 (C5), 114.73, 123.93 (t, J = 259.2 Hz, C2′), 140.13, 145.97 (C6), 157.69 (C2), 164.83 (C4), 176.00. ^{19}F NMR (CD_3OD) δ −120.09 (br d, J = 239.6 Hz, 1F), −119.16 (dd, J = 10.9, 239.9 Hz, 1F). MS (ESI$^+$) m/z 430 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{29}F_2N_3NaO_5$ [M + Na]$^+$, 452.1967; found, 452.1982. Anal. Calcd for $C_{20}H_{29}F_2N_3O_5{\cdot}0.5H_2O$ (438.47): C, 54.79; H, 6.90; N, 9.58. Found: C, 54.48; H, 6.53; N, 9.21.

4-*N*-(12-Tridecenoyl)-2′-deoxy-2′,2′-difluorocytidine (6). Treatment of **2a** (30 mg, 0.1 mmol) with commercially available 12-tridecenoic acid (23 mg, 0.11 mmol) by procedure A gave 43.1 mg of the crude product, which was then column chromatographed (70 → 80% EtOAc/hexane) to give **6** (20.1 mg, 44%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.27−1.38 (m, 14H, 7 × CH_2), 1.66 (quin, J = 6.9 Hz, 2H, CH_2), 2.04 (dd, J = 14.3, 6.7 Hz, 2H, CH_2), 2.45 (t, J = 7.4 Hz, 2H, CH_2), 3.81 (dd, J = 12.4, 2.8 Hz, 1H, H5′), 4.07−3.88 (m, 2H, H5″, H4′), 4.31 (dt, J = 20.8, 10.4 Hz, 1H, H3′), 4.89−5.00 (m, 2H, CH_2), 5.80 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H, CH), 6.26 (t, J = 7.2 Hz, 1H, H1′), 7.50 (d, J = 7.6 Hz, 1H, H5), 8.34 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.94, 30.11, 30.15, 30.20, 30.39, 30.53 (2 × CH_2), 30.62, 34.87, 38.16, 60.30, 70.24 (t, J = 23.1 Hz, C3′), 82.83 (C4′), 86.46 (t, J = 32.2 Hz, C1′), 98.26 (C5), 114.67, 123.1 (t, J = 260.1 Hz, C2′), 140.14, 145.95 (C6), 157.68 (C2), 164.82 (C4), 176.0. ^{19}F NMR (CD_3OD) δ −120.13 (br d, J = 239.4 Hz, 1F), −119.21 (dd, J = 9.3, 239.3 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{22}H_{33}F_2N_3NaO_5$ [M + Na]$^+$, 480.2280; found, 480.2289.

4-*N*-(11-Hydroxyundecanoyl)-2′-deoxy-2′,2′-difluorocytidine (7). Treatment of **2a** (58 mg, 0.194 mmol) with commercially available 11-hydroxyundecanoic acid (43 mg, 0.213 mmol) by procedure A gave 75.5 mg of the crude product, which was then column chromatographed (7.5% MeOH/$CHCl_3$) to give **7** (35 mg, 40%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.33 (br s, 12H, 6 × CH_2), 1.49−1.54 (m, 2H, CH_2), 1.66 (quin, J = 7.2 Hz, 2H, CH_2), 2.45 (t, J = 7.4 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 3.81 (dd, J = 3.1, 12.8 Hz, 1H, H5′), 3.94−3.99 (m, 2H, H4′, H5′), 4.26−4.34 (m, 1H, H3′), 6.26 (t, J = 7.3 Hz, 1H, H1′), 7.49 (d, J = 7.6 Hz, 1H, H5), 8.33 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.93, 26.94, 30.13, 30.37, 30.48, 30.53, 30.64, 33.65, 38.17, 60.30 (C5′), 63.01, 70.23 (t, J = 23.0 Hz, C3′), 82.88 (d, J = 9.0 Hz, C4′), 86.47 (dd, J = 27.0, 37.6 Hz, C1′), 98.25 (C5), 123.91 (t, J = 258.9 Hz, C2′), 145.95 (C6), 157.67 (C2), 164.82 (C4), 176.00. ^{19}F NMR (CD_3OD) δ −120.16 (br d, J = 239.0 Hz, 1F), −119.21 (dd, J = 10.5, 242.6 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{31}F_2N_3NaO_6$ [M + Na]$^+$, 470.2073; found, 470.2073.

4-*N*-(11-Fluoroundecanoyl)-2′-deoxy-2′,2′-difluorocytidine (8). Treatment of **2a** (69.8 mg, 0.233 mmol) with 11-fluoroundecanoic acid (**S4**, 52 mg, 0.256 mmol) by procedure A gave 82.7 mg of the crude product, which was then column chromatographed (70% EtOAc/hexane) to give **8** (42.1 mg, 41%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.35 (br s, 12H, 6 × CH_2), 1.62−1.74 (m, 4H, 2 × CH_2), 2.47 (t, J = 7.4 Hz, 2H, CH_2), 3.83 (dd, J = 3.0, 12.8 Hz, 1H, H5′), 3.96−4.02 (m, 2H, H5″, H4′), 4.32 (dt, J = 8.6, 12.2 Hz, 1H, H3′), 4.42 (dt, J = 6.1, 47.5 Hz, 2H, CH_2), 6.28 (t, J = 7.2 Hz, 1H, H1′), 7.51 (d, J = 7.6 Hz, 1H, H5), 8.35 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.95, 26.35, 30.16, 30.38, 30.48, 30.59, 31.50, 31.69, 38.17, 60.29(C5′), 70.20 (t, J = 23.0 Hz, C3′), 82.85 (dd, J = 2.3, 3.6 Hz, C4′), 84.89 (d, J = 163.8 Hz, CH_2F), 86.47 (dd, J = 29.6, 34.7 Hz, C1), 98.29 (C5), 123.94 (t, J = 259.2 Hz, C2′), 145.96 (C6), 157.69 (C2), 164.83 (C4), 176.01. ^{19}F NMR (CD_3OD) δ −219.87 (tt, J = 24.7, 47.5 Hz, 1F), −120.09 (br d, J = 239.0 Hz, 1F), −119.17 (br dd, J = 10.2, 239.0 Hz, 1F). MS (ESI) m/z 450 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{30}F_3N_3NaO_5$ [M + Na]$^+$, 472.2023; found, 472.2011.

