SI Appendix

Selective Incorporation of Proteinaceous over Non-Proteinaceous Cationic Amino Acids in Model Prebiotic Oligomerization Reactions

Moran Frenkel-Pinter[a,b,c], Jay W. Haynes[a,b], Martin C[a,b], Anton S. Petrov[a,b,c], Bradley T. Burcar[a,b], Ramanarayanan Krishnamurthy[a,d], Nicholas V. Hud[a,b], Luke J. Leman[a,d*] and Loren Dean Williams[a,b,c*]

[a] NSF-NASA Center for Chemical Evolution, Atlanta, GA 30332 (USA)

[b] School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 (USA)

[c] NASA Center for the Origins of Life, Georgia Institute of Technology, Atlanta, GA 30332 (USA)

[d] Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037 (USA)

*Co-corresponding authors

*Corresponding Authors:

Prof. Loren Dean Williams

School of Chemistry and Biochemistry

Georgia Institute of Technology

315 Ferst Drive NW

Atlanta, GA 30332-0400

Ph: (+1) (404) 385-6258

Fax: (+1) (404) 894-2295

loren.williams@chemistry.gatech.edu

Prof. Luke J. Leman

Department of Chemistry

The Scripps Research Institute

10550 North Torrey Pines Road

BCC104

La Jolla, CA 92037

Ph: (+1) (858) 784-2711

Fax: (+1) (858) 784-2798

lleman@scripps.edu

www.pnas.org/cgi/doi/10.1073/pnas.1904849116

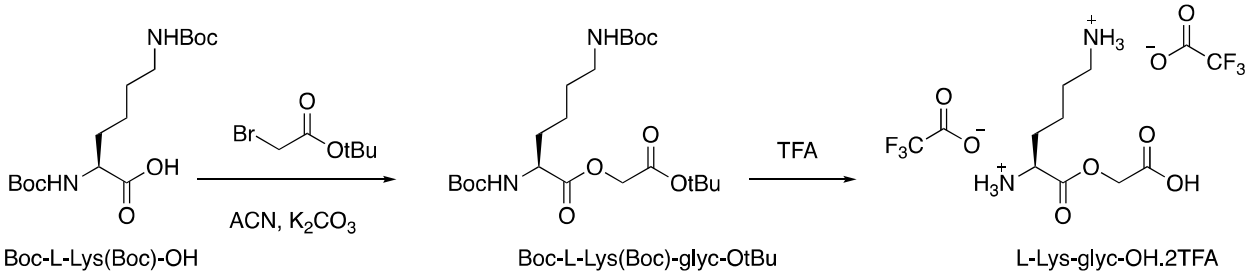
Table of Contents

I. Materials and Methods

Materials

Glycolic acid was purchased from Sigma-Aldrich (#124737). L-lactic acid was purchased from TCI America (L0165). L-Arginine monohydrochloride was purchased from Alfa Aesar (#A14730). L-Histidine monohydrochloride monohydrate (H8125 Sigma), L-Lysine monohydrochloride (L5626 Sigma-Aldrich), D-Lysine monohydrochloride (L5876 Sigma-Aldrich), L-2-Amino-3-guanidinopropionic acid hydrochloride (A5402 Sigma-Aldrich) and L-Ornithine monohydrochloride (O2375 Sigma) were purchased from Sigma-Aldrich. L-2,4-diaminobutyric acid dihydrochloride (Dab) was purchased from BeanTown Chemical (#223705). L-2,3-Diaminopropionic acid monohydrochloride was purchased from Alfa Aesar (#H62128). Cyclic 2,4-diaminobutyric acid (3-Aminopyrrolidin-2-one) (#EN300-119051) and H-Dpr(Me)$_2$-OH·2HCl (#EN300-365749) were purchased from Enamine. Cyclic Ornithine (3-Aminopiperidine-2-one) was purchased from TCI America (#A3171). H-Lys(Me)$_2$-OH·HCl was purchased from BACHEM (#E-1810). H-Lys(Me)$_3$-OH·HCl was purchased from Chem-Impex International, Inc.

Synthesis of L-Lys-glycolic acid



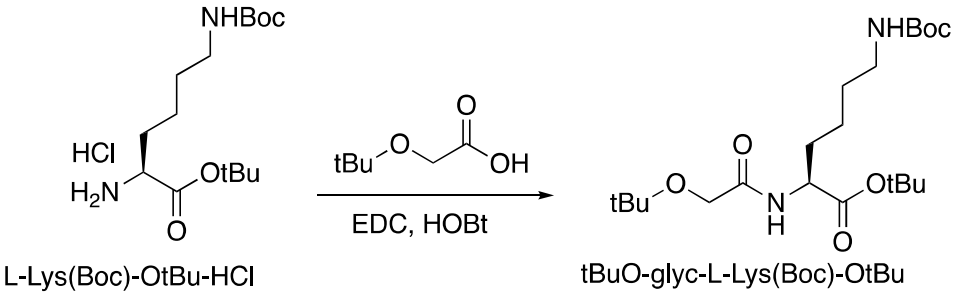
Boc-L-Lys(Boc)-OH Boc-L-Lys(Boc)-glyc-OtBu L-Lys-glyc-OH.2TFA

The dicyclohexylammonium salt Boc-L-Lys(Boc)-OH.DCHA (Novabiochem, 0.528 g, 1.0 mmol) was first converted to the free acid as follows. The compound was suspended in EtOAc (10 mL). Cold aqueous 5% H_3PO_4 was added until the pH of the aqueous layer was ~3. The mixture was shaken to dissolve the solid, and the organic layer was removed. The aqueous layer was extracted twice with 5 mL EtOAc, and the organic layers were combined, washed twice with 1 % H3PO4 and 3X with water, and dried over MgSO4. The solvent was removed by evaporation to give the free acid in quantitative yield as a clear oil. The compound was dissolved in acetonitrile (30 mL). Next, tert-butylbromoacetate (0.205 g, 1.05 mmol) was added, followed by K_2CO_3 (0.746 g, 5.4 mmol). The suspension became a thick slurry that was stirred vigorously for 13 h at room temperature. TLC (10% MeOH/DCM, $KMnO_4$ stain) indicated the reaction was complete. The suspension was filtered and rotovapped to yield an oil. This material was dissolved in EtOAc, washed twice with sat $NaHCO_3$, twice with 0.1 N HCl, and once with brine. The organic layer was dried over $MgSO_4$ and rotovapped to give 0.43 g of an oil. Neat TFA (10 mL) was added, and the solution

was allowed to stand for 45 min. After this time, the solvent was evaporated, water (10 mL) was added, and the material was lyophilized to yield 0.37 g (86% yield).

