Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Total Synthesis, NMR Solution Structure and Binding Model of the Potent Histone Deacetylase Inhibitor FR235222

Manuela Rodriquez,[a,b] Stefania Terracciano,[a] Elena Cini,[b] Giulia Settembrini,[b] Ines Bruno,[a] Giuseppe Bifulco,[a] Maurizio Taddei[b,*] and Luigi Gomez-Paloma[a,*]

[a] Professors L. Gomez-Paloma, I. Bruno, G. Bifulco, Doctors M. Rodriquez and S. Terracciano, Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, 84084 Fisciano (Salerno), Italy. Fax: (+39)-089-962828. E-mail: **gomez@unisa.it**
[b] Professor Maurizio Taddei, E. Cini and G. Settembrini, Dipartimento Farmaco Chimico Tecnologico, Università di Siena, via A. Moro 2, 53100, Siena, Italy. Fax: +39-0577-234333. Email: taddei.m@unisi.it

Abbreviations:
AA: amino acid
Bn: benzyl;
Boc: *tert*-butyloxycarbonyl;
ClTrt-Cl: 2-chlorotrityl chloride-resin;
DCC: *N,N'* dicyclohexylcarbodiimide;
DCM: dichloromethane;
DIEA: *N,N*-diisopropylethylamine;
DMF: *N,N*-dimethylformamide;
Fmoc: 9-fluorenylmethyloxycarbonyl;
N-α-Boc-*O*-TBDMS-Ahoda: *N*-α-Boc-9-*O*-TBDMS-8-oxodecanoic acid;
Fmoc-*D*-4-MePro-OH: *N*-α-Fmoc-D-4-methylproline;
Fmoc-*L*-Phe-OH: *N*-α-Fmoc-*L*-phenylalanine;
Fmoc-*L*-Iva-OH: *N*-α-Fmoc-*L*-isovaline;
HATU:*O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
HBTU:*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
HOBt: *N*-hydroxybenzotriazole;
TBDMS: *tert*-butyldimethylsilyl;
TFA: trifluoroacetic acid;
TFE: 2,2,2-trifluoroethanol;
TIS: triisopropylsilane.

General Experimental Data

Proton detected (^{1}H, HMBC, HSQC, TOCSY, ROESY) and carbon detected NMR spectra were recorded on Bruker instruments of Avance series operating at 300, 400, 600 MHz and 75, 100 and 150 MHz, respectively. Chemical shifts are expressed in parts per million (ppm) on the delta (δ) scale. The solvent peak was used as internal reference: for ^{1}H NMR $CDCl_3$ = 7.25 ppm, DMSO-d_6: 2.49 ppm; for ^{13}C NMR: $CDCl_3$ = 77.0 ppm, DMSO-d_6: 39.5 ppm. Multiplicities are reported as follows: (s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublets; dt = doublet of triplets; b = broad). Mass spectra were recorded on a LCQ DECA TermoQuest (San Josè, California, USA) mass spectrometer using an electrospray ion source (ES-MS).
Analytical and semipreparative reverse phase HPLC purifications were performed on a Jupiter C-18 column (250 x 4.60mm, 5μ, 300 Å; 250 x 10.00mm, 10μ, 300 Å, respectively). For estimation of Fmoc amino acids loading on the resin, absorbance at 301 nm was read employing a Shimadzu UV 2101 PC spectrophotometer.
All the reaction solvents were previously dried following standard procedures. Analytical TLC was performed on silica gel 60 F254 (Merck) plates. Visualization was done under UV (λ = 254 nm). Flash chromatography was done using a Sepacore® system or 60/230-400 mesh silica gel.

