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Synthesis and anti-HIV activity of glucose-containing prodrugs derived from saquinavir, indinavir and nelfinavir

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Abstract

With the aim at improving the transport of the current HIV protease inhibitors across the intestinal and blood brain barriers and their penetration into the central nervous system, the synthesis of various acyl and carbamatoyl glucose-containing prodrugs derived from saquinavir, indinavir and nelfinavir, their in vitro stability with respect to hydrolysis, and their anti-HIV activity have been investigated. D-Glucose, which is actively transported across these barriers, was connected through its 3-hydroxyl to these antiproteases *via* a linker. The liberation of the active free drug during the incubation time of the prodrugs with the cells was found to be crucial for HIV inhibition. The labile ester linking of the glucose-containing moiety to the peptidomimetic hydroxyl of saquinavir or to the indinavir C-8 hydroxyl, which is not part of the transition state isostere, is not an obstacle for anti-HIV activity. This is not the case for its stable carbamate linking to the peptidomimetic hydroxyl of saquinavir, indinavir and nelfinavir. The chemical stability with respect to hydrolysis of some of the saquinavir and indinavir prodrugs reported here, the liberation rate of the active free drug and the HIV inhibitory potency are acceptable for an in vivo use of these prodrugs. These glucose-linked ester and carbamate prodrugs display a promising therapeutic potential provided that their bioavailability, penetration into the HIV sanctuaries, and/or the liberation of the active free drug from the carbamate prodrugs are improved. Furthermore, no cytotoxicity was detected for the prodrugs for concentrations as high as 10 or even 100 μM, thus indicating an encouraging therapeutic index.

Keywords: HIV; Protease inhibitors; D-Glucose prodrugs; Indinavir; Saquinavir; Nelfinavir

1. Introduction

Polytherapies which rely on the use of HIV reverse transcriptase and protease inhibitor combinations are the most efficient chemotherapies against AIDS known at present. Recent clinical trials have clearly established their efficacy in drastically reducing the plasma viral load below its detection threshold and in substantially increasing the level of T4 lymphocytes [for a recent overview, see Ref. 1]. Despite such polytherapies, HIV replication continues indicating the existence of

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reservoirs or sanctuaries for the virus (such as the lymphatic system and central nervous system (CNS)) in which the antiviral agents do not penetrate at an efficient inhibitory level or do not penetrate at all.[2–4] This is particularly the case of the protease inhibitors [for a recent overview, see Ref. 4]. In order to reduce total body viral replication, an attractive alternative is to improve the pharmacological properties, safety and pharmacokinetic profiles, and, consequently, the therapeutic potential of the current antiproteases (APs) used in clinics, i.e. indinavir, saquinavir, ritonavir, nelfinavir, amprenavir, lopinavir. This implies, among others, to increase their oral bioavailability and delivery into the CNS sanctuary by facilitating, for example, their active permeation through the intestinal and blood–brain barriers. Aiming at these goals, different strategies which have been used with some success for several drugs may be applied. Thus, the diffusion through the intestinal and blood–brain barriers of pharmacologically significant amounts of drugs can be facilitated via the nutrient (D-glucose, aminoacids) carrier-mediated transport systems located at these barriers.[5–9]

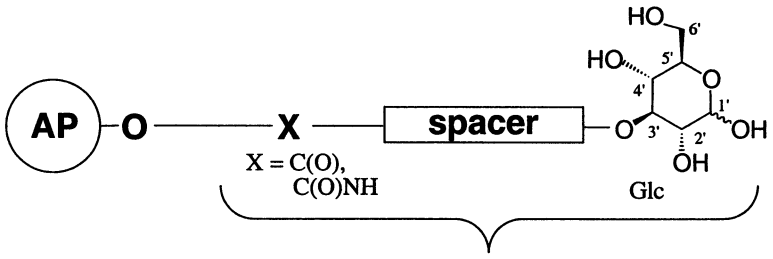
This paper is devoted to the synthesis of various D-glucose-containing prodrugs derived from saquinavir, indinavir and nelfinavir (Scheme 1), to their chemical stability with respect to hydrolysis under physiological conditions and to their in vitro anti-HIV evaluation, which are prerequisites for further in vitro and in vivo investigations. The connection of D-glucose onto the hydroxyl functions of the APs has been performed, via different spacer units, through ester or chemically more stable carbamate bonds which should be hydrolysed by cellular enzymes, thus liberating the active free drug within the cells. This glucose prodrug strategy is mainly aimed at improving the penetration of the APs into the up to now preserved CNS sanctuary. D-Glucose, which is actively transported across the intestinal and blood–brain barriers, was connected through its 3-hydroxyl to the APs via a linker. This connection should preserve its recognition and transport capability by the GLUT-1 transporter system located at these barriers.[5,6,10] This work is in continuation of a recent paper from our laboratory which was dedicated to saquinavir and indinavir prodrugs.[11] These antiproteases were conjugated to fatty acid chains, amino-acids or hydrophilic polyethylene glycol polymers with a view to facilitate their passive or active diffusion across the physiological barriers, or to avoid their binding to plasma proteins and their inactivation and rapid elimination from the blood circulation, respectively.

2. Results and discussion

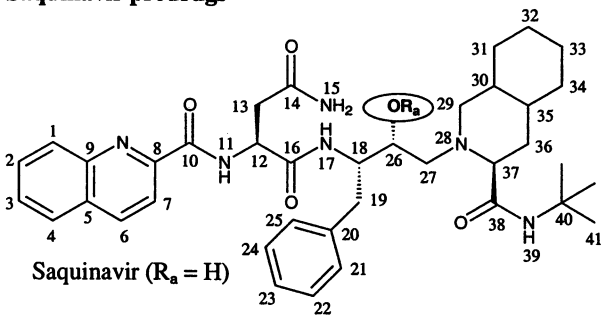
Syntheses.—The syntheses of the prodrugs were performed using a two-step procedure which consisted of the condensation of an acid or isocyanate derivative of the isopropylidene protected sugar and the appropriate antiprotease, followed by TFA-deprotection of the isopropylidene groups, as shown in Scheme 2.

Concerning the ester-linked prodrugs, acylation of the antiproteases was conducted using conventional EDC–DMAP as coupling reagent.[12] Actually, EDC was preferred to DCC due to easier work-up and purification leading to higher yields in the target compounds.

The unique hydroxyl function of saquinavir was acylated in the 72–77% yield range. Deacetalation was performed using aqueous TFA[13] and gave **Saq-C(O)C4Glc(2TFA)** in 80% yield. Although these deprotection conditions were previously used to prepare sugar esters without any particular difficulty,[14] deprotection of the succinate **Saq-C(O)C2C(O)GlcP** and carboxymethyl **Saq-C(O)C1GlcP** prodrugs was most difficult and rapid degradation occurred during work-up and silica gel purification. The deprotection of **Saq-C(O)C2C(O)GlcP** was further poorly reproducible and only possible on very low amounts giving **Saq-C(O)-C2C(O)Glc(2TFA)** in 41% yield. All our attempts using more dilute TFA media, lower temperature or $FeCl_3$–6 H_2O[15] failed. These trials produced either hydrolysis of only the 5,6-isopropylidene group that is well-known as to be the easier group to be cleaved,[16] or several degradation compounds, the major one being **Saq-C(O)C2C(O)OH** resulting from

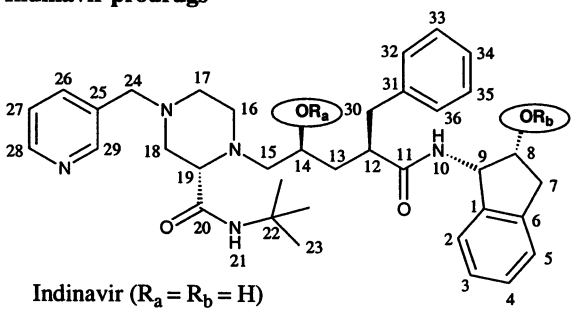


Saquinavir prodrugs



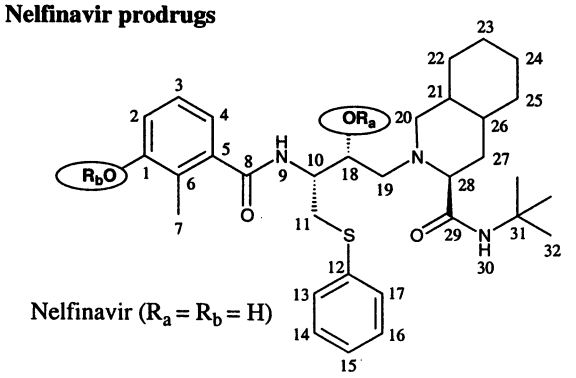
R_a	Code name
$C(O)(CH_2)_2C(O)$-Glc	Saq-C(O)C2C(O)Glc(2TFA)
$C(O)CH_2$-Glc	Saq-C(O)C1Glc(xTFA)
$C(O)(CH_2)_4$-Glc	Saq-C(O)C4Glc(2TFA)
$C(O)NH(CH_2)_4$-Glc	Saq-C(O)NC4Glc(4TFA)

Indinavir prodrugs



R_a	R_b	Code name
H	$C(O)(CH_2)_2C(O)$-Glc	Ind(8)-C(O)C2C(O)Glc(2TFA)
H	$C(O)(CH_2)_4$-Glc	Ind(8)-C(O)C4Glc(1TFA)
$C(O)NH(CH_2)_4$-Glc	H	Ind(14)-C(O)NC4Glc(2TFA)

Nelfinavir prodrugs

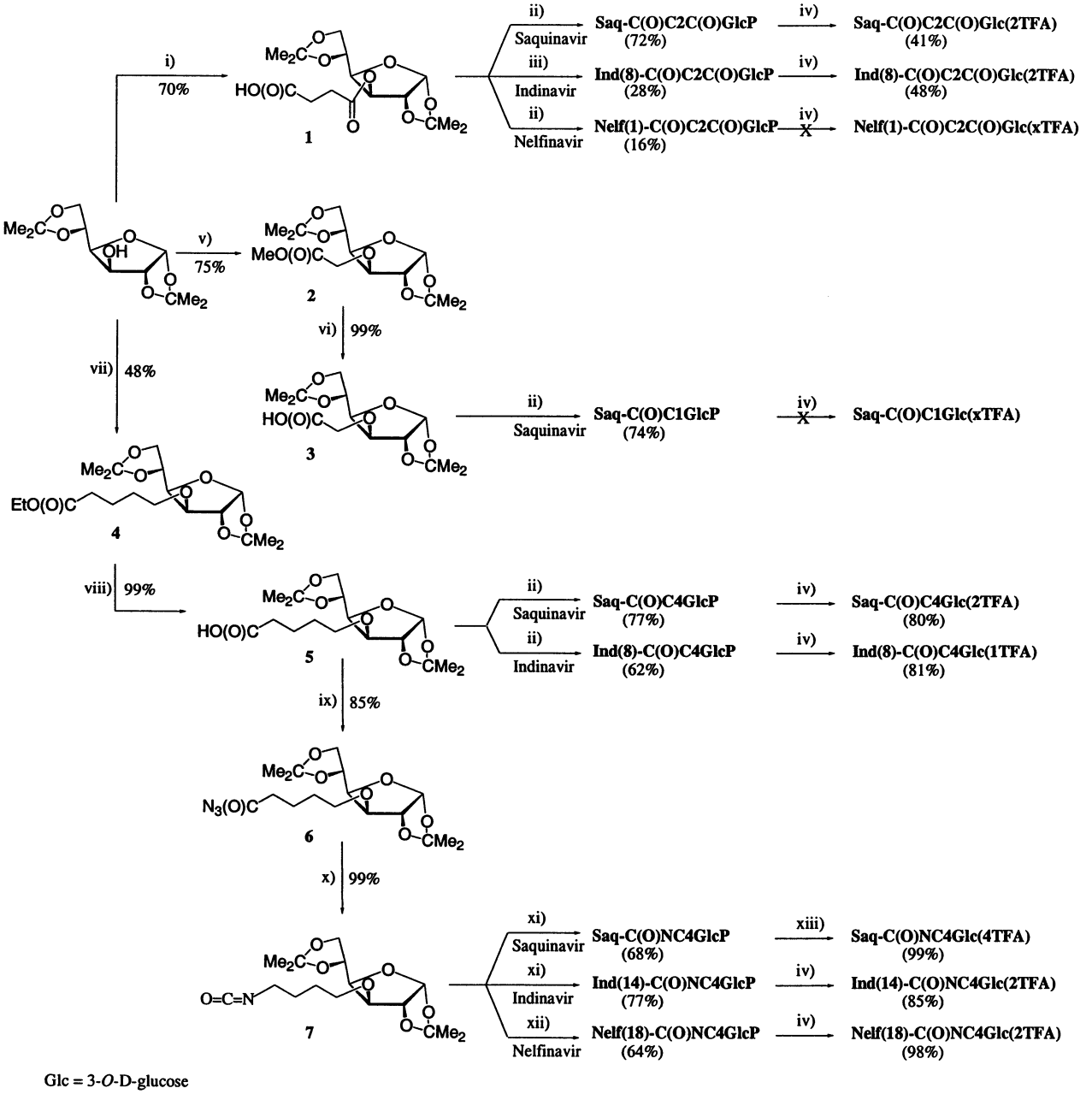


R_a	R_b	Code name
H	$C(O)(CH_2)_2C(O)$-Glc	Nelf(1)-C(O)C2C(O)Glc(xTFA)
$C(O)NH(CH_2)_4$-Glc	H	Nelf(18)-C(O)NC4Glc(2TFA)

Scheme 1. Chemical structures and code names of the glucose-containing antiprotease (AP = saquinavir, indinavir, and nelfinavir) prodrugs described in this study and atom numbering used in the description of their NMR spectra.

the cleavage of the ester bond α to glucose. Concerning the deacetalation of **Saq-C(O)-C1GlcP**, saquinavir was quantitatively regenerated, likely as a result of an internal lactonisation involving one of the deprotected sugar hydroxyl and the ester linkage, by analogy with previously described intramolecular reactions occurring in ester-linked glucose–peptide adducts.[17]

For the acylation of the C-8 'external' hydroxyl of indinavir (Scheme 2), a stochastic reaction was anticipated to be as efficient as a protection/deprotection strategy of its 'internal' C-14 hydroxyl. Furthermore, the C-8 hydroxyl of indinavir was found to be more easily acylated than its C-14 hydroxyl.[11] Thus, reacting indinavir with 1 equiv of acid **1** or **5** resulted mainly in the formation of the mo-



Scheme 2. Synthetic pathway to the glucose-containing saquinavir, indinavir and nelfinavir prodrugs: (i) succinic anhydride–Et_3N–CH_2Cl_2; (ii) EDC–DMAP–CH_2Cl_2; (iii) DCC–DMAP–CH_2Cl_2; (iv) 9:1 TFA–H_2O; (v) NaH–methyl bromoacetate–THF; (vi) NaOH (1 M); (vii) NaH–ethyl 5-bromopentanoate–THF; (viii) NaOH (1 M)–THF; (ix) Et_3N–ethyl chloroformate–NaN_3–acetone; (x) toluene–Δ; (xi) CuCl–CH_2Cl_2; (xii) CuCl–DMF; (xiii) 7:3 TFA–water.

noester **Ind(8)-C(O)C2C(O)GlcP** (28% yield) and **Ind(8)-C(O)C4GlcP** (62% yield), respectively, this latter derivative being accompanied by a low amount of diester **Ind-[C(O)C4-GlcP]2** (14%). Deacetalation of the C-8 prodrugs afforded **Ind(8)-C(O)C2C(O)Glc(3TFA)** and **Ind(8)-C(O)C4Glc(1TFA)** in 48 and 81% yields, respectively. Deprotection of **Ind(8)-C(O)C2C(O)GlcP** was achieved with the same difficulties as for its saquinavir analogue.

