p-Nitromandelic Acid (Pnm) as a Highly Acid-Stable Safety-Catch Linker for Solid Phase Synthesis of Peptide and Depsipeptide Acids

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Supporting Information

General

Commercial compounds

Commercial grade reagents and solvents were used without further purification. AM-resin (aminomethylated polystyrene, 100-200 mesh, f = 1.1 mmol/g), PyBOP for in situ neutralization and HOBt were purchased from NovaBiochem, lactic acid, leucic acid and diisopropylcarbodiimide (DIC) from Aldrich, Boc-amino acids and Fmoc-Leu-OH from Iris Biotech and DIEA from Merck.

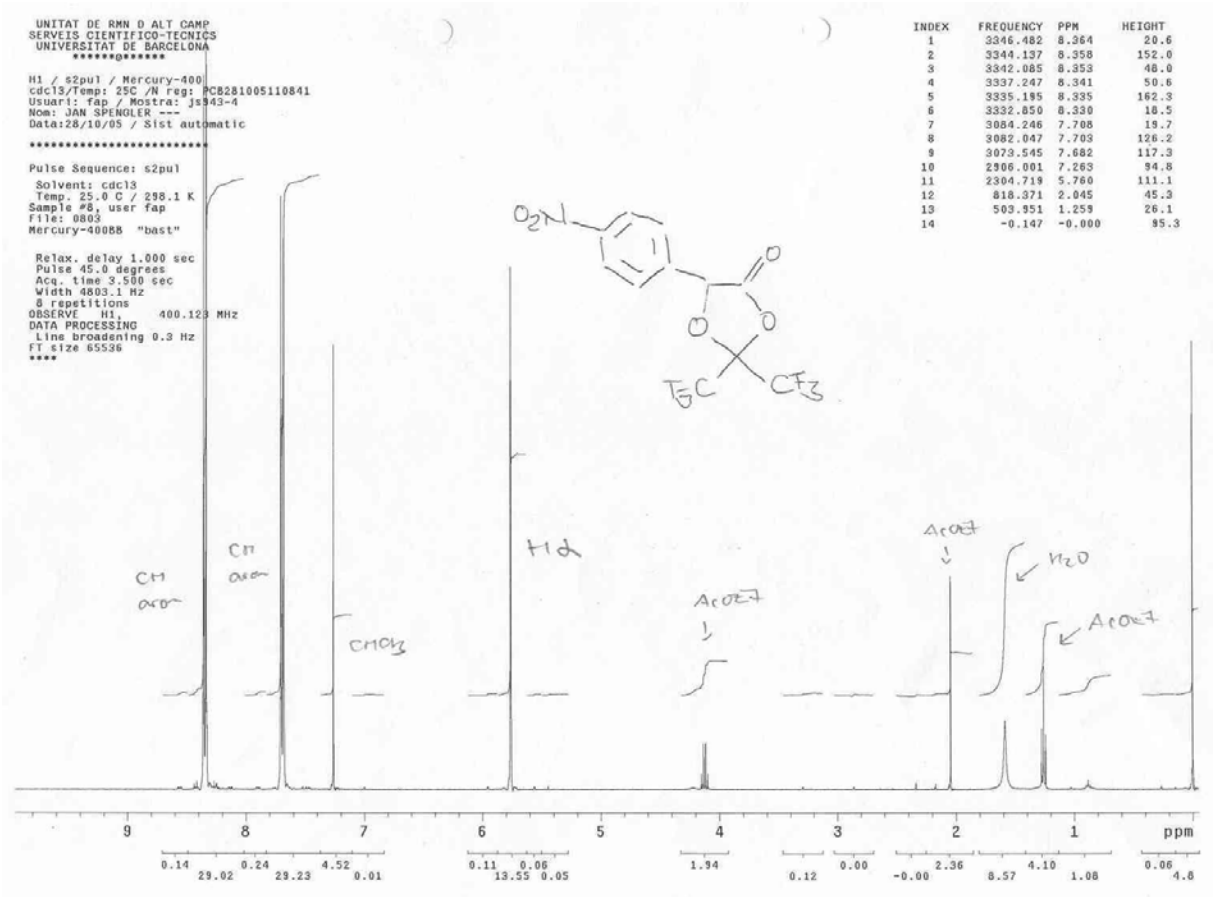
Apparatus

Analytical HPLC was carried out with a Waters instrument on a C8-column. Linear gradients (given in the table) of CH_3CN (0.036% TFA) into H_2O (0.045% TFA) were run at a flow rate of 1.0 mL/min. UV detection was performed at 220 nm. The mass signals in the HPLC-MS spectra were obtained with an electrospray detector (Waters micromass ZQ). NMR spectra were acquired with a Mercury-400 spectrometer (^1H at 400.125 MHz, ^{13}C at 100.625 MHz with TMS as internal reference) (High-field NMR Unit, Barcelona Science Park).
The following abbreviations are used to indicate multiplicity: s, singlet; d, doublet, dd, double doublet; t, triplet; dt, double triplet; m, multiplet, br s, broad signal. Microwave experiments were performed with a CEM Discover apparatus. Temperature was measured with a non-contact infrared sensor.

