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Prodrugs of HIV protease inhibitors—saquinavir, indinavir and nelfinavir—derived from diglycerides or amino acids: synthesis, stability and anti-HIV activity †

Bérangère Gaucher,[a] Marielle Rouquayrol,[a] Dominique Roche,[a] Jacques Greiner,[a] Anne-Marie Aubertin[b] and Pierre Vierling*[a]

[a] *Laboratoire de Chimie Bioorganique, UMR 6001 CNRS, Université de Nice Sophia-Antipolis, Parc Valrose, 06108 Nice Cédex 2, France. E-mail: vierling@unice.fr; Fax: (33) (0)4 92 07 61 51; Tel: (33) (0)4 92 07 61 43*

[b] *INSERM U74, Institut de Virologie, 3 rue Koeberlé, 67000 Strasbourg, France*

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With the aim of improving the pharmacological properties of current protease inhibitors (PIs), the synthesis of various acyl and carbamate amino acid- or diglyceride-containing prodrugs derived from saquinavir, indinavir and nelfinavir, their *in vitro* stability with respect to hydrolysis and their anti-HIV activity in CEM-SS and MT4 cells have been investigated. L-Leucine (Leu) and L-phenylalanine (Phe) were connected through their carboxyl to the PIs while L-tyrosine (Tyr) was conjugated through its aromatic hydroxyl *via* various spacer units. Hydrolysis of the prodrug with liberation of the active free drug was crucial for antiviral activity. The Leu- and Phe-PI prodrugs released the active free drug very rapidly (half-lives of hydrolysis in buffer at 37 °C of 3–4 h). The Tyr-PI conjugates with a $-C(O)(CH_2)_4-$ linker exhibited half-lives in the 40–70 h range and antiviral activities in the 21–325 nM range (from 2 to 22 nM for the free PIs). The chemically very stable carbamate "peptidomimetic" Tyr-PI prodrugs (no hydrolysis detected after 7 days in buffer) displayed a very low anti-HIV activity or were even inactive (EC_{50} from 2300 nM to >10 μM). A very low antiviral activity was measured for the diglyceride-substituted saquinavir and for all of the disubstituted indinavir and nelfinavir prodrugs. All these prodrugs probably released the active parent PI too slowly under the antiviral assay conditions. These results combined with those from transepithelial transport studies (Rouquayrol *et al.*, *Pharm. Res.*, 2002, **19**, 1704–1712) indicate that conjugation of amino acids (through their carboxyl) to the PIs constitutes a most appealing alternative which could improve the intestinal absorption of the PIs and reduce their recognition by efflux carriers.

Introduction

Highly active antiretroviral therapies (HAART) involving combinations of HIV protease inhibitors (PIs) and reverse transcriptase inhibitors have dramatically improved the treatment of AIDS and HIV-1 infection. Although viral RNA is not detectable in plasma, replication of the virus still continues despite these HAARTs, indicating, among others, the existence of reservoirs or sanctuaries for the virus, such as the lymphatic and central nervous system (CNS), in which the antivirals do not penetrate at an efficient inhibitory level or do not penetrate at all.[1–3] The low penetration into the CNS of more particularly the current PIs used in clinical trials is worth noting. Most of these PIs display further disadvantageous physicochemical and pharmacological properties. To overcome these suboptimal pharmacokinetics, high daily doses must be ingested, often with food and fluid restrictions. This complicates patient adherence to the prescribed regimen, and contributes to resistance issues and to the appearance of serious long-term metabolic complications, such as cardiovascular disturbances, hyperlipidemia, lipodystrophy, insulin resistance, osteopenia, and diabetes,[4–8] and to lower the viral treatment outcome.[9]

As HIV has so far proved to be intractable to the vaccine approach,[10] and as HIV strains have emerged that are resistant to the available drugs, the search for new antivirals must continue.[11] Another attractive alternative to reduce viral replication with the current FDA approved PIs (indinavir, saquinavir, ritonavir, nelfinavir, amprenavir, lopinavir) and their metabolic complications is to modify these PIs with a view to improve their short- and long-term safety, their pharmacokinetic profiles, and, consequently, their therapeutic potency. This generation of "modified" PIs should display a higher water solubility, an increased bioavailability (plasma concentration, blood circulation time), and/or an improved delivery into HIV sanctuaries.[12] One should further be able with such "modified" PIs to inhibit their possible transport by the multi-drug-resistant P-glycoprotein (P-gp) responsible for their limited oral bioavailability and brain penetration.[9]

Aiming at these goals, different strategies which have been used with some success for several drugs may be applied to the current PIs. The use of colloidal drug carrier and targeting systems, such as liposomes or nanoparticles,[13] and the "prodrug" approach constitute the most popular strategies.[12] The prodrug approach is indeed widely used to obtain enhanced oral delivery of poorly membrane permeable compounds.[14] Potential approaches for facilitating the delivery of the current PIs could involve modulation of their lipophilic/hydrophilic balance or the utilisation of the body's own nutrient (*e.g.* D-glucose, amino acids) carrier-mediated transport systems for increasing their passive or active transport across cell membranes, the intestinal and/or the blood–brain barriers.[15] This could potentially be achieved by conjugation of the PIs to fatty acid chains, glycerides, amino acids or D-glucose. The connection of these moieties to the PIs must further be performed through *in vivo* labile functions. The prodrug being not pharmacologically active, the critical requirement for the prodrug strategy is to conjugate the various substituents to the

† Electronic supplementary information (ESI) available: description of the synthesis and of the analytical data of the starting material *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu and *N*-Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu, and of all the protected amino acid-derived prodrugs. See http://www.rsc.org/suppdata/ob/b3/b313119j/

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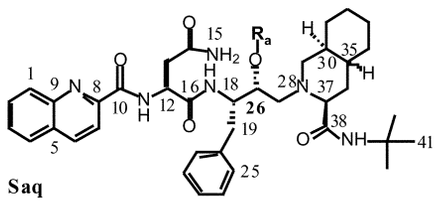
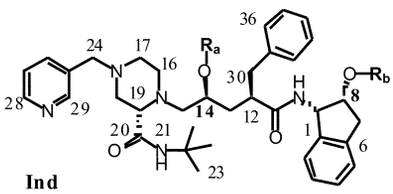
PROTEASE INHIBITORS (PI)



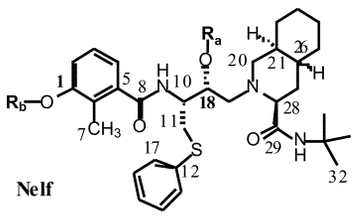
Saq

R_a = H (saquinavir)



Ind

R_a = H; R_b = H (indinavir)



Nelf

R_a = H; R_b = H (nelfinavir)

FIRST GENERATION of PI PRODRUGS

R_a and/or Rb =

-C(O)CH(CHMe$_2$)NH$_2$ **PI-Val**: PI = Saq, Ind(8), Ind(14)

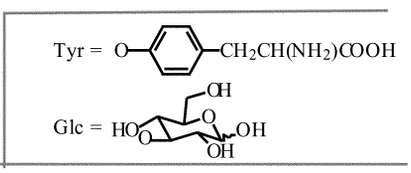
-C(O)CH$_2$-Tyr **PI-C(O)C1Tyr**: PI = Saq, Ind(14)

-C(O)(CH$_2$)$_7$CH=CH(CH$_2$)$_7$CH$_3$ **PI-Oleoyl**: PI = Saq, Ind(8)

-C(O)(CH$_2$)$_{12}$CH$_3$

-C(O)(CH$_2$)$_2$C(O)(OC$_2$H$_4$)$_n$OCH$_3$ (n = 7, 44; PEG)

-C(O)NH(CH$_2$)$_4$Glc



SECOND GENERATION of PI PRODRUGS (this work)

Ester PI-prodrugs

R_a and/or R_b =

-C(O)CH(CH$_2$CHMe$_2$)NH$_2$ **PI-Leu**: PI = Saq, Ind(8)

-C(O)CH(CH$_2$Ph)NH$_2$ **PI-Phe**: PI = Saq, Ind(8)

-C(O)(CH$_2$)$_n$-Tyr n = 1 or 4 **PI-C(O)CnTyr**: PI = Saq (n = 4), Ind(8) (n = 1 or 4), Ind(14) (n = 4), Nelf(1) (n = 4)

PI-[C(O)CnTyr]2: PI = Ind (n = 4)

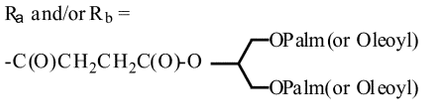
Carbamate PI-prodrugs

R_a =

-C(O)NH(CH$_2$)$_4$-Tyr **PI-C(O)NC4Tyr**: PI = Saq, Ind(14), Nelf(18)

Glyceride PI-prodrugs

R_a and/or R_b =



Palm = -C(O)(CH$_2$)$_{14}$CH$_3$

Oleoyl = -C(O)(CH$_2$)$_7$CH=CH(CH$_2$)$_7$CH$_3$

PI-C(O)C2C(O)GlyPalm
PI-C(O)C2C(O)GlyOleoyl
PI = Saq, Ind(8)

PI-[C(O)C2C(O)GlyPalm]2
PI-[C(O)C2C(O)GlyOleoyl]2
PI = Ind

Fig. 1 Chemical structures and code names of the protease inhibitor (PI) prodrugs used in this study and atom numbering of the PIs used in the description of their NMR spectra. All amino acids are of L-configuration. The first generation PI prodrugs are described in refs. 16 and 17.

parent drug in a bioreversible manner. Among the strategies explored up to now, the most successful one was the "hydrophilic" prodrug approach which has led to the discovery of fosamprenavir, a water-soluble phosphate ester prodrug of the sparingly water-soluble amprenavir, which has reached phase III clinical trials (for a review, see ref. 12). This success gives strong support for the search of PI prodrugs as a therapeutic alternative in addition to the development of new and well-tolerated PIs.

As part of our contribution into this field, our laboratory has reported on various ester PI prodrugs derived from saquinavir, indinavir or nelfinavir connected to fatty acids, L-valine, L-tyrosine, polyethylene glycol (PEG), or D-glucose (first generation of PI prodrugs in Fig. 1).[16,17] The most encouraging results obtained with the L-valine PI prodrugs[18] prompted us to extend this series to L-leucine and L-phenylalanine which, as L-valine, are known as being involved too in carrier-mediated transport systems. In continuation of these works, we also extended these series to new ester PI prodrugs that derived from L-tyrosine or that are linked to diglycerides (Fig. 1). Concerning the tyrosine derivatives, we introduced different spacer units, and extended these series to nelfinavir and to chemically more stable carbamate PI prodrugs. The spacer and/or carbamate modifications were undertaken with a view to enhance the chemical stability of the first generation of tyrosine ester PI prodrugs.[16] Concerning more particularly the "glyceride" approach, this strategy is known to facilitate the delivery of drugs after oral administration from the gastrointestinal tract directly into the lymphatic system,[19–21] which is a viral reservoir.[22] This approach, which allows to bypass the plasma protein inactivation and first-pass hepatic metabolism, is therefore particularly attractive for the HIV PIs. Moreover, conjugation of the PIs to glycerides could also improve their brain delivery.[23]

We describe here the synthesis of these various new amino acid- and glyceride- containing prodrugs which derived from saquinavir, indinavir or nelfinavir (second generation of PI prodrugs in Fig. 1). We report also on the chemical stability with respect to hydrolysis under physiological conditions and on the *in vitro* anti-HIV activity of some selected prodrugs. Permeation studies across polarised monolayers formed of the human intestinal Caco-2 cells (which are widely approved models of the intestinal epithelium) were also performed with several of the PI prodrugs reported here. This screening, which was aimed at evaluating drug absorption, selecting and detecting the most promising prodrugs that could improve the intestinal and/or CNS absorption of the parent PIs, reduce their recognition by the efflux P-gp carrier,[24–26] and for further *in vivo* studies, was reported and discussed elsewhere.[18] The results from this study are however very briefly presented here.

Results

Synthesis

The synthesis of the ester saquinavir- and indinavir-glyceride, -Leu or -Phe prodrugs (Fig. 2) was performed by acylation of

the PIs with the appropriate glyceride-containing succinic acid derivatives (*i.e.* HOC(O)C2C(O)GlyPalm and HOC(O)C-2C(O)GlyOleoyl), or *N*-Boc protected amino acids which were activated using EDC/DMAP. This step was followed in the case of the amino acid derivatives by the quantitative Boc-deprotection using CF_3CO_2H (TFA) in CH_2Cl_2. The synthesis of the various ester PI-C(O)C1Tyr or PI-C(O)C4Tyr prodrugs (PI = saquinavir, indinavir or nelfinavir) (Fig. 3A) was achieved following a similar procedure but using *N*-Boc-Tyr(OCH_2CO-OH)-O^{t}Bu or *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu as starting materials, respectively. The one-step *N*-Boc- and *O*-^{t}Bu-deprotection of tyrosine was also performed with TFA in yields ranging from 34 to 99%.

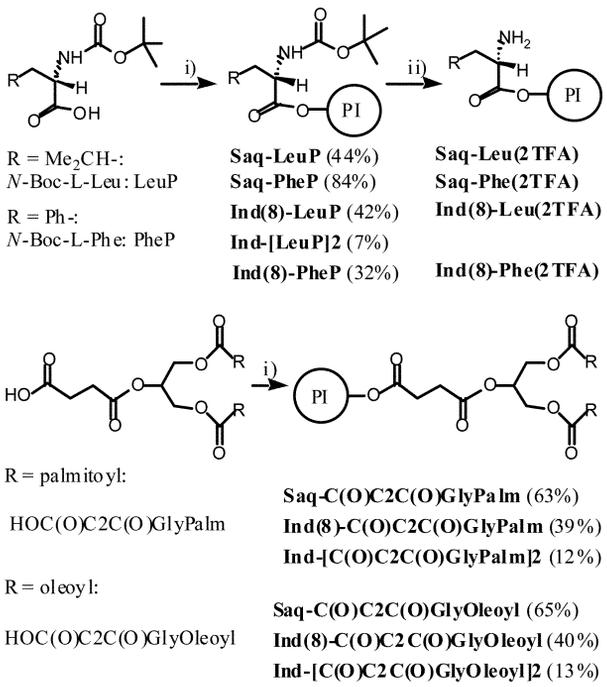


Fig. 2 Synthetic pathway to the ester PI prodrugs derived from L-leucine, L-phenylalanine or from 1,3-diglycerides: i) PI (= Saq or Ind)/EDC/DMAP, CH_2Cl_2 or DMF; ii) CF_3CO_2H (TFA)/CH_2Cl_2, quantitative. Mono- and di-esters of indinavir are obtained during the same reaction. [palmitoyl = $CH_3(CH_2)_{14}$-; oleoyl = (*Z*) $CH_3(CH_2)_7CH$=$CH(CH_2)_7$-].

