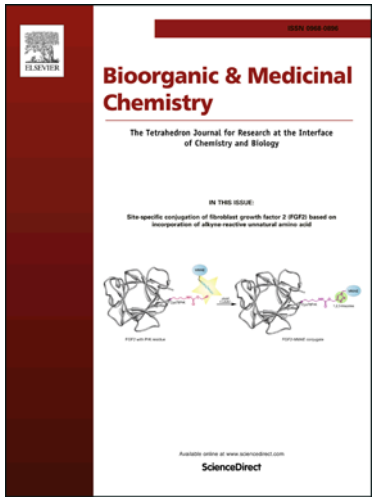

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Linker structure-activity relationships in fluorodeoxyglucose chlorambucil conjugates for tumor-targeted chemotherapy

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Abstract

Nitrogen mustards, such as chlorambucil (CLB), can cause adverse side-effects due to ubiquitous distribution in non-target organs. To minimize this toxicity, strategies of tumor-targeting drug delivery have been developed, where a cytotoxic warhead is linked to a tumor-cell-specific small ligand. Malignant cells exhibit marked glucose avidity and an accelerated metabolism by aerobic glycolysis, known as the Warburg effect, and recognized as a hallmark of cancer. A targeting approach exploiting the Warburg effect by conjugation of CLB to 2-fluoro-2-deoxyglucose (FDG) was previously reported and identified two peracetylated glucoconjugates **2** and **3** with promising antitumor activities *in vivo*. These results prompted us to investigate the importance of the spacer in this tumor-targeting glucose-based conjugates. Here we report the chemical synthesis and an *in vitro* cytotoxicity evaluation, using a 5-member panel of human carcinoma cell lines and human fibroblasts, of 16 new CLB glucoconjugates in which the alkylating drug is attached to the C-1 position of FDG *via* different linkages. We studied the structure-activity relationships in the linker, and evidenced the positive impact of an aromatic linker on *in vitro* cytotoxicity: compound **51** proved to be the most active FDG-CLB glucoside, characterized by a bis-aromatic spacer tethered to CLB through an amide function.

Keywords :

Chlorambucil, 2-fluoro-2-deoxy-D-glucose derivatives, tumor-targeting drug delivery, *in vitro* cytotoxicity

1. Introduction

Targeted chemotherapy is one key issue for a successful outcome in cancer therapy. The main limitations of conventional cancer chemotherapy arise from the suboptimal biodistribution of cytotoxics in the body: lack of drug-specific affinity for tumor cells and systemic toxicity lead to many negative, sometimes life-threatening side effects. If a drug can instead be selectively delivered to the targeted tumor, with less drug reaching critical normal tissues, then cancer therapy and patients' quality of life can be substantially improved. Much effort has accordingly been made to develop new drug delivery systems that mediate drug release selectively at the tumor site.

Active drug delivery systems range widely, and use various kinds of targeting carriers, such as antibodies or small-molecule ligands.[1] Although immunoconjugates show substantial clinical promise, as represented by several approved monoclonal antibody-drug conjugates for treating cancer (e.g. lymphoma and breast cancer),[2] they present major drawbacks, including immunogenic responses and (or) suboptimal biodistribution, leading to unwanted side-effects. Low-molecular-weight molecules targeting specific tumor biomarkers have now been well-documented as an alternative to antibodies for targeted drug delivery,[3] given the inherent advantages of their small size, nonimmunogenic nature, and much more manageable synthesis. To date, the most clinically advanced low-molecular-weight ligands are folate-conjugated drugs,[4,5] such as vintafolide and EC-0225. Other small ligands that have also stirred much interest in recent years for tumor targeting are glucose derivatives.[6–12]

To fuel cell growth and division, cancer cells can reprogram their glucose metabolism with increased glucose uptake and need for glycolysis, known as Warburg effect[13] and recognized as one of the hallmarks of cancer.[14] This metabolic transformation is driven by increased surface expression of the glucose transporters[15,16] and enhanced activity of glycolytic enzymes (hexokinase and phosphofructokinase).[17,18] Glucose is transported into cells via two classes of hexose transporters: the SGLT family (sodium-dependent glucose transporter, SLC5A family) and GLUT family (SLC2A family). GLUT1 is the most common glucose transporter in humans and it is aberrantly expressed in several tumors, including hepatic, pancreatic, breast, esophageal, brain, lung, cutaneous, colorectal cancers. But other GLUT and SGLT isoforms have also been reported for cancers with tissue specific expression differences: GLUT2 (gastric, breast, colon, and liver carcinomas), GLUT3 (B-cell non-Hodgkin's lymphoma), GLUT12 (prostate adenocarcinomas), and SGLT1–2 (colorectal and metastatic lung carcinomas).[16,19] The high demand for glucose in cancer cells is the basis for the detection and

staging of tumors using a radiolabeled analogue of glucose (2-^{18}F-fluorodeoxyglucose, 2-[^{18}F]FDG)[20] in PET imaging. Glucose transporters, especially GLUT1 and GLUT3, and hexokinase seem to play a crucial role on the 2-FDG accumulation. Some tumors do not accumulate 2-FDG, which is a substrate for GLUTs but not for SGLTs, increasing interest in the expression of SGLTs in cancer. This Na^+-dependent co-transporter was reported in the literature to mediate the uptake of a glucose-isophosphoramide mustard conjugate (glufosfamide), the first-in-class glucose conjugate reaching advanced clinical trial (Phase III for treatment of metastatic pancreatic cancer)[21] in a strategy using the high demand for glucose into tumor cells to deliver chemotherapeutic agents.

Indeed, targeting the Warburg effect has been developed in the last decades in therapeutic area using several glucose-based conjugates designed to more specifically deliver the attached drug to cancer cells. The synthesis and biological evaluation of glycoconjugate anticancer therapeutics was firstly reported in the literature in 1995 by Wiessler *et al*[22] with glufosfamide. Since the introduction of glufosfamide, there has been many examples of glucose-conjugated drugs in preclinical or clinical evaluation[6] (paclitaxel[23,24], adriamycin[25], DNA alkylator including chlorambucil[7,11,26,27], cyclophosphamide[28], platinum[29–31]).

In the study reported here, FDG was used as active tumor-specific drug carrier. The choice of FDG was motivated by the markedly increased uptake and retention of this fluorinated glucose analogue in malignant cells, and by its potential to inhibit glycolysis. Also, 2-deoxy-D-glucose (2-DG) has been described as a potential potent antitumor agent through depletion of cellular energy, increased oxidative stress, interference with *N*-linked glycosylation, and induction of autophagy.[32] Neoplastic cells treated with 2-DG accelerate their own demise, and 2-DG also enhances the cytotoxic effects of anticancer agents when used in combination.[33,34]

We have previously reported preclinical results of this targeting approach,[35–37] mainly with chlorambucil (CLB). CLB served as basis for our FDG-glycolysis-mediated targeting proof-of-concept, because its cell uptake occurs through passive diffusion, and like all alkylating agents, its lack of tumor specificity causes serious side-effects, such as nausea, bone marrow suppression and anemia. Three peracetylated glucoconjugates **1-3** demonstrated an increased cytotoxicity *in vitro* compared with the free CLB (Figures 1 and 2) for all the cell lines tested.[35] *In vivo*, a single-dose-finding study to determine the maximum tolerated dose (MTD) using the intraperitoneal route (IP) showed that the three peracetylated glucoconjugates **1-3** were less toxic than CLB itself. When given to tumor-bearing mice (melanoma and colon carcinoma models), according to a q4d × 3 schedule (i.e. three doses at 4-day intervals), compounds **2** and **3** demonstrated promising antitumor activities, with log cell kill (LCK)

values higher than 1.3 in both syngenic colon carcinoma and melanoma models (CT-26 and B16F0).[36] The difference in *in vivo* antitumor activity between conjugated forms **2-3** and CLB, and their better tolerance, make them promising candidates for further work to develop new highly active antineoplastic compounds.

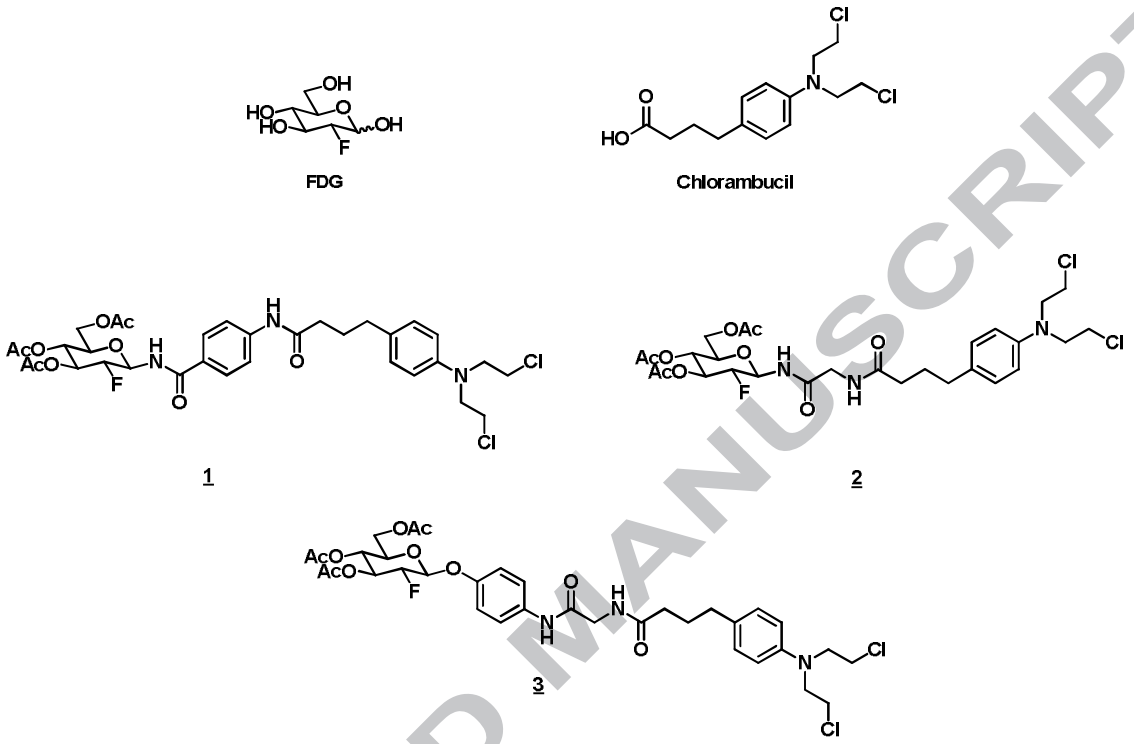


Figure 1. Structures of FDG, chlorambucil (CLB) and CLB-peracetylated FDG conjugates **1**, **2** and **3.**

The success of a tumor-targeting drug delivery system depends on the nature of the cytotoxic warhead and the tumor-seeking moiety, but also on the chemical scaffold linking these two parts. We therefore undertook a more thorough pharmacomodulation investigation of this linker. The composition of the linker can be designed to contain stable or cleavable functional groups, and at the same time residues that may influence physical properties such as the hydrophilic-lipophilic balance of the designed conjugate. The nature of the linker thus influences how successful drug delivery is, and its outcome. Ideally, as the conjugate is meant to reach the malignant site, the spacer arm has to be designed in such a way as to ensure its stability in blood, while also allowing the action of the active cytotoxic warhead addressed to tumor cells. Too-stable linkers can curb the activity of the associated drug, resulting in a low-potency conjugate. Conversely, a too-labile linking moiety can lead to poor target specificity

and high systemic toxicity. Several chemical functions can form the linker. The most common ones are amide, reverse amide, ester and carbamate subjected to enzymatic and non-enzymatic hydrolysis, or labile linkers that rely on more specific modes: enzymatic, reductive or pH-controlled release. In our study, the CLB provides a carboxyl group that can be appropriately functionalized to bind to the linker or to the FDG residue by the formation of an ester or an amide bond, largely used in targeted drug strategies.

In this report, we extended the pharmacomodulation of the previously selected compounds **1-3**, with the synthesis, characterization and *in vitro* cytotoxicities of new glycoconjugate analogs to provide more knowledge on the structure-activity relationships between the nature of the linker and the *in vitro* cytotoxicity of linked drugs.

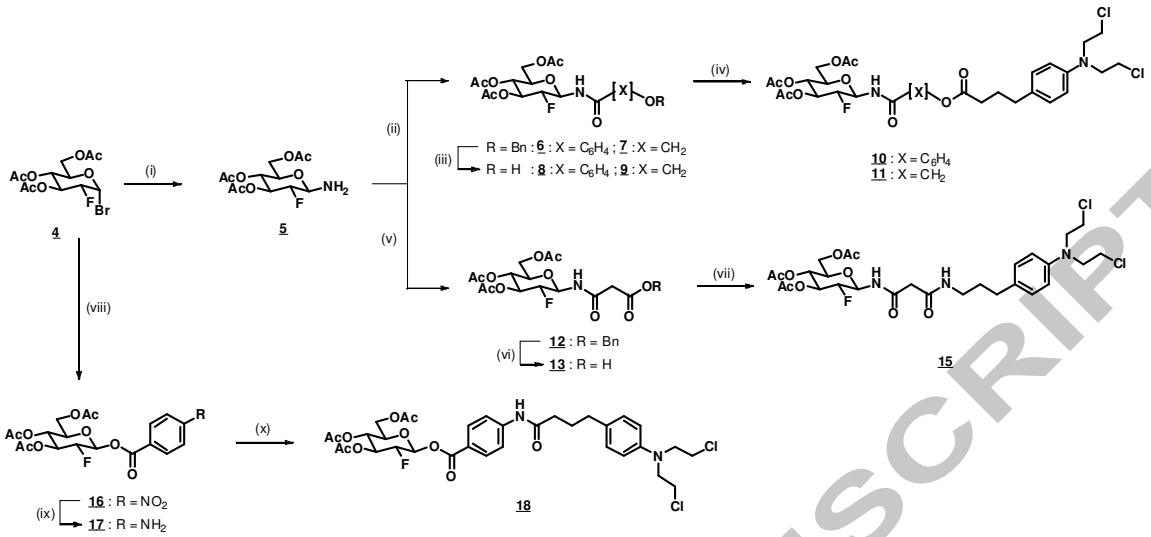
2. Results and Discussion

2.1. Chemistry

As stated above, the purpose of this study was to pursue the pharmacomodulation of the lead compounds **1-3**, chosen on the basis of their *in vitro* and *in vivo* activity, and their tolerance in tumor-bearing mice.[35,36] We also designed analogues of these derivatives, particularly of compound **3**, which presented the most promising antitumor activity, and can be retained as a highly active antitumor drug on the basis of the NCI criteria in two solid tumor models. Compound **3** exhibited LCK values higher than 1.3 (1.52 in the colon carcinoma xenograft model and 1.81 in the melanoma model). Schemes 1-5 describe the syntheses of the targeted CLB-FDG conjugates analogues of compounds **1**, **2** and **3** with the following linker modifications: CLB was attached to a spacer arm through amide, ester, peptide and reverse peptide bonds, and the FDG moiety was linked *via* its C-1 position to the spacer arm through amide, ester, carbamate or glycosidic bonds. Published results suggest that a β-conformation for glucose conjugates is preferred for GLUT-1 transport.[6] We accordingly synthesized all the compounds in the β-conformation.

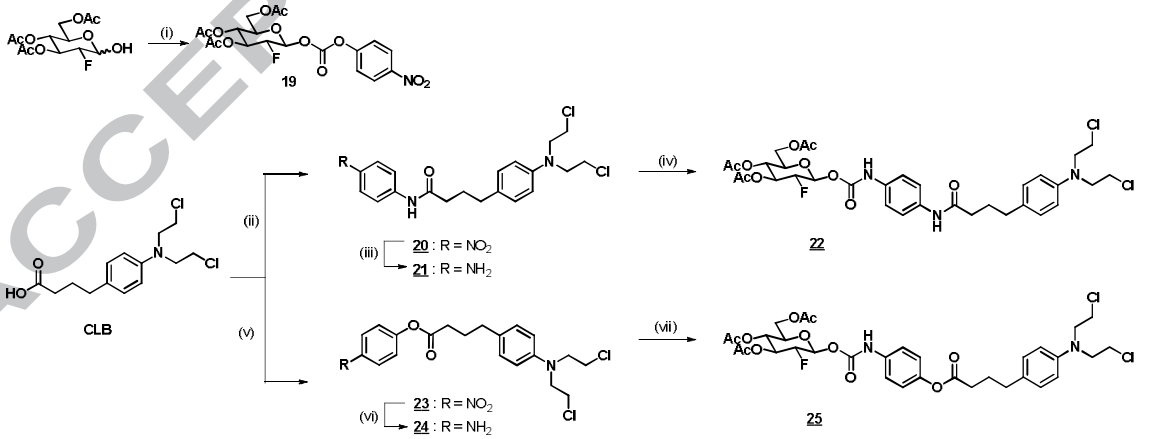
For compounds **1** and **2**, the spacer between FDG and CLB was made up of two amide linkages and an aromatic or glycyl group. Also, for the analogues of these two derivatives, the amide was replaced by an ester, a reverse amide or a carbamate bond in compounds **10**, **11**, **15**, **18**, **22** and **25**. The syntheses are described in schemes 1 and 2.

Scheme 1[a]



[a] Reagents and conditions: (i) (1) NaN_3, acetone/water, rt, 1 h, 84%; (2) H_2, Pd/C, THF/MeOH, rt, 1 h, 98%; (ii) DCC, HOBt, DMF, rt: 4 d from 4-benzyloxybenzoic acid, (**6**), 45%; 21 h from benzyloxyacetic acid, (**7**), 83%; (iii) H_2, Pd/C, rt, 5 h, THF, 46% (**8**), THF/MeOH, 99% (**9**); (iv) DCC, DMAP, DCM, rt, 20 h, 55% (**10**), 81% (**11**); (v) malonic acid monobenzyl ester, DCC, HOBt, DMF, rt, 48 h, 57%; (vi) LiOH, EtOH/H_2O, rt, 12 h, 89%; (vii) 4-(3-aminopropyl)-*N,N*-bis(2-chloroethyl)aniline **14**, DCC, HOBt, DMF, rt, 24 h, 13%; (viii) 4-nitrobenzoic acid, NaOH, rt, 4 h 30, 37%; (ix) H_2, Pd/C, THF/EtOH, rt, 10 h, quantitative yield; (x) CLB, TEA, $ClCO_2Et$, DCM, 30 h, 50%.

Scheme 2[a]



[a] Reagents and conditions: (i) 4-nitrophenylchloroformate, TEA, THF, rt, 24 h, 77% yield for α- and β-anomers; 35% yield for β-anomer; (ii) 4-nitroaniline, $ClCO_2Et$, TEA, DCM, rt, 17 h, 24%; (iii) H_2, Pd/C, THF/EtOH, rt, 3.5 h, 75%; (iv) **19**, TEA, DMF, rt, 25 h, 42%; (v) 4-

nitrophenol, DCC, DMAP, DCM, 24 h, rt, 93%; (vi) H_2, Pd/C, EtOH, 20 h, rt, 93%; (vii) **19**, TEA, DMF, rt, 40 h, 14%.