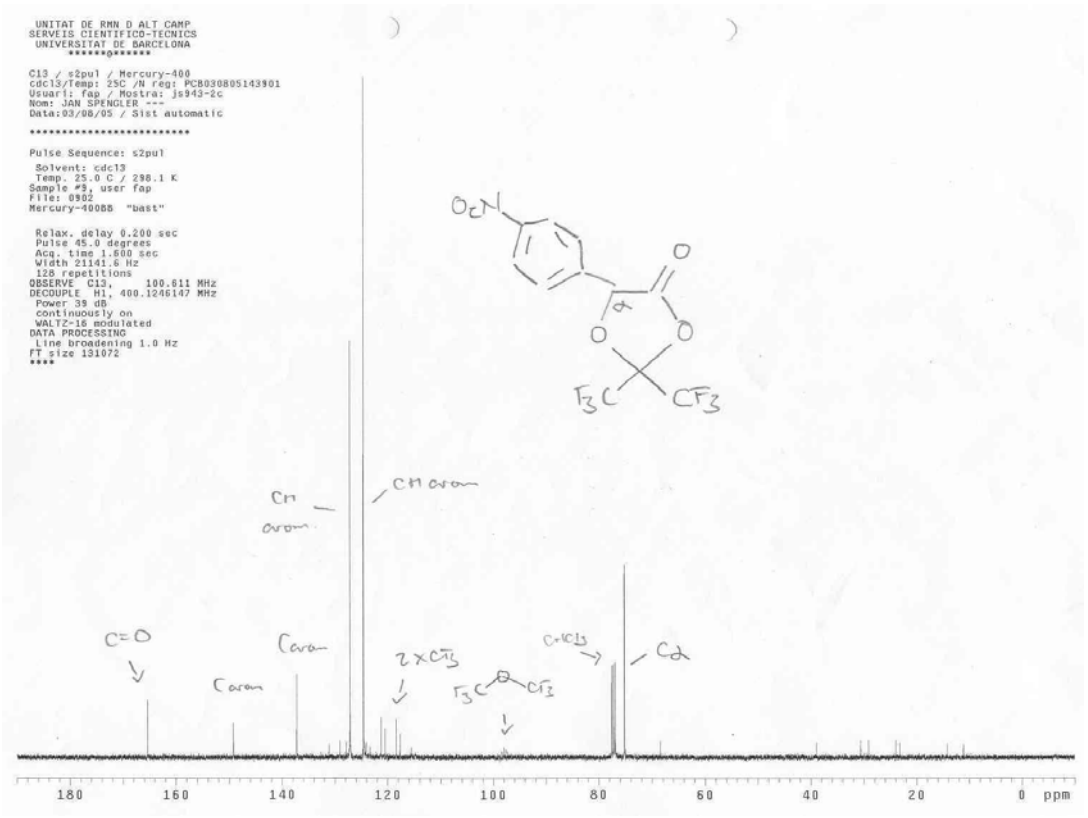
2,2-Bis(trifluoromethyl)-1,3-dioxolan-5-(4-nitrophenyl)-4-one (HFA-Pnm)

p-Nitromandelic acid was synthesized from *p*-nitrobenzaldehyde (32.5 mmol, 4.9 g) and potassium cyanide. The crude nitrile was transformed in the methyl ester as described earlier.[1] The crude methyl ester (3.8 g) was then saponified by refluxing with glacial HOAc / conc. HCl mixture (5 : 1), until no starting material was detected by TLC. Note: saponification with LiOH gave no desired product. After evaporation, a mixture of products was obtained, which was dissolved in DMSO and directly subjected to reaction with HFA. The extracted product (1.7 g) was purified by flash chromatography (hexane / ethyl acetate 4 : 1, $R_f = 0.6$) 2,2-bis(trifluoromethyl)-1,3-dioxolan-5-(4-nitrophenyl)-4-one [HFA(Pnm), 0.54 g, 5% overall yield from *p*-nitrobenzaldehyde]. It spectroscopical data are in agreement with those already described for such compounds.[2] ^{1}H NMR ($CDCl_3$): δ (ppm) = 5.75 (s, 1H), 7.69 (d, J = 8.67 Hz, 2H), 8.34 (d, J = 8.91 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 74.9, 97.8 (m), 118.8 (q, J = 285 Hz), 119.5 (q, J = 288 Hz), 124.3, 126.9, 137.0, 148.9, 165.1. ^{19}F NMR ($CDCl_3$): δ (ppm) = -81.0 (q, J = 7.75 Hz), -80.45 (q, J = 7.75 Hz). IR (film): ν = 1855, 1531, 1352, 1238, 1134 cm^{-1}.

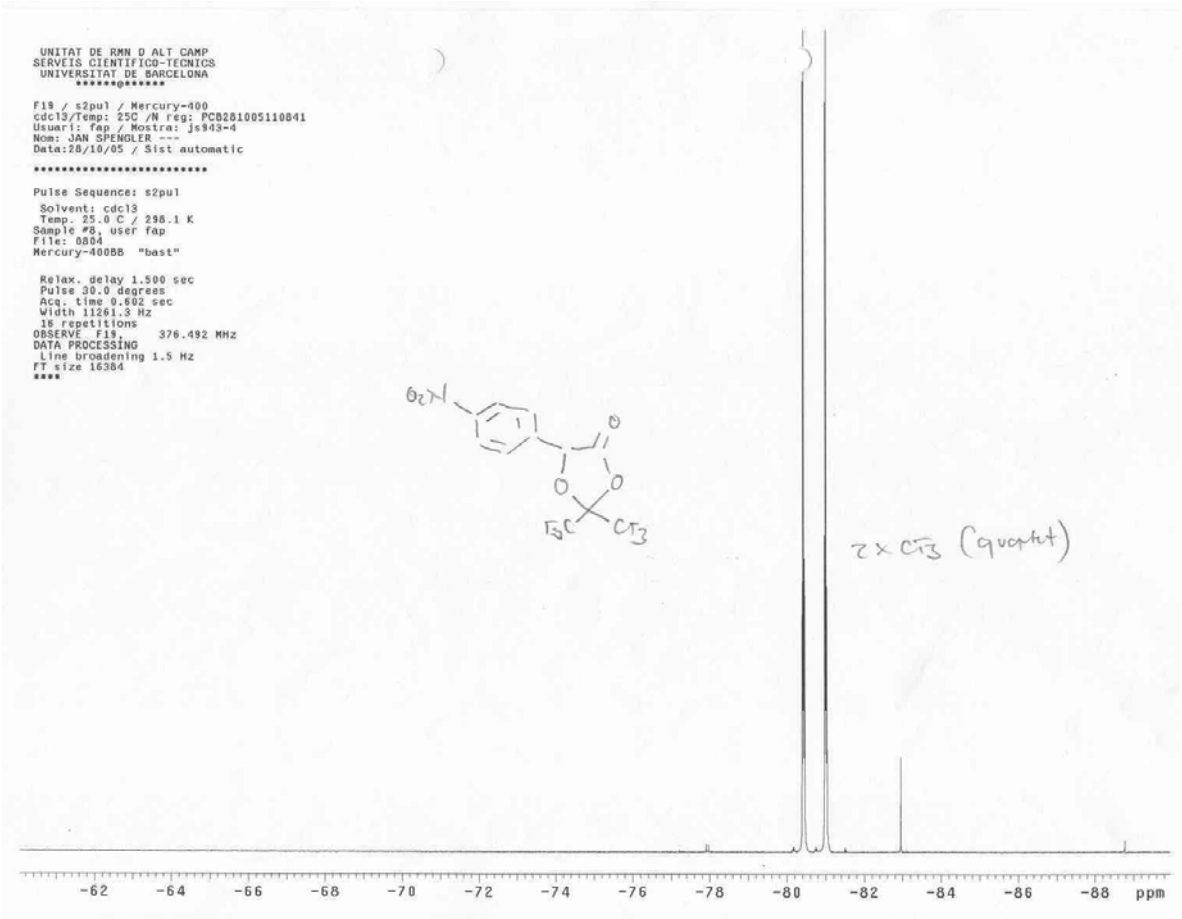
^{1}H NMR:



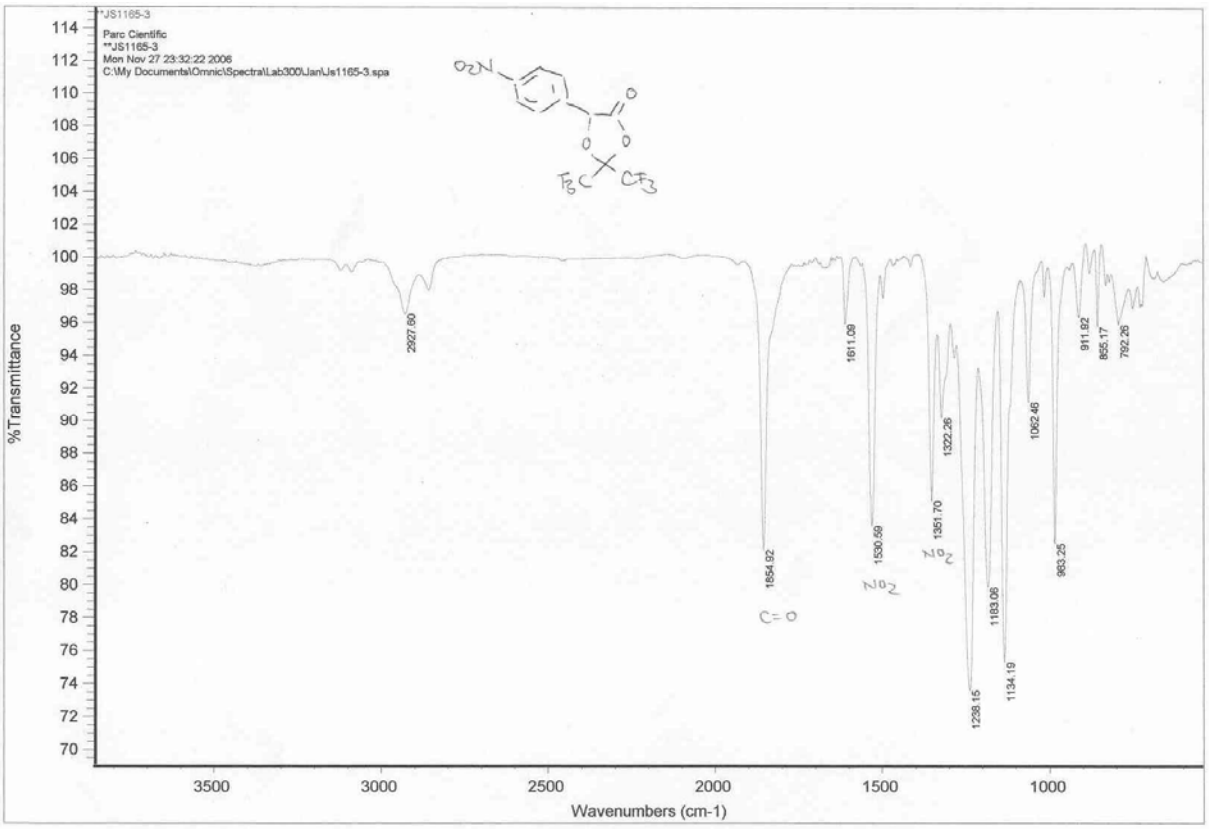
^{13}C NMR:



^{19}F NMR:



IR (film):



Preparation of H-Pnm-AM-resin and depsipeptide synthesis

4 eq of HFA-Pnm were dissolved in THF and added to aminomethylated polystyrene (which turned blue) and the mixture was left to shake until the ninhydrin-test was negative. The filtrate and washing solutions with the excess of HFA-Pnm were then evaporated and stored for future reuse. The depsipeptides were then synthesized by standard protocols. Attachment of the first amino acid: symmetric anhydride generated with DIC, cat. amounts of DMAP. Third position after an ester bond: in-situ neutralization using the PyBOP-reagent.[3] Lactic and leucic acid were coupled without α-OH protection schemes with DIC/HOBt.

For side-chain deprotection:

The resins were treated with 150 µL of thioanisol at 0 ºC followed by addition of 1 mL of TFA and 10 min of stirring. 100 µL of Trifluoromethanesulfonic acid (TFMSA) were then added and the resins were stirred 2 h at rt. Finally, the following washings were carried out: TFA (3 x 1 min), DCM (5 x 1 min), isopropanol (5 x 1 min) and DMF (5 x 1min).