The acylation of the unique hydroxyl of saquinavir was achieved in yields ranging from 44 to 84%. In the case of indinavir, esterification of its C-8 hydroxyl occurred mainly when the reaction was performed with one equivalent of acid (yields ranging from 18 to 42%). Besides these "C-8" monoester derivatives, the "C-14" monoester and the C-8/C-14 diester derivatives were also detected, though the diester Ind[C(O)C4TyrP]2 was obtained in much larger amounts (22%) than the other diester derivatives (≤ 13%). In the case of nelfinavir, and with one equivalent of acid, one observed mainly esterification of the C-1 hydroxyl (36%), the monoester "C-18" and diester derivatives being isolated in much lower amounts (yields ≤ 3%).

The carbamate PI-C(O)NC4Tyr prodrugs (PI = saquinavir, indinavir or nelfinavir) (Fig. 3B) were obtained in 31 to 73% yields using a procedure that was described for the synthesis of carbamate glucose-conjugated PIs.[17] This procedure consisted of the condensation of the appropriate PI with the isocyanate *N*-Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu in the presence of a catalytic amount of CuCl. This condensation was then followed by the one-step *N*-Boc- and *O*-^{t}Bu-deprotection of tyrosine which was also performed with TFA (yields from 25 to 64%). In the case of the bis-hydroxyl indinavir and nelfinavir, the condensation step was performed with one equivalent of isocyanate. Under these conditions, the reaction of the C-14 indinavir (31%) and C-18 nelfinavir (73%) hydroxyl with the isocyanate function occurred almost selectively.

The acid *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu starting material (Fig. 3) was obtained by condensation of ethyl 5-bromopentanoate on *N*-Boc-Tyr(OH)-O^{t}Bu followed by base hydrolysis. Its isocyanate *N*-Boc-Tyr[$O(CH_2)_4NCO$]O^{t}Bu derivative was prepared in two steps (85% overall yield) from acid *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu using a conventional two-step

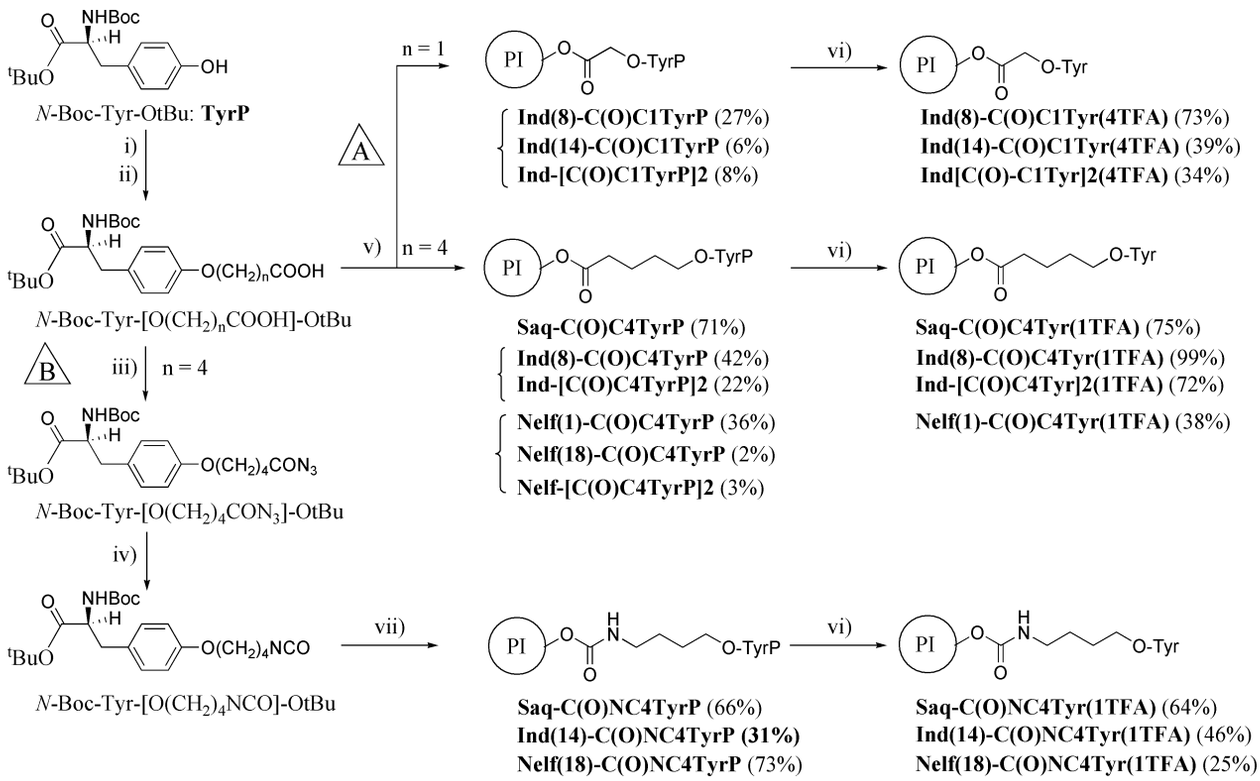


Fig. 3 Synthetic pathway to the (A) ester and (B) carbamate PI prodrugs derived from L-tyrosine: i) $Br(CH_2)_nCO_2Et$ (n = 1 or 4), K_2CO_3, DMF; ii) LiOH, THF; iii) EtOC(O)Cl, NaN_3, acetone; iv) toluene, Δ; v) PI (= Saq, Ind or Nelf)/EDC/DMAP, CH_2Cl_2 or DMF; vi) CF_3CO_2H (TFA)/CH_2Cl_2; vii) PI (= Saq, Ind, or Nelf), CuCl, CH_2Cl_2 or DMF.

procedure consisting of the formation of the acyl azide *N*-Boc-Tyr[O(CH_2)$_4$$CON_3$]-O^{t}Bu and then a Curtius rearrangement of this fairly stable azide.[27] The acyl azide was produced by reacting NaN_3 with the mixed anhydride prepared *in situ* from *N*-Boc-Tyr[O(CH_2)$_4$$CO_2$H]-O^{t}Bu and ethyl chloroformate.[28] Azide *N*-Boc-Tyr[O(CH_2)$_4$$CON_3$]-O^{t}Bu could be only be recovered contaminated by the isocyanate *N*-Boc-Tyr[O(CH_2)$_4$NCO]O^{t}Bu. This was attested by ^{1}H and ^{13}C NMR which showed the presence of the methylene CH_2NCO signals in addition to the methylene $CH_2C(O)N_3$ ones.

The ^{1}H and ^{13}C NMR spectra of the various PI prodrugs are in full agreement with the proposed structures. Acylation of the C-26 hydroxyl of saquinavir is more particularly attested by the deshielding of H26 ($|\Delta\delta|$ = 1.13–1.45 ppm) and C26 ($|\Delta\delta|$ = 6.3–9.4 ppm), as expected.[16,17,29,30] 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, is unambiguously attested by ^{13}C NMR. Except for the C-1-substituted nelfinavir derivatives, the resonances of these carbon atoms and of their vicinal carbon atoms are deshielded ($|\Delta\delta|$ = 1.6–7.2 ppm) and shielded ($|\Delta\delta|$ = 0–4.9 ppm), respectively, in comparison with those of the parent PI. For the C-1-substituted nelfinavir derivatives and as expected for phenyl esters,[31,32] the opposite trends, *i.e.* shielding ($|\Delta\delta|$ = 5.5–5.7 ppm) of the aromatic C-1 carbon atom and deshielding of the vicinal carbon atoms ($|\Delta\delta|$ = 5.9–9.3 ppm) with respect to nelfinavir, are observed. Furthermore, the resonances of the carbon atom bearing the remaining hydroxyl group and of its vicinal carbon atoms are almost not affected.[29,30] For the di-esters of indinavir and nelfinavir, acylation of the two hydroxyls of these PIs is confirmed by the shielding of both the C14 and C8 carbons of indinavir and of the C18 carbon of nelfinavir, and by the deshielding of the C1 carbon of nelfinavir.

Chemical stability

The sensitivity to hydrolysis of some of the ester and carbamate prodrugs was checked by incubating the selected prodrug in a pH 7.3 buffer at 37 °C and in the absence of serum in order to facilitate the detection and analyses by HPLC.[16,17] This is an important issue to consider for further *in vitro* and *in vivo* investigations and for the evaluation of their antiviral activity (which reflects the release rate of the active parent drug, see next section). If the nature of the incubation medium has an impact on the hydrolysis rates of the prodrugs (which are most probably faster in the biological medium owing to the presence of esterases), one can reasonably assume that it should not affect the sequence of stability found for the prodrugs. The prodrug hydrolysis half-lives, which were measured or calculated from the time course of prodrug disappearance and/or drug appearance are collected in Table 1. The ester amino acid-PI conjugates wherein the Phe or Leu amino acid was linked through its acid function to the PIs display half-lives which do not exceed 4 h. The Tyr conjugates PI-C(O)C4Tyr(*n*TFA) which are linked to the PIs through the aromatic hydroxyl and a C(O)(CH_2)$_4$ spacer were found to be very stable with half-lives in the 50–70 h range. These Tyr conjugates are particularly more stable than the Saq-C(O)C1Tyr(3TFA) or Ind(14)-C(O)C1Tyr(4TFA) analogues of the first generation which posses a shorter C(O)CH_2 spacer (half-life of 1.5 and 3.3 h, respectively).[16] Concerning the three carbamate-linked Tyr-PI prodrugs, Saq-C(O)NC4Tyr(1TFA), Ind(14)-C(O)NC4Tyr-(1TFA), and Nelf(18)-C(O)NC4Tyr(1TFA), no hydrolysis was detected after 7 days of incubation. The chemical stability of the glyceride-PI conjugates could not be investigated using this protocol owing to their extremely low aqueous solubility.

Antiviral activity

The HIV inhibition levels and cytotoxicities of some of the amino acid- and glyceride-containing saquinavir, indinavir, and nelfinavir prodrugs were evaluated *in vitro* in CEM-SS and MT4 cells against HIV-1 according to published procedures.[33–35] The EC_{50} data are collected in Table 1 together with those of their parent PI and of some of the first generation of PI prodrugs. In these antiviral assays, the antiviral activity levels measured reflect to some extent the *in vivo* release rate of the parent PI from the prodrugs which were incubated for 4 days at 37 °C in the culture medium that contains serum, virus and cells. These data will be presented and discussed in the next section. No cytotoxicity (CC_{50} > 100 μM, data not shown) was detected for any of these new prodrugs.

Discussion

In previous studies from our laboratory,[16–18] the indinavir, saquinavir, and nelfinavir PIs were conjugated to long chain fatty acids, L-valine, L-tyrosine, D-glucose or hydrophilic polyethylene glycol polymers with the aim of facilitating their passive or active diffusion across the physiological barriers, or avoiding their binding to plasma proteins and their inactivation and rapid elimination from the blood circulation, respectively. These studies have more particularly shown that conjugation of L-valine to the PIs led to most promising PI prodrugs. Indeed, and as indicated by the absorption apparent permeability coefficients collected in Table 1, conjugation of the PIs to L-valine resulted into a 2- to 6-fold increase of the absorption of PI-equivalents across a Caco-2 cell monolayers used as a model of the intestinal epithelium.[18] These studies have also shown that the chemical stability of the first generation of L-tyrosine-PI conjugates (*i.e.* Saq-C(O)C1Tyr(3TFA) and Ind(14)-C(O)-C1Tyr(4TFA) which displayed half-lives of hydrolysis of 1.5 and 3.3 h, respectively) should be improved.[16] All these results prompted us (i) to extend these series to other amino acids such as L-phenylalanine and L-leucine (which are also actively transported across biological barriers by the nutrient carrier-mediated transport systems), and (ii) to modify the chemical nature (length and carbamate connecting function) of the spacer linking L-tyrosine to the PIs. By contrast to L-valine, L-phenylalanine and L-leucine which were conjugated to the PIs through their carboxyl, L-tyrosine, which is actively transported across the blood-brain barrier by the large amino acid transporter system, was connected through its aromatic hydroxyl to the PIs in order to preserve its recognition and transport capability by this transporter.[36,37] To improve the uptake of the PIs from the gastrointestinal tract directly into the lymphatic system,[19–21] which is a viral reservoir,[1] we extended the PI prodrug series to 1,3-diglyceride-PI conjugates containing palmitoyl or oleoyl chains, these 1,3-diglycerides being linked to the PIs through an ester bond by means of a succinyl connecting unit.