4-*N*-[11-(1*H*-Benzotriazol-1-yloxy)-undecanoyl]-2′-deoxy-2′,2′-difluorocytidine (9). Treatment of **2a** (50 mg, 0.167 mmol) with commercially available 11-bromoundecanoic acid (48.7 mg, 0.184 mmol) by procedure A gave 85.5 mg of the crude product, which was then column chromatographed (5% MeOH/EtOAc) to give **9** (50 mg, 53%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.28 (br s, 10H, CH_2), 1.45−1.57 (m, 4H, CH_2), 1.73−1.80 (m, 2H, CH_2), 2.40 (t, J = 7.3 Hz, 2H, CH_2), 3.66 (br d, J = 13.6 Hz, 1H, H5′), 3.80 (br d, J = 13.6 Hz, 1H, H5″), 3.89 (dt, J = 2.7, 8.4 Hz, 1H, H4′), 4.20 (br dt, J = 9.1, 12.6 Hz, 1H, H3′), 4.55 (t, J = 6.5 Hz, 2H, CH_2), 5.35 (br t, J = 4.6 Hz, 1H, OH), 6.17 (t, J = 7.5 Hz, 1H, H1′), 6.39 (br s, 1H, OH), 7.28 (d, J = 7.6 Hz, 1H, H5), 7.48 (t, J = 7.6 Hz, 1H, Ar), 7.64 (t, J = 7.6 Hz, 1H, Ar), 7.82 (d, J = 8.4 Hz, 1H, Ar), 8.07 (d, J = 8.4 Hz, 1H, Ar), 8.25 (d, J = 7.6 Hz, 1H, H6), 10.99 (br s, 1H, NH). ^{13}C NMR (CD_3OD) δ 25.90, 26.64, 29.12, 30.06, 30.24, 30.28, 30.35, 30.40, 38.14, 58.32, 60.30, 70.23 (t, J = 23.1 Hz, C3′), 82.32, 82.89 (m, C4′), 98.25 (C5), 110.16, 120.50, 123.92, 126.38, 128.72, 129.55, 144.49, 145.95, 157.66, 164.81, 175.99. ^{19}F NMR (CD_3OD) δ −120.09 (br d, J = 239.0 Hz, 1F), −119.14 (dd, J = 243.7, 12.3 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{26}H_{34}F_3N_6NaO_6$ [M + Na]$^+$, 587.2406; found, 587.2442.

4-*N*-(11-Chloroundecanoyl)-2′-deoxy-2′,2′-difluorocytidine (10). *Method A.* TMSCl (79 μL, 68 mg, 0.630 mmol) was added to a suspension of **2a** (150 mg, 0.500 mmol) in Pyr/MeCN (3:1, 2 mL) at 0 °C under Ar and stirred for 2.5 h, resulting in a clear solution. In a separate vessel, carbonyldiimidazole (CDI, 22.5 mg, 0.138 mmol) was added to a solution of 11-bromoundecanoic acid (36.5 mg, 0.138 mmol) in MeCN (1 mL) portionwise, and the mixure was stirred at ambient temperature. After 30 min, the latter solution was combined with the previously prepared solution of transiently protected nucleoside, and the new reaction mixture was stirred at 65 °C overnight. After 19 h, EtOH (2 mL) was added to the mixture followed by H_2O (4 mL), and the solution was stirred at 65 °C for 20 min. The volatiles were then evaporated under reduced pressure, the residue was partitioned between EtOAc and H_2O, the pH was adjusted to 2.0 with phosphoric acid, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with saturated $NaHCO_3/H_2O$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure, and the resulting residue (47.2 mg) was column chromatographed (70% EtOAc/hexane) to give **10** (11 mg, 5%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.34 (br s, 10H, 2 × CH_2), 1.41−1.49 (m, 2H, CH_2), 1.66−1.71 (m, 2H, CH_2), 1.73−1.82 (m, 2H, CH_2), 2.47 (t, J = 7.5 Hz, 2H, CH_2), 3.56 (t, J = 6.7 Hz, 2H, CH_2), 3.83 (dd, J = 12.7, 3.1 Hz, 1H, H5′), 3.96−4.03 (m, 2H, H5″, H4′), 4.27−4.37 (m, 1H, H3′), 6.28 (t, J = 7.2 Hz, 1H, H1′), 7.51 (d, J = 7.6

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Hz, 1H, H5), 8.36 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.94, 27.93, 29.94, 30.13, 30.35, 30.43, 30.51, 33.83, 38.15, 45.74, 60.31 (C5′), 70.25 (C3′), 82.89 (C4′), 86.81 (C1′), 98.26 (C5), 123.93 (t, J = 258.0 Hz, C2′), 145.97 (C6), 157.71 (C2), 164.86 (C4), 176.02 (CO). ^{19}F NMR (CD_3OD) δ −120.13 (br d, J = 240.2 Hz, 1F), −119.2 (br dd, J = 10.9, 240.2 Hz, 1F). MS (ESI$^+$) m/z 466 (100, [M + H]$^+$ for ^{35}Cl), 468 (100, [M + H]$^+$ for ^{37}Cl). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{30}{}^{35}ClF_2N_3NaO_5$ [M + Na]$^+$, 488.1734; found, 488.1742.

Method B. Et_3N (28 μL, 0.200 mmol) was added to a mixture of 11-bromoundecanoic acid (26.6 mg, 0.100 mmol) in THF (1 mL), and the mixture was stirred at ambient temperature under Ar. The reaction mixture was then cooled to −15 °C followed by the dropwise addition of a solution of $ClCO_2Et$ (19 μL, 0.200 mmol) in THF (0.5 mL) with continued stirring. After 15 min, a solution of **2a** (30 mg, 0.100 mmol) in DMF/DMSO (2.5 mL, 1.5:1) was added dropwise, and the reaction mixture was allowed to warm to ambient temperature and was kept stirring overnight. After 24 h, the reaction was treated with $NaHCO_3$ and extracted with EtOAc (3×). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure, and the residue was column chromatographed (70% EtOAc/hexane) to give **10** (7 mg, 15%) with data as above.

4-*N*-(11-Bromoundecanoyl)-3′,5′-di-*O*-(*tert*-butoxycarbonyl)-2′-deoxy-2′,2′-difluorocytidine (11). A solution of **2b**[46] (35.5 mg, 0.077 mmol) and $NaHCO_3$ (400 mg, 4.76 mmol) in CH_2Cl_2 (0.5 mL) was added to a stirred solution of 11-bromoundecanoyl chloride (**S5**, 0.1 mL, 122 mg, 0.43 mmol) in CH_2Cl_2 (1 mL) at 0 °C under Ar. After 15 min, the reaction mixture was allowed to warm to ambient temperature and was kept stirring for 3 h. The reaction mixture was quenched by addition of saturated $NaHCO_3/H_2O$, the mixture was partitioned with water, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure, and the resulting residue (141.0 mg) was chromatographed (25% EtOAc/hexane) to give **11** (18 mg, 33%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.30 (br s, 10H, 5 × CH_2), 1.40−1.45 (m, 2H, CH_2), 1.53 (s, 18H, 6 × CH_3), 1.68 (quin, J = 7.3 Hz, 2H, CH_2), 1.86 (quin, J = 7.3 Hz, 2H, CH_2), 2.48 (t, J = 7.5 Hz, 2H, CH_2), 3.42 (t, J = 6.9 Hz, 2H, CH_2), 4.37−4.50 (m, 3H, H4′, H5′,5″), 5.14 (dt, J = 4.5, 11.2 Hz, 1H, H3′), 6.46 (dd, J = 7.3, 9.5 Hz, 1H, H1′), 7.51 (d, J = 7.6 Hz, 1H, H5), 7.85 (d, J = 7.6 Hz, 1H, H6), 9.05 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 24.77, 27.54, 27.70, 28.14, 28.72, 28.96, 29.22, 29.26, 29.33, 32.82, 34.07, 37.58, 63.87 (C5′), 72.64 (dd, J = 17.2, 34.0 Hz, C3′), 77.79 (C4′), 83.37, 84.21 (m, C1′), 84.83, 97.02 (C5), 120.40 (dd, J = 260.7, 267.3 Hz, C2′), 145.27 (C6), 151.42, 152.91, 153.94 (C2), 163.40 (C4), 174.17. ^{19}F NMR ($CDCl_3$) δ −120.00 (br d, J = 246.9 Hz, 1F), −115.57 (dt, J = 11.4, 246.9 Hz, 1F). MS (ESI$^+$) m/z 710 (100, [M + H]$^+$ for ^{79}Br), 712 (100, [M + H]$^+$ for ^{81}Br).