For final cleavage, the resins were treated with:

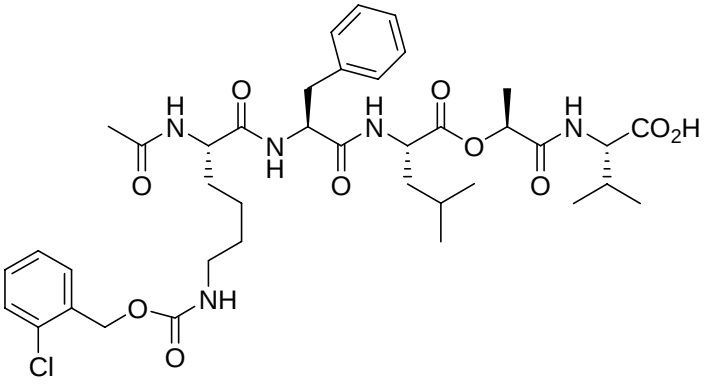
i) 6 M $SnCl_2$, 1.6 mM HCl/dioxane in DMF, 1 h at rt.

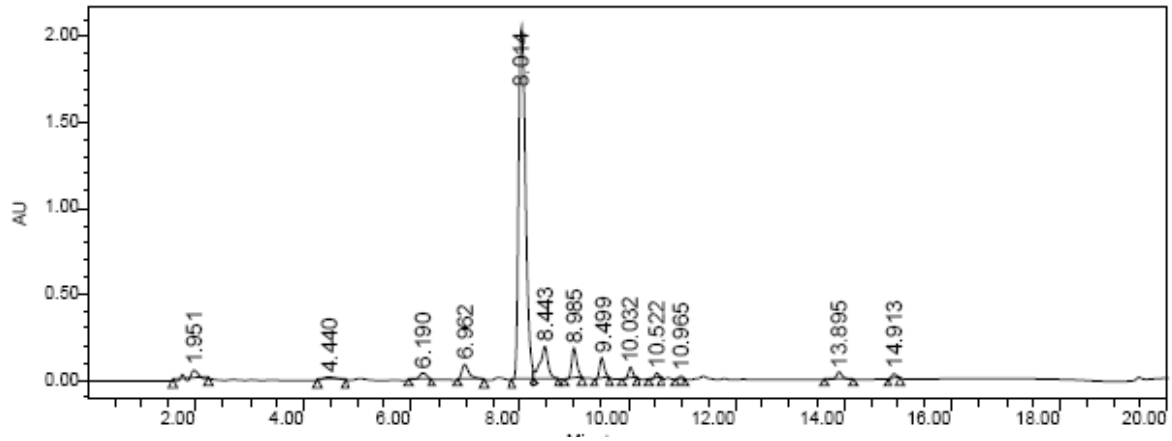
Washings: 5 x 1 min. with DMF and 5 x 30 seconds with dioxane.

ii) 5% TFA in dioxane 1h at 50 ºC (Microwave heating).

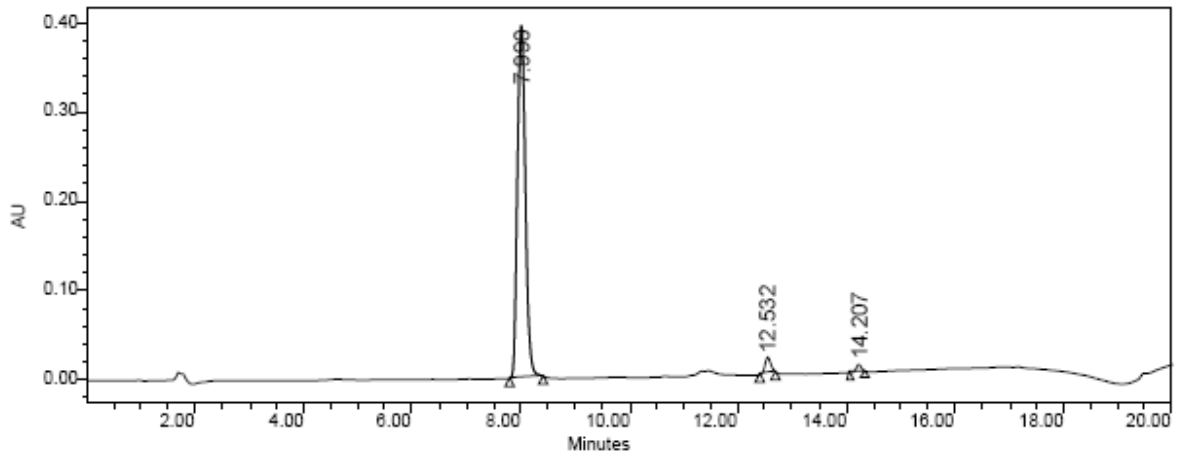
Ac-Lys(2ClZ)-Phe-Leu-Lac-Val-OH (5b) :

HRMS (ESI-MS, ES^+): $C_{39}H_{53}ClN_5O_{10}$ ($M+H^+$) calcd.: 789.34721, found: 789.36602.