(*S*)-2-Dibenzylamino-6-oxo-hexanoic acid benzyl ester (4).
Methoxymethyltriphenylphosphomium chloride (0.77 g, 2.25 mmol) was dissolved in dry THF (8 mL) and to this solution, cooled to 0 °C, $LiN(SiMe_3)_2$ (2.4 mL of a 1 M solution in THF) was slowly added. After 1 h at 0 °C, aldehyde **2** (0.60 g, 1.5 mmol) was added in dry THF (8 mL). The mixture was stirred overnight at room temperature. Water was added and the two phases separated. The organic layer was washed several times with Et_2O, the collected organic fractions, were dried and evaporated. The crude product was purified using a Sepacore® system (silica gel column, petroleum ether 60-80/ EtOAc 3:1) to give compound **3** (0.48 g, 75% yield). This product was dissolved in EtOAc (8 mL) and then a solution of HCl 6N (4 mL) was added. The mixture was stirred at room temperature for 20 min, and then a saturated solution of Na_2CO_3 was added. The organic phase was separated and the aqueous layer washed several times with EtOAc. The organic fractions were collected and dried, the solvent evaporated to give crude compound **4** (0.436 g. 94% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60-1.80 (m, 4H), 2.05 (m, 2H), 2.23 (t-like, 1H), 3.41 (d-like, 2H), 3.80 (d-like, 2H), 5.12 (AB system, 2H), 7.15-7.40 (m, 15 H), 9.57 (s-like, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.6, 28.9, 42.2, 53.4, 53.7, 59.1, 60.2, 125.2 (3C), 126.3 (6C), 128.6 (6C), 134.2 (2C), 136.2, 174.3, 202.1. ES-MS *m/z* 416 $[M+H]^+$.

(*2S,9R,E*)-2-Dibenzylamino-9-(*tert*-butyldimethylsilyloxy)-8-oxo-6-decenoic acid benzyl ester (5).
To a solution of (*R*)-dimethyl-(2-[*tert*-butyldimethylsilyloxy)-1-oxo-propyl] phosphonate (0.293 g, 0.94 mmol) in dry MeCN (5 mL), dry LiCl (42.7 mg, 0.94 mmol) and then freshly distilled DIEA (98.0 mg, 0.78 mmol) were added. After stirring for 2 h at rt, aldehyde **4** (0.30 g, 0.72 mmol) in MeCN was added and the mixture was stirred at room temperature for 72 h. A saturated solution of NaCl was added and the organic layer separated, dried and purified using a Sepacore® system (silica gel column, petroleum ether 60-80/ EtOAc 6:1) to give compound **5** (0.37 g, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.12 (s, 3H) and 0.15 (s, 3H), 0.89 (s, 9H), 1.28 (d, *J* = 7 Hz, 3H), 1.40 (m, 1H), 1.59 (m, 1H), 1.70 (m, 1H), 1.77 (m, 1H), 2.01 (m, 2H), 3.24 (X part of an ABX system, 1H), 3.49 (d-like, 2H), 3.87 (d-like, 2H), 4.21 (q, *J* = 7 Hz, 1H), 5.13 (d, *J* = 9 Hz, 1H), 5.25 (d, *J* = 9Hz, 1H), 6.58 (d, *J* = 13 Hz, 1H), 6.89 (dt, ^{A}*J* = 13 Hz, ^{B}*J* = 8 Hz, 1H), 7.20-7.40 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: -4.9, -4.8, 18.1, 21.1, 24.4 (3C), 25.7, 28.9, 32.1, 54.2, 54.4, 60.2, 64.0, 74.3, 124.2, 126.9, 127.0, 128.2 (2C), 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.8 (2C), 129.0 (2C), 132.7, 134.6, 135.1, 139.4, 148.2, 172.3, 201.7. ES-MS 600 *m/z* $[M+H]^+$.

***(2S,9R)*-2-(*tert*-Butoxycarbonylamino)-9-(*tert*-butyldimethylsilyloxy)-8-oxodecanoic acid (6).**
$Pd(OH)_2$ on C, (20 mg) was dissolved in dry MeOH (4 mL) and put in a pressure bottle connected with a Parr apparatus. Two cycles of vacuum-nitrogen were performed and compound **5** (0.200 g, 0.334 mmol) dissolved in dry MeOH (2 mL) was added followed by addition of Boc_2O (0.145 g, 0.66 mmol). The bottle was filled with H_2 at 6 atm and shaken at room temperature for 12 h. The bottle was degassed; the catalyst filtered (*attention: the residue Pd may be pyrophoric*) and washed several times with MeOH. The solvent was evaporated and the crude product was purified using the Sepacore® system (silica gel column, $CHCl_3$: MeOH 98:2) giving **6** (0.108 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.10 (s, 6H), 0.87 (s, 9H), 1.21 (d, *J* = 7Hz, 3H), 1.45 (s, 9H), 1.4-1.9 (m, 8H), 2.55 (m, 2H), 4.12 (q, *J* = 7Hz, 1H), 5.20 (m, 1H), 5.90 (bs, 1H), 11.2 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -3.7, -3.5, 18.1, 21.1 (3C), 24.3 (3C), 25.0, 25.7, 28.9, 29.2, 32.1, 33.4, 54.7, 75.3, 80.6, 158.2, 172.5, 211.3. ES-MS *m/z* 430 $[M-H]^-$. Anal Calcd. for $C_{21}H_{41}NO_6Si$: C, 58.43; H, 9.57; N, 3.25. Found C, 58.33, H, 9.52, N, 3.23.