For the ester PI-conjugates, the conjugation of the various acid functionalities (1,3-diglyceride-succinic acid, L-Leu, L-Phe, Boc-Tyr[O(CH_2)$_n$COOH]-O^{t}Bu with n = 1 or 4) to saquinavir, indinavir or nelfinavir was performed using a conventional one-step (acylation) or two-step process (acylation and deprotection) which was applied to the synthesis of the first generation of PI prodrugs.[16] In the case of indinavir or nelfinavir, which possesses two hydroxyls, the synthesis of the mono-ester indinavir C-8 and nelfinavir C-1 conjugates was performed in a non-specific manner, *i.e.* directly from the non-protected indinavir or nelfinavir, the different mono- and di-ester conjugates being separated by column chromatography. Indeed, when one equivalent of the acid derivative was used, one observed mainly the esterification of the more reactive indinavir C-8 hydroxyl or nelfinavir C-1 hydroxyl. It should be noted that the non-specific two-step process (acylation then deprotection) we used here for the ester-indinavir conjugates gave almost the same overall yields of the monoester compound than a more selective three-step synthesis which implies a protection step of one of the two reactive hydroxyls.[16]

Table 1 Anti-HIV activity (EC_{50}) data for saquinavir, indinavir, and nelfinavir prodrugs[a] in CEM-SS and MT4 cell cultures infected by HIV-1 LAI and HTLV IIIB, respectively, together with their half-life of hydrolysis ($t_{1/2}$)[b] and apparent permeability coefficient[c] measured from transepithelial transport experiments across a monolayer of Caco-2 cells

	EC_{50}(nM)			Permeability coefficient (± SD) (cm $s^{-1} \times 10^{-8}$)[c]	
Compound	CEM-SS	MT4	$t_{1/2}$	Secretion	Absorption
Saquinavir ($MeSO_3H$)	9	18		398 (117)	110 (31)
Saq-Oleoyl[d]	1300	2700	>30 days		
Saq-C(O)C2C(O)GlyPalm[c]	110	3500			
Saq-C(O)C2C(O)GlyOleoyl[c]	800	1700			
Saq-Val(3TFA)[d]	140	290	40 h	343 (22)	232 (22)
Saq-PheP	28	197			
Saq-Phe(2TFA)	nt[f]	nt[f]	3 h	840 (313)	332 (31)
Saq-LeuP	137	485			
Saq-Leu(2TFA)	nt[f]	nt[f]	4 h	428 (25)	265 (0)
Saq-C(O)C1Tyr(3TFA)[d]	33	85	1.5 h		
Saq-C(O)C4Tyr(1TFA)	39	160	40 h		
Saq-C(O)NC4Tyr(1TFA)	2300	6000	>7 days	nd	nd
Indinavir (H_2SO_4)	≤10	22		1550 (234)	103 (10)
Ind(8)-Oleoyl[d]	110	250	24 days		
Ind(8)-C(O)C2C(O)GlyPalm[c]	2600	5300			
Ind-[C(O)C2C(O)GlyPalm]2[c]	4600	$>10^4$			
Ind(8)-C(O)C2C(O)GlyOleoyl[c]	2100	6900			
Ind-[C(O)C2C(O)GlyOleoyl]2[c]	$>10^4$	$>10^4$			
Ind(8)-Val(4TFA)	17[d]	150[d]	46 h	737 (26)	664 (44)
Ind(14)-Val(4TFA)	90[d]	27[d]	68 h	708 (147)	513 (50)
Ind(8)-PheP	430	1830			
Ind(8)-Phe(2TFA)	nt[f]	nt[f]	4 h		
Ind-LeuP	685	1800			
Ind(8)-Leu(2TFA)	nt[f]	nt[f]	4 h		
Ind(14)-C(O)C1Tyr(4TFA)[d]	10	80	3.3 h	162 (26)	44 (6)
Ind(8)-C(O)C4TyrP	220	325			
Ind(8)-C(O)C4Tyr(1TFA)	9	47	50 h	nd[g]	nd[g]
Ind-[C(O)C4TyrP]2	$>10^3$	$>10^3$			
Ind-[C(O)C4Tyr]2(1TFA)	810	1400	70 h	nd[g]	nd[g]
Ind(14)-C(O)NC4Tyr(1TFA)	6400	12000	>7 days	nd[g]	nd[g]
Nelfinavir ($MeSO_3H$)[e]	2			251 (68)	103 (66)
Nelf(18)-C(O)C4TyrP	540	2050			
Nelf(1)-C(O)C4TyrP	29	116			
Nelf(1)-C(O)C4Tyr(1TFA)	21	193	60 h	nd[g]	nd[g]
Nelf-[C(O)C4TyrP]2	2200	4700			
Nelf-[C(O)C4Tyr]2	nt[f]	nt[f]		nd[g]	nd[g]
Nelf(18)-C(O)NC4Tyr(1TFA)	$>10^4$	$>10^4$	>7 days	nd[g]	nd[g]

[a] These data reflect the amount of parent drug that was released during the 4 day's time-span of the experiments (see discussion). [b] $t_{1/2}$, which corresponds to the time at which 50% of hydrolysis was observed, was determined from hydrolysis experiments performed by incubating the prodrugs in a pH 7.3 DMEM/MeOH solution at 37 °C (for the amount of MeOH, see Materials and methods section). [c] From ref. 18; the absorption and secretion permeability coefficients correspond to the absorptive (from apical to basolateral compartment) and secretory (from basolateral to apical) translocation across the Caco-2 monolayer, respectively. Owing to the very poor aqueous solubility of the diglyceride conjugates, their translocation could not be investigated. [d] From ref. 16. [e] From ref. 48. [f] nt: not tested. [g] nd: not detected; neither the prodrug nor the parent drug was detected in the acceptor chamber, *i.e.* below the detection limit which is 0.1 μM, 0.4 μM, and 0.08 μM for the saquinavir, indinavir, and nelfinavir derivatives, respectively.

Concerning the synthesis of the carbamate tyrosine-based PI prodrugs (*i.e.* Saq-C(O)NC4TyrP, Ind(14)-C(O)NC4TyrP, and Nelf(18)-C(O)NC4TyrP), these derivatives were best obtained by reacting the respective PI with the isocyanate *N*-Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu in the presence of a catalytic amount of CuCl, as previously described for the synthesis of carbamate glucose-conjugated PIs.[17] No reaction occurred between the PIs and the isocyanate *N*-Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu using conventional condensation procedures,[38] such as the base-catalyzed addition with DMAP,[39] Et_3N, or pyridine,[40] or the acid-catalyzed addition with $BF_3 \cdot Et_2O$.[41] In the case of the bis-hydroxyl indinavir and nelfinavir, the reactions were further performed with the non-protected PIs. One observed almost selectively the condensation of the indinavir C-14 and nelfinavir C-18 hydroxyl with the isocyanate function, as for the synthesis of carbamate glucosylated-indinavir prodrugs.[17] These data confirm the higher reactivity of the indinavir C-14 and nelfinavir C-18 hydroxyl for the carbamate formation which contrasts with that of the indinavir C-8 and nelfinavir C-1 hydroxyl for the ester formation. This regioselectivity could tentatively be attributed to the formation of a complex between Cu(I) and indinavir (or nelfinavir) activating the C-14 indinavir (or C-18 nelfinavir) hydroxyl.

The binding of diverse substituents has been performed onto the hydroxyl function of the PIs which is part (*i.e.* all Saq-, Ind(14)- and Nelf(18)-derivatives) or not (*i.e.* the mono-functionalized Ind(8)- and Nelf(1)-derivatives) of the peptido-mimetic noncleavable transition state isostere responsible for the HIV inhibitory potency of the parent PIs.[42] Thus, the prodrug concept concerns strictly all saquinavir prodrugs and all

indinavir C-14 and nelfinavir C-18 conjugates. This concept does apply successfully only if these hydroxyls become accessible and at a rate that is compatible with a pharmacological/therapeutic benefit as compared with the parent drug: a too rapid breakdown of the prodrug will not allow significant modulation of the bioavailability, biodisponibility and/or delivery of the parent drug, while a too slow release rate will compromise its inhibitory potency. The prodrug concept can also apply to the mono-substituted indinavir C-8 and nelfinavir C-1 conjugates if the substituent that has been introduced onto these positions must be cleaved for their recognition by the HIV protease and for recovering the antiviral activity of the parent PI. However, such conjugates can act as classical drugs if they remain substrates of the HIV protease, the indinavir C-8 and nelfinavir C-1 hydroxyls being indeed not involved in the key nonscissile transition state isostere.

In a previous study from this laboratory, a close correlation between antiviral activity level and hydrolysis rate was established for the PI prodrugs wherein the binding of the diverse substituents has been performed onto the unique saquinavir hydroxyl or C-14 indinavir hydroxyl: the faster the prodrug was hydrolysed, the closest was the measured EC_{50} value to that of the parent drug. Therefore, the antiviral activities of all PI-conjugates for which a very short half-life (<4 h) was measured in buffer were not investigated, these conjugates being likely hydrolysed even faster in the antiviral assay conditions (4 days of incubation at 37 °C in a medium that contains serum, cells and viruses). Conversely, the more stable the PI prodrugs were, the lower were the measured antiviral activities, as expected. In line with these results, the chemically very stable "peptidomimetic-carbamate" Tyr-PI prodrugs, *i.e.* Saq-C(O)-NC4Tyr(1TFA), Ind(14)-C(O)NC4Tyr(1TFA) and Nelf(18)-C(O)NC4Tyr(1TFA) (no hydrolysis detected after 7 days in buffer), were found to display a very low anti-HIV activity or even to be inactive (EC_{50} from 2300 nM to > 10^4 nM). A very low antiviral activity was also measured for the 1,3-diglyceride-substituted saquinavir and for all of the disubstituted indinavir and nelfinavir prodrugs. All these prodrugs release the active parent PI probably too slowly under the antiviral assay conditions.

For the mono-substituted indinavir C-8 and nelfinavir C-1 derivatives, we found that a relatively stable masking of the indinavir C-8 hydroxyl, as in Ind(8)-C(O)C4Tyr(1TFA), or of the nelfinavir C-1 hydroxyl, as in Nelf(1)-C(O)C4Tyr(1TFA), was not detrimental to their inhibitory potency, in line with our previous results concerning Ind(8)-Val(4TFA) and Ind(8)-Oleoyl. This is however not the case for the C-8 diglyceride conjugates. The very low anti-HIV activity found for these derivatives indicates that the active parent drug is likely to be released too slowly and that the prodrug itself is not an active anti-HIV drug.

Concerning the release rate of the parent drug from the ester amino acid-conjugates, the much lower chemical stability of the leucine- and phenylalanine-based prodrugs as compared with their valine analogues should be emphasized. Their half-lives were indeed in the 3–4 h range in comparison with the 40–70 h range for the valine-based prodrugs (Table 1). Electronic and/or steric effects may account for the increased stability of the ester bond in the valine conjugates. For the tyrosine-based prodrugs, lengthening the spacer from one methylene, as in Saq-C(O)-C1Tyr(3TFA) or Ind(14)-C(O)C1Tyr(4TFA), to four methylenes, as in Saq-C(O)C4Tyr(1TFA), Ind(8)-C(O)C4Tyr(1TFA) and Nelf(1)-C(O)C4Tyr (1TFA), resulted in a substantial (10- to 20-fold) increase of chemical stability. This increase of resistance to hydrolysis is related to the removal of the electro-attracting Tyr-O group from the ester-connecting bond. Although these latter prodrugs displayed half-lives in the 40–60 h range, their chemical stability remained compatible with a high antiviral activity (EC_{50} in the 9 to 40 nM and 47–193 nM range in CEM-SS and MT4 cells, respectively).

Importantly, conjugation of L-valine, L-leucine or L-phenylalanine (through their carboxyl) to the PIs was found to constitute a most appealing alternative which could improve the intestinal absorption of the PIs and reduce their recognition by efflux carriers. Indeed, the transepithelial transport studies of the L-amino acid-conjugated PIs across a Caco-2 cell monolayer showed that the L-valyl, L-phenylalanyl and L-leucyl esters of saquinavir (despite the extensive hydrolysis during the transport experiments of the two latter conjugates) display a 2- to 3-fold enhanced absorptive flux of saquinavir equivalents as compared with saquinavir (Table 1). An even larger enhancement (5- to 6-fold) was found upon conjugation of L-valine to indinavir.[18] These absorptive transport enhancements were attributed (i) to an active transport mechanism, indicating that these amino acid derivatives are substrates of the amino acid carriers located at the brush border side of the Caco-2 cell monolayer, and/or (ii) to a substantial decrease of their efflux owing to a lower affinity of the efflux carriers for these conjugates, and/or (iii) to an increase of passive diffusion. The lower affinity of the efflux carriers for these conjugates was more particularly supported by the similar absorptive and secretory flux found for the Val-indinavir prodrugs, and by the slightly asymmetric absorptive and secretory flux measured for the Val-, Leu- and Phe-saquinavir conjugates, by contrast to the highly asymmetric absorptive and secretory flux of their respective parent PI. These results are further of interest considering that drug translocation across Caco-2 monolayers is commonly assumed to be lower as compared to their transport across the human intestine.[43] The two- to six-fold absorption enhancement resulting from the conjugation of the PIs to these amino acids indicates that lower doses of PIs (as their conjugates) can potentially be administered orally to HIV(+) patients. To confirm that this approach effectively improves intestinal absorption remains to be explored by investigating the bioavailability after oral administration of these L-valine, L-leucine or L-phenylalanine PI prodrugs. These studies are in progress and will be reported in due course.

By contrast, conjugation of L-tyrosine through its aromatic hydroxyl to the PIs was found to inhibit their translocation across the Caco-2 cell monolayer: no PI or PI-conjugate was detected in the opposite chamber of the donor one containing any of the tyrosine-PI conjugates (Table 1).[18] These data were taken to indicate that translocation of the PIs across the human intestine will not be improved upon their conjugation to L-tyrosine. Whether it could improve their delivery into the CNS can however not be excluded and remains to be investigated.

Concerning the 1,3-diglyceride prodrugs, their transepithelial transport across a Caco-2 cell monolayer could unfortunately not be investigated using our experimental protocol owing to their very poor aqueous solubility; the maximum concentration in the donor chamber that could be attained was too low to allow their detection in the acceptor chamber. Whether conjugation of the PIs to diglycerides constitutes an approach to improve their uptake from the intestine directly into the lymphatic system remains however worth to be explored *in vivo*.