To access compounds **10**, **11** and **15**, with FDG anchored to the spacer through an amide bond, the synthetic methods depicted in Scheme 1 were designed starting from β-aminosugar **5**, easily produced from the α-bromo compound **4** through the β-anomeric azid[35]. The *N*-acylation of the β-aminosugar **5** with 4-benzyloxybenzoic acid, benzyloxyacetic acid or benzyl malonate half ester, the latter prepared by reaction of Meldrum's acid with benzyl alcohol at 120 °C,[38] led respectively to derivatives **6**, **7** and **12** in moderate yields. Cleavage of the benzyl protecting group by catalytic hydrogenation provided the corresponding hydroxyl derivatives **8**, **9** and **13**. Intermediates **8** and **9** were then directly esterified using CLB activated by DCC and DMAP to produce compounds **10** and **11**. Derivative **15** was obtained by coupling acid **13** with 4-(3-aminopropyl)-*N,N*-bis(2-chloroethyl)aniline **14**,[39] synthesized *via* the Curtius rearrangement.

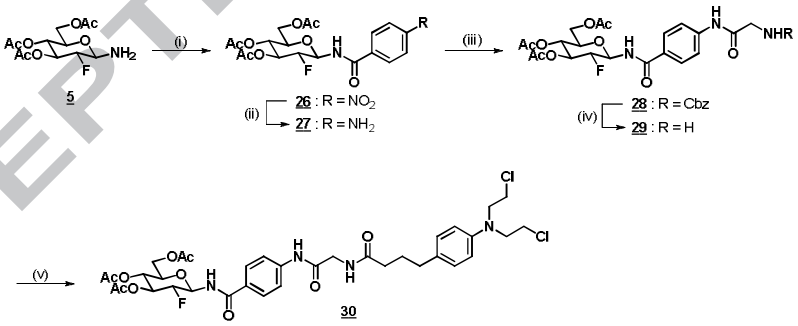
For compound **18**, with FDG anchored to the spacer through an ester bond, a first synthetic route starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α,β-D-glucose[35] was considered. Despite repeated attempts, compound **16** was always isolated as an inseparable mixture of the two α/β anomers. Another reaction pathway was therefore tested starting from α-bromo compound **4**. Reaction with 4-nitrobenzoic acid successfully gave compound **16** exclusively as the β-form, but with a moderate yield (37%). The β-stereochemistry of **16** was assigned based on its ^{1}H NMR signature, in particular on the multiplicity at and near the anomeric center: the C1 anomeric proton signal appears at 6.05 ppm as a doublet of doublets ($J_{1\text{-}2} = J_{ax\text{-}ax}$ = 8.0 Hz, $J_{1\text{-}F}$ = 3.0 Hz) and the C2 proton signal appears at 4.66 ppm as a doublet of doublets ($J_{1,2} = J_{ax\text{-}ax}$ = 8.0 Hz, $J_{2\text{-}3} = J_{ax\text{-}ax}$ = 9.1 Hz, $J_{2\text{-}F}$ = 50.8 Hz). For the α-anomer, the $J_{1\text{-}2}$ coupling constant appears smallest (4 Hz), characteristic of a $J_{ax\text{-}eq}$ coupling constant. Finally, reduction to amine **17** followed by coupling using ethylchloroformate and CLB gave compound **18** in 49% yield.

For compounds **22** and **25**, with FDG anchored to the spacer through a carbamate bond (scheme 2), the synthetic pathway designed required the production of carbonate **19**, where the anomeric hydroxyl group of the sugar was activated with 4-nitrophenylchloroformate, followed by substitution with an amine group included in the linker unit. The sequence chosen had to ensure a β-configuration of the carbamoyl linkage at the anomeric center. During the activation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α,β-D-glucose with 4-

nitrophenylchloroformate, we faced the problem of stereospecificity. This reaction, performed in THF at room temperature in the presence of TEA, gave an anomeric mixture of carbonate **19** (α/β = 20/80) as already described for glucuronate derivatives.[40] Other carbamoylation methods such as activation through *N,N*-carbonyldiimidazole[41] or utilization of isocyanate derivatives[42,43] also lead to αβ mixtures. However, the difference in solubility of the two anomers in THF and diisopropryl ether allowed us to isolate the pure β-anomer in 35% yield. The β-stereochemistry of **19** was confirmed by coupling constants observed in the ^{1}H NMR spectra ($J_{1\text{-}2}$ = 8.0 Hz, $J_{2\text{-}3}$ = 9.2 Hz, typical of ax-ax coupling). The β-carbonate derivative **19** was then treated with two different synthetic aromatic amines **21** and **24**[35] in DMF in the presence of TEA to afford compounds **22** and **25** in 14% and 42% yield respectively (enantiomerically pure as shown by ^{1}H NMR analyses).

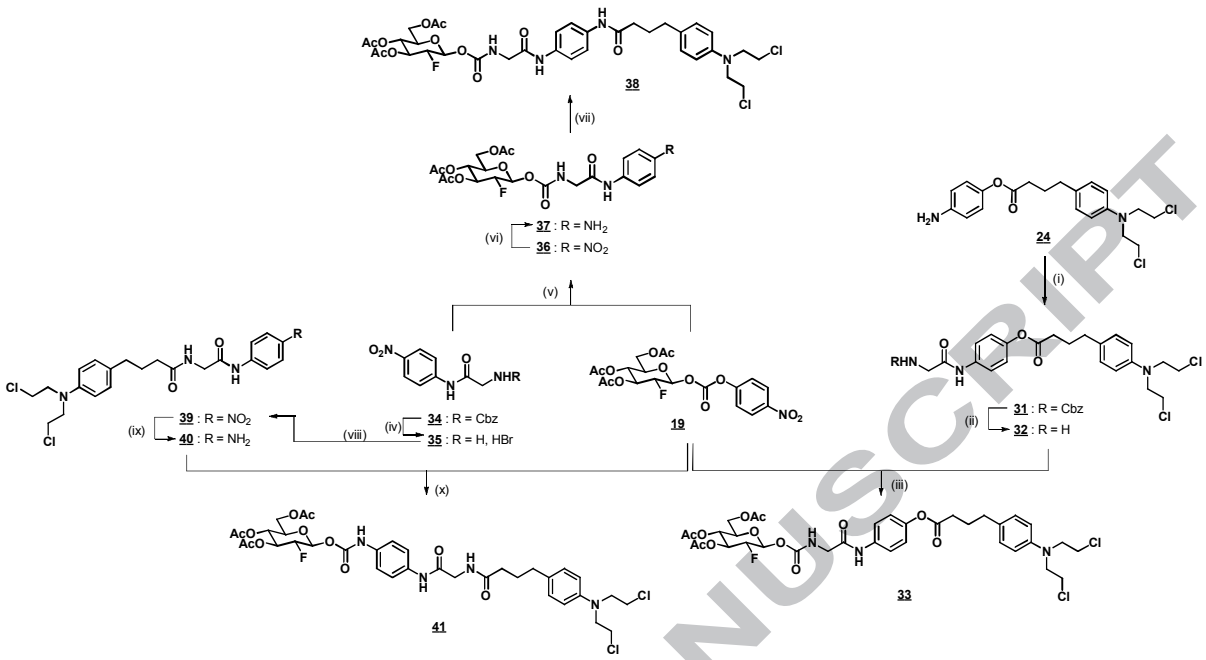
In an attempt to improve the antitumor efficacy of our lead *O*-glycoside **3**, we modulated the spacer arm of this derivative. To evaluate the importance of the type of linkage binding the spacer domain, the FDG moiety and the drug, and also the importance of the nature of the spacer domain (lipophilicity, length), we synthesized compounds with FDG anchored through an amide bond (**30**, scheme 3) or a carbamate bond (**33**, **38**, **41**) (scheme 4) and other *O*-glycosides with several spacer arms (**45**, **48**, **51** and **56**) (scheme 5).

Scheme 3[a]



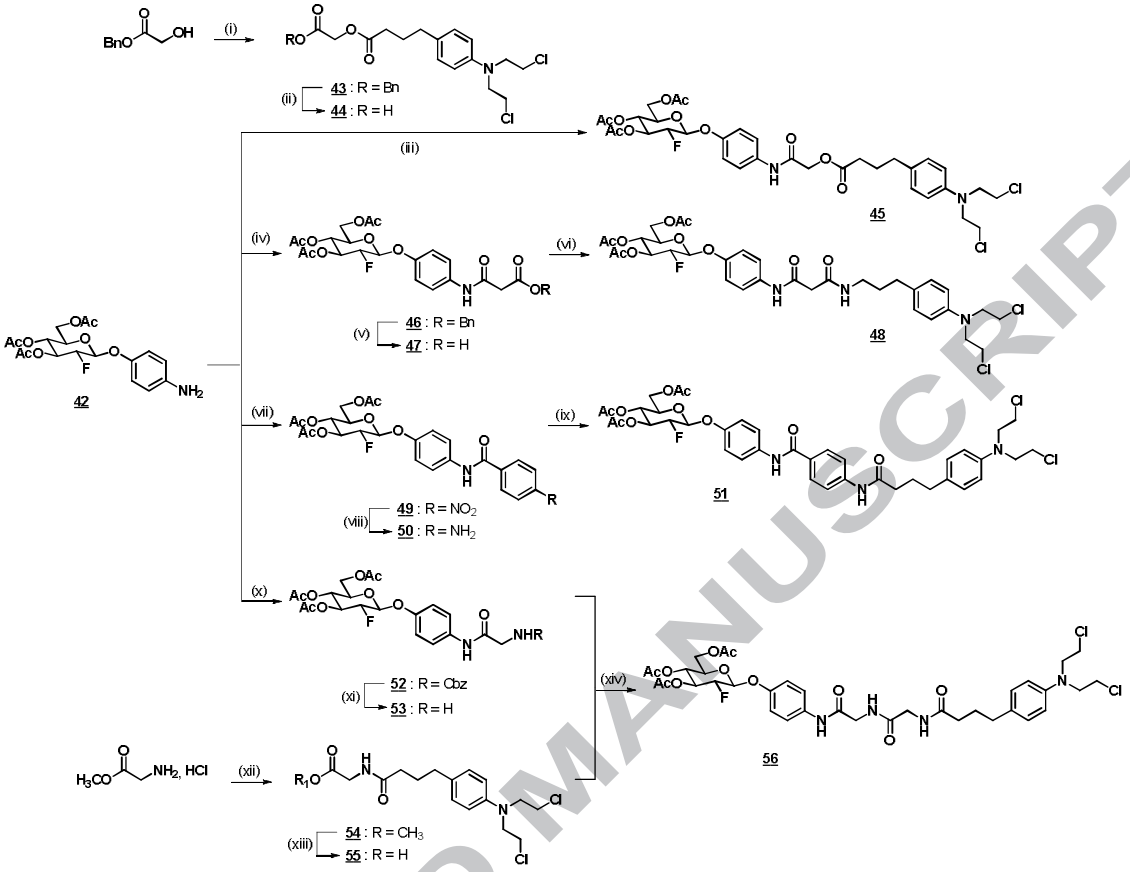
[a] Reagents and conditions: (i) 4-nitrobenzoyl chloride, TEA, THF, rt, 4 h, 67%; (ii) H_2, Pd/C, THF/MeOH, rt, 3 h, 91%; (iii) *N*-Cbz-glycine, $ClCO_2Et$, TEA, DCM, rt, 22 h, 50%; (iv) H_2, Pd/C, THF/EtOH, rt, 5 h, quant. yield; (v) CLB, DCC, HOBt, DMF, rt, 24 h, 58%.

Scheme 4[a]



[a] Reagents and conditions: (i) *N*-Cbz-glycine, DCC, HOBt, DMF, rt, 24 h, 90%; (ii) H_2, Pd/C, THF/EtOH, rt, 4 h, 99%; (iii) TEA, DMF, rt, 24 h, 59%; (iv) HBr/AcOH, AcOH, rt, 2 h 45, 87%; (v) (a) from **35**, TEA, DMF, rt, 1.5 h, (b) TEA, DMF, rt, 5 h, 89%; (vi) H_2, Pd/C, THF/EtOH, 5 h, rt, 93%; (vii) CLB, $ClCO_2Et$, TEA, DCM, rt, 4 h, 86%; (viii) (a) TEA, DCM, rt, 2.5 h, (b) CLB, $ClCO_2Et$, TEA, DCM, rt, 3 d, 92%; (ix) H_2, Pd/C, THF/EtOH, rt, 24 h, 88%; (x) TEA, DMF, rt, 20 h, 37%.

Scheme 5[a]



[a] Reagents and conditions: (i) CLB, DCC, DMAP, DCM, rt, 48 h, 85%; (ii) H_2, Pd/C, THF/EtOH, rt, 2 h, 97%; (iii) ClCOOEt, TEA, DCM, rt, 18 h, 48%; (iv) malonic acid monobenzyl ester, DCC, HOBt, DMF, rt, 22 h, 61%; (v) H_2, Pd/C, THF/MeOH, rt, 2 h, quant. yield; (vi) 4-(3-aminopropyl)-*N,N*-bis(2-chloroethyl)aniline (**14**), DCC, HOBt, DMF, rt, 39 h, 56%; (vii) 4-nitrobenzoic chloride, TEA, THF, 0 °C, 30 mn, rt, 20 h, 40%; (viii) H_2, Pd/C, THF/MeOH, rt, 24 h, 88%; (ix) CLB, $ClCO_2Et$, TEA, DCM, rt, 15 h, 43%; (x) *N*-Cbz-Gly, DCC, HOBt, DMF, rt, 28 h, 89%; (xi) HCO_2NH_4, Pd/C, THF/EtOH, 70 °C, 50 mn, 98%; (xii) CLB, $ClCO_2Et$, TEA, DCM, rt, 24 h, 54%; (xiii) LiOH, EtOH/H_2O, rt, 5 h, 99%; (xiv) DCC, HOBt, DMF, rt, 24 h, 28%.

The synthetic method for the preparation of compound **30** with an amide bond instead of the glucoside bond is depicted in Scheme 3. Starting from the β-aminosugar **5**, *N*-acylation with 4-nitrobenzoyl chloride preceded reduction of the nitro function to provide amino derivative **27**.[35] *N*-Acylation with *N*-Cbz-glycine activated by ethylchloroformate and

triethylamine, followed by cleavage of the benzyloxycarbonyl (Cbz) protecting group by Pd/C catalytic hydrogenation provided the corresponding amino derivative **29**. Final coupling with CLB afforded compound **30** in 18% overall yield.

For compounds **33**, **38** and **41**, with the FDG moiety grafted to the spacer through a carbamate bond, syntheses were carried out starting from compound **19** and different amines **32**, **35** and **40** (scheme 4). Compound **33** was obtained in three steps in 53% overall yield from amine **24**: *N*-acylation with *N*-Cbz-glycine activated by DCC/HOBt, cleavage of the benzyloxycarbonyl protecting group by catalytic hydrogenation before final formation of the carbamate linkage by reaction with carbonate derivative **19**. The synthesis of derivatives **38** and **41** differed from the CLB-glycyl-aromatic sequence, but 2-amino-*N*-(4-nitrophenyl)acetamide **35** was used as common key intermediate. This compound was obtained by reaction of 4-nitroaniline with *N*-Cbz-glycine, activated by ethylchloroformate followed by selective cleavage of the Cbz protecting group of the glycine residue by hydrogen bromide in acetic acid. The synthesis of compound **38** was carried out according to the following sequence: reaction of amine **35** with the carbonate derivative **19**, then reduction of the aromatic nitro function and coupling of CLB. Using amine **35**, the same sequence of reactions was used, but in reverse, to obtain derivative **41**: coupling of CLB with amine **35**, reduction of the aromatic nitro function and finally reaction with the carbonate derivative **19**.

The introduction of a spacer *via* a glycosidic bond needed the synthesis of compound **42** (Scheme 5), following the protocol of Reux *et al.*[35] For the synthesis of compound **45**, the requisite acid **44** was readily accessible in 85% yield from benzyl glycolate[44] through a two-step sequence: reaction of CLB with benzyl glycolate, using DCC and DMAP as condensing reagents and hydrolysis of the benzyl ester with hydrogen using Pd/C. Activation of **44** with ethylchloroformate in the presence of triethylamine before reaction with the *O*-glucoside **42** provided derivative **45** in 48% yield. DCC/HOBt coupling of **42** with benzyl malonate half ester[38] gave compound **46**. Hydrogenolytic removal of the benzyl carbamate led in quantitative yield to acid **47**, and finally a further DCC/HOBt coupling with 4-(3-aminopropyl)-*N,N*-bis(2-chloroethyl)aniline **14** afforded expected derivative **48**.

Compound **51** was obtained in three steps starting from the *O*-glucoside **42**: *N*-acylation with 4-nitrobenzoyl chloride, reduction of the nitro function by catalytic hydrogen atmosphere and finally coupling with CLB using ethylchloroformate instead of DCC/HOBt to avoid problems typically faced during purification by silica gel column chromatography due to the presence of dicyclohexylurea.

Finally in this set of compounds with FDG anchored to the spacer through a glucosidic bond, we performed the synthesis of glycoside **56** for which two glycine moieties were condensed next to the aromatic of *O*-glucoside **24** to extend the spacer domain.

The synthetic route designed for compound **56** used derivative **53**, a key intermediate in the production of the lead compound **3**.[35] In a first attempt, we designed a three-step reaction pathway including (i) coupling of compound **53** to *N*-Cbz-glycine (65% or 53% yield respectively under the standard DCC/HOBt or ethylchloroformate coupling conditions), (ii) hydrogenolytic removal of the benzyl carbamate (quantitative yield), and finally (iii) *N*-acylation to introduce CLB. Unfortunately, our attempts to introduce CLB under the standard DCC/HOBt or ethylchloroformate coupling conditions all failed to produce derivative **56** in the high chemical purity required for further biological evaluation. Consequently, a convergent pathway was preferred: acid **55** was readily accessible from glycine methyl ester through a two-step sequence: coupling of glycine methyl ester with CLB (54% yield) and hydrolysis of the methyl ester with aqueous lithium hydroxide (quantitative yield). Finally, compound **56** was obtained by DCC/HOBt condensation of acid **55** and amine **53** in 28% yield.

2.2. *In vitro* cytotoxicity

The antiproliferative activity (i.e., IC_{50}) of all the target fluoroglycosides was evaluated relative to CLB, using a 5-member panel of human carcinoma cell lines from prostate (PC3), breast (MCF-7), lung (A549), melanoma (M4Beu), ovarian (PA1) and a primary culture of human fibroblasts (see Figure 2). On the basis of this study, whatever the cell line considered, almost all the compounds were more potent than CLB, which is only moderately cytotoxic in these conditions (with IC_{50} values higher than 40 μM except for PA1 cells, which showed high sensitivity in all cases. The cytotoxicity of FDG or peracetylated FDG alone, or in combination with CLB at 50 µM was assessed on A549 cells and no additive effect was observed for concentrations up to 200 µM (data not shown).