4-*N*-(11-Bromoundecanoyl)-2′-deoxy-2′,2′-difluorocytidine (12). Compound **11** (32 mg, 0.045 mmol) was dissolved in TFA (1.0 mL), and the mixture was stirred at 20 °C. After 4 h, the reaction mixture was diluted with toluene, the volatiles were evaporated, and the residue was coevaporated with a fresh portion of toluene. The resulting residue (32 mg) was column chromatographed (80 → 100% EtOAc/hexane) to give **12** (19.9 mg, 86%) as a colorless solid. ^{1}H NMR (CD_3OD) δ 1.31−1.41 (m, 10H, 5 × CH_2), 1.41−1.52 (m, 2H, CH_2), 1.63−1.73 (m, 2H, CH_2), 1.81−1.89 (m, 2H, CH_2), 2.47 (t, J = 7.4 Hz, 2H, CH_2), 3.45 (t, J = 6.7 Hz, 2H, CH_2), 3.75−3.89 (m, 1H, H5″), 3.93−4.05 (m, 2H, H4′, H5″), 4.32 (dt, J = 8.5, 12.2 Hz, 1H, H3′), 6.28 (t, J = 7.3 Hz, 1H, H1′), 7.51 (d, J = 7.6 Hz, 1H, H5), 8.35 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 25.94, 29.17, 29.80, 30.13, 30.34, 30.43, 30.48, 34.01, 34.42, 38.17, 60.32 (C5′), 70.25 (dd, J = 22.2, 23.6 Hz, C3′), 82.88 (d, J = 8.6 Hz, C4′), 86.48 (dd, J = 26.6, 37.6 Hz, C1), 98.29 (C5), 123.93 (t, J = 259.9 Hz, C2′), 145.98 (C6), 157.69 (C2), 164.84 (C4), 176.03. ^{19}F NMR (CD_3OD) δ −120.10 (br d, J = 240.0 Hz, 1F), −119.17 (ddd, J = 3.9, 12.1, 240.0 Hz, 1F). MS (ESI$^+$) m/z 510 (100, [M + H]$^+$ for ^{79}Br), 512 (100, [M + H]$^+$ for ^{81}Br). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{30}{}^{79}BrF_2N_3NaO_5$ [M + Na]$^+$, 532.1229; found, 532.1239.

4-*N*-(11-Hydroxyundecanoyl)-3′,5′-di-*O*-(*tert*-butoxycarbonyl)-2′-deoxy-2′,2′-difluorocytidine (13). Treatment of **2b** (39 mg, 0.084 mmol) with 11-hydroxyundecanoic acid (29 mg, 0.14 mmol) by procedure A gave 102 mg of the crude product, which was then column chromatographed (55 → 65% EtOAc/hexane) to give **13** (20 mg, 37%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.30 (br s, 12H, 6 × CH_2), 1.53 (s, 18H, 6 × CH_3), 1.58 (quin, J = 6.9 Hz, 2H, CH_2), 1.69 (quin, J = 7.4 Hz, 2H, CH_2), 2.47 (t, J = 7.5 Hz, 2H, CH_2), 3.65 (t, J = 6.6 Hz, 2H, CH_2), 4.37−4.50 (m, 3H, H4′, H5′,5″), 5.14 (″dt, J = 4.8, 11.1 Hz, 1H, H3′), 6.46 (dd, J = 7.2, 9.5 Hz, 1H, H1′), 7.51 (d, J = 7.6 Hz, 1H, H5), 7.85 (d, J = 7.6 Hz, 1H, H6), 9.08 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 24.75, 25.65, 27.53, 27.69, 28.88, 29.11, 29.16, 29.27, 29.38, 32.75, 37.78, 62.99, 63.88 (C5′), 72.67 (dd, J = 17.0, 33.8 Hz, C3′), 77.73 (C4′), 83.33, 84.16 (dd, J = 18.2, 37.9 Hz, C1′), 84.77, 97.08 (C5), 120.42 (t, J = 263.8 Hz, C2′), 144.78 (C6), 151.44, 152.93, 154.67 (C2), 162.93 (C4), 173.46. ^{19}F NMR ($CDCl_3$) δ −120.00 (br d, J = 246.7 Hz, 1F), −115.58 (dt, J = 11.4, 246.7 Hz, 1F); MS (ESI$^+$) m/z 648 (100, [M + H]$^+$).

Treatment of **13** (4.0 mg, 0.008 mmol) with TFA as descibed for **12** gave **7** (3.1 mg, 87%) with data as reported above.

4-*N*-(11-Fluoroundecanoyl)-3′,5′-di-*O*-(*tert*-butoxycarbonyl)-2′-deoxy-2′,2′-difluorocytidine (14). A chilled (−78 °C) solution of DAST (6.2 μL, 7.6 mg, 0.048 mmol) in CH_2Cl_2 (500 μL) was added to a stirred solution of **13** (9.8 mg, 0.016 mmol) in CH_2Cl_2 (1.5 mL) at −78 °C. After 30 min, the reaction mixture was allowed to warm to ambient temperature and was kept stirring. After 2 h, the reaction mixture was then poured into a separatory funnel containing a chilled solution of $NaHCO_3/H_2O$ (10 mL, pH 8) and was then extracted with $CHCl_3$ (3 × 10 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure, and the resulting residue (14 mg) was column chromatographed (5% MeOH/$CHCl_3$) to give **14** (4.2 mg, 40%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (br s, 12H, 6 × CH_2), 1.51 (s, 9H, *t*-Bu), 1.52 (s, 9H, *t*-Bu), 1.60−1.78 (m, 4H, 2 × CH_2), 2.45 (t, J = 7.4 Hz, 2H, CH_2), 4.38−4.47 (m, 3H, H4′, H5′, H5″), 4.44 (dt, J = 6.2, 47.3 Hz, 2H, CH_2), 5.12−5.15 (m, 1H, H3′), 6.43 (t, J = 7.3 Hz, 1H, H1′), 7.51−7.54 (m, 1H, H5), 7.87 (d, J = 7.0 Hz, 1H, H6). ^{19}F NMR ($CDCl_3$) δ −217.97 (dt, J = 25.0, 47.4 Hz, 2F), −120.27 (br d, J = 240.7 Hz, 1F), −115.77 (dt, J = 10.9, 247.4 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{30}H_{46}F_3N_3NaO_9$ [M + Na]$^+$, 672.3078; found, 672.3096.