Materials and methods: chemistry

Unless otherwise indicated, the reactions were performed under anhydrous nitrogen using dry solvents and reagents. Anhydrous solvents (dimethylformamide (DMF), toluene and dichloromethane) and anhydrous triethylamine (Aldrich) were prepared by standard methods. Copper(I) chloride, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP), lithium hydroxide, sodium pentanesulfonate, anhydrous potassium carbonate, potassium hydrogen sulfate, sodium acetate, sodium azide, sodium carbonate, sodium chloride, sodium hydrogen carbonate, and sodium sulfate were purchased from Aldrich, and ethyl 5-bromopentanoate, ethyl chloroformate, and tri-

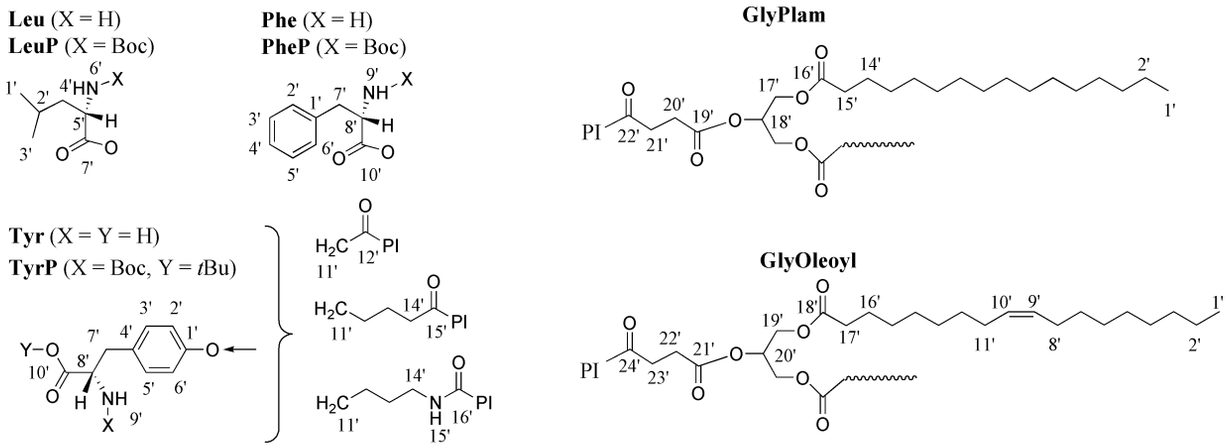


Fig. 4 Atom numbering of the substituent linked to the PI used for the description of the NMR spectra of the PI prodrugs.

fluoroacetic acid (TFA) from Fluka. *N*-α-*t*-Boc-L-phenylalanine, *N*-α-*t*-Boc-L-leucine, L-tyrosine *t*-butyl ester, and di-*tert*-butyl dicarbonate came from Novabiochem. All these materials were used without further purification. Saquinavir, indinavir and nelfinavir (as their methanesulfonate salt or sulfate salt) were a gift from Hoffmann-La Roche, Merck, and Agouron, respectively, and were deprotonated prior to their use in the synthetic processes ($CHCl_3$ or AcOEt extraction of the free base from a $NaHCO_3$ or Na_2CO_3 10% solution of the PI). Boc-Tyr(OCH_2COOH)-O^{t}Bu was prepared, as previously described.[16] The glyceride derivatives HOC(O)C2C(O)GlyPalm and HOC(O)C2C(O)GlyOleoyl were prepared from 1,3-dihydroxyacetone according to published procedures.[44–47]

If not specified, column chromatography purifications were carried out on Silica Gel 60 (Merck, 70–230 mesh). The purity of all new compounds was checked by TLC, HPLC and NMR. TLC analyses were performed on precoated Silica Gel F254 plates (Merck) with detection by UV, ninhydrin and/or 1 : 1 H_2SO_4/MeOH. HPLC analyses (flow of 1 mL min^{-1}) were performed using a HP1100 apparatus using a Lichrospher 100 RP-18 (5 μm)-packed column (250 × 4 mm) (column I) or Lichrospher 100 RP-18 (5 μm) column (250 × 3.2 mm) (column II) and H_2O/CH_3CN (v : v) 0.1% TFA gradient as eluent (solvent A: from 80 : 20 to 0 : 100 over 30 min; solvent B: from 70 : 30 to 0 : 100 over 30 min; UV detection) or isocratic H_2O (15 mM sodium acetate and 15 mM sodium pentanesulfonate)/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 (t_R) of indinavir and saquinavir are of 10.7 and 16.6 min, respectively, while with column I and solvent E, t_R of nelfinavir is of 12.9 min.

^{1}H, ^{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 indirectly to the signal of $CHCl_3$ (δ 7.27 for ^{1}H and δ 76.9 for ^{13}C), and to internal reference $CFCl_3$ for ^{19}F. The coupling constants are given in Hz. Concerning the description of the NMR spectra, the atoms of the PI part are depicted as Hx and Cy, those of the substituent as Hx′ and Cy′ (see Figs. 1 and 4 for numbering, respectively). For the diester prodrug derivatives, the Hx'a (Cy'a) and Hx'b (Cy'b) notation refers to the chemical shift of the hydrogen atom (carbon atom) of the substituent linked to the C14/C18 and C8/C1 hydroxyl of indinavir/nelfinavir, respectively. Electron-spray ionization mass spectra in positive or negative mode [ESI(+) MS or ESI(−) MS, respectively] were recorded on a Finnigan MAT TSQ 7000 apparatus equipped with an atmospheric pressure ionization 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. Amino acid conjugated prodrugs consisted of TFA salts and the TFA anion quantification was assessed by ^{19}F NMR using 3,3,3-trifluoroethanol as internal standard. COSY ^{1}H/^{1}H, ^{1}H/^{13}C NMR correlation, ^{13}C DEPT, and/or MS data fully confirm the signal assignments and structure of the isolated materials.

General procedure for the preparation of ester-conjugated PI prodrugs ("ester procedure")

EDC (1.1 equiv) was added to a mixture of anhydrous CH_2Cl_2 at 0 °C containing the PI (1 equiv), the *N*-protected amino acid derivative or the glyceride derivative (1.1 equiv), and DMAP (1.1 equiv). After stirring at room temperature until PI consumption (TLC monitoring), the solution diluted with CH_2Cl_2 was washed until neutrality. The organic phase was dried over Na_2SO_4, filtered then evaporated. The crude residue was purified through silica gel column chromatography affording the ester prodrug as a white solid unless otherwise indicated. For the synthesis of the nelfinavir prodrugs, the reaction was performed in DMF. For the specific preparation of the diester Ind-[C(O)C4TyrP]2, 1 equiv of indinavir was reacted with 2.2 equivs of the *N*-protected amino acid derivative, DMAP, and EDC.

General procedure for the preparation of the carbamate-conjugated PI prodrugs ("carbamate procedure")

Two equivs of isocyanate then 2 equivs of CuCl were added to 1 equiv of saquinavir in anhydrous CH_2Cl_2 at room temperature. After stirring for 24 h, the solution diluted with CH_2Cl_2 was washed with aqueous saturated NaCl. The organic phase was dried over Na_2SO_4, filtered then evaporated. The crude residue was purified through silica gel column chromatography giving the carbamate-conjugated PI prodrug as a white solid. A similar procedure was applied for the indinavir and nelfinavir carbamate prodrug synthesis, except that reactives and reagents were used in 1 : 1 stoichiometric amount. The synthesis of the nelfinavir derivative was performed in a 4 : 1 CH_2Cl_2/DMF solution.

General deprotection procedure

The *O*-^{t}Bu and/or *N*-Boc amino acid-protected prodrugs in anhydrous 1 : 1 TFA/CH_2Cl_2 (concentration range of 12–48 mmol L^{-1}) were stirred for 1–5 h at 0 °C then for a further 4–24 h period at room temperature until disappearance of the starting material (TLC monitoring). The reaction mixture was then evaporated under reduced pressure, followed by several co-evaporation with CH_2Cl_2 or toluene, and the crude residue was purified by silica gel column chromatography giving the desired PI prodrugs as TFA salts. Stirring at 0 °C for 2–4 h was sufficient to deprotect Saq-LeuP and Saq-PheP in 1 : 10 TFA/CH_2Cl_2, and Ind(8)-LeuP and Ind(8)-PheP in 1 : 7 and 1 : 5 TFA/CH_2Cl_2, respectively. The deprotection of Nelf(18)-

C(O)NC4TyrP and Nelf(1)-C(O)C4TyrP was performed in 4 : 1 and 2 : 1 CH_2Cl_2/DMF, respectively.

Synthesis of leucine-derived prodrugs

Saq-LeuP. The ester procedure when applied to 297 mg (1.19 mmol) of *N*-α-*t*-Boc-L-leucine and 400 mg (0.59 mmol) of saquinavir gave after purification by chromatography (1 : 1 to 0 : 1 hexane/AcOEt) 231 mg (0.26 mmol, 44%) of Saq-LeuP.

Saq-Leu(2TFA). The general deprotection procedure when applied to 51 mg (0.05 mmol) of Saq-LeuP and 0.35 mL of TFA gave after purification by chromatography (1 : 0 to 7 : 3 AcOEt/MeOH) 58 mg (0.05 mmol, 99%) of Saq-Leu(2TFA): R_f (9 : 1 AcOEt/MeOH) = 0.35; t_R (column II, solvent D, 240 nm) = 14.0 min; ^{1}H NMR (CD_3OD): 0.96 (*d*, 6H, *J* 5.6, H1′, H3′), 1.33 (*s*, 9H, H41), 1.35–2.40 (*m*, 17H, H19, H30–36, H2′, H4′), 2.55–3.50 (*m*, 7H, H13, H27, H29, H37), 3.59 (*m*, 1H, H5′), 4.05 (*m*, 1H, H18), 4.65 (*m*, 1H, H12), 5.49 (*m*, 1H, H26), 6.85 (*t*, *J* 7.5, 1H, H23), 6.99 (*t*, *J* 7.5, 2H, H22, H24), 7.15 (*d*, *J* 7.5, 2H, H21, H25), 7.61 (*dd*, *J* 7.1, 8.1, 1H, H3), 7.75 (*dd*, *J* 7.1, 8.5, 1H, H2), 7.91 (*d*, *J* 8.1, 1H, H4), 8.03 (*d*, *J* 8.5, 1H, H1), 8.10 (*d*, *J* 8.6, 1H, H6), 8.38 (*d*, *J* 8.6, 1H, H7); ^{13}C NMR (CD_3OD): 21.9, 27.2, 27.3, 31.4 (C31–34), 22.7, 22.9 (C1′, C3′), 26.0 (C2′), 29.0 (C41), 32.0 (C36), 34.9 (C35), 35.0 (C19), 37.3 (C30), 38.1 (C13), 43.3 (C4′), 51.8 (C18), 52.1 (C40), 53.0, 53.3 (C12, C5′), 55.9 (C27), 59.8 (C29), 71.0 (C37), 76.0 (C26), 119.8 (C7), 127.2 (C23), 129.1 (C3), 129.6 (C4), 130.2 (C22, C24), 130.5 (C5), 130.9 (C21, C25), 131.7 (C1, C2), 139.1 (C6), 139.6 (C20), 148.0 (C9), 152.2 (C8), 166.3 (C10), 172.5 (C38), 175.0, 175.2 (C14, C16), 176.8 (C7′); ESI(+) MS: m/z = 784.4 $(M + H)^+$ and 806.4 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{44}H_{61}N_7O_6$.

Ind(8)-LeuP and Ind-[LeuP]2. The ester procedure when applied to 208 mg (0.83 mmol) of *N*-α-*t*-Boc-L-leucine and 465 mg (0.76 mmol) of indinavir gave after purification by chromatography (1 : 0 to 9 : 1 AcOEt/MeOH) 260 mg (0.31 mmol, 42%) of Ind(8)-LeuP and 55 mg (0.053 mmol, 7%) of Ind-[LeuP]2.

Ind(8)-Leu(2TFA). The general deprotection procedure when applied to 132 mg (0.16 mmol) of Ind(8)-LeuP and 1.5 mL of TFA gave after purification by chromatography (1 : 0 to 7 : 3 AcOEt/MeOH) 150 mg (0.16 mmol, 98%) of Ind(8)-Leu(2TFA): R_f (4 : 1 AcOEt/MeOH) = 0.40; t_R (column II, solvent C, 210 nm) = 20.6 min; ^{1}H NMR (CD_3OD): 0.80 (*d*, *J* 5.6, 6H, H1′, H3′), 1.29 (*s*, 9H, H23), 1.30–1.70 (*m*, 5H, H13, H2′, H4′), 2.55–3.40 (*m*, 14H, H7, H12, H15–19, H30), 3.76 (*m*, 3H, H14, H24), 3.95 (*m*, 1H, H5′), 5.52 (*m*, 1H, H8), 5.63 (*d*, *J* 5.0, 1H, H9), 7.15–7.33 (*m*, 9H, H2–5, H32–36), 7.46 (*dd*, *J* 5.0, 7.8, 1H, H27), 7.89 (*d*, *J* 7.8, 1H, H26), 8.49 (*m*, 1H, H28), 8.51 (*s*, 1H, H29); ^{13}C NMR (CD_3OD): 22.2, 22.6 (C1′, C3′), 25.5 (C2′), 28.8 (C23), 37.8 (C7), 38.3 (C13), 40.6 (C4′, C30), 45.4 (C12), (C18 hindered by solvent), 51.8, 55.2 (C16, C17), 52.6 (C5′, C22), 59.8 (C24), 56.8 (C9), 62.9 (C15), 67.0 (C19), 67.5 (C14), 79.7 (C8), 125.2 (C27), 125.5 (C5), 126.1 (C34), 127.7, 128.6 (C3, C4), 129.5 (C2, C33, C35), 130.3 (C32, C36), 133.9 (C25), 139.7 (C26), 140.5, 140.7 (C1, C6), 141.1 (C31), 149.4 (C28), 150.9 (C29), 169.8 (C20), 170.7 (C7′), 178.2 (C11); ESI(+) MS: m/z = 727.5 $(M + H)^+$ and 749.4 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{42}H_{58}N_6O_5$.

Synthesis of phenylalanine-derived prodrugs

Saq-PheP. The ester procedure when applied to 316 mg (1.20 mmol) of *N*-α-*t*-Boc-L-phenylalanine and 400 mg (0.60 mmol) of saquinavir afforded after purification by chromatography (1 : 1 to 3 : 7 hexane/AcOEt) 460 mg (0.50 mmol, 84%) of Saq-PheP.