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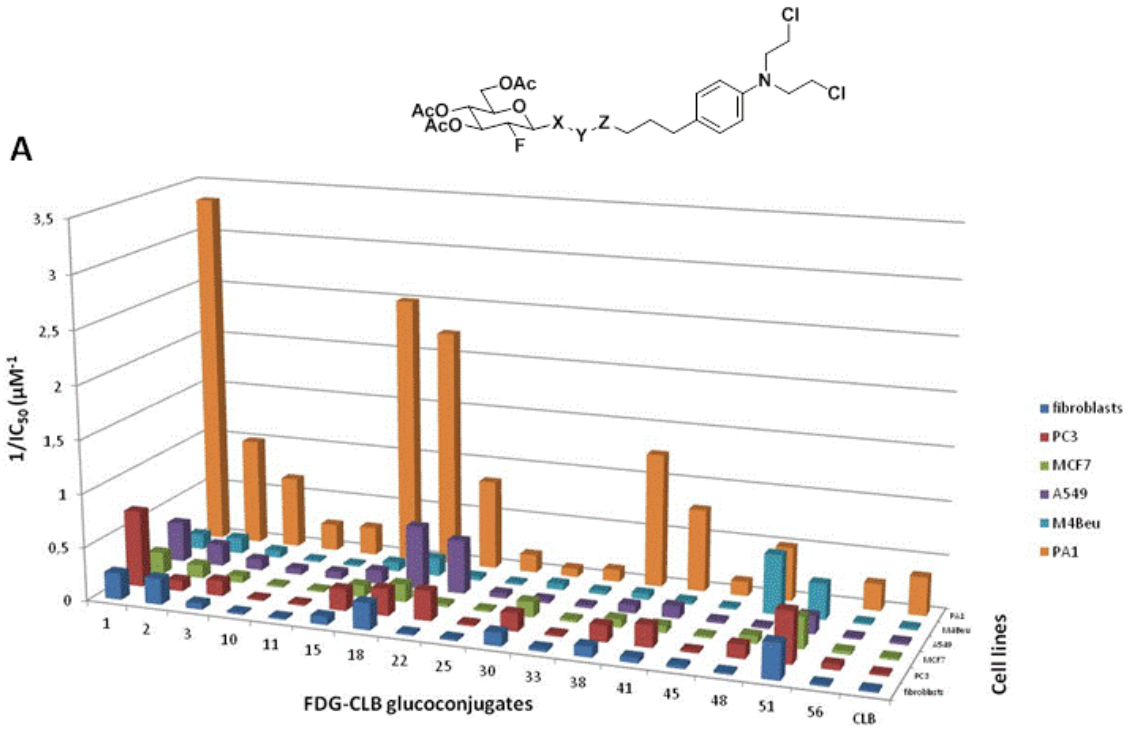

B

Compd	Type of bond between FDG and CLB X	Y	Z	IC50 (µM) fibroblasts	PC3	MCF7	A549	M4Beu	PA1
1	NHCO	*p*-C_6H_4	NHCO	4±2	1.4±0.3	5±2	2.7±0.6	7±3	0.3±0.1
2	NHCO	CH_2	NHCO	4.1±0.1	10±2	8±2	5±2	6.8±0.9	1.0±0.1
3	O	Ph-NHCO-CH_2	NHCO	17±6	7±2	16±3	10±5	15±6	1.5±0.5
10	NHCO	*p*-C_6H_4	OCO	>50	>50	>50	19±9	>50	4±1
11	NHCO	CH_2	OCO	>50	>50	>50	15±5	>50	3.8±0.3
15	NHCO	CH_2	CONH	13±3	5±3	9±3	8±4	12±3	0.4±0.1
18	OCO	*p*-C_6H_4	NHCO	4±1	4±2	6±2	1.7±0.1	6±2	0.45±0.04
22	OCONH	*p*-C_6H_4	NHCO	>50	3.6±0.9	35±4	2±1	29±4	1.2±0.2
25	OCONH	*p*-C_6H_4	OCO	>50	>50	>50	22±1	>50	6±2
30	NHCO	Ph-NHCO-CH_2	NHCO	8±1	6±1	7±4	30±2	17.8±0.9	13±4
33	OCONH	CH_2-NHCO-Ph	OCO	32±9	>50	39.5±0.7	39±4	30±18	9±1
38	OCONH	CH_2-NHCO-Ph	NHCO	10±3	6.5±0.8	13±3	13±4	18±6	0.8±0.2
41	OCONH	Ph-NHCO-CH_2	NHCO	21±9	4.9±0.9	15±3	9±1	31±10	1.3±0.2
45	O	Ph-NHCO-CH_2	OCO	32.5±0.5	> 50	> 50	39±4	> 50	7.6±2.1
48	O	Ph-NHCO-CH_2	CONH	>50	8±5	14±4	>50	13.8±0.4	2±0.3
51	O	Ph-NHCO-Ph	NHCO	3±1	2.1±0.1	3.4±0.4	6±3	3±2	ND
56	O	Ph-NHCO-CH_2-NHCO-CH_2	NHCO	>50	20±3	32±4	>50	>50	4±1
CLB				99±2	>100	>100	43±14	>100	2.8±0.4

Figure 2. Cytotoxicity induced by the newly synthesized CLB-peracetylated FDG-conjugates on human normal and tumor cells. (A) Summary of IC_{50} data from cytotoxicity assay of all fluoroglucoconjugates (reciprocal values displayed). Comparisons were made against previously identified compounds **1**, **2** and **3**, and CLB. (B) IC_{50} data and error values are

given. Human cell lines tested included PC3 (prostate), MCF-7 (breast), A549 (lung), M4Beu (melanoma), PA1 (ovarian) and a primary culture of fibroblasts.

Only compounds **10**, **11**, **25** and **45** did not reveal any cytotoxic effect, with IC_{50} values above 50 µM, except in A549 and PA1 cells. These four compounds with CLB linked *via* an ester bond showed a drop in activity compared with amide bond analogues **1**, **2**, **22** and **3**. The negative impact of the ester bond was already observed with compound **33,** showing overall IC_{50} values above 30 µM, compared with compound **38**, with an IC_{50} values ranging from 6 to 18 µM, i.e. approximately half. As the ester bond opens a route for CLB release due to its significant susceptibility to hydrolysis when exposed to aqueous media and cellular medium *in vitro*, we surmise that the decreased potencies of these glucoconjugates may be due to hydrolysis and early release of CLB. In the literature, we can find several examples where drugs linked with the spacer via an ester bond are released due to hydrolysis or enzymatic degradation.[45–47] The rate of release generally decreases in the order ester > amide. Such an early hydrolysis could not be excluded *in vivo* in the blood circulation, preventing any active targeting.

However, an ester bond in another position, namely the anomeric position of FDG, given an ester-type glycoside linkage did not induce a negative impact on cytotoxic activity, with IC_{50} values in the same range (0.3 to 6 µM) for glycosyl ester **18** and amide **1**. Considering these results, namely that the ester bond induced an unchanged or decreased activity, and difficulties in synthesis of pure β-anomer of compound **18**, no other ester compound was synthesized. Amide or reverse amide bond led to barely any change in cytotoxicity, as shown by compounds **2** and **15**. Regarding compounds **1** and **22** differing only in one connection, an amide or a carbamate function grafted to the FDG moiety, a difference in cytotoxicity patterns was observed. Interestingly, carbamate **22** did not induce cytotoxicity on human fibroblasts (IC_{50} > 50 µM), but was still active on tumor cell lines, especially prostatic adenocarcinoma PC3, lung carcinoma A549 and ovary adenocarcinoma PA1 cell lines (IC_{50} < 4 µM). These preliminary results suggest a different activity between tumor and these healthy cells.

Since compound **3** presented the most promising antitumor activity in two *in vivo* solid tumor models, we further designed analogues in an attempt to understand the impact of each fragment of the linker in term of cytotoxicity. For our lead compound **3**, an extend structure-activity relationship study was considered: the glycoside bond was replaced by an amide function, affording compound **30** or replaced by a carbamate, leading to compounds **38** and

41. For amide **30**, quite no change was obtained in term of activity on all cancer cell lines. For derivatives **38** and **41**, the carbamate function was chosen in order to obtain similar results as thus obtained for carbamate **22**, where a several fold higher IC_{50} value was observed in fibroblast cells as compared to tumour cells. Unfortunately, no differencial activity was observed with these derivatives **38** and **41**, having a quite similar potency on every cell lines, *i.e.* tumor cells or fibroblasts.

We also inverted methylene and phenyl groups in compound **38** and no difference in IC_{50} values was observed. Regarding compound **51**, a spacer arm with two aromatic groups seemed to be beneficial for the cytotoxic activity (IC_{50} values were similar to that of compound **1**, the most active compound in this *in vitro* study). Extension of the linker, with two glycine fragments, afforded derivative **56**, which clearly appears unpromising in terms of cytotoxicity, with $IC_{50} > 20$ µM. Finally, inversion of the amide bond (compound **48**) seemed to favor a specific pattern, with no activity on normal human fibroblasts and lung carcinoma A549, unlike the observation made above for compound **15**, with IC_{50} values below 13 µM for all cell lines tested.

3. Conclusions

The synthesis of this set of glycoconjugates focused on the use of FDG designed to enhance sugar-mediated uptake, and led to overall improvement in antiproliferative effect compared with CLB. The present study revealed that *in vitro* antitumoral efficiency was optimized using an aromatic spacer, which seemed to favor the cytotoxicity on all human carcinoma cell lines tested as previously described.[35] With IC_{50} values similar to that of lead compound **1**, compound **51** appears as one of the most active FDG-CLB glycoside in this series in terms of *in vitro* cytotoxicities. However, compounds with CLB tethered through an ester bond lose their overall cytotoxicities, perhaps by early hydrolysis. Concerning the cell lines, no real sensitivity or resistance was evident for this set of compounds, except for the two derivatives **22** and **48**, which did not show any activities on human fibroblasts ($IC_{50} > 50$ µM), but maintained their effect on tumor cell lines. To conclude, this work adds to our understanding of the structure-activity relationships concerning the linker, and highlights the positive impact of an aromatic linker on *in vitro* cytotoxicity, but raises questions about the precise mechanisms of cytotoxicity. These will be addressed in further experiments, including: (i) fluoroglycoside–DNA alkylation assessment, (ii) transport/receptor-mediated uptake (GLUT or SGLT), (iii) biodistribution of [^{14}C]-radiolabeled compound **51** in tumor-bearing

mice in order to study its tumor uptake, (iv) stability of the alkylating agent, and (v) *in vivo* antitumor activity in tumor-bearing mice.

4. Materials and Methods

4.1. Chemistry

General

Solvents and reagents were purchased from Aldrich, Acros Organics, or Carlo Erba, and used without further purification. Column chromatography was performed with silica gel A normal phase (Chromagel, 35–70 µm, SDS) or neutral aluminum oxide 90 standardized (63–200 µm, Fluka) using the indicated solvent mixture expressed as volume/volume ratios. Analytical thin-layer chromatography (TLC) was conducted on precoated silica gel aluminum plates (SDS, 60 F_{254}, 0.2 mm thick) or neutral aluminum oxide aluminum plates (Fluka, 60F_{254}, 0.2 mm thick). The plates were visualized with ultraviolet light (254 nm) and (or) by development with vanillin in sulfuric acid. Melting points were determined on an electrothermal digital apparatus (Reichert), are uncorrected, and only given for purified compounds. Infrared spectra were recorded in KBr pellets or with NaCl plates on an FTIR-Nicolet Impact 410 spectrophotometer. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were performed on a Bruker AM 200 spectrometer (200 MHz for ^{1}H, 50 MHz for ^{13}C) or a Bruker DRX 500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) (Bruker Biospin SAS, Wissembourg, France). Chemical shifts are reported in parts per million relative to the internal tetramethylsilane standard for ^{1}H NMR and the solvent for ^{13}C NMR (acetone-d_6, δ = 29.8 ppm; DMSO-d_6, δ = 39.5 ppm; $CDCl_3$, δ = 77.2 ppm). The abbreviations used for signal patterns are: br, broad; s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; dt, doublet of triplets; q, quadruplet; qt, quintuplet; m, multiplet. ^{19}F NMR spectra (470 MHz) were recorded on a Bruker DRX 500 apparatus using tetrafluorotoluene as internal reference (δ -63 ppm); δ values were expressed in ppm, coupling constants (*J* values) are in hertz. Mass spectra were recorded on a Bruker Esquire-LC spectrometer. Electrospray ionization mass spectrometry (ESI-MS) was used in positive mode. Microanalyses were performed by the Central Analysis Service (CNRS, Vernaison, France) for C, H, and N; the results were within ± 0.4% of the theoretical values.

Abbreviations for reactions, reagents and protecting groups: Ac, acetyl; Cbz, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DMAP, *N*,*N*-4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; HOBt, 1-hydroxybenzotriazole; TEA, triethylamine; THF, tetrahydrofuran.

General procedure for DCC/HOBt coupling

The amine was dissolved in dry DMF and the suitable acid (0.8-1.5 equiv), DCC (1.1 equiv), and HOBt (1.1 equiv) were added. The solution was stirred for several hours at room temperature, filtered, concentrated *under vacuo*, and purified by silica gel chromatography to give the expected compound.

General procedure for DCC/DMAP coupling

The suitable acid (1.1 equiv), DCC (1.1 equiv) and a catalytic amount of DMAP (0.1-0.2 equiv.) were added to a solution of the alcohol in anhydrous DCM, and stirred at room temperature for several hours. The solid was removed by filtration, and the filtrate was washed successively with a 1N aqueous acetic acid solution and water, dried over $MgSO_4$, filtered, and concentrated under *vacuum*. The resulting residue was purified by silica gel chromatography to yield the expected compound.

General procedure for ethylchloroformate coupling

A stirred solution of the acid in anhydrous DCM was treated with triethylamine. The mixture was cooled to 0-10 °C, treated with ethyl chloroformate, and stirred at room temperature for 2 h. The amine was then added, and the mixture was stirred for several hours at room temperature, followed by tlc monitoring. The quantities of reagents used are reported for each reaction. The mixture was suspended in saturated aqueous Na_2CO_3 and extracted twice with ethyl acetate. The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure before purification by column chromatography (SiO_2 or Al_2O_3) to give the expected compound.

General reduction or N-Cbz/OBn deprotection procedure using hydrogen

To a suspension of 10% palladium on charcoal (25 wt %) in THF or THF/MeOH or THF/EtOH was added the nitro or protected compound. The mixture was hydrogenated at atmospheric pressure for several hours, filtered on celite® 545, and concentrated to yield the reduced or deprotected product, which was used in the next step without further purification unless otherwise specified.

General carbamoylation procedure

To a solution of carbonate **19** in DMF, were added the suitable amine (0.8-1.3 equiv.) and TEA (1.4-1.5 equiv.) and stirring was maintained at room temperature for several hours. After

evaporation of the volatiles, the crude product was chromatographed on silica gel to give the title β-anomer.

N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl)-4-(benzyloxy)benzamide ***(6)***. Coupling of amino-sugar **5** (500 mg, 1.63 mmol) with 4-benzyloxybenzoic acid (410 mg, 1.79 mmol) was carried out according to the DCC/HOBt general procedure in DMF (44 mL) and 4 days stirring. After filtration and evaporation under *vacuum*, the crude residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 5/5, v/v) to give compound **6** (382 mg, 45%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.82 (d, 2H, J = 8.8 Hz, H_{Ar}), 7.42-7.36 (m, 5H, H_{Ar}), 7.20 (d, 1H, J = 9.3 Hz, NH), 7.00 (d, 2H, J = 8.8 Hz, H_{Ar}), 5.61 (td, 1H, $J_{1,NH} = J_{1,2}$ = 8.8 Hz, $J_{1,F}$ = 1.4 Hz, H-1), 5.43 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 13.7 Hz, H-3), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.06 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.55 (td, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 49.9 Hz, H-2), 4.40 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.0 Hz, H-6a), 4.04 (m, 1H, H-6b), 3.91 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$ = 1.8 Hz, H-5), 2.08, 2.06, 2.04 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.77, 170.16, 169.93, 167.14 (CO), 162.18 ($C_{Ar}O$), 136.27 ($\underline{C}_{Ar}CH_2$), 129.53, 128.81, 128.37, 127.60 (CH_{Ar}), 125.60 ($\underline{C}_{Ar}CO$), 114.88 (CH_{Ar}), 88.51 (C-2, $J_{2,F}$ = 190.5 Hz), 78.18 (C-1, $J_{1,F}$ = 22.4 Hz), 73.80-73.50 (C-3, C-5), 70.27 ($\underline{C}H_2Ph$), 68.11 (C-4, $J_{4,F}$ = 6.1 Hz), 61.63 (C-6), 20.73 (CH_3); ^{19}F NMR ($CDCl_3$) δ −197.67.

N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl)-2-benzyloxyacetamide ***(7)***. DCC/HOBt coupling of amino-sugar **5** (350 mg, 1.14 mmol) with 4-benzyloxyacetic acid (284 mg, 1.71 mmol) was carried out with DCC (352 mg, 1.71 mmol) and HOBt (31 mg, 0.23 mmol) in DMF (12 mL) and 21 h stirring, to give after silica gel purification (petroleum ether/ethyl acetate, 6/4 then 5/5, v/v), compound **7** (429 mg, 83%) as a pale green solid: mp 149 °C; IR (NaCl) ν 3355, 3066, 3032, 1751, 1700, 1524, 1368, 1230, 1097, 1070, 1036 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.41-7.23 (m, 5H, H_{Ar}), 5.45-5.28 (m, 2H, H-1, H-3), 5.04 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 4.58 (s, 2H, $C\underline{H}_2Ph$), 4.34 (td, 1H, $J_{1,2} = J_{2,3}$ = 9.1 Hz, $J_{2,F}$ = 50.5 Hz, H-2), 4.30 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.11-4.03 (m, 3H, H-6b, $COCH_2$), 3.84 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$ = 2.1 Hz, H-5), 2.08, 2.05, 2.04 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.30, 169.70 (CO), 137.02 ($C_{Ar}CH_2$), 128.81, 128.15, 128.20 (CH_{Ar}), 88.70 (C-2, $J_{2,F}$ = 191.0 Hz), 76.81 (C-1, $J_{1,F}$ = 23.0 Hz), 74.05 (C-5), 73.51 (C-3, $J_{3,F}$

= 19.1 Hz), 73.57 ($\underline{C}H_2Ph$), 69.80 (C-4, $J_{4,F}$ = 7.0 Hz), 66.92 ($CO\underline{C}H_2$), 20.78 (CH_3); ^{19}F NMR ($CDCl_3$) δ −198.10.