The stochastic strategy for the acylation of nelfinavir with acid **1** led mainly to **Nelf(1)-C(O)C2C(O)GlcP** which corresponds to the acylation of the nelfinavir aromatic C-1 hydroxyl function. Neither the monoester corresponding to the acylation of the C-18 nor the diester corresponding to the acylation of both the C-1 and C-18 hydroxyls were detected. However, **Nelf(1)-C(O)C2C(O)GlcP** was obtained with poor yield (16%) owing to partial degradation which occurred during silica gel chromatography purification. Unfortunately, all our attempts to deprotect **Nelf(1)-C(O)C2C(O)GlcP** failed, the expected **Nelf(1)-C(O)C2C(O)Glc** compound could even not be detected in the reaction medium.

Concerning the carbamate-linked **Saq-C(O)NC4GlcP**, **Ind(14)-C(O)NC4GlcP**, and **Nelf(18)-C(O)NC4GlcP** prodrugs, these derivatives were obtained by CuCl-catalysed addition of the corresponding antiprotease to isocyanate **7** in the 64–77% yield range.[18] It is noticeable that no reaction occurred between these antiproteases and isocyanate **7** using more conventional procedures,[19] including the base-catalysed addition with DMAP,[20] Et_3N, or pyridine,[21] or the acid-catalysed addition involving $BF_3{\cdot}Et_2O$,[22] the antiproteases being recovered quantitatively. These glucose-protected carbamates were further converted with TFA into **Saq-C(O)NC4Glc(4TFA)**, **Ind(14)-C(O)NC4Glc(2TFA)** and **Nelf(18)-C(O)NC4-Glc(2TFA)**, respectively, in high yields (85–99%).

It is furthermore noteworthy that only one carbamate derivative was produced with indinavir and nelfinavir but, in contrast to the formation of the ester derivatives, the carbamate bond was formed surprisingly and quasi exclusively on the internal C-14 indinavir and C-18 nelfinavir hydroxyl. One expected indeed a reaction between the isocyanate and the more accessible, hence more reactive C-8 indinavir and C-1 nelfinavir hydroxyl, as observed for their acylation. The surprising issue of the reaction between the isocyanate group and indinavir or nelfinavir, which occurred only in the presence of CuCl, indicates likely a complex formation between Cu(I), and indinavir or nelfinavir and, consequently, activation of the C-14 indinavir and C-18 nelfinavir hydroxyl.

The ^1H and ^{13}C NMR spectra of the protected and deprotected prodrugs are in full agreement with the proposed structures.[23–25] The prodrugs consist further into mutarotated mixtures of D-glucose anomers in approximately equal amounts (3:2 to 1:1) as shown by the anomeric proton integration H-1′α [4.99–5.16 ppm ($J_{1',2'}$ 2.7–3.2 Hz)] and H-1′β [4.37–4.53 ppm ($J_{1',2'}$ 7.2–8.7 Hz)].

That formation of the esters occurred on the C-8 indinavir and C-1 nelfinavir hydroxyl and formation of the carbamates on the C-14 indinavir and C-18 nelfinavir hydroxyl, was unambiguously confirmed by ^{13}C NMR spectroscopy. As expected, the resonances of these carbon atoms are deshielded ($|\Delta\delta| = 2–4.5$ ppm) [resp. shielded for **Nelf(1)-C(O)C2C-(O)GlcP** ($|\Delta\delta| \sim 7$ ppm)] in comparison with those of the free antiprotease (see examples given in Table 1), while the signals of their respective vicinal β-carbon atoms are shielded ($|\Delta\delta| = 1.5–3.2$ ppm) [resp. deshielded for **Nelf(1)-C(O)C2C(O)GlcP** ($|\Delta\delta| \sim 7$ ppm)]. Furthermore, the resonances of the carbon atoms bearing the remaining 'free' hydroxyl group and of their vicinal β-carbon atoms are almost not affected.[24,25]

Concerning the synthesis of the glucose starting materials depicted in Scheme 2, condensation of commercial 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose with succinic anhydride, methyl bromoacetate or ethyl 5-bromopentanoate afforded **1**, **2** or **4** in 70, 75 and 48% yield, respectively.[26,27] Saponification of the two latter esters afforded quantitatively their corresponding acids **3** and **5**. The isocyanate derivative **7** was prepared in two steps from acid **5** (84% overall yield) which consisted first into formation of the acyl azide **6**, then into a Curtius rearrangement of this

Table 1
^{13}C chemical shifts of the indinavir C-14 and C-8, of the nelfinavir C-18 and C-1 carbons bearing a hydroxyl, acyl or carbamate functionality, and of their C-β vicinal carbons

Compound	^{13}C chemical shift (in ppm)					
	C-13(β)	C-14(ORa)	C-15(β)	C-7(β)	C-8(ORb)	C-9(β)
Indinavir (Ra = Rb = H)	39.2	65.8	61.5	38.1	73.0	57.5
Ind(14)-C(O)NC4GlcP (Ra = C(O)NC4GlcP; Rb = H)	**36.4**	**70.3**	**59.9**	39.1	73.3	57.9
Ind(8)-C(O)C4GlcP (Ra = H; Rb = C(O)C4GlcP)	38.2	65.8	61.4	**37.6**	**75.8**	**54.9**
	C-10(β)	C-18(ORa)	C-19(β)	C-6(β)	C-1(ORb)	C-2(β)
Nelfinavir (Ra = Rb = H)	54.2	70.5	59.6	123.4	156.8	116.8
Nelf(18)-C(O)NC4GlcP (Ra = C(O)NC4GlcP; Rb = H)	**51.0**	**72.4**	**56.5**	123.3	156.0	116.3
Nelf(1)-C(O)C2C(O)GlcP (Ra = H; Rb = C(O)C2C(O)GlcP)	54.8	70.4	58.8	**129.4**	**149.8**	**123.9** (125.7)

fairly stable azide.[28] This azide was obtained by reacting NaN_3 with the mixed anhydride prepared from **5** and ethyl chloroformate.[29] Azide **6** was recovered contaminated by isocyanate **7**, as shown by ^1H and ^{13}C NMR [$\sim$2.36 vs. $\sim$3.33 ppm for $CH_2\alpha C(O)N_3$ and $CH_2\alpha N{=}C(O)$, respectively], and was converted quantitatively into isocyanate **7** by heating.

Biological activity and chemical stability.—The saquinavir hydroxyl, the indinavir C-14 hydroxyl—and not the C-8—and the nelfinavir C-18 hydroxyl—and not the C-1—are involved in the peptidomimetic noncleavable transition state isostere responsible for the protease inhibitory potency of saquinavir, indinavir, and nelfinavir, respectively.[11] Therefore, it is most important that these hydroxyls be accessible for antiviral activity.

In the previous study dedicated to the ester prodrugs of saquinavir and indinavir, a close correlation between their anti-HIV activity and the hydrolysis of their acylated 'peptidomimetic' hydroxyl, hence the liberation of the active free drug during the time of incubation, was found: the faster the hydrolysis, the closer the anti-HIV activity level to that of the respective parent drug.[11] Concomitantly, the level of HIV inhibition was very low for the prodrugs for which hydrolysis of this peptidomimetic hydroxyl was very slow. On the other hand, no correlation was found between the hydrolysis rate of the acylated C-8 indinavir prodrugs (which is not part of the transition state isostere) and their anti-HIV activity.[11]

The HIV inhibition levels and cytotoxicities of the new glucose-containing saquinavir, indinavir, and nelfinavir prodrugs were evaluated in vitro in CEM-SS and MT4 cells against HIV-1 according to published procedures.[30–32] The data are collected in Table 2 together with those of saquinavir and nelfinavir[33] (as their methanesulfonate salt), and indinavir (as its sulfate salt).

The sensitivity to hydrolysis of some of the glucose-containing ester and carbamate prodrugs and their stability was also checked using the same hydrolysis protocol as that described in the previous study.[11] The hydrolysis experiments were performed in a pH 7.3 buffer at 37 °C and in the absence of serum, cells and virus using a prodrug concentration in the 146–239 μM range. A prodrug hydrolysis half-life of 180 min was measured for the representative ester **Saq-C(O)C4-Glc(2TFA)**, while no hydrolysis was detected for the three carbamate prodrugs after 7 days of incubation (Table 2). This stability of the carbamate function was in accordance with literature data.[9,34]

In the antiviral assays, and based on the prodrug hydrolysis data measured for the representative saquinavir ester and the saquinavir, indinavir C-14- and nelfinavir C-18 carbamate derivatives, the antiviral activity levels appear to be correlated to the amount of the parent drug released during the 4 day time-span of the antiviral experiments. Indeed, the glucose-containing ester saquinavir prodrugs display high HIV inhibitory levels (IC_{50} from ~ 10 to 120 nM on CEM-SS cells, from 40 to 560 nM on MT4 cells). For these ester prodrugs, e.g., **Saq-C(O)C1-GlcP**, **Saq-C(O)-C2C(O)-GlcP**, **Saq-C(O)C2C(O)-Glc(2TFA)**, and **Saq-C(O)C4-Glc(2TFA)**, an anti-HIV activity (as shown by the *R* values from 1 to 8 listed in Table 1) close to that of free saquinavir is detected, likely indicating a relatively fast hydrolysis rate for these saquinavir derivatives. This is confirmed by the short half-life (180 min) of **Saq-C(O)C4-Glc(2TFA)** for which the highest anti-HIV activity was measured.

By contrast, the anti-HIV efficiency of saquinavir, indinavir, and nelfinavir is considerably reduced when glucose is conjugated to the peptidomimetic hydroxyl of these antiproteases through a carbamate functionality: the chemically stable carbamate **Saq-C(O)-NC4-Glc(4TFA)**, **Ind(14)-C(O)NC4-Glc-(2TFA)** and **Nelf(18)-C(O)NC4-Glc(2TFA)** prodrugs with IC_{50} from 3600 nM to >10 μM are indeed far less active than their respective parent drug (*R* values from 400 to $\geq 10^4$).

As far as the two C-8 indinavir ester prodrugs, i.e., **Ind(8)-C(O)C2C(O)-GlcP** and **Ind-(8)-C(O)C4-Glc(1TFA)** are concerned, one expects that the masking of the C-8 hydroxyl, which is not part of the transition state isostere, and its liberation rate should not modify drastically the antiviral activity of indinavir. This is indeed the case as evidenced by their *R*-values ranging from 5 to 25. These results suggest also that these C-8 indinavir prodrugs themselves may possess an antiviral activity, which is lower than that of indinavir, and/or that their hydrolysis rate may however also contribute to increasing their intrinsic antiviral potency.

In conclusion, it appears that the labile ester masking of the peptidomimetic hydroxyl of the antiproteases is not an obstacle for anti-HIV activity. This is however not the case for its stable carbamate masking. The chemical stability with respect to hydrolysis of some of the saquinavir and indinavir prodrugs re-

Table 2
Anti-HIV activity (IC_{50}) and cytotoxicity (CC_{50}) data for saquinavir, indinavir and nelfinavir prodrugs* in CEM-SS and MT-4 cell cultures infected with HIV-1 LAI and HTLV IIIB, respectively, together with their hydrolysis half life ($t_{1/2}$) [a]

Compound	IC_{50} (nM) CEM-SS	*R* [d]	IC_{50} (nM) MT-4	*R* [d]	CC_{50} (M) CEM-SS	CC_{50} (M) MT-4	$t_{1/2}$ hydrolysis
Saquinavir ($MeSO_3H$)	9		18		$>10^{-5}$	$>10^{-5}$	
Saq-C(O)C2C(O)GlcP	18	~ 2	150	~ 8	$>10^{-5}$	8×10^{-6}	[b]
Saq-C(O)C2C(O)Glc(2TFA)	32	~ 3.5	109	~ 6	$>10^{-5}$	$>10^{-5}$	[b]
Saq-C(O)C1GlcP	19	~ 2	41	~ 2	$>10^{-5}$	$>10^{-5}$	[b]
Saq-C(O)C4Glc(2TFA)	<11	<1.2	62	~ 3.5	$>10^{-5}$	$>10^{-5}$	180 min
Saq-C(O)NC4Glc(4TFA)	3600	~ 400	7100	~ 395	$>10^{-5}$	$>10^{-5}$	[c]
Indinavir (H_2SO_4)	≥ 10		22		$>10^{-4}$	$>10^{-4}$	
Ind(8)-C(O)C2C(O)GlcP	120	≥ 12	560	~ 26	$>10^{-5}$	$>10^{-5}$	[b]
Ind(8)-C(O)C4Glc(1TFA)	48	≥ 4.8	190	~ 9	$>10^{-5}$	$>10^{-5}$	[b]
Ind(14)-C(O)NC4Glc(2TFA)	52,000	≥ 5200	42000	~ 1910	$>10^{-4}$	$>10^{-4}$	[c]
Nelfinavir ($MeSO_3H$)	2 [e]						
Nelf(18)-C(O)NC4Glc(2TFA)	8200	4100	$>10^4$		$>10^{-5}$	$>10^{-5}$	[c]

[a] $t_{1/2}$, which corresponds to the time at which 50% of hydrolysis is observed, has been determined from hydrolysis experiments performed by incubating the prodrugs in a pH 7.3 DMEM solution at 37 °C.
[b] Not tested.
[c] No hydrolysis detected after 7 days of incubation.
[d] R is the ratio of the prodrug IC_{50} to that of its parent compound.
[e] Data from Ref. 33.

ported here, the liberation rate of the active free drug and the HIV inhibitory potency are acceptable for an in vivo use of these prodrugs. These glucose-linked ester and carbamate prodrugs display a promising therapeutic potential provided that their bioavailability, their penetration into the HIV sanctuaries, and/or the liberation of the active free drug from the carbamate prodrugs are improved. Furthermore, no cytotoxicity was detected for the prodrugs for concentrations as high as 10 or even 100 μM (Table 2), thus indicating a promising therapeutic index for these new derivatives. Studies concerning their pharmacological properties (binding to plasma proteins, permeation across intestinal and blood–brain barrier models), and their safety and pharmacokinetic profiles are now in progress.

3. Experimental

Syntheses

General.—Unless otherwise indicated, the reactions were performed under anhyd nitrogen using dry solvents and reagents. Anhydrous solvents were prepared by standard methods.