Treatment of **14** (4.0 mg, 0.008 mmol) with TFA as descibed for **12** gave **8** (2.9 mg, 82%) with data as reported above.

4-*N*-(10-Fluoroundecanoyl)-2′-deoxy-2′,2′-difluorocytidine (15). Chilled hydrogen fluoride/pyridine (70%, 1.0 mL) was added to **5** (20 mg, 0.044 mmol) in an HDPE vessel at 0 °C and stirred. After 2 h, the reaction mixture was treated with saturated $NaHCO_3/H_2O$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure, and the resulting residue (24.6 mg) was then column chromatographed (70% EtOAc/hexane) to give **15** (19 mg, 91%; isomeric mixture of **15**/**16**/**17** in a 75:20:5 ratio) as a white solid. UV (CH_3OH) λ_{max} 250 nm (ε 13 250), 298 nm (ε 5350), λ_{min} 226 nm (ε 4650), 279 nm (ε 4700). Major isomer **15** had: ^{1}H NMR (DMSO-d_6) δ 1.22 (br d, J = 6.1 Hz, 2H, CH_2), 1.27 (br s, 8H, 4 × CH_2), 1.29 (br s, 2H, CH_2), 1.44−1.62 (m, 5H, CH_2, CH_3), 2.40 (t, J = 7.3 Hz, 2H, CH_2), 3.66 (dt, J = 12.5, 4.3 Hz, 1H, H5″), 3.81 (br d, J = 12.0 Hz, 1H, H5′), 3.89 (br d, J = 8.5 Hz, 1H, H4′), 4.19 (sep, J = 6.4 Hz, 1H, H3′), 4.64 (dsex, J = 49.0, 6.0 Hz, 1H, CH), 5.31 (t, J = 5.1 Hz, 1H, OH), 6.17 (t, J = 7.5 Hz, 1H, H1′), 6.33 (d, J = 5.8 Hz, 1H, OH), 7.29 (d, J = 7.6 Hz, 1H, H5), 8.24 (d, J = 7.6 Hz, 1H, H6), 10.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 24.30, 24.42, 24.46, 28.37, 28.57, 28.71, 28.74, 36.13, 36.35, 58.78 (C5′), 68.37 (t, J = 22.5 Hz, C3′), 81.01 (t, J = 3.9 Hz, C4′), 84.50 (d, J = 82.2 Hz, C1′), 90.53 (d, J = 162.9 Hz), 95.87 (C5), 124.18 (d, J = 260.1 Hz, C2′), 144.68 (C6), 154.17 (C2), 162.85 (C4), 174.06. ^{19}F NMR (DMSO-d_6) δ −170.27 (symmetric m, 0.75F), δ −116.91 (br s, 2F). MS (ESI$^+$) m/z 450 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{30}F_3N_3NaO_5$ [M + Na]$^+$, 472.2030; found, 472.2048. Anal. Calcd for $C_{20}H_{30}F_3N_3O_4{\cdot}H_2O{\cdot}0.33CH_3CN$

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(481.03): C, 51.59; H, 6.91; N, 9.70. Found: C, 51.36; H, 6.89; N, 9.97.

Minor isomers **16** [4-*N*-(9-fluoroundecanoyl)] and **17** [4-*N*-(8-fluoroundecanoyl)] had the following distinguishable peaks. ^{1}H NMR (DMSO-d_6) δ 4.41 (d quin, J = 49.6, 5.8 Hz, 0.25, CHF). ^{19}F NMR (DMSO-d_6) δ −179.79 (symmetric m, 0.20F), −178.83 (m, 0.05F), −116.91 (br s, 2F).

4-*N*-(*p*-Toluenosulfonyl)-3′,5′-di-*O*-(*tert*-butoxycarbonyl)-2′-deoxy-2′,2′-difluorocytidine (18). Et_3N (1.45 mL, 10.5 mmol) and TsCl (997 mg, 5.2 mmol) were added to a solution of **2b** (242 mg, 0.52 mmol) in dry 1,4-dioxane (4.0 mL) and stirred at ambient temperature under Ar. The tightly sealed reaction mixture was then gradually heated to 65 °C and kept stirring. After 24 h, the reaction mixture was diluted with EtOAc and partitioned with a saturated $NaHCO_3/H_2O$ solution, and the aqueous layer was then extracted with EtOAc (2×). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure, and the resulting residue (403 mg) was then column chromatographed (35% EtOAc/hexane) to give **18** (146 mg, 45%) as a colorless, solidifying oil. ^{1}H NMR δ 1.49 (s, 9H, 3 × CH_3), 1.52 (s, 9H, 3 × CH_3), 2.43 (s, 3H, CH_3), 4.46−4.32 (m, 3H, H4′, H5′, 5″), 5.11 (ddd, J = 4.0, 5.3, 12.8 Hz, 1H, H3′), 5.80 (br s, 1H, H5), 6.24 (dd, J = 6.6, 10.6 Hz, 1H, H1′), 7.31 (d, J = 8.1 Hz, 2H, Ar), 7.48 (dd, J = 1.9, 8.1, Hz, 1H, H6), 7.84 (d, J = 8.3 Hz, 2H, Ar), 10.96 (br s, 1H, NH). ^{13}C NMR δ 21.54, 27.51, 27.65, 63.80 (C5′), 72.40 (dd, J = 16.9, 33.8 Hz, C3′), 78.02 (dd, J = 2.2, 4.7 Hz, C4′), 83.31 (dd, J = 20.6, 38.7 Hz, C1′), 83.41, 84.99, 98.41 (C5), 120.38 (dd, J = 260.2, 266.5 Hz, C2′), 126.71 (Ar), 129.58 (Ar), 138.26 (d, J = 3.4 Hz, Ar), 139.88 (d, J = 2.2 Hz, C6), 143.74 (Ar), 147.16 (C2), 151.35, 152.82, 154.82 (C4). ^{19}F NMR δ −120.59 (br d, J = 247.6 Hz, 1F), −115.80 (br d, J = 247.6 Hz, 1F). MS (ESI$^+$) m/z 618 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{26}H_{33}F_2N_3NaO_{10}S$ [M + Na]$^+$, 640.1747; found, 640.1754.