N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl)-4-hydroxybenzamide ***(8)***. Hydrogenation at atmospheric pressure for 5 h of a suspension of compound **6** (118 mg, 0.228 mmol) and 10% palladium on charcoal (30 mg) in THF (7 mL) yielded phenol **8** (45 mg, 46%) as a white solid after purification by silica gel chromatography (petroleum ether/ethyl acetate, 5/5, v/v): mp 155 °C; IR (KBr) ν 3361, 1757, 1646, 1511, 1369, 1232, 1074, 1031 cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 9.17 (br s, 1H, OH), 8.47 (d, 1H, J = 9.2 Hz, NH), 7.86 (d, 2H, J = 8.7 Hz, H_{Ar}), 6.92 (d, 2H, J = 8.7 Hz, H_{Ar}), 5.73 (td, 1H, $J_{1,NH} = J_{1,2}$ = 9.2 Hz, $J_{1,F}$ = 2.5 Hz, H-1), 5.55 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.2 Hz, H-3), 5.01 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.65 (td, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.4 Hz, H-2), 4.27 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.6 Hz, H-6a), 4.15-4.02 (m, 2H, H-5, H-6b), 2.04, 2.02, 1.99 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, acetone-d_6) δ 170.17, 167.35 (CO), 161.82 ($C_{Ar}O$), 130.46 (CH_{Ar}), 125.65 ($\underline{C}_{Ar}CO$), 115.96 (CH_{Ar}), 89.47 (C-2, $J_{2,F}$ = 187.2 Hz), 78.77 (C-1, $J_{1,F}$ = 22.7 Hz), 74.28 (C-3, $J_{3,F}$ = 19.7 Hz), 73.98 (C-5), 69.10 (C-4, $J_{4,F}$ = 7.4 Hz), 62.68 (C-6), 20.59 (CH_3); ^{19}F NMR (acetone-d_6) δ −198.11.

N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyransyl)-2-hydroxyacetamide ***(9)***. Deprotection of compound **7** (400 mg, 0.879 mmol) using standard hydrogenation conditions in THF/MeOH (15/15 mL) with 3.5 h stirring, yielded alcohol **9** (320 mg, 99%) as white solid which was used in the next step without further purification: IR (NaCl) ν 3537, 3343, 1760, 1663, 1516, 1369, 1252, 1232, 1068, 1045 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.42 (d, 1H, $J_{1,NH}$ = 9.5 Hz, NH), 5.51-5.31 (m, 2H, H-1, H-3), 5.05 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 4.39 (td, 1H, $J_{1,2} = J_{2,3}$ = 9.1 Hz, $J_{2,F}$ = 50.6 Hz, H-2), 4.34-4.06 (m, 4H, H-6, $C\underline{H}_2OH$), 3.87 (ddd, 1H, $J_{6a,6b}$ = 10.1 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 2.11, 2.09, 2.05 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.31, 170.70, 169.90, 169.69 (CO), 88.40 (C-2, $J_{2,F}$ = 191.3 Hz), 76.98 (C-1, $J_{1,F}$ = 23.1 Hz), 73.91 (C-5), 73.36 (C-3, $J_{3,F}$ = 19.1 Hz), 67.84 (C-4, $J_{4,F}$ = 6.9 Hz), 62.33, 61.61 (C-6, CH_2OH), 20.74, 20.69, 20.60 (CH_3); ^{19}F NMR ($CDCl_3$) δ −198.07.

4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosylcarbamoyl)phenyl 4-{4-[bis(2-chloroethyl)amino]phenyl}butanoate ***(10)***. A standard DCC/DMAP procedure starting from

compound **7** (262 mg, 0.612 mmol) and CLB (204 mg, 0.673 mmol) in DCM (27 mL) and 16 h stirring, followed by purification of the crude product by silica gel chromatography (petroleum ether/ethyl acetate, 6/4, v/v) yielded **10** (242 mg, 55%) as a white solid: mp 103 °C; IR (NaCl) ν 3376, 1749, 1676, 1520, 1367, 1235, 1036 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.86 (d, 2H, J = 8.6 Hz, H_{Ar}), 7.18-7.07 (m, 5H, H_{Ar}, NH), 6.64 (d, 2H, J = 8.7 Hz, H_{Ar}), 5.59 (td, 1H, $J_{1,2} = J_{1,NH}$ = 9.2 Hz, $J_{1,F}$ = 1.7 Hz, H-1), 5.43 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 13.6 Hz, H-3), 5.06 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 4.50 (td, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.5 Hz, H-2), 4.38 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.3 Hz, H-6a), 4.07 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 1.9 Hz, H-6b), 3.91 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.76-3.58 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.65, 2.59 (each t, 2×2H, J = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2$), 2.08-1.96 (m, 11H, OAc, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.77, 170.75, 170.23, 169.93, 166.85 (CO), 153.88 ($C_{Ar}O$), 144.38 ($C_{Ar}N$), 130.61 ($\underline{C}_{Ar}$), 129.86, 129.10, 121.93, 112.57 (CH_{Ar}), 88.36 (C-2, $J_{2,F}$ = 190.7 Hz), 78.18 (C-1, $J_{1,F}$ = 22.5 Hz), 73.77-73.41 (C-3, C-5), 68.08 (C-4, $J_{4,F}$ = 6.7 Hz), 61.59 (C-6), 53.78 (NCH_2), 40.52 (CH_2Cl), 34.00, 33.75 ($\underline{C}H_2CH_2\underline{C}H_2$), 26.64 ($CH_2\underline{C}H_2CH_2$), 20.74 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ –197.82; MS (ESI) m/z 713.49 $[M+H]^+$ (Exact Mass: 713.20); Anal. ($C_{33}H_{39}Cl_2FN_2O_{10}$) C, H, N.

*2-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosylamino)-2-oxoethyl 4-{4-[bis(2-chloroethyl)amino]phenyl}butanoate (**11**).* A standard DCC/DMAP procedure starting from compound **9** (319 mg, 0.870 mmol) and CLB (345 mg, 1.14 mmol) in DCM/DMF (5/5 mL) and 24 h stirring, followed by purification of the crude product by silica gel chromatography (7/3 to 5/5, v/v, petroleum ether/ethyl acetate gradient), yielded **11** (461 mg, 81%) as a beige solid: mp 73 °C; IR (NaCl) ν 3348, 1748, 1519, 1367, 1229, 1070, 1032 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.09-7.02 (m, 3H, H_{Ar}, NH), 6.64 (d, 2H, J = 8.7 Hz, H_{Ar}), 5.48-5.31 (m, 2H, H-1, H-3), 5.04 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 4.61 (s, 2H, $COCH_2O$), 4.38 (td, 1H, $J_{1,2} = J_{2,3}$ = 9.1 Hz, $J_{2,F}$ = 50.5 Hz, H-2), 4.32 (dd, 1H, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 12.7 Hz, H-6a), 4.05 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.87 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.75-3.58 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.59 and 2.43 (each t, 2H, J = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2$), 2.08, 2.06, 2.04 (each s, 3×3H, OAc), 1.95 (qt, 2H, ^{3}J = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.94, 170.63, 169.88, 169.74, 167.78 (CO), 144.62 ($C_{Ar}N$), 130.18 ($\underline{C}_{Ar}CH_2$), 129.84, 112.42 (CH_{Ar}), 88.45 (C-2, $J_{2,F}$ = 191.6 Hz), 77.09 (C-1, $J_{1,F}$ = 23.0 Hz), 74.11 (C-5), 73.40 (C-3, $J_{3,F}$ = 19.3 Hz), 67.86 (C-4, $J_{4,F}$ = 7.0 Hz), 62.84, 61.67 (C-6, $CO\underline{C}H_2O$), 53.72 (NCH_2), 40.63

(CH_2Cl), 33.90, 33.20 ($\underline{C}H_2CH_2\underline{C}H_2$), 26.46 ($CH_2\underline{C}H_2CH_2$), 20.82, 20.75, 20.67 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ −198.10; MS (ESI) m/z 651.43 $[M+H]^+$ (exact mass = 651.19). Anal. ($C_{28}H_{37}Cl_2FN_2O_{10}$) C, H, N.

*Benzyl (3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosylamino]-3-oxopropanoic acid (**12**).* Coupling of amine **5** (500 mg, 1.63 mmol) with malonic acid monobenzyl ester (320 mg, 1.80 mmol) was carried out according to the DCC/HOBt general procedure in DMF (25 mL) and 2 days stirring. Chromatography on silica gel (cyclohexane/ethyl acetate, 6/4, v/v) yielded compound **12** (450 mg, 57%) as a pale oil: IR (KBr) ν 3368, 1751, 1560, 1382, 1230, 1036 cm^{-1}; NMR ^1H (200 MHz, $CDCl_3$) δ 8.12 (d, 1H, J = 9.0 Hz, NH), 7.37 (s, 5H, Ph), 5.45-5.29 (m, 2H, H-1, H-3), 5.19 (s, 2H, $OC\underline{H}_2Ph$), 5.05 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.35 (td, 1H, $J_{1,2} = J_{2,3}$ = 8.9 Hz, $J_{2,F}$ = 50.6 Hz, H-2), 4.29 (dd, 1H, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.08 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 1.9 Hz, H-6b), 3.83 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.44 (s, 2H, $COCH_2CO$), 2.09, 2.08, 2.04 (3s, 3×3H, OAc); NMR ^{13}C (50 MHz, $CDCl_3$) δ 170.71, 169.98, 169.72, 169.18 (COO), 165.60 (NHCO), 134.79 ($C_{Ar}CH_2$), 128.88, 128.63 (CH_{Ar}), 88.50 (C-2, $J_{2,F}$ = 191.2 Hz), 77.29 (C-1, $J_{1,F}$ = 23.2 Hz), 73.87 (C-5), 73.46 (C-3, $J_{3,F}$ = 19.5 Hz), 67.92 (C-4, $J_{4,F}$ = 6.5 Hz), 61.70 (C-6), 40.70 ($CO\underline{C}H_2CO$), 20.84, 20.78, 20.69 (CH_3).

*3-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosylamino)-3-oxopropanoic acid (**13**).* Benzyl ester **12** (430 mg, 0.889 mmol) in ethanol/water (20/10 mL) was treated with lithium hydroxide (80 mg, 3.34 mmol) for 12 h at room temperature. After addition of water (10 mL) and DCM (30 mL) and separation of the layers, the aqueous one was acidified with HCl 1M (2 mL) and extracted with DCM (3×25 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield the acid **13** (310 mg, 89%) as an oil which was used in the next step without further purification: IR (KBr) ν 3350, 1740, 1708, 1655, 1568, 1381, 1238, 1044 cm^{-1}; NMR ^1H (200 MHz, $CDCl_3$) δ 8.14 (d, 1H, J = 8.4 Hz, NH), 6.71 (br.s, 1H, COOH), 5.45-5.31 (m, 2H, H-1, H-3), 5.05 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 4.55 (t, ½ H, $J_{1,2} = J_{2,3}$ = 8.9 Hz, H-2), 4.29 (m, 1.5H, ½ H-2, H-6a), 4.09 (m, 1H, H-6b), 3.46 (m, 1H, H-5), 3.46 (s, 2H, $COCH_2CO$), 2.08, 2.05 (each s, 9H, OAc); NMR ^{13}C (50 MHz, DMSO-d_6) δ 170.02, 169.55, 169.36, 168.70, 166.53 (CO), 88.38 (C-2, $J_{2,F}$ = 186.1 Hz), 76.47 (C-1, $J_{1,F}$ = 22.8 Hz), 72.68 (C-3, $J_{3,F}$ = 19.0 Hz), 71.95 (C-5), 67.74 (C-4, $J_{4,F}$ = 7.3 Hz), 61.58 (C-6), 42.91 ($CO\underline{C}H_2CO$), 20.54, 20.45, 20.38 (CH_3), ^{19}F NMR ($CDCl_3$) δ −197.45.

*N-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)-N'-[3-{4-[bis(2-chloroethyl)amino]phenyl}propyl]propanediamide (**15**).* Coupling of 4-(3-aminopropyl)-*N,N*-bis(2-chloroethyl)aniline **14** (100 mg, 0.363 mmol) with acid **13** (140 mg, 0.356 mmol) was carried out according to the general DCC/HOBt procedure in DMF (5 mL). After 24 h at room temperature, 10 mL of water were added and the reaction medium was extracted with DCM (3×10 mL). The organic extracts were combined, washed with saturated aqueous $NaHCO_3$ (2×5 mL) and aqueous acetic acid 1N (2×5 mL) solution, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (cyclohexane/ethyl acetate, 3/7 to 5/5, v/v) provided compound **15** (30 mg, 13%) as a white powder: mp 180 °C; IR (KBr) ν 3466, 1753, 1654, 1520, 1367, 1232, 1069, 1038 cm^{-1}; NMR ^1H (200 MHz, $CDCl_3$) δ 8.50 (d, 1H, J = 8.9 Hz, C_1NH), 7.05 (d, 2H, J = 8.4 Hz, H_{Ar}), 6.61 (d, 3H, J = 8.4 Hz, H_{Ar}, N<u>H</u>CH_2), 5.44-5.28 (m, 2H, H-1, H-3), 5.04 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.37 (td, 1H, $J_{1,2} = J_{2,3}$ = 9.1 Hz, $J_{2,F}$ = 50.7 Hz, H-2), 4.27 (m, 1H, H-6a), 4.07 (m, 1H, H-6b), 3.83 (m, 1H, H-5), 3.72-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 3.28 (q, 2H, J = 6.6 Hz, NHC<u>H</u>$_2$), 3.21 (s, 2H, $COCH_2CO$), 2.56 (t, 2H, J = 7.4 Hz, C<u>H</u>$_2$Ph), 2.08, 2.07, 2.04 (each s, 3×3H, OAc), 1.80 (qt, 2H, J = 7.3 Hz, $CH_2CH_2CH_2$); NMR ^{13}C (50 MHz, $CDCl_3$) δ 170.72, 170.03, 169.68 (<u>C</u>OCH_3), 168.06, 166.96 (NHCO), 144.53 ($C_{Ar}N$), 130.28 ($C_{Ar}CH_2$), 129.67, 112.43 (CH_{Ar}), 88.43 (C-2, $J_{2,F}$ = 190.9 Hz), 77.63 (C-1), 73.84 (C-5), 73.47 (C-3, $J_{3,F}$ = 19.3 Hz), 67.93 (C-4, $J_{4,F}$ = 7.1 Hz), 61.70 (C-6), 53.71 (NCH_2), 42.79 (CO<u>C</u>H_2CO), 40.62 (CH_2Cl), 39.53 (CH_2NH), 32.11 (<u>C</u>H_2Ph), 31.03 (CH_2<u>C</u>H_2CH_2), 20.84, 20.78, 20.69 (CH_3); ^{19}F NMR ($CDCl_3$) δ −197.98; MS (ESI) m/z 650.24 $[M+H]^+$ (exact mass = 650.20).

*3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-1-(4-nitrobenzyl)-β-D-glucopyranose (**16**).* 4-nitrobenzoic acid (580 mg, 3.47 mmol) was dissolved in MeOH (30 mL) and NaOH (140 mg, 3.5 mmol) was added. The mixture was stirred for 1h30 at room temperature. After evaporation under *vacuum*, the residue was dissolved in DMF (15 mL) and bromo sugar **4** (700 mg, 1.88 mmol) was added. The mixture was kept at room temperature for 3h. DMF was evaporated and the crude product was dissolved in water (60 mL) before extraction with DCM (5 × 40 mL). The organic extracts were combined, washed with saturated aqueous $NaHCO_3$ solution (2 × 30 mL), dried over $MgSO_4$, filtered and then evaporated to afford, after purification by silica gel chromatography (cyclohexane/ethyl acetate, 5/5, v/v) and precipitation in diisopropyl ether, the pure product **16** (320 mg, 37%) as a white powder: mp 109 °C ; IR (KBr) ν 1744, 1535, 1367, 1242, 1084, 1033 cm^{-1}; NMR ^1H (500 MHz, $CDCl_3$) δ

8.33 (d, 2H, J = 8.8 Hz, H_{Ar}), 8.28 (d, 2H, J = 8.8 Hz, H_{Ar}), 6.05 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{1,F}$ = 3.0 Hz, H-1), 5.46 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, $J_{3,F}$ = 14.4 Hz, H-3), 5.15 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 4.66 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.8 Hz, H-2), 4.33 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, $J_{6a,5}$ = 4.6 Hz, H-6a), 4.15 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.98 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 2.12, 2.08, 2.07 (each s, 3×3H, OAc); RMN ^{13}C (50 MHz, $CDCl_3$) δ 170.58, 169.95, 169.63 (CO), 162.85 (COPh), 151.26 ($C_{Ar}NO_2$), 133.98 ($C_{Ar}CO$), 131.49, 123.68 (CH_{Ar}), 92.49 (C-1, $J_{1,F}$ = 24.2 Hz), 88.36 (C-2, $J_{2,F}$ = 191.4 Hz), 73.19 (C-5), 72.74 (C-3, $J_{3,F}$ = 19.6 Hz), 67.68 (C-4, $C_{4,F}$ = 7.1 Hz), 61.41 (C-6), 20.76, 20.68 (CH_3).

*3,4,6-Tri-O-acetyl-1-(4-aminobenzyl)-2-deoxy-2-fluoro-β-D-glucopyranose (**17**).* Reduction of nitro compound **16** (300 mg, 0.65 mmol) using standard conditions in THF/EtOH (10/5 mL) during 10 h, yielded aniline **17** (280 mg, quant.) as a white solid: IR (KBr) ν 3478, 3383, 1747, 1603, 1368, 1243, 1069, 1040 cm^{-1}; NMR ^{1}H (200 MHz, $CDCl_3$) δ 7.90 (d, 2H, J = 8.7 Hz, H_{Ar}), 6.64 (d, 2H, J = 8.7 Hz, H_{Ar}), 6.01 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{1,F}$ = 3.1 Hz, H-1), 5.44 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, $J_{3,F}$ = 14.3 Hz, H-3), 5.13 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 4.61 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.7 Hz, H-2), 4.33 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.4 Hz, H-6a), 4.18 (br.s, 2H, NH_2), 4.11 (dd, 1H, $J_{6a,6b}$, $J_{6b,5}$ = 2.2 Hz, H-6b), 3.93 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 2.10, 2.06, 2.05 (3s, 3×3H, OAc); NMR ^{13}C (50 MHz, $CDCl_3$) δ 170.71, 170.03, 169.71 (CO), 164.41 (COPh), 151.95 ($C_{Ar}NH_2$), 132.60 (CH_{Ar}), 117.69 ($C_{Ar}CO$), 113.88 (CH_{Ar}), 91.57 (C-1, $J_{1,F}$ = 24.0 Hz), 88.53 (C-2, $J_{2,F}$ = 190.9 Hz), 73.08 (C-3, $J_{3,F}$ = 19.7), 72.79 (C-5), 67.91 (C-4, $C_{4,F}$ = 7.0 Hz), 61.60 (C-6), 20.80, 20.70 (CH_3).