D-*N,N*-dibenzyl-γ-methylglutamic acid dimethyl ester (8).
A solution of (D)-*N,N*-dibenzyl glutamic acid dimethyl ester (**7**, 1.10 g, 3.08 mmol) in dry THF (15 mL) was cooled to –78 °C under nitrogen and magnetic stirring. Iodomethane (1.32 g, 9.29 mmol) was added followed by a slow addition of a solution of KHDMS (12.39 mL of a 0.5 M solution in THF, 6.19 mmol). After the addition, the mixture was stirred at –78 °C for 30 min. A saturated solution of NH_4Cl (5 mL) was added with a syringe and the mixture was slowly warmed

to room temperature. The organic layer was separated and the aqueous layer extracted several times with AcOEt. The collected organic layers were washed with a saturated solution of $NaHCO_3$ and brine. After evaporation of the solvent, a silica gel chromatography (eluent hexane: AcOEt 9: 1) gave product **8** as a mixture of diastereoisomers approximatively 1:4 (1.06 g, 92% yield). The spectroscopic data of the major isomer were following reported. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15 (d, *J* = 7Hz, 3H), 1.75 (m, 1H), 2.01 (m, 1H), 2.75 (m, 1 H), 3.48 (m, 1H), 3.55 (d-like, 2H), 3.60 (s, 3H), 3.75 (s, 3H), 3.95 (d-like, 2H), 7.30 (m, 10 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.6, 33.6, 36.8, 50.2, 51.6, 57.5, 58.9, 63.9, 127.5 (2C), 128.9 (4C), 129.3 (4C), 134.9 (2C), 172.3, 174.5. ES-MS m/z 370 $[M+H]^+$.

(5*R*, 3*S*)-1-*tert*-Butoxycarbonyl-5-carboxymethyl, 3-methyl-2-pyrrolidinone (9).
Compound **8** (0.410 g, 1.11 mmol) was dissolved in dry MeOH (8 mL) and the solution poured in the pressure bottle of a Parr hydrogenation apparatus. $Pd(OH)_2$ 20% on C was added (50 mg) and the mixture shaken under 6 atm of H_2 for 12 h. The bottle was degassed, the catalyst filtered (*attention: the residue Pd may be pyrophoric*) and washed several times with MeOH. The solvent was evaporated and the crude product was dissolved in dry MeCN (15 mL). Boc_2O (0.186, 0.85 mmol) was added followed by addition of Et_3N (0.9 g, 0.9 mmol) and DMAP (64 mg, 0.07 mmol). The mixture was stirred at rt for 48 h. The solvent was evaporated under vacuum and the residue purified by column chromatography (eluent hexane: AcOEt 1:1) to give trans isomer **9** as major compound (rf = 0.35, 110 mg), together with a small amount of cis isomer (rf = 0.30, 32 mg). ^{1}H NMR data of **9** (300 MHz, $CDCl_3$) δ: 1.15 (d, *J* = 7Hz, 3H,), 1.45 (s, 9H), 1.85 (m, 1H), 2.20 (m, 1H), 2.60 (m, 1 H), 3.71 (s, 3H), 4.50 (d-like, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.6, 28.2 (3C), 33.6, 36.8, 51.6, 52.6, 70.3, 158.2, 164.5, 172.9. ES-MS m/z 258 $[M+H]^+$.