4-*N*-(10-Undecenyl)-2′-deoxy-2′,2′-difluorocytidine (19). In a tightly sealed vessel, a mixture of **18** (40 mg, 0.065 mmol) and 1-amino-10-undecene (0.50 mL, 404 mg, 2.4 mmol) was stirred at 60 °C. After 30 h, the volatiles were evaporated, and the resulting residue was column chromatographed (8% MeOH/EtOAc) to give **19** (9.5 mg, 36%) as colorless viscous oil. UV (CH_3OH) λ_{max} 268 nm (ε 11 600), λ_{min} 228 nm (ε 7800). ^{1}H NMR (CD_3OD) δ 1.43−1.30 (m, 12H, 6 × CH_2), 1.65−1.56 (m, 2H, CH_2), 2.03−2.09 (m, 2H, CH_2), 3.39 (t, J = 7.1 Hz, 2H, CH_2), 3.80 (dd, J = 3.3, 12.6 Hz, 1H, H5′), 3.89 (td, J = 2.8, 8.3 Hz, 1H, H4′), 3.95 (d, J = 12.6 Hz, 1H, H5″), 4.26 (dt, J = 8.3, 12.1 Hz, 1H, H3′), 4.91−5.02 (m, 2H, CH_2), 5.82 (tdd, J = 6.7, 10.3, 17.0 Hz, 1H, CH), 5.87 (d, J = 7.6 Hz, 1H, H5), 6.23 (t, J = 8.0 Hz, 1H, H1′), 7.74 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 28.01, 29.98, 30.12, 30.19, 30.42, 30.51, 30.63, 34.88, 41.75, 60.56 (C5′), 70.67 (dd, J = 22.4, 23.8 Hz, C3′), 82.26 (dd, J = 3.6, 5.0 Hz, C4′), 85.94 (dd, J = 26.0, 38.0 Hz, C1), 97.33 (C5), 114.68, 124.05 (t, J = 258.4 Hz, C2′), 140.16, 140.77 (C6), 158.30 (C2), 165.37 (C4). ^{19}F NMR (CD_3OD) δ −119.89 (br d, J = 240.1 Hz, 1F), −118.80 (br d, J = 240.1 Hz, 1F). MS (ESI$^+$) m/z 416 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{31}F_2N_3NaO_4$ [M + Na]$^+$, 438.2175; found, 438.2178. Anal. Calcd for $C_{20}H_{31}F_2N_3O_4 \cdot 0.5H_2O \cdot 0.5CH_3CN$ (445.01): C, 56.68; H, 7.59; N, 11.02. Found: C, 56.93; H, 7.77; N, 10.76.

4-*N*-(11-Hydroxyundecanyl)-2′-deoxy-2′,2′-difluorocytidine (21). 11-Amino-1-undecanol (**S7**; 88 mg, 0.47 mmol) and Et_3N (0.5 mL) were added to a solution of **18** (23.2 mg, 0.038 mmol) in 1,4-dioxane (0.5 mL) and stirred at ambient temperature under Ar. The reaction mixture was then gradually heated to 65 °C (oil bath) and kept stirring overnight. After 40 h, the volatiles were evaporated, and the residue (97 mg) was column chromatographed (1 → 3% MeOH/EtOAc) to give monoprotected product **20** (9.5 mg, 47%). ^{1}H NMR (CD_3OD) δ 1.32 (br s, 12H, 6 × CH_2), 1.49 (s, 9H, *t*-Bu), 1.49−1.61 (m, 4H, 2 × CH_2), 3.37 (t, J = 7.1 Hz, 2H, CH_2), 3.49−3.62 (m, 2H, CH_2), 4.17 (dt, J = 9.9, 19.4 Hz, 1H, H4′), 4.02−4.09 (m, 1H, H3′), 4.48 (dd, J = 2.6, 12.4 Hz, 1H, H5′), 4.33 (dd, J = 4.3, 12.4 Hz, 1H, H5″), 5.86 (d, J = 7.6 Hz, 2H, H5), 6.25 (t, J = 8.2 Hz, 2H, H1′), 7.51 (d, J = 7.6 Hz, 2H, C6). MS (ESI$^+$) m/z 534 (100, [M + H]$^+$)] followed by **21** (4 mg, 24%) of 90% purity. Compound **20** (9.5 mg, 0.018 mmol) was dissolved in TFA (1.0 mL), and the reaction mixture was stirred at 18 °C. After 5 h, the reaction mixture was diluted with toluene (2 mL), the volatiles were evaporated, and the residue was coevaporated with a toluene (2 × 1 mL). The resulting residue (17 mg) was then column chromatographed (1% MeOH/EtOAc) to give **21** (2.2 mg, 29% from **20**; 38% overall from **18**) as a colorless oil. ^{1}H NMR (CD_3OD) δ 1.30−1.41 (m, 14H, 7 × CH_2), 1.50−1.57 (m, 2H, CH_2), 1.58−1.64 (m, 2H, CH_2), 3.39 (t, J = 7.1 Hz, 2H, CH_2), 3.55 (t, J = 6.6 Hz, 2H, CH_2), 3.80 (dd, J = 3.3, 12.6 Hz, 1H, H5′), 3.89 (td, J = 2.8, 8.3 Hz, 1H, H4′), 3.95 (br dd, J = 2.0, 12.6, 1H, H5″), 4.26 (dt, J = 8.3, 12.1 Hz, 1H, H3′), 5.87 (d, J = 7.6 Hz, 1H, H5), 6.23 (t, J = 8.0 Hz, 1H, H1′), 7.74 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 26.94, 28.01, 29.97, 30.42, 30.58, 30.63, 30.66, 30.71, 33.67, 41.74, 60.56 (C5′), 63.03, 70.63 (dd, J = 22.0, 24.8 Hz, C3′), 82.23 (dd, J = 3.8, 5.0 Hz, C4′), 85.82 (C1), 97.32 (C5), 124.04 (t, J = 259.8 Hz, C2′), 140.77 (C6), 158.29 (C2), 165.37 (C4). ^{19}F NMR (CD_3OD) δ −119.90 (br d, J = 239.2 Hz, 1F), −118.83 (dd, J = 11.6, 239.2 Hz, 1F). MS (ESI$^+$) m/z 434 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{33}F_2N_3NaO_5$ [M + Na]$^+$, 456.2280; found, 456.2287.