*N-[4-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl-oxycarbonyl)phenyl]-4-{4-[bis(2-chloroethyl)amino]phenyl}butanamide (**18**).* Ethylchloroformate coupling, starting from amine **17** (260 mg, 0.61 mmol), CLB (220 mg, 0.72 mmol), TEA (140 µL, 1.01 mmol) and ethylchloroformate (84 µL, 0.88 mmol) in DCM (20 mL) and with a reaction time of 30 min, afforded compound **18** (290 mg, 50%) as a white powder after chromatography on silica gel (ethyl acetate/DCM, 15/85, v/v): mp 87 °C; IR (KBr) ν 3366, 1746, 1597, 1519, 1367, 1243, 1070, 1043 cm^{-1}; NMR ^{1}H (200 MHz, $CDCl_3$) δ 8.05 (d, 2H, J = 8.8 Hz, H_{Ar}), 7.61 (d, 2H, J = 8.8 Hz, H_{Ar}), 7.34 (s, 1H, NH), 7.08 (d, 2H, J = 8.6 Hz, H_{Ar}), 6.63 (d, 2H, J = 8.6 Hz, H_{Ar}), 6.03 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{1,F}$ = 3.0 Hz, H-1), 5.45 (td, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.1 Hz, $J_{3,F}$ = 14.3 Hz, H-3), 5.14 (t,1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 4.64 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 50.7 Hz, H-

2), 4.33 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, $J_{6a,5}$ = 4.3 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6a}$, $J_{6b,H5}$ = 1.9 Hz, H-6b), 3.95 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.74-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.63 (t, 2H, ^3J = 7.3 Hz, $PhC\underline{H}_2$), 2.38 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 2.11-2.10 (m, 11H, OAc, $CH_2C\underline{H}_2CH_2$); NMR ^{13}C (50 MHz, $CDCl_3$) δ 171.44 (CONH), 170.69, 170.02, 169.68 (CO), 163.95 ($\underline{C}OPh$), 144.62, 143.20 ($C_{Ar}N$, $C_{Ar}NH$), 131.73 (CH_{Ar}), 130.35 ($\underline{C}_{Ar}CH_2$), 129.86 (CH_{Ar}), 123.73 ($\underline{C}_{Ar}CO$), 118.80, 112.40 (CH_{Ar}), 91.88 (C-1, $J_{1,F}$ = 24.1 Hz), 88.46 (C-2, $J_{2,F}$ = 191.2 Hz), 72.96 (C-3, $J_{3,F}$ = 19.7), 72.93 (C-5), 67.83 (C-4, $J_{4,F}$ = 7.2 Hz), 61.53 (C-6), 53.72 (NCH_2), 40.65 (CH_2Cl), 37.03 ($CO\underline{C}H_2$), 34.01 ($\underline{C}H_2Ph$), 26.91 ($CH_2\underline{C}H_2CH_2$), 20.81, 20.78, 20.69 (CH_3); ^{19}F NMR ($CDCl_3$) δ −200.69; MS (ESI) m/z 713.29 $[M+H]^+$ (Exact Mass: 713.20); Anal. ($C_{33}H_{39}Cl_2FN_2O_{10}$) C, H, N.

*3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl 4-nitrophenylcarbonate (**19**).* To a solution of compound 3,4,6-tri-*O*-acetyl-2-desoxy-2-fluoro-α,β-D-glucose (4.4 g, 14.3 mmol) in THF (70 mL), cooled to 0 °C were added 4-nitrophenylchloroformate (2.87 g, 14.2 mmol) and TEA (2.4 mL, 17.3 mmol). The reaction mixture was stirred at room temperature for 24 h. The formed white solid was filtered, dissolved in ethyl acetate (150 mL) and the resulting solution was washed successively with water (4×40 mL) and brine (60 mL). After drying over $MgSO_4$, filtration and concentration under reduced pressure, a white solid corresponding to the β-anomer of compound **19** (1.5 g, 3.17 mmol) was obtained. The former filtrate was evaporated under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane/ethyl acetate, 6/4, v/v) to afford carbonate **19** (3.7 g, 7.82 mmol) as a mixture of α- and β-anomers. From this mixture, a supplementary fraction of β-anomer (890 mg, 1.88 mmol) can be obtained by crystallization in diisopropryl ether: 77% yield for α- and β-anomers; 35% yield for β-anomer; mp 191 °C (β-anomer); IR (KBr) ν 1779, 1740, 1526, 1346, 1241, 1079, 1040 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.32 (d, 2H, J = 9.1 Hz, H_{Ar}), 7.44 (d, 2H, J = 9.1 Hz, H_{Ar}), 5.75 (dd, 1H, $J_{1,F}$ = 3.3 Hz, $J_{1,2}$ = 8.0 Hz, H-1), 5.44 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.3 Hz, H-3), 5.13 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 4.57 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.6 Hz, H-2), 4.34 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.4 Hz, H-6a), 4.17 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.93 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 2.12, 2.10, 2.06 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.59, 169.93, 169.56 ($\underline{C}OCH_3$), 154.99, 150.95 ($O\underline{C}O\underline{C}_{Ar}O$), 145.96 ($C_{Ar}NO_2$), 125.59, 121.84 ($CH_{Ar}$), 95.58 (C-1, $J_{1\text{-}F}$ = 24.4 Hz), 88.09 (C-2, $J_{2\text{-}F}$ = 191.9 Hz), 73.19 (C-5), 72.41 (C-3, $J_{3\text{-}F}$ = 19.6 Hz), 67.43 (C-4, $J_{4\text{-}F}$ = 7.3 Hz), 61.26 (C-6), 20.79, 20.74, 20.65 (CH_3); ^{19}F NMR ($CDCl_3$) δ −200.81.

*4-{4-[bis(2-chloroethyl)amino]phenyl}-N-(4-nitrophenyl)butanamide (**20**).* Prepared from *p*-nitroaniline (240 mg, 1.74 mmol) and CLB (500 mg, 1.64 mmol) in the presence of TEA (380 µL, 2.73 mmol) and ethylchloroformate (170 µL, 1.78 mmol) in DCM (16 mL) according to the standard ethylchloroformate coupling. After stirring for 17 h, purification by chromatography on alumina (cyclohexane/ethyl acetate, 7/3, v/v) yielded compound **20** (170 mg, 24%) as a yellow powder: mp 105 °C; IR (KBr) ν 3331, 1681, 1520, 1508, 1349 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.16 (d, 3H, *J* = 9.0 Hz, H_{Ar}, NH), 7.73 (d, 2H, *J* = 9.0 Hz, H_{Ar}), 7.05 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 6.59 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 3.72-3.55 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.60 (t, 2H, *J* = 7.3 Hz, $C\underline{H}_2Ph$), 2.43 (t, 2H, *J* = 7.4 Hz, $COCH_2$), 2.01 (qt, 2H, *J* = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.63 (CO), 144.66, 143.87, 143.54 ($C_{Ar}N$, $C_{Ar}NO_2$, $C_{Ar}NH$), 130.18 ($\underline{C}_{Ar}CH_2$), 129.85, 125.24, 119.05, 112.38 (CH_{Ar}), 53.69 (NCH_2), 40.66 ($\underline{C}H_2Cl$), 36.99 ($CO\underline{C}H_2$), 33.97 ($\underline{C}H_2Ph$), 26.86 ($CH_2\underline{C}H_2CH_2$).

*N-(4-aminophenyl)-4-{4-[bis(2-chloroethyl)amino]phenyl}butanamide (**21**).* Reduction of nitro compound **20** (360 mg, 0.848 mmol) in THF/EtOH (10/10 mL) for 4 h using standard conditions, yielded amine **21** (250 mg, 75%) as a light yellow solid after purification by silica gel chromatography (cyclohexane/ethyl acetate, 4/6, v/v): mp 130 °C; IR (KBr) ν 3463, 3279, 1647, 1517 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.24 (d, 2H, *J* = 8.4 Hz, H_{Ar}), 7.08 (d, 3H, *J* = 8.7 Hz, H_{Ar}, NH), 6.62 (2d, 2×2H, *J* = 8.4 Hz, *J* = 8.7 Hz, H_{Ar}), 3.74-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.60 (t, 2H, *J* = 7.3 Hz, $C\underline{H}_2Ph$), 2.30 (t, 2H, *J* = 7.4 Hz, $COCH_2$), 1.99 (qt, 2H, *J* = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.98 (CO), 144.47, 143.34 ($C_{Ar}N$, $C_{Ar}NH_2$), 130.71 ($\underline{C}_{Ar}CH_2$), 129.84 (CH_{Ar}), 129.39 ($C_{Ar}NH$), 122.10, 115.48, 112.30 (CH_{Ar}), 53.70 (NCH_2), 40.67 (CH_2Cl), 36.75 ($CO\underline{C}H_2$), 34.11 ($\underline{C}H_2Ph$), 27.34 ($CH_2\underline{C}H_2CH_2$).

*(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 4-(4-{4-[bis(2-chloroethyl) amino]phenyl}butanamido)phenylcarbamate (**22**)* was prepared according to the standard carbamoylation procedure, starting from amine **21** (100 mg, 0.254 mmol) and β-carbonate **19** (118 mg, 0.249 mmol) in DMF (10 mL) with a reaction time of 25 h. Chromatography on silica gel (DCM/ethyl acetate, 9/1, v/v) afforded β anomer **22** (77 mg, 42%) as a white powder: mp 119 °C; IR (KBr) ν 3422, 1752, 1519, 1222, 1071, 1037 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.43 (d, 3H, *J* = 8.8 Hz, H_{Ar}, NH), 7.31 (d, 2H, *J* = 8.8 Hz, H_{Ar}), 7.24 (br s, 1H, NH), 7.07 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 6.61 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 5.83 (dd, 1H, $J_{1,2}$ = 8.1

Hz, $J_{1,F}$ = 2.9 Hz, H-1), 5.41 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.3 Hz, H-3), 5.09 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.48 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 50.8 Hz, H-2), 4.33 (dd, 1H, $J_{6a,6b}$ = 12.8 Hz, $J_{5,6a}$ = 4.3 Hz, H-6a), 4.10 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 3.1 Hz, H-6b), 3.89 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.74-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.60 (t, 2H, J = 7.3 Hz, $C\underline{H}_2Ph$), 2.33 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.10-1.96 (m, 11H, OAc, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.43 (NHCO), 170.71, 170.00, 169.70 ($\underline{C}OCH_3$), 150.92 (OCONH), 144.51 ($C_{Ar}N$), 134.40, 133.17 ($C_{Ar}NH$), 130.52 ($\underline{C}_{Ar}CH_2$), 129.79, 120.95, 119.90, 112.32 (CH_{Ar}), 92.49 (C-1, $J_{1,F}$ = 23.8 Hz), 88.27 (C-2, $J_{2,F}$ = 191.3 Hz), 72.83 (C-3, $J_{3,F}$ = 19.0 Hz), 72.65 (C-5), 67.74 (C-4, $J_{4,F}$ = 7.4 Hz), 61.44 (C-6), 53.65 (NCH_2), 40.67 (CH_2Cl), 36.77 ($CO\underline{C}H_2$), 34.08 ($\underline{C}H_2Ph$), 27.18 ($CH_2\underline{C}H_2CH_2$), 20.74, 20.63 (CH_3); ^{19}F NMR ($CDCl_3$) δ −200.71; MS (ESI) m/z 728.25 $[M+H]^+$ (Exact Mass: 728.20); Anal. ($C_{33}H_{40}Cl_2FN_3O_{10}$) C, H, N.

*3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 4-(4-{4-[bis(2-chloroethyl)amino] phenyl}butanoyloxy)phenylcarbamate (**25**).* Carbamoylation of amine **24**[35] (280 mg, 0.708 mmol) with β-anomer **19** (250 mg, 0.528 mmol) was carried out in DMF (15 mL) with 40 h stirring, according to the standard procedure. The crude product was chromatographed on silica gel (DCM/ethyl acetate, 19/1, v/v) to give compound **25** (54 mg, 14%) as a white powder: mp 130 °C; IR (KBr) ν 3361, 1753, 1519, 1218, 1071, 1038 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.38 (d, 2H, J = 8.8 Hz, H_{Ar}), 7.10 (d, 3H, J = 8.7 Hz, NH, H_{Ar}), 7.01 (d, 2H, J = 8.8 Hz, H_{Ar}), 6.64 (d, 2H, J = 8.7 Hz, H_{Ar}), 5.84 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{1,F}$ = 2.9 Hz, H-1), 5.42 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.2 Hz, H-3), 5.09 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.49 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 50.8 Hz, H-2), 4.34 (dd, 1H, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.10 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.0 Hz, H-6b), 3.90 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.75-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.64 (t, 2H, J = 7.2 Hz, $PhC\underline{H}_2$), 2.56 (t, 2H, J = 7.4 Hz, $COC\underline{H}_2$), 2.10-1.95 (m, 11H, OAc, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.30 ($\underline{C}OCH_2$), 170.69, 169.97, 169.71 ($\underline{C}OCH_3$), 150.76 (OCONH), 147.03 ($C_{Ar}O$), 144.53 ($C_{Ar}N$), 134.51 ($C_{Ar}NH$), 130.33 ($\underline{C}_{Ar}CH_2$), 129.85, 122.27, 120.09, 112.27 (CH_{Ar}), 92.49 (C-1, $J_{1\text{-}F}$ = 23.9 Hz), 88.23 (C-2, $J_{2\text{-}F}$ = 191.3 Hz), 72.80 (C-3, $J_{3\text{-}F}$ = 19.4 Hz), 72.67 (C-5), 67.69 (C-4, $J_{4\text{-}F}$ = 7.2 Hz), 61.41 (C-6), 53.65 ($N\underline{C}H_2$), 40.62 ($\underline{C}H_2Cl$), 34.01, 33.68 ($CO\underline{C}H_2CH_2\underline{C}H_2Cl$), 26.76 ($CH_2\underline{C}H_2CH_2$), 20.76, 20.74, 20.65 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ −200.73; MS (ESI) m/z 729.19 $[M+H]^+$ (Exact Mass: 729.20); Anal. ($C_{33}H_{39}Cl_2FN_2O_{11}$) C, H, N.

Benzyl 2-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosylcarbamoyl)phenyl amino]-2-oxoethylcarbamate (***28***) was prepared according to the standard ethylchloroformate coupling, starting from *N*-Cbz-glycine (215 mg, 1.03 mmol), amine **27**[35] (400 mg, 0.938 mmol), TEA (141 µL, 1.01 mmol) and ethylchloroformate (100 µL, 1.05 mmol) in DCM (25 mL). After 20 h stirring, chromatography on alumina (ethyl acetate/ethanol, 9/1, v/v) yielded compound **28** (290 mg, 50%) as a white powder: mp 225 °C; IR (KBr) ν 3374, 1745, 1677, 1656, 1525, 1367, 1242, 1067, 1036 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.28 (s, 1H, PhNHCO), 9.23 (d, 1H, J = 9.1 Hz, C_1NH), 7.88 (d, 2H, J = 8.7 Hz, H_{Ar}), 7.71 (d, 2H, J = 8.7 Hz, H_{Ar}), 7.60 (br t, 1H, J = 6.3 Hz, $CH_2N\underline{H}$), 7.37 (m, 5H, Ph), 5.74-5.51 (m, 2H, $J_{3,F}$ = 14.3 Hz, H-1, H-3), 5.05 (s, 2H, $C\underline{H}_2$Ph), 4.90 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 4.67 (td, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.9 Hz, $J_{2,F}$ = 50.2 Hz, H-2), 4.21-4.16 (m, 2H, 2H-6), 3.98 (m, 1H, H-5), 3.84 (d, 2H, J = 6.1 Hz, $C\underline{H}_2$NH), 2.05, 2.00, 1.98 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, DMSO-d_6) δ 170.53, 170.08, 169.88, 169.08, 166.60 (CO), 157.14 (NHCOO), 142.91 (C_{Ar}NH), 137.57 ($\underline{C}_{Ar}CH_2$), 129.16, 128.90, 128.34, 128.26 (CH_{Ar}), 127.93 ($\underline{C}_{Ar}$CO), 118.83 (CH_{Ar}), 88.70 (C-2, $J_{2,F}$ = 185.1 Hz), 77.71 (C-1, $J_{1,F}$ = 22.8 Hz), 73.41 (C-3, $J_{3,F}$ = 19.6 Hz), 72.49 (C-5), 68.36 (C-4, $J_{4,F}$ = 7.8 Hz), 66.04 ($\underline{C}H_2$Ph), 62.16 (C-6), 44.73 (CO$\underline{C}H_2$NH), 21.06, 20.99, 20.92 (CH_3) ; ^{19}F NMR (DMSO-d_6) δ −198.84.

*N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl)-4-(2-aminoacetamido) benzamide (**29**).* The deprotection of the *N*-Cbz protected compound **28** (390 mg, 0.631 mmol) was performed under standard hydrogenation conditions in THF/EtOH (40/10 mL) and yielded compound **29** (305 mg, 100%) as a white solid, used directly for the next step: IR (KBr) ν 3446, 1747, 1653, 1540, 1249, 1036 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.77 (br s, 1H, NH), 7.80 (d, 2H, J = 7.7 Hz, NH, H_{Ar}), 7.61 (d, 2H, J = 7.7 Hz, H_{Ar}), 5.59-5.32 (m, 2H, H-1, H-3), 5.07 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 4.63 (td, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.5 Hz, $J_{2,F}$ = 50.4 Hz, H-2), 4.36-3.87 (m, 3H, H-5, 2H-6), 3.56 (s, 2H, $C\underline{H}_2NH_2$), 2.74 (br s, 2H, NH_2), 2.07, 2.03 (each s, 9H, OAc); ^{13}C NMR (50 MHz, DMSO-d_6) δ 171.64, 170.01, 169.56, 169.38, 166.08 (CO), 142.16 (C_{Ar}NH), 128.63 (CH_{Ar}), 127.47 ($\underline{C}_{Ar}$CO), 118.31 (CH_{Ar}), 88.24 (C-2, $J_{2,F}$ = 185.9 Hz), 77.15 (C-1, $J_{1,F}$ = 22.8 Hz), 72.87 (C-3, $J_{3,F}$ = 19.3 Hz), 72.00 (C-5), 67.84 (C-4, $J_{4,F}$ = 7.6 Hz), 61.62 (C-6), 45.03 ($\underline{C}H_2NH_2$), 20.53, 20.46, 20.39 (CH_3); ^{19}F NMR (DMSO-d_6) δ −198.88.

