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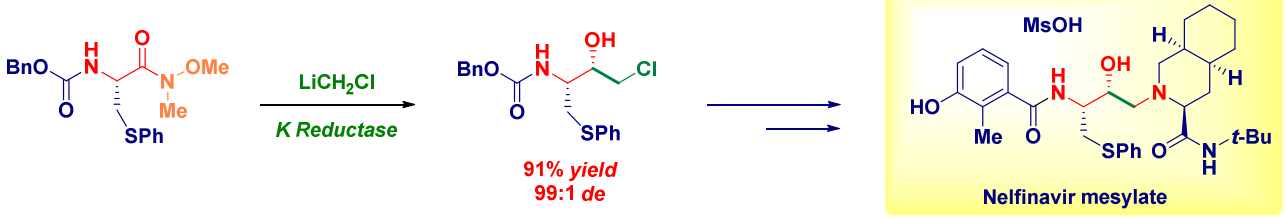
Chemoenzymatic Approach to Nelfinavir



Merging Lithium Carbenoid Homologation and Enzymatic Reduction: A Combinative Approach to the HIV-Protease Inhibitor Nelfinavir

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Abstract An effective stereocontrolled synthesis of the HIV protease inhibitor Nelfinavir is reported. Two transformations were identified crucial for achieving success: the formation of a densely functionalized α-chloroketone *via* the homologation of a Weinreb amide with chloromethyllithium ($LiCH_2Cl$), followed by its *erythro* selective reduction into the corresponding chiral chlorohydrin. A commercially available enzyme P2-C02 was particularly well suited for this purpose, affording the key alcohol (in an excellent 99% *de*), which was then smoothly converted into the active biologically active agent.

Introduction

The pharmaceutical treatment of HIV diseases ranks at the frontier of modern medicine as a consequence of the important and dramatic repercussions it causes within the society (*e.g.* 40 M infected individuals worldwide).[1] As such, the development of active and resistant-free drugs continues to represent an actively pursued research area for medicinal chemistry.[2] The highly structural differentiation among currently employed drugs constitutes a significant task in synthetic methodology for ensuring rapid and straightforward routes to agents active *via* progressively new identified biological targets, as exemplified by 3'-azido-3'-deoxythymidine (AZT, the first approved nucleoside analogue) and HIV protease inhibitors (Figure 1).[3] Undoubtedly, the introduction of these agents in the mid 1990s[4] benefited considerably the treatment of the pathology *via* the inhibition of the virus replication guaranteed by the not cleavable peptide bioisosteric hydroxyethylene unit.[5] A prominent example of the class is Nelfinavir, whose mesylate salt (Viracept®) conjugates an effective anti-HIV activity with a convenient pharmacokinetic behaviour (oral administration), thus improving patient compliance.[6]

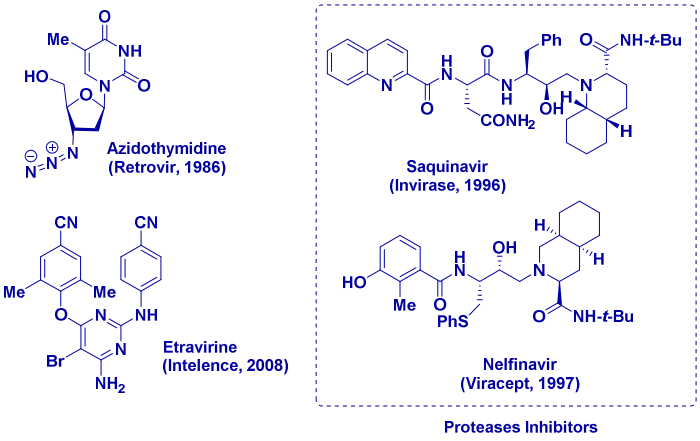
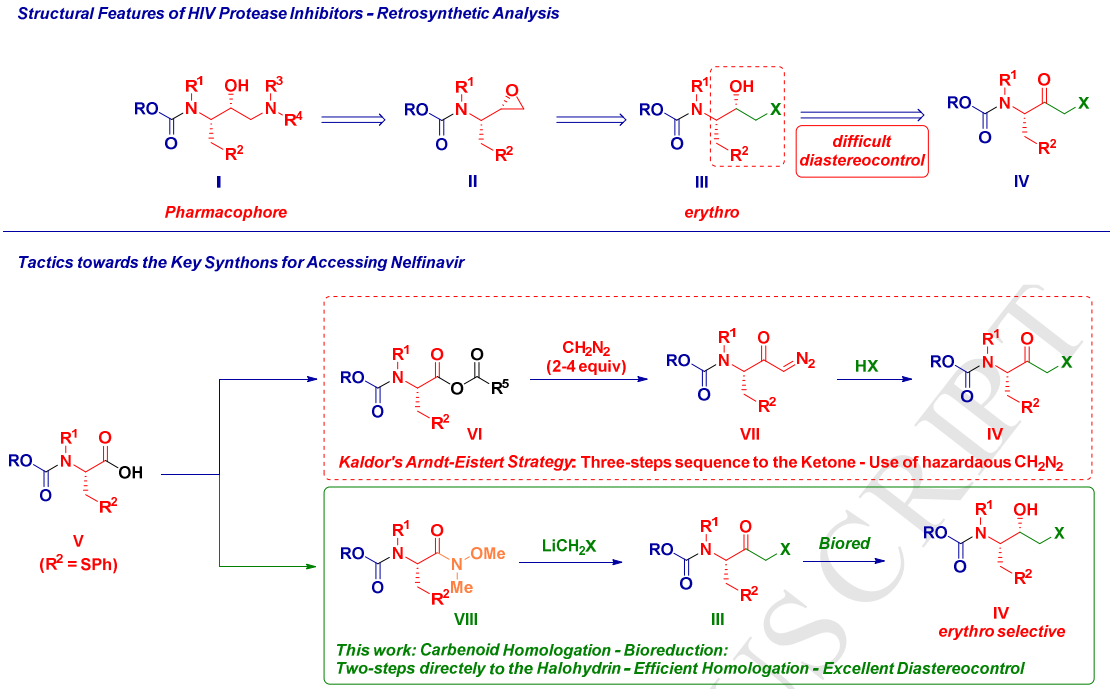