4-*N*-(*p*-Toluenosulfonyl)-2′-deoxy-2′,2′-difluorocytidine (22). TMSCl (5.1 mL) was added to a suspension of **2a** (600 mg, 2.0 mmol) in anhydrous pyridine (10 mL), and the mixture was stirred at ambient temperature under Ar. After 2 h, TsCl (3.8 g, 20.027 mmol) was added, and the reaction mixture was gradually heated to 60 °C (oil-bath) and kept stirring. After 20 h, volatiles were evaporated under reduced pressure, and the resulting residue was treated with MeOH/NH_3 (10 mL) and stirred at ambient temperature overnight. After 24 h, volatiles were evaporated under reduced pressure, and the resulting residue was column chromatographed (90% EtOAc/hexane) to give **22** (808 mg, 96%) as a white solid. ^{1}H NMR (CD_3OD) δ 2.42 (s, 3H, CH_3), 3.78 (dd, J = 3.4, 12.8 Hz, 1H, H5′), 3.90−3.95 (m, 2H, H4′, H5″), 4.28 (dt, J = 8.4, 12.0 Hz, 1H, H3′), 6.13 (dd, J = 5.3, 9.5 Hz, 1H, H1′), 6.65 (d, J = 8.2 Hz, 1H, H5), 7.36 (d, J = 8.0 Hz, 2H, Ar), 7.79 (d, J = 8.3 Hz, 2H, Ar), 7.99 (d, J = 8.1 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 21.43, 60.34 (C5′), 70.21 (dd, J = 18.8, 27.2 Hz, C3′), 82.99 (d, J = 8.4, C4′), 85.46 (dd, J = 23.9, 41.3 Hz, C1′), 98.46 (C5), 123.84 (t, J = 258.7 Hz, C2′), 127.58 (Ar), 130.52 (Ar), 140.71 (Ar), 142.62 (C6), 144.66 (Ar), 150.21 (C2), 160.54 (C4). ^{19}F NMR (CD_3OD) δ −120.17 (br s, 1F), −119.41 (dd, J = 4.1, 12.7 Hz, 1F). MS (ESI$^+$) m/z 440 (100, [M + Na]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{16}H_{17}F_2N_3NaO_6S$ [M + Na]$^+$, 440.0698; found, 440.0711.

4-*N*-(11-Benzyloxyundecanyl)-2′-deoxy-2′,2′-difluorocytidine (23). In a tightly sealed container, a solution of **22** (158 mg, 0.383 mmol), 11-(benzyloxy)undecanyl amine (**S11**; 945 mg, 3.41 mmol), and TEA (2 mL) in 1,4-dioxane was stirred at 75 °C. After 96 h, the volatiles were evaporated under reduced pressure, and the resulting residue was column chromatographed (1% MeOH/EtOAc) to give **23** (122 mg, 61%). ^{1}H NMR (CD_3OD) δ 1.28 (br s, 10H, 5 × CH_2), 1.32 (br s, 4H, 2 × CH_2), 1.54−1.61 (m, 4H, 2 × CH_2), 3.36 (t, J = 7.1 Hz, 2H, CH_2), 3.47 (t, J = 6.6 Hz, 2H, CH_2), 3.79 (dd, J = 3.3, 12.6, 1H, H5′), 3.88−3.96 (m, 2H, H4′, H5″), 4.25 (dt, J = 8.3, 12.0 Hz, 1H, H3′), 4.48 (s, 2H, CH_2), 5.89 (d, J = 7.6 Hz, 1H, H5), 6.21 (t, J = 8.0 Hz, 1H, H1′), 7.32 (br s, 5H, Ar), 7.70 (d, J = 7.6 Hz, 1H, H6). ^{13}C NMR (CD_3OD) δ 27.23, 28.00, 29.96, 30.42, 30.48, 30.60, 30.63, 30.65, 30.71, 41.74, 60.54, 68.14 (C3′), 71.44, 73.86, 82.24 (t, J = 2.95 Hz, C4′), 85.92 (dd, J = 26.7, 37.7 Hz, C1′), 97.31 (C5), 124.04 (t, J = 258.7 Hz, C2′), 128.62, 128.84, 129.35, 139.87, 140.75, 158.27, 165.34. ^{19}F NMR (CD_3OD) δ −119.47 (br d, J = 236.7 Hz, 1F), −118.42 (dd, J = 8.6, 236.7 Hz, 1F). HRMS (ESI$^+$) m/z calcd for $C_{27}H_{39}F_2N_3NaO_5$ [M + Na]$^+$, 546.2750; found, 546.2774.

4-*N*-(11-Benzyloxyundecanyl)-3′,5′-di-*O*-benzoyl-2′-deoxy-2′,2′-difluorocytidine (24). BzCl (50 μL, 0.49 mmol) was added to a solution of **23** (117 mg, 0.22 mmol), 2,6-lutidine (64 μL, 0.89 mmol), and 4-dimethylaminopyridine (27 mg, 0.22 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at 35 °C under Argon. After 20 h, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and partitioned with H_2O, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were sequentially washed with 1 M HCl (20 mL), saturated $NaHCO_3/H_2O$ (20 mL), and brine (20 mL), dried over Na_2SO_4, and evaporated under reduced

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pressure, and the resulting residue (157 mg) was column chromatographed (1% MeOH/$CHCl_3$) to give **24** (50.6 mg, 60%) as a mixture of rotamers (80:20). The major rotamer had the following peaks. ^{1}H NMR (CD_3OD) δ 1.24 (br s, 12H, 6 × CH_2), 1.51−1.62 (m, 4H, 2 × CH_2), 3.20 (t, J = 7.1 Hz, 2H, CH_2), 3.39 (t, J = 12.3 Hz, 2H, CH_2), 4.48 (s, 2H, CH_2), 4.49−4.53 (m, 1H, H5′), 4.63−4.67 (m, 1H, H5′), 4.73−4.79 (m, 1H, H4′), 5.54 (d, J = 7.6 Hz, 1H, H5), 5.57−5.61 (m, 1H, H3′), 6.60−6.65 (m, 1H, H1′), 7.26−7.33 (m, 5H, Ar), 7.41−7.49 (m, 4H, Ar), 7.55−7.64 (m, 2H, Ar), 8.02−8.08 (m, 4H, Ar). ^{19}F NMR (CD_3OD) δ −120.48 (br d, J = 246.7 Hz, 1F), −115.34 (dt, J = 13.6, 246.7 Hz, 1F). MS (ESI$^+$) m/z 754 (100, [M + Na]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{41}H_{47}F_2N_3NaO_7$ [M + Na]$^+$, 754.3274; found, 754.3303.

The minor rotamer had the following distinguishable peaks. ^{1}H NMR (CD_3OD): δ 5.70 (d, J = 7.8, 1H, H5). ^{19}F NMR (CD_3OD): δ −115.82 (dt, J = 13.4, 246.7, 1F).