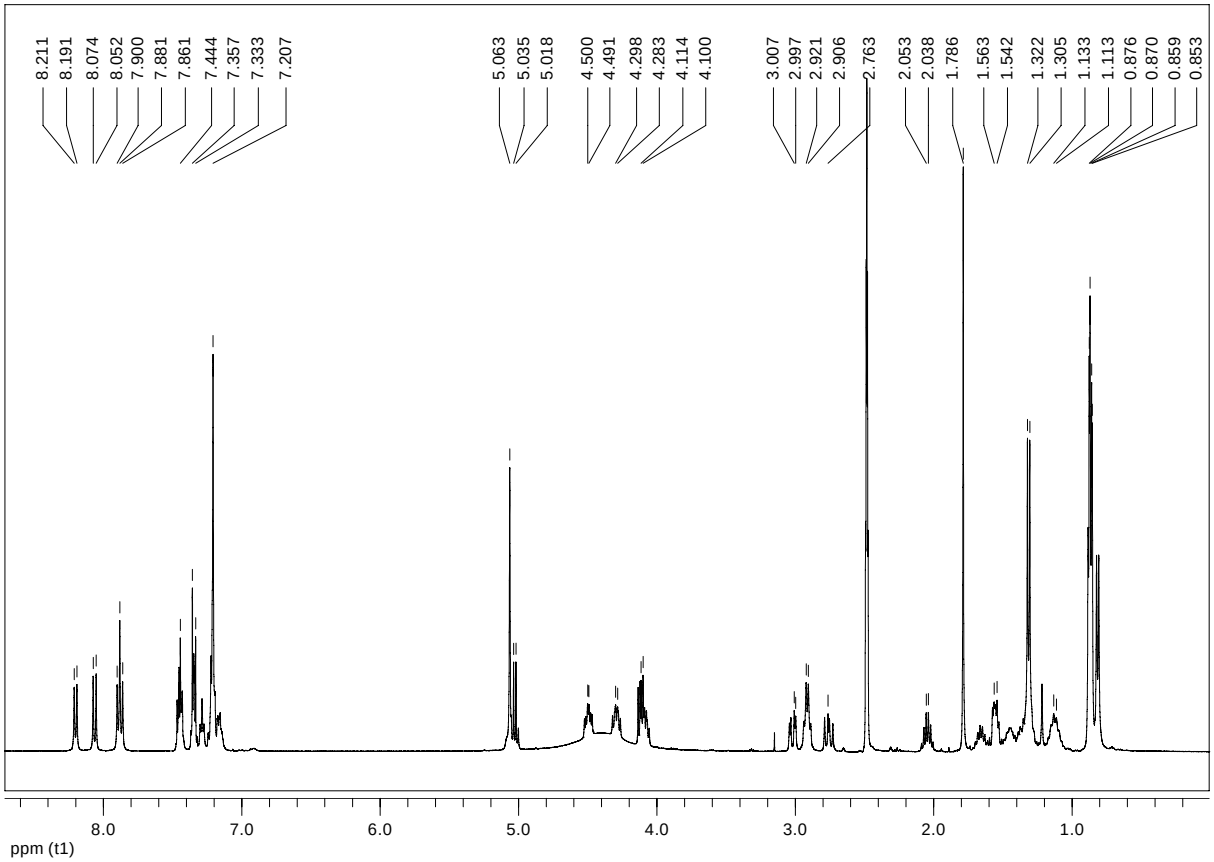


The depsipeptide **5b** (Ac-Lys(2ClZ)-Phe-Leu-Lac-Val-OH) (25 mg) was purified by semipreparative HPLC



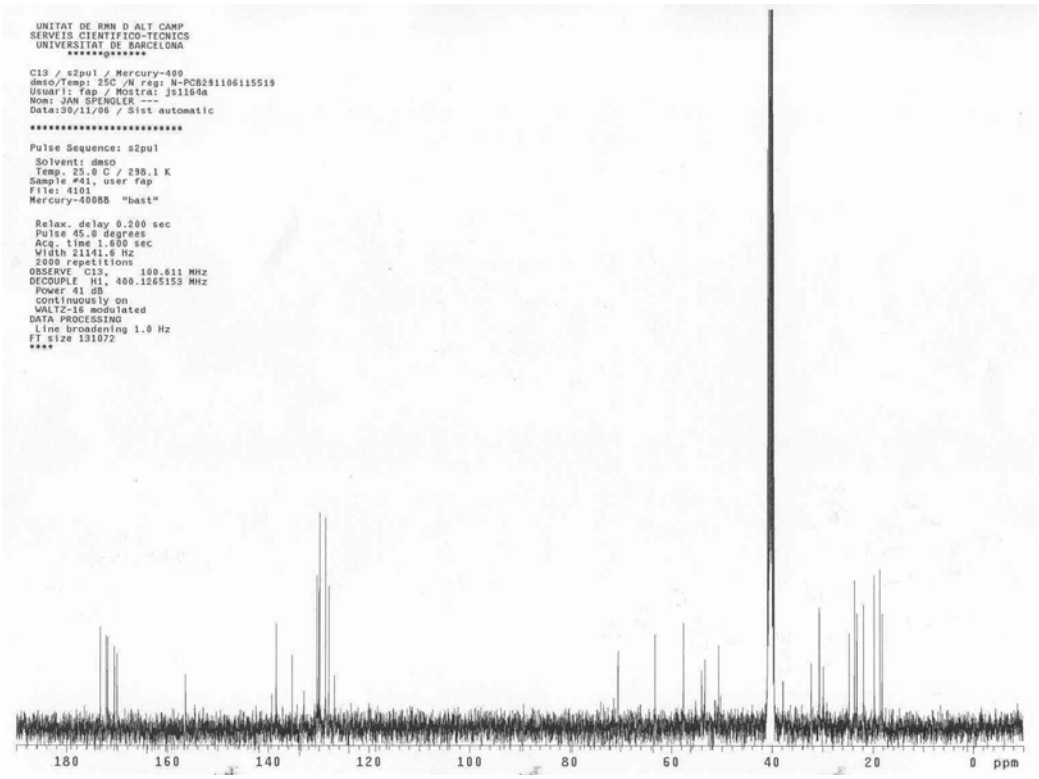
^{1}H NMR of **5b** (400 MHz, DMSO):

8.21 (d, *J*= 7.79 Hz, NH from Leu), 8.06 (d, *J*= 8.62 Hz, NH from Val), 7.88 (m, 2H, NH from Phe and Lys), 7.45 (dd, *J*= 9.2 Hz, *J'*= 5.6 Hz, 2 CH ar.), 7.35 (m, 2 CH ar.), 7.28 (t, *J*= 5.5 Hz, εNH from Lys), 7.2 (m, 4 CH ar.), 7.16 (m, CH ar.), 5.06 (s, CH_2 from 2-Cl-Z), 5.03 (q, *J*= 6.8 Hz, CH from lactic acid), 4.50 (m, αCH from Phe), 4.28 (m, αCH from Leu), 4.10 (m, αCH from Val and Lys), 3.02 (dd, *J*= 14.0 Hz, *J*= 4.0 Hz, 1H, CH_2 from Phe), 2.91 (m, εCH_2 from Lys), 2.76 (dd, *J*= 14.0 Hz, *J*= 9.8 Hz, 1H, CH_2 from Phe), 2.05 (m, βCH_2 from Val), 1.79 (s, acetyl CH_3), 1.66 (m, βCH Leu), 1.56 (m, CH_2 from Leu), 1.45 (m, 1H, βCH_2 from Lys), 1.36 (m, 1H, βCH_2 from Lys), 1.31 (d, *J*= 6.8 Hz, CH_3 from lactic acid), 1.31 (m, δCH_2 from Lys), 1.12 (m, γCH_2 from Lys), 0.87 (m, 9H, 2CH_3 from Val, CH_3 from Leu), 0.81 (d, *J*= 6.3 Hz, CH_3 from Leu).