Figure 1. Commonly used anti-HIV agents.

The rational drug design individuates - *inter alia* - the presence of a chiral substituted 1,3-diamino-butan-2-ol (**I**, Scheme 1) featuring an *anti* (*erythro*) configuration for observing optimal therapeutic activity.[5,7] Accordingly, the retrosynthetic analysis depicted in Scheme 1 highlights the fundamental role displayed by the epoxide-synthon **II** (derived from the halohydrin **III**) whose efficient obtainment in terms of both chemical yield and optical purity represents the most intriguing and relevant event within the whole production process.[5,8] The classical approach towards it is paved on a three-steps sequence – involving a suitable α-aminoalkyl-α'-halomethylketone **IV** as the key synthon[7,9] - constituted by: *i*) carboxylic acid derivative homologation, followed by *ii*) stereocontrolled reduction to a chiral halohydrin **III**, direct precursor – under retentive conditions - of the targeted epoxide **II**.[5] Some points merit mention: a) Focusing on the homologation event it is quite interesting how the usual method employed for synthesizing the ketone **IV** still relies on the Arndt-Eistert procedure[10], involving the use of highly toxic and hazardous diazomethane.[11] Since the first preparation by Kaldor in 1997, this formally *direct* homologation requires three different synthetic operations: 1) activation of the carboxylic acid **V** to a more reactive anhydride **VI**; 2) formal CH_2N_2 homologation reaction and, 3) acydolysis[12] to convert the intermediate diazoketone **VII** to the requested α-haloketone **IV**. Notably, a significant excess of CH_2N_2 is required to obtain good yields of diazoketone - [(73% with 4 equiv),[6b] (62% with 2 equiv)[13]] - while the adoption of microfluidic techniques enables to get directly ketone **IV** in 73% yield as elegantly introduced by Kappe.[14] b) Controlling the diastereoselectivity in the reduction to the halohydrin **III** is troublesome and, in the case of Nelfinavir, no available completely stereoselective methods are known.[5,13]



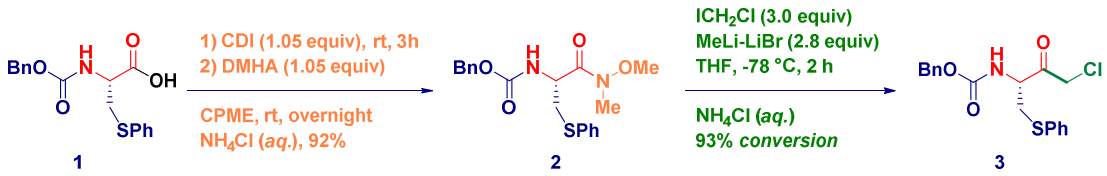
Scheme 1. General context of the presented work.

With the aim to design a diazomethane-free homologation strategy and direct carbenoid mediated tactic towards Nelfinavir, herein we detail an effective route in which the key haloketone is in turn reduced to the chiral halohydrine with excellent stereocontrol *via* a straightforward enzymatic reduction finally conducting to this important drug.

In recent years our group reported highly effective and reliable tactics for enabling the formal insertion of a CH_2X fragment into an acyl type electrophile by using lithium carbenoid reagents[15] (*i.e.* $LiCH_2X$, X = Cl, Br, I, F, OR, CN).[16] Such a direct operation presents the intrinsic advantage of installing the nucleophilic unit with the exact degree of functionalization, therefore affording the targeted structure through a single experimental operation. Among the portfolio of electrophiles studied, we introduced Weinreb amides[17] as privileged acylating manifolds for such reagents:[18] the formation of a stable (isolable)[19] tetrahedral intermediate guarantees excellent levels of chemoselectivity and, as a consequence, no undesired overaddition processes could be observed.

Results and Discussion.