4-*N*-(11-Hydroxyundecanyl)-3′,5′-di-*O*-benzoyl-2′-deoxy-2′,2′-difluorocytidine (25). Ammonium cerium(IV) nitrate (63 mg, 0.115 mmol) was added to a solution of **24** (106 mg, 0.145 mmol) in CH_3CN/H_2O (9:1, 5 mL), and the mixture was stirred at ambient temperature overnight. Additional portions of CAN (240 mg) were added to the reaction mixture every 24 h until no starting material could be detected by TLC. After 72 h, the reaction was quenched by the addition of saturated $NaHSO_3$ (20 mL), the volatiles were evaporated under reduced pressure, and the resulting aqueous residue was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure, and the resulting residue (103.5 mg) was then column chromatographed (1% MeOH/EtOAc) to give **25** (66 mg, 70%) as a mixture of rotamers (72:28). The major rotamer had the following peaks. ^{1}H NMR (CD_3OD) δ 1.24 (br s, 14H, 7 × CH_2), 1.51−1.64 (m, 4H, 2 × CH_2), 3.20 (t, J = 7.0 Hz, 2H, CH_2), 3.63 (t, J = 6.6 Hz, 2H, CH_2), 4.50−4.58 (m, 1H, H4′), 4.64−4.71 (m, 1H, H5′), 4.75−4.82 (m, 1H, H5″), 5.57−5.62 (m, 1H, H3′) 5.60 (d, J = 7.6 Hz, 1H, H5), 6.58−6.61 (m, 1H, H1′), 7.31 (d, J = 7.6 Hz, 1H, H6), 7.41−7.65 (m, 6H, Ar), 8.02−8.11 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ 25.79, 26.93, 29.20, 29.29, 29.44, 29.47, 29.53, 29.57, 32.78, 41.14, 62.90, 63.01, 71.86 (m, C3′), 77.74 (C4′), 83.51 (br s, C1′), 96.55 (C5), 120.98 (t, J = 256.3 Hz, C2′), 128.10, 128.70, 128.81, 129.39, 129.81, 130.27, 133.60, 134.28, 139.86, 155.75, 163.59, 165.05, 166.09. ^{19}F NMR δ −115.36 (dt, J = 13.7, 246.3 Hz, 1F), −120.50 (br d, 1F). MS (ESI$^+$) m/z 664 (100, [M + Na]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{34}H_{41}F_2N_3NaO_7$ [M + Na]$^+$, 664.2805; found, 664.2837.

The minor rotamer had the following distinguishable peaks. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40−3.41 (m, 2H, CH_2), 5.71 (d, J = 7.8 Hz, 1H, H5). ^{19}F NMR δ −115.85 (dt, J = 12.4, 246.0 Hz, 1F).

4-*N*-(11-Fluoroundecanyl)-3′,5′-di-*O*-benzoyl-2′-deoxy-2′,2′-difluorocytidine (26). A chilled (−78 °C) solution of DAST (14 μL, 17.2 mg, 0.107 mmol) in CH_2Cl_2 (250 μL) was added to a stirred solution of **25** (21.7 mg, 0.034 mmol) in CH_2Cl_2 (1 mL) at −78 °C. After 30 min, the reaction mixture was allowed to warm to ambient temperature and was kept stirring. After 3 h, the reaction mixture was poured into a separatory funnel containing an ice-cold solution of Na_2HCO_3 in H_2O (10 mL, pH 8) and was extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure, and the resulting oily residue (20.5 mg) was then column chromatographed (40% EtOAc/hexane) to give **26** (10.6 mg, 48%) as a mixture of rotamers (76:24). The major rotamer had the following peaks. ^{1}H NMR ($CDCl_3$) δ 1.27 (br s, 14H, 7 × CH_2), 1.55−1.69 (m, 4H, 2 × CH_2), 3.47−3.52 (m, 2H, CH_2), 4.43 (dt, J = 6.2, 47.4 Hz, 2H, CH_2), 4.51−4.55 (m, 1H, H4′), 4.67 (dd, J = 4.5, 12.3 Hz, 1H, H5′), 4.79 (dd, J = 3.2, 12.3 Hz, 1H, H5″), 5.54 (d, J = 7.6 Hz, 1H, H5), 5.58−5.63 (m, 1H, H3′), 6.61 (brs, 1H, H1′), 7.32 (d, J = 7.5 Hz, 1H, H6), 7.42−7.66 (m, 6H, Ar), 8.03−8.16 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ 25.30, 27.01, 29.24, 29.32, 29.49, 29.56, 29.83, 30.44, 30.63, 41.28, 63.02 (C5′), 71.87 (br s, C3′), 79.16 (br s, C4′), 83.56 (br s, C1′), 84.37 (d, J = 164.0 Hz′), 96.17 (C5), 121.88 (t, J = 255.7 Hz, C2′), 128.14, 128.73, 128.84, 129.43, 129.84, 130.31, 133.59, 134.29, 140.10, 155.46, 163.39, 165.06, 166.10. ^{19}F NMR ($CDCl_3$) δ −217.94 (tt, J = 24.9, 47.3 Hz, 1F), −120.62 (br d, J = 203.1, 1F), −115.40 (dt, J = 14.1, 247.3 Hz, 1F). MS (ESI$^+$) m/z 644 (100, [M + H]$^+$). HRMS (ESI-TOF$^+$) m/z calcd for $C_{34}H_{40}F_3N_3NaO_6$ [M + Na]$^+$, 666.2761; found, 666.2763.

The minor rotamer had the following distinguishable peaks. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20−3.21 (m, 2H, CH_2N), 5.71 (d, J = 7.8 Hz, 1H, H5). ^{19}F NMR δ −115.98 (dt, J = 12.9, 247.5 Hz, 1F)

4-*N*-(11-Fluoroundecanyl)-2′-deoxy-2′,2′-difluorocytidine (27). *Method A.* Compound **26** (10.6 mg, 0.017 mmol) was dissolved in methanolic ammonia (2 mL) and stirred at ambient temperature. After 2 h, volatiles were evaporated under reduced pressure, and the resulting residue was chromatographed (5% MeOH/EtOAc) to give **27** (6.5 mg, 90%) as a clear oil. ^{1}H NMR (CD_3OD) δ 1.32 (brs, 14H, 7 × CH_2), 1.55−1.73 (m, 4H, 2 × CH_2), 3.37 (t, J = 7.1 Hz, 2H, CH_2), 3.78 (dd, J = 3.3, 12.6 Hz, 1H, H5′), 3,87 (dt, J = 3.0, 8.28 Hz, 1H, H3′), 3.93 (d, J = 13.3 Hz, 1H, H5″), 4.20−4.28 (m, 1H, H4′), 4.40 (dt, J = 6.1, 47.6 Hz, 1H, CH_2), 5.85 (d, J = 7.6 Hz, 1H, H5), 6.21 (t, J = 7.96 Hz, H1′), 7.73 (d, J = 7.6 Hz, 1H, H6). ^{19}F NMR δ −219.94 (tt, J = 25.5, 47.3 Hz, 1F, CH_2F), −119.60 (br s, 1F), −119.14 (br s, 1F). MS (ESI) m/z 436 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{32}F_3N_3NaO_4$ [M + Na]$^+$, 458.2237; found, 458.2256.