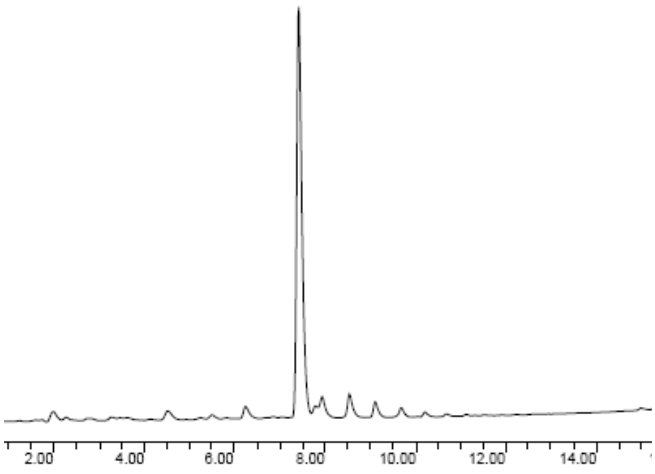


^{13}C-NMR of **5b** (100 MHz):

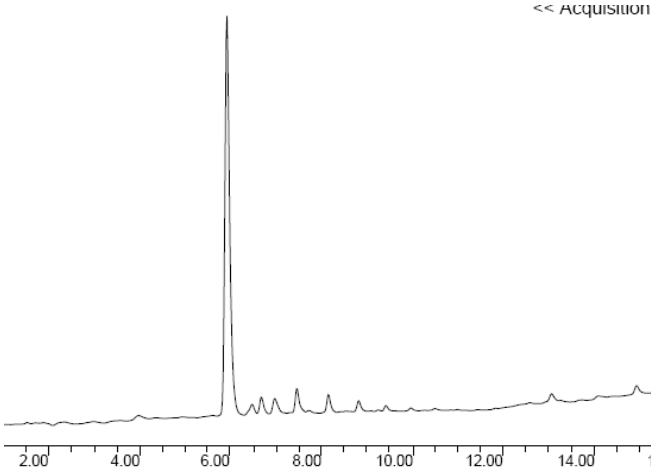
173.39, 172.27, 172.25, 171.94, 170.59, 170.05 , 156.42, 138.44 , 135.30, 130.35, 129.93, 129.83, 128.66, 127.98, 126.89, 70.49, 63.21, 57.59, 54.03, 53.39, 50.66, 37.81, 32.22, 30.62, 29.85, 24.69, 23.62, 23.20, 23.15, 21.84, 19.72, 18.58, 18.13.



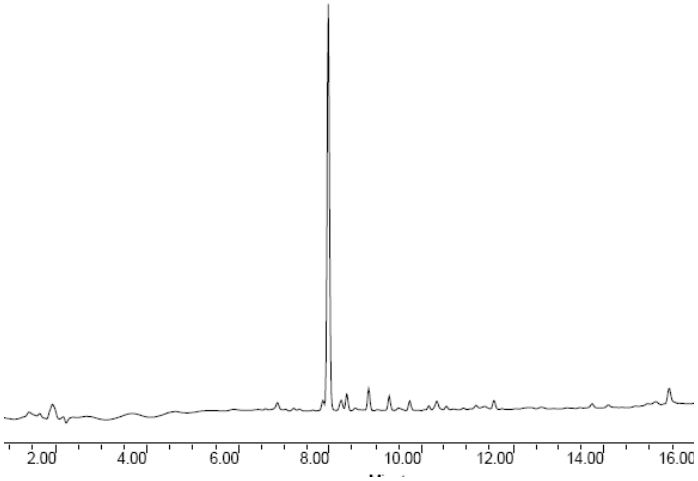
Ac-Glu(OBzl)-Phe-Leu-Lac-Val-OH (5a): HRMS (ESI-MS, ES^+): $C_{37}H_{50}N_4O_{10}$ ($M+H^+$) calcd.: 711.35997, found: 711.35844.



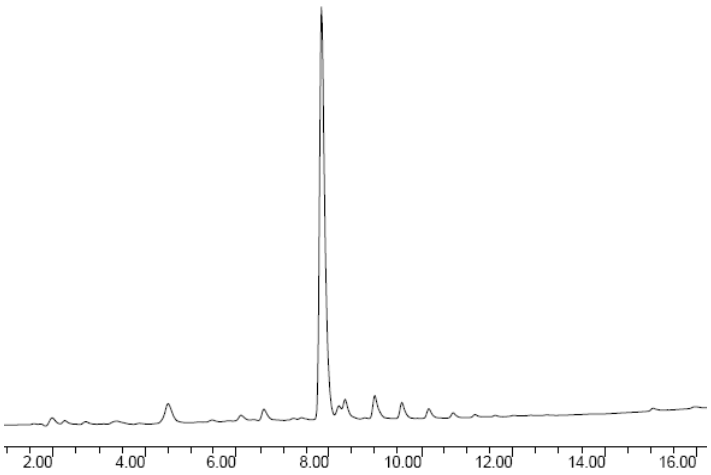
Ac-Glu-Phe-Leu-Lac-Val-OH (6a): HRMS (ESI-MS, ES^+): $C_{30}H_{44}N_4O_{10}$ ($M+H^+$) calcd.: 621.31302, found: 621.31208.