(2*R*, 4*S*)-1-*tert*-Butoxycarbonyl-2-carboxymethyl, 4-methyl-pyrrolidine (10).
Compound **9** (0.10 g, 0.39 mmol) was dissolved in dry THF (5 mL) and the solution heated at 40 °C. $BH_3.Me_2S$ (1 mL of a 1 M solution in THF, 1 mmol) was added and the solution stirred at 40 °C for 12 h. The solvent was evaporated under vacuum and the mixture was subject to a silica gel chromatography on (eluent petroleum ether 40-60 : AcOEt 7: 1) to give 56 mg of **10** (60% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15 (d, *J* = 7Hz, 3H), 1.45 (s, 9H), 1.80 (m, 1H), 2.10 (m, 1H), 2.45 (m, 1H), 2.90 (m, 1H), 3.72 (m, 1H), 3.74 (s, 3H), 4.31 (dd, ^{A}*J* = 9 Hz, ^{B}*J* = 4Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.6, 27.6, 29.1, 35.3, 52.4, 56.8, 82.1, 150.3, 171.4, 176.3. ES-MS m/z 244 $[M+H]^+$.

(2*R*, 4*S*)-1-(9-Fluorenylmethoxycarbonyl)-4-methyl-pyrrolidin-2-carboxylic acid (12).
To a solution of compound **10** (0.14 g, 0.58 mmol) in THF (5 mL) LiOH (0.049 g, 1.16 mmol in 2 mL of H_2O) was added and the mixture was stirred at rt for 10 h. The solvent was evaporated *in vacuo* and the crude residue was dissolved in water. HCl 0.5 N was added dropwise until precipitation of a white solid (**11**) that was filtered off. The acid (**11**) was dissolved in dry CH_2Cl_2 (2.5 mL) under argon atmosphere and at rt, followed by addition of TIS (0.39 g, 2.47 mmol) and TFA (2.45 mL 32 mmol). After 3 h the solvent was evaporated *in vacuo* and the crude product was triturated with Et_2O to obtain a powder. The amino acid was dissolved in H_2O (8 mL) followed by addition of TEA (0.22 mL, 1.6 mmol) and FmocOSu in acetonitrile (0.26 g, 0.77 mmol). Finally TEA was progressively added over 45 min to obtain a stable basic solution (pH 8.5-9). After addition of HCl 1N, ethyl acetate was used to extract the desired product **12** (144 mg, 70% yield). ^{1}H NMR (300 MHz, DMSO-d_6, 353 K) δ: 1.03 (d, *J* = 6.5 Hz, 3H), 1.87 (m, 1H), 2.08 (m, 1H), 2.34 (m, 1H), 2.95 (bt, *J* = 9.4 Hz, 1H), 3.59 (dd, ^{A}*J* =9.4 Hz, ^{B}*J* = 7.7 Hz, 1H), 4.29 (m, 4H), 7.35 (t, *J* = 7.9 Hz, 2H), 7.43, (t, *J* = 7.9 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 353 K) δ: 18.3, 32.7, 38.1, 47,9, 54.0, 59.8, 67.9, 121.2 (2C), 126.3 (2C), 128.3 (2C), 128.8 (2C), 141.9 (2C), 145.0 (2C), 154.9, 174.9. ES-MS *m/z* 351 $[M+H]^+$.

Fmoc-L-Iva
The Fmoc-group was introduced by stirring L-Iva (0.50 g, 3.7 mmol), 9-fluorenylmethylchloroformate (1.053 g, 4.07 mmol), and Na_2CO_3 (1.176 g, 11.1 mmol) in 50% acetone/water (75 mL) overnight; after evaporation of acetone, the aqueous solution was washed with ether and acidified with 10% citric acid. The precipitated acid, washed with water, was dissolved in ethyl acetate, dried ($MgSO_4$), and evaporated. Hexane was added to precipitate the pure acid. After being dried in vacuo, Fmoc-L-Iva-OH was obtained in 79% yield. ES-MS *m/z* 362 $[M+Na]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.80 (CH_3, 3H, m), 1.50 (CH_3, 3H, s) 1.80-2.10 (CH_2, 2H, m), 4.2-4.4 (CH, CH_2, 3H, m), 7.25-7.76 (aromatic protons, 8H, m)