Saq-Phe(2TFA). The general deprotection procedure when applied to 239 mg (0.26 mmol) of Saq-PheP and 0.9 mL of TFA gave after purification by chromatography (1 : 0 to 7 : 3 AcOEt/MeOH) 270 mg (0.26 mmol, 99%) of Saq-Phe(2TFA): R_f (9 : 1 AcOEt/MeOH) = 0.40; t_R (column II, solvent D, 240 nm) = 14.2 min; ^{1}H NMR (CD_3OD): 1.34 (*s*, 9H, H41), 1.35–2.40 (*m*, 14H, H19, H30–36), 2.60–3.40 (*m*, 9H, H13, H27, H29, H37, H7′), 4.20 (*m*, 2H, H18, H8′), 4.55 (*m*, 1H, H12), 5.30 (*m*, 1H, H26), 6.80 (*t*, *J* 7.3, 1H, H23), 6.94 (*dd*, *J* 6.8, 7.3, 2H, H22, H24), 7.18–7.38 (*m*, 7H, H21, H25, H2′–6′), 7.60 (*dd*, *J* 7.5, 8.0, 1H, H3), 7.79 (*dd*, *J* 7.5, 8.0, 1H, H2), 7.95 (*d*, *J* 8.0, 1H, H4), 8.10 (*m*, 1H, H1), 8.13 (*d*, *J* 8.1, 1H, H6), 8.41 (*d*, *J* 8.1, 1H, H7); ^{13}C NMR (CD_3OD): 21.8, 26.6, 27.1, 31.5 (C31–34), 28.9 (C41), 32.0 (C36), 35.0 (C19, C35), 37.3 (C30), 38.0 (C13), 38.5 (C7′), 51.7 (C18), 52.1 (C40), 52.3 (C12), 55.8 (C8′), 59.5, 59.9 (C27, C29), 70.9 (C37), 76.5 (C26), 119.8 (C7), 127.0, 127.1 (C23, C4′), 128.7 (C3), 129.1 (C4), 129.2 (C22, C24), 130.0 (C3′, C5′), 130.4 (C2′, C6′), 130.8 (C5), 130.9 (C21, C25), 131.7 (C1, C2), 139.0 (C6), 139.4 (C20), 139.7 (C1′), 148.0 (C9), 150.2 (C8), 166.2 (C10), 172.8 (C38), 175.1, 175.2, 175.8 (C14, C16, C10′); ESI(+) MS: m/z = 818.4 $(M + H)^+$ in agreement with the calculated mass for [M] = $C_{47}H_{59}N_7O_6$.

Ind(8)-PheP. The ester procedure when applied to 221 mg (0.83 mmol) of *N*-α-*t*-Boc-L-phenylalanine and 465 mg (0.76 mmol) of indinavir gave after purification by chromatography (1 : 0 to 9 : 1 AcOEt/MeOH) 210 mg (0.24 mmol, 32%) of Ind(8)-PheP.

Ind(8)-Phe(2TFA). The general deprotection procedure when applied to 240 mg (0.28 mmol) of Ind(8)-PheP and 2 mL of TFA gave after purification by chromatography (1 : 0 to 7 : 3 AcOEt/MeOH) 274 mg (0.28 mmol, 99%) of Ind(8)-Phe(2TFA): R_f (4 : 1 AcOEt/MeOH) = 0.45; t_R (column II, solvent C, 210 nm) = 22.4 min; ^{1}H NMR (CD_3OD): 1.30 (*s*, 9H, H23), 1.50 (*m*, 2H, H13), 2.65–3.70 (*m*, 16H, H7, H12, H15–19, H30, H7′), 3.84 (*m*, 3H, H14, H24), 3.97 (*m*, 1H, H8′), 5.34 (*m*, 1H, H8), 5.60 (*d*, *J* 5.0, 1H, H9), 6.93 (*m*, 2H, H3′, H5′), 7.20–7.29 (*m*, 12H, H2–5, H32–36, H2′, H4′, H6′), 7.49 (*dd*, *J* 5.0, 7.8, 1H, H27), 7.92 (*d*, *J* 7.8, 1H, H26), 8.52 (*d*, *J* 5.0, 1H, H28), 8.57 (*s*, 1H, H29); ^{13}C NMR (CD_3OD): 28.8 (C23), 37.1 (C7), 37.9 (C13), 38.9 (C7′), 40.7 (C30), 45.9 (C12), (C18 hindered by solvent), 51.4, 54.8 (C16, C17), 52.7 (C22), 55.1 (C8′), 56.7 (C9), 59.5 (C24), 62.8 (C15), 66.8 (C19), 67.3 (C14), 79.9 (C8), 125.2 (C27), 125.5 (C5), 126.1 (C34), 127.5, 128.5 (C3, C4), 128.9 (C4′), 129.5 (C33, C35), 129.7 (C2), 130.1 (C3′, C5′), 130.3, 130.4 (C32, C36, C2′, C6′), 133.5 (C25), 135.4 (C1′), 140.1 (C26), 140.3, 140.8 (C1, C6), 141.0 (C31), 149.5 (C28), 150.9 (C29), 168.9 (C10′), 169.8 (C20), 177.8 (C11); ESI(+) MS: m/z = 761.4 $(M + H)^+$ and 783.4 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{45}H_{56}N_6O_5$.

Synthesis of tyrosine-derived prodrugs

Ester conjugates. *Saq-C(O)C4TyrP.* The ester procedure when applied to 261 mg (0.61 mmol) of *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu and 200 mg (0.30 mmol) of saquinavir gave after purification by chromatography (3 : 7 to 0 : 1 hexane/AcOEt) 232 mg (0.21 mmol, 71%) of Saq-C(O)C4TyrP as a colorless oil.

Saq-C(O)C4Tyr(1TFA). The general deprotection procedure when applied to 220 mg (0.20 mmol) of Saq-C(O)C4TyrP and 3 mL of TFA gave after purification by chromatography (1 : 0 to 3 : 2 AcOEt/MeOH) 158 mg (0.15 mmol, 75%) of Saq-C(O)C4Tyr(1TFA): R_f (3 : 2 AcOEt/MeOH) = 0.5; t_R (column II, solvent D, 240 nm) = 5.8 min; ^{1}H NMR (CD_3OD): 1.31 (*s*, 9H, H41), 1.40–2.10 (*m*, 12H, H31–34, H12′, H13′), 2.25–3.30 (*m*, 17H, H13, H19, H27, H29, H30, H35–37, H7′, H14′), 3.74 (*m*, 1H, H8′), 3.87 (*m*, 2H, H11′), 4.44 (*m*, 1H, H18), 4.80 (*m*, 1H, H12), 5.29 (*m*, 1H, H26), 6.80 (*m*, 3H, H23,

H2′, H6′), 6.99 (*t*, *J* 7.4, 2H, H22, H24), 7.19 (*m*, 4H, H21, H25, H3′, H5′), 7.62 (*m*, 1H, H3), 7.72 (*m*, 1H, H2), 7.92 (*d*, *J* 8.0, 1H, H4), 8.07 (*d*, *J* 8.5, 1H, H1), 8.09 (*d*, *J* 8.5, 1H, H6), 8.37 (*d*, *J* 8.5, 1H, H7); ^{13}C NMR (CD_3OD): 22.1, 27.2, 31.4 (C31–34), 29.1 (C41), 29.8 (C12′), 31.9 (C36), 34.8 (C35), 35.0 (C14′), 35.7 (C19), 37.1 (C30), 37.4 (C13), 38.1 (C7′), 51.8 (C18), 52.1 (C40), 53.2 (C12), 56.9 (C27), 57.7 (C8′), 60.0 (C29), 68.7 (C11′), 70.8 (C37), 74.8 (C26), 116.1 (C2′, C6′), 119.8 (C7), 127.2 (C23), 129.05 (C4′), 129.1 (C3), 129.3 (C22, C24), 129.5 (C4), 130.4 (C21, C25), 130.8 (C5), 130.9 (C1, C2), 131.6 (C3′, C5′), 139.0 (C6), 139.4 (C20), 147.9 (C9), 150.3 (C8), 159.9 (C1′), 166.1 (C10), 172.4 (C38), 173.9 (C10′), 174.8, 175.1, 175.7 (C14, C16, C15′); ESI(+) MS: *m*/*z* = 949.64 (M^+), 950.63 $(M + H)^+$ and 972.56 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{53}H_{71}N_7O_9$.

Ind(8)-C(O)C1TyrP, Ind(14)-C(O)C1TyrP and Ind-[C(O)-C1TyrP]2. The general condensation procedure when applied to 302 mg (0.76 mmol) of *N*-Boc-Tyr(OCH_2CO_2H)-O^{t}Bu and 389 mg (0.63 mmol) of indinavir gave, after purification by four successive chromatographies (1 : 0 to 9 : 1 AcOEt/MeOH), 170 mg (0.17 mmol, 27%) of Ind(8)-C(O)C1TyrP, 40.0 mg (0.04 mmol, 6%) of Ind(14)-C(O)C1TyrP and 70.0 mg (0.05 mmol, 8%) of Ind-[C(O)C1TyrP]2.

Ind(8)-C(O)C1Tyr(4TFA), Ind(14)-C(O)C1Tyr(4TFA), and Ind-[C(O)C1Tyr]2 (4TFA). The general deprotection procedure when applied to 26 mg (0.03 mmol) of Ind(8)-C(O)C1TyrP, 28 mg (0.03 mmol) of Ind(14)-C(O)C1TyrP or 60 mg (0.04 mmol) of Ind-[C(O)C1TyrP]2, and 1 mL of TFA gave 26 mg (0.02 mmol, 73%) of Ind(8)-C(O)C1Tyr(4TFA), 14 mg (0.01 mmol, 39%) of Ind(14)-C(O)C1Tyr(4TFA), or 25 mg (0.016 mmol, 34%) of Ind-[C(O)C1Tyr]2(4TFA), after precipitation with ether or purification by chromatography (4 : 1 to 0 : 1 AcOEt/MeOH for Ind(8)-C(O)C1Tyr(4TFA) or 2 : 3 to 0 : 1 AcOEt/MeOH for Ind-[C(O)C1Tyr]2(4TFA)), respectively.

Ind(8)-C(O)C1Tyr(4TFA). R_f (2 : 3 AcOEt/MeOH) = 0.2; t_R (column I, solvent A, 210 nm) = 10.4 min; ^{1}H NMR (CD_3OD): 1.31 (s, 3H, H23), 1.51, 1.90 (2m, 2H, H13), 2.65–3.34 (m, 18H, H7, H12, H15–19, H24, H30, H7′), 4.14 (m, 2H, H14, H8′), 4.40 and 4.51 (AB system, $^2J_{AB}$ 16.4, 2H, H11′), 5.47 (td, *J* 4.9, 1.6, 1H, H8), 5.63 (m, 1H, H9), 6.87 (d, *J* 8.7, 2H, H2′, H6′), 7.12–7.37 (m, 11H, H2–5, H32–36, H3′, H5′), 7.94 (dd, *J* 8.0, 5.1, 1H, H27), 8.47 (d, *J* 8.0, 1H, H26), 8.75 (d, *J* 5.1, 1H, H28), 8.81 (*bs*, 1H, H29); ^{13}C NMR (CD_3OD): 28.8 (C23), 36.5 (C7), 38.0 (C7′), 38.5 (C13), 40.5 (C30), 45.9 (C12), 50.7 (C18), 52.8 (C22), 54.7 (C16, C17), 55.3 (C9), 56.8 (C8′), 58.5 (C24), 61.5 (C15), 65.9 (C11′), 66.9 (C14, C19), 78.1 (C8), 116.3 (C2′, C6′), 125.1 (C27), 126.0 (C5), 127.3 (C34), 127.6, 128.3 (C3, C4), 128.6 (C2), 129.4 (C33, C35, C4′), 130.2 (C32, C36), 131.7 (C3′, C5′), 140.5, 140.7 (C1, C6), 141.2 (C31), 144.8 (C28), 146.0 (C29), 158.9 (C1′), 166.6 (C20), 170.2 (C12′), 171.3 (C10′), 177.2 (C11). ESI(+) MS: data identical to that described for Ind(14)-C(O)C1Tyr(4TFA) in ref. 16.

Ind(14)-C(O)C1Tyr(4TFA). Data identical to that described in ref. 16.

Ind-[C(O)C1Tyr]2(4TFA). R_f (MeOH) = 0.1; t_R (column I, solvent A, 210 nm) = 10.0 min; ^{1}H NMR (CD_3OD): 1.27 (s, 9H, H23), 1.40, 1.61 (m, 2H, H13), 2.10–3.34 (m, 18H, H7, H12, H15–19, H30, H7′), 3.55 (s, 2H, H24), 3.73 (m, 2H, H8′), 4.47 (s, 2H, H11′b), 4.70 (s, 2H, H11′a), 5.25 (m, 1H, H14), 5.52 (td, *J* 5.2, 1.2, 1H, H8), 5.62 (d, *J* 5.2, 1H, H9), 6.81 (d, *J* 8.6, 2H, H2′, H6′), 6.90 (d, *J* 8.6, 2H, H2′, H6′), 7.10–7.46 (m, 15H, H2–5, H21, H27, H32–36, H3′, H5′), 7.81 (d, *J* 7.9, 1H, H26), 8.46 (m, 2H, H28, H29); ^{13}C NMR (CD_3OD): 29.0 (C23), 35.3 (C13), 37.3 (C7′), 38.0 (C7), 40.6 (C30), 45.4 (C12), 50.3 (C18), 52.2 (C22), 53.1, 56.2 (C16, C17), 56.8 (C9), 57.6 (C8′), 59.5 (C24), 60.4 (C15), 66.1, 66.3 (C11′), 68.0 (C19), 72.4 (C14), 78.2 (C8), 116.1, 116.2 (C2′, C6′), 125.2 (C27), 126.0 (C5), 127.7 (C34), 128.4, 129.3 (C3, C4), 129.4 (C2), 129.5 (C33, C35), 130.2 (C32, C36), 131.5, 131.6, 131.7 (C3′–5′), 135.1 (C25), 139.3 (C26), 140.3, 140.8 (C1, C6), 141.2 (C31), 149.1 (C28), 150.9 (C29), 158.5, 158.7 (C1′), 170.2 (C20), 170.8, 172.7 (C12′), 173.7, 173.8 (C10′), 177.4 (C11). ESI(+) MS: *m*/*z* = 1056.50 $(M + H)^+$ in agreement with the calculated mass for [M] = $C_{58}H_{69}N_7O_{12}$

Ind(8)-C(O)C4TyrP and Ind-[C(O)C4TyrP]2. The ester procedure, when applied to 235 mg (0.54 mmol) of *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu and 300 mg (0.49 mmol) of indinavir, gave after purification by chromatography (1 : 0 to 9 : 1 AcOEt/MeOH) 213 mg (0.20 mmol, 42%) of Ind(8)-C(O)C4TyrP and 156 mg (0.11 mmol, 22%) of Ind-[C(O)C4TyrP]2. When applied to 235 mg (0.54 mmol) of Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu and 150 mg (0.24 mmol) of indinavir, 199 mg (0.14 mmol, 56%) of Ind-[C(O)C4TyrP]2 were obtained.