Acetic acid sodium salt trihydrate (ACS), copper(I) chloride, dicyclohexylcarbodiimide (DCC), 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP), 1-pentane sulfonic acid sodium salt (PSA), anhyd potassium carbonate, potassium hydrogen sulfate, sodium azide, sodium chloride, sodium hydride, sodium hydrogencarbonate, sodium hydroxide, succinic anhydride, triethylamine, were purchased from Aldrich, methyl bromoacetate from Acros, and ethyl chloroformate, ethyl 5-bromopentanoate, and trifluoroacetic acid (TFA) from Fluka. All these materials, except triethylamine, were used without further purification. Saquinavir, indinavir and nelfinavir (as their methanesulfonate salt or sulfate salt) were a gift from Hoffmann–La Roche, E. Merck, and Agouron, respectively, and were deprotonated prior to their use in the synthetic processes ($CHCl_3$ or EtOAc extraction of the free base from a $NaHCO_3$ or Na_2CO_3 10% solution of the antiprotease).

If not specified, column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70–230 mesh). The purity of all new compounds was checked by TLC, NMR, MS and HPLC. TLC analyses were performed on precoated Silica Gel F254 plates (E. Merck) with detection by UV and by charring with 50% MeOH–H_2SO_4 solution, ninhydrin or $KMnO_4$. HPLC analyses (flow of 1 mL/min) were performed using a HP1100 apparatus using a Lichrospher 100 RP-18 (5 μm)-packed column (250 × 4 mm) (column I) or a Nucleosil 120-3C8 column (100 × 4.6 mm) (column II) or a Lichrospher 100 RP-18 (5 μm) column (250 × 3.2 mm) (column III) and water–CH_3CN (v/v) 0.1% TFA gradient as eluent (solvent A: from 4:1 to 0:1 over 30 min; solvent B: from 7:3 to 0:1 over 30 min; UV detection) or isocratic water (15 mM ACS and 15 mM PSA)–CH_3CN (v/v) pH 6.0 buffer (solvent C: v/v = 59:41; solvent: D v/v = 41:59; solvent E: v/v = 45:55; UV detection). With column I and solvent A, retention times (R_t) of indinavir and saquinavir are 10.7 and 16.6 min, respectively, while with column I and solvent E, R_t of nelfinavir is 12.9 min. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded with a Brucker AC 200 spectrometer at 200, 50.3, and 188.3 MHz, respectively. Chemical shifts (δ) are given in ppm relative to the signal (i) for internal reference Me_4Si or indirectly to $CHCl_3$ (δ 7.27) for ^1H; (ii) for internal reference Me_4Si or indirectly to $CDCl_3$ (δ 76.9) for ^{13}C; (iii) to internal reference $CFCl_3$ for ^{19}F. Concerning the description of the prodrug NMR spectra, the atoms of the antiprotease part are depicted as C-*x* and H-*y* whereas those of the sugar part are depicted as C-*x*′ and H-*y*′ according to their standard nomenclature numbering (see Scheme 1 for numbering). COSY $^1H/^1H$, $^1H/^{13}C$ NMR correlation, ^{13}C DEPT, and/or mass spectrometry data fully confirm the signal assignments and structure of the isolated materials. The glucose-deprotected target compounds consisted of TFA salts and the TFA anion quantification was assessed by ^{19}F NMR using 3,3,3-trifluoroethanol as internal standard. Electron-spray ionisation–mass spectra (ESI–MS) were recorded on a Finnigan MAT TSQ 7000 apparatus equipped with an atmospheric pres-

sure ionisation source. This method used in positive mode gives either M^+, $[M+H]^+$, $[M+Na]^+$, $[M-H+Na]^+$ and/or $[M-H+K]^+$ signals. IR spectra were recorded on a Brucker FT-IFS 45 spectrometer as Nujol films.

*Synthesis of the 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose derivatives*

*1,2;5,6-Di-*O*-isopropylidene-3-*O*-(3-carboxypropanoyl)-α-*D*-glucofuranose* (**1**).—1,2;5,6-Di-*O*-isopropylidene-α-D-glucofuranose (5.0 g, 19.2 mmol) and succinic anhydride (5.8 g, 57.6 mmol) were stirred with Et_3N (6.7 mL, 48.0 mmol) in CH_2Cl_2 (80 mL) at rt for 30 h. The reaction mixture was washed with 5% $KHSO_4$, then water until neutrality. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The brown oil obtained was chromatographed on silica gel (EtOAc) and the solid obtained was centrifuged in CH_2Cl_2. After evaporation of the solvent, **1** (4.8 g, 70%) was obtained as a white powder; R_f 0.85 (9:1 EtOAc–EtOH); ^{1}H NMR ($CDCl_3$): δ 10.03 [bs, 1 H, C(O)OH], 5.85 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 5.24 (d, $J_{3,4}$ 1.9 Hz, 1 H, H-3), 4.48 (d, 1 H, H-2), 4.22–4.15 (m, 2 H, H-4, H-5), 4.11–3.97 (m, 2 H, H-6a,6b), 2.66 (m, 4 H, 2 CH_2), 1.50, 1.39, 1.30, 1.28 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 177.6 [C(O)OH], 170.9 [O*C*(O)CH_2], 112.4, 109.5 (CH_3*C*), 105.1 (C-1), 83.3 (C-2), 79.8 (C-4), 76.6 (C-3), 72.5 (C-5), 67.3 (C-6), 28.9, 28.8 (CH_2), 26.9, 26.8, 26.2, 25.3 (*C*H_3C).

*1,2;5,6-Di-*O*-isopropylidene-3-*O*-(methoxycarbonylmethyl)-α-*D*-glucofuranose* (**2**).—Sodium hydride (240 mg, 10 mmol) was added portionwise to a solution of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (1.0 g, 3.8 mmol) in THF (10 mL) at 0 °C. After the initial reaction had subsided, the stirred mixture was refluxed for 1.5 h, then cooled down to 0 °C and methyl bromoacetate (1.8 mL, 19 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h, then at rt for 24 h. After hydrolysis of excess NaH, the mixture was evaporated under diminished pressure. The residual oil was extracted with CH_2Cl_2 (3 × 30 mL) that was washed with cold water, dried over Na_2SO_4, and evaporated under diminished pressure. The residue purified by chromatography on silica gel (1:0–0:1 hexane–EtOAc) afforded the white solid **2** (0.96 g, 75%); R_f 0.62 (3:2 hexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 5.89 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.71 (d, 1 H, H-2), 4.37–4.29 (m, 1 H, H-5), 4.26 (s, 2 H, CH_2), 4.14–4.06 (m, 2 H, H-4, H-6a), 4.02–3.98 (m, 1 H, H-6b), 3.94 (d, $J_{3,4}$ 2.8 Hz, 1 H, H-3), 3.75 (s, 3 H, OCH_3), 1.48, 1.41, 1.34, 1.30 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 170.7 [*C*(O)OCH_3], 112.0, 109.1 (CH_3*C*), 105.3 (C-1), 83.8 (C-3), 83.4 (C-2), 81.2 (C-4), 72.7 (C-5), 68.4 (CH_2), 67.4 (C-6), 52.0 (CH_3), 26.9, 26.3, 25.4 (*C*H_3C).

*1,2;5,6-Di-*O*-isopropylidene-3-*O*-(carboxymethyl)-α-*D*-glucofuranose* (**3**).—Compound **2** (960 mg, 2.9 mmol) in 1 M NaOH aq solution (10 mL) was refluxed for 1.5 h, then stirred at rt for 12 h. After addition of $CHCl_3$, the reaction mixture was made acid up to pH 2–3 with 1 M HCl. The organic layer was then washed until neutrality, dried over Na_2SO_4, filtered and evaporated. Colourless oil **3** was obtained quantitatively; R_f 0.56 (9:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.89 [bs, 1 H, C(O)OH], 5.92 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.57 (d, 1 H, H-2), 4.33 (m, 1 H, H-5), 4.31 and 4.14 [AB system, J_{AB} 17.3 Hz, 2 H, CH_2C(O)], 4.21–4.12 (m, 2 H, H-4, H-6a), 4.02 (m, 1 H, H-6b), 3.94 (d, $J_{3,4}$ 3.5 Hz, 1 H, H-3), 1.47, 1.44, 1.36, 1.30 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 171.7 [C(O)OH], 112.4, 110.0 (CH_3*C*), 105.8 (C-1), 84.1 (C-3), 82.5 (C-2), 81.0 (C-4), 73.2 (C-5), 67.9 [CH_2], 67.4 (C-6), 26.9, 26.7, 26.3, 25.0 (*C*H_3C).

*1,2:5,6-Di-*O*-isopropylidene-3-*O*-(4-ethoxycarbonylbutyl)-α-*D*-glucofuranose* (**4**).—The same process described for the preparation of **2** was applied to 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (3.0 g, 11.5 mmol), NaH (553 mg, 23 mmol) and ethyl 5-bromopentanoate (9.23 mL, 57.6 mmol). After treatment, the residue was chromatographed on silica gel (3:0–3:1 hexane–EtOAc) to afford **4** (2.15 g, 48%) as a colourless oil; R_f 0.7 (3:2 hexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 5.85 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.50 (d, 1 H, H-2), 4.30–4.15 (m, 1 H, H-5), 4.10–3.90 (m, 5 H, H-4, H-6a,6b, $CH_3C$$H_2$), 3.85 (d, $J_{3,4}$ 3.0 Hz, 1 H, H-3), 3.65–3.40 [m, 2 H, OCH_2], 2.30 [t, *J* 7.0 Hz, 2 H, CH_2C(O)], 1.75–1.55 [m, 4 H, $OCH_2CH_2CH_2$], 1.50, 1.40, 1.35, 1.30 (4 s, 4 × 3 H, CH_3C), 1.25 (t, *J* 7.1 Hz, 3 H,

CH_3CH_2); ^{13}C NMR ($CDCl_3$): δ 173.6 [C(O)], 111.9, 109.1 (CH_3C), 105.5 (C-1), 82.7 (C-3), 82.4 (C-2), 81.4 (C-4), 72.6 (C-5), 70.3 [OCH_2CH_2], 67.5 (C-6), 60.4 (CH_3CH_2), 34.1 [$CH_2C(O)$], 27.0, 26.9, 26.4, 25.5 (CH_3C), 29.3 [OCH_2CH_2], 21.6 [$CH_2CH_2C(O)$], 14.4 (CH_3CH_2).

1,2:5,6-Di-O-*isopropylidene-3*-O-*(4-carboxybutyl)*-α-D-*glucofuranose* (**5**).—The same process described for the preparation of **3** was applied to **4** (1.6 g, 4.1 mmol) in THF (12 mL) and 1 M NaOH solution (10 mL). After treatment, **5** (1.9 g, 99%) was obtained as a colourless oil; R_f 0.6 (3:2 CH_2Cl_2–EtOAc); ^1H NMR ($CDCl_3$): δ 9.70 [bs, 1 H, C(O)OH], 5.82 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.48 (d, 1 H, H-2), 4.30–4.20 (m, 1 H, H-5), 4.08–4.00 (m, 2 H, H-4, H-6a), 3.93 (dd, $J_{6',6}$ 8.6, $J_{6',5}$ 5.8 Hz, 1 H, H-6b), 3.80 (d, $J_{3,4}$ 3.0 Hz, 1 H, H-3), 3.57 and 3.50 [AB part of an ABX_2 system, J_{AB} 9.3, J_{AX} 5.6, J_{BX} 5.9 Hz, 2 H, OCH_2CH_2], 2.33 [t, J 7.0 Hz, 2 H, $CH_2C(O)$], 1.62 [m, 4 H, $OCH_2CH_2CH_2$], 1.44, 1.37, 1.29, 1.26 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 179.1 [C(O)OH], 111.8, 109.0 (CH_3C), 105.3 (C-1), 82.6 (C-3), 82.3 (C-2), 81.3 (C-4), 72.5 (C-5), 70.0 [OCH_2CH_2], 67.3 (C-6), 33.6 [$CH_2C(O)$], 29.0 [OCH_2CH_2], 26.9, 26.8, 26.3, 25.3 (CH_3C), 21.4 [$CH_2CH_2C(O)$].

1,2:5,6-Di-O-*isopropylidene-3*-O-*(4-azidocarbonylbutyl)*-α-D-*glucofuranose* (**6**).—Derivative **5** (2.4 g, 6.7 mmol) was suspended in water (1.2 mL) and a sufficient amount of acetone was added to complete the solution. The solution was cooled down to 0 °C and successively Et_3N (1.1 mL, 7.7 mmol) in acetone (13 mL) and ethyl chloroformate (0.83 mL, 8.7 mmol) in acetone (4 mL) were added. After a stirring period of 30 min at 0 °C, NaN_3 (653 mg, 10 mmol) in water (2.3 mL) was added dropwise. After another stirring period of 2 h at 0 °C, the reaction mixture was poured into ice water. The oil which separated was extracted with Et_2O (3 × 30 mL). The organic layer was washed with cold water, dried over Na_2SO_4, and evaporated to afford **6** (2.1 g, 85%) as a yellow oil contaminated with ca. 10% of isocyanate derivative **7** (see NMR data below); IR (ν cm^{-1}, Nujol): 1713 (C=O), 2041 (N=N); ^1H NMR ($CDCl_3$): δ 5.85 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.51 (d, 1 H, H-2), 4.30–4.19 (m, 1 H, H-5), 4.15–3.90 (m, 3 H, H-4, H-6a,6b), 3.84 (d, $J_{3,4}$ 2.8 Hz, 1 H, H-3), 3.65–3.40 [m, 2 H, OCH_2CH_2], 2.36 [t, J 6.3 Hz, 2 H, $CH_2C(O)$], 1.75–1.55 [m, 4 H, $OCH_2CH_2CH_2$], 1.48, 1.41, 1.33, 1.30 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 111.9, 109.1 (CH_3C), 105.4 (C-1), 82.6 (C-3), 82.3 (C-2), 81.3 (C-4), 72.5 (C-5), 69.9 [OCH_2CH_2], 67.4 (C-6), 36.5 [$CH_2C(O)$], 29.0 [OCH_2CH_2], 26.95, 26.9, 26.3, 25.4 (CH_3C), 21.5 [$CH_2CH_2C(O)$].

1,2:5,6-Di-O-*isopropylidene-3*-O-*(4-isocyanatobutyl)*-α-D-*glucofuranose* (**7**).—A solution of **6** (2.0 g, 5.2 mmol) in toluene (4 mL) was refluxed until no more nitrogen evolved. Removal of toluene under reduced pressure afforded **7** (1.8 g, 99%) as an orange oil; IR (ν cm^{-1}, Nujol): 2262 (N=C=O); ^1H NMR ($CDCl_3$): δ 5.85 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.51 (d, $J_{2,1}$ 3.7 Hz, 1 H, H-2), 4.30–4.15 (m, 1 H, H-5), 4.10–3.90 (m, 3 H, H-4, H-6a,6b), 3.84 (d, $J_{3,4}$ 3.0 Hz, 1 H, H-3), 3.53 and 3.64 (AB part of an ABX_2 system, J_{AB} 9.4, J_{AX} 5.4, J_{BX} 5.7 Hz, 2 H, OCH_2CH_2), 3.32 (t, J 6.3 Hz, 2 H, CH_2N), 1.75–1.55 (m, 4 H, $CH_2CH_2CH_2N$), 1.48, 1.40, 1.33, 1.30 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 111.9, 109.1 (CH_3C), 105.4 (C-1), 82.6 (C-3), 82.3 (C-2), 81.3 (C-4), 72.5 (C-5), 69.7 (OCH_2), 67.5 (C-6), 42.8 (CH_2N), 28.1 (OCH_2CH_2), 26.8 (CH_2CH_2N), 26.95, 26.9, 26.3, 25.4 (CH_3C).