Final stages of the FR235222 synthesis.
Solid Phase Synthesis of the linear precursor and cyclization of **cyclo[-(2*S*,9*R*)-Ahoda-L-Iva-L-Phe-(2*R*,4*S*)-4-MePro-]** (**1**)

a) Loading of the 2-ClTrt-Cl resin :
The 2-ClTrt-Cl resin was placed in a 25 mL polypropylene ISOSOLUTE syringe on a VAC MASTER system, swollen in DMF (3 mL) for 1 h, and then washed with 2x3 mL of DCM.
A solution of Fmoc-D-4-MePro-OH (48 mg, 0.14mmol) and DIEA (18 µl, 0.4 mmol) in 2.5 mL of dry DCM was added and the mixture was stirred for 2 h with a N_2 stream. The mixture was then removed, and the resin was washed with 3x

DCM/MeOH/DIEA (17:2:1) and successively with: DCM 3 x 3 mL, DMF 2 x 3 mL, DCM 2 x 3 mL (1.5 min each). The loading of the resin was determined by UV quantification of the Fmoc-piperidine adduct.
The assay was performed on a duplicate samples: 0.4 mL of piperidine and 0.4 mL of DCM were added to two dried samples of Fmoc-amino acid-resin in two volumetric flasks of 25 mL. The reaction was allowed to proceed for 30 min at rt and than 1.6 mL of MeOH were added and the solutions were diluted to 25 mL volume with DCM. A reference solution was prepared in a 25 mL volumetric flask using 0.4 mL of piperidine, 1.6 mL of MeOH and DCM to volume. The solutions were shaken and the absorbance of the samples versus the reference solution was measured at 301 nm. The substitution level (expressed in mmol of amino acid/g of resin) was calculated from the equation: mmol g^{-1} = (A_{301}/7800) x (25 mL g^{-1} of resin).

b) Fmoc deprotection conditions:
After Fmoc-D-4-MePro-*O*-2ClTrt-resin swelling (1 h with 3 mL of DMF), removal of the Fmoc protecting group was carried out using 20% piperidine in DMF (3 mL, 1 x 1.5 min), 20% piperidine in DMF (3 mL, 1 x 10 min or 1 x 5 min); washings in DMF 2 x 3 mL, DCM 2 x 3 mL, DMF 2 x3 mL (1.5 min each).

c) Peptide coupling conditions:
The coupling reaction was promoted by a HOBt/HBTU in DMF coupling protocol:
Fmoc-amino acid (3-4 eq), HOBt (3-4 eq), HBTU (3-4 eq) and NMM or DIEA (4-5 eq) were stirred under N_2 in DMF (2.5 mL) for 2 h. After each coupling, washings were carried out with DMF (3 mL, 3 x 1.5 min), and DCM (3 mL, 3 x 1.5 min) and the Kaiser test was used to assess coupling efficiency.
1st coupling: Fmoc-L-Phe-OH (156.5 mg, 0.4 mmol), HOBt (61.9 mg, 0.4 mmol), HBTU (153.1 mg, 0.4 mmol), and NMM (55 µl, 0.49 mmol). After incorporation of Fmoc-Phe-OH, the Fmoc protecting group was removed according to general procedure b).
2nd coupling: Fmoc-L-Iva-OH (180.3 mg, 0.5 mmol), HOBt (77.3 mg, 0.5mmol), HBTU (191.4 mg, 0.5 mmol), and DIEA (175 µl, 1.01 mmol), followed by *N*-a-Fmoc deprotection.
3rd coupling: *N*-α-Boc-Ahoda-O-(TBDMS)-OH (217.7 mg, 0.5 mmol), HOBt (77.3 mg, 0.5 mmol), HBTU (191.4 mg, 0.5 mmol), and DIEA (176 µl, 1.01 mmol).

d) Cleavage conditions:
The dried resin was treated for 2 h, under stirring, with the AcOH/TFE/DCM (2:2:6; 10 µL x 1 mg of resin) cleavage mixture. Then the resin was filtered off and washed with neat cleavage mixture (3 mL, 3 x 1.5 min). After addition of hexane (15 times volume), to remove acetic acid as an azeotrope, the filtrate was concentrated and lyophilized.