*N-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl)-4-[2-(4-{4-[bis(2-chloro ethyl)amino]phenyl}butanamido)acetamido]benzamide (**30**).* Coupling of amine **29** (140 mg, 0.290 mmol) with CLB (97 mg, 0.319 mmol) was carried out in DMF (15 mL) according to the DCC/HOBt general procedure to yield after 24 h stirring and purification on silica gel (cyclohexane/ethyl acetate, 3/7, v/v) the title compound **30** (130 mg, 58%) as a white solid: mp 190 °C; IR (KBr) ν 3385, 1748, 1654, 1522, 1507, 1244, 1039 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.28 (s, 1H, PhNHCO), 9.23 (d, 1H, J = 8.8 Hz, C_1NH), 8.19 (br.t, 1H, J = 5.8 Hz, $CH_2N\underline{H}$), 7.88 (d, 2H, J = 8.9 Hz, H_{Ar}), 7.70 (d, 2H, J = 8.9 Hz, H_{Ar}), 7.04 (d, 2H, J = 8.6 Hz, H_{Ar}), 6.66 (d, 2H, J = 8.6 Hz, H_{Ar}), 5.73-5.50 (m, 2H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.1 Hz, H-1, H-3), 4.89 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 4.66 (td, 1H, $J_{1,2} = J_{2,3}$ = 9.0 Hz, $J_{2,F}$ = 50.2 Hz, H-2), 4.20-3.93 (m, 3H, 2H-6, H-5), 3.89 (d, 2H, J = 5.6 Hz, $C\underline{H}_2NH$), 3.70 (s, 8H, $N(CH_2CH_2Cl)_2$), 2.47 (m, 2H, $C\underline{H}_2Ph$), 2.16 (t, 2H, J = 7.3 Hz, $COC\underline{H}_2CH_2$), 2.05, 2.00, 1.98 (each s, 3×3H, OAc), 1.75 (qt, 2H, ^3J = 7.3 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.61, 169.96, 169.52, 169.34, 168.47, 166.04 (CO), 144.39 ($C_{Ar}N$), 142.32 ($C_{Ar}NH$), 129.97 ($\underline{C}_{Ar}CH_2$), 129.33, 128.58 (CH_{Ar}), 127.41 ($\underline{C}_{Ar}CO$), 118.30, 111.90 (CH_{Ar}), 88.23 (C-2, $J_{2,F}$ = 185.8 Hz), 77.09 (C-1, $J_{1,F}$ = 22.9 Hz), 72.83 (C-3, $J_{3,F}$ = 18.9 Hz), 71.96 (C-5), 67.79 (C-4, $J_{4,F}$ = 7.8 Hz), 61.58 (C-6), 52.23 (NCH_2), 42.76 (CH_2NH), 41.16 (CH_2Cl), 34.58, 33.56 ($\underline{C}H_2CH_2\underline{C}H_2$), 27.32 ($CH_2\underline{C}H_2CH_2$), 20.51, 20.44, 20.37 ($CH_3$); ^{19}F NMR (DMSO-d_6) δ −198.87; MS (ESI) m/z 769.35 $[M+H]^+$ (Exact Mass: 769.23); Anal. ($C_{35}H_{43}Cl_2FN_4O_{10}$) C, H, N.

*4-(2-(benzyloxycarbonylamino)acetamido)phenyl 4-(4-(bis(2-chloroethyl)amino)phenyl) butanoate (**31**).* Coupling of compound **24** (1.19 g, 3.01 mmol) with *N*-Cbz-glycine (640 mg, 3.06 mmol) was carried out according to the DCC/HOBt general procedure in DMF (70 mL) and with stirring during 24 h, to yield after purification on silica gel (petroleum ether/ethyl acetate, 5/5, v/v) the title compound **31** (1.60 g, 90%) as a white powder: IR (KBr) ν 3337, 1746, 1692, 1670 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.37 (br.s, 1H, $N\underline{H}Ph$), 7.47 (d, 2H, J_o = 8.8 Hz, H_{Ar}), 7.34 (m, 5H, H_{Ar}), 7.10 (d, 2H, J'_o = 8.5 Hz, H_{Ar}), 6.97 (d, 2H, J_o, H_{Ar}), 6.63 (d, 2H, J'_o, H_{Ar}), 5.70 (br t, 1H, $N\underline{H}CH_2$), 5.14 (s, 2H, $PhC\underline{H}_2CO$), 3.98 (d, 2H, ^3J = 5.5 Hz, $NHC\underline{H}_2CO$), 3.75-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.64, 2.55 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2CH_2C\underline{H}_2$), 2.02 (qt, 2H, ^3J = 7.3 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.42, 167.42, 157.04 (CO), 147.08 ($C_{Ar}O$), 144.53 ($C_{Ar}N$), 136.08, 135.20 ($C_{Ar}NH$, $C_{Ar}CH_2O$), 130.33 ($C_{Ar}CH_2CH_2$), 129.84, 128.69, 128.40, 128.10, 122.06, 121.05, 112.28 (CH_{Ar}), 67.45

(Ph<u>C</u>H$_2$O), 53.65 (N<u>C</u>H$_2$CH$_2$), 45.41 (NH<u>C</u>H$_2$CO), 40.63 (CH$_2$Cl), 34.01, 33.79 (<u>C</u>H$_2$CH$_2$<u>C</u>H$_2$), 26.75 (CH$_2$<u>C</u>H$_2$CH$_2$).

*4-(2-aminoacetamido)phenyl 4-{4-[bis(2-chloroethyl)amino]phenyl}butanoate (**32**).* Cbz cleavage in THF/EtOH (3/3 mL) using standard hydrogenation conditions (4 h), starting from compound **31** (300 mg, 0.511 mmol) yielded the unprotected product **32** (230 mg, 99%) as a colorless oil which was used in the next step without further purification: IR (NaCl) ν 3348, 1746, 1654, 1235 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 9.43 (br s, 1H, N<u>H</u>Ph), 7.61 (d, 2H, *J* = 8.8 Hz, H$_{Ar}$), 7.11 (d, 2H, *J* = 8.5 Hz, H$_{Ar}$), 7.02 (d, 2H, *J* = 8.8 Hz, H$_{Ar}$), 6.64 (d, 2H, *J* = 8.5 Hz, H$_{Ar}$), 3.75-3.58 (m, 8H, N(CH$_2$CH$_2$Cl)$_2$), 3.46 (s, 2H, NH$_2$C<u>H</u>$_2$), 2.64, 2.56 (t, 2H, *J* = 7.4 Hz, C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$), 2.10-1.99 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 172.29, 170.94 (CO), 146.76 (C$_{Ar}$O), 144.45 (C$_{Ar}$N), 135.37 (C$_{Ar}$NH), 130.28 (C$_{Ar}$CH$_2$), 129.77, 122.00, 120.38, 112.20 (CH$_{Ar}$), 53.57 (N<u>C</u>H$_2$CH$_2$), 45.04 (NH$_2$<u>C</u>H$_2$CO), 40.59 (CH$_2$Cl), 33.95, 33.63 (<u>C</u>H$_2$CH$_2$<u>C</u>H$_2$), 26.71 (CH$_2$<u>C</u>H$_2$CH$_2$).

*4-[2-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxycarbonylamino] acetamido)phenyl 4-{4-[bis(2-chloroethyl)amino]phenyl}butanoate (**33**).* Carbamoylation of amine **32** (500 mg, 1.11 mmol) with β-anomer **19** (490 mg, 1.04 mmol) in DMF (50 mL) was carried out according to the standard procedure after 24 h stirring. The crude product was chromatographed on silica gel (cyclohexane/ethyl acetate, 5/5, v/v) to give carbamate β-anomer **33** (480 mg, 59%) as a white powder: mp 110 °C; IR (KBr) ν 3359, 1751, 1519, 1508, 1233, 1074, 1037 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 8.10 (br s, 1H, N<u>H</u>Ph), 7.48 (d, 2H, *J* = 8.8 Hz, H$_{Ar}$), 7.10 (d, 2H, *J* = 8.5 Hz, H$_{Ar}$), 7.00 (d, 2H, *J* = 8.8 Hz, H$_{Ar}$), 6.64 (d, 2H, *J* = 8.5 Hz, H$_{Ar}$), 5.93 (br t, 1H, ^3J = 5.0 Hz, N<u>H</u>CH$_2$), 5.82 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{1,F}$ = 2.9 Hz, H-1), 5.43 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.2 Hz, H-3), 5.08 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 4.45 (td, 1H, $J_{1,2}$ ≈ $J_{2,3}$, $J_{2,F}$ = 50.8 Hz, H-2), 4.30 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.10 (dd, 1H, $J_{6a,6b}$, $J_{5,6a}$ = 2.4 Hz, H-6b), 4.01 (d, 2H, ^3J = 5.3 Hz, NHC<u>H</u>$_2$), 3.89 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.75-3.57 (m, 8H, N(CH$_2$CH$_2$Cl)$_2$), 2.64, 2.56 (each t, 2H, *J* = 7.3 Hz, C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$), 2.10-1.98 (m, 11H, OAc, CH$_2$C<u>H</u>$_2$CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 172.49 (PhO<u>C</u>O), 170.76, 170.13, 169.66 (<u>C</u>OCH$_3$), 166.68 (CH$_2$<u>C</u>ONH), 154.26 (OCONH), 147.31 (C$_{Ar}$O), 144.58 (<u>C</u>$_{Ar}$N), 135.01 (<u>C</u>$_{Ar}$NH), 130.42 (<u>C</u>$_{Ar}$CH$_2$), 129.87, 122.17, 121.23, 112.42 (CH$_{Ar}$), 92.86 (C-1, $J_{1\text{-}F}$ = 24.2 Hz), 88.33 (C-2, $J_{2\text{-}F}$ = 191.5 Hz), 72.82 (C-3, $J_{3\text{-}F}$ = 19.4 Hz), 72.62 (C-5), 67.73 (C-4, $J_{4\text{-}F}$ = 7.0 Hz), 61.46 (C-6), 53.74 (N<u>C</u>H$_2$CH$_2$), 45.10 (NH<u>C</u>H$_2$CO),

40.65 ($\underline{C}H_2Cl$), 34.05, 33.73 ($\underline{C}H_2CH_2\underline{C}H_2$), 26.76 ($CH_2\underline{C}H_2CH_2$), 20.78, 20.65 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ −200.70; MS (ESI) m/z 786.37 $[M+H]^+$ (Exact Mass: 786.22); Anal. ($C_{35}H_{42}Cl_2FN_3O_{12}$) C, H, N.

*Benzyl 2-(4-nitrophenylamino)-2-oxoethylcarbamate (**34**).* Coupling of *N*-Cbz-glycine (1.5 g, 7.17 mmol) with *p*-nitroaniline (1.11 g, 8.03 mmol) in DCM (60 mL) was carried out according to the general ethylchloroformate coupling, in the presence of TEA (1.05 mL, 7.55 mmol) and ethylchloroformate (0.75 mL, 7.84 mmol). After stirring for 21 h, purification on alumina (cyclohexane/ethyl acetate, 4/6, v/v) provided compound **34** (1.35 g, 57%) as a dark yellow solid: mp 172 °C; IR (KBr) ν 3327, 3285, 1678, 1519, 1344 cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 9.84 (br s, 1H, N$\underline{H}$Ph), 8.22 (d, 2H, *J* = 9.2 Hz, H_{Ar}), 7.91 (d, 2H, *J* = 9.2 Hz, H_{Ar}), 7.39-7.29 (m, 5H, Ph), 6.79 (br t, 1H, $CH_2N\underline{H}$), 5.12 (s, 2H, $OC\underline{H}_2Ph$), 4.06 (d, 2H, *J* = 6.1 Hz, $C\underline{H}_2NH$); ^{13}C NMR (50 MHz, acetone-d_6) 169.63 (NHCO), 157.71 (NHCOO), 145.77 ($C_{Ar}NH$), 143.99 ($C_{Ar}NO_2$), 138.08 ($C_{Ar}CH_2$), 129.24, 128.71, 125.61, 119.87 (CH_{Ar}), 67.06 ($O\underline{C}H_2Ph$), 45.76 ($CO\underline{C}H_2NH$).

*2-amino-N-(4-nitrophenyl)acetamide hydrobromide (**35**).* To a stirred suspension of **34** (502 mg, 1.52 mmol) in acetic acid (3 mL) was added dropwise 2.6 mL (15.1 mmol) of 33% hydrogen bromide in acetic acid. The resulting solution was stirred at room temperature for 2h45 before careful addition to cold diethyl ether (30 mL). The white solid formed was filtered off and washed with diethyl ether to yield compound **35** (365 mg, 87%) as a white solid: mp 255 °C; IR (KBr) ν 3030, 2595, 1691, 1557, 1346 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 8.18 (d, 2H, *J* = 8.9 Hz, H_{Ar}), 7.64 (d, 2H, *J* = 8.9 Hz, H_{Ar}), 4.04 (s, 2H, $C\underline{H}_2CO$); ^{13}C NMR (100 MHz, DMSO-d_6) 165.79 (NHCO), 144.18 ($C_{Ar}NH$), 142.64 ($C_{Ar}NO_2$), 125.11, 118.92 (CH_{Ar}), 41.31 (CH_2NH_2).

*(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 2-(4-nitrophenylamino)-2-oxoethylcarbamate (**36**)* was prepared according to the standard carbamoylation procedure, starting from amine hydrobromide **35** (300 mg, 1.09 mmol) and carbonate **19** (510 mg, 1.08 mmol) in DMF (20 mL) with 5 h stirring. Chromatography on silica gel (cyclohexane/ethyl acetate, 4/6, v/v) provided compound **36** (510 mg, 89%) as a white powder: mp 177 °C; IR

(KBr) ν 3349, 1747, 1558, 1513, 1345, 1236, 1074, 1038 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.50 (s, 1H, NHPh), 8.22 (d, 2H, J = 9.2 Hz, H_{Ar}), 7.72 (d, 2H, J = 9.2 Hz, H_{Ar}), 5.89-5.83 (m, 2H, $J_{1,2}$ = 8.2 Hz, $J_{1,F}$ = 3.0 Hz, H-1, NHCH$_2$), 5.46 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{3\text{-}F}$ = 14.1 Hz, H-3), 5.10 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 4.46 (td, 1H, $J_{1,2}$≈$J_{2,3}$, $J_{2,F}$ = 50.9 Hz, H-2), 4.31 (dd, 1H, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 4.1 Hz, H-6a), 4.18-4.07 (m, 3H, J = 5.7 Hz, H-6b, NHCH$_2$), 3.92 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$ = 2.2 Hz, H-5), 2.12, 2.08, 2.05 (each s, 3×3H, OAc); ^{19}F NMR ($CDCl_3$) δ −200.76.

*(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 2-(4-aminophenylamino)-2-oxo ethylcarbamate (**37**).* Reduction of the nitro compound **36** (500 mg, 0.944 mmol) in THF/EtOH (10/10 mL) and 5 h stirring, using standard conditions gave the amine **37** (440 mg, 93%) as a solid which was used in the next step without further purification: IR (KBr) ν 3367, 1747, 1681, 1516, 1237, 1074, 1037 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.02 (s, 1H, NHPh), 7.22 (d, 2H, J = 8.5 Hz, H_{Ar}), 6.62 (d, 2H, J = 8.5 Hz, H_{Ar}), 6.11 (br t, 1H, J = 5.2 Hz, NHCH$_2$), 5.81 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{1,F}$ = 3.0 Hz, H-1), 5.42 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.2 Hz, H-3), 5.08 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 4.45 (td, 1H, $J_{1,2}$≈$J_{2,3}$, $J_{2,F}$ = 50.4 Hz, H-2), 4.28 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 3.9 Hz, H-6a), 4.08 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 1.8 Hz, H-6b), 3.98 (d, 2H, J = 5.2 Hz, NHCH$_2$), 3.86 (m, 1H, H-5), 3.14 (br s, 2H, NH_2), 2.10, 2.06, 2.03 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.73, 170.09, 169.66 (COCH$_3$), 166.09 (CONH), 154.15 (OCONH), 143.99 ($C_{Ar}NH_2$), 128.37 (C_{Ar}NH), 122.42, 115.54 (CH_{Ar}), 92.85 (C-1, $J_{1,F}$ = 23.7 Hz), 88.34 (C-2, $J_{2,F}$ = 191.0 Hz), 72.84 (C-3, $J_{3,F}$ = 19.7 Hz), 72.65 (C-5), 67.74 (C-4, $J_{4,F}$ = 7.3 Hz), 61.45 (C-6), 45.08 (NHCH$_2$), 20.80, 20.67 (CH_3); ^{19}F NMR ($CDCl_3$) δ −200.71.

*(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 2-[4-(4-{4-[bis(2-chloroethyl) amino]phenyl}butanamido)phenylamino]-2-oxoethylcarbamate (**38**).* The general ethylchloroformate procedure was carried out starting from CLB (244 mg, 0.802 mmol), amine **37** (200 mg, 0.400 mmol), TEA (160 µL, 1.15 mmol) and ethylchloroformate (82 µL, 0.858 mmol) in DCM (10 mL). After 1 h stirring, purification by chromatography on silica gel (cyclohexane/ethyl acetate, 4/6, v/v) provided compound **38** (270 mg, 86%) as a dark solid: mp 114 °C; IR (KBr) ν 3350, 1750, 1676, 1517, 1236, 1074, 1037 cm^{-1};^1H NMR (200 MHz, $CDCl_3$) δ 8.30 (s, 1H, NH), 7.56 (s, 1H, NH), 7.31 (s, 4H, H_{Ar}), 7.07 (d, 2H, J = 8.6 Hz, H_{Ar}), 6.61 (d, 2H, J = 8.6 Hz, H_{Ar}), 6.12 (br t, 1H, J = 5.2 Hz, NHCH$_2$), 5.81 (dd, 1H, $J_{1,2}$ =

8.0 Hz, $J_{1,F}$ = 2.9 Hz, H-1), 5.40 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.1 Hz, H-3), 5.08 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.45 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 50.9 Hz, H-2), 4.29 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 4.1 Hz, H-6a), 4.14-4.00 (m, 3H, NHC$\underline{H}_2$, H-6b), 3.88 (m, 1H, H_5), 3.73-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.60 (t, 2H, J = 7.3 Hz, C$\underline{H}_2$Ph), 2.35 (t, 2H, J = 7.0 Hz, $COCH_2$), 2.10-1.96 (m, 11H, OAc, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.88 (PhNHC$\underline{O}$), 170.79, 170.13, 169.68 ($\underline{C}OCH_3$), 166.86 ($CH_2\underline{C}ONH$), 154.24 (OCONH), 144.53 ($C_{Ar}N$), 134.40, 133.79 ($C_{Ar}NH$), 130.47 ($\underline{C}_{Ar}CH_2$), 129.81, 121.51, 121.21, 112.29 (CH_{Ar}), 92.79 (C-1, J_{1-F} = 24.1 Hz), 88.35 (C-2, $J_{2,F}$ = 191.1 Hz), 72.77 (C-3, $J_{3,F}$ = 20.8 Hz), 72.56 (C-5), 67.69 (C-4, $J_{4,F}$ = 6.8 Hz), 61.44 (C-6), 53.65 (NCH_2), 44.94 (NH$\underline{C}H_2$), 40.68 (CH_2Cl), 36.71 ($CO\underline{C}H_2CH_2$), 34.11 ($\underline{C}H_2Ph$), 27.24 ($CH_2\underline{C}H_2CH_2$), 20.79, 20.66 (CH_3); ^{19}F NMR ($CDCl_3$) δ −200.66; MS (ESI) m/z 785.60 $[M+H]^+$ (Exact Mass: 785.24); Anal. ($C_{35}H_{43}Cl_2FN_4O_{11}$) C, H, N.