Synthesis of the ester prodrug derivatives

*Synthesis of succinic acid 1(*R*)-[3(*S*)*-tert-*butylcarbamoyloctahydro - 4a(*S*),8a(*S*) - iso - quinolin - 2 - ylmethyl)] - 2(*S*) - [3 - carbamoyl - 2(*S*) - [(quinoline - 2 - carbonyl) - amino] - propionylamino] - 3 - phenyl - propyl ester 4 - [3 - O - D - glucose] - butyl ester (bis trifluoroacetic acid salt)* [**Saq-C(O)C2C(O)Glc(2TFA)**]

*Succinic acid 1(*R*)-[3(*S*)*-tert-*butylcarbamoyloctahydro - 4a(*S*),8a(*S*) - isoquinolin - 2 - ylmethyl)] - 2(*S*) - [3 - carbamoyl - 2(*S*) - [(quinoline - 2 - carbonyl) - amino] - propionylamino] - 3 - phenyl - propyl ester 4 - [3 - O - (1,2;5,6 - di - O - isopropylidene - α - D - glucofuranose)] - butyl ester* [**Saq-C(O)C2C(O)GlcP**]:—Compound **1** (200 mg, 0.6 mmol) and DMAP (67 mg, 0.6 mmol) were added to saquinavir (185 mg, 0.3 mmol) in 3 mL CH_2Cl_2. Then, EDC (106 mg, 0.6 mmol) was added to the mixture at 0 °C and the solution was stirred for 15 min at 0 °C and for

19 h at rt. The reaction mixture was diluted with CH_2Cl_2 and was washed. The organic layer dried over Na_2SO_4 was filtered, and evaporated under diminished pressure. Purification by chromatography on silica gel (EtOAc) gave the title compound (203 mg, 72%) as a white solid; R_f 0.35 (EtOAc); R_t 19.6 min (column II, solvent B, 210 nm); ^{1}H NMR ($CDCl_3$): δ 9.11 (d, J 7.5 Hz, 1 H, H-11), 8.20 (d, $J_{7,6}$ 8.5 Hz, 1 H, H-7), 8.11 (d, 1 H, H-6), 8.08 (d, $J_{1,2}$ 8.3 Hz, 1 H, H-1), 7.80 (d, $J_{4,3}$ 8.0 Hz, 1 H, H-4), 7.72 (ddd, $J_{2,3}$ 7.0, $J_{2,1}$ 8.3, $J_{2,4}$ 1.8 Hz, 1 H, H-2), 7.63 (d, J 8.9 Hz, 1 H, H-17), 7.57 (ddd, $J_{3,4}$ 8.0, $J_{3,1}$ 1.8 Hz, 1 H, H-3), 7.11 (d, J 7.0 Hz, 2 H, H-21, H-25), 7.02 (t, J 7.0 Hz, 2 H, H-22, H-24), 6.90 (t, J 7.0 Hz, 1 H, H-23), 6.57 (bs, 1 H, H-15), 6.37 (bs, 1 H, H-15), 5.90 (bs, 1 H, H-39), 5.84 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.35 (m, 1 H, H-26), 5.23 (d, $J_{3',4'}$ 2.4 Hz, 1 H, H-3′), 4.80 (m, 1 H, H-12), 4.48 (d, 1 H, H-2′), 4.38 (m, 1 H, H-18), 4.23–4.19 (m, 2 H, H-4′, H-5′), 4.14–3.95 (m, 2 H, H-6′a,6′b), 3.00–2.20 [m, 17 H, H-13, H-19, H-27, H-29, H-30, H-35–37, C(O)CH_2], 1.90–1.60 (m, 8 H, H-31–34), 1.31 (s, 9 H, H-41), 1.47, 1.37, 1.29, 1.25 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 173.7, 173.5 (C-14, C-16), 171.9, 171.3 [*C*(O)CH_2], 170.3 (C-38), 164.6 (C-10), 149.1 (C-8), 146.6 (C-9), 137.4 (C-6), 137.2 (C-20), 130.2, 130.1 (C-1, C-2), 129.4 (C-5), 129.1 (C-21, C-25), 128.5 (C-22, C-24), 128.1 (C-4), 127.7 (C-3), 126.5 (C-23), 118.7 (C-7), 112.3, 109.4 (CH_3*C*), 105.1 (C-1′), 83.3 (C-2′), 79.7 (C-4′), 76.5 (C-3′), 73.9 (C-26), 72.5 (C-5′), 70.4 (C-37), 67.2 (C-6′), 59.4 (C-29), 56.5 (C-27), 51.8 (C-12), 51.0 (C-40), 49.7 (C-18), 37.5 (C-13), 35.7 (C-30), 35.1 (C-19), 33.2 (C-35), 30.8 (C-36), 29.3, 29.1 [C(O)*C*H_2], 28.8 (C-41), 26.9, 26.8, 26.3, 25.4 (*C*H_3C), 30.7, 26.2, 25.8, 20.7 (C-31–34); ESI-MS: m/z 1013.5 $[M]^+$, 1035.4 $[M - H + Na]^+$ in agreement with the calculated mass for $[M] = C_{54}H_{72}N_6O_{13}$.

Saq-C(O)C2C(O)Glc(2TFA):—**Saq-C(O)C2C(O)GlcP** (43 mg, 0.04 mmol) in a 9:1 TFA–water mixture (5 mL) was stirred for 10 min at rt. The residue obtained after evaporation of the solvents was purified by chromatography (10:0 to 4:1 $CHCl_3$–MeOH) to give **Saq-C(O)C2C(O)Glc(2TFA)** (20 mg, 41%) as a white solid; R_f 0.24 (9:1 EtOAc–MeOH); R_t 10.7 min (column II, solvent B, 210 nm); ^{1}H NMR (CD_3OD): δ 8.47 (d, $J_{7,6}$ 8.5 Hz, 1 H, H-7), 8.16 (d, 1 H, H-6), 8.15 (d, $J_{1,2}$ 8.2 Hz, 1 H, H-1), 8.00 (d, $J_{4,3}$ 8.1 Hz, 1 H, H-4), 7.83 (dd, $J_{2,3}$ 7.2, $J_{2,1}$ 8.2 Hz, 1 H, H-2), 7.69 (dd, $J_{3,4}$ 8.1, $J_{3,2}$ 7.2 Hz, 1 H, H-3), 7.24 (d, J 7.4 Hz, 2 H, H-21, H-25), 6.99 (t, J 7.4 Hz, 2 H, H-22, H-24), 6.83 (t, J 7.4 Hz, 1 H, H-23), 5.30 (m, 1 H, H-26), 5.15 (d, $J_{1',2'}$ 3.5 Hz, 0.5 H, H-1′α), 4.64 (m, 1 H, H-18), 4.45 (d, $J_{1',2'}$ 9.4 Hz, 0.5 H, H-1′β), 3.90–3.61 (m, 2 H, H6′a,6′b), 3.61–3.30 (m, 4 H, H-2′–5′), 3.15–2.15 [m, 17 H, H-13, H-19, H-27, H-29, H-30, H-35–37, C(O)CH_2], 2.15–1.39 (m, 8 H, H-31–34), 1.33 (s, 9 H, H-41); ^{13}C NMR (CD_3OD): δ 175.2, 175.1 [Saq-*C*(O) α,β], 175.7, 174.0 (C-14, C-16), 174.5, 174.3 [Glc-*C*(O) α,β], 172.3 (C-38), 166.1, 166.2 (C-10), 149.0 (C-8), 147.0 (C-9), 139.4 (C-20), 138.9 (C-6), 131.6, 130.8 (C-1, C-2), 130.9 (C-5), 130.3 (C-21, C-25), 129.5 (C-4), 129.2 (C-22, C-24), 129.0 (C-3), 127.1 (C-23), 119.7 (C-7), 98.2 (C-1′β), 94.0 (C-1′α), 79.8 (C-3′β), 77.8, 77.7 (C-5′β, C-3′α), 74.9 (C-26), 74.6 (C-2′β), 72.9 (C-5′α), 72.2 (C-2′α), 70.7 (C-37), 69.9, 69.8 (C-4′α, C-4′β), 62.5, 62.4 (C-6′α, C-6′β), 59.8 (C-29), 56.6 (C-27), 53.1 (C-12), 52.0 (C-40), 51.6 (C-18), 38.1 (C-13), 37.1 (C-30), 35.2 (C-19), 34.9 (C-35), 31.9 (C-36), 30.5, 30.3 [C(O)*C*H_2], 29.0 (C-41), 31.5, 27.2, 27.0, 22.0 (C-31–34).

*Synthesis of [3-*O*-(1,2;5,6-di-*O*-isopropylidene-α-*D*-glucofuranose)]acetic acid 1(*R*)-[3(*S*)-*tert*-butylcarbamoyloctahydro-4a(*S*),8a-(*S*)-isoquinolin-2-ylmethyl)]-2(*S*)-[3-carbamoyl-2(*S*)-[(quinoline-2-carbonyl)-amino]-propionylamino]-3-phenyl-propyl ester* [**Saq-C(O)C1GlcP**].—The same process described for the preparation of **Saq-C(O)C2C(O)GlcP** was applied to **3** (157 mg, 0.5 mmol), DMAP (61 mg, 0.5 mmol), saquinavir (166 mg, 0.25 mmol) and EDC (95 mg, 0.5 mmol) in CH_2Cl_2 (3 mL). After treatment, the residue was chromatographed on silica gel (10:0–9:1 $CHCl_3$–MeOH) to give **Saq-C(O)C1GlcP** (180 mg, 74%) as a white solid; R_f 0.81 (9:1 $CHCl_3$–MeOH); R_t 18.6 min (column II, solvent B, 210 nm); ^{1}H NMR ($CDCl_3$): δ 9.14 (d, J 6.9 Hz, 1 H, H-11), 8.19 (d, $J_{7,6}$ 8.5 Hz, 1 H, H-7), 8.11 (d, 1 H, H-6), 8.09 (d, $J_{1,2}$ 8.5 Hz, 1 H, H-1), 7.91 (d, J 8.9 Hz, 1 H, H-17), 7.78 (d,

$J_{4,3}$ 7.5 Hz, 1 H, H-4), 7.71 (ddd, $J_{2,1}$ 8.5, $J_{2,3}$ 7.0, $J_{2,4}$ 1.4 Hz, 1 H, H-2), 7.55 (dd, $J_{3,4}$ 7.5, $J_{3,2}$ 7.0 Hz, 1 H, H-3), 7.17–6.99 (m, 5 H, H-21–25), 6.55 (bs, 1 H, H-15), 6.37 (bs, 1 H, H-15), 6.06 (bs, 1 H, H-39), 5.88 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.48 (m, 1 H, H-26), 4.75 (m, 1 H, H-12), 4.69 (d, $J_{2',1'}$ 3.7 Hz, 1 H, H-2′), 4.44–3.94 [m, 8 H, H-18, $CH_2C(O)$, H-3′–6′], 3.00–2.23 (m, 13 H, H-13, H-19, H-27, H-29, H-30, H-35–37), 1.85–1.60 (m, 8 H, H-31–34), 1.33 (s, 9 H, H-41), 1.46, 1.42, 1.27, 1.23 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 173.8, 173.6 (C-14, C-16), 170.2, 170.1 [C-38, $CH_2C(O)$], 164.4 (C-10), 149.1 (C-8), 146.6 (C-9), 137.4 (C-6), 137.1 (C-20), 130.2, 130.1 (C-1, C-2), 129.4 (C-5), 129.0 (C-21, C-25), 128.6 (C-22, C-24), 128.1 (C-4), 127.6 (C-3), 126.6 (C-23), 118.6 (C-7), 112.0, 109.2 (CCH_3), 105.3 (C-1′), 83.2 (C-3′), 83.1 (C-2′), 81.1 (C-4′), 74.4 (C-26), 72.7 (C-5′), 70.8 (C-37), 67.9 [$CH_2C(O)$], 67.2 (C-6′), 59.8 (C-29), 56.6 (C-27), 51.4 (C-12), 51.0 (C-40), 49.3 (C-18), 37.7 (C-13), 35.8 (C-30), 34.8 (C-19), 33.1 (C-35), 30.9 (C-36), 28.8 (C-41), 26.9, 26.4, 25.5 (CH_3C), 30.7, 26.3, 25.9, 20.6 (C-31–34). ESI-MS: m/z 971.4 $[M]^+$, 993.4 $[M - H + Na]^+$, in agreement with the calculated mass for $[M] = C_{52}H_{70}N_6O_{12}$.

*Synthesis of 5-[3-*O*-(*D*-glucose)]pentanoic acid 1(*R*)-[3(*S*)-*tert*-butylcarbamoyloctahydro-4a(*S*),8a(*S*)-isoquinolin-2-ylmethyl)]-2(*S*)-[3-carbamoyl-2(*S*)-[(quinoline-2-carbonyl)-amino]-propionylamino]-3-phenyl-propyl ester (bis trifluoroacetic acid salt)* [**Saq-C(O)C4Glc(2TFA)**]

*5-[3-*O*-(1,2;5,6-Di-*O*-isopropylidene-α-*D*-glucofuranose)]pentanoic acid 1(*R*)-[3(*S*)-tert-butylcarbamoyloctahydro-4a(*S*),8a(*S*)-isoquinolin-2-ylmethyl)]-2(*S*)-[3-carbamoyl-2(*S*)-[(quinoline-2-carbonyl)-amino]-propionylamino]-3-phenyl-propyl ester* [**Saq-C(O)C4GlcP**]:—The same process described for the preparation of **Saq-C(O)C2C(O)GlcP** was applied to **5** (122 mg, 0.34 mmol), DMAP (63 mg, 0.5 mmol), saquinavir (163 mg, 0.24 mmol) and EDC (94 mg, 0.5 mmol) in CH_2Cl_2 (3 mL). After treatment, the residue was chromatographed on silica gel (EtOAc) to give **Saq-C(O)C4GlcP** (188 mg, 77%) as a white solid; R_f 0.55 (EtOAc); R_t 21.3 min (column I, solvent A, 240 nm); ^{1}H NMR ($CDCl_3$): δ 9.15 (d, J 7.3 Hz, 1 H, H-11), 8.18 (d, $J_{7,6}$ 8.5 Hz, 1 H, H-7), 8.09 (d, 1 H, H-6), 8.06 (d, $J_{1,2}$ 8.4 Hz, 1 H, H-1), 7.78 (d, $J_{4,3}$ 8.1 Hz, 1 H, H-4), 7.69 (dd, $J_{2,3}$ 7.3, 1 H, H-2), 7.67 (d, J 8.4 Hz, 1 H, H-17), 7.54 (dd, 1 H, H-3), 7.10 (d, J 7.0 Hz, 2 H, H-21, H-25), 7.02 (t, J 7.0 Hz, 2 H, H-22, H-24), 6.89 (t, J 7.0 Hz, 1 H, H-23), 6.66 (bs, 1 H, H-15), 6.43 (bs, 1 H, H-15), 5.88 (bs, 1 H, H-39), 5.81 (d, $J_{1',2'}$ 3.6 Hz, 1 H, H-1′), 5.29 (m, 1 H, H-26), 4.78 (m, 1 H, H-12), 4.48 (d, 1 H, H-2′), 4.36 (m, 1 H, H-18), 4.31–4.20 (m, 1 H, H-5′), 4.10–3.99 (m, 2 H, H-4′, H-6′a), 3.97 (dd, $J_{6'b,6'a}$ 8.5, $J_{6'b,5'}$ 5.8 Hz, 1 H, H-6′b), 3.81 (d, $J_{3',4'}$ 2.8 Hz, 1 H, H-3′), 3.54 [m, 2 H, CH_2Glc], 2.88–2.15 [m, 19 H, H-13, H-19, H-27, H-29, H-30, H35–37, C(O)CH_2], 1.98–1.56 [m, 12 H, H-31–34, $CH_2CH_2CH_2$Glc], 1.29 (s, 9 H, H-41), 1.44, 1.37, 1.25, 1.21 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 173.7, 173.5 (C-14, C-16), 173.1 [C(O)CH_2], 170.3 (C-38), 164.6 (C-10), 149.1 (C-8), 146.6 (C-9), 137.4 (C-6), 137.1 (C-20), 130.2, 130.1 (C-1, C-2), 129.3 (C-5), 129.1 (C-21, C-25), 128.5 (C-22, C-24), 128.1 (C-4), 127.6 (C-3), 126.5 (C-23), 118.7 (C-7), 111.8, 109.0 (CCH_3), 105.3 (C-1′), 82.6 (C-3′), 82.3 (C-2′), 81.2 (C-4′), 73.4 (C-26), 72.6 (C-5′), 70.6 (C-37), 70.2 [CH_2Glc], 67.3 (C-6′), 59.6 (C-29), 56.9 (C-27), 51.8 (C-12), 50.9 (C-40), 49.7 (C-18), 37.4 (C-13), 35.7 (C-30), 35.3 (C-19), 34.1 [C(O)CH_2], 33.2 (C-35), 30.8 (C-36), 29.3 [CH_2CH_2Glc], 28.8 (C-41), 26.9, 26.8, 26.3, 25.5 (CH_3C), 21.5 [C(O)CH_2CH_2], 30.7, 26.1, 25.9, 20.7 (C-31–34).

Saq-C(O)C4Glc(2TFA):—**Saq-C(O)C4GlcP** (44 mg, 0.04 mmol) in a 9:1 TFA–water v/v mixture (2 mL) was stirred for 10 min at rt. The residue obtained after evaporation of the solvents was purified by chromatography (10:0–4:1 EtOAc–MeOH) to give **Saq-C(O)C4Glc(2TFA)** (40 mg, 80%) as a white solid; R_f 0.25 (9:1 EtOAc–MeOH); R_t 12.5 min (column I, solvent A, 240 nm); ^{1}H NMR (CD_3OD): δ 8.37 (d, $J_{7,6}$ 8.5 Hz, 1 H, H-7), 8.07 (d, 1 H, H-6), 8.05 (d, $J_{1,2}$ 8.4 Hz, 1 H, H-1), 7.90 (d, $J_{4,3}$ 8.0 Hz, 1 H, H-4), 7.74 (ddd, $J_{2,3}$ 7.2, $J_{2,1}$ 8.4, $J_{2,4}$ 1.3 Hz, 1 H, H-2), 7.59 (ddd, $J_{3,1}$ 1.3 Hz, 1 H, H-3), 7.14 (d, J 7.3 Hz, 2 H, H-21, H-25), 6.92 (t, J 7.3 Hz, 2 H, H-22, H-24), 6.77 (t, J 7.3 Hz, 1 H, H-23), 5.20 (m, 1 H, H-26), 4.99 (d, $J_{1',2'}$ 3.1 Hz, 0.5 H, H-1′α), 4.37 (m, 1.5 H, H-18, H-1′β), 3.75–

3.13 (m, 8 H, H-2′–6′, CH_2Glc), 3.10–2.10 [m, 17 H, H-13, H-19, H-27, H-29, H-30, H-35–37, C(O)CH_2], 2.00–1.40 [m, 12 H, H-31–34, CH_2CH_2CH$_2$Glc], 1.25 (s, 9 H, H-41); ^{13}C NMR (CD_3OD): δ 175.6, 175.5 [*C*(O)CH$_2$, α,β], 175.2, 175.1 (C-14, C-16), 172.4 (C-38), 166.1 (C-10), 148.6 (C-8), 146.5 (C-9), 139.3 (C-20), 139.0 (C-6), 130.9 (C-5), 131.6, 130.8 (C-1, C-2), 130.3 (C-21, C-25), 129.5 (C-4), 129.2 (C-22, C-24), 129.0 (C-3), 127.2 (C-23), 119.7 (C-7), 98.3 (C-1′β), 94.1 (C-1′α), 86.5 (C-3′β), 83.6 (C-3′α), 78.0 (C-5′β), 76.3 (C-2′β), 74.5 (C-26), 73.9 (C-5′α), 73.6, 73.5 [*C*H$_2$Glc α,β], 73.1 (C-2′α), 71.6, 71.5 (C-4′α, C-4′β), 70.7 (C-37), 62.9, 62.7 (C-6′α, C-6′β), 59.9 (C-29), 56.8 (C-27), 53.1 (C-12), 52.0 (C-40), 51.5 (C-18), 38.0 (C-13), 37.1 (C-30), 35.5 (C-19), 35.0 [C(O)*C*H$_2$], 34.8 (C-35), 31.8 (C-36), 30.7 [*C*H$_2$CH$_2$Glc], 29.0 (C-41), 22.6 [C(O)CH$_2$*C*H$_2$], 31.5, 27.2, 27.1, 22.0 (C-31–34). ESI-MS: m/z 933.5 $[M]^+$, 955.5 $[M - H + Na]^+$, 971.4 $[M - H + K]^+$ in agreement with the calculated mass for [M] = $C_{49}H_{68}N_6O_{12}$.

*Synthesis of succinic acid 1(*S*)-[2-(*R*)-benzyl-5-(2(*S*)-*tert*-butylcarbamoyl-4-pyridin-3-ylmethyl-piperazin-1-yl)-4(*S*)-hydroxy-pentanoylamino]-indan-2(*R*)-yl ester 4-[3-*O*-D-glucose]-butyl ester (tris trifluoroacetic acid salt)* [**Ind(8)-C(O)C2C(O)Glc(3TFA)**]

*Succinic acid 1(*S*)-[2-(*R*)-benzyl-5-(2(*S*)-tert-butylcarbamoyl-4-pyridin-3-ylmethyl-piperazin-1-yl)-4(*S*)-hydroxy-pentanoylamino]-indan-2(*R*)-yl ester 4-[3-O-(1,2;5,6-di-O-isopropylidene-α-D-glucofuranose)]-butyl ester* [**Ind(8)-C(O)C2C(O)GlcP**]:—The same process described for the preparation of **Saq-C(O)C2C(O)GlcP** was applied to **1** (131 mg, 0.36 mmol), DMAP (44 mg, 0.36 mmol), indinavir (222 mg, 0.36 mmol) and EDC (75 mg, 0.36 mmol) in CH_2Cl_2 (10 mL). After treatment, the residue was chromatographed on silica gel (10:0–9:1 EtOAc–MeOH) to give **Ind(8)-C(O)C2C(O)GlcP** (97 mg, 28%) as a white solid; R_f 0.59 (4:1 EtOAc–MeOH); R_t 9.6 min (column I, solvent B, 254 nm); ^{1}H NMR ($CDCl_3$): δ 8.55 (m, 2 H, H-28, H-29), 7.67 (bs, 1 H, H-21), 7.59 (dt, J 7.8, J 2.0 Hz, 1 H, H-26), 7.30–7.15 (m, 10 H, H-2–5, H-27, H-32–36), 6.37 (d, J 9.1 Hz, 1 H, H-10), 5.77 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.63 (dd, J 5.2, J 9.1 Hz, 1 H, H-9), 5.29 (td, J 5.2, J 1.8 Hz, 1 H, H-8), 5.19 (d, $J_{3',4'}$ 2.0 Hz, 1 H, H-3′), 4.28 (d, $J_{2',1'}$ 3.7 Hz, 1 H, H-2′), 4.15–4.08 (m, 2 H, H-4′, H-5′), 4.04–3.96 (m, 2 H, H-6′a,6′b), 3.81 (m, 1 H, H-14), 3.48 (s, 2 H, H-24), 3.18–1.90 [m, 20 H, H-7, H-12, H-13, H-30, H-15–19, C(O)CH$_2$], 1.32 (s, 9 H, H-23), 1.49, 1.37, 1.28, 1.25 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 175.1 (C-11), 171.3, 171.9 [*C*(O)CH$_2$], 169.4 (C-20), 150.6 (C-29), 149.1 (C-28), 141.0 (C-31), 139.8, 139.3 (C-1, C-6), 136.9 (C-26), 132.5 (C-25), 129.1 (C-32, C-36), 128.4 (C-33, C-35), 128.1 (C-2), 127.1, 126.3 (C-3, C-4), 125.1 (C-34), 123.6, 123.5 (C-5, C-27), 112.4, 109.5 (CH$_3$*C*), 105.1 (C-1′), 83.4 (C-2′), 79.8 (C-4′), 76.5, 76.4 (C-8, C-3′), 72.5 (C-5′), 67.3 (C-6′), 65.9 (C-14), 64.4 (C-19), 61.6 (C-15), 60.3 (C-24), 55.1 (C-9), 54.9, 52.7 (C-16, C-17), 51.2 (C-22), 48.2 (C-18), 46.1 (C-12), 39.4 (C-30), 38.1 (C-13), 37.6 (C-7), 29.1 (C-23), 28.8 [C(O)*C*H$_2$], 26.9, 26.8, 26.6, 25.3 (*C*H$_3$C).

Ind(8)-C(O)C2C(O)Glc(3TFA):—**Ind(8)-C(O)C2C(O)GlcP** (97 mg, 0.1 mmol) in a 9:1 TFA–water v/v mixture (10 mL) was stirred for 10 min at rt. The residue obtained after evaporation of the solvents was purified by chromatography (10:0 to 4:1 $CHCl_3$–MeOH) to give **Ind(8)-C(O)C2C(O)Glc(3TFA)** (58 mg, 48%) as a white solid; R_f 0.45 (7:3 EtOAc–MeOH); R_t 5.4 min (column I, solvent B, 254 nm); ^{1}H NMR (CD_3OD): δ 8.50 (m, 2 H, H-28, H-29), 7.82 (d, J 7.8 Hz, 1 H, H-26), 7.50–7.07 (m, 10 H, H-2–5, H-27, H-32–36), 5.56 (d, J 5.1 Hz, 1 H, H-9), 5.38 (m, 1 H, H-8), 5.10 (d, $J_{1',2'}$ 3.5 Hz, 0.5 H, H-1′α), 4.53 (d, $J_{1',2'}$ 8.7 Hz, 0.5 H, H-1′β), 3.93–1.90 [m, 29 H, H-7, H-12–19, H-24, H-30, C(O)CH$_2$, H-2′, H-3′, H-4′, H-5′, H-6′a,6′b], 1.32 (s, 9 H, H-23); ^{13}C NMR (CD_3OD): δ 178.0 (C-11), 174.2, 174.0 [Ind-C(O), α,β], 173.6, 173.5 [Glc*C*(O) α,β], 173.1 (C-20), 150.9 (C-29), 149.1 (C-28), 141.7 (C-31), 140.9, 140.8 (C-1, C-6), 139.3 (C-26), 135.2 (C-25), 130.2 (C-32, C-36), 129.5 (C-33, C-35), 129.3 (C-2), 128.1, 127.4 (C-3, C-4), 126.0 (C-34), 125.2, 125.0 (C-5, C-27), 98.1 (C-1′β), 93.9 (C-1′α), 79.6 (C-3′β), 77.7, 77.6 (C-5′β, C-3′α), 77.2 (C-8), 74.5 (C-2′β), 72.9 (C-5′α), 72.1 (C-2′α), 69.9, 69.8 (C-4′α, C-4′β), 68.2 (C-14), 67.8 (C-19), 63.3 (C-15), 62.6, 62.4 (C-6′α, C-6′β), 60.4

(C-24), 56.6 (C-9), 56.4, 53.1 (C-16, C-17), 52.1 (C-18, C-22), 46.2 (C-12), 40.5 (C-30), 38.8 (C-13), 38.1 (C-7), 30.2 [C(O)CH_2], 29.0 (C-23).

*Synthesis of 5-[3-*O*-(*D*-glucose)]pentanoic acid 1(*S*)-[2-(*R*)-benzyl-5-(2(*S*)*-tert*-butylcarbamoyl-4-pyridin-3-ylmethyl-piperazin-1-yl)-4(*S*)-hydroxy-pentanoylamino]-indan-2(*R*)-yl ester (trifluoroacetic acid salt)* [**Ind(8)-C(O)-C4Glc(1TFA)**]

*5-[3-*O*-(1,2;5,6-Di-*O*-isopropylidene-α-*D*-glucofuranose)]pentanoic acid 1(*S*)-[2-(*R*)-benzyl-5-(2(*S*)-tert-butylcarbamoyl-4-pyridin-3-ylmethyl-piperazin-1-yl)-4(*S*)-hydroxy-pentanoylamino]-indan-2(*R*)-yl ester* [**Ind(8)-C(O)-C4GlcP**]:—The same process described for the preparation of **Saq-C(O)C2C(O)GlcP** was applied to **5** (120 mg, 0.33 mmol), DMAP (42 mg, 0.35 mmol), indinavir (177 mg, 0.29 mmol) and EDC (66 mg, 0.35 mmol) in CH_2Cl_2 (4 mL). After treatment, the residue was chromatographed three times on silica gel (10:0–9:1 EtOAc–MeOH) to give **Ind(8)-C(O)C4GlcP** (160 mg, 62%) and 5-[3-*O*-(1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose)] pentanoic acid 1(*S*)-[(2(*S*)-*tert*-butylcarbamoyl-4-pyridin-3-ylmethyl-piperazin-1-yl-methyl]-3-(*R*)-[2-(*R*)-5-[3-*O*-(1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose)]pentanoyl]-indan-2(*S*)-ylcarbamoyl]-4-phenyl-butyl ester [**Ind-[C(O)C4GlcP]2**] (46.5 mg, 14%) as white solids.