The commercially available *N*-Carbobenzoxy-*S*-phenyl-L-cysteine **1** activated with carbonyldiimidazole (CDI) provided the corresponding Weinreb amide **2** upon reaction with *N*,*O*-dimethylhydroxylamine hydrochloride (DMHA).[18a,20] Interestingly, the reaction highly benefited from using cyclopentyl methyl ether (CPME)[16f,21]: it proved by far to be the optimal choice compared to other organic solvents (classical dichloromethane or the green solvent 2-MeTHF,[22] 78% and 53% respectively) affording the requested synthon – just after recrystallization - in an excellent 92% yield. With the Weinreb amide **2** in hands, we next focused our attention on the halomethylation procedure. With the goal of rapidly assembling the haloketone **3**, pleasingly we found $LiCH_2Cl$ - generated from ICH_2Cl (3.0 equiv) and MeLi-LiBr (2.8 equiv) – suitable for attacking the Weinreb amide **2** (Scheme 2).



Scheme 2. Preparation and homologation of the Weinreb amide to the key α-chloroketone.

Although ^{1}H-NMR analysis of the reaction crude showed an excellent conversion into **3** (93%), purification through silica gel chromatography provided it pure in a modest 41% isolated yield (Table 1). Analogously troublesome purification was obtained switching to aluminium oxide (neutral, basic or acidic) - Brockmann degree 1, 3 and 4 (entries 2-8).[19] Deactivation of silica gel with triethylamine (2-5%) allowed to increase the isolated yield up to 48% and 55% isolated yields (entries 9-10), respectively. Finally, deactivating silica with TMSCl maximized the isolated yield up to 84% (entry 12), while, using Florisil, decomposition phenomena again were predominant, allowing to recover the desired target in substantially decreased yield (entry 14). Importantly, despite the use of notoriously basic reagents as lithium carbenoids are, full preservation of the optical purity was observed [α_D (20 °C) = + 10.9 (*c* 0.3 $CHCl_3$), lit.[14b] + 12.7 (*c* 1.01 $CHCl_3$)].

Table 1. Chromatographic purification of ketone **3**: Study of the stationary phase.

Entry	Stationary phase	Deactivating agent (*w/w* %)	Isolated Yield of **3** (%)[a]
1	SiO_2	-	41
2[a]	Alox*N*-BG1	H_2	16
3[a]	Alox*N*-BG3	H_2O	24
4[a]	Alox*B*-BG1	-	20
5[a]	Alox*B*-BG3	H_2O	29
6[a]	Alox*B*-BG4	H_2O	34
7[a]	Alox*A*-BG1	-	11
8[a]	Alox*A*-BG4	-	20
9	SiO_2	NEt_3 (2%)	48
10	SiO_2	NEt_3 (5%)	55
11	SiO_2	TMSCl (1%)	73
12	SiO_2	TMSCl (2%)	84
13	SiO_2	TMSCl (5%)	82
14	Florisil	-	43

BG = Brockmann grade. [a] Deactivation of Alox *N, B, A* was realized according to ref. 19.

Previous studies on homologation chemistry by Izawa with $LiCH_2X$ reagents pointed out important difficulties in reactions involving common amino acid derivatives such as esters:[2a,5] in fact, the halogen-lithium exchange responsible for the carbenoid formation event starting from XCH_2Cl (X = Br, I) could be interfered by the presence of an acidic NH functionality. Because of the needing of using Barbier type conditions in analogous processes,[23] full substitution on the nitrogen was considered the unique available strategy. Accordingly, Barluenga proposed the use of *N,N*-dibenzyl esters,[24] while Izawa introduced *N*-protected 3-oxazolidin-5-ones[25] or, alternatively, *N*-imino protected amino acid esters.[26] Additionally, scientists at Roche described the use of *in situ* protected carbamates formed upon reaction of TMSCl with the corresponding lithium amide base, prior to the generation of the carbenoid.[27] It should be highlighted none of these techniques has been adapted

to the synthesis of Nelfinavir[28] thus, restricting homologation-based tactics towards it to the previously discussed CH_2N_2 paved ones.[5,6b,7,13]

With the key α-amino-α'-chloroketone **3** in hand, we then considered the stereoselective reduction to the crucial (*R*, *S*)-chlorohydrin **4** required for accomplishing the synthesis of Nelfinavir. Indeed, the reduction *via* standard chemical methods (*e.g.* $NaBH_4$) has been reported to proceed with incomplete stereocontrol – giving variable mixtures of the *erythro* (**4**) and *threo* (**5**) products - thus, making challenging a protocol for the selective *erythro* reduction.[13,28c,29] In this sense, nowadays biocatalytic reductions represent a reliable and effective tool for organic synthesis able to overcome serious issues associated with the employment of classical chemistry, including not only a favourable comparison in term of eco-compatibility, but mainly the possibility to achieve excellent levels of stereocontrol.[30] Cognizant of the recent work by De Souza and Kappe who developed a whole-cells based protocol for accessing enantiopure halohydrins,[31] we investigated the *erythro*-selective reduction by means of a commercially available set of engineered ketoreductases (Codex® KRED Screening Kit, provided by Codexis),[32] in the presence of the corresponding cofactor NAD(P)H, which was continuously regenerated *via* the *i*-propanol-acetone oxidative cycle. As indicated in Table 2, the screened enzymes[33] are able to induce a different stereocontrol at variable conversion rates under standardized conditions (24 h, 30 °C, pH 7). Few KRED enzymes (P2-B02, P2-D11, P2-D12, P2-G3, – entries 4, 6-8) showed modest selectivity (*de* 51-64%) and, therefore were not further screened. Albeit, only low to moderate conversions were evidenced, two enzymes P1-A04 (entry 1) and P2-C02 (entry 5) resulted very promising since excellent opposite selectivities (*de* 99%) were achieved, affording the *threo* (*R,R*) and the desired *erythro* (*R,S*) products, respectively. Presumably, the not optimal conversions were consequence of the limited solubility of ketone **3** in the reaction medium (*i*-propanol-acetone) and, as such, we analysed the effect of a cosolvent. The addition of DMSO (5% *v/v*) provoked the reversal of selectivity (*erythro* to *threo* - entries 9-11) for the KREDs P1-B02, P1-C01 and P2-C02, while in the case of P2-D11 it did not modify the stereochemical outcome (entry 12 *vs* 6). However, this cosolvent conducted consistently to the *threo* chlorohydrin **5** not suitable as precursor of Nelfinavir.

THF (5% *v/v*) had a positive effect on conversions (98-99% - entries 13-16) in the case of P1-B02, P1-C01, P2-C02 and P2-D11. This solvent showed a significant preference for the targeted *erythro* product **4** and, remarkably P2-C02 resulted again the best KRED giving 96% *de*. Further screening of this same KRED in the presence of sustainable cosolvents such as 2-methyltetrahydrofuran (2-MeTHF – entry 17) or, tetrahydrofurfuryl alcohol (THFA – entry 18) confirmed the pattern in terms of both *erythro* selectivity (97 – 96%, respectively) and conversion (>96%). Finally, by running the reaction in the presence of cyclopentyl methyl ether (CPME, 5% *v/v*, entry 19) maximized both crucial parameters, thus affording the transformation with full stereocontrol – desired *erythro* product **4** - (*de* 99%) and conversion (99%). With our delight the transformation was amenable for scale-up (1 mmol scale): analytically and optically pure (*de* 99%) compound **4** was obtained in an excellent 91% isolated yield after chromatography on silica gel (entry 20).

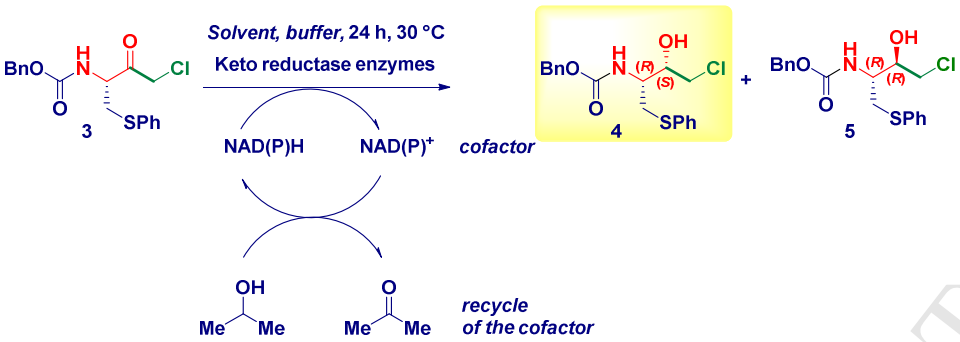
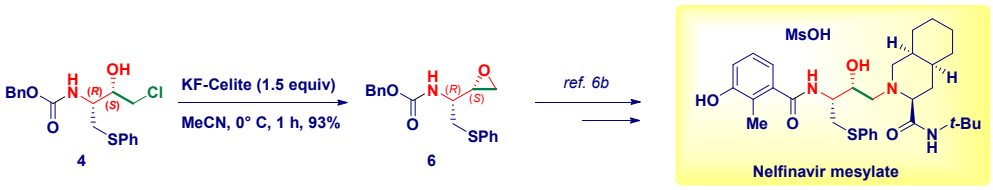


Table 2. Screening of KREDs and optimization of the reduction process.

Entry	Biocatalyst (KRED)	Conversion [%][a]	Cosolvent (*v/v*) %	*de* [%][a]
1	P1-A04	31	-	99 (*R,R*)
2	P1-B02	99	-	84 (*R,S*)
3	P1-C01	67		78 (*R,S*)
4	P2-B02	99		63 (*S,S*)
5	P2-C02	95		99 (*R,S*)
6	P2-D11	99		64 (*R,R*)
7	P2-D12	99		52 (*R,R*)
8	P2-G03	51		51 (*R,S*)
9	P1-B02	73	DMSO (5%)	94 (*R,R*)
10	P1-C01	95	DMSO (5%)	82 (*R,R*)
11	P2-C02	84	DMSO (5%)	84 (*R,R*)
12	P2-D11	74	DMSO (5%)	98 (*R,R*)
13	P1-B02	99	THF (5%)	50 (*R,S*)
14	P1-C01	98	THF (5%)	85 (*R,S*)
15	P2-C02	98	THF (5%)	96 (*R,S*)
16	P2-D11	98	THF (5%)	56 (*R,S*)
17	P2-C02	97	2-MeTHF (5%)	97 (*R,S*)
18	P2-C02	96	THFA (5%)	96 (*R,S*)
19	***P2-C02***	***99***	***CPME (5%)***	***99 (R,S)***
20[c]	***P2-C02***	99[d]	CPME (5%)	99 (*R,S*)

(*R,R*) refers to the *threo* product **5**. (*R,S*) refers to the – desired - *erythro* product **4**. [a] Otherwise stated NADPH was the cofactor. [b] NADH was the cofactor. [c] Reaction run at 1 mmol scale. [d] 91% Isolated yield.

Having established the conditions for preparing with full stereocontrol the key halohydrin **4**, a formal synthesis conducting to Nelfinavir mesylate was realized (Scheme 3). Interestingly, the reactive epoxide **6** was directly prepared from the chlorohydrin through a heterogeneous base (KF-Celite)[34]-mediated ring-closure we reported in 2011.[35] Finally, by applying the reported procedure Nelfinavir mesylate was obtained in 75% yield (starting from epoxide **6**).



Scheme 3. Formal synthesis of Nelfinavir mesylate.

Conclusions.

In conclusion, we developed an efficient strategy for accessing the HIV protease inhibitor Nelfinavir in high chemical yield under excellent stereocontrol. The tactic is paved on a straightforward preparation of an α-chloroketone through the reaction of an easily accessible Weinreb amide and the carbenoid chloromethyllithium. Such a homologation event proceeds with efficiency and full retention of the stereochemical information. Subsequently, a biocatalytic reduction performed with the commercially available P2-C02 enzyme in the presence of CPME, considered a green cosolvent, provides the *erytrho* chlorohydrin product – requested for the synthesis of the drug – in an excellent 99% *de*. The transformation of such an alcohol into an intermediate epoxide, followed by known procedures, finally affords Nelfinavir mesylate in 75% yield.

Experimental part.

General Methods.

Melting Points were determined on a Reichert-Kofler hot-stage microscope and are uncorrected. Mass spectra were obtained on a Shimadzu QP 1000 instrument (EI, 70 eV) and on a Bruker MaXis 4G instrument (ESI-TOF, APCI, HRMS). ^{1}H, ^{13}C, and ^{15}NMR spectra were recorded on a Bruker Avance III 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 40 MHz for ^{15}N) or on a Bruker Avance 200 spectrometer (200 MHz for ^{1}H, 50 MHz for ^{13}C) at 297 K using a directly detecting broadband observe (BBFO) probe. The centre of the (residual) solvent signal was used as an internal standard which was related to TMS with δ 7.26 ppm (^{1}H in $CDCl_3$), δ 77.00 ppm (^{13}C in $CDCl_3$). ^{15}N NMR spectra were referenced against external nitromethane (0.0 ppm). Spin-spin coupling constants (*J*) are given in Hz. In nearly all cases, full and unambiguous assignment of all resonances could be performed by combined application of standard NMR techniques, such as APT, HSQC, HMBC, COSY and NOESY experiments. Solutions were evaporated under reduced pressure with a rotary evaporator.

All the reactions involving organolithium reagents were carried out under inert atmosphere of argon. THF was distilled over Na / benzophenone. Chemicals were purchased from Sigma-Aldrich, Fluorochem Acros, Alfa Aesar and TCI Europe.

The mixture of alcohols **4** and **5** for HPLC traces was obtained *via* chemical reduction ($NaBH_4$).

Benzyl [(2*R*)-1-[methoxy(methyl)amino]-1-oxo-3-(phenylsulfanyl)-2-propanyl]carbamate (2)

To a solution of *N*-Carbobenzoxy-*S*-phenyl-L-cysteine **1** (3.0 g, 9 mmol, 1.0 equiv) in CPME (30 mL) was added 1,1'-carbonyldiimidazole (CDI, 1.61 g, 9.9 mmol, 1.05 equiv) in one portion and it was allowed to stir for 3 h at rt. Then, *N,O*-dimethylhydroxylamine hydrochloride (DMHA, 1.05 g, 10.8 mmol, 1.05 equiv) was added all at once, turning the solution a cloudy white. The reaction mixture was stirred overnight at rt and then quenched with 10 mL of a saturated aq. solution of NH_4Cl and, the aqueous layer was extracted with diethyl ether (2 x 15 mL). The combined organic layers were washed with water (30 mL) and brine (40 mL) then, dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was then purified by recrystallization from *i*-

propanol washed with *n*-hexane (20 mL) to afford the amide as a pale yellow solid (3.13 g, 92%; mp: 79-80 °C). $[\alpha]_D^{20}$: +12.2° (*c* 0.3 $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (m, 2H, SPh H-2,6), 7.35 (m, 4H, Ph H-2,3,5,6), 7.33 (m, 1H, Ph H-4), 7.28 (m, 2H, SPh H-3,5), 7.20 (m, 1H, SPh H-4), 5.66 (br d, *J* = 8.4 Hz, 1H, NH), 5.10 and 5.09 (AB-System, $^2J_{AB}$ = 12.3 Hz, 2H, CH_2O), 4.89 (m, 1H, NHC<u>H</u>), 3.58 (s, 3H, OCH_3), 3.32 (dd, *J* = 13.9, 4.7 Hz, 1H, SCH_2), 3.10 (dd, *J* = 13.9, 7.1 Hz, 1H, SCH_2), 3.07 (s, 3H, NCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 170.3 (C=O), 155.8 (OC=O), 136.2 (Ph C-1), 134.9 (SPh C-1), 130.9 (SPh C-2,6), 128.9 (SPh C-3,5), 128.5 (Ph C-2,6), 128.1 (Ph C-4), 128.0 (Ph C-3,5), 126.8 (SPh C-4), 66.9 (CH_2O), 61.5 (OCH3), 50.6 (NHCH), 36.9 (SCH_2), 32.0 (NCH_3). **^{15}N NMR** (40 MHz, $CDCl_3$) δ: -296.7 (NHCH), -196.1 (NCH_3). **IR (neat):** 3290, 1721, 1650, 1535, 1264, 1194, 778 cm^{-1}. **HRMS (ESI)**, *m/z*: calcd. for $C_{19}H_{22}N_2NaO_4S$: 397.1192 $[M+Na]^+$; found. 397.1201.

Benzyl [(2*R*)-4-chloro-3-oxo-1-(phenylsulfanyl)-2-butanyl]carbamate[14b] (3)

To a solution of benzyl [(2*R*)-1-[methoxy(methyl)amino]-1-oxo-3-(phenylsulfanyl)-2-propanyl]carbamate **2** (0.374 g, 1.0 mmol, 1.0 equiv) in THF, ICH_2Cl (0.22 mL, 3.0 mmol, 3.0 equiv) was added and the solution was cooled at -78 °C for 10 minutes. Then MeLi-LiBr (1.5 M, 1.87 mL, 2.8 equiv) was added dropwise over 30 minutes. After 2 hours the reaction was quenched with saturated aq. NH_4Cl (3.0 mL), the cooling bath was removed and the reaction was left under stirring until reached rt. The mixture was extracted with diethyl ether (2 x 20 mL) and the organic phase was further washed with brine (10 mL). The combined organic layers were washed with water (20 mL) and then dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography on silica gel – previously deactivated with 2% (*v/v*) TMSCl - using *n*-hexane / ethyl acetate (6:4, *v/v*) as the eluent. Analytically pure compound **3** was obtained as a white solid (0.305 g, 84%; mp: 89-91 °C - lit.[14b] 91-92 °C). $[\alpha]_D^{20}$: +10.8° (*c* 0.4 $CHCl_3$), lit.:[14b] $[\alpha]_D^{20}$: +12.7° (*c* 1.01 $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (m, 2H, SPh H-2,6), 7.37 – 7.30 (m, 5H, Ph H-2,3,4,5,6), 7.28 (m, 2H, SPh H-3,5), 7.25 (m, 1H, SPh H-4), 5.57 (br d, *J* = 6.3 Hz, 1H, NH), 5.08 (s, 2H, CH_2O), 4.73 (m, 1H, NHC<u>H</u>), 4.20 (part A of an AB-System, $^2J_{AB}$ = 15.9 Hz, 2H, CH_2Cl), 4.14 (part B of an AB-System, $^2J_{AB}$ = 15.9 Hz, 2H, CH_2Cl), 3.42 (dd, *J* = 14.0, 5.4 Hz, 1H, SCH_2), 3.33 (dd, *J* = 14.0, 6.1 Hz, 1H, SCH_2). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 199.6 (C=O), 155.7 (OC=O), 135.8 (Ph C-1), 133.8 (SPh C-1), 130.9 (SPh C-2,6), 129.4 (SPh C-3,5), 128.6 (Ph C-2,6), 128.4 (Ph C-4), 128.1 (Ph C-3,5), 127.5 (SPh C-4), 67.4 (CH_2O), 57.0 (NHCH), 46.9 (CH_2Cl), 35.7 (SCH_2). **^{15}N NMR** (40 MHz, $CDCl_3$) δ: -296.7 (NHCH). **IR (neat):** 3352, 1691, 1553, 1523, 1241 cm^{-1}. **HRMS (ESI)**, *m/z*: calcd. for $C_{18}H_{18}ClNNaO_3S$: 386.0588 $[M+Na]^+$; found 386.0593.

Benzyl [(2*R*,3*S*)-4-chloro-3-hydroxy-1-(phenylsulfanyl)-2-butanyl]carbamate[28c] (4)

Benzyl [(2*R*)-4-chloro-3-oxo-1-(phenylsulfanyl)-2-butanyl]carbamate **3** (0.018 g, 0.05 mmol) was dissolved in *i*-propanol (2 mL). Contemporaneously, ketoreductase enzyme P1-C02 (0.01 g) was suspended in an eppendorf containing KRED recycle mix P (0.030 g) solubilized in H_2O (0.8 mL). The solution of substrate **3** in *i*-propanol was added to the latter suspension and, the eppendorf was positioned in a shaker at 200 rpm/ 30 °C and monitored by TLC. After 24 h AcOEt (1.0 mL) was added, the eppendorf was shaked with vortex for 30 s and the phases separated by centrifugation (10 min 16000 rpm). The procedure is repeated and the organic phase was transferred into another eppendorf and dried on $NaSO_4$. Then, the organic phase is filtered, transferred in a vial and concentrated under reduced pressure. The (*R,S*)-isomer has been isolated as white solid (17 mg, 95%, mp: 111-113 °C, lit.:[28c] 113-114 °C) after chromatography on silica gel (*n-h*exane – ethyl acetate 8/2). $[\alpha]_D^{20}$: -69.3° (*c* 0.7 MeOH), lit.:[28c] $[\alpha]_D^{20}$: -73.4° (*c* 0.96 MeOH).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (m, 2H, SPh H-2,6), 7.36 – 7.30 (m, 5H, Ph H-2,3,4,5,6), 7.27 (m, 2H, SPh H-3,5), 7.20 (m, 1H, SPh H-4), 5.15 (br s, 1H, NH), 5.07 (s, 2H, CH_2O), 3.93 (m, 1H, OHC<u>H</u>), 3.92 (m, 1H, NHC<u>H</u>), 3.68 (dd, *J* = 11.4, 3.5Hz, 1H, CH_2Cl), 3.60 (dd, *J* = 11.4, 3.5 Hz, 1H, CH_2Cl), 3.30 (m, 2H, SCH_2), 2.81 (br s, 1H,

OH). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 156.1 (OC=O), 136.0 (Ph C-1), 135.2 (SPh C-1), 130.1 (SPh C-2,6), 129.2 (SPh C-3,5), 128.6 (Ph C-3,5), 128.3 (Ph C-4), 128.1 (Ph C-2,6), 126.8 (SPh C-4), 72.9 (CHOH), 67.2 (CH_2O), 53.3 (NHCH), 47.2 (CH_2Cl), 35.3 (SCH_2). **^{15}N NMR** (40 MHz, $CDCl_3$) δ: -295.5 (NHCH). **IR (neat):** 3312, 2939, 1660, 1532, 1261, 749 cm^{-1}. **HRMS (ESI)**, *m/z*: calcd. for $C_{18}H_{20}ClNNaO_3S$: 388.0745 $[M+Na]^+$; found 388.0748. The sample was analyzed by HPLC (Chiralpak IA Column, λ 220 nm, 90 *n*-hexane/ 10 *i*-propanol, 1ml/min, 20 °C; t_R (*R,R*): 14,85 min; t_R (*R,S*): 18,47 min, *de* 99:1.

Synthesis of compound 4 in 1 mmol scale (entry 20 – Table 2)

By following an analogous procedure, starting from compound **3** (0.364 g, 1.0 mmol) in *i*-propanol (40 mL) containing CMPE 5% (*v/v*), KRED-P1-C02 (0.2 g) and KRED recycle mix P (0.60 g in 16 mL of H_2O), compound 4 was obtained as a white solid (332 mg) in 91% yield. Spectroscopic data, optical rotation power and HPLC charts perfectly match with those ones reported above.

Benzyl [(2*R*,3*R*)-4-chloro-3-hydroxy-1-(phenylsulfanyl)-2-butanyl]carbamate[13] (5)

Benzyl [(2*R*)-4-chloro-3-oxo-1-(phenylsulfanyl)-2-butanyl]carbamate **3** (0.018 g, 0.05 mmol) was dissolved in *i*-propanol (2 mL) containing CMPE 5% (*v/v*). Contemporaneously, ketoreductase enzyme P1-A04 (0.01 g) was suspended in an eppendorf containing KRED recycle mix P (0.030 g) solubilized in H_2O (0.8 mL). The solution of substrate **3** in *i*-propanol was added to the latter suspension and, the eppendorf was positioned in a shaker at 200 rpm/ 30 °C and monitored by TLC. After 24 h AcOEt (1.0 mL) was added, the eppendorf was shaked with vortex for 30 sec and the phases separated by centrifugation (10 min, 16000 rpm). The procedure is repeated and the organic phase was transferred into another eppendorf and dried over $NaSO_4$. Then, the organic phase is filtered, transferred in a vial and concentrated under reduced pressure. Compound **5** was isolated as pale yellow oil (6 mg, 31%). $[\alpha]_D^{20}$: +26.2° (*c* 0.2 MeOH) **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.41 (m, 2H, SPh H-2,6), 7.39 – 7.32 (m, 5H, Ph H-2,3,4,5,6), 7.30 (m, 2H, SPh H-3,5), 7.21 (m, 1H, SPh H-4), 5.26 (d, *J* = 8.9 Hz, 1H, NH), 5.10 (part A of an AB System, $^2J_{AB}$ = 12.2 Hz, 1H, CH_2O), 5.09 (part B of an AB System, $^2J_{AB}$ = 12.2 Hz, 1H, CH_2O), 4.19 (m, 1H, OHC<u>H</u>), 3.87 (m, 1H, NHC<u>H</u>), 3.52 (m, 2H, CH_2Cl), 3.27 (dd, *J* = 13.8, 6.1 Hz, 1H, SCH_2), 3.12 (dd, *J* = 13.8, 8.1 Hz, 1H, SCH_2), 2.83 (d, *J* = 2.8 Hz, 1H, OH) . **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 156.3 (OC=O), 136.1 (Ph C-1), 135.0 (SPh C-1), 129.5 (SPh C-2,6), 129.2 (SPh C-3,5), 128.6 (Ph C-3,5), 128.3 (Ph C-4), 128.1 (Ph C-2,6), 126.6 (SPh C-4), 70.8 (CHOH), 67.2 (CH_2O), 52.3 (NHCH), 47.5 (CH_2Cl), 35.6 (SCH_2). **^{15}N NMR** (40 MHz, $CDCl_3$) δ: -299.2 (NHCH). **HRMS (ESI)**, *m/z*: calcd. for $C_{18}H_{20}ClNNaO_3S$: 388.0745 $[M+Na]^+$; found 388.0749. The sample was analysed by HPLC (Chiralpak IA Column, λ 220 nm, 90 *n*-hexane / 10 *i*-propanol, 1mL/min, 20 °C; Rt (*R,R*): 14,85 min; Rt (*R,S*): 18,47 min. *de* 1:99.

Benzyl [(1*R*)-1-[(2*S*)-2-oxiranyl]-2-(phenylsulfanyl)ethyl]carbamate (6).[6b,13]

To a solution of benzyl [(2*R,3S*)-4-chloro-3-hydroxy-1-(phenylsulfanyl)-2-butanyl]carbamate **4** (365 mg, 1.0 mmol, 1.0 equiv) in MeCN (5 mL) at 0 °C, potassium fluoride supported on Celite (50% *w/w*, 232 mg, 2.0 mmol, 2.0 equiv) was added and the mixture was stirred for 1 h. Then, the mixture was filtered under reduced pressure and washed with dichloromethane (10 mL). The resulting organic solution was concentrated *in vacuo* to afford pure epoxide **6** as colourless crystals (306 mg, 93%, mp: 61 °C, lit.[28e] mp 61-62°C). $[\alpha]_D^{20}$: -24.4 ° (*c* 1.0 $CHCl_3$), lit.:[28e] -26.2 (*c* 1.01 $CHCl_3$).

^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.50 – 7.09 (m, 10H), 5.09 (s, 3H), 3.71 (dtd, *J* = 12.1, 6.4, 2.8 Hz, 2H), 3.22 (d, *J* = 5.7 Hz, 2H), 3.00 (dt, *J* = 6.5, 3.3 Hz, 1H), 2.77 (d, *J* = 3.2 Hz, 2H). **^{13}C NMR** (50 MHz, $CDCl_3$) δ: 155.8, 136.1, 135.1, 130.0, 129.1, 128.5, 128.4, 128.1, 126.8, 67.0, 52.6, 52.3, 46.9, 36.1. **IR (neat):** 3318, 3076, 1685, 1552, 1275, 759 cm^{-1}. **HRMS (ESI)**, *m/z*: calcd. for $C_{18}H_{19}NNaO_3S$: 352.0978 $[M+Na]^+$; found 352.0981.

Nelfinavir mesylate - (3*S*,4a*S*,8a*S*)-*N*-(*tert*-butyl)-2-((2*R*,3*R*)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylthio)butyl)decahydroisoquinoline-3-carboxamide (as mesylate salt)

By applying a reported procedure[6b] Nelfinavir mesylate was prepared from compound **6** in 75% isolated yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (br s, 1H), 7.99 (br s, 1H), 7.40 – 7.35 (m, 3H), 7.23 (m, 3H), 7.15 (m, 1H), 6.85 (m, 1H), 6.78 (m, 1H), 6.68 (m, 1H), 5.74 (br s, 1H), 4.25 (m, 3H), 3.47 – 3.21 (m, 5H), 2.68 (s, 3H), 2.14 (br s, 3H), 2.05 (br s, 4H), 1.78 – 1.26 (complex, 13H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 172.2, 167.3, 155.1, 136.8, 135.3, 129.8, 129.1, 126.5, 126.4, 122.1, 118.4, 117.1, 68.5, 66.8, 60.1, 59.8, 52.9, 52.2, 39.5, 34.1, 31.1, 31.0, 29.8, 28.7, 28.3, 25.6, 24.3, 20.0, 12.8. **HRMS (ESI)**, *m/z*: calcd. for $C_{32}H_{45}N_3NaO_4S$: 590.3023 $[M+Na]^+$; found 590.3023.

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