Method B. In a tightly sealed cylindrical pressure vessel with screw cap, a solution of KF (1.6 mg, 0.028 mmol), K_2CO_3 (3.8 mg, 0.028 mmol), Kryptofix 2.2.2 (10.5 mg, 0.028 mmol), and **28** (5.0 mg, 0.007 mmol) in CH_3CN (1 mL) was stirred at 110 °C. After 18 min, the reaction mixture was quickly cooled in a water bath and vacuum filtered into another pressure vessel. The effluent containing crude **26** was concentrated under reduced pressure, and the resulting residue was treated with 0.5 CH_3ONa/MeOH (1 mL), stirred, and heated at 100 °C. After 8 min, the reaction mixture was neutralized with 1 N HCl and evaporated under reduced pressure to dryness. The crude sample was then dissolved in 45% CH_3CN/H_2O to a total volume of 4.5 mL, passed through a 0.2 μm PTFE syringe filter, and then injected into a semipreparative HPLC column (Phenomenex Gemini RP-C18 column; 5 μ, 25 cm × 1 cm) via a 5 mL loop. The HPLC column was eluted with an isocratic mobile phase mixture of 45% CH_3CN/H_2O at a flow rate of 5 mL/min to give **27** (1.9 mg, 62% overall yield from **28**, t_R = 13.1 min) with spectral properties as above.

4-*N*-[11-(Methanesulfoxy)undecanyl]-3′,5′-di-*O*-benzoyl-2′-deoxy-2′,2′-difluorocytidine (28). Et_3N (3.8 μL, 2.7 mg, 0.027 mmol) and MsCl (1.5 μL, 2.3 mg, 0.020 mmol) were sequentially added to a stirred solution of **25** (11.6 mg, 0.018 mmol) in CH_2Cl_2 at 0 °C. After 5 min, the reaction mixture was allowed to warm to ambient temperature and was kept stirring. After 3 h, the reaction mixture was then partitioned between H_2O and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure, and the resulting residue (12.1 mg) was column chromatogramphed (50% EtOAc/hexane) to give **28** (11.7 mg, 90%) as a mixture of rotamers (71:29). The major rotamer had the following peaks. ^{1}H NMR ($CDCl_3$) δ 1.25 (brs, 14H, 7 × CH_2), 1.55−1.77 (m, 4H, 2 × CH_2), 2.99 (s, 3H, CH_3), 3.46−3.52 (m, 2H, CH_2), 4.21 (t, J = 6.6 Hz, 2H, CH_2), 4.51−4.58 (m, 1H, H4′), 4.64−4.81 (m, 2H, H5′, H5″), 5.55 (d, J = 7.6 Hz, 1H, H5), 5.59−5.63 (m, 1H, H3′), 6.55−6.67 (m, 1H, H1′), 7.32 (dd, J = 1.6, 7.5 Hz, 1H, H6), 7.43−7.51 (m, 4H, Ar), 7.57−7.66 (m, 2H, Ar), 8.03−8.10 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ 21.15, 25.47, 26.94, 29.00, 29.22, 29.30, 29.39, 29.46, 29.55, 37.50, 41.18, 63.02 (C5′), 70.39, 71.96 (dd, J = 17.3, 35.8 Hz, C3′), 77.36 (C4′), 84.00 (br s, C1′), 96.22 (C5), 120.93 (t, J = 262.8 Hz, C2′), 128.13, 128.71, 128.82, 129.41, 129.82, 130.28, 133.61, 134.28, 139.97 (C6), 155.66, 163.55, 165.05, 166.08. ^{19}F NMR δ −120.61 (brd, J = 261.9 Hz, 1F), −115.38 (dt, J = 14.1, 246.7 Hz, 1F). MS (ESI) m/z 720 (100, [M + H]$^+$). HRMS (ESI$^+$) m/z calcd for $C_{35}H_{43}F_2N_3NaO_9S$ [M + Na]$^+$, 742.2580; found, 742.2603.

The minor rotamer had the following distinguishable peaks. ^{1}H NMR ($CDCl_3$) δ 3.23−3.25 (m, CH_2N), 5.77 (d, J = 7.9 Hz, H5). ^{19}F NMR δ −115.98 (dt, J = 13.1, 234.1 Hz, 1F).

Cytostatic Activity Assays.[33] The compounds tested were added to murine leukemia L1210, human T-lymphocyte CEM, human

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cervical carcinoma HeLa, and human breast carcinoma MCF-7 cell cultures in 96-well microtiter plates. After 2 (L1210), 3 (CEM), or 4 (HeLa, MCF-7) days of incubation at 37 °C, the number of living cells was determined by a Coulter counter. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to inhibit cell proliferation by 50%.

dCK and TK-2 Activity Assays. The activity of recombinant mitochondrial thymidine kinase (TK-2) and cytosolic 2′-deoxycytidine kinase (dCK) and the 50% inhibitory concentration of the test compounds were assayed in a 50 μL reaction mixture containing 50 mM Tris/HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM CHAPS, 3 mg/mL bovine serum albumin, 2.5 mM ATP, 1 μM [5-^{3}H]dCyd or [CH_3-^{3}H]dThd, and enzyme. The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations (5-fold dilutions) of the test compounds. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE-81 filter-paper disks. The filters were washed three times for 5 min each in 1 mM ammonium formate, once for 1 min in water, and once for 5 min in ethanol. The radioactivity retained on the filter discs was determined by a scintillation counter. To evaluate substrate activity against TK-2 and dCK, the tested compounds were added to the enzyme reaction mixture at 100 μM, and conversion to their 5′-monophosphates was monitored by HPLC on an anion-exchange Partisil Sax column.

Human Serum and Murine Liver Extract Stability Assays. The compounds tested were exposed to 50% human serum in phosphate buffered saline (PBS) or murine liver extract in PBS at 100 μM concentrations and incubated for 0, 60, and 240 min (human serum) or 0, 30, and 120 min (murine liver extract) at 37 °C. At each time point (0, 60, and 240 min), an aliquot was withdrawn and subjected to HPLC analysis on a reverse-phase RP-18 column (mobile phase: acetonitrile/H_2O). Elution times were 13.2 and 16.4 min for dFdU and gemcitabine, respectively, and 22.8 and 22.7 min for compounds **7** and **21**, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, representative HPLC chromatograms, and characterization data for 11-fluoroundecanoic, 11-bromoundecanoyl, 11-aminoundecanol, and 11-(benzyloxy)-undecan-1-amine. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

dFdC, 2′,2′-difluoro-2′-deoxycytidine (Gemcitabine); hENT1, human equilibrative nucleoside transport protein 1; dCK, deoxycytidine kinase; RNR, ribonucleotide reductase; CTP, cytidine triphosphate; dFdU, 2′,2′-difluorouridine; CDA, cytidine deaminase; SQgem, 4-*N*-squalenoylgemcitabine; [^{18}F]-FAC, 1-(2′-deoxy-2′-^{18}F-fluoro-β-D-arabinofuranosyl)-cytosine; L-^{18}F-FMAC, 1-(2′-deoxy-2′-^{18}F-fluoro-β-L-arabino-furanosyl)-5-methylcytosine; EDCI, *N*-dimethylaminopropyl)-*N*′-ethyl-carbodiimide; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; CDI, 1,1′-carbonyldiimidazole; TEA, triethylamine; DAST, (diethylamino)sulfur trifluoride; HDPE, high-density polyethylene; TK-2, thymidine kinase

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