Ind(8)-C(O)C4GlcP: R_f 0.57 (4:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 8.52 (dd, *J* 4.8, *J* 1.6 Hz, 1 H, H-28), 8.49 (d, *J* 1.6 Hz, 1 H, H-29), 7.75 (bs, 1 H, H-21), 7.59 (dt, *J* 7.8, *J* 1.6 Hz, 1 H, H-26), 7.31–7.12 (m, 10 H, H-2–5, H-27, H-32–36), 6.26 (d, *J* 9.1 Hz, 1 H, H-10), 5.82 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.62 (dd, *J* 5.2, *J* 9.1 Hz, 1 H, H-9), 5.23 (td, *J* 5.2, *J* 1.5 Hz, 1 H, H-8), 4.48 (d, 1 H, H-2′), 4.22 (dd, $J_{5',6'a}$ 5.9, $J_{5',6'b}$ 6.0 Hz, 1 H, H-5′), 4.08 (d, $J_{4',3'}$ 3.0 Hz, 1 H, H-4′), 4.06–3.90 (m, 2 H, H-6′a,6′b), 3.88–3.73 (m, 1 H, H-14), 3.80 (d, $J_{3',4'}$ 3.0 Hz, 1 H, H-3′), 3.48 [m, 4 H, H-24, CH_2Glc], 3.19–2.43 (m, 14 H, H-7, H-12, H-15–19, H-30), 2.32 (m, 1 H, H-13b), 2.05–1.80 [m, 3 H, H-13a, Ind-C(O)CH_2], 1.50 [m, 4 H, CH_2CH_2CH_2Glc], 1.32 (s, 9 H, H-23), 1.47, 1.39, 1.29, 1.24 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 174.8 (C-11), 172.4 [Ind-*C*(O)], 169.2 (C-20), 150.5 (C-29), 149.1 (C-28), 141.0 (C-31), 139.7, 139.3 (C-1, C-6), 136.8 (C-26), 132.4 (C-25), 128.9 (C-32, C-36), 128.3 (C-33, C-35), 128.0 (C-2), 127.0, 126.3 (C-3, C-4), 125.0 (C-34), 123.7, 123.4 (C-5, C-27), 111.7, 108.9 (CCH_3), 105.2 (C-1′), 82.5 (C-3′), 82.2 (C-2′), 81.2 (C-4′), 75.8 (C-8), 72.4 (C-5′), 70.1 [CH_2Glc], 67.3 (C-6′), 65.8 (C-14), 63.9 (C-19), 61.4 (C-15), 60.2 (C-24), 54.9 (C-9), 54.6, 52.7 (C-16, C-17), 51.2 (C-22), 47.8 (C-18), 46.2 (C-12), 39.3 (C-30), 38.2 (C-13), 37.6 (C-7), 33.6 [Ind-C(O)CH_2], 29.6 [CH_2CH_2Glc], 29.0 (C-23), 26.9, 26.8, 26.2, 25.4 (CH_3C), 21.3 [C(O)CH_2CH_2]. ESI-MS: *m*/*z* 956.5 $[M]^+$, 978.6 $[M-H+Na]^+$, in agreement with the calculated mass for [M] = $C_{53}H_{73}N_5O_{11}$.

Ind-[C(O)C4GlcP]2: R_f 0.79 (4:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 8.50 (dd, *J* 4.8, *J* 1.7 Hz, 1 H, H-28), 8.49 (d, *J* 1.7 Hz, 1 H, H-29), 7.62 (dt, *J* 7.9, *J* 1.7 Hz, 1 H, H-26), 7.28–7.11 (m, 10 H, H-2–5, H-27, H-32–36), 6.90 (bs, 1 H, H-21), 6.10 (d, *J* 9.2 Hz, 1 H, H-10), 5.84 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.82 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.63 (dd, *J* 5.2, *J* 9.2 Hz, 1 H, H-9), 5.28 (td, *J* 5.2, *J* 1.5 Hz, 1 H, H-8), 4.50 (m, 1 H, H-14), 4.49 (d, 2 H, H-2′), 4.23 (m, 2 H, H-5′), 4.11–3.92 (m, 6 H, H-4′, H-6′a,6′b), 3.83 (d, $J_{3',4'}$ 3.4 Hz, 1 H, H-3′), 3.81 (d, $J_{3',4'}$ 3.4 Hz, 1 H, H-3′), 3.53 [m, 4 H, CH_2Glc], 3.46 (s, 2 H, H-24), 3.25–2.00 [m, 20 H, H-7, H-12, H13, H-15–19, H-30, C(O)CH_2], 1.70–1.50 [m, 8 H, CH_2CH_2CH_2-Glc], 1.49, 1.40, 1.32, 1.31 (4s, 33 H, H-23, CH_3C); ^{13}C NMR ($CDCl_3$): δ 174.0 (C-11), 173.4, 172.5 [*C*(O)CH_2], 170.2 (C-20), 150.4 (C-29), 148.9 (C-28), 140.6 (C-31), 139.3, 139.0 (C-1, C-6), 136.7 (C-26), 132.9 (C-25), 128.8 (C-32, C-36), 128.5 (C-33, C-35), 128.2 (C-2), 127.2, 126.6 (C-3, C-4), 125.0 (C-34), 123.8, 123.4 (C-5, C-27), 105.3 (C-1′), 82.6 (C-3′), 82.2, 82.1 (C-2′), 81.2 (C-4′), 75.9 (C-8), 72.4 (C-5′), 70.1, 70.0 [CH_2Glc], 69.8 (C-14), 67.3, 67.2 (C-6′), 67.0 (C-19), 59.9 (C-24), 58.9 (C-15), 55.1 (C-9), 55.8, 52.2 (C-16, C-17), 50.9 (C-22), 49.9 (C-18), 45.7 (C-12), 39.7 (C-30), 37.6 (C-7), 35.5 (C-13), 34.1, 33.6 [C(O)CH_2], 29.7, 29.2 [CH_2CH_2Glc], 28.8 (C-23), 26.8, 26.3, 25.5, 25.4 (CH_3C), 21.7, 21.3 [C(O)CH_2CH_2]. ESI-MS: *m*/*z* 1298.6 $[M]^+$, 1320.6 $[M-H+Na]^+$, in agreement with the calculated mass for [M] = $C_{70}H_{99}N_5O_{18}$.

Ind(8)-C(O)C4Glc(1TFA):—A 9:1 TFA–water mixture (1 mL) was added dropwise to a solution of **Ind(8)-C(O)C4GlcP** (47 mg, 0.05 mmol) in CH_3CN. The solution was stirred for 3 h at 0 °C and for 1 h at rt. Then, a 9:1 TFA–water mixture (1 mL) was added dropwise to the reaction solution at rt and the mixture stirred for another 30 min. The residue obtained after evaporation of the solvents was purified twice by chromatography (10:0–7:3 EtOAc–MeOH) to give **Ind(8)-C(O)C4Glc(1TFA)** (39.5 mg, 81%) as a white solid; R_f 0.63 (7:3 EtOAc–MeOH); R_t 8.6 min (column I, solvent A, 254 nm); ^{1}H NMR (CD_3OD): δ 8.58 (m, 2 H, H-28, H-29), 7.59 (dt, J 7.9 Hz, 1 H, H-26), 7.31–7.17 (m, 10 H, H-2–5, H-27, H-32–36), 5.58 (d, J 5.0 Hz, 1 H, H-9), 5.37 (td, J 5.0, J 1.5 Hz, 1 H, H-8), 5.08 (d, $J_{1',2'}$ 3.2 Hz, 0.5 H, H-1′α), 4.47 (d, $J_{1',2'}$ 7.2 Hz, 0.5 H, H-1′β), 4.11–3.23 [m, 11 H, H-14, H-24, H-2′-6′, CH_2Glc], 3.22–2.62 (m, 14 H, H-7, H-12, H-15–19, H-30), 2.30–1.82 [m, 4 H, H-13, C(O)CH_2], 1.60 [m, 4 H, CH_2CH_2CH$_2$Glc], 1.32 (s, 9 H, H-23); ^{13}C NMR (CD_3OD): δ 177.6 (C-11), 174.9, 174.8 [C-20, *C*(O)CH$_2$ α,β], 150.6 (C-29), 149.4 (C-28), 141.7 (C-31), 141.1, 140.5 (C-1, C-6), 140.4 (C-26), 130.2 (C-32, C-36), 129.6 (C-33, C-35), 129.3 (C-2), 128.2, 127.7 (C-3, C-4), 126.1 (C-34), 125.8 (C-5), 125.1 (C-27), 98.4 (C-1′β), 94.1 (C-1′α), 86.5 (C-3′β), 83.6 (C-3′α), 78.0 (C-5′β), 76.9 (C-8), 76.3 (C-2′β), 73.9 (C-5′α), 73.6, 73.5 [*C*H$_2$Glc α,β], 73.1 (C-2′α), 71.7, 71.6 (C-4′α, C-4′β), 67.2, 67.1 (C-14, C-19), 62.9, 62.8 (C-6′α, C-6′β), 62.7 (C-15), 59.3 (C-24), 56.7 (C-9), 54.6 (C-16, C-17), 52.8 (C-22), 51.1 (C-18), 46.1 (C-12), 40.6 (C-30), 38.5 (C-13), 38.3 (C-7), 34.8 [C(O)*C*H$_2$], 30.7 [*C*H$_2$CH$_2$Glc], 28.8 (C-23), 22.6 [C(O)CH$_2$-*C*H$_2$]. ESI-MS: m/z 876.5 $[M]^+$, 898.5 $[M - H + Na]^+$, in agreement with the calculated mass for $[M] = C_{47}H_{65}N_5O_{11}$.

Synthesis of succinic acid 3-[3-[3(S)-tert-*butylcarbamoyloctahydro - 4a*(S),*8a*(S) *- iso - quinolin - 2 - yl] - 2*(R)*-hydroxy - 1*(S)*-phenylsulfanylmethyl - propylcarbamoyl] - 2 - methyl - phenyl ester 4-[3*-O-*(1,2;5,6-di*-O-*isopropylidene*-α-D-*glucofuranose)]-butyl ester* [**Nelf(1)-C(O)C2C(O)GlcP**].—The same process as that used for **Saq-C(O)C2C(O)GlcP** was applied to **1** (118 mg, 0.33 mmol), DMAP (40 mg, 0.33 mmol), nelfinavir (186 mg, 0.33 mmol) and EDC (68 mg, 0.33 mmol) in DMF (10 mL). After treatment, the residue was chromatographed twice on silica gel (10:0–2:3 hexane–EtOAc, then 10:0–9:1 $CHCl_3$–MeOH) to give **Nelf(1)-C(O)C2C(O)GlcP** (49 mg, 16%) as a white solid; R_f 0.72 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.45–7.00 (m, 9 H, H-2–4, H13-17, H-9 or H-30), 5.84 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.53 (bs, 1 H, H-9 or H-30), 5.28 (d, $J_{3',4'}$ 2.0 Hz, 1 H, H-3′), 4.51 (d, J 3.7 Hz, 1 H, H-2′), 4.50–4.40 (m, 1 H, H-10), 4.31–4.01 (m, 4 H, H-4′–6′ and H-18), 3.80 and 3.69 (m, 2 H, H-11a,b), 2.90 (dd, J_{AB} 9.7, J_{AX} 4.4 Hz, 1 H, H-20a), 3.00–2.75 (m, 2 H, H-19a, H-28), 2.70–2.38 (m, 2 H, H-19b, H-20b), 2.28 (s, 3 H, H-7), 2.25–1.30 [m, 16 H, H-21–27, C(O)CH$_2$CH$_2$C(O)], 1.53, 1.42, 1.31, 1.29 (4 s, 4 × 3 H, CH_3C), 1.17 (s, 9 H, H-32); ^{13}C NMR ($CDCl_3$): δ 174.0 (C-8), 170.8, 170.7 [*C*(O)CH$_2$], 170.3 (C-29), 149.8 (C-1), 137.9, 135.8 (C-5, C-12), 130.2 (C-13, C-17), 129.4 (C-6), 129.1 (C-14, C-16), 126.5 (C-3, C-15), 125.7, 123.9 (C-2, C-4), 112.4, 109.5 (CH_3*C*), 105.2 (C-1′), 83.3 (C-2′), 79.8 (C-4′), 76.7 (C-3′), 72.5 (C-5′), 70.8 (C-28), 70.4 (C-18), 67.4 (C-6′), 59.8 (C-20), 58.8 (C-19), 54.8 (C10), 51.3 (C-31), 36.2 (C-21), 35.5 (C-11), 33.8 (C-26), 31.1 (C-27), 29.1, 29.0 [C(O)*C*H$_2$], 28.5 (C-32), 31.0, 26.4, 26.1, 20.6 (C-22–5), 26.9, 26.8, 26.3, 25.3 (*C*H$_3$C), 13.3 (C-7).