^{1}H NMR (500 MHz, DMSO-d6): δ = 8.61 (br s, 3 H), 7.92 (br s, 3 H), 4.79-4.69 (q, 2 H), 4.13 (br t, 1 H), 2.79-2.73 (m, 2 H), 1.92-1.80 (m, 2 H), 1.61-1.41 (m, 4 H). 13C NMR (126 MHz, DMSO-d6): δ = 169.73, 168.83, 62.16, 51.93, 38.80, 29.89, 26.78, 21.45. High resolution ESI-TOF MS (m/z): calc'd 205.1183 for C8H17N2O4, obs'd 205.1188.

Synthesis of tBuO-glyc-L-Lys(Boc)-OtBu

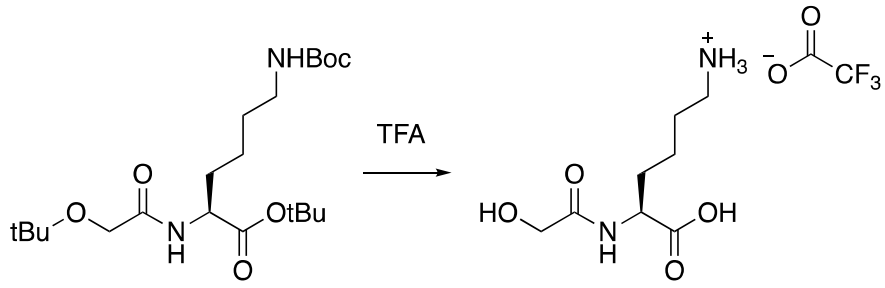


L-Lys(Boc)-OtBu-HCl tBuO-glyc-L-Lys(Boc)-OtBu

Tert-butoxy acetic acid (Combi-Blocks, 0.145 g, 1.1 mmol) was dissolved in DCM (4 mL). Next, HOBt.H_2O (0.168 g, 1.1 mmol) and EDC.HCl (0.211 g, 1.1 mmol) were added. This was followed by the addition of H-Lys(Boc)-OtBu.HCl (Chem-Impex, 0.339 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol). The yellowish solution was stirred at room temperature for 14 h. The solution was diluted with DCM (50 mL), washed twice with sat. NaHCO3, twice with sat. $KHSO_4$, and brine. After drying over $MgSO_4$, the solvent was evaporated to a clear oil. The compound was purified by preparative HPLC and lyophilized to give 0.40 g of an oil (96% yield).

^{1}H NMR (500 MHz, DMSO-d6): δ = 7.48-7.46 (d, 1H), 6.77-6.75 (t, 1H), 4.20-4.16 (m, 1H), 3.82 (s, 2H), 2.91-2.86 (m, 2H), 1.73-1.61 (m, 2H), 1.41 (s, 9H), 1.39-1.33 (m, 11H), 1.26-1.21 (m, 2H), 1.18 (s, 9H). 13C NMR (126 MHz, DMSO-d6): δ = 171.42, 170.45, 156.04, 81.35, 77.76, 74.54, 62.27, 52.32, 31.39, 29.48, 28.73, 28.08, 27.58, 22.90.

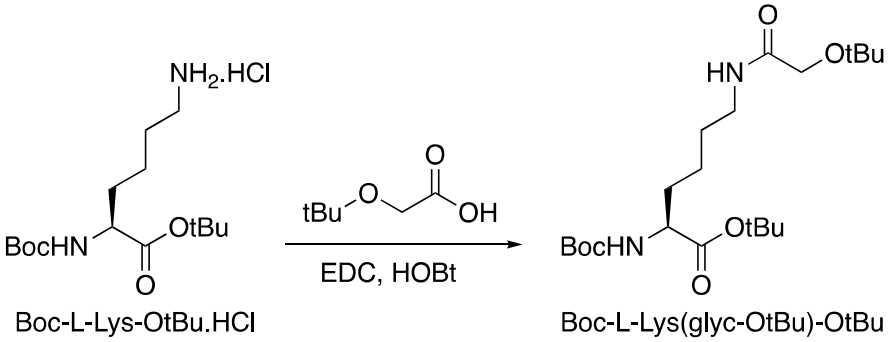
Synthesis of glyc-L-Lys-OH.TFA



tBuO-glyc-L-Lys(Boc)-OtBu (0.40 g) was dissolved in TFA (2 mL) and allowed to stand at room temperature for 1 h. The solvent was evaporated, and 5 mL water was added to residue. After lyophilization, 307 mg product was obtained (quantitative yield).