*4-{4-[bis(2-chloroethyl)amino]phenyl}-N-[2-(4-nitrophenylamino]-2-oxoethyl)butanamide (**39**).* Amine **35** as hydrobromide salt (305 mg, 1.09 mmol) was beforehand treated with TEA (230 µL, 1.67 mmol) at ambient temperature for 2.5 h in DCM (5 mL). Then a general ethylchloroformate procedure was carried out starting from CLB (300 mg, 0.986 mmol) in the presence of TEA (150 µL, 1.08 mmol) and ethylchloroformate (100 µL, 1.05 mmol) in DCM (10 mL). After 3 days stirring and evaporation of the reaction mixture, the residue was suspended in saturated aqueous Na_2CO_3 solution (20 mL) and extracted twice with ethyl acetate (2 × 20 mL). During this extraction, an insoluble compound (i.e. amide **39**) was filtered off. The organic extracts were combined, washed twice with water, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a white powder. Both solids were combined and washed with diethyl ether. After drying, compound **39** (435 mg, 92%) was obtained as a yellow powder, analytically pure, and used without other purification for the following stage: IR (KBr) ν 3394, 3280, 3250, 3225, 1702, 1643, 1508, 1337 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.78 (br s, 1H, N$\underline{H}$Ph), 7.38 (d, 3H, J = 9.0 Hz, H_{Ar}, NH), 6.99 (d, 2H, J = 9.0 Hz, H_{Ar}), 6.194 (d, 2H, J = 8.3 Hz, H_{Ar}), 5.82 (d, 2H, J = 8.3 Hz, H_{Ar}), 3.09 (d, 2H, J = 5.4 Hz, C$\underline{H}_2$NH), 2.85 (s, 8H, $N(CH_2CH_2Cl)_2$), 1.63 (t, 2H, J = 6.9 Hz, C$\underline{H}_2$Ph), 1.33 (t, 2H, J = 6.8 Hz, $COCH_2$), 0.91 (qt, 2H, J = 6.8 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.66, 169.01 (CO), 145.10, 144.40 ($2C_{Ar}N$), 142.14 ($C_{Ar}NO_2$), 129.98 ($\underline{C}_{Ar}CH_2$), 129.31, 125.00, 118.71, 111.91 (CH_{Ar}), 52.23 (CH_2N), 42.87 ($COCH_2NH$), 41.15 (CH_2Cl), 34.54, 33.52 ($\underline{C}H_2CH_2\underline{C}H_2$), 27.28 ($CH_2\underline{C}H_2CH_2$).

*N-[2-(4-aminophenylamino)-2-oxoethyl]-4-{4-[bis(2-chloroethyl)amino]phenyl}butanamide (**40**).* Reduction of the nitro compound **39** (354 mg, 0.735 mmol) in THF/EtOH (4/4 mL) during 24 h according to the standard procedure yielded compound **40** (291 mg, 88%) as a pale pink solid: IR (KBr) ν 3377, 3307, 1686, 1630, 1517 cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 8.88 (br s, 1H, NH), 7.30 (d, 3H, J = 8.9 Hz, NH, H_{Ar}), 7.09 (d, 2H, J = 8.9 Hz, H_{Ar}), 6.72 (d, 2H, J = 8.9 Hz, H_{Ar}), 6.60 (d, 2H, J = 8.9 Hz, H_{Ar}), 4.45 (br s, 2H, NH_2), 3.95 (d, 2H, J = 5.7 Hz, $C\underline{H}_2NH$), 3.83-3.66 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.55 (t, 2H, J = 7.6 Hz, $C\underline{H}_2Ph$), 2.28 (t, 2H, J = 7.4 Hz, $COC\underline{H}_2CH_2$), 1.88 (qt, 2H, J = 7.5 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.44, 166.90 (CO), 144.39, 144.18 ($C_{Ar}NH_2$, $C_{Ar}N$), 130.02, 128.41 ($\underline{C}_{Ar}CH_2$, $C_{Ar}NH$), 129.33, 120.88, 114.12, 111.90 (CH_{Ar}), 52.25 (CH_2N), 42.49 ($COCH_2NH$), 41.17 (CH_2Cl), 34.67, 33.59 ($\underline{C}H_2CH_2\underline{C}H_2$), 27.33 ($CH_2\underline{C}H_2CH_2$).

*(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) 4-[2-(4-{4-[bis(2-chloroethyl) amino]phenyl}butanamido)acetamido]phenylcarbamate (**41**).* Carbamoylation of amine **40** (270 mg, 0.598 mmol) with β-anomer **19** (340 mg, 0.718 mmol) was carried out according to the standard procedure in DMF (20 mL) and stirring for 20 h. The crude product was chromatographed on silica gel (cyclohexane/ethyl acetate, 3/7, v/v) to give the title compound **41** (175 mg, 37%) as a white powder: mp 104 °C; IR (KBr) ν 3350, 1752, 1653, 1519, 1221, 1071, 1037 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.91 (br s, 1H, NH), 7.47 (d, 2H, J = 8.6 Hz, H_{Ar}), 7.29 (d, 2H, J = 8.6 Hz, H_{Ar}), 7.06-7.00 (m, 3H, H_{Ar}, NH), 6.61 (d, 3H, J = 8.2 Hz, H_{Ar}, NH), 5.85 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{1,F}$ = 2.9 Hz, H-1), 5.43 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.4 Hz, H-3), 5.10 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 4.51 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.6 Hz, H-2), 4.34 (dd, 1H, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 3.9 Hz, H-6a), 4.15-4.10 (m, 3H, $C\underline{H}_2NH$, H-6b), 3.92 (m, 1H, H-5), 3.72-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.56 (t, 2H, J = 7.3 Hz, $C\underline{H}_2Ph$), 2.30 (t, 2H, J = 7.3 Hz, $COC\underline{H}_2$), 2.11, 2.06, 2.05 (each s, 3×3H, OAc), 1.95 (qt, 2H, J = 7.3 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.24 (NHCO), 170.69, 170.00, 169.69 ($\underline{C}OCH_3$), 167.28 (NHCO), 150.81 (OCONH), 144.55 ($C_{Ar}N$), 134.26, 133.24 ($C_{Ar}NH$), 130.40 ($\underline{C}_{Ar}CH_2$), 129.78, 120.89, 119.83, 112.34 (CH_{Ar}), 92.57 (C-1, $J_{1,F}$ = 23.8 Hz), 88.30 (C-2, $J_{2,F}$ = 191.0 Hz), 72.85 (C-3, $J_{3,F}$ = 19.8 Hz), 72.73 (C-5), 67.72 (C-4, $J_{4,F}$ = 7.2 Hz), 61.43 (C-6), 53.69 (NCH_2), 44.52 ($CO\underline{C}H_2NH$), 40.69 (CH_2Cl), 35.60, 34.10 ($\underline{C}H_2CH_2\underline{C}H_2$), 27.36 ($CH_2\underline{C}H_2CH_2$), 20.78, 20.68 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ −200.68; MS (ESI) m/z 785.34 $[M+H]^+$ (Exact Mass: 785.25); Anal. ($C_{35}H_{43}Cl_2FN_4O_{11}$) C, H, N.

*2-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyloxy)acetic acid (**44**).* A standard DCC/DMAP procedure in DCM (25 mL) starting from benzyl glycolate (252 mg, 1.52 mmol) and CLB (500 mg, 1.64 mmol), with stirring during 48 h, followed by purification of the crude product by silica gel chromatography (petroleum ether/ethyl acetate, 9/1, v/v) provided compound **43** (572 mg, 85%) as a dark yellow syrup: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 (*s*, 5H, Ph), 7.08 (d, 2H, *J* = 8.6 Hz, H_{Ar}), 6.64 (d, 2H, *J* = 8.6 Hz, H_{Ar}), 5.20 (s, 2H, $PhCH_2O$), 4.66 (s, 2H, $COCH_2O$), 3.74-3.60 (m, 8H, $N(CH_2CH_2)_2Cl$), 2.59, 2.43 (each t, 2H, *J* = 7.4 Hz, $CH_2CH_2CH_2$), 1.94 (qt, 2H, *J* = 7.3 Hz, $CH_2CH_2CH_2$). This benzyl compound **43** (552 mg. 1.22 mmol) was hydrogenated at atmospheric pressure for 2 h in THF/EtOH (7/7 mL) to yield the acid **44** (427 mg, 97%) as a brown oil which was used in the next step without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.07 (d, 2H, *J* = 8.6 Hz, H_{Ar}), 6.63 (d, 2H, *J* = 8.6 Hz, H_{Ar}), 4.66 (s, 2H, $COCH_2O$), 3.75-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.59, 2.43 (each t, 2H, *J* = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2$), 1.94 (qt, 2H, *J* = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.03, 172.59 (CO), 144.09 ($C_{Ar}N$), 131.20 ($C_{Ar}CH_2$), 129.94, 112.78 (CH_{Ar}), 60.23 ($CO\underline{C}H_2O$), 53.97 (NCH_2), 40.47 (CH_2Cl), 33.92, 33.11 ($\underline{C}H_2CH_2\underline{C}H_2$), 26.69 ($CH_2\underline{C}H_2CH_2$).

*2-{[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenyl]amino}-2-oxoethyl 4-{4-[bis(2-chloroethyl)amino]phenyl}butanoate (**45**).* Coupling of compound **44** (414 mg, 1.14 mmol) with amine **42** (511 mg, 1.28 mmol) in the presence of TEA (1.05 mL, 7.55 mmol) and ethychloroformate (0.75 mL, 7.84 mmol) was carried out according to the general ethylchloroformate coupling in DCM (10 mL) during 18 h. Chromatography on silica gel (cyclohexane/ethyl acetate, 5/5, v/v) gave expected compound **45** (404 mg, 48%) as a white solid: IR (KBr) ν 3364, 1751, 1511, 1367, 1225, 1068, 1045 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.75 (br s, 1H, NH), 7.47 (d, 2H, *J* = 9.0 Hz, H_{Ar}), 7.08 (d, 2H, *J* = 8.7 Hz, H_{Ar}), 7.05 (d, 2H, *J* = 9.0 Hz, H_{Ar}), 6.63 (d, 2H, *J* = 8.7 Hz, H_{Ar}), 5.41 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.5 Hz, H-3), 5.10 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 5.09 (dd, 1H, $J_{1,F}$ = 3.0 Hz, $J_{1,2}$ = 7.7 Hz, H-1), 4.67 (s, 2H, $COC\underline{H}_2O$), 4.56 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.4 Hz, H-2), 4.30 (dd, 1H, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.15 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.4 Hz, H-6b), 3.86 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.75-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.61, 2.48 (each t, 2×2H, *J* = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2$), 2.12, 2.08, 2.06 (each s, 3×3H, OAc), 1.99 (qt, 2H, *J* = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.07 ($O\underline{C}OCH_2$), 170.63, 170.11, 169.64 ($\underline{C}OCH_3$), 165.15 (NHCO), 153.82 ($C_{Ar}O$), 144.66 ($C_{Ar}N$), 132.57 ($C_{Ar}NH$), 130.15 ($C_{Ar}CH_2$), 129.83, 121.90, 118.27, 112.42 (CH_{Ar}), 99.34 (C-1, $J_{1,F}$ = 23.2 Hz), 89.13 (C-2, $J_{2,F}$ = 190.8

Hz), 72.80 (C-3, $J_{3,F}$ = 19.8 Hz), 72.22 (C-5), 68.21 (C-4, $J_{4,F}$ = 7.0 Hz), 63.26 (CO<u>C</u>H_2O), 61.95 (C-6), 53.72 (NCH_2), 40.63 (CH_2Cl), 33.93, 33.32 (<u>C</u>H_2CH_2<u>C</u>H_2), 26.54 (CH_2<u>C</u>H_2CH_2), 20.80, 20.68 (CH_3); ^{19}F NMR ($CDCl_3$) δ −199.53; MS (ESI) m/z 743.27 $[M+H]^+$ (Exact Mass: 743.21); Anal. ($C_{34}H_{41}Cl_2FN_2O_{11}$) C, H, N.

*Benzyl 3-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenylamino]-3-oxopropanoate (**46**).* Coupling of amine **42** (475 mg, 1.19 mmol) with malonic acid monobenzyl ester (254 mg, 1.31 mmol) was carried out according to the DCC/HOBt general procedure in DMF (20 mL) with a reaction time of 22 h. Chromatography on silica gel (cyclohexane/ethyl acetate, 5/5, v/v) provided compound **46** (0.420 g, 61%) as a pale yellow oil: IR (KBr) ν 3367, 1754, 1689, 1544, 1370, 1229, 1067, 1048 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 7.48 (d, 2H, J = 9.0 Hz, H_{Ar}), 7.39 (s, 5H, Ph), 7.05 (d, 2H, J = 9.0 Hz, H_{Ar}), 5.39 (td, 1H, $J_{3,F}$ = 14.5 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 5.23 (s, 2H, OC<u>H</u>$_2$Ph), 5.09 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.08 (dd, 1H, $J_{1,F}$ = 3.0 Hz, $J_{1,2}$ = 7.6 Hz, H-1), 4.56 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.4 Hz, H-2), 4.30 (dd, 1H, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.15 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.4 Hz, H-6b), 3.85 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.52 (s, 2H, $COCH_2CO$), 2.10, 2.08, 2.07 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.29, 170.76, 170.57, 170.30 (COO), 163.37 (NHCO), 154.17 (C_{Ar}O), 135.53 (C_{Ar}NH), 134.04 ($C_{Ar}CH_2$), 129.55, 129.26, 122.37, 118.84 (CH_{Ar}), 100.07 (C-1, $J_{1,F}$ = 23.5 Hz), 89.78 (C-2, $J_{2,F}$ = 191.0 Hz), 73.49 (C-3, $J_{3,F}$ = 20.0 Hz), 72.86 (C-5), 68.90 (C-4, $J_{4,F}$ = 7.5 Hz), 68.42 (<u>C</u>H_2Ph), 62.63 (C-6), 42.15 (CO<u>C</u>H_2CO), 21.44, 21.33 (CH_3); ^{19}F NMR ($CDCl_3$) δ −199.50.

*3-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenylamino]-3-oxopropanoic acid (**47**).* Hydrolysis of benzyl ester **46** (370 mg, 0.643 mmol) was carried out by hydrogenation during 2 h according to the standard procedure in THF/MeOH (3/3 mL) to yield the acid **47** (339 mg, quant. yield) as a oil which was used in the next step without further purification: IR (KBr) ν 1763, 1727, 1670, 1558, 1366, 1233, 1080, 1068, 1040 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 9.51 (br s, 1H, NH), 7.62 (d, 2H, J = 9.0 Hz, H_{Ar}), 7.09 (d, 2H, J = 9.0 Hz, H_{Ar}), 5.53 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.5 Hz, H-3), 5.52 (dd, 1H, $J_{1,F}$ = 2.7 Hz, $J_{1,2}$ = 7.6 Hz, H-1), 5.07 (t, 1H, $J_{3,4}$ = $J_{4,5}$= 9.5 Hz, H-4), 4.59 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 51.1 Hz, H-2), 4.33-4.13 (m, 3H, 2H-6, H-5), 3.47 (s, 2H, $COCH_2CO$), 2.06, 2.02, 2.01 (each s, 3×3H, OAc); ^{13}C NMR (50 MHz, acetone-d_6) δ 170.63, 170.26, 170.10, 169.18 (COO), 165.69 (NHCO), 154.01 (C_{Ar}O), 135.02 (C_{Ar}NH), 121.68, 118.01 (CH_{Ar}), 99.06 (C-1,

$J_{1,F}$ = 23.0 Hz), 90.40 (C-2, $J_{2,F}$ = 188.5 Hz), 73.37 (C-3, $J_{3,F}$ = 19.5 Hz), 72.58 (C-5), 69.13 (C-4, $J_{4,F}$ = 7.5 Hz), 62.64 (C-6), 20.60 (CH_3); ^{19}F NMR (acetone-d_6) δ −200.35.

*N-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenyl]-N'-[3-{4-[bis(2-chloroethyl)amino]phenyl}propyl]propanediamide (**48**).* Coupling of acid **47** (150 mg, 0.309 mmol) with amine **14** (102 mg, 0.371 mmol) was carried out according to the DCC/HOBt general procedure in DMF (5 mL) with a reaction time of 39 h, to give after silica gel purification (pentane/ethyl acetate, 3/7, v/v), compound **48** (129 mg, 56%) as an oil: IR (KBr) ν 3307, 1755, 1675, 1649, 1519, 1509, 1367, 1225, 1068, 1043 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.50 (s, 1H, PhNHCO), 7.50 (d, 2H, J = 9.0 Hz, H_{Ar}), 7.05 (d, 2H, J = 8.5 Hz, H_{Ar}), 7.02 (d, 2H, J = 9.0 Hz, H_{Ar}), 6.69 (br t ,1H, N<u>H</u>CH_2), 6.61 (d, 2H, J = 8.5 Hz, H_{Ar}), 5.41 (td, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.5 Hz, H-3), 5.09 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 5.06 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{1,F}$ = 3.1 Hz, H-1), 4.56 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 50.2 Hz, H-2), 4.29 (dd, 1H, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.14 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.85 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.74-3.56 (m, 8H, N(CH_2CH_2Cl)$_2$), 3.31 (q, 2H, J = 7.2 Hz, NHC<u>H</u>$_2$), 3.30 (s, 2H, $COCH_2CO$), 2.57 (t, 2H, J = 7.5 Hz, C<u>H</u>$_2$Ph), 2.11, 2.08, 2.05 (each s, 3×3H, OAc), 1.83 (qt, 2H, J = 7.3 Hz, $CH_2CH_2CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.28, 170.77, 170.28 (<u>C</u>OCH_3), 168.44, 165.70 (NHCO), 154.08 ($C_{Ar}O$), 145.22 ($C_{Ar}N$), 134.24 ($C_{Ar}NH$), 130.95 ($C_{Ar}CH_2$), 130.31, 122.29, 118.75, 113.07 (CH_{Ar}), 100.00 (C-1, $J_{1,F}$ = 23.5 Hz), 89.78 (C-2, $J_{2,F}$ = 191.0 Hz), 73.47 (C-3, $J_{3,F}$ = 20.0 Hz), 72.81 (C-5), 68.86 (C-4, $J_{4,F}$ = 7.5 Hz), 62.59 (C-6), 54.34 (NCH_2), 44.41 (CO<u>C</u>H_2CO), 41.26 (CH_2Cl), 40.22 (CH_2NH), 32.77 (<u>C</u>H_2Ph), 31.70 (CH_2<u>C</u>H_2CH_2), 21.44, 21.32 (CH_3); ^{19}F NMR ($CDCl_3$) δ −199.49; MS (ESI) m/z 742.27 $[M+H]^+$ (Exact Mass: 742.23); Anal. ($C_{34}H_{42}Cl_2FN_3O_{10}$) C, H, N.