Synthesis of the carbamate prodrug derivatives

Synthesis of [4-(3-O-D-*glucose)-butyl]-carbamic acid 1*(R)*-[3*(S)-tert-*butylcarbamoyloctahydro - 4a*(S),*8a*(S) *- isoquinolin - 2 - ylmethyl)] - 2*(S)*-[3 - carbamoyl - 2*(S)*-[(quinoline - 2 - carbonyl) - amino] - propionylamino] - 3 - phenyl - propyl ester (tetrakis trifluoroacetic acid salt)* [**Saq-C(O)NC4Glc(4TFA)**]

[4-[3-O-*(1,2;5,6-Di*-O-*isopropylidene*-α-D-*glucofuranose)]-butyl]-carbamic acid 1*(R)*-[3*(S)- tert *- butylcarbamoyloctahydro - 4a*(S),*8a*-*(S) - isoquinolin - 2 - ylmethyl] - 2*(S)*-[3 - carbamoyl - 2*(S)*-[(quinoline - 2 - carbonyl) - amino] - propionylamino] - 3 - phenyl - propyl ester* [**Saq-C(O)NC4GlcP**]:—Derivative **7** (214 mg, 0.60 mmol) was added at rt to a stirred solution of saquinavir (200 mg, 0.30 mmol) in CH_2Cl_2 (2 mL), followed by CuCl (59 mg, 0.60 mmol). After 5 h stirring at rt, the reaction mixture

was extracted with CH_2Cl_2 (3×20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (9:0–9:1 EtOAc–MeOH) to afford **Saq-C(O)NC4GlcP** (208 mg, 68%) as a white powder; R_f 0.30 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 9.12 (d, J 7.6 Hz, 1 H, H-11), 8.15 (d, $J_{7,6}$ 8.6 Hz, 1 H, H-7), 8.06 (d, 1 H, H-6), 8.02 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1), 7.74 (d, $J_{4,3}$ 8.1 Hz, 1 H, H-4), 7.65 (dd, $J_{2,3}$ 7.8, 1 H, H-2), 7.50 (dd, 1 H, H-3), 7.26–6.92 (m, 4 H, H-21, H-22, H-24, H-25), 6.84 (m, 1 H, H-23), 6.60 (bs, 1 H, H-17), 6.08 (bs, 2 H, H-15), 5.79 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.39 (m, 1 H, H-39), 5.25 [m, 1 H, N*H*C(O)O], 5.02 (m, 1 H, H-26), 4.82 (m, 1 H, H-12), 4.45 (d, $J_{2',1'}$ 3.7 Hz, 1 H, H-2′), 4.31 (m, 1 H, H-18), 4.20 (m, 1 H, H-5′), 4.10–3.85 (m, 3 H, H-4′, H-6′a,6′b), 3.77 (d, $J_{3',4'}$ 2.9 Hz, 1 H, H-3′), 3.48 (m, 2 H, CH_2Glc), 3.02 (m, 2 H, CH_2NH), 3.00–2.25 (m, 13 H, H-13, H-19, H-27, H-29, H-30, H-35–37), 1.80–1.45 (m, 12 H, H-31–34, CH_2CH_2CH$_2$NH), 1.26 (s, 9 H, H-41), 1.41, 1.34, 1.25, 1.22 (4 s, 4×3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 174.0, 173.5 (C-14, C-16), 170.4 (C-38), 164.5 (C-10), 156.3 [NH*C*(O)O], 149.1 (C-8), 146.5 (C-9), 137.3 (C-6, C-20), 130.1 (C-1, C-2), 129.3 (C-5), 129.2 (C-21, C-25), 128.3 (C-22, C-24), 128.0 (C-4), 127.6 (C-3), 126.3 (C-23), 118.7 (C-7), 111.7, 108.9 (CH_3C), 105.2 (C-1′), 82.5 (C-3′), 82.2 (C-2′), 81.1 (C-4′), 73.7 (C-26), 72.5 (C-5′), 70.2 (C-37), 70.1 (*C*H$_2$Glc), 67.2 (C-6′), 59.9 (C-29), 57.1 (C-27), 52.1 (C-12), 50.8 (C-40), 49.9 (C-18), 40.8 (*C*H$_2$NH), 37.5 (C-13), 35.9 (C-30), 35.6 (C-19), 33.2 (C-35), 30.9 (C-36), 29.6 (*C*H$_2$CH$_2$Glc), 28.7 (C-41), 27.0 (*C*H$_2$CH$_2$NH), 26.8, 26.2, 25.4 (*C*H$_3$C), 30.7, 26.5, 25.6, 20.7 (C-31–34).

Saq-C(O)NC4Glc(4TFA):—A 7:3 TFA–water mixture (10 mL) was added at 0 °C to a solution of **Saq-C(O)NC4GlcP** (100 mg, 0.097 mmol) in CH_3CN (2 mL). After 2 h at 0 °C then 24 h at rt, the reaction mixture was evaporated and chromatographed on silica gel (10:0–4:1 CH_2Cl_2–MeOH) to afford **Saq-C(O)NC4Glc(4TFA)** (135 mg, 99%) as a white powder; R_f 0.25 (9:1 EtOAc–MeOH); R_f 0.35 (9:1 CH_2Cl_2–MeOH); R_t 3.2 min (column I, solvent D, 240 nm); 3.8 min (column III, solvent D, 240 nm); ^{1}H NMR (CD_3OD): δ 8.42 (d, $J_{7,6}$ 8.6 Hz, 1 H, H-7), 8.15 (d, 1 H, H-6), 8.10 (d, $J_{1,2}$ 8.7 Hz, 1 H, H-1), 7.95 (d, $J_{4,3}$ 7.9 Hz, 1 H, H-4), 7.80 (dt, $J_{2,3}$ 7.9, $J_{2,1}$ 8.7 Hz, 1 H, H-2), 7.65 (t, 1 H, H-3), 7.20 (d, J 7.4 Hz, 2 H, H-21, H-25), 6.98 (t, J 7.4 Hz, 2 H, H-22, H-24), 6.81 (t, J 7.4 Hz, 1 H, H-23), 5.16 (d, $J_{1',2'}$ 2.7 Hz, 0.5 H, H-1′α), 5.20–5.00 (m, 2 H, H-12, H-26), 4.52 (d, 0.5 H, $J_{1',2'}$ 7.2 Hz, H-1′β), 4.35 (m, 1 H, H-18), 3.70–3.30 (m, 8 H, H-2′–6′, CH_2Glc), 3.25-2.20 (m, 12 H, H-13, H-19, H-27, H-29, H-30, H-35–37, CH_2NH), 2.10–1.40 (m, 12 H, H-31–34, CH_2CH_2Glc), 1.32 (s, 9 H, H-41); ^{13}C NMR (CD_3OD): δ 175.6 (C-14, C-16), 172.4 (C-38), 166.5 (C-10), 158.6 [NH*C*(O)O], 150.1 (C-8), 148.0 (C-9), 139.3 (C-20), 139.1 (C-6), 130.9 (C-5), 131.7, 130.8 (C-1, C-2), 130.2 (C-21, C-25), 129.7 (C-4), 129.3 (C-22, C-24), 129.1 (C-3), 127.3 (C-23), 119.9 (C-7), 98.3 (C-1′β), 94.1 (C-1′α), 86.3 (C-3′β), 83.5 (C-3′α), 78.1 (C-5′β), 76.2 (C-2′β), 75.3 (C-26), 73.8 (*C*H$_2$Glc), 73.7 (C-5′α), 73.2 (C-2′α), 71.5 (C-4′α,β), 71.1 (C-37), 62.8, 62.6 (C-6′α,β), 60.7 (C-29), 58.0 (C-27), 53.6 (C-12), 52.3 (C-40), 51.5 (C-18), 41.9 (*C*H$_2$NH), 38.3 (C-13), 37.2 (C-30), 35.4 (C-19), 34.7 (C-35), 31.9 (C-36), 29.0 (C-41), 28.6 (*C*H$_2$CH$_2$NH), 27.5 (*C*H$_2$CH$_2$Glc), 31.6, 27.2, 26.7, 21.9 (C-31–34). ESI-MS: m/z 948.5 $[M]^+$, 970.4 $[M - H + Na]^+$, in agreement with the calculated mass for $[M] = C_{49}H_{69}N_7O_{12}$.

*Synthesis of [3-*O*-(*D*-glucose)]-carbamic acid 1(*S*)-(2(*S*)-*tert*-butylcarbamoyl-4-pyridin-3-yl-methyl-piperazin-1-ylmethyl)-3(*R*)-(2(*R*)-hydroxy-indan-1(*S*)-ylcarbamoyl)-4-phenyl-butyl ester (bis trifluoroacetic acid salt)* [**Ind(14)-C(O)NC4Glc(2TFA)**]

*[4-[3-*O*-(1,2;5,6-Di-*O*-isopropylidene-α-*D*-glucofuranose)]-butyl]-carbamic acid 1(*S*)-(2(*S*)-tert-butylcarbamoyl-4-pyridin-3-yl-methyl-piperazin-1-ylmethyl)-3(*R*)-(2(*R*)-hydroxy-indan-1(*S*)-ylcarbamoyl)-4-phenyl-butyl ester* [**Ind(14)-C(O)NC4GlcP**]:—The same process described for the preparation of **Saq-C(O)NC4GlcP** was applied to **7** (134 mg, 0.37 mmol), indinavir (230 mg, 0.37 mmol), CuCl (37 mg, 0.37 mmol) in CH_2Cl_2 (2 mL). After treatment, the residue was chromatographed on silica gel (9:0–4:1 EtOAc–MeOH) to afford **Ind(14)-C(O)NC4GlcP** (280 mg, 77%) as

a white powder; R_f 0.6 (4:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.65 (d, J 7.8 Hz, 1 H, H-26), 7.40–7.15 (m, 12 H, H-2–5, H-27–29, H-32–36), 6.90 (bs, 1 H, H-21), 6.05 (d, J 8.6 Hz, 1 H, H-10), 5.84 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.28 (dd, J 4.7, J 8.6 Hz, 1 H, H-9), 5.10–4.95 [m, 2 H, H-14, N*H*C(O)O], 4.50 (d, 1 H, H-2′), 4.30–4.20 (m, 2 H, H-8, H-4′), 4.15–3.95 (m, 3 H, H-5′, H-6′), 3.84 (d, $J_{3',4'}$ 3.0 Hz, 1 H, H-3′), 3.49 (s, 2 H, H-24), 3.55 (m, 2 H, CH_2CGlc), 3.21 (m, 2 H, CH_2NH), 3.00–2.25 (m, 16 H, H-7, H-12, H-13, H-15–19, H-30), 1.59 (s, 4 H, CH_2CH_2CH$_2$NH), 1.36 (s, 9 H, H-23), 1.50, 1.43, 1.34, 1.31 (4 s, 4 × 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 174.1 (C-11), 170.6 (C-20), 156.3 [NH*C*(O)O], 150.5 (C-29), 149.0 (C-28), 140.3 (C-31), 140.1, 139.7 (C-1, C-6), 136.9 (C-26), 129.1 (C-32, C-36), 128.7 (C-33, C-35), 127.9 (C-2), 126.9, 126.8 (C-3, C-4, C-34), 125.0, 124.6 (C-5, C-27), 111.9, 109.0 (CH_3C), 105.3 (C-1′), 82.4 (C-3′), 82.2 (C-2′), 81.1 (C-4′), 73.3 (C-8), 72.5 (C-5′), 70.3 (C-14), 69.9 (*C*H$_2$Glc), 67.4 (C-6′), 59.9 (C-15), 59.6 (C-24), 57.9 (C-9), 56.0, 52.2 (C-16, C-17), 50.9 (C-18), 50.8 (C-22), 46.4 (C-12), 40.9 (*C*H$_2$NH), 40.1 (C-30), 39.1 (C-7), 36.4 (C-13), 28.8 (C-23), 26.9 (*C*H$_2$-CH$_2$Glc), 26.8, 26.3, 25.5 (*C*H$_3$C), 26.6 (*C*H$_2$CH$_2$NH).

Ind(14)-C(O)NC4Glc(2TFA):—A 9:1 TFA–water mixture (1 mL) was added at 0 °C to a solution of **Ind(14)-C(O)NC4GlcP** (160 mg, 0.16 mmol) in CH_3CN (1 mL). After 10 min stirring at 0 °C, the reaction mixture was evaporated under diminished pressure and chromatographed on silica gel (49:1–4:1 CH_2Cl_2–MeOH) to afford **Ind(14)-C(O)-NC4Glc(2TFA)** (157 mg, 85%) as a white powder; R_f 0.40 (4:1 CH_2Cl_2–MeOH); R_t 4.0 min (column I, solvent C, 210 nm); 5.1 min (column III, solvent C, 210 nm); ^{1}H NMR (CD_3OD): δ 7.96 (m, 2 H, H-28, H-29), 7.60 (m, 1 H, H-26), 7.35–7.15 (m, 10 H, H-2–5, H-27, H-32–36), 5.24 (m, 1 H, H-9), 5.05 (d, J 2.7 Hz, 0.5 H, H-1′α), H-14 masked by solvent, 4.45–4.35 (m, 1.5 H, H-1′β, H-8), 4.11 (m, 1 H), 3.85–3.25 (m, 11 H, H-24, H-3′–6′, CH_2Glc), 3.25–2.65 (m, 16 H, H-7, H-12, H-15–19, H-30, CH_2NH), 2.02 (m, 2 H, H-13), 1.55 (m, 4 H, CH_2CH_2CH$_2$NH), 1.31 (s, 9 H, H-23); ^{13}C NMR (CD_3OD): δ 177.6 (C-11), 170.7 (C-20), 158.9 [NH*C*(O)O], 151.5 (C-29), 150.5 (C-28), 142.2 (C-31), 140.9 (C-26), 141.9, 140.5 (C-1, C-6), 130.3 (C-32, C-36), 129.6 (C-33, C-35), 128.9 (C-2), 127.8, 127.6 (C-3, C-4), 126.2 (C-34), 125.9 (C-5), 98.3 (C-1′β), 94.2 (C-1′α), 86.5 (C-3′β), 83.6 (C-3′α), 78.0 (C-5′β), 76.3 (C-2′β), 74.2 (C-8), 73.9 (C-5′α), 73.8, 73.6 (*C*H$_2$CGlc), 73.2 (C-2′α), 71.7, 71.6 (C-4′α, β), 71.1 (C-14), 62.9, 62.7 (C-6′α, β), 60.0 (C-15), 59.2 (C-24), 58.8 (C-9), 53.0 (C-16, C-17), 52.8 (C-22), 52.2 (C-18), 46.0 (C-12), 41.9 (*C*H$_2$NH), 40.9 (C-30), 40.7 (C-7), 35.6 (C-13), 29.0 (C-23), 28.6 (*C*H$_2$CH$_2$Glc), 27.7 (*C*H$_2$CH$_2$NH). ESI-MS: m/z 892.0 $[M+H]^+$, 914.0 $[M+Na]^+$, in agreement with the calculated mass for [M] = $C_{47}H_{66}N_6O_{11}$.

*Synthesis of [4-(3-*O-D-*glucose)-butyl]-carbamic acid 1(*R*)-[3(*S*)-*tert*-butylcarbamoyloctahydro-4a(*S*),8a(*S*)-isoquinolin-2-ylmethyl]-2(*S*)-(3-hydroxy-2-methyl-benzoylamino)-3-phenylsulfanyl-propyl ester (bis trifluroacetic acid salt)* [**Nelf(18)-C(O)NC4Glc(2TFA)**]

*[4-[3-*O*-(1,2;5,6-Di-*O*-isopropylidene-α-*D*-glucofuranose)]-butyl]-carbamic acid 1(*R*)-[3(*S*)-*tert*-butylcarbamoyloctahydro-4a(*S*),8a-(*S*)-isoquinolin-2-ylmethyl]-2(*S*)-(3-hydroxy-2-methyl-benzoylamino)-3-phenylsulfanyl-propyl ester* [**Nelf(18)-C(O)NC4GlcP**]:—The same process described for the preparation of **Saq-C(O)NC4Glc** was applied to **7** (95 mg, 0.26 mmol), nelfinavir (150 mg, 0.26 mmol), CuCl (26 mg, 0.26 mmol) in 2:0.5 CH_2Cl_2–DMF (2.5 mL). After treatment, the residue was chromatographed on silica gel (3:0–3:2 CH_2Cl_2–EtOAc) to afford **Nelf(18)-C(O)-NC4GlcP** (155 mg, 63.5%) as a white powder; R_f 0.5 (3:2 CH_2Cl_2–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.88 (bs, 1 H, H-9 or H-30), 7.41 (dd, J 1.5 Hz and J 7.0 Hz, 2 H, H-13, H-17), 7.33–7.14 (m, 5 H, H-14-16), 6.96–6.75 (m, 3 H, H-2–4), 6.11 (bs, 1 H, H-9 or H-30), 5.82 (d, $J_{1',2'}$ 3.7 Hz, 1 H, H-1′), 5.30–5.12 [m, 2 H, H-18, N*H*C(O)O], 4.55 (m, 1 H, H-10), 4.49 (d, 1 H, H-2′), 4.31–4.21 (m, 1 H, H-5′), 3.91–4.14 (m, 3 H, H-4′, H-6′), 3.80 (d, $J_{3',4'}$ 3.0 Hz, 1 H, H-3′), 3.60–3.32 (m, 4 H, H-11, CH_2Glc), 3.10 (m, 2 H, CH_2NH), 2.95 (m, 1 H, H-20a), 2.70–2.40 (m, 2 H, H-19a, H-28), 2.35–2.10 (m, 2 H, H-19b, H-20b), 2.22 (s, 3 H, H-7), 2.00-1.10 (m, 16 H, H-21–27,

$CH_2CH_2CH_2NH$), 1.46, 1.39, 1.31, 1.26 (4 s, 4×3 H, CH_3C), 1.13 (s, 9 H, H-32); ^{13}C NMR ($CDCl_3$): δ 174.1 (C-8), 170.5 (C-29), 156.0 (C-1), 155.2 [NH*C*(O)O], 138.3, 136.0 (C-5, C-12), 129.8 (C-13, C-17), 129.0 (C-14, C-16), 126.3 (C-3, C-15), 122.3 (C-6), 118.6 (C-4), 116.3 (C-2), 111.7, 108.9 (CH_3C), 105.2 (C-1′), 82.4 (C-3′), 82.1 (C-2′), 81.1 (C-4′), 72.5 (C-5′), 72.4 (C-18), 70.3 (CH_2CGlc), 70.0 (C-28), 67.2 (C-6′), 59.4 (C-20), 56.5 (C-19), 51.1 (C-31), 51.0 (C10), 40.8 (CH_2NH), 35.9 (C-21), 35.6 (C-11), 33.7 (C-26), 31.0 (C-27), 28.5 (C-32), 26.9 (CH_2CH_2Glc), 26.5 (CH_2CH_2NH), 30.9, 26.4, 25.8, 20.7 (C-22–25), 26.8, 26.2, 25.4 (CH_3C), 12.7 (C-7).