e) Boc and TBDMS deprotection conditions:
N-terminus and side-chain deprotections were carried out by treatment with TFA/H_2O/TIS 95:4:1 for 20 min, under stirring. The crude linear peptide (**15**) was purified by semipreparative RP HPLC on a Jupiter C-18 column using a 40 min gradient from 27:85 to 50:50 of CH_3CN/H_2O (each containing 0.1% TFA) at a flow rate of 4 mL/min and UV detection at 240 nm. The HPLC analysis showed a main peak t_R = 10.93 min that was identified as the linear deprotected peptide (60% yield) on the basis of ES-MS and ^{1}H-NMR data. Note that the ^{1}H-NMR spectrum contained many broad resonances at rt, preventing the observation of clear signal multiplicities. For the same reason, exchangeable protons, including amide signals, could not be unambiguously assigned.
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.28-7.19 (aromatic protons, 5H, m), 4.81 (1H, m) 4.32 (1H, m), 4.27 (1H, m), 4.01 (1H, m), 3.83 (1H, m), 2.98 (2H, m), 2.48 (2H, m), 2.31 (2H, m), 2.01-1.94 (2H, m), 1.81-1.61 (4H, m), 1.54 (1H, m), 1.43 (2H, m), 1.37 (3H, m), 1.32 (4H, m), 0.82 (3H, m), 0.75 (3H, m). ES-MS *m/z* 575 $[M+H]^+$, 597 $[M+Na]^+$.

f) Cyclization conditions:
The cyclization was performed in solution at a concentration of 7.7 x 10^{-5} M with HATU (18.8 mg, 0.05 mmol) and DIEA (11 µl, 0.062 mmol) in DCM. The solution was stirred at 4 °C for 1h and then allowed to warm to room temperature for 1 h. The solvent was removed under reduced pressure.
The crude cyclopeptide (36.9 mg) was purified by semipreparative RP HPLC on a Jupiter C-18 column using the following gradient: from 5% B to 100% B over 30 min at a flow rate of 4 mL/min. The binary solvent system (A/B) was as follows: 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The absorbance was detected at 240nm.
The HPLC analysis showed one main peak t_R = 19.20 min that was identified as pure FR235222 (**1**) (68 % isolated yield) on the basis of ES-MS and NMR data.
^{1}H NMR (600 MHz, $CDCl_3$) δ: **Ahoda** 7.20 (NH, d, J=9.8 Hz, 1H), 4.19 (H-2, m, 1H), 1.81 (H-3, m, 1H), 1.62 (H-3, m, 1H), 1.32 (H_2-4, m, 2H), 1.33 (H_2-5, m, 2H), 1.63 (H_2-6, m, 2H), 2.44 (H-7', m, 1H), 2.50 (H-7'', m, 1H), 4.22 (H-9, m, 1H), 1.38 (H_3-10, d, *J* = 7.0 Hz, 3H); **4-MePro** 4.68 (H-13, d, *J* = 8.0 Hz, 1H), 2.39 (H-14, m, 1H), 1.37 (H-14, m, 1H), 2.61 (H-15, m, 1H), 4.04 (H-16, dd, *J* = 9.0, 7.5 Hz, 1H), 2.73 (H-16, t, *J* = 9.0 Hz, 1H), 0.88 (H_3-18, d, *J* = 6.0, 7.0 Hz, 3H); **Phe** 7.54 (NH, d, J=9.8 Hz, 1H), 5.16 (H-20, ddd, *J* = 9.5, 9.5, 6.0 Hz, 1H), 3.24 (H-21, dd, *J* = 13.3, 9.5 Hz, 1H), 2.96 (H-21, dd, *J* = 13.3, 6.0 Hz, 1H), 7.23 (H-23/H-27, m, 2H), 7.27 (H-24/H-26, m, 2H), 7.20 (H-25, m, 1H); **Iva** 5.82 (NH, bs, 1H), 2.33 (H-31, m, 1H), 2.15 (H-31, dq, *J* = 13.9, 7.0 Hz, 1H), 0.84 (H_3-32, t, *J* = 7.0 Hz, 3H), 1.29 (H_3-33, s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: **Ahoda** 19.7, 23.2, 25.4, 28.8 (2C), 37.3, 54.5, 72.5, 174.2, 212.4; **4-MePro** 18.2, 32.9, 33.1, 53.8, 58.1, 171.9; **Phe** 35.8, 53.2, 126.7, 128.6 (2C), 129.1 (2C), 137.0, 173.2; **Iva** 8.4, 22.5, 27.7, 63.0, 175.5.
HRES-MS *m/z* 557.3343 $[M+H]^+$, $C_{30}H_{44}N_4O_6$.