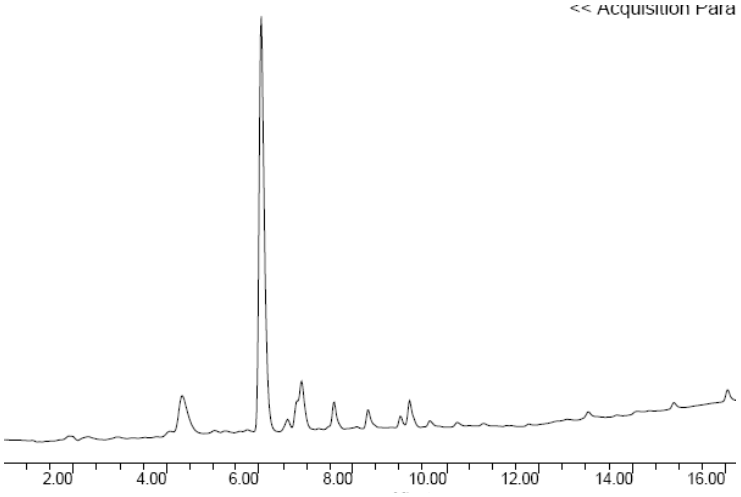
Ac-Lys-Phe-Leu-Lac-Val-OH (6b): HRMS (ESI-MS, ES^+): $C_{31}H_{49}N5O_8$ ($M+H^+$) calcd.: 621.34941, found: 621.36843.



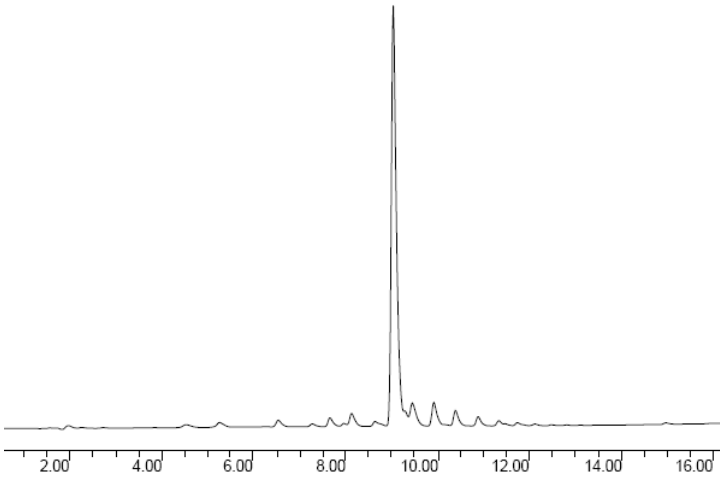
Ac-Thr(Bzl)-Phe-Leu-Lac-Val-OH (5c): HRMS (ESI-MS, ES^+): $C_{36}H_{50}N_4O_9$ ($M+H^+$) calcd.: 683.36506, found: 683.36511.



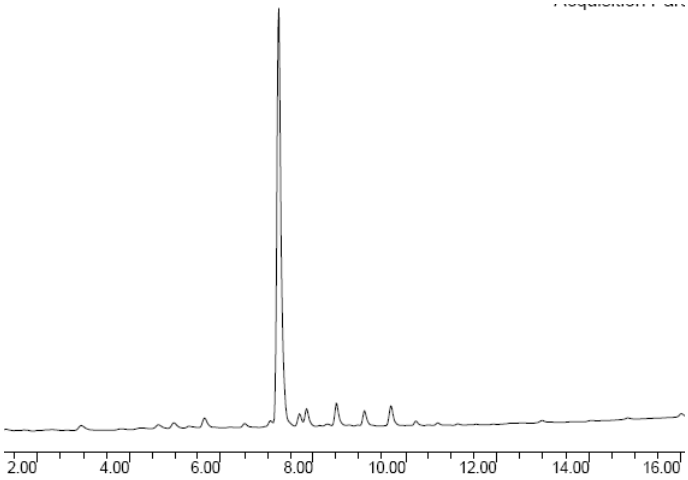
Ac-Thr-Phe-Leu-Lac-Val-OH (6c): HRMS (ESI-MS, ES^+): $C_{29}H_{44}N_4O_9$ ($M+H^+$) calcd.: 593.31811, found: 593.31692.



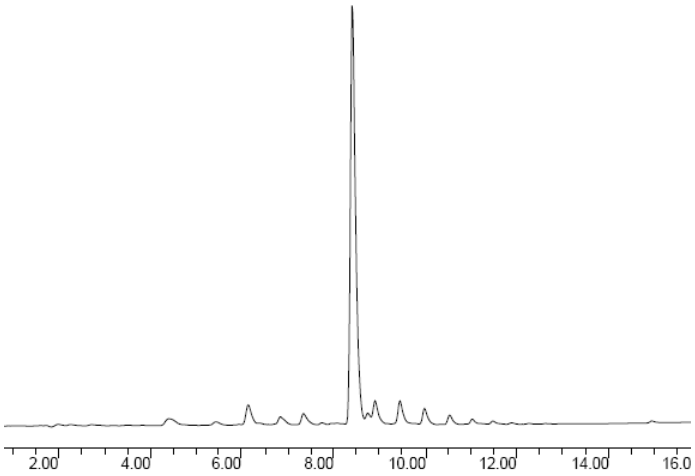
Ac-Tyr(Bzl)-Phe-Leu-Lac-Val-OH (5d): HRMS (ESI-MS, ES^+): $C_{41}H_{52}N_4O_9$ ($M+H^+$) calcd.: 745.38071, found: 745.38043.