Ind(8)-C(O)C4Tyr(1TFA) and Ind-[C(O)C4Tyr]2. The general deprotection procedure when applied to 105 mg (0.10 mmol) of Ind(8)-C(O)C4TyrP or 215 mg (0.15 mmol) of Ind-[C(O)C4TyrP]2, and 1.5 mL or 3 mL of TFA gave after purification by chromatography (1 : 0 to 3 : 7 CH_2Cl_2/MeOH or 7 : 3 to 3 : 7 AcOEt/MeOH, respectively) 100 mg (0.10 mmol, 99%) of Ind(8)-C(O)C4Tyr(1TFA) or 133 mg (0.11 mmol, 72%) of Ind-[C(O)C4Tyr]2(1TFA), respectively.

Ind(8)-C(O)C4Tyr(1TFA). R_f (1 : 1 AcOEt/MeOH) = 0.25; t_R (column II, solvent C, 210 nm) = 8.5 min; ^{1}H NMR (CD_3OD): 1.38 (*s*, 9H, H23), 1.67 (*m*, 4H, H12′, H13′), 2.01 (*m*, 2H, H13), 2.15–2.20 (*m*, 2H, H14′), 2.40–3.30 (*m*, 16H, H7, H12, H15–19, H30, H7′), 3.64 (*s*, 2H, H24), 3.73 (*m*, 1H, H8′), 3.84 (*m*, 3H, H14, H11′), 5.37 (*m*, 1H, H8), 5.55 (*m*, 1H, H9), 6.80 (*d*, *J* 8.5, 2H, H2′, H6′), 7.14–7.22 (*m*, 11H, H2–5, H32–36, H3′, H5′), 7.42 (*dd*, *J* 7.8, 4.9, 1H, H27), 7.84 (*d*, *J* 7.8, 1H, H26), 8.45 (*d*, *J* 4.9, 1H, H28), 8.49 (*s*, 1H, H29); ^{13}C NMR (CD_3OD): 22.6 (C13′), 29.8 (C12′), 29.0 (C23), 34.6 (C14′), 37.3 (C7′), 38.3 (C7), 38.7 (C13), 40.7 (C30), 46.2 (C12), 52.2 (C18), 52.3 (C22), 52.7, 56.1 (C16, C17), 56.7 (C9), 57.6 (C8′), 60.2 (C24), 63.2 (C15), 67.7 (C14), 67.9 (C19), 68.7 (C11′), 77.0 (C8), 116.0 (C2′, C6′), 125.1 (C27), 125.3 (C5), 126.1 (C34), 127.5, 128.2 (C3, C4), 128.9 (C4′), 129.3 (C2), 129.5 (C33, C35), 130.2 (C32, C36), 131.6 (C3′, C5′), 134.6 (C25), 139.4 (C26), 140.8, 141.1 (C1, C6), 141.8 (C31), 149.3 (C29), 151.0 (C28), 159.9 (C1′), 171.8 (C20), 173.6 (C10′), 174.5 (C15′), 177.8 (C11). ESI(+) MS: *m*/*z* = 877.6 $(M + H)^+$ and 899.56 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{50}H_{64}N_6O_8$.

Ind-[C(O)C4Tyr]2(1TFA). R_f (MeOH) = 0.5; t_R (column II, solvent C, 210 nm) = 8.5 min; ^{1}H NMR (CD_3OD): 1.25 (*s*, 9H, H23), 1.60–2.40 (*m*, 14H, H13, H12′–14′), 2.45–4.00 (*m*, 24H, H7, H12, H15–19, H24, H30, H7′, H11′), 4.20 (*m*, 2H, H8′), 5.15 (*m*, 1H, H14), 5.45 (*t*, *J* 4.7, 1H, H8), 5.60 (*d*, *J* 5.1, 1H, H9), 6.82 (*d*, *J* 8.4, 4H, H2′, H6′), 7.10–7.45 (*m*, 14H, H2–5, H27, H32–36, H5′, H6′), 7.85 (*d*, *J* 7.8, 1H, H26), 8.45 (*m*, 2H, H28, H29); ^{13}C NMR (CD_3OD): 22.6, 22.9 (C13′), 29.7, 29.8 (C12′), 29.4 (C23), 35.1, 34.7 (C14′), 37.1, 37.3 (C7′), 38.2 (C7), 40.3 (C13), 40.5 (C30), 46.1 (C12), 52.2 (C18), 52.3 (C22), 52.7, 56.1 (C16, C17), 56.7 (C9), 57.6 (C8′), 60.2 (C24), 65.8 (C15), 67.7 (C19), 68.5 (C11′), 70.4 (C14), 76.9 (C8), 116.2 (C2′, C6′), 124.9 (C27), 125.2 (C5), 126.1 (C34), 127.5, 128.1 (C3, C4), 129.1 (C4′), 129.1 (C2), 129.5 (C33, C35), 130.4 (C32, C36), 131.5 (C3′, C5′), 134.3 (C25), 139.1 (C26), 140.8, 141.2 (C1, C6), 141.6 (C31), 149.2 (C29), 150.9 (C28), 159.4 (C1′), 171.4 (C20), 173.1 (C15′), 174.8 (C11), 177.5 (C10′); ESI(+) MS: *m*/*z* = 1140.8 M^+, 1141.7 $(M + H)^+$ and 1163.5 $(M + Na)^+$, in agreement with the calculated mass for [M] = $C_{64}H_{81}N_7O_{12}$.

Nelf(1)-C(O)C4TyrP, Nelf(18)-C(O)C4TyrP and Nelf-[C(O)C4TyrP]2. The ester procedure when applied to 385 mg (0.88 mmol) of *N*-Boc-Tyr[$O(CH_2)_4CO_2H$]-O^{t}Bu and 500 mg (0.88 mmol) of nelfinavir gave after purification by chromatography (7 : 3 to 1 : 1 cyclohexane/AcOEt) 313 mg (0.32 mmol, 36%) of Nelf(1)-C(O)C4TyrP, 19 mg (0.02 mmol, 2%) of Nelf(18)-C(O)C4TyrP, and 32 mg (0.02 mmol, 3%) of Nelf-[C(O)C4TyrP]2.

Nelf(1)-C(O)C4Tyr(1TFA). The general deprotection procedure when applied to 250 mg (0.25 mmol) of Nelf(1)-C(O)C4TyrP and 5 mL of TFA gave after purification by chromatography (1 : 0 to 4 : 1 CH_2Cl_2/MeOH) 91 mg (0.10 mmol, 38%) of Nelf(1)-C(O)C4Tyr(1TFA): R_f (9 : 1 CH_2Cl_2/MeOH) = 0.2; t_R (column II, solvent E, 210 nm) = 9.6 min; ^{1}H NMR (CD_3OD): 1.14 (*s*, 9H, H32), 1.20–2.20 (*m*, 16H, H11, H21–27, H12′, H13′), 2.22 (*s*, 3H, H7), 2.55–2.80 (*m*, 4H, H7′, H14′), 2.85–3.40 (*m*, 5H, H19, H20, H28), 3.43, 3.54 (*m*, 2H, H11), 3.75 (*m*, 1H, H8′), 3.95–4.15 (*m*, 3H, H18, H11′), 4.45 (*m*, 1H, H10), 6.89 (*d*, *J* 8.2, 2H, H3′, H5′), 7.05–7.40 (*m*, 8H, H2–4, H14–16, H2′, H6′), 7.50 (*d*, *J* 7.2, 2H, H13, H17); ^{13}C NMR (CD_3OD): 13.7 (C7), 22.9 (C13′), 29.9 (C12′), 21.8, 27.2, 27.6, 31.8 (C22–25), 28.9 (C32), 32.3 (C11), 34.6 (C27), 35.3 (C26), 35.6 (C14′), 37.6 (C7′), 37.7 (C21), 52.0 (C31), 54.7 (C10), 57.7 (C8′), 59.8 (C19), 60.5 (C20), 68.8 (C11′), 70.7 (C18), 71.1 (C28), 116.2 (C2′, C6′), 124.7, 126.3 (C2, C4), 127.2 (C15), 127.7 (C3), 129.3, 129.5 (C6, C4′), 130.1 (C14, C16), 130.9 (C13, C17), 131.7 (C3′, C5′), 137.8, 140.4 (C5, C12), 151.2 (C1), 160.0 (C1′), 172.4 (C10′), 173.4 (C29, C15′), 176.2 (C8); ESI(+) MS: m/z = 831.9 $(M + H)^+$ in agreement with the calculated mass for [M] = $C_{46}H_{62}N_4O_8S$.

Carbamate conjugates. *Saq-C(O)NC4TyrP.* The carbamate procedure when applied to 194 mg (0.44 mmol) of Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu and 150 mg (0.22 mmol) of saquinavir gave after purification by chromatography (1 : 0 to 9 : 1 AcOEt/MeOH) 162 mg (0.15 mmol, 66%) of Saq-C(O)NC4TyrP.

Saq-C(O)NC4Tyr(1TFA). The general deprotection procedure when applied to 150 mg (0.13 mmol) of Saq-C(O)-NC4TyrP and 1.5 mL of TFA gave after purification by chromatography (1 : 0 to 0 : 1 AcOEt/MeOH) 92 mg (0.09 mmol, 64%) of Saq-C(O)NC4Tyr(1TFA): R_f (1 : 1 AcOEt/MeOH) = 0.35; t_R (column II, solvent D, 240 nm) = 4.3 min; ^{1}H NMR (CD_3OD): 1.36 (*s*, 9H, H41), 1.40–2.20 (*m*, 14H, H31–34, H12′–14′), 2.50–3.30 (*m*, 15H, H13, H19, H27, H29, H30, H35–37, H7′), 3.78 (*m*, 1H, H8′), 3.95 (*m*, 2H, H11′), 4.37 (*m*, 1H, H18), (H12 hindered by water), 5.16 (*m*, 1H, H26), 6.87 (*m*, 3H, H23, H2′, H5′), 7.01 (*t*, *J* 7.1, 2H, H22, H24), 7.21 (*m*, 4H, H21, H25, H3′, H5′), 7.70 (*dd*, *J* 7.7, 7.0, 1H, H3), 7.85 (*td*, *J* 1.6, 8.0, 1H, H2), 8.00 (*d*, *J* 8.0, 1H, H4), 8.15 (*d*, *J* 7.7, 1H, H1), 8.17 (*d*, *J* 8.4, 1H, H6), 8.46 (*d*, *J* 8.5, 1H, H7); ^{13}C NMR (CD_3OD): 22.1, 26.8, 27.2, 31.5 (C31–34), 27.7, 27.8 (C12′, C13′), 29.2 (C41), 31.8 (C36), 34.5 (C35), 36.2 (C19), 36.9 (C30), 37.4 (C13), 38.0 (C7′), 41.9 (C14′), 51.7 (C18), 52.4 (C40), 53.8 (C12), 57.7 (C8′), 58.3 (C27), 60.3 (C29), 68.8 (C11′), 70.5 (C37), 75.1 (C26), 116.3 (C2′, C6′), 119.9 (C7), 127.3 (C23), 128.9 (C4′), 129.1 (C3), 129.4 (C22, C24), 129.7 (C4), 130.4 (C21, C25), 130.8, 131.7 (C1, C2), 131.0 (C5), 131.6 (C3′, C5′), 139.2 (C6, C20), 148.1 (C9), 150.4 (C8), 158.7 (C16′), 160.1 (C1′), 166.3 (C10), 172.7 (C38), 173.8 (C10′), 175.1 (C14, C16); ESI(−) MS: m/z = 947.6 $(M - H)^-$ and 1061.3 $(M - H + CF_3CO_2H)^-$ in agreement with the calculated mass for [M] = $C_{52}H_{68}N_8O_9$.

Ind(14)-C(O)NC4TyrP. The carbamate procedure when applied to 420 mg (0.97 mmol) of Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu and 593 mg of indinavir gave after purification by chromatography (1 : 0 to 9 : 1 AcOEt/MeOH) 313 mg (0.30 mmol, 31%) of Ind(14)-C(O)NC4TyrP.