*N-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenyl]-4-nitrobenzamide (**49**).* To a ice cold solution of the amine **42** (1.99 g, 4.98 mmol) and triethylamine (2 mL, 14.4 mmol) in anhydrous THF (90 mL) stirred over 4 Å molecular sieves, was added dropwise a solution of 4-nitrobenzoyl chloride (1.4 g, 7.54 mmol) in anhydrous THF (15 mL). The mixture was stirred at 0 °C for 30 min and at room temperature for 20 h. After evaporation under reduced pressure, the residue was diluted with ethyl acetate (200 mL) and washed successively with 1N aqueous HCl solution (50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and water (2 × 50 mL). Then the organic layer was dried over $MgSO_4$,

filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (cyclohexane/ethyl acetate, v/v, 5/5) to yield compound **49** (1.09 g, 40%) as a white solid: mp 231 °C; IR (KBr) ν 3355, 1759, 1744, 1672, 1515, 1349, 1220, 1288, 1053, 1024 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.56 (s, 1H, NH), 8.37 (d, 2H, J = 8.9 Hz, H_{Ar}), 8.18 (d, 2H, J = 8.9 Hz, H_{Ar}), 7.74 (d, 2H, J = 9.1 Hz, H_{Ar}), 7.08 (d, 2H, J = 9.1 Hz, H_{Ar}), 5.67 (dd, 1H, $J_{1,F}$ = 2.7 Hz, $J_{1,2}$ = 7.6 Hz, H-1), 5.57 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.5 Hz, H-3), 4.99 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 4.70 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 51.4 Hz, H-2), 4.30-4.05 (m, 3H, 2H-6, H-5), 2.07, 2.02 (each s, 9H, OAc); ^{13}C NMR (50 MHz, DMSO-d_6) δ 169.91, 169.62, 169.33 ($\underline{C}OCH_3$), 163.61 (NH$\underline{C}$O), 152.60 ($C_{Ar}O$), 149.13 ($C_{Ar}NO_2$), 140.55 ($\underline{C}_{Ar}CO$), 133.84 ($C_{Ar}NH$), 129.15, 123.54, 121.96, 116.52 (CH_{Ar}), 96.86 (C-1, $J_{1,F}$ = 22.2 Hz), 89.03 (C-2, $J_{2,F}$ = 187.6 Hz), 72.05 (C-3 , $J_{3,F}$ = 18.9 Hz), 70.77 (C-5), 67.84 (C-4, $J_{4,F}$ = 8.1 Hz), 61.51 (C-6), 20.36, 20.41, 20.47 (CH_3); ^{19}F NMR (DMSO-d_6) δ −201.27.

*4-amino-N-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenyl] benzamide (**50**).* Reduction of the nitro compound **49** (502 mg, 0.915 mmol) according to the standard procedure in THF/MeOH (37/37 mL) during 24 h, provide the amine **50** (416 mg, 88%), as a white solid which was used in the next step without further purification: IR (KBr) ν 3350, 1511 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.74 (s, 1H, NH), 7.70 (d, 2H, J = 8.5 Hz, H_{Ar}), 7.69 (d, 2H, J = 9.1 Hz, H_{Ar}), 7.01 (d, 2H, J = 9.1 Hz, H_{Ar}), 6.59 (d, 2H, J = 8.5 Hz, H_{Ar}), 5.75 (s, 2H, NH_2), 5.62 (dd, 1H, $J_{1,F}$ = 2.4 Hz, $J_{1,2}$ = 7.5 Hz, H-1), 5.56 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, $J_{3,F}$ = 14.5 Hz, H-3), 4.98 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 4.68 (ddd, 1H, $J_{1,2}$, $J_{2,3}$, $J_{2,F}$ = 51.4 Hz, H-2), 4.29-4.04 (m, 3H, 2H-6, H-5), 2.06, 2.01 (each s, 9H, OAc); ^{13}C NMR (50 MHz, DMSO-d_6) δ 169.92, 169.62, 169.34 ($\underline{C}OCH_3$), 165.08 (NH$\underline{C}$O), 152.06, 151.77 ($C_{Ar}O$, $C_{Ar}NH_2$), 135.01 ($C_{Ar}NH$), 129.25, 121.47 (CH_{Ar}), 121.05 ($\underline{C}_{Ar}CO$), 116.40, 112.54 (CH_{Ar}), 97.03 (C-1, $J_{1,F}$ = 22.7 Hz), 89.04 (C-2, $J_{2,F}$ = 187.3 Hz), 72.08 (C-3 , $J_{3,F}$ = 18.7 Hz), 70.72 (C-5), 67.86 (C-4, $J_{4,F}$ = 7.4 Hz), 61.53 (C-6), 20.42, 20.37 (CH_3); ^{19}F NMR (DMSO-d_6) δ −198.71.

*4-(4-{4-[bis(2-chloroethyl)amino]phenyl}butamido)-N-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenyl]benzamide (**51**).* Coupling of CLB (60 mg, 0.197 mmol) with amine **50** (90 mg, 0.174 mmol) in the presence of TEA (30 µL, 0.216 mmol) and ethylchloroformate (20 µL, 0.209 mmol) in DCM (20 mL) according to the general

ethylchloroformate procedure with a time reaction of 15 h, followed by chromatography on silica gel (cyclohexane/ethyl acetate, 4/6, v/v) provided compound **51** (60 mg, 43%) as a white powder: mp 205 °C; IR (KBr) ν 3397, 1752, 1647, 1512, 1221, 1068, 1045 cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 8.10 (s, 1H, NH), 7.80 (d, 2H, J = 8.6 Hz, H_{Ar}), 7.64-7.55 (m, 5H, NH, H_{Ar}), 7.06 (d, 2H, J = 8.5 Hz, H_{Ar}), 7.05 (d, 2H, J = 8.8 Hz, H_{Ar}), 6.61 (d, 2H, J = 8.6 Hz, H_{Ar}), 5.41 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.6 Hz, H-3), 5.14-5.05 (m, 2H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, $J_{1,2}$ = 7.4 Hz, $J_{1,F}$ = 3.1 Hz, H-1, H-4), 4.56 (td, 1H, $J_{1,2} \approx J_{2,3}$, $J_{2,F}$ = 50.5 Hz, H-2), 4.30 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 5.4 Hz, H-6a), 4.14 (dd, 1H, $J_{6a,6b}$, $J_{5,6b}$ = 2.0 Hz, H-6b), 3.86 (ddd, 1H, $J_{4,5}$, $J_{5,6a}$, $J_{5,6b}$, H-5), 3.74-3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.60 (t, 2H, J = 7.2 Hz, CH_2Ph), 2.37 (t, 2H, J = 7.3 Hz, $COCH_2$), 2.11-1.97 (m, 11H, $CH_2CH_2CH_2$, 3×OAc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.42, 170.45, 169.93, 169.54, 165.17 (CO), 153.79 ($C_{Ar}O$), 145.15 ($C_{Ar}N$), 141.54, 134.28 ($C_{Ar}NH$), 130.99, 130.53 ($C_{Ar}CH_2$, $C_{Ar}CO$), 129.90, 128.35, 122.20, 119.69, 118.66, 113.30 (CH_{Ar}), 99.84 (C-1, $J_{1,F}$ = 23.7 Hz), 89.46 (C-2, $J_{2,F}$ = 190.4 Hz), 73.17 (C-3, $J_{3,F}$ = 20.3 Hz), 72.56 (C-5), 68.81 (C-4, $J_{4,F}$ = 7.2 Hz), 62.31 (C-6), 54.09 (NCH_2), 40.88 (CH_2Cl), 37.12 ($COCH_2$), 34.25 (CH_2Ph), 27.00 ($CH_2CH_2CH_2$), 20.67, 20.64, 20.56 (CH_3); ^{19}F NMR ($CDCl_3$) δ −199.47; MS (ESI) m/z 804.35 $[M+H]^+$ (Exact Mass: 804.24); Anal. ($C_{39}H_{44}Cl_2FN_3O_{10}$) C, H, N.

*Methyl (3-{4-[Bis(2-chloroethyl)amino]phenyl}propyl)carbamoylacetic acid (**54**).* Starting from glycine methyl ester (133 mg, 1.50 mmol) and CLB (500 mg, 1.64 mmol) according to the standard ethylchloroformate conditions with TEA (0.28 mL, 1.97 mmol) and ethylchloroformate (0.14 mL, 1.73 mmol) in DCM (9 mL) and with a reaction time of 24 h, compound **54** (305 mg, 54%) was obtained pure after silica gel chromatography (40 to 60% ethyl acetate in petroleum ether): mp 82 °C ; IR (NaCl) ν 3309, 1751, 1654, 1519, 1208, 1181 cm^{-1}; NMR ^1H (200 MHz, $CDCl_3$) δ 7.07 (d, 2H, J = 8.5 Hz, H_{Ar}), 6.62 (d, 2H, J = 8.5 Hz, H_{Ar}), 6.12 (m, 1H, NH), 4.04 (d, 2H, J = 5.2 Hz, CH_2NH), 3.76 (s, 3H, CH_3), 3.72-3.57 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.57 (t, 2H, J = 7.4 Hz, CH_2Ph), 2.24 (t, 2H, J = 7.4 Hz, $COCH_2$), 1.93 (qt, 2H, $CH_2CH_2CH_2$); NMR **^{13}C** (200 MHz, $CDCl_3$) δ 173.10, 170.73 (CO), 144.30 ($C_{Ar}N$), 130.77 ($C_{Ar}CH_2$), 129.74, 112.33 (CH_{Ar}), 53.66 (NCH_2), 52.37 (CH_3), 41.20 (CH_2NH), 40.56 (CH_2Cl), 35.47 ($COCH_2$), 33.98 (CH_2Ph), 27.22 ($CH_2CH_2CH_2$).

*(3-{4-[Bis(2-chloroethyl)amino]phenyl}propyl)carbamoyl acetic acid (**55**).* Methyl ester **54** (305 mg, 0.813 mmol) in ethanol/water (16/8 mL) was treated with lithium hydroxide (49 mg,

2.03 mmol) for 5 h at ambient temperature. After addition of water/DCM and separation of the layers, the aqueous one was acidified with aqueous HCl 1M solution (2 mL) and extracted with DCM (4 × 15 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the acid **55** (290 mg, 99%) as a white solid which was used in the next step without further purification: IR (NaCl) ν 3600-2500, 1731, 1650, 1519 cm^{-1}; NMR ^1H 200 MHz, $CDCl_3$) δ 9.66 (br.s, 1H, OH), 7.04 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 6.60 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 6.50 (br.t, 1H, *J* = 4.9 Hz, NH), 4.01 (d, 2H, *J* = 4.9 Hz, $C\underline{H}_2NH$), 3.73-3.55 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.54 (t, 2H, *J* = 7.3 Hz, $C\underline{H}_2Ph$), 2.26 (t, 2H, *J* = 7.4 Hz, $COCH_2$), 1.89 (qt, 2H, *J* = 7.4 Hz, $CH_2C\underline{H}_2CH_2$); NMR **^{13}C** (200 MHz, $CDCl_3$) δ 174.63, 172.73 (CO), 144.50 ($C_{Ar}N$), 130.47 ($\underline{C}_{Ar}CH_2$), 129.79, 112.34 (CH_{Ar}), 53.67 (NCH_2), 41.63 (CH_2NH), 40.68 (CH_2Cl), 35.50 ($CO\underline{C}H_2$), 34.00 ($\underline{C}H_2Ph$), 27.28 ($CH_2\underline{C}H_2CH_2$).

*4-{4-[Bis(2-chloroethyl)amino]phenyl}-N-(2-{2-[4-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyloxy)phenylamino]-2-oxoethylamino}-2-oxoethyl)butanamide (**56**).* Coupling of amine **53** (160 mg, 0.351 mmol) with acid **55** (160 mg, 0.443 mmol) was carried out according to the DCC/HOBt general procedure in DMF (10 mL) during 24 h. Purification by silica gel chromatography (ethyl acetate/EtOH, 95/5, v/v) afforded compound **56** (80 mg, 28%) as an oil: IR (KBr) ν 3306, 1752, 1654, 1510, 1367, 1225, 1068, 1045 cm^{-1}; NMR ^1H (200 MHz, $CDCl_3$) δ 8.89 (s, 1H, PhNHCO), 7.50 (d, 3H, *J* = 8.8 Hz, H_{Ar}, $N\underline{H}CH_2$), 7.00-6.87 (m, 5H, H_{Ar}, $N\underline{H}CH_2$), 6.57 (d, 2H, *J* = 8.4 Hz, H_{Ar}), 5.39 (td, 1H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, $J_{3,F}$ = 14.6 Hz, H-3), 5.13-5.00 (m, 2H, H-1, H-4), 4.54 (td, 1H, $J_{2,3}$, $J_{1,2}$ = 8.3 Hz, $J_{2,F}$ = 50.6 Hz, H-2), 4.27 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.17 (m, 5H, H-6b, $2NHC\underline{H}_2$), 3.86-3.76 (m, 1H, H-5), 3.66-3.59 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.10, 2.04 (each s, 9H, OAc), 1.93 (m, 2H, $CH_2C\underline{H}_2CH_2$); NMR ^{13}C (50 MHz, $CDCl_3$) δ 174.68, 170.68, 170.23, 170.15, 169.65, 167.32 (CO), 153.42 ($C_{Ar}O$), 144.56 ($C_{Ar}N$), 133.54 ($C_{Ar}NH$), 130.43 ($C_{Ar}CH_2$), 129.73, 121.71, 117.92, 112.31 (CH_{Ar}), 99.16 (C-1, $J_{1,F}$ = 23.3 Hz), 89.22 (C-2, $J_{2,F}$ = 190.3 Hz), 72.79 (C-3, $J_{3,F}$ = 19.7 Hz), 72.06 (C-5), 68.15 (C-4, $J_{4,F}$ = 7.3 Hz), 61.87 (C-6), 53.66 (NCH_2), 43.95, 43.71 (CH_2NH), 40.70 (CH_2Cl), 35.48, 34.08 ($C\underline{H}_2CH_2\underline{C}H_2$), 27.26 ($CH_2\underline{C}H_2CH_2$), 20.80, 20.68 ($CH_3$); ^{19}F NMR ($CDCl_3$) δ −199.31; MS (ESI) m/z 799.47 $[M+H]^+$ (Exact Mass: 799.25); Anal. ($C_{36}H_{45}Cl_2FN_4O_{11}$) C, H, N.

4.2. Biological Assays.

4.2.1. Cell culture. Normal human fibroblasts were purchased from Promocell (Heidelberg, Germany). This frozen culture was obtained from foreskin waste from a 6-year old Caucasian male and the cells used in this work were from the seventh to twelfth passage of the culture. M4Beu, a human melanoma cell line, was established in the laboratory of Pr. J. F. Doré (INSERM, Unit 218, Lyon, France) from metastatic biopsy specimens and maintained in cell culture for almost 20 years in our lab. Breast cancer adenocarcinoma MCF 7, prostatic adenocarcinoma PC 3, colon adenocarcinoma DLD-1, lung non-small cell carcinoma A 549, ovary adenocarcinoma PA1 human cell lines and L 929 murine cell line were purchased from the European Collection of Cell Cultures (ECACC; Salisbury, United Kingdom).

Stock cell cultures were maintained as monolayers in 75 cm^2 culture flasks in Glutamax Eagle's minimum essential medium with Earle's salts (MEM; Invitrogen, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (Sigma, Saint-Quentin-Fallavier, France), 1 mM sodium pyruvate (Invitrogen), 1X vitamins solution (Invitrogen), 1X non essential amino acids solution (Invitrogen) and 4 µg/ml of gentamicine (Invitrogen). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2.

4.2.2. Cell growth inhibition assay. Exponentially growing cells were plated at a density of 5 $\times 10^3$ cells per well in 96-well microplates (Nunclon™, Nunc, Roskilde, Denmark) in 150 µL of culture medium and were allowed to adhere for 16 h before treatment with the compound tested. A stock solution of each compound was prepared in dimethylsulfoxide (DMSO) and kept at - 20 °C until use. The percentage of DMSO was kept at 0.5% (v/v) whatever the concentration tested and each treatment was tested in triplicate. Fifty µL of a 4X solution in MEM was then added and a 48 h continuous drug exposure protocol was used. The cytotoxic effect of compounds on tumor cells was then tested using the Resazurin reduction test.

Resazurin Reduction Test. The resazurin reduction test (RRT) was carried out according to the protocol described previously[48]. Briefly, plates were rinsed with 200 µL PBS (37° C, Gibco). Then 150 µL of a 25 µg/mL solution of resazurin in MEM without SVF or phenol red was added to each well. The plates were incubated for 1 h at 37 °C in a humidified atmosphere with 5% of CO_2 for fluorescence development by living cells. Fluorescence was then measured on the automated 96-well plate reader Fluoroskan Ascent FL™ (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. The

fluorescence is proportional to the number of living cells in the well and IC_{50} (drug concentration required to decrease final cell population by 50%) was calculated from the curve of concentration-dependent cell number decrease, defined as the fluorescence in experimental wells as a percentage of that in control wells, with blank values subtracted.

Available Supporting Information: ^{1}H NMR and ^{13}C NMR spectra of compounds (6-22, 25, 28-41, 43-51, 54-56), Elemental analysis data of target compounds.

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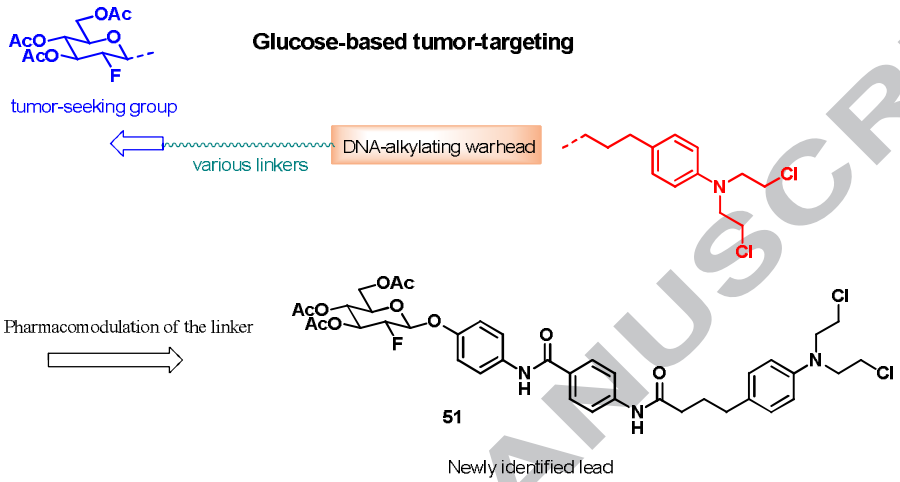
Graphical abstract

Pharmacomodulation of the linker tethered the FDG tumor-seeking group and the alkylating *N*-mustard leads to compound **51**, identified as the most active FDG-CLB glucoside by *in vitro* cytotoxicity assays against different human normal and tumor cell lines.



Highlights

- Chlorambucil was conjugated to FDG for tumor-targeting drug delivery.
- A novel series of sixteen fluoroglucoconjugates of *N*-mustard was synthesized.
- *In vitro* cytotoxicities against a panel of human tumor cell lines were evaluated.
- The study highlights the positive impact of an aromatic amide linker
- Compound **51** was identified as the most potent cytotoxic glucoside