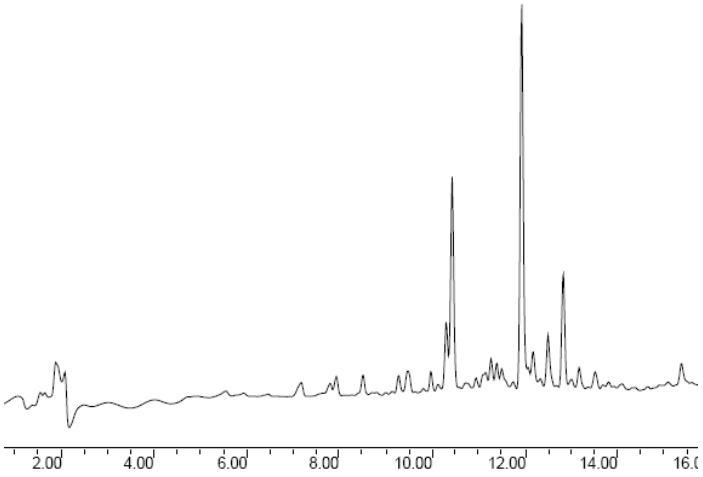
Ac-Tyr-Phe-Leu-Lac-Val-OH (6d): HRMS (ESI-MS, ES^+): $C_{34}H_{46}N_4O_9$ ($M+H^+$) calcd.: 655.33376, found: 655.33270.



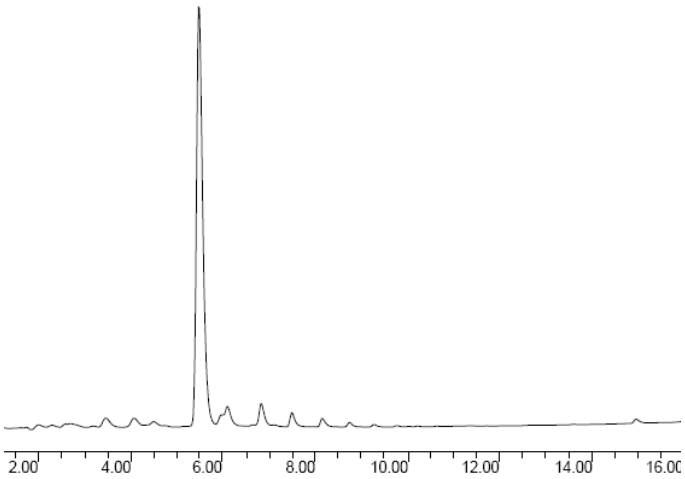
Ac-Cys(pMeBzl)-Phe-Leu-Lac-Val-OH (5e): HRMS (ESI-MS, ES^+): $C_{36}H_{50}N_4O_8S$ ($M+H^+$) calcd.: 699.34221, found: 699.34108.



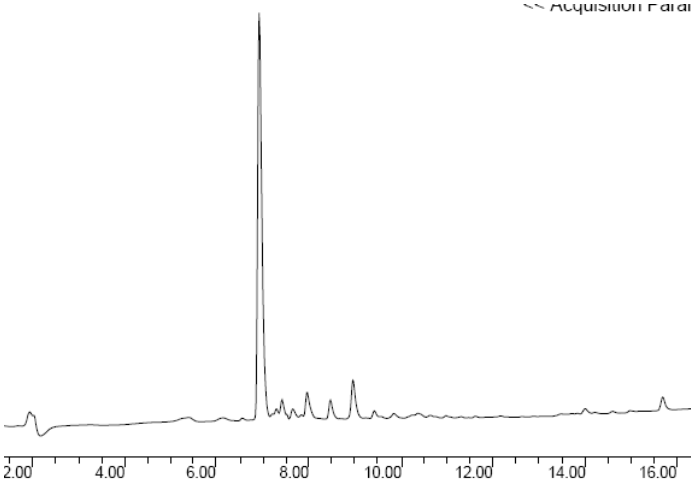
Ac-Cys-Phe-Leu-Lac-Val-OH (6e): HRMS (ESI-MS, ES^+): $C_{28}H_{42}N_4O_8S$ ($M+H^+$) calcd.: 595.27961, found: 595.27728.



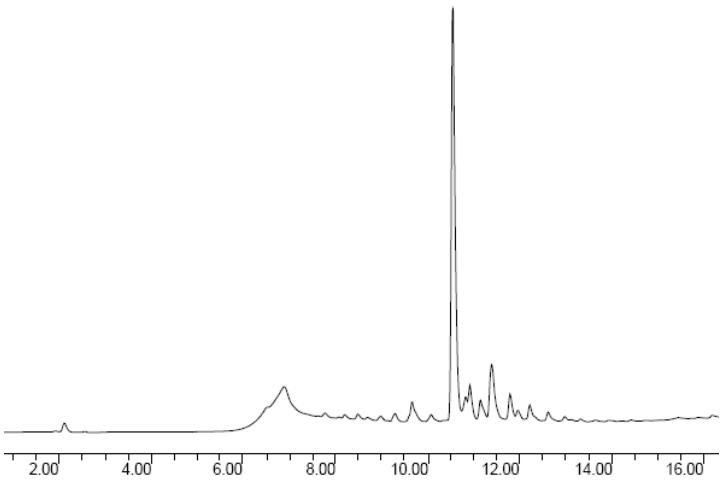
Ac-Arg(Tos)-Phe-Leu-Lac-Val-OH 5f : HRMS (ESI-MS, ES^+): $C_{38}H_{55}N_7O_{10}S$ ($M+H^+$) calcd.: 802.38039, found: 802.38051.



Ac-Arg-Phe-Leu-Lac-Val-OH 6f : HRMS (ESI-MS, ES^+): $C_{31}H_{49}N_7O_8$ ($M+H^+$) calcd.: 648.37154, found: 648.37143.



Ac-Asn(Xan)-Phe-Leu-Lac-Val-OH 5g : HRMS (ESI-MS, ES^+): $C_{29}H_{43}N_5O_9$ ($M+H^+$) calcd.: 606.31335, found: 606.31338.



Quantification of resin functionalization

The Fmoc-group was cleaved with 20% piperidine in DMF (3 x 5 min). The solutions were diluted with DMF and UV-absorbance at 290 nm was measured.

[1] Fosdick, L. S.; Wessinger, G. D. *J. Am. Chem. Soc.* **1938**, *60*, 1465-1466.
[2] Pumpor, K.; Windeisen, E.; Burger K. *J. Heterocyclic Chem.* **2003**, 40, 435-442.
[3] Gairi, M.; Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1990**, *31*, 7363-7366.