Nelf(18)-C(O)NC4Glc(2TFA):—TFA deprotection as described for the preparation of **Ind(14)-C(O)NC4Glc(2TFA)** was applied to **Nelf(18)-C(O)NC4GlcP** (100 mg, 0.11 mmol) in CH_3CN (1 mL). After treatment, the residue was chromatographed on silica gel (9:1–4:1 CH_2Cl_2–MeOH) to afford **Nelf(18)-C(O)NC4Glc(2TFA)** (113 mg, 97%) as a white powder; R_f 0.2 (9:1 CH_2Cl_2–MeOH); R_t 5.2 min (column I, solvent E, 210 nm); 5.8 min (column III, solvent E, 210 nm); ^1H NMR (CD_3OD): δ 7.48 (m, 2 H, H-13, H-17), 7.28 (d, *J* 7.1 Hz, 2 H, H-14, H-16), 7.17 (m, 1 H, H-15), 7.00 (t, *J* 7.5 Hz, 1 H, H-3), 6.82 (m, 2 H, H-2, H-4), 5.22 (m, 1 H, H-18), 5.06 (m, 0.6 H, H-1′α), 4.51–4.42 (m, 1.4 H, H-10, H-1′β), 3.85–3.00 (m, 13 H, H-11, H-20a, H-2′–6′, CH_2Glc, CH_2NH), 2.75–2.60 (m, 2 H, H-19a, H-28), 2.35–2.15 (m, 2 H, H-19b, H-20b), 2.22 (s, 3 H, H-7), 2.15–1.10 (m, 16 H, H-21–27, $CH_2CH_2CH_2$NH), 1.16 (s, 9 H, H-32); ^{13}C NMR (CD_3OD): δ 173.6 (C-8, C-29), 158.3 [NH*C*(O)O], 157.1 (C-1), 140.0, 137.2 (C-5, C-12), 131.3 (C-13, C-17), 130.1 (C-14, C-16), 127.5, 127.4 (C-3, C-15), 123.4 (C-6), 119.4 (C-4), 117.1 (C-2), 98.3 (C-1′β), 94.1 (C-1′α), 86.4 (C-3′β), 83.6 (C-3′α), 78.1 (C-5′β), 76.3 (C-2′β), 73.8 (CH_2Glc), 73.6 (C-5′α, C-28), 73.2 (C-2′α), 71.8, 71.6 (C-4′α,β), 71.0 (C-18), 63.0, 62.9 (C-6′α, β), 60.6 (C-20), 57.6 (C-19), 52.2 (C-31), 52.1 (C-10), 41.9 (CH_2NH), 37.5 (C-21), 35.5 (C-11), 35.0 (C-26), 32.2 (C-27), 29.0 (C-32), 28.6 (CH_2CH_2Glc), 27.6 (CH_2CH_2NH), 32.9, 27.5, 27.0, 21.8 (C-22–25), 15.6 (C-7). ESI-MS: *m*/*z* 845.9 $[M+H]^+$, 867.9 $[M+Na]^+$, in agreement with the calculated mass for $[M] = C_{43}H_{64}N_4O_{11}S$.

Hydrolysis kinetics.—Hydrolysis experiments were performed by incubating 22 mL of a 9:1 DMEM–MeOH solution (pH 7.3) of the prodrug (156, 146, 239 and 174 μM for **Saq-C(O)C4Glc(2TFA)**, **Saq-C(O)NC4Glc(4TFA)**, **Ind(14)-C(O)NC4Glc(2TFA)**, **Nelf(18)-C(O)-NC4Glc(2TFA)**, respectively) at 37 °C with stirring. Hydrolysis was followed by HPLC monitoring of either the disappearance of the prodrug and appearance of the parent drug, by injecting 60 μL of the solution onto the HPLC column. HPLC analysis was performed using a HP1100 apparatus with a Kromasil C18 (100–5 μm)-packed column (150 × 3 mm) for the saquinavir ester prodrug (column IV), or with a Lichrospher 100 RP-18 (5 μm)-packed column (250 × 3.2 mm) for the carbamate prodrugs (column I). The mobile phase consisted of solvent D for the saquinavir derivatives, solvent C for the indinavir derivatives, and solvent E for the nelfinavir derivatives. The prodrugs and/or drugs were detected by measuring their UV absorption at 210 or 240 nm and the signals were interpreted by the software provided. Under their respective HPLC conditions, the retention times measured for the different compounds were 7.3 min for saquinavir and 4.4 min for **Saq-C(O)C4Glc(2TFA)** (column IV), 5.1 min for saquinavir and 3.2 min for **Saq-C(O)-NC4Glc(4TFA)**, 11.6 min for indinavir and 4.0 min **for Ind(14)-C(O)NC4Glc(2TFA)**, 12.9 min for nelfinavir and 5.2 min for **Nelf(18)-C(O)NC4Glc(2TFA)** (column I). The prodrug and/or drug concentration was determined from HPLC calibration curves. These curves were established under the same HPLC conditions, using standard calibrated prodrug and drug solutions that were prepared in the same hydrolysis medium as the sample under investigation. The calibration curves were linear (correlation coefficient in the 0.9995–1 range) in a concentration range of 0.00149–0.3737 mM for saquinavir, 0.0022–0.22 mM for **Saq-C(O)C4Glc(2TFA)**, 0.0007–0.1780 mM for **Saq-C(O)NC4Glc(4TFA)**, 0.0016–0.4073 for indinavir, 0.0009–0.2234 mM for **Ind(14)-C(O)NC4Glc(2TFA)**, 0.0017–0.4403 mM for nelfinavir and 0.0009–0.2330 mM for **Nelf-**

(18)-C(O)NC4Glc(2TFA). Each sample was analysed in triplicate and each calibration curve was determined in triplicate and repeated on the same day of analysis.

For **Saq-C(O)C4Glc(2TFA)**, $\ln([\text{prodrug}]_0 - [\text{prodrug}(t)])$ and of $\ln[\text{drug}(t)]$ against time was plotted and its half-life of hydrolysis ($t_{1/2}$) was measured from this curve. For the three carbamate prodrugs investigated, no hydrolysis was observed after 7 days of incubation.

Antiviral assays.—The in vitro antiviral activity of the compounds was determined according to published procedures.[30–32] Briefly, CEM-SS cells were infected with a dose of HIV-1 (LAI strain) infecting 50% of the cells. Four days latter, the growing of HIV-1 was evaluated by measuring the reverse transcriptase (RT) which expresses the presence of the virus in the supernatant culture medium. The tested compounds were added to the cell cultures after viral infection. RT inhibition % was measured in comparison with the non treated cells.

The growing of HIV-1 [HTLV-I (IIIB)] was followed by the cytopathogenic effect induced by the virus. MT4 cells were infected with a virus dose allowing 4 days latter the death of 90%. The tested compounds were added in the cell culture medium after viral infection. Cell viability was measured by the colorimetric MTT test (see below). The percentage of protection was calculated as the ratio Δ:

$$\Delta = [(\text{OD of treated infected cells} - \text{OD of untreated infected cells}) / (\text{OD of non infected cells} - \text{OD of untreated infected cells})] \times 100$$

The prodrug IC_{50} values were determined from the curves of the RT inhibition % (CEM-SS cells) or the protection percentage Δ (MT4 cells) against prodrug concentration.

Their cytotoxicity was evaluated in parallel with the antiviral activity, and was based on the viability of the non-infected cells as monitored by the MTT method [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide]. This colorimetric MTT test is based on the capacity of living cells to reduce MTT to formazan. The produced quantity of formazan (OD at 540 nm) is directly proportional to the number of living cells. Cell viability of the prodrugs, as estimated by the CC_{50} values, was measured after 5 days of incubation at 37 °C with various concentrations of the tested product. The prodrug CC_{50} values were determined from the curves of the % of living cells against prodrug concentration.

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References

1. Gulick, R. M. In *Protease Inhibitors in AIDS Therapy*; Ogden, R. C.; Flexner, C. W., Eds. Protease Inhibitors: Clinical Efficacy; Marcel Dekker: New York, 2001; pp. 179–236 and references therein.
2. Chun, T. W.; Stuyver, L.; Mizell, S. B.; Ehler, L. A.; Mican, J. A. M.; Baseler, M.; Lloyd, A. L.; Nowak, M. A.; Fauci, A. S. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3193–3197.
3. Pialoux, G.; Fournier, S.; Moulignier, A.; Poveda, J. D.; Clavel, F.; Dupont, B. *AIDS* **1997**, *11*, 1302–1303.
4. Flexner, C. W. In *Protease Inhibitors in AIDS Therapy*; Ogden, R. C.; Flexner, C. W., Eds. Pharmacology and Drug Interactions of HIV Protease Inhibitors; Marcel Dekker: New York, 2001; pp. 139–159 and references therein.
5. Pardridge, W. M. *Adv. Drug Deliv. Rev.* **1995**, *15*, 5–36.
6. Halmos, T.; Santarromana, M.; Antonakis, K.; Scherman, D. *Eur. J. Pharmacol.* **1996**, *318*, 477–484.
7. Bilsky, E. J.; Egleton, R. D.; Mitchell, S. A.; Palian, M. M.; Davis, P.; Huber, J. D.; Jones, H.; Yamamura, H. I.; Janders, J.; Davis, T. P.; Porreca, F.; Hruby, V. J.; Polt, R. *J. Med. Chem.* **2000**, *43*, 2586–2590.
8. Battaglia, G.; La Russa, M.; Bruno, V.; Arenare, L.; Ippolito, R.; Copani, A.; Bonina, F.; Nicoletti, F. *Brain Res.* **2000**, *860*, 149–156.
9. Vlieghe, P.; Bihel, F.; Clerc, T.; Pannecouque, C.; Witvrouw, M.; De Clercq, E.; Salles, J. P.; Chermann, J. C.; Kraus, J. L. *J. Med. Chem.* **2001**, *44*, 777–786.
10. Jolliet-Riant, P.; Tillement, J.-P. *Fundam. Clin. Pharmacol.* **1999**, *13*, 16–26.
11. Farese-Di Giorgio, A.; Rouquayrol, M.; Greiner, J.; Aubertin, A.-M.; Vierling, P.; Guedj, R. *Antivir. Chem. Chemother.* **2000**, *11*, 97–110.
12. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer Verlag: Berlin, 1984.
13. Christensen, J. E.; Goodman, L. *Carbohydr. Res.* **1968**, *7*, 510–512.

14. Manfredi, A.; Abouhilale, S.; Greiner, J.; Riess, J. G. *Bull. Soc. Chim. Fr.* **1989**, 872–878.
15. Sen, S. E.; Roach, S. L.; Boggs, J. K.; Ewing, G. J.; Magrath, J. *J. Org. Chem.* **1997**, *62*, 6684–6686.
16. Schmidt, O. T. *Methods Carbohydr. Chem.* **1963**, *2*, 318–325.
17. Jeric, I.; Horvat, S. *Eur. J. Org. Chem.* **2001**, 1533–1539.
18. Duggan, M. E.; Imagire, J. S. *Synthesis* **1989**, 131–132.
19. Satchell, D. P. N.; Satchell, R. S. *Chem. Soc. Rev.* **1975**, *4*, 231–250.
20. Hofle, G.; Steglich, W.; Vorbruggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.
21. Hauser, F. M.; Rhee, R. P.; Ellenberger, S. R. *J. Org. Chem.* **1984**, *49*, 2236–2240.
22. Ibuka, T.; Chu, G. N.; Aoyagi, T.; Kitada, K.; Tsukida, T.; Yoneda, F. *Chem. Pharm. Bull.* **1985**, *33*, 451–453.
23. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
24. Breitmaier, E.; Voelter, W. *^{13}C NMR Spectroscopy*; VCH: Weinheim, 1987.
25. Hesse, M.; Meier, H.; Zeeh, B. *Spectroscopic Methods in Organic Chemistry*; Thieme: Stuttgart, 1995.
26. Chrusciel, R. A.; Bauer, L.; Kaminski, J. M.; Zaneveld, L. J. D. *Tetrahedron* **1991**, *47*, 8831–8854.
27. Majumdar, S.; Bhattachaijya, A. *J. Org. Chem.* **1999**, *64*, 5682–5685.
28. Lwowski, W. In *The Chemistry of the Azido Group*; Patai, S., Ed. Acyl Azides; Interscience: New York, 1971; pp. 503–554.
29. Weinstock, J. *J. Org. Chem.* **1961**, *26*, 3511.
30. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309–321.
31. Génu-Dellac, C.; Gosselin, G.; Puech, F.; Henry, J. C.; Aubertin, A-M.; Aubert, G.; Kirn, A.; Imbach, J.-L. *Nucleosides Nucleotides* **1991**, *10*, 1345–1376.
32. Moog, C.; Wick, A.; Le Ber, P.; Kirn, A.; Aubertin, A-M. *Antivir. Res.* **1994**, *24*, 275–288.
33. West, M. L; Fairlie, D. P. *Trends Pharmacol. Sci.* **1995**, *16*, 67–75.
34. Silverman, R. B. In *The Organic Chemistry of Drugs Design and Drug Action*. Prodrugs and Drug Delivery Systems; Academic Press: San Diego, 1992; pp. 352–401.