Ind(14)-C(O)NC4Tyr(1TFA). The general deprotection procedure when applied to 200 mg (0.19 mmol) of Ind(14)-C(O)NC4TyrP and 2 mL of TFA gave after purification by chromatography (1 : 0 to 3 : 2 AcOEt/MeOH) 88 mg (0.09 mmol, 46%) of Ind(14)-C(O)NC4Tyr(1TFA): R_f (1 : 1 AcOEt/MeOH) = 0.5; t_R (column II, solvent C, 210 nm) = 8.0 min; ^{1}H NMR (CD_3OD): 1.28 (*s*, 9H, H23), 1.35–1.85 (*m*, 6H, H13, H12′, H13′), 2.00–3.40 (*m*, 18H, H7, H12, H15–19, H30, H7′, H14′), 3.56 (*s*, 2H, H24), 3.73 (*m*, 1H, H8′), 3.87 (*m*, 2H, H11′), 4.35 (*m*, 1H, H8), 4.90 (*m*, 1H, H14), 5.23 (*m*, 1H, H9), 6.81 (*d*, *J* 7.4, 2H, H2′, H6′), 7.10–7.30 (*m*, 12H, H2–5, H27, H32–36, H3′, H5′), 7.34 (*m*, 1H, H26), 7.74–8.00 (*m*, 2H, H28, H29); ^{13}C NMR (CD_3OD): 29.4 (C12′, C13′), 30.7 (C23), 37.3 (C13), 38.9 (C7′), 42.2 (C7), 42.4 (C30), 43.2 (C14′), 47.4 (C12), 53.8 (C18, C22), 54.8, 57.7 (C16, C17), 60.4 (C9, C8′), 61.8 (C24), 62.3 (C15), 69.7 (C19), 70.3 (C11′), 73.0 (C14), 75.8 (C8), 117.6 (C2′, C6′), 127.4, 127.6 (C5, C27), 129.1, 129.4 (C3, C4, C34), 130.3 (C2), 130.6 (C4′), 131.1 (C33, C35), 131.8 (C32, C36), 133.2 (C3′, C5′), 141.0 (C26), 142.2, 143.3, 143.8 (C1, C6, C31), 150.9 (C28), 152.6 (C29), 160.3 (C16′), 161.4 (C1′), 174.3 (C10′), 179.0 (C11), C20 could not be localized; ESI(+) MS: m/z = 892.7 $(M + H)^+$ and 914.7 $(M + Na)^+$, and ESI(−) MS: m/z = 890.8 $(M - H)^-$ in agreement with the calculated mass for [M] = $C_{50}H_{65}N_7O_8$.

Nelf(18)-C(O)NC4TyrP. The carbamate procedure when applied to 112 mg (0.31 mmol) of Boc-Tyr[$O(CH_2)_4NCO$]-O^{t}Bu and 178 mg of nelfinavir gave after purification by chromatography (1 : 0 to 3 : 2 CH_2Cl_2/AcOEt) 210 mg (0.23 mmol, 73%) of Nelf(18)-C(O)NC4TyrP.

Nelf(18)-C(O)NC4Tyr(1TFA). The general deprotection procedure when applied to 140 mg (0.14 mmol) of Nelf(18)-C(O)NC4TyrP and 1.5 mL of TFA gave after purification by chromatography (1 : 0 to 4 : 1 AcOEt/MeOH) 36 mg (0.04 mmol, 25%) of Nelf(18)-C(O)NC4Tyr(1TFA): R_f (4 : 1 AcOEt/MeOH) = 0.5; t_R (column II, solvent E, 210 nm) = 7.6 min; ^{1}H NMR (CD_3OD): 1.20 (*s*, 9H, H32), 1.20–2.15 (*m*, 16H, H21–27, H12′, H13′), 2.24 (*s*, 3H, H7), 2.60–3.65 (*m*, 11H, H11, H19, H20, H28, H7′, H14′), 3.74 (*m*, 1H, H8′), 3.95 (*m*, 2H, H11′), 4.55 (*m*, 1H, H10), 5.25 (*m*, 1H, H18), 6.75–7.35 (*m*, 4H, H2–4, H14–16, H2′, H3′, H5′, H6′), 7.52 (*d*, *J* 7.9, 2H, H13, H17); ^{13}C NMR (CD_3OD): 13.2 (C7), 21.8, 27.2, 27.6, 31.9 (C22–25), 27.8 (C12′, C13′), 29.0 (C32), 31.9 (C24), 32.2 (C11), 35.1 (C26), 35.5 (C27), 37.5 (C7′), 37.6 (C21), 41.7 (C14′), 52.1 (C31), 52.2 (C10), 57.4 (C8′), 57.8 (C19), 60.7 (C20), 68.7 (C11′), 71.2 (C28), 73.7 (C18), 116.1 (C2′, C6′), 117.0 (C2), 119.4 (C4), 123.3 (C6), 127.4 (C3, C15), 129.1 (C4′), 130.1 (C14, C16), 131.2, 131.5 (C13, C17, C3′, C5′), 137.2, 140.0 (C5, C12), 157.0 (C1), 158.3 (C16′), 160.0 (C1′), 173.5 (C29, C10′), 176.1 (C8); ESI(−) MS: m/z = 844.6 $(M - H)^-$ in agreement with the calculated mass for [M] = $C_{46}H_{63}N_5O_8S$.

Synthesis of glyceride-derived prodrugs. *Saq-C(O)C2C(O)-GlyPalm.* The ester procedure when applied to 101 mg (0.15 mmol) of HOC(O)C2C(O)GlyPalm and 92 mg (0.14 mmol) of saquinavir gave after purification by chromatography (AcOEt) 113 mg (0.09 mmol, 63%) of Saq-C(O)C2C(O)GlyPalm as an oil: R_f (AcOEt) = 0.65; ^{1}H NMR ($CDCl_3$): 0.85 (*t*, *J* 6.4, 6H, H1′), 1.22 (*s*, 48H, H2′–13′), 1.31 (*s*, 9H, H41), 1.40–1.90 (*m*, 12H, H31–34, H14′), 2.29 (*t*, *J* 7.5, 4H, H15′), 2.30–2.95 (*m*, 17H, H13, H19, H27, H29, H30, H35–37, H20′, H21′), 4.16 (*dd*, *J* 5.6, 12.0, 1H, H17′$_A$), 4.26 (*dd*, *J* 4.6, 12.0, 1H, H17′$_B$), 4.40 (*m*, 1H, H18), 4.82 (*m*, 1H, H12), 5.23 (*m*, 1H, H18′), 5.35 (*m*, 1H, H26), 5.87 (*bs*, 1H, H39), 6.38 (*bs*, 1H, H15), 6.46 (*bs*, 1H, H15), 6.89 (*m*, 1H, H23), 7.02–7.12 (*m*, 4H, H21, H22, H24, H25), 7.61 (*m*, 2H, H3, H17), 7.73 (*td*, *J* 1.6, 8.0, 1H, H2), 7.82 (*d*, *J* 8.0, 1H, H4), 8.10 (*d*, *J* 8.0, 1H, H1), 8.14 (*d*, *J* 8.5, 1H, H6), 8.24 (*d*, *J* 8.5, 1H, H7), 9.12 (*d*, *J* 7.4, 1H, H11); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 20.7, 25.8, 26.2, 30.7 (C31–34), 22.7 (C2′), 24.9 (C14′), 29.1, 29.2, 29.3, 29.4, 29.5, 29.7 (C4′–13′, C20′, C21′), 28.8 (C41), 30.9 (C36), 32.0 (C3′), 33.2 (C35), 34.1 (C15′), 35.0 (C19), 35.8 (C30), 37.6 (C13), 49.7 (C18), 51.0 (C40), 51.8 (C12), 56.7 (C27), 59.5 (C29), 61.9 (C17′), 69.8 (C18′), 70.7 (C37), 73.9 (C26), 118.8 (C7), 126.5 (C23), 127.7 (C3), 128.1 (C4), 128.5 (C22, C24), 129.2 (C21, C25), 129.4 (C5), 130.2 (C1, C2), 137.2 (C20), 137.4 (C6), 146.7 (C9), 149.2 (C8), 164.6 (C10), 170.3 (C38), 171.8, 171.9 (C19′, C22′), 173.4, 173.6 (C14, C16, C16′); ESI(+) MS: m/z = 1321.88 $(M + H)^+$ and 1343.83 $(M + Na)^+$ in agreement with the calculated mass for [M] = $C_{77}H_{120}N_6O_{12}$.

Saq-C(O)C2C(O)GlyOleoyl. The ester procedure when applied to 141 mg (0.19 mmol) of HOC(O)C2C(O)GlyOleoyl

and 120 mg (0.18 mmol) of saquinavir gave after purification by chromatography (3 : 7 to 0 : 7 hexane/AcOEt) 160 mg (0.12 mmol, 65%) of Saq-C(O)C2C(O)GlyOleoyl: R_f (3 : 7 hexane/AcOEt) = 0.4; ^{1}H NMR ($CDCl_3$): 0.84 (*t*, *J* 6.4, 6H, H1′), 1.25 (*s*, 40H, H2′–7′, H12′–15′), 1.30 (*s*, 9H, H41), 1.45–1.85 (*m*, 12H, H31–34, H16′), 1.98 (*m*, 8H, H8′, H11′), 2.28 (*t*, *J* 7.5, 4H, H17′), 2.20–3.00 (*m*, 17H, H13, H19, H27, H29, H30, H35–37, H22′, H23′), 4.15, 4.25 (*2m*, 4H, H19′), 4.39 (*m*, 1H, H18), 4.82 (*m*, 1H, H12), 5.15–5.40 (*m*, 6H, H26, H9′, H10′, H20′), 5.97 (*bs*, 1H, H39), 6.39 (*bs*, 1H, H15), 6.53 (*bs*, 1H, H15), 6.91 (*m*, 1H, H23), 7.00–7.15 (*m*, 4H, H21, H22, H24, H25), 7.58 (*m*, 2H, H3, H17), 7.71 (*td*, *J* 1.6, 8.0, 1H, H2), 7.80 (*d*, *J* 8.0, 1H, H4), 8.09 (*d*, *J* 8.0, 1H, H1), 8.13 (*d*, *J* 8.3, 1H, H6), 8.22 (*d*, *J* 8.3, 1H, H7), 9.12 (*d*, *J* 7.4, 1H, H11); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 20.7, 25.8, 26.2, 30.7 (C31–34), 22.7 (C2′), 24.9 (C16′), 27.3 (C8′, C11′), 28.8 (C41), 29.1, 29.2, 29.4, 29.6, 29.8 (C4′–7′, C12′–15′, C22′, C23′), 30.9 (C36), 31.9 (C3′), 33.2 (C35), 34.1 (C17′), 35.0 (C19), 35.8 (C30), 37.5 (C13), 49.7 (C18), 50.9 (C40), 51.7 (C12), 56.6 (C27), 59.5 (C29), 61.9 (C19′), 69.8 (C20′), 70.6 (C37), 73.8 (C26), 118.7 (C7), 126.5 (C23), 127.6 (C3), 128.1 (C4), 128.5 (C22, C24), 129.1 (C21, C25), 129.3 (C5), 129.8, 130.1 (C9′, C10′), 130.2 (C1, C2), 137.2 (C20), 137.3 (C6), 146.6 (C9), 149.1 (C8), 164.5 (C10), 170.3 (C38), 171.8, 171.9 (C21′, C24′), 173.3, 173.4, 173.6 (C14, C16, C18′); ESI(+) MS: *m*/*z* = 1374.09 $(M + H)^+$ in agreement with the calculated mass for $[M] = C_{81}H_{124}N_6O_{12}$.

Ind(8)-C(O)C2C(O)GlyPalm and Ind-[C(O)C2C(O)-GlyPalm]2. The ester procedure when applied to 234 mg (0.35 mmol) of HOC(O)C2C(O)GlyPalm and 195 mg (0.32 mmol) of indinavir gave after purification by chromatography (1 : 0 to 0 : 1 hexane/AcOEt) 157 mg (0.12 mmol, 39%) of Ind(8)-C(O)C2C(O)GlyPalm and 73 mg (0.04 mmol, 12%) of Ind-[C(O)C2C(O)GlyPalm]2.

Ind(8)-C(O)C2C(O)GlyPalm. R_f (10 : 1 AcOEt/MeOH) = 0.5; ^{1}H NMR ($CDCl_3$): 0.85 (*t*, *J* 6.4, 6H, H1′), 1.23 (*s*, 48H, H2′–13′), 1.30 (*s*, 9H, H23), 1.45–1.70 (*m*, 6H, H13, H14′), 2.20–3.15 (*m*, 22H, H7, H12, H15–19, H30, H15′, H20′, H21′), 3.45 (*s*, 2H, H24), 3.85 (*m*, 1H, H14), 3.90–4.20 (*m*, 4H, H17′), 4.98 (*m*, 1H, H18′), 5.30 (*m*, 1H, H8), 5.64 (*dd*, *J* 5.0, 9.1, 1H, H9), 6.43 (*d*, *J* 9.1, 1H, H10), 7.12–7.30 (*m*, 10H, H2–5, H27, H32–36), 7.60 (*d*, *J* 7.8, 1H, H26), 7.68 (*bs*, 1H, H21), 8.50 (*m*, 2H, H28, H29); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 22.7 (C2′), 24.9 (C14′), 29.1, 29.2, 29.3, 29.4, 29.5, 29.7 (C4′–13′, C20′, C21′), 28.9 (C23), 32.0 (C3′), 34.1 (C15′), 37.6 (C7), 38.3 (C13), 39.4 (C30), 46.1 (C12), 48.0 (C18), 51.2 (C22), 52.8, 54.8 (C16, C17), 55.2 (C9), 60.3 (C24), 61.5 (C15), 61.7 (C17′), 64.4 (C19), 65.9 (C14), 69.7 (C18′), 77.2 (C8), 123.5 (C27), 123.7 (C5), 125.1 (C34), 126.3, 127.0 (C3, C4), 128.1 (C2), 128.4 (C33, C35), 129.1 (C32, C36), 132.6 (C25), 136.8 (C26), 139.4, 139.9 (C1, C6), 141.0 (C31), 149.2 (C28), 150.6 (C29), 169.4 (C20), 170.8, 171.7 (C19′, C22′), 173.3, 173.4 (C16′), 175.1 (C11); ESI(+) MS: *m*/*z* = 1265.03 $(M + H)^+$ and 1287.89 $(M + Na)^+$ in agreement with the calculated mass for $[M] = C_{75}H_{117}N_5O_{11}$.

Ind-[C(O)C2C(O)GlyPalm]2. R_f (AcOEt) = 0.4; ^{1}H NMR ($CDCl_3$): 0.85 (*t*, *J* 6.4, 12H, H1′), 1.20 (*s*, 96H, H2′–13′), 1.30 (*s*, 9H, H23), 1.45–1.70 (*m*, 10H, H13, H14′), 2.25–3.10 (*m*, 30H, H7, H12, H15–19, H30, H15′, H20′, H21′), 3.44 (*s*, 2H, H24), 3.65–4.25 (*m*, 8H, H17′), 4.73 (*m*, 1H, H18′a), 4.95 (*m*, 1H, H18′b), 5.16 (m, 1H, H14), 5.30 (*m*, 1H, H8), 5.71 (*dd*, *J* 4.8, 9.5, 1H, H9), 6.53 (*d*, *J* 9.5, 1H, H10), 6.86 (*bs*, 1H, H21), 7.13–7.34 (*m*, 10H, H2–5, H27, H32–36), 7.61 (*d*, *J* 7.8, 1H, H26), 8.47 (*m*, 2H, H28, H29); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 22.9 (C2′), 24.9 (C14′), 29.2, 29.4, 29.6, 29.8 (C4′–13′, C20′, C21′), 28.9 (C23), 32.0 (C3′), 34.0 (C15′), 35.3 (C13), 37.5 (C7), 40.0 (C30), 45.2 (C12), 50.5 (C18), 50.9 (C22), 52.2, 55.9 (C16, C17), 55.3 (C9), 59.1 (C15), 60.0 (C24), 61.2, 61.4, 61.7 (C17′), 67.4 (C19), 69.6 (C18′), 70.3 (C14), 76.8 (C8), 123.4 (C27), 124.1 (C5), 124.9 (C34), 126.4, 127.2 (C3, C4), 128.1 (C2), 128.5 (C33, C35), 129.0 (C32, C36), 133.1 (C25), 136.7 (C26), 139.2, 139.3 (C1, C6), 140.5 (C31), 148.9 (C28), 150.4 (C29), 170.5 (C20), 171.1, 171.6, 172.0, 172.1 (C16′), 173.0, 173.2 (C19′, C22′), 174.4 (C11); ESI(+) MS: *m*/*z* = 1915.17 $(M)^+$ and 1916.24 $(M + H)^+$ in agreement with the calculated mass for $[M] = C_{114}H_{187}N_5O_{18}$.

Ind(8)-C(O)C2C(O)GlyOleoyl and Ind-[C(O)C2C(O)-GlyOleoyl]2. The ester procedure when applied to 252 mg (0.35 mmol) of HOC(O)C2C(O)GlyOleoyl and 180 mg (0.29 mmol) of indinavir gave after purification by chromatography (1 : 0 to 0 : 1 hexane/AcOEt) 155 mg (0.12 mmol, 40%) of Ind(8)-C(O)C2C(O)GlyOleoyl and 77 mg (0.04 mmol, 13%) of Ind-[C(O)C2C(O)GlyOleoyl]2.

Ind(8)-C(O)C2C(O)GlyOleoyl. R_f (10 : 1 AcOEt/MeOH) = 0.5; ^{1}H NMR ($CDCl_3$): 0.85 (*t*, *J* 6.0, 6H, H1′), 1.20 (*s*, 9H, H23), 1.25 (*s*, 24H, H2′–7′), 1.30 (*s*, 16H, H12′–15′), 1.50–1.65 (*m*, 6H, H13, H16′), 1.99 (*m*, 8H, H8′, H11′), 2.20–3.20 (*m*, 22H, H7, H12, H15–19, H30, H17′, H22′, H23′), 3.47 (*s*, 2H, H24), 3.80 (*m*, 1H, H14), 3.95–4.21 (*m*, 4H, H19′), 4.97 (*m*, 1H, H20′), 5.31 (*m*, 5H, H8, H9′, H10′), 5.63 (*dd*, *J* 5.1, 9.1, 1H, H9), 6.45 (*d*, *J* 9.1, 1H, H10), 7.12–7.29 (*m*, 10H, H2–5, H27, H32–36), 7.58 (*d*, *J* 7.8, 1H, H26), 7.65 (*bs*, 1H, H21), 8.50 (*m*, 2H, H28, H29); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 22.7 (C2′), 24.9 (C16′), 27.2 (C8′, C11′), 29.1, 29.2, 29.4, 29.6, 29.7, 29.8 (C4′–7′, C12′–15′, C22′, C23′), 29.0 (C23), 31.9 (C3′), 34.0 (C17′), 37.6 (C7), 38.2 (C13), 39.4 (C30), 46.1 (C12), 48.1 C18), 51.2 (C22), 52.8, 54.8 (C16, C17), 55.2 (C9), 60.3 (C24), 61.6 (C15), 61.7 (C19′), 64.4 (C19), 65.9 (C14), 69.7 (C20′), 77.4 (C8), 123.5 (C27), 123.7 (C5), 125.1 (C34), 126.3, 127.0 (C3, C4), 128.1 (C2), 128.4 (C33, C35), 129.0 (C32, C36), 129.8, 130.1 (C9′, C10′), 132.6 (C25), 136.8 (C26), 139.3, 139.9 (C1, C6), 141.0 (C31), 149.1 (C28), 150.6 (C29), 169.4 (C20), 170.8, 171.7 (C21′, C24′), 173.2, 173.3 (C18′), 175.1 (C11); ESI(+) MS: *m*/*z* = 1316.91 $(M + H)^+$ and 1338.91 $(M + Na)^+$ in agreement with the calculated mass for $[M] = C_{79}H_{121}N_5O_{11}$.

Ind-[C(O)C2C(O)GlyOleoyl]2. R_f (AcOEt) = 0.4; ^{1}H NMR ($CDCl_3$): 0.84 (*t*, *J* 6.4, 12H, H1′), 1.20 (*s*, 9H, H23), 1.25 (*s*, 48H, H2′–7′), 1.30 (*s*, 32H, H12′–15′), 1.45–1.70 (*m*, 10H, H13, H16′), 1.90–2.15 (*m*, 16H, H8′, H11′), 2.20–3.15 (*m*, 30H, H7, H12, H15–19, H30, H17′, H22′, H23′), 3.43 (*s*, 2H, H24), 3.60–4.25 (*m*, 8H, H19′), 4.71 (*m*, 1H, H20′a), 4.93 (*m*, 1H, H20′b), 5.30 (*m*, 9H, H8, H9′, H10′), 5.68 (*dd*, *J* 5.0, 9.3, 1H, H9), 6.55 (*d*, *J* 9.3, 1H, H10), 6.86 (*bs*, 1H, H21), 7.11–7.33 (*m*, 10H, H2–5, H27, H32–36), 7.60 (*d*, *J* 7.8, 1H, H26), 8.46 (*m*, 2H, H28, H29); ^{13}C NMR ($CDCl_3$): 14.2 (C1′), 22.7 (C2′), 24.9 (C16′), 27.3 (C8′, C11′), 28.9 (C23), 29.2, 29.3, 29.4, 29.6, 29.8 (C4′–7′, C12′–15′, C22′, C23′), 31.9 (C3′), 34.0 (C17′), 35.3 (C13), 37.5 (C7), 39.9 (C30), 45.1 (C12), 50.6 (C18), 50.9 (C22), 52.2, 55.9 (C16, C17), 55.2 (C9), 59.1 (C15), 59.9 (C24), 61.2, 61.4, 61.6 (C19′), 67.3 (C19), 69.6 (C20′), 70.3 (C14), 76.8 (C8), 123.4 (C27), 124.1 (C5), 124.9 (C34), 126.4, 127.2 (C3, C4), 128.1 (C2), 128.5 (C33, C35), 129.0 (C32, C36), 129.7, 130.1 (C9′, C10′), 133.1 (C25), 136.7 (C26), 139.2, 139.3 (C1, C6), 140.5 (C31), 148.8 (C28), 150.4 (C29), 170.5 (C20), 171.1, 171.6, 172.0 (C18′), 173.0, 173.2 (C21′, C24′), 174.4 (C11); ESI(+) MS: *m*/*z* = 2019.45 $(M + H)^+$ in agreement with the calculated mass for $[M] = C_{122}H_{195}N_5O_{18}$.

Hydrolysis kinetics

The hydrolysis experiments were performed by incubating 20 mL of a DMEM/MeOH solution (pH 7.3) of the prodrug (250 μg mL^{-1}) at 37 °C under stirring. The MeOH amount (v/v) of these solutions was 6% for Saq-Phe(2TFA), 6% for Saq-Leu(2TFA), 5.1% for Saq-C(O)C4Tyr(1TFA) and Saq-C(O)NC4Tyr(1TFA), 5% for Ind(8)-Val(4TFA), Ind(14)-Val(4TFA), Ind(8)-Phe(2TFA), Ind(8)-Leu(2TFA), Ind-[C(O)C4Tyr]2 (1TFA), and Nelf(1)-C(O)C4Tyr(1TFA), 4% for Nelf(18)-C(O)NC4Tyr(1TFA), 3% for Ind(8)-C(O)C4Tyr-(1TFA), and 2% for Ind(14)-C(O)NC4Tyr(1TFA). Hydrolysis was followed by HPLC monitoring of the disappearance of the prodrug and appearance of the parent drug [this was the case

for Saq-Phe(2TFA), Saq-Leu(2TFA), Ind(8)-Val(4TFA), Ind(14)-Val(4TFA), Ind(8)-Phe(2TFA), Ind(8)-Leu(2TFA), Ind(8)-C(O)C4Tyr (1TFA), and Ind-[C(O)C4Tyr]2(1TFA), Nelf(1)-C(O)C4Tyr(1TFA)] or only of the disappearance of the prodrug [this was the case for Ind(14)-C(O)NC4Tyr(1TFA), Nelf(18)-C(O)NC4Tyr(1TFA), Saq-C(O)C4Tyr (1TFA), and Saq-C(O)NC4Tyr(1TFA)], by injecting 40 μL for nelfinavir and its prodrugs or 60 μL for all other compounds of the solution onto the HPLC column. HPLC analysis was performed using a HP1100 apparatus equipped with a Lichrospher 100 RP-18 (5 mm)-packed column (250 × 3.2 mm). The mobile phase consisted of a 15 mM sodium acetate and 15 mM sodium pentanesulfonate aqueous solution (pH 6) and CH_3CN (59/41, v/v, for indinavir and its prodrugs; 41/59, v/v, for saquinavir and its prodrugs; 45/55, v/v, for nelfinavir and its prodrugs, with a flow rate of 1 mL min^{-1}). The prodrugs and/or drugs were detected by measuring their UV absorption at 240 (saquinavir and its prodrugs) or 210 nm (for the other drugs and prodrugs) and the signals (peak integration) were computerized by the software provided. Under their respective HPLC conditions, the retention times were of 11.5 min for indinavir, 5.7 min for saquinavir, 12.5 min for nelfinavir, 14.2 min for Saq-Phe(2TFA), 14.0 min for Saq-Leu(2TFA), 5.8 min for Saq-C(O)C4Tyr-(1TFA), 4.3 min for Saq-C(O)NC4Tyr(1TFA), 5.1 min for Ind(8)-Val(4TFA), 5.4 min for Ind(14)-Val(4TFA), 22.4 min for Ind(8)-Phe(2TFA), 20.6 min for Ind(8)-Leu(2TFA), 8.5 min for Ind(8)-C(O)C4Tyr(1TFA), 8.5 min for Ind-[C(O)C4Tyr]-2(1TFA), 8.0 min for Ind(14)-C(O)NC4Tyr(1TFA), 9.6 min for Nelf(1)-C(O)C4Tyr(1TFA), and 7.6 min for Nelf(18)-C(O)-NC4Tyr(1TFA). The prodrug and/or drug concentrations were determined from HPLC calibration curves. These curves were established under the same HPLC conditions and using standard calibrated prodrug and drug solutions which were prepared in the same hydrolysis medium than the sample under investigation. The calibration curves are linear (correlation coefficient in the 0.9909–0.9997 range) in a concentration range of 1.6 to 410 μM for indinavir, 1.5 to 375 μM for saquinavir, 1.8 to 440 μM for nelfinavir, 0.9 to 240 μM for Saq-Phe(2TFA), 1.0 to 250 μM for Saq-Leu(2TFA), 1.1 to 270 μM for Saq-C(O)C4Tyr-(1TFA), 1.1 to 265 μM for Saq-C(O)NC4Tyr(1TFA), 0.8 to 214 μM for Ind(8)-Val(4TFA), 0.8 to 214 μM for Ind(14)-Val(4TFA), 1.0 to 255 μM for Ind(8)-Phe(2TFA), 1.0 to 265 μM for Ind(8)-Leu(2TFA), 1.1 to 285 μM for Ind(8)-C(O)C4Tyr-(1TFA), 0.8 to 220 μM for Ind-[C(O)C4Tyr]2(1TFA), 1.1 to 280 μM for Ind(14)-C(O)NC4Tyr(1TFA), 1.2 to 305 μM for Nelf(1)-C(O)C4Tyr(1TFA), 1.2 to 295 μM for Nelf(18)-C(O)-NC4Tyr(1TFA). Each sample was analyzed in triplicate and each calibration curve was determined in triplicate and repeated the same day of analysis.

Plots of $\ln([\text{prodrug}]_o - [\text{prodrug}(t)])$ and of $\ln[\text{drug}(t)]$ against time are linear in the concentration range studied, indicating that the hydrolysis is a first order process with respect to the prodrug. The half-lives of hydrolysis ($t_{1/2}$) were measured, when possible, or calculated from these plots; $t_{1/2}$ is related to the slope, K, of these curves by the relation of $t_{1/2} = (\ln 2)/K$.

Materials and methods: virology (antiviral assays)

The *in vitro* antiviral activity and cytotoxicity assays were performed as previously described according to published procedures.[33–35] Briefly, CEM-SS cells were infected with a dose of HIV-1 (LAI strain) infecting 50% of the cells. Four days later, the growth 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 growth of HIV-1 [HTLV-I (IIIB)] was followed by the cytopathogenic effect induced by the virus in MT4 cells. MT4 cells were infected with a virus dose allowing 4 days later the death of 90%. The tested compounds were added in the cell culture medium after viral infection and cell viability was measured by the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] test. The percentage of protection was calculated as the ratio Δ.

Δ = [(OD of treated infected cells − OD of untreated infected cells)/(OD of non infected cells − OD of untreated infected cells)]×100.

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

The effect of the prodrugs on cell viability was measured on non-infected cells using the colorimetric MTT test after 5 days of incubation at 37 °C with various concentrations of the tested product.

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