^{1}H NMR (500 MHz, DMSO): δ = 7.75-7.74 (m, 4H), 4.28-4.24 (m, 1H), 3.85 (s, 2H), 2.80-2.74 (m, 2H), 1.80-1.65 (m, 2H), 1.59-1.48 (m, 2H), 1.37-1.29 (m, 2H). 13C NMR (126 MHz, DMSO): δ = 173.79, 172.30, 61.67, 51.48, 39.07, 31.07, 27.05, 22.69. High resolution ESI-TOF MS (m/z): calc'd 205.1183 for C8H17N2O4, obs'd 205.1183

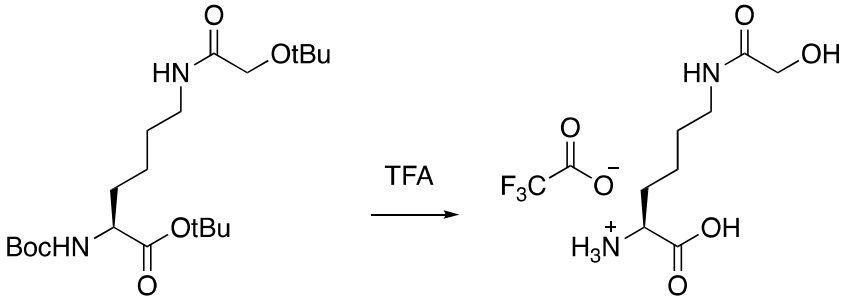
Synthesis of Boc-L-Lys(glyc-OtBu)-OtBu



Tert-butoxy acetic acid (Combi-Blocks, 0.126 g, 0.95 mmol) was dissolved in DMF (4 mL). Next were added HOBt.H_2O (0.145 g, 0.95 mmol), Boc-L-Lys-OtBu.HCl (0.288 g, 0.85 mmol), and triethylamine (0.18 mL, 1.3 mmol). EDC.HCl (0.182 g, 0.95 mmol) was added, and the clear yellowish solution was stirred at room temperature for 16 h. The solvent was evaporated and the residue was taken up in DCM (30 mL). This was washed twice with sat. NaHCO3, twice with sat. $KHSO_4$, and once with brine. After drying over $MgSO_4$, the solution was filtered and evaporated to a clear oil that was purified by preparative HPLC. Lyophilization yielded 106 mg pure compound (30% yield).

^{1}H NMR (500 MHz, DMSO): δ = 7.50-7.48 (t, 1H), 7.07-7.06 (d, 1H), 3.75-3.68 (m, 3H), 3.12-3.06 (m, 2H), 1.60-1.52 (m, 2H), 1.39-1.38 (m, 18H), 1.31-1.25 (m, 2H), 1.17 (s, 9H). 13C NMR (126 MHz, DMSO): δ = 172.40, 170.32, 156.03, 80.58, 78.42, 74.25, 62.45, 54.82, 38.21, 30.79, 29.28, 28.66, 28.11, 27.51, 23.37. ESI-LCMS (m/z): calc'd 439.55 for C21H40N2O6Na, obs'd 439.0.

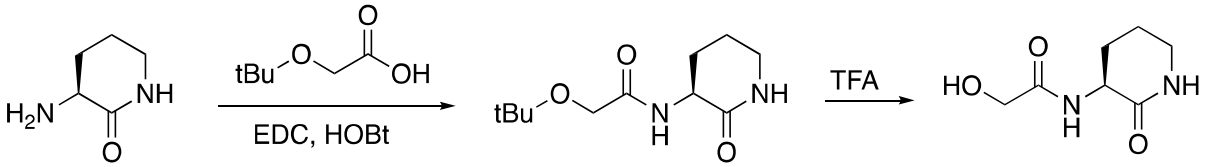
Synthesis of L-Lys(glyc)-OH.TFA



Boc-L-Lys(glyc-OtBu)-OtBu (106 mg) was dissolved in TFA (2 mL) and allowed to stand at room temperature for 1 h. The solvent was evaporated, and 5 mL water was added to residue. After lyophilization, 71 mg product (88% yield) was obtained.

^{1}H NMR (500 MHz, DMSO): δ = 8.26 (br s, 3H), 7.74-7.72 (t, 1H), 3.78 (br s, 1H), 3.74 (s, 2H), 3.11-3.07 (q, 2H), 1.83-1.71 (m, 2H), 1.47-1.26 (m, 4H). 13C NMR (126 MHz, DMSO): δ = 172.13, 171.54, 61.88, 52.37, 38.07, 30.15, 29.21, 22.16. High resolution ESI-TOF MS (m/z): calc'd 205.1183 for C8H17N2O4, obs'd 205.1185.

Synthesis of glyc-L-Orn lactam

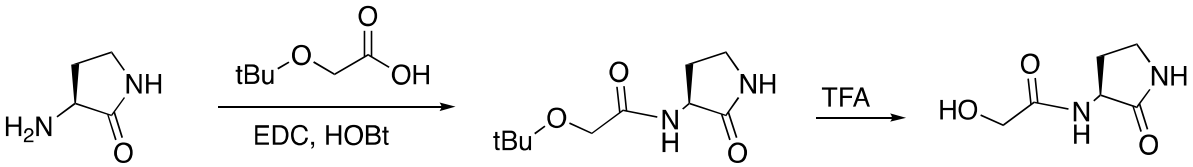


Tert-butoxy acetic acid (Combi-Blocks, 0.145 g, 1.1 mmol) was dissolved in DCM (4 mL). Next, HOBt.H_2O (0.168 g, 1.1 mmol) and EDC.HCl (0.211 g, 1.1 mmol) were added. This was followed by the addition of H-L-Orn lactam (TCI-America #A3171, 0.114 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol). The solution was stirred at room temperature for 14 h. The solution was diluted with DCM (50 mL), washed twice with sat. $NaHCO_3$, twice with sat. $KHSO_4$, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield a clear oil. The crude product was purified by preparative HPLC and concentrated

to a clear oil. The residue was dissolved in neat TFA and allowed to stand at RT for 1 h. After evaporation of the solvent, the desired compound was purified by preparative HPLC. Lyophilization yielded 0.090 g of a white solid (53% yield over two steps).

^{1}H NMR (500 MHz, DMSO) δ 7.81-7.79 (d, 1H), 7.63 (br s, 1H), 4.17-4.12 (m, 1H), 3.82 (s, 2H), 3.15-3.12 (m, 2H), 2.10-2.04 (m, 1H), 1.82-1.70 (m, 2H), 1.67-1.59 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 171.99, 170.38, 61.82, 49.19, 41.23, 27.76, 21.49. High resolution ESI-TOF MS (m/z): calc'd 173.0926 for $C_7H_{12}N_2O_3$ [M+H], obs'd 173.0925.

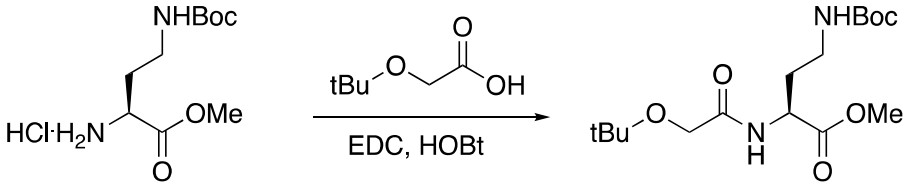
Synthesis of glyc-L-Dab lactam



Tert-butoxy acetic acid (Combi-Blocks, 0.145 g, 1.1 mmol) was dissolved in DCM (4 mL). Next, HOBt.H2O (0.168 g, 1.1 mmol) and EDC.HCl (0.211 g, 1.1 mmol) were added. This was followed by the addition of H-L-Dab lactam (Enamine, 0.100 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol). The solution was stirred at room temperature for 12 h. The solution was diluted with DCM (50 mL), washed twice with sat. $NaHCO_3$, twice with sat. $KHSO_4$, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield a clear oil. The crude product was purified by preparative HPLC and concentrated to a clear oil. The residue was dissolved in neat TFA and allowed to stand at RT for 1 h. After evaporation of the TFA, toluene was added to the residue and then evaporated to azeotropically remove residual TFA. The residue was then dissolved in water and lyophilized to yield 0.104 g of a white solid (66% yield over two steps).

^{1}H NMR (500 MHz, DMSO) δ 7.87-7.85 (d, 1H), 7.80 (br s, 1H), 4.35-4.29 (m, 1H), 3.83 (s, 2H), 3.19-3.15 (m, 2H), 2.31-2.26 (m, 1H), 1.96-1.88 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 174.84, 172.41, 61.84, 49.60, 38.51, 28.63. High resolution ESI-TOF MS (m/z): calc'd 159.0770 for $C_6H_{10}N_2O_3$ [M+H] obs'd 159.0766.

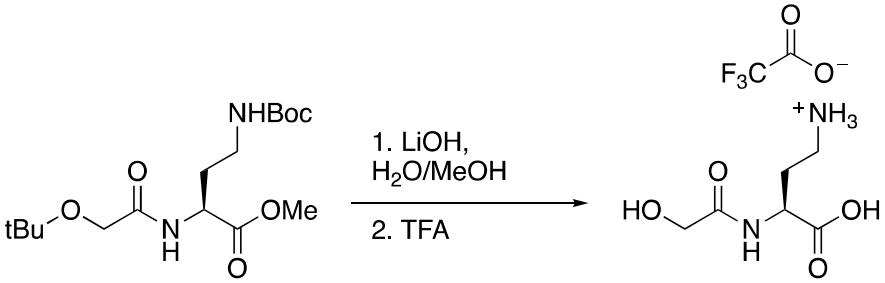
Synthesis of tBuO-glyc-L-Dab(Boc)-OMe



Tert-butoxy acetic acid (Combi-Blocks, 0.145 g, 1.1 mmol) was dissolved in DCM (4 mL). Next, HOBt.H2O (0.168 g, 1.1 mmol) and EDC.HCl (0.211 g, 1.1 mmol) were added. This was followed by the addition of H-L-Dab(Boc)-OMe (Chem-Impex, 0.269 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol). The solution was stirred at room temperature for 12 h. The solution was diluted with DCM (50 mL), washed twice with sat. $NaHCO_3$, twice with sat. $KHSO_4$, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield a clear oil. The crude product was purified by preparative HPLC and lyophilized to yield 0.33 g of a white foamy solid (95% yield).

^{1}H NMR (500 MHz, DMSO) δ 7.81-7.80 (d, 1H), 6.80-6.78 (t, 1H), 4.37-4.33 (m, 1H), 3.82 (s, 2H), 3.63 (s, 3H), 3.03-2.96 (m, 1H), 2.93-2.86 (m, 1H), 1.93-1.78 (m, 2H), 1.37 (s, 9H), 1.19 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 172.54, 170.83, 156.02, 78.09, 74.57, 62.27, 52.43, 49.64, 37.08, 31.33, 28.69, 27.52. ESI-TOF MS (m/z): calc'd 369.2 for $C16H30N2O6Na$ [M+Na], obs'd 369.2.

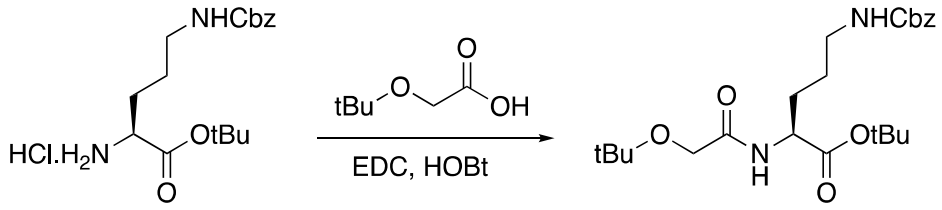
Synthesis of glyc-L-Dab-OH.TFA



tBuO-glyc-L-Dab(Boc)-OMe (0.3 g, 0.87 mmol) was dissolved in MeOH (5 mL). Separately, LiOH monohydrate (0.33 g, 7.9 mmol) was dissolved in water (5 mL). These solutions were combined and the cloudy suspension was stirred at RT for 16 h, after which the solvent was removed by rotary evaporation. To the residue, EtOAc and 1 N HCl were added. The organic layer was removed, and the aqueous layer was extracted again with EtOAc. The organic layers were combined, dried over $MgSO_4$, and evaporated to yield an oil. This oil was dissolved in TFA (5 mL) and allowed to stand at RT for 1 h. After this time, the TFA was removed by rotary evaporation, water was added to the residue, and the solution was lyophilized to yield 0.21 g (83 % yield for two steps) of a pale yellow oil.

^{1}H NMR (500 MHz, DMSO) δ 8.02-8.01 (d, 1H), 7.82 (br s, 3H), 4.40-4.34 (m, 1H), 3.87 (s, 2H), 2.85-2.78 (m, 2H), 2.13-2.05 (m, 1H), 1.98-1.89 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 172.90, 172.72, 61.70, 49.46, 36.75, 29.57. High resolution ESI-TOF MS (m/z): calc'd 177.0875 for $C6H10N2O3$ [M+H], obs'd 177.0879.

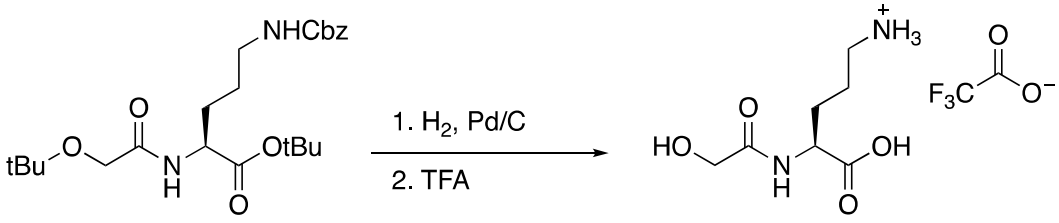
Synthesis of tBuO-glyc-L-Orn(Cbz)-OtBu



Tert-butoxy acetic acid (Combi-Blocks, 0.145 g, 1.1 mmol) was dissolved in DCM (4 mL). Next, HOBt.H_2O (0.168 g, 1.1 mmol) and EDC.HCl (0.211 g, 1.1 mmol) were added. This was followed by the addition of H-L-Orn(Cbz)-OtBu (Combi-Blocks, 0.359 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol). The solution was stirred at room temperature for 12 h. The solution was diluted with DCM (50 mL), washed twice with sat. $NaHCO_3$, twice with sat. $KHSO_4$, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield a clear oil. The crude product was purified by preparative HPLC and lyophilized to yield 0.42 g of a white solid (96% yield).

^{1}H NMR (500 MHz, DMSO) δ 7.50-7.49 (d, 1H), 7.38-7.27 (m, 6H), 5.01 (s, 2H), 4.22-4.18 (m, 1H), 3.82 (s, 2H), 3.01-2.97 (m, 2H), 1.77-1.62 (m, 2H), 1.41 (m, 11H), 1.18 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 171.30, 170.47, 156.55, 137.73, 128.80, 128.21, 128.18, 81.40, 74.54, 65.58, 62.27, 52.18, 29.05, 28.07, 27.57, 26.15. ESI-TOF MS (m/z): calc'd 437.5 for $C_{23}H_{37}N_2O_6$ [M+H], obs'd 437.2.

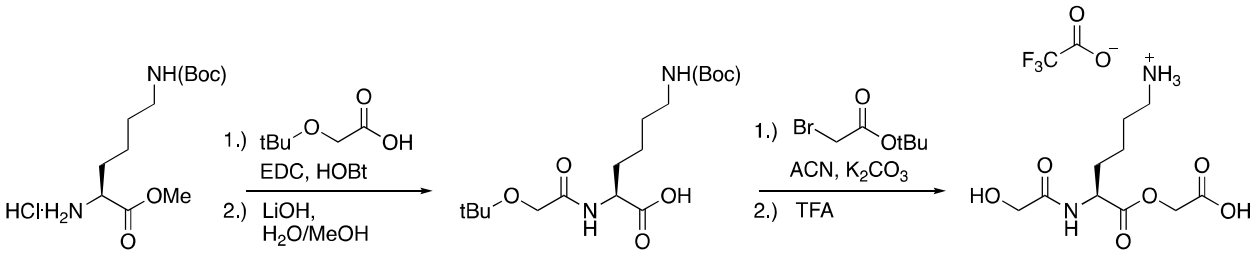
Synthesis of glyc-L-Orn-OH.TFA



tBuO-glyc-L-Orn(Cbz)-OtBu (0.42 g, 0.96 mmol) was dissolved in 200 proof EtOH (10 mL). This solution was added to a flask containing Pd/C (0.1 g, 5 wt%, wet support, Degussa E101 NOW). (CAUTION: Hydrogenation reactions pose a significant fire hazard due to the use of flammable reagents, solvents, and catalysts. Such reactions should only be carried out by trained personnel.) Hydrogen gas was introduced at atmospheric pressure, and the reaction was stirred at room temperature for 20 minutes, after which time HPLC indicated complete removal of the Cbz group. The mixture was filtered through Celite and the solvent was evaporated to yield a clear oil. The desired product was purified by preparative HPLC, concentrated to an oil, and dissolved in TFA (5 mL). After 1 h at room temperature, the TFA was removed by evaporation and the residue was lyophilized to yield 0.256 g of the desired compound as an oil (88% yield for 2 steps).

^{1}H NMR (500 MHz, DMSO) δ 7.81-7.76 (m, 4H), 4.31-4.27 (m, 1H), 3.86 (s, 2H), 2.83-2.76 (m, 2H), 1.88-1.81 (m, 1H), 1.75-1.67 (m, 1H), 1.59-1.53 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 173.53, 172.32, 61.67, 51.21, 38.96, 28.57, 24.09. High resolution ESI-TOF MS (m/z): calc'd 191.1032 for $C_7H_{15}N_2O_4$ [M+H], obs'd 191.1031.

Synthesis of glyc-L-Lys-glyc.TFA



Tert-butoxy acetic acid (Combi-Blocks, 0.436 g, 3.13mmol) was dissolved in DCM (12 mL). Next, HOBt.H_2O (0.505 g, 3.3 mmol) and EDC.HCl (0.633 g, 3.3 mmol) were added. This was followed by the addition of H-L-Lys(Boc)-OMe.HCl (Combi-Blocks, 0.890 g, 3.0 mmol) and triethylamine (0.62 mL, 4.5 mmol). The solution was stirred at room temperature for 16 h. The solution was diluted with DCM (150 mL), washed 4X with sat. $NaHCO_3$, 3X with 1N HCl, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield 1.1 g (98%) of tBuO-glyc-L-Lys(Boc)-OMe as a pale yellow oil, which was used directly in the next step. The obtained compound was dissolved in MeOH (8 mL). To this solution was added LiOH.H_2O (0.42 g, 10 mmol) dissolved in water (8 mL). The suspension was stirred at room temperature for 1 h, after which time HPLC indicated complete removal of the methyl ester. The solvent was evaporated, and the residue was taken up in 1 N HCl and EtOAc. The organic layer was removed, and the aqueous layer was extracted once more with EtOAc. The organic layers were combined, washed with brine, and dried over $MgSO_4$. Removal of the solvent yielded 1.08 g of an oil (quant. yield). This oil was dissolved in acetonitrile (60 mL), to which was added tert-butyl bromoacetate (0.614 g, 3.15 mmol) and K_2CO_3 (2.24 g, 16.2 mmol). The suspension was stirred vigorously for 14 h at room temperature. The mixture was filtered and the supernatant was removed by evaporation. The residue was taken up in EtOAc, washed twice with sat. $NaHCO_3$, twice with 0.1 N HCl, and brine. After drying over $MgSO_4$, the solvent was evaporated to yield 1.228 g of tBuO-glyc-L-Lys(Boc)-glyc-OtBu as an oil (86%). A portion of the product (0.13 g, 0.27 mmol) was dissolved in TFA (3 mL) and allowed to stand at room temperature for 1 h. After this time, the solvent was removed by evaporation and the residue was purified by preparative HPLC. After evaporation of the solvent, the compound was lyophilized to yield 0.083 g (81% yield, 68% over 4 steps) of glyc-L-Lys-glyc.TFA as an oil.

^{1}H NMR (500 MHz, DMSO) δ 7.97-7.96 (d, 1H), 7.76 (br s, 3H), 4.66-4.56 (q, 2H), 4.43-4.39 (m, 1H), 3.87 (s, 2H), 2.80-2.74 (m, 2H), 1.86-1.72 (m, 2H), 1.60-1.49 (m, 2H), 1.45-1.33 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 172.56, 171.96, 169.26, 61.64, 61.42, 51.38, 39.05, 30.76, 26.98, 22.58. High resolution ESI-TOF MS (m/z): calc'd 263.1243 for $C_{10}H_{19}N_2O_6$ [M+H], obs'd 263.1246.

Dry-down reactions and NMR spectroscopy

A description of the dry-down reactions and NMR spectroscopy is presented in the Materials and Methods section in the main text.

IR spectroscopy

IR data was obtained on a Thermo Nicolet 4700 FTIR Spectrometer. Prior to analysis, samples (5 µl, 100 mM) were placed on hydrophilic PVDF Membranes with a pore size of 0.2µm (Pall Laboratory, #66477) and allowed to dry. Dried samples were analyzed in an Attenuated Total Reflectance (ATR) sample chamber. Spectra were background-subtracted from 400 to 4000 cm^{-1} and signal-averaged (16 scans per spectrum). Data processing was performed using Excel software.

Degradation assays using hydrolytic enzymes

To confirm the existence of both esters and amides within the product mixtures, glycolic acid and Lysine were dried at 85˚C for seven days and the resulting depsipeptides were subjected to incubation with the trypsin peptidase (T1763 Sigma, Sigma-Aldrich) or an esterase from porcine liver (E3019 sigma, Sigma-Aldrich) for 19 hr at 37˚C. Samples were analyzed before and after treatment with the enzymes by C18-HPLC column.

HPLC analysis

HPLC analyses were conducted on an Agilent 1260 Infinity HPLC system. Products of dry-down reactions were separated using either a Kinetex XB-C18 column (150 × 2.1 mm, 2.6 µm particle size) or SeQuant ZIC-HILIC column (150 × 2.1 mm, 3.5 µm particle size). For the C18 column, the flow rate was 0.3 mL min^{-1} and the column temperature was held at 25 °C. The mobile phase was water (0.1% formic acid) /acetonitrile. The gradient method started with 100% water for the first 5 minutes and ramped to 55% acetonitrile in 25 minutes. The acetonitrile concentration ramped to 100% immediately and was held as such for 10 minutes before set back to 100% water for column equilibration for 15 min. For the SeQuant ZIC-HILIC column, the flow rate was 0.2 mL min^{-1} and the column temperature was held at 40 °C. The mobile phase was water (0.5% formic acid) /acetonitrile. The gradient method started with 5% water and ramped to 50% water over 25 min. The mobile phase composition was held at 50% water for 5 min before

returned to 5% water for column equilibration for 15 min. Samples (10 ul for C18 and 5 ul for HILIC) were injected and peptides/depsipeptides were detected at 210 nm. For separation using the HILIC column, the aqueous sample solutions were diluted 2-fold with acetonitrile to 50% v/v acetonitrile/water.

Preparative reverse-phase (RP)-HPLC for purification of NMR standards involved a Thermo BioBasic-18 C18 column connected to a Hitachi D-7000 HPLC system. Binary gradients of solvent A (99% H_2O, 0.9% acetonitrile, 0.1% TFA) and solvent B (90% acetonitrile, 9.9% H_2O, 0.07% TFA) were employed for preparative HPLC.

Processing of HPLC spectra was carried out using Igor Pro 6.3. Species identity was verified using LCMS on a 6130 Single Quadrupole Mass Spectrometer attached to an Agilent 1200 HPLC system using the Kinetex XB-C18 column or the SeQuant ZIC-HILIC column.

Ninhydrin assay

An aliquot of 50 µl of the dry-down reaction that contained glycolic acid and Lysine (100 mM referring to original Lysine monomer concentration) was diluted with water to 10 mM and dialyzed using a Micro Float-A-Lyzer with a cutoff of 0.5-1.0 kD (Spectrum Laboratories, #F235063) at RT four times against 4 L of water. As a negative control, similar protocol was followed for equimolar solution of Lysine monomer, to ensure that monomeric Lysine is dialyzed away. Following dialysis, samples were lyophilized and resuspended in 50 µl water, vortexed and sonicated in ice. For ninhydrin assay, dialyzed samples as well as negative controls (water, fresh or dried glycolic acid) and positive controls (Lysine dilutions) were tested in triplicates. Samples (15 µl) were placed in a 96-well plate and diluted with water (25 µl) prior to addition of the ninhydrin reagent (5% m/v) to each well (10 µl). The plate was covered with a sealing tape and boiled in water for three minutes. Prior to analysis, samples were diluted 2-fold with water and the absorbance was measured 480 nm using a BioTek Synergy H4 Hybrid plate reader.

II. Supplemental Figures Referenced in Main Text

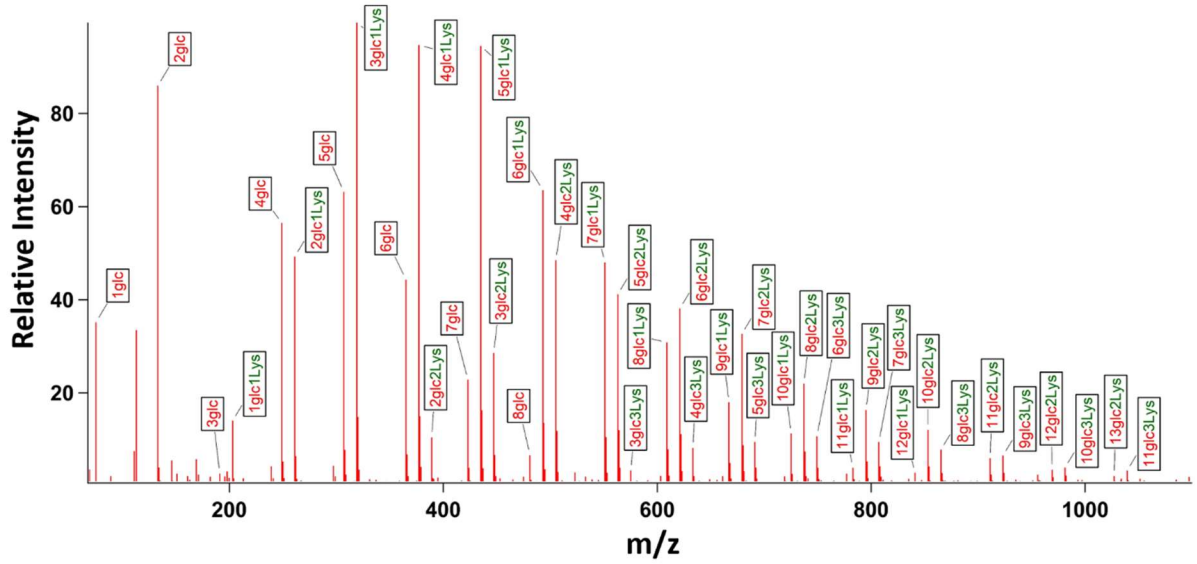


Figure S1. ESI-MS of a dry-down reaction of glc and Lys supports the formation of depsipeptides. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS, indicating a variety of depsipeptides. glc is labeled in red, Lys is labeled in green. All labeled species correspond to $[M-H]^-$ ions.

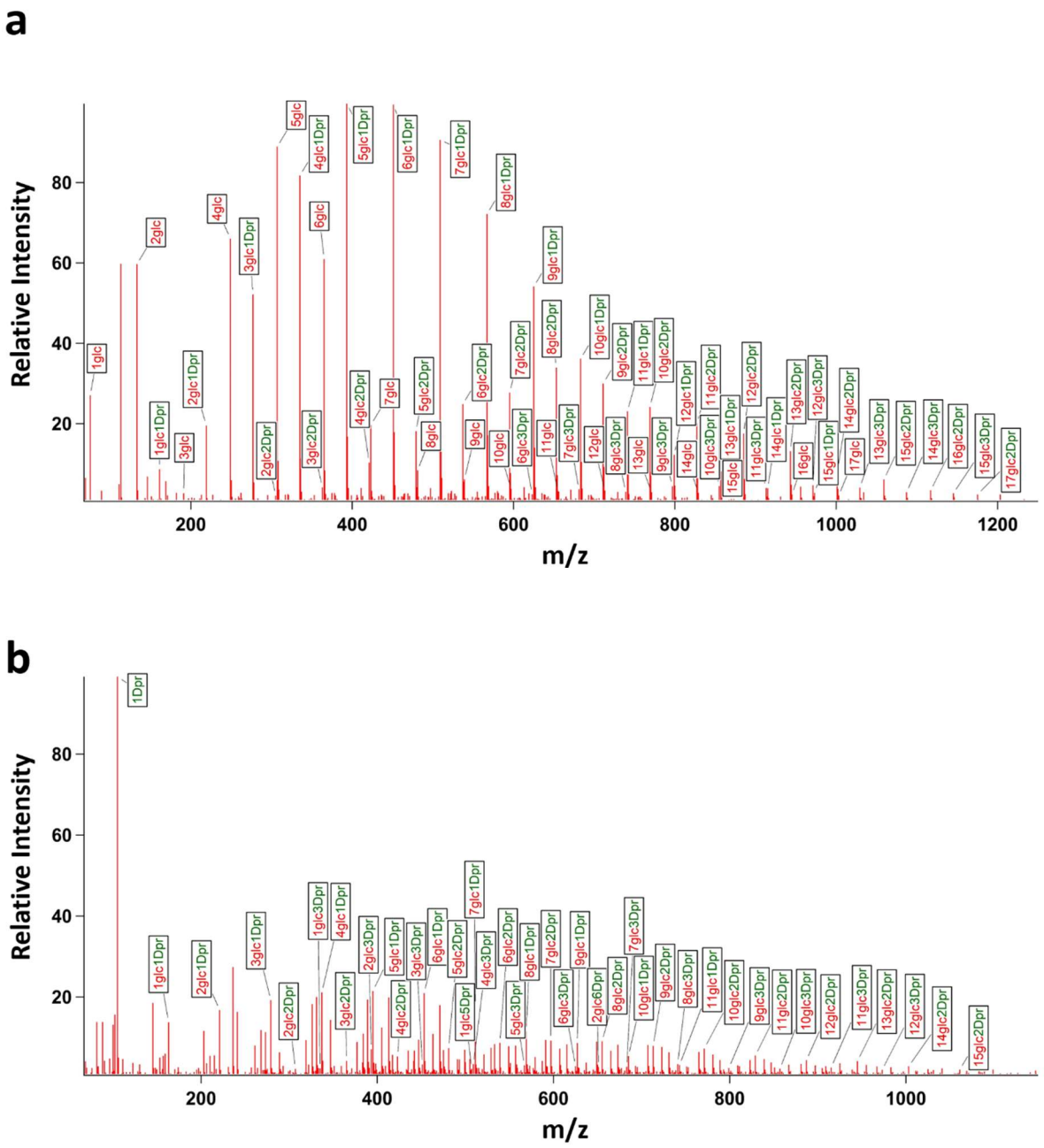


Figure S2. ESI-MS of a dry-down reaction of glc and Dpr supports the formation of depsipeptides. glc and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. glc is labeled in red, Dpr is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

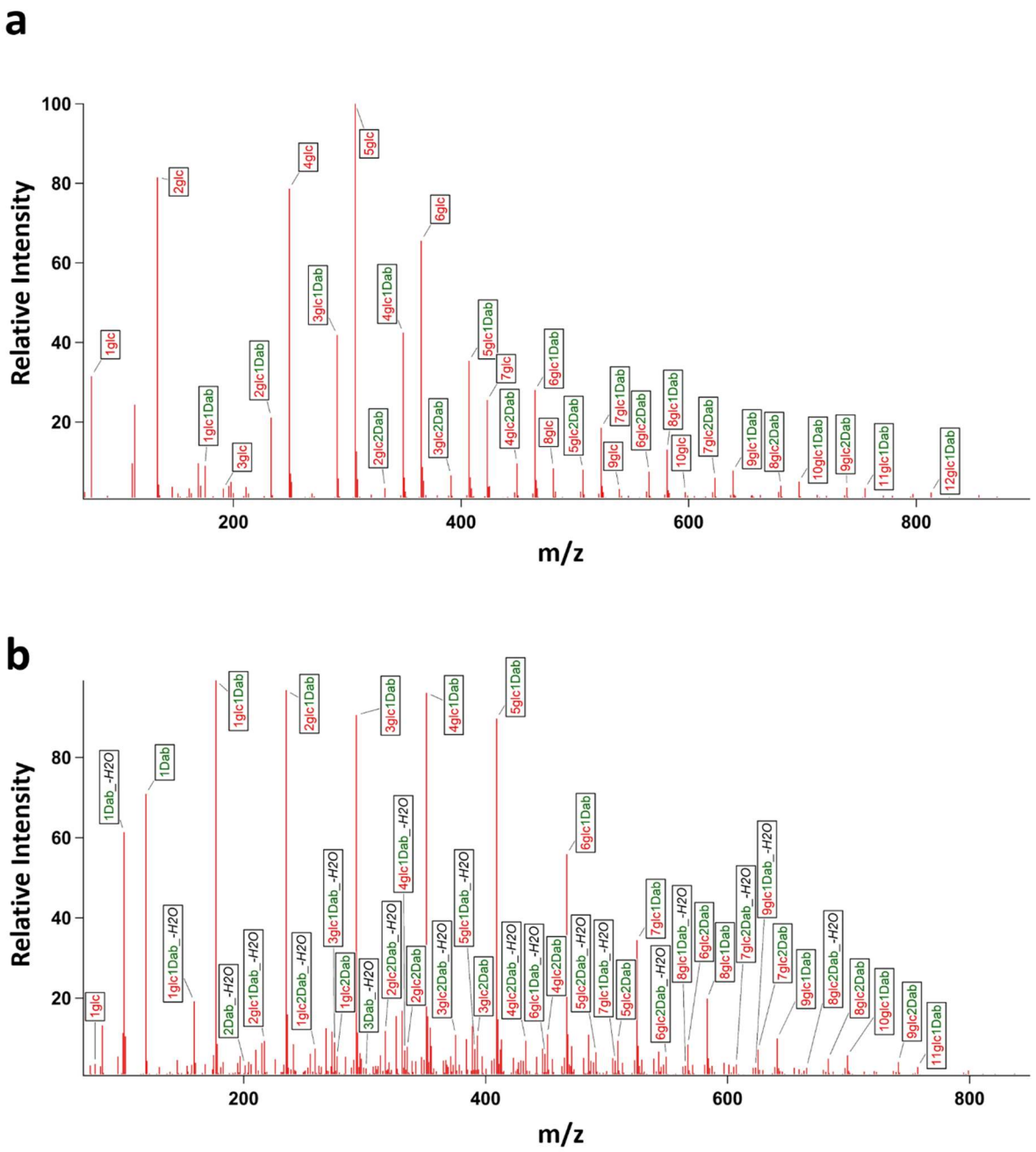


Figure S3. ESI-MS of a dry-down reaction of glc and Dab supports the formation of depