Supporting information

Impact of Stereochemistry on Ligand Binding: X-ray Crystallographic Analysis of an Epoxide Based HIV Protease Inhibitor

Fabio Benedetti,[*§] Federico Berti,[§] Pietro Campaner,[§] Lidia Fanfoni,[§] Nicola Demitri,[§] Folasade M. Olajuyigbe,[§†] Matteo De March,[§] and Silvano Geremia[*§]

[§]Department of Chemical and Pharmaceutical Sciences, Centre of Excellence in Biocrystallograpy, University of Trieste, Via Giorgeri 1, 34127 Trieste, Italy. [‡]Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure 340001, Ondo State, Nigeria.

Table of Contents

Expression and Purification of HIV-1 Protease

Expression of HIV-1 protease was done using wild type HIV-PR (Genbank HIVHXB2CG), stabilized by five mutations (Q7K, L33I and L63I to minimize auto-proteolysis, and C67A and C95 to prevent aggregation by the formation of disulfide bonds) was expressed in *E.coli* BL21-Gold strain (DE3) pLysS competent cells. The expressed HIV-PR was purified from inclusion bodies using a modified method of Louis et al[1] on FPLC with eluting buffer, 50 mM 2-(N-morpholino) ethanesulfonic acid (MES) pH 6.5, 8 M urea. [2] The eluted HIV-PR fractions were serially diluted in a ratio of 1:1000 with 50 mM formic acid pH 2.5, than concentrated over 5kD cut-off membrane filter (Amicon Ultra, Milipore, USA) to 2–5 mg/mL and stored at 4°C.

Refolding and Cocrystallization of HIV-1 Protease with the Epoxide Inhibitor

The pulsed dilution technique was used to refold the HIV-PR. 200 μL of the HIV-PR in formic acid was added drop-wise to the refolding buffer (10 ml of solution obtained mixing 1 mL of 2.5 10^{-3} M inhibitor in DMSO with a buffer solution 50 mM sodium acetate pH 4.5) and gently swirled. This was left at room temperature for 1 hour for refolding. At the end of the 1 hour, another 200 μL was added and left also for 1 hour. This procedure was repeated until a total of 1 mL of HIV-PR has been added to the refolding buffer. The refolded protease-inhibitor complex solution was then concentrated over 5kD cut-off membrane filter (Amicon Ultra, Milipore, USA) to 1.1 mg/mL (about 100-200 μL of final clean solution with a percentage yield of the renaturation process of about 10%). The crystallization drops were formed using 1 μL of reservoir solution (0.25 M sodium citrate pH 6, 10% DMSO, and 40–60% saturated ammonium sulphate) and 1 μL of a solution of protein with inhibitor. Crystals of typical dimension $0.4 \cdot 0.2 \cdot 0.3$ mm^3 grew at 20 °C by vapor diffusion using the hanging drop method.

X-Ray Data Collection and Crystallographic analysis of PR/1b

X-ray data were collected for PR/**1b** crystals on XRD1 diffraction beam-line of Synchrotron ELETTRA, Trieste – Italy. Crystals were cryoprotected with 20% glycerol, mounted on a nylon loop and flash-frozen in liquid nitrogen. Data collection was performed at 100 K on a MAR-CCD detector. Data were processed using MOSFLM and CCP4 suite.[3,4] Structure refinement was conducted using REFMAC,[5] starting from isomorphic crystal structures (PDB code: 2NMZ) and refitted with program WinCoot.[6] Alternative conformations for residues were modeled where appropriate, and water molecules were inserted in the model based on peaks greater than 3s in F_{obs}-F_{calc} maps. The type of ion and other solvent molecules were identified by the shape of the 2Fo-Fc electron density map and inter-atomic distances. All figures were made using PyMol.[7] The coordinates for the PR/**1b** structure have been deposited in Protein Data Bank (PDB code: 3TOF).

Crystallization of Pure 1a, X-ray Data Collection and Crystallographic analysis

Pure epoxide crystals (dimensions ~$0.5 \cdot 0.1 \cdot 0.1$ mm^3) were grown in a dichloromethane solution. Ethyl ether was stratified on it and slow evaporation gave needle shaped single crystals suitable for X-ray structure determination. Data collection was performed at the X-ray diffraction beamline of ELETTRA Synchrotron (λ = 1.000 Å) using a Pilatus 6M image plate detector with Φ scan strategy. The **1a** crystals were mounted in a drop-loop of Paratone N and flash frozen at 100 K with nitrogen stream. The diffraction data were indexed and integrated using MOSFLM[3,8] and scaled with SCALA[3] from CCP4*i* platform. The structures were solved by direct methods using SIR2002[9] and Fourier analyzed and refined by the full-matrix least-squares based on F^2 using SHELXL-97[10].

In the final refinement, all non-hydrogen atoms were treated anisotropically and the hydrogen atoms were included at calculated positions with isotropic U factors = 1.2 U_{eq}. The experimental data has been corrected empirically for X-ray absorption using XABS2 program.[11] CCDC 843044 contains the crystallographic data for this paper.

Table S1. Refinement statistics for the HIV-PR/**1b** crystal

HIV-PR / 1b complex	
	Orthorhombic
PDB_{ID}	3TOF
Reflections (I/σ>2)	30233 (23666)
R_free reflections	1512
Restrains	20916
Parameters	15687
R_factor (I/σ>2) %	18 (17)
R_free (%)	24.6
Final model	
Protein atoms	1512
Inhibitor atoms	38
Water molecules	177
Other atoms	16
RMS Deviation	
Bond lengths (Å)	0.023
Bond angles (Å)	0.024
B-factor ($Å^2$)	
Protein main chain	11.0
Protein side chains	14.6
Inhibitor	19.5
Water molecules	25.1
Other molecules	20.7

*****(PDB code: 3TOF)

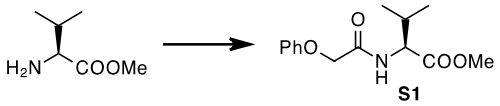
Table S2: Data reduction and refinement statistics for **1a** crystal

Space Group	$P2_12_12_1$
a (Å)	5.016
b (Å)	18.392
c (Å)	30.423
V ($Å^3$)	2806.65
Z	4
Dc ($g\ cm^{-3}$)	1.22
F (000)	1103.8
M (mm^{-1})	0.149
θ min,max (°)	1.82 – 32.36
Resolution (Å)	0.98
Reflections collected	11976
Reflections independent	1864
Reflections Obs. [Fo>4σ(Fo)]	4876
R merge (all data)	0.133
R merge (max Reso.)	0.364
I/σ(I) (all data)	11.9
I/σ(I) (max Reso.)	4.4
Completeness (all data) %	98.1
Completeness (max Reso.) %	78.9
Multiplicity (all data)	6.4
Multiplicity (max resolution)	4.8
Data / Restraints / Parameters	1836 / 0 / 155
R [I > 2.0σ (I)]	0.0692
R (all data)	0.0857
wR2 [I > 2.0σ (I)]	0.184
GooF	1.081

CCDC 843044 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the (CCDC) Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

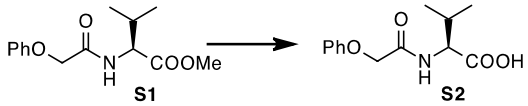
SYNTHESIS OF THE INHIBITOR

Melting points were recorded on a Büchi 510 capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded at 25°C, in $CDCl_3$ with TMS as internal standard, at 400 MHz and 100.1 MHz, respectively, unless otherwise stated. Optical rotations were measured at 25°C on a Perkin-Elmer 261 polarimeter at λ = 581 nm. ESI-MS was performed on a API 1 Perkin-Elmer SICIEX spectrometer. FT-IR spectra were recorded in Nujol, unless otherwise stated, with a ATAVAR 320 spectrophotometer. TLC were performed using Silica Gel 60-F254 coated Merck plastic sheets and light petroleum-ethyl acetate or dichloromethane-methanol mixtures as eluents. Flash chromatography was run on Merck silica gel 60 230-400 mesh.



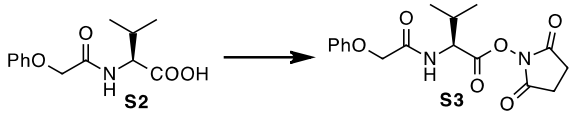
S1

(*S*)-Methyl-3-methyl-2-(2-phenoxyacetamido)butanoate (S1). HOBt (3.081 g, 22.8 mmol), N-methylmorpholine (5 mL, 45.6 mmol) and L-valine methylester hydrochloride (3.830 g, 22.8 mmol) were added, in the order, to a solution of phenoxyacetic acid (3.469 g, 22.8 mmol) in dry CH_3CN. The mixture was cooled to 0 °C and EDC (5.070 g, 26.4 mmol) was added. The mixture was stirred at 0 °C for 1h and at room temperature overnight. The solvent was removed in *vacuo*, the residue was dissolved in AcOEt and extracted with 10% aqueous citric acid, sat. $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure giving a yellow oil (97% yield) that was used without further purification. [□]$_D$ = - 4.1 (c=0.7, MeOH). ^{1}H NMR: δ 0.87 (*d*, 3H, *J*=6.8 Hz, *C*H$_3$); 0.92 (*d*, 3H, *J*=6.8 Hz, *C*H$_3$); 2.20 (*m*, 1H, C*H*(CH$_3$)$_2$); 3.74 (*s*, 3H, OC*H*$_3$); 4.54 (*d*, 2H, *J*=3.6 Hz C*H*$_2$O); 4.62 (*dd*, 1H, *J*$_1$=4.8 Hz, *J*$_2$=9.2 Hz C*H*NH); 6.95 (*d*, 2H, *J*=8.4 Hz, Ar orto); 7.03 (*t*, 1H, *J*=7.2 Hz, Ar para); 7.32 (*t*, 2H, *J*=7.2 Hz Ar meta). ^{13}C NMR: 17.7 (*C*H$_3$); 18.9 (*C*H$_3$); 31.3 [*C*H(CH$_3$)$_2$]; 52.2 (O*C*H$_3$); 56.7 (*C*HNH); 67.3 (*C*H$_2$O); 114.8 (Ar orto); 122.2 (Ar para); 129.8 (Ar meta); 157.2 (Ar-O); 168.2 (*C*O); 171.9 (*C*O). MS, m/z: 266 [MH]$^+$; 288 [MNa]$^+$.



S1 **S2**

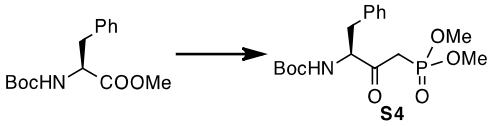
(*S*)-3-methyl-2-(2-phenoxyacetamido)butanoic acid (S2). The methyl ester **S1** (3.672 g, 13.8 mmol) was stirred, at 25 °C, in a 1:1 mixture of THF and 0.5 M aqueous LiOH (100 mL) and the reaction was monitored by TLC (CH_2Cl_2/MeOH 9:1). The reaction mixture was acidified to pH 4 with 10% HCl and extracted three times with AcOEt. The combined organic phases were extracted with brine and dried over anhydrous Na_2SO_4. The solvent was removed in *vacuo* giving the acid (88%), mp 128-130 °C. [□]$_D$ = + 18 (c=0.6, MeOH). IR: 3372 and 3345 (NH); 1712 (C=O acid); 1625 (C=O amide); 1562; 1459 cm^{-1} (Ar). ^{1}H NMR: δ 0.93 (*d*, 3H, *J*=6.8 Hz, C*H*$_3$); 0.97 (*d*, 3H, *J*=7.2 Hz, C*H*$_3$); 2.29 (*m*, 1H, C*H*(CH$_3$)$_2$); 4.57 (*d*, 2H, *J*=2 Hz C*H*$_2$O); 4.64 (*dd*, 1H, *J*$_1$=5.2 Hz, *J*$_2$=8.8 Hz, C*H*NH); 6.95 (*d*, 2H, *J*=8.8 Hz, Ar orto); 7.04 (*m*, 2H, Ar para + N*H*); 7.32 (*t*, 2H, *J*=8 Hz, Ar meta). ^{13}C NMR (125.68 MHz): 17.5 (*C*H$_3$); 18.9 (*C*H$_3$); 30.9 [*C*H(CH$_3$)$_2$]; 56.6 (*C*HNH); 67.2 (*C*H$_2$O); 114.8 (Ar orto); 122.3 (Ar para); 129.8 (Ar meta); 157.0 (Ar-O); 168.8 (*C*O); 174.9 (*C*O). MS, m/z: 252 [MH]$^+$; 274 [MNa]$^+$; 290 [MK]$^+$; 206 [M-CO$_2$].



S2 **S3**

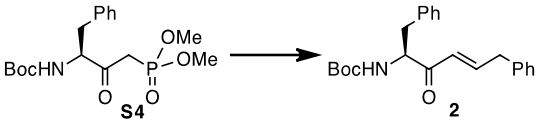
(*S*)-2,5-dioxopyrrolidin-1-yl-3methyl-2-(2-phenoxyacetamido)butanoate (S3) DCC (1.23 g, 6 mmol) was added, under argon, to a solution of the acid **S2** (1,5 g, 6 mmol) and N-hydroxysuccinimide (0.69 g, 6 mmol) in dry dioxane (20 mL) and the mixture was stirred at room temperature for 2 h. Dicyclohexylurea was filtered off, the solvent was evaporated and ethyl acetate (5 mL) was added to the residue: a second portion of dicyclohexylurea was filtered off and the solvent was evaporated giving the crude product (100 %) as a viscous oil that was used without further purification. IR (film): 3450 and 3350 (NH); 1810 (C=O ester); 1770 and 1730 (imide); 1680 (C=O amide); 1600 and 1580 cm^{-1} (Ar). ^{1}H-NMR: δ 1.03 (*d*, 3H, *J*=5.6 Hz, C*H*$_3$); 1.04 (*d*, 3H, *J*=5.6 Hz, C*H*$_3$); 2.38 (*m*, 1H, C*H*(CH$_3$)$_2$); 2.84 (*br*, 4H, C*H*$_2$-C*H*$_2$); 4.57 (*s*, 2H, OC*H*$_2$); 5.00 (*dd*, 1H, *J*$_1$=4.8 Hz, *J*$_2$=9.2 Hz, C*H*-NH); 6.95 (*d*, 2H, *J*=8 Hz, Ar

orto); 7.04 (*m*, 2H, Ar para + N*H*); 7.3 (*dd*, J_1=7.6 Hz, J_2=8.8 Hz 2H, Ar meta). ^{13}C-NMR: δ 17.3 (*C*H_3); 18.7 (*C*H_3); 25.6 (*C*H_2-*C*H_2); 31.6 [*C*H(CH_3)$_2$]; 54.9 (*C*HNH); 67.2 (*C*H_2O); 114.8 (Ar orto); 122.3 (Ar para); 129.8 (Ar meta); 157.0 (Ar-O); 167.2 (*C*O); 168.2 (*C*O); 168.5 (*C*O).



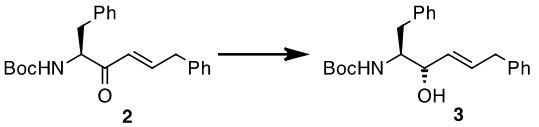
S4

Dimethyl [(3S)-4-phenyl-3-[N-(*tert*-butyloxycarbonyl)amino]-2-oxobutyl]phosphonate (S4) 100 mL of nBuLi in hexane (1.6 M, 162 mmol) was added dropwise to a stirred solution of dimethyl methanephosphonate (17.3 mL, 162 mmol) in THF (75 mL) under an argon atmosphere, at - 78 °C and the resulting solution was stirred for 1 h at -78 °C. N-Boc-L-phenylalanine methyl ester (7.55 g, 27 mmol) in THF (60 mL) was added dropwise and stirring was continued for 2 h at -78 °C. The mixture was allowed to warm up to 25°C; 20% aqueous citric acid was added and the organic layer was separated; the aqueous layer was extracted with AcOEt (2 x 100 mL) and the combined organic phases were extracted with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in *vacuo*. The phosphonate was crystallized from petroleum ether giving 9.42 g (yield 94%) of a white solid, mp 74–76 °C. $[\alpha]_D$ = - 52 (c=0.4, MeOH). IR (KBR)): 3280 (NH); 1723; 1713 (C=O); 1225 cm^{-1} (P=O). ^{1}H NMR: δ 1.39 (*s*, 9H, C(C*H*$_3$)$_3$); 2.93 (*dd*, 1H, J_1=8.4 Hz, J_2=13.6 Hz, C*H*$_2$Ph); 3.10 (*dd*, 1H, J_1=14 Hz, J_2=22.4 Hz, C*H*$_2$PO); 3.16 (*dd*, 1H, J_1=8.8 Hz, J_2=14.8 Hz, C*H*$_2$Ph); 3.25 (*dd*, 1H, J_1=14.4 Hz, J_2=22.4 Hz, C*H*$_2$PO); 3.75 (*d*, 3H, *J*=7.2 Hz, OC*H*$_3$); 3.77 (*d*, 3H, *J*=7.2 Hz, OC*H*$_3$); 4.55 (*m*, 1H, C*H*NH); 5.30 (*d*, 1H, *J*=7.2 Hz, N*H*); 7.17-7.32 (*m*, 5H, Ar). ^{13}C NMR (125.68 MHz): δ 28.2 (C(*C*H$_3$)$_3$); 36.9 (*C*H$_2$Ph); 38.5 (*d*, *J*=130 Hz, *C*H$_2$P); 53.15 (*d*, *J*=3.8 Hz, 2 x *C*H$_3$O); 61.2 (*C*HNH); 80.1 (*C*(CH$_3$)$_3$); 126.9-129.3 (*C* Ar); 136.4 (*C* Ar); 155.3 (*C*O Boc); 201.0 (*d*, *J*= 6.3 Hz, *C*O). MS, m/z: 410 [MK]$^+$, 394 [MNa]$^+$, 372 [MH]$^+$.



S4 **2**

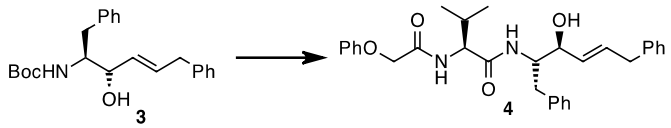
(*S*,*E*)-*tert*-butyl-3-oxo-1,6-diphenylhex-4-en-2-ylcarbamate (2) K_2CO_3 (1.87 g, 13 mmol) was added in small portions, over 15 min, to a stirred solution of phosphonate **S4** (5 g, 13 mmol) in dry EtOH (156 mL) under argon atmosphere at 25 °C. Phenylacetaldehyde (1.6 mL, 13 mmol) in dry EtOH (33 mL) was then added dropwise; the mixture was stirred at 25 °C for 20 min and filtered. The solution was neutralized with glacial acetic acid and the solvent was removed in *vacuo*. The residue was partitioned between AcOEt and sat. aqueous $NaHCO_3$ and the aqueous phase was extracted twice with AcOEt. The combined organic phases were extracted with sat. aqueous $NaHSO_3$ and brine and dried over anhydrous Na_2SO_4. The solvent was removed in *vacuo* and the product was purified by column chromatography with petroleum ether/AcOEt as eluent giving a white solid (50%), mp 75–78 °C. $[\alpha]_D$ = + 77 (c=1, MeOH). IR: 3360 (NH); 1690 (CO); 1623 (C=C); 1602 cm^{-1} (Ph). ^{1}H NMR: δ 1.40 (*s*, 9H, C(C*H*$_3$)$_3$); 2.97 (*dd*, 1H, J_1=7.2 Hz, J_2=13.6 Hz, C*H*$_2$Ph); 3.06 (*dd*, 1H, J_1=7.2 Hz, J_2=13.6 Hz, C*H*$_2$Ph); 3.48 (*d*, 2H, *J*=6.4 Hz, =CH-C*H*$_2$); 4.78 (*m*, 1H, C*H*NH); 5.25 (*d*, 1H, *J*=7.6 Hz, N*H*); 6.09 (*d*, 1H, *J*=15.6 Hz, =C*H*-CO); 7.09 (*m*, 5H, Ar orto + =C*H*-CH$_2$); 7.20-7.33 (*m*, 6H, Ar meta and para). ^{13}C NMR: δ 28.3 [C(*C*H$_3$)$_3$]; 38.5 (*C*H$_2$); 38.8 (*C*H$_2$); 58.4 (*C*HNH); 79.7 [*C*(CH$_3$)$_3$]; 126.8 (Ar); 126.9 (Ar); 128.0 (=*C*H-CO); 128.4 (Ar); 128.7 (Ar); 128.8 (Ar); 129.5 (Ar); 136.1 (Ar); 147.7 (=*C*H-CH$_2$); 155.1 (*C*ONH); 197.4 (*C*O). MS, m/z: 338 [MNa]$^+$.



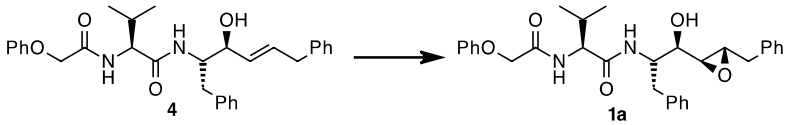
2 **3**

***Tert*-butyl (2*S*,3*S*,*E*)-3-hydroxy-1,6-diphenylhex-4-en-2-ylcarbamate (3).** L-selecride (10.3 mL of a 1.0 M solution in THF, 10.3 mmol) was added dropwise, at – 78 °C and under an Ar atmosphere, to a solution of the enone **2** (1.29 g, 3.53 mmol) in dry MeOH. The mixture was stirred at – 78 °C for 3.5 h, acidified to pH=5 with 1N HCl, and the solvent was evaporated *in vacuo*. The residue was partitioned between AcOEt and sat. aqueous $NaHCO_3$ and the aqueous phase was extracted twice with AcOEt. The combined organic phases were extracted with sat. aqueous $NaHSO_3$ and brine and dried over anhydrous Na_2SO_4. The solvent was removed in *vacuo* giving a mixture of diastereoisomers, in a 9:1 ratio, which were separated by column chromatography with petroleum ether and AcOEt as eluent (90:10 to 74:26 gradient). The major (*S*,*S*) isomer **3** was obtained as a white solid (77%), mp 99-100 °C.

[□]$_D$ = - 59.9 (c=0.39, MeOH). ^{1}H NMR: δ 1.39 (*s*, 9H, C(C*H*$_3$)$_3$); 2.89 (*m*, 2H, C*H*$_2$Ph); 3.36 (*d*, 1H, *J*=6.4 Hz, C*H*$_2$Ph); 3.78 (*br*, 1H, C*H*NH); 4.09 (*m*, 1H, C*H*OH); 4.80 (*d*, 1H, *J*=6.8 Hz, N*H*); 5.59 (*dd*, 1H, J_1=6 Hz, J_2=15.2 Hz, =C*H*-CHOH); 5.84 (*dt*, 1H, J_1=7.2 Hz, J_2=15.2 Hz, =C*H*-CH$_2$); 7.14-7.28 (*m*, 10H, Ar). ^{13}C NMR: δ 28.28 [C(*C*H$_3$)$_3$]; 38.15 (*C*H$_2$); 38.8 (*C*H$_2$); 56.40 (*C*HNH); 79.48 [*C*(CH$_3$)$_3$]; 126.22 (Ar); 126.42 (Ar); 128.54 (=*C*H-CO); 128.65 (Ar); 129.47 (Ar); 131.64 (Ar); 138.50 (Ar); 140.02 (=*C*H-CH$_2$); 156.21 (*C*ONH). MS, m/z: 368 [MH]$^+$; 390 [MNa]$^+$; 312 [MH-C$_4$H$_8$]$^+$; 268 [MH-Boc]$^+$.

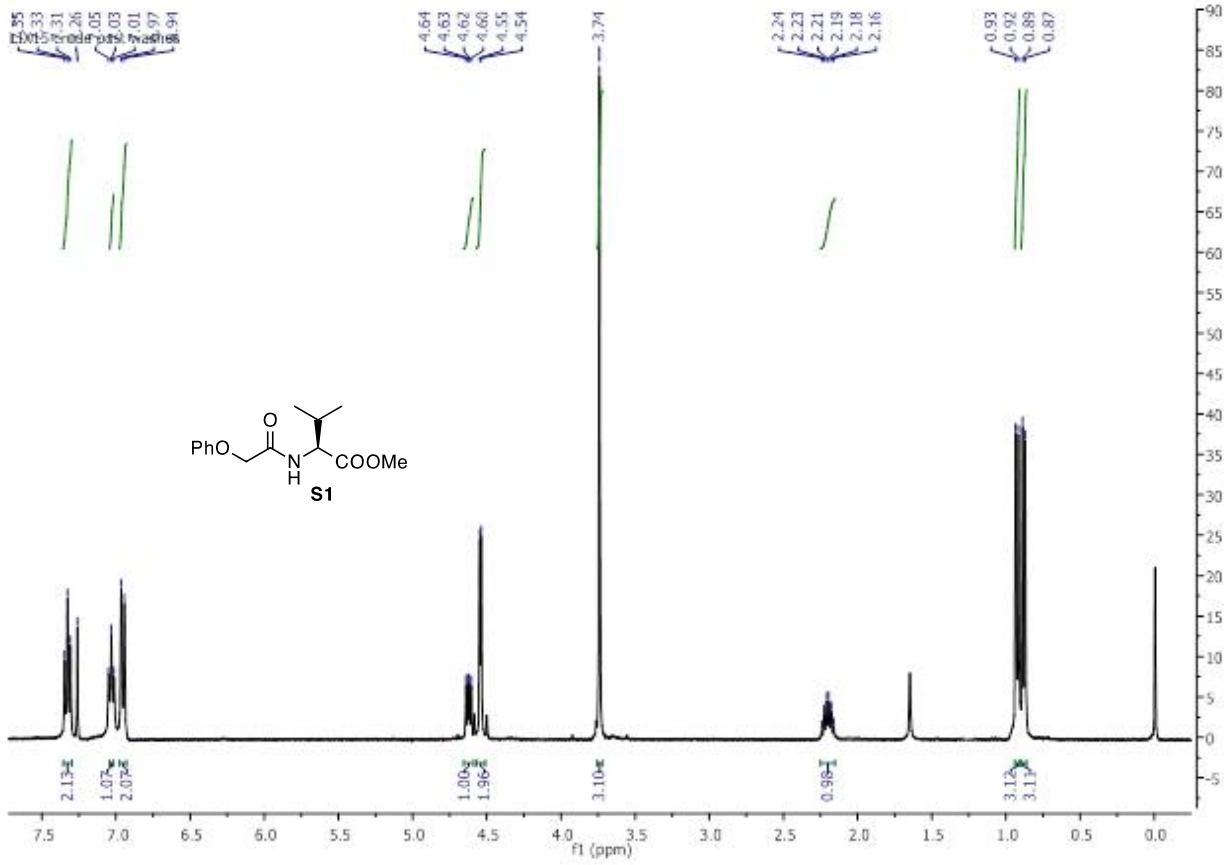


(*S*)-N-((2*S*,3*S*,*E*)-3-hydroxy-1,6-diphenylhex-4-en-2-yl)-3-methyl-2-(2-phenoxyacetamido) butanamide (4). N-Boc aminoalcohol **3** (413 mg, 1.12 mmol) was deprotected with 4 mL of a 40% solution of TFA in CH_2Cl_2 at 25 °C for 20 min. The solvent was removed in *vacuo* and the brown residue was co-evaporated several times with CH_2Cl_2 and Et_2O. The deprotected amine (1.12 mmol) and the activated acid **S3** (414 mg, 1.19 mmol) were dissolved in CH_2Cl_2; Et_3N (331 μL, 2.38 mmol) was added at 25 °C and the mixture was stirred overnight. The orange solution was extracted with 1M HCl, sat. $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with a 5% gradient of AcOEt in petroleum spirit as eluent, giving a white solid (396 mg, 96%). [α]$_D$ = -40 (c=0.3, MeOH). ^{1}H-NMR (500 MHz): δ 0.79 (*d*, 3H, *J*=7 Hz, C*H*$_3$); 0.87 (*d*, 3H, *J*=7 Hz, C*H*$_3$); 2.07 (*m*, 1H, C*H*(CH$_3$)$_2$); 2.55 (*d*, 1H, *J*=2.5 Hz, O*H*); 2.84 (*dd*, 1H, J_1=7 Hz, J_2=7.5 Hz, C*H*$_2$Ph); 2.94 (*dd*, 1H, J_1=7 Hz, J_2=7.5 Hz, C*H*$_2$Ph); 3.32 (br, 2H, =CH-C*H*$_2$) 4.16-4.22 (*m*, 3H, C*H*OH, C*H*NH, C*H*NH); [4.43 (d, 1H; *J*=15.0 Hz) e 4.51 (d, 1H; *J*=15.0 Hz) C*H*$_2$O]; 5.51 (dd, 1H, , J_1=5 Hz, J_2=15 Hz, =C*H*-CHOH); 5.84 (dt, 1H, J_1=5 Hz, J_2=15 Hz, =C*H*-CH$_2$); 6.32 (d, 1H, N*H*; *J*=10 Hz); 6.92-7.34 (m, 16H, Ar + NH). ^{13}C-NMR (125.68 MHz): δ: 18.0 (*C*H$_3$); 19.4 (*C*H$_3$); 30.7 [*C*H(CH$_3$)$_2$]; 38.0 (*C*H$_2$Ph); 38.8 (*C*H$_2$Ph); 55.1 (*C*HNH); 58.6 (*C*HNH); 67.2 (*C*H$_2$O); 71.9 (*C*HOH); 114.8 (Ar); 122.3 (Ar); 126.3-129.9 (Ar); 131.3 (=*C*H-CHOH); 132.1 (=*C*H-CH$_2$); 137.9 (Ar C$_1$); 139.7 (Ar C$_1$); 157.2 (Ar C$_1$-O); 168.5 (CO); 170.5 (CO). MS, m/z: 501.2 [MH]$^+$, 523.2 [MNa]$^+$, 539.1 [MK]$^+$

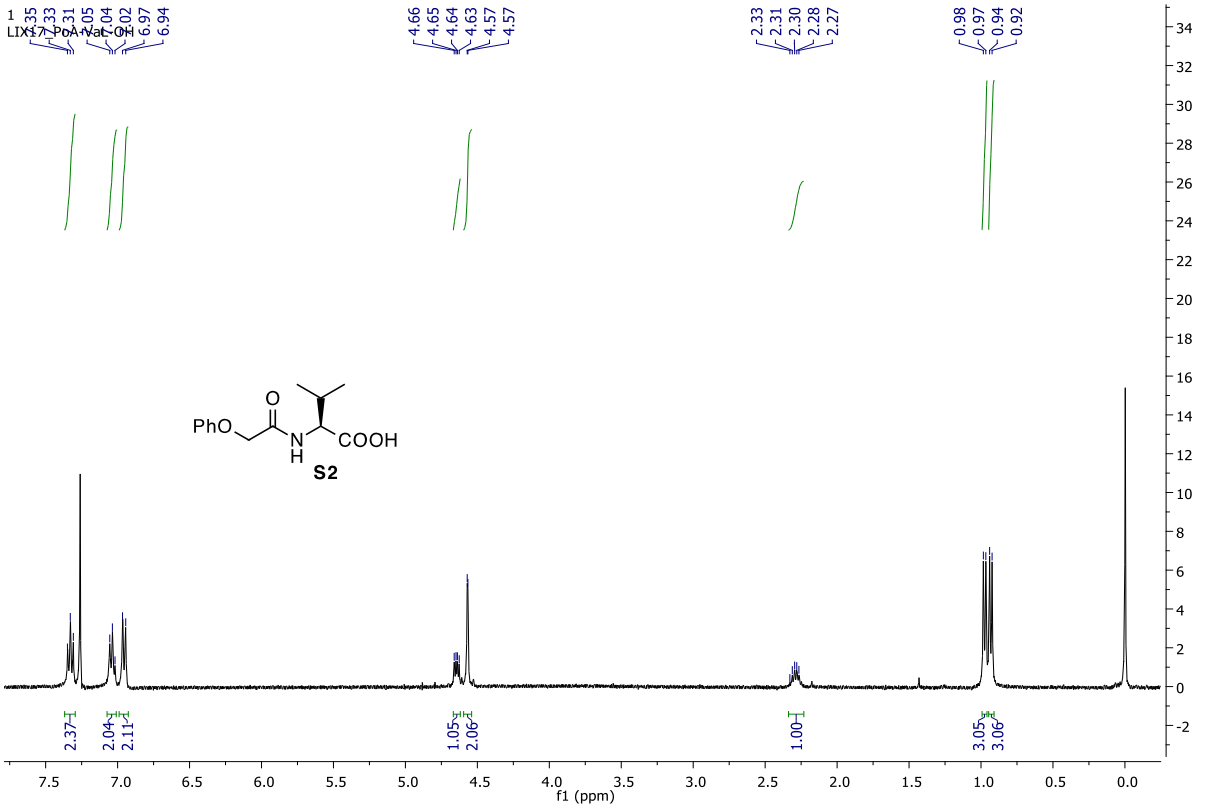


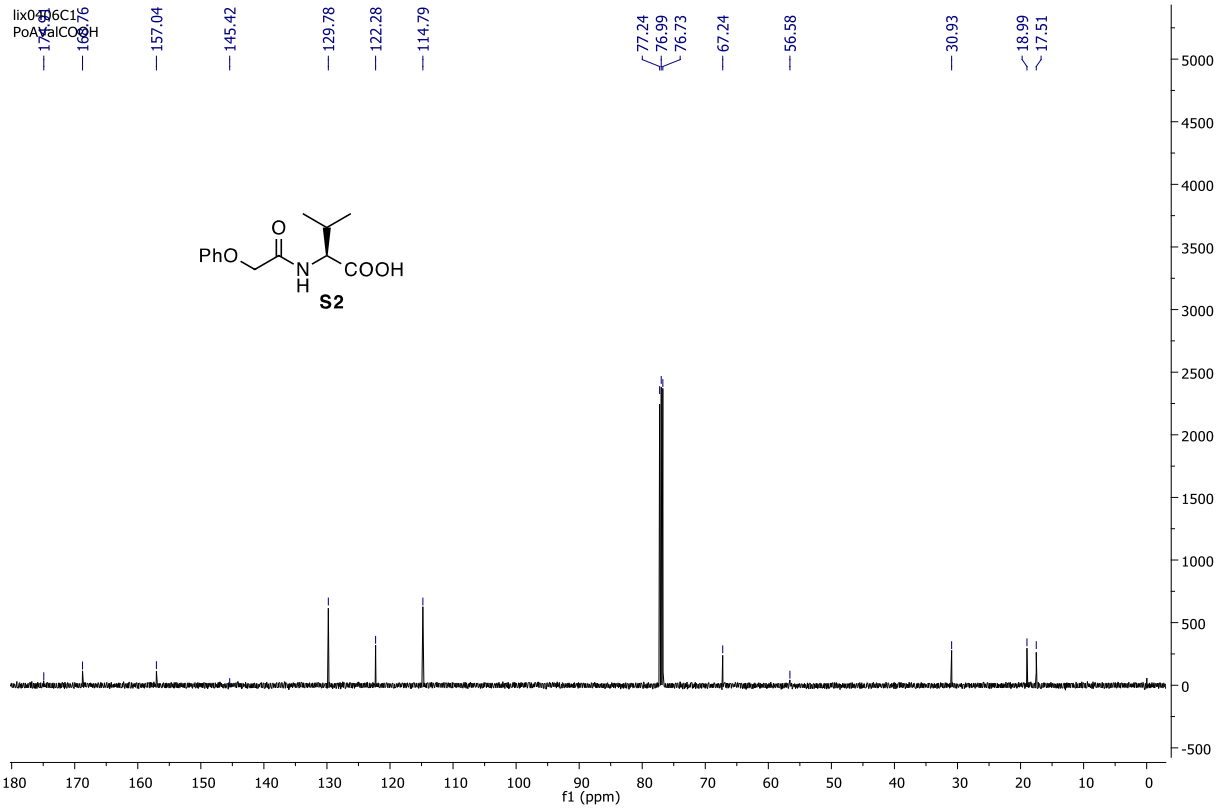
(*S*)-N-((1*R*,2*S*)-1-((2*S*,3*R*)-3-benzyloxiran-2-yl)-1-hydroxy-3-phenylpropan-2-yl)-3-methyl-2-(2-phenoxyacetamido)butanamide (1a). 3-Chloroperoxybenzoic acid (1.8 mmol) in CH_2Cl_2 (10 mL) was added, at 0 °C to the allylic alcohol **4** (751 mg, 1.5 mmol) in 10 mL CH_2Cl_2. The mixture was stirred for 16h, diluted with CH_2Cl_2 (20 mL), extracted with 10% aqueous $Na_2S_2O_4$, sat. $NaHCO_3$ and brine, and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was crystallized from Et_2O giving epoxide **1a** (480 mg, 0.93 mmol, 62%). [α]$_D$ = -47 (c=0.28, MeOH). ^{1}H-NMR (500 MHz): δ 0.81 (*d*, 3H, *J*=7 Hz, C*H*$_3$); 0.89 (*d*, 3H, *J*=7 Hz, C*H*$_3$); 2.16 [*m*, 1H, C*H*(CH$_3$)$_2$]; 2.55 (*d*, 1H, *J*=6 Hz, O*H*); 2.76 (*dd*, 1H, J_1=6 Hz, J_2=14.5 Hz, C*H*$_2$Ph) 2.84-2.94 (*m*, 4H, 1 x C*H*$_2$Ph + 2 x C*H*$_2$Ph + OC*H*-CH); 3.01 (*m*, 1H, OC*H*-CH$_2$); 3.51 (*m*, 1H, C*H*OH); 4.20 (*m*, 1H, C*H*NH); 4.29 (*m*, 1H, C*H*NH) 4.48 (*dd*, 2H, J_1=15 Hz, J_2=34 Hz, C*H*$_2$O); 6.31 (*d*, 1H, *J*=9 Hz, N*H*) 6.91-7.35 (*m*, 16H, Ar + N*H*). ^{13}C-NMR (125.68 MHz): δ: 17.8 (*C*H$_3$); 19.4 (*C*H$_3$); 30.2 [*C*H(CH$_3$)$_2$]; 37.6 (*C*H$_2$); 37.8 (*C*H$_2$); 53.6 (*C*HNH); 56.2 (*C*HO-CH$_2$); 58.6 (*C*HNH); 59.2 (*C*HO-CH); 67.1 (*C*H$_2$O); 70.2 (*C*HOH); 114.84 (Ar); 122.3-129.89 (Ar); 136.90 (Ar C$_1$); 137.74 (Ar C$_1$); 157.16 (Ar C-O); 168.8 (CO); 170.71 (CO).

^{1}H AND ^{13}C NMR SPECTRAL DATA

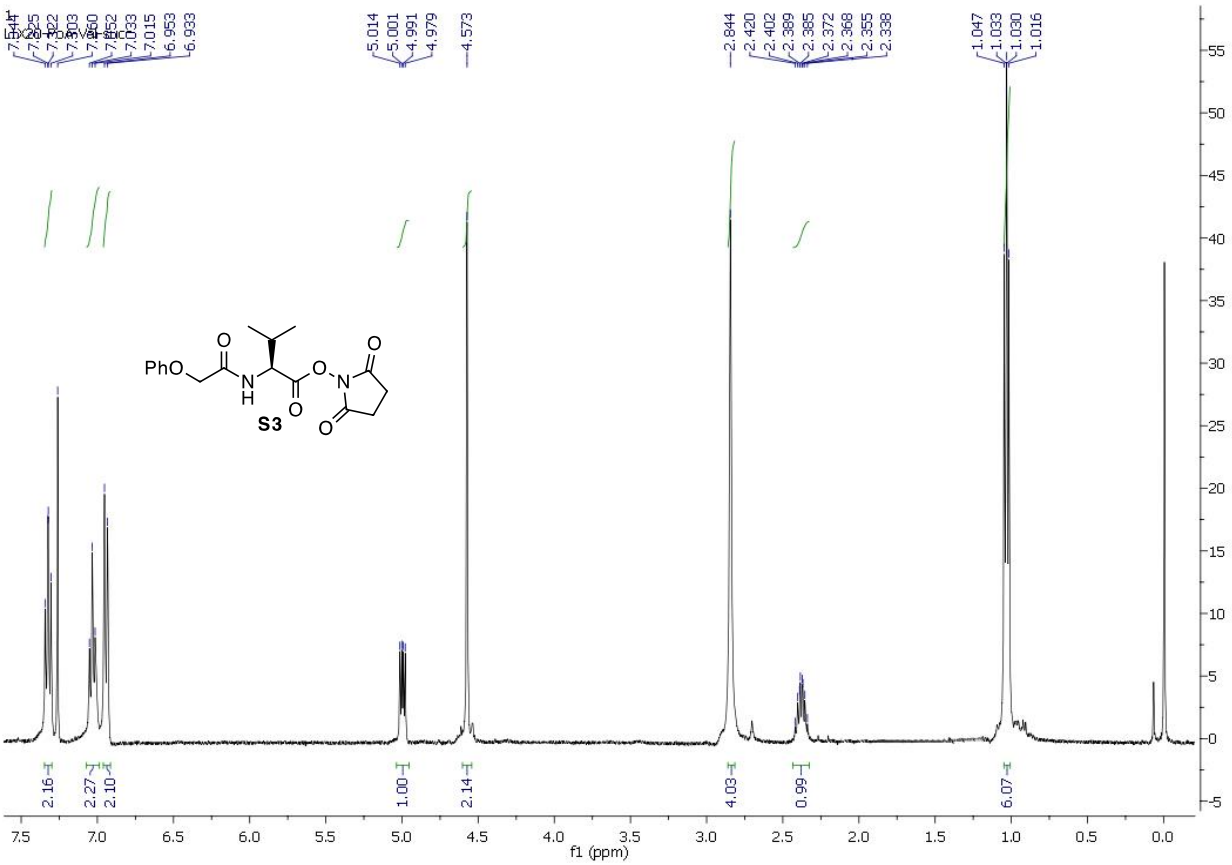


(*S*)-methyl 3-methyl-2-(2-phenoxyacetamido)butanoate (S1)

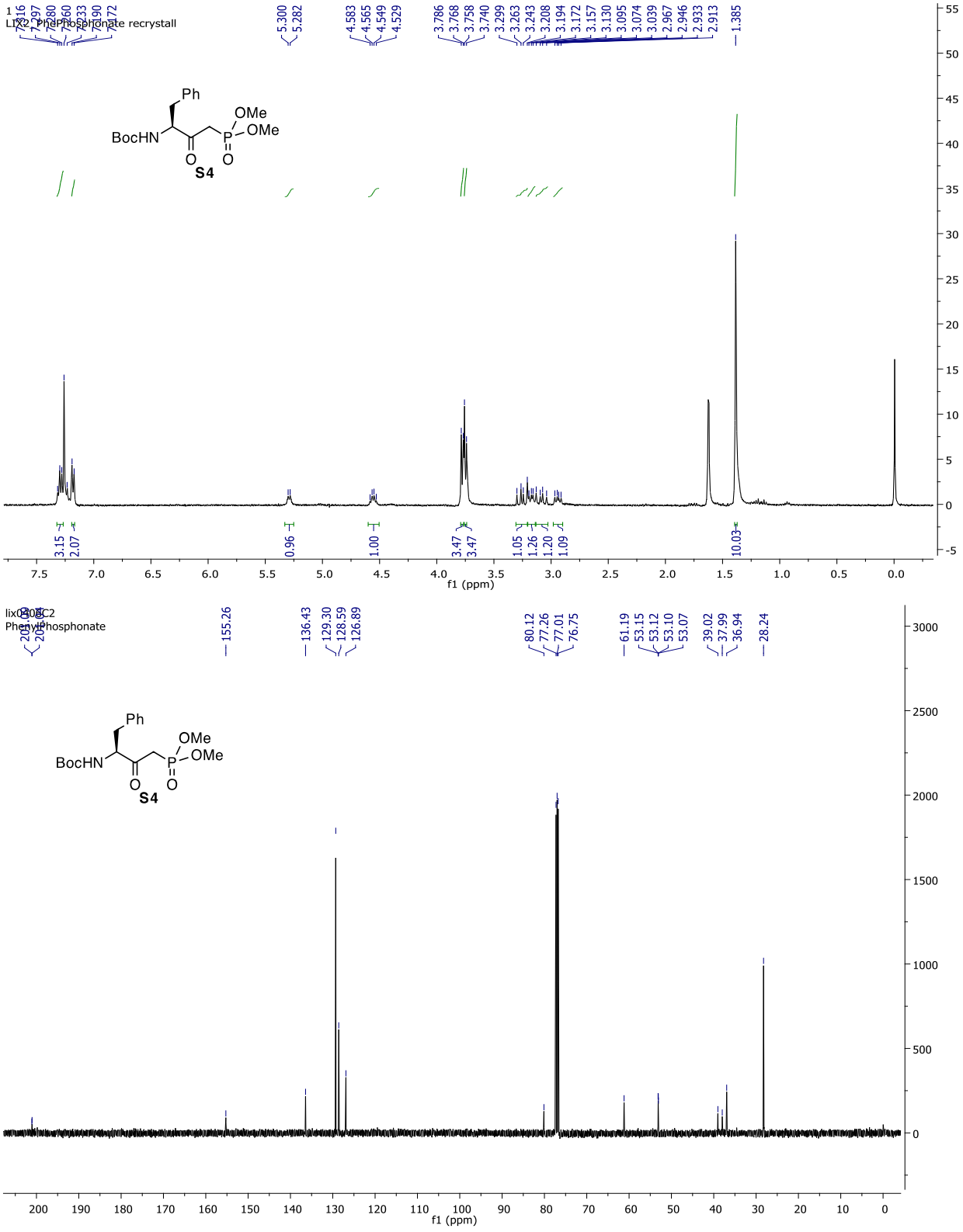




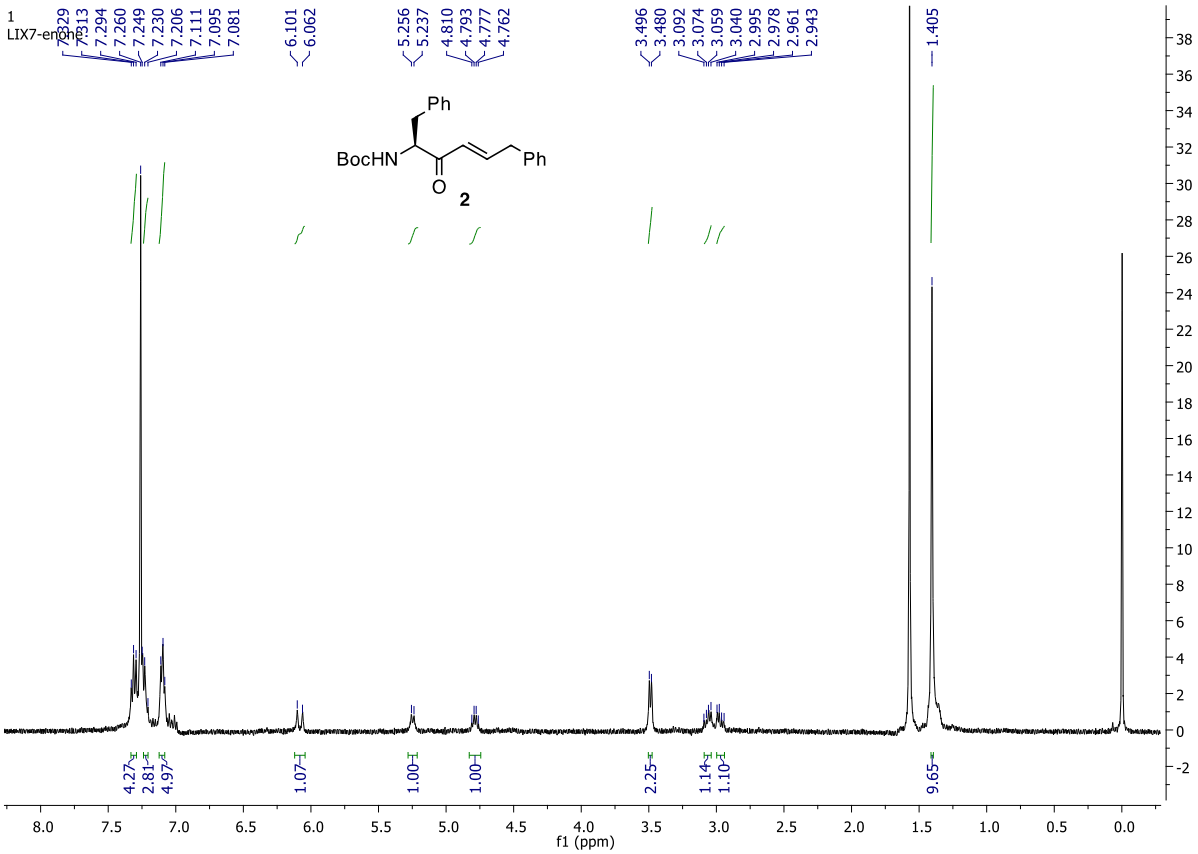
(*S*)- 3-methyl-2-(2-phenoxyacetamido) butanoic acid (S2)



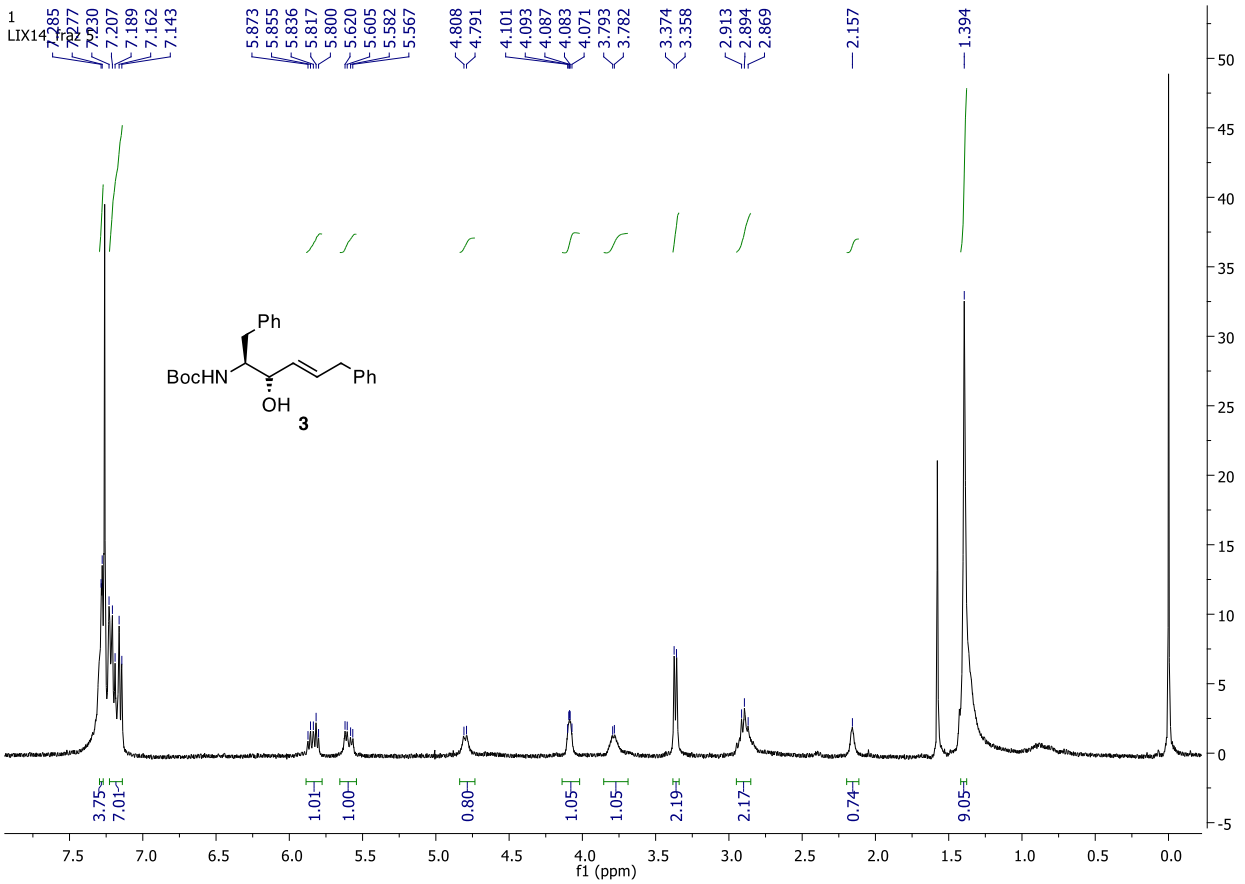
(*S*)-2,5-Dioxopyrrolidin-1-yl-3methyl-2-(2-phenoxyacetamido)butanoate (S3)



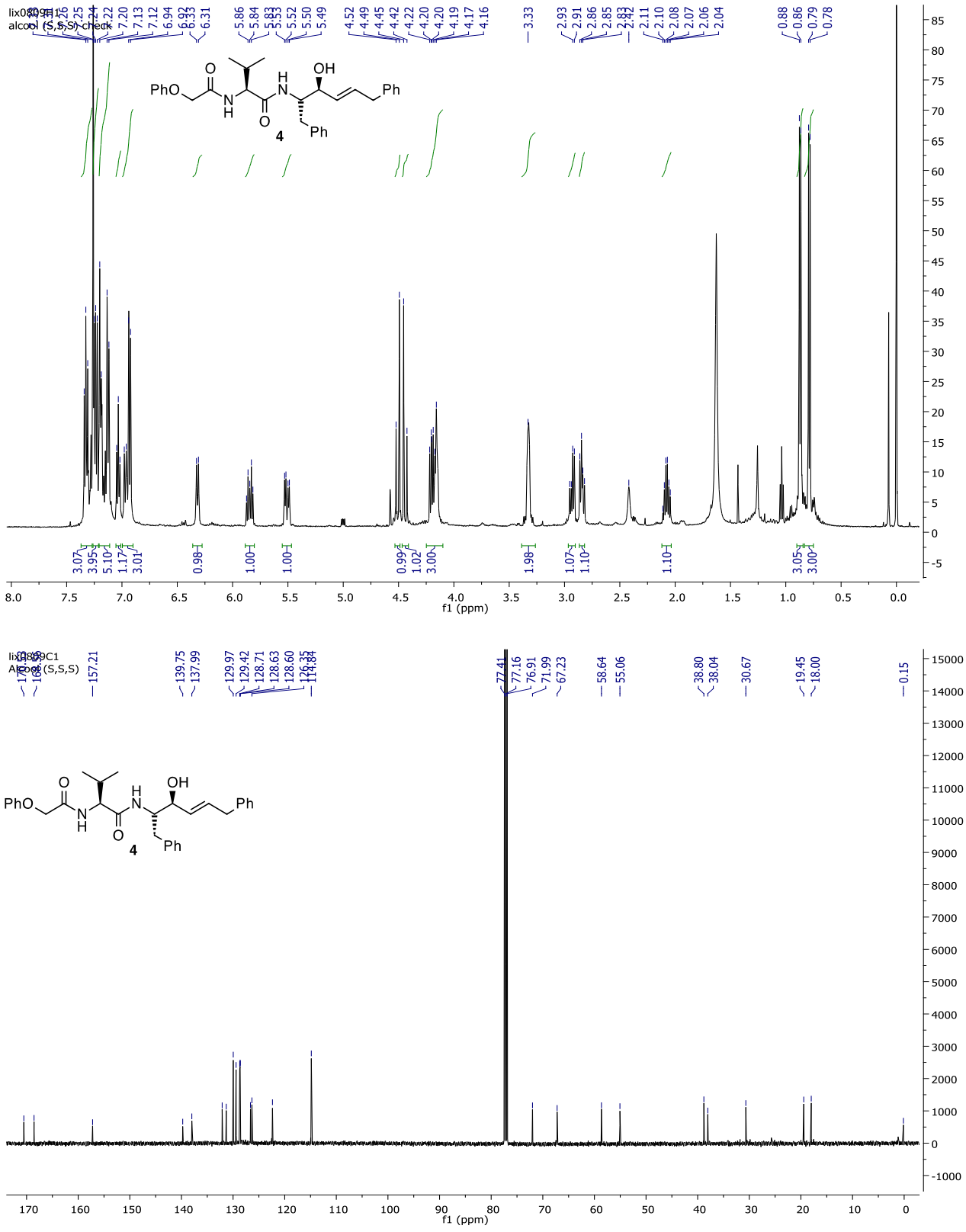
Dimethyl [(3S)-4-phenyl-3-[N-(*tert*-butyloxycarbonyl)amino]-2-oxobutyl]phosphonate (S4)



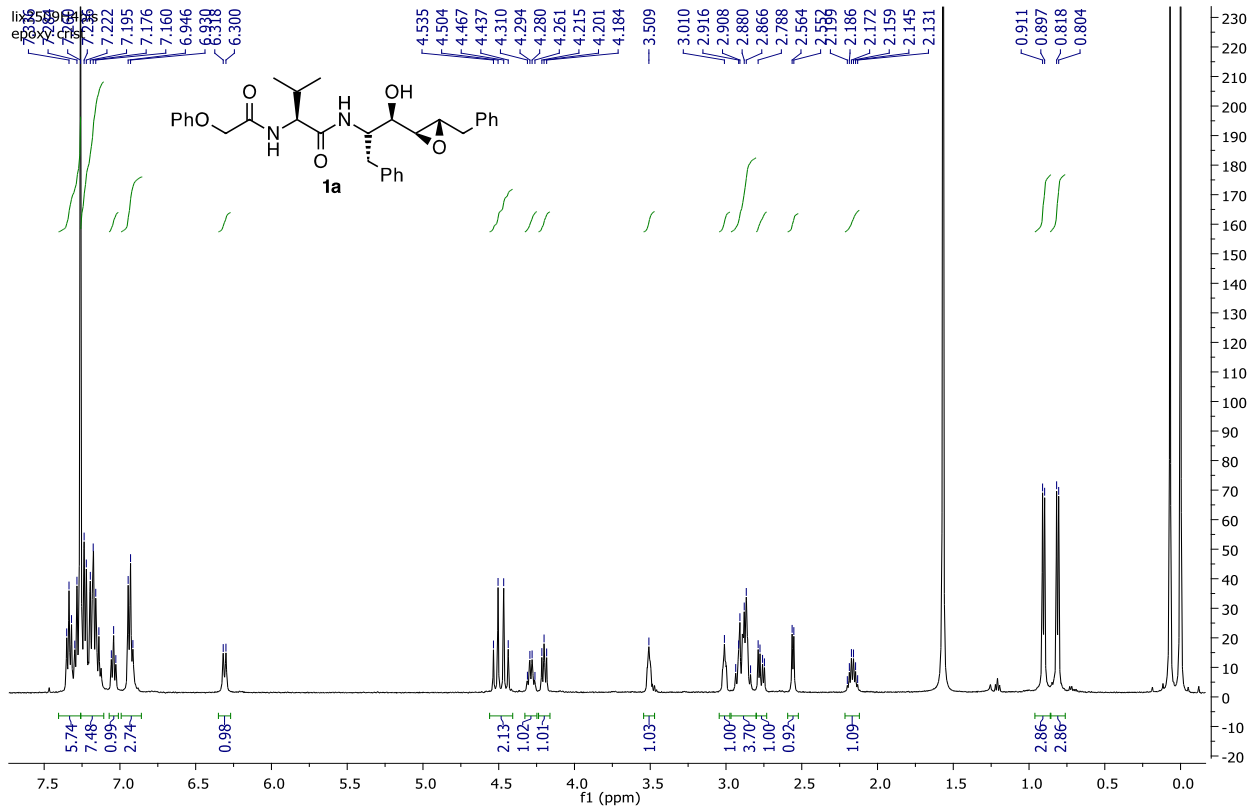
(*S*,*E*)-*tert*-butyl-3-oxo-1,6-diphenylhex-4-en-2-ylcarbamate (2)

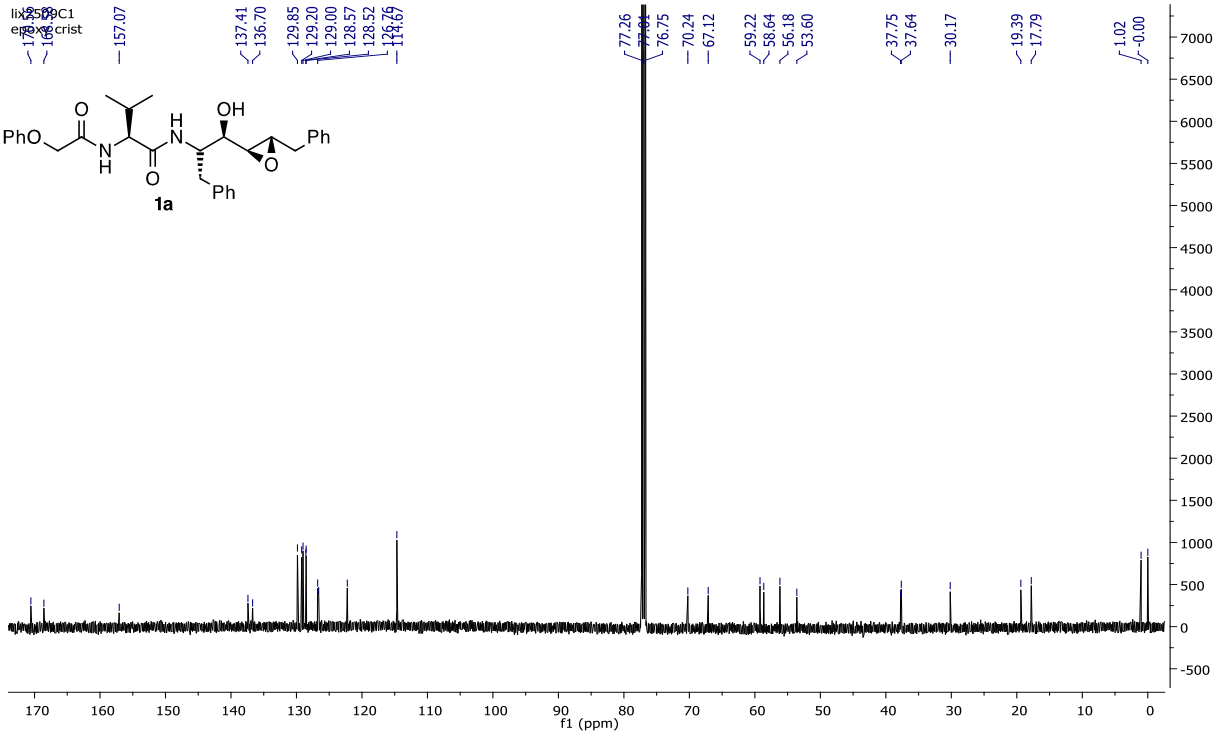


(2*S*,3*S*,*E*)-*Tert*-butyl 3-hydroxy-1,6-diphenylhex-4-en-2-ylcarbamate (3)



(*S*)-N-((2*S*,3*S*,*E*)-3-hydroxy-1,6-diphenylhex-4-en-2-yl)-3-methyl-2-(2-phenoxyacetamido) butanamide (4)





(*S*)-N-((1*R*,2*S*)-1-((*2S*,3*R*)-3-benzyloxiran-2-yl)-1-hydroxy-3-phenylpropan-2-yl)-3-methyl-2-(2-phenoxyacetamido)butanamide (1a)

INHIBITION OF RECOMBINANT HIV-PR BY EPOXIDE 1a

Solution A: 10 µl of a substrate stock solution in DMSO (10 mg/ml, 10.6 mM) were diluted in 1.99 ml 100 mM MES buffer, pH 5.5, containing 400 mM NaCl, 1 mM EDTA, 1 mM DTT, and 1 mg/ml BSA, to a final concentration of 53mM.

Solution B: 10 µL of a protease stock solution (0.4 mg/ml) in 10 mM sodium phosphate buffer, pH = 6.5, containing 1 mM EDTA, 10% glycerol, 0.05% mercaptoethanol, 50 mM NaCl, were diluted 100 times with the MES dilution buffer, pH 5.5, to a final concentration of 0.004 mg/ml.

Assay: 114 µL of solution A, 11 µL of solution B and 75 µL of the MES dilution buffer were pre-incubated in a cuvette, at 25 °C. After 1 min, 2 µL of the inhibitor from 100 times concentrated reference solutions in DMSO were added and the fluorence was recorded at 325 nm excitation and 420 nm emission. Final concentrations in the assay were 1.2 nM protease, 30 µM substrate and 0.5 nM – 5 mM inhibitor. IC50 were obtained by measuring the relative residual enzyme activity (ratio of the increase of fluorescence velocities before and after the addition of inhibitor) and by fitting the residual activity vs. inhibitor concentration semilog plots to a tetraparametric logistic function (Sigma plot 2001, SPSS Inc.). The experiments were run in triplicate: results are in Table S3 and in figure S1. The IC50 was found to be 670±190 nM.

Table S3

1a $molL^{-1}$	Residual activity
3.44E-09	100±7
3.44E-08	84±15
3.44E-07	64±13
1.75E-06	35±17
3.44E-06	17±9
3.44E-05	4±3
3.44E-04	0.5±0.5
6.87E-04	0.5±0.5

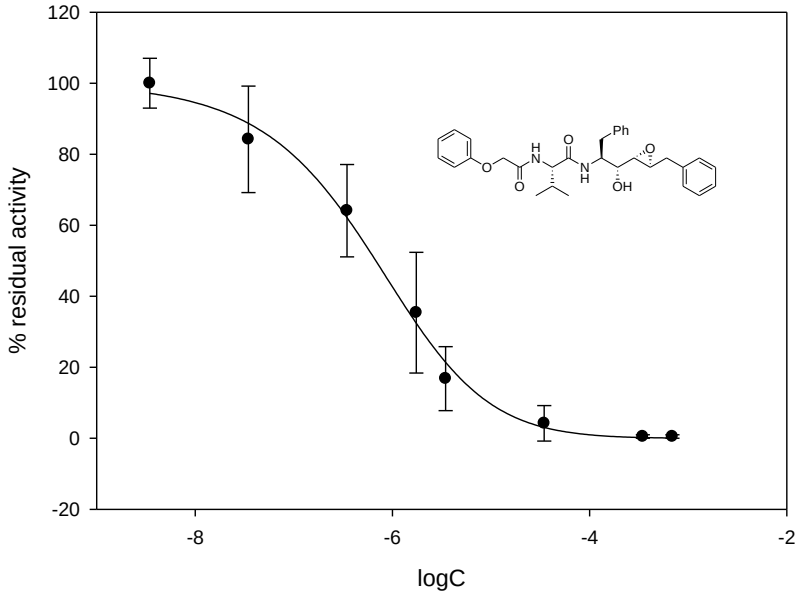


Figure S1

References

1 J. M. Louis, G. M. Clore and A. M. Gronenborn, *Nat. Struct. Biol.,* 1999, 6, 868-75.

2 F. Olajuyigbe, N. Demitri and S. Geremia, Cryst. Growth Des., 2011, 11, 4378–4385.

3 A. G. W. Leslie, *Joint CCP4 + ESF-EAMCB Newsletter on Prot. Crystallography,* 1992, 26.

4 The CCP4 suite: programs for protein crystallography. *Acta Cryst.,* 1994, D50, 760-763.

5 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Cryst.*1997, D53, 240-255.

6 P. Emsley and K. Cowtan, *Acta Cryst.,* 2004, D60, 2126-2132.

7 W. L. DeLano, *DeLano Scientific LLC* San Carlos, CA, USA, http://www.pymol.org.

8 A. G. Leslie, *Acta Cryst.,* 1999, D55, 1696-702.

9 M. C. Burla, M. Camalli, B. Carrozzini, G. L. Cascarano, C. Giacovazzo, G. Polidori and R. Spagna, *J. Appl. Crystallogr.,* 2003, 36, 1103.

10 G. M. Sheldrick and T. R. Schneider, *Meth. Enzymol.,* 1997, 277, 319-343.

11 S. Parkin, B. Moezzi and H. Hope, *J. Appl. Crystallogr.,* 1995, 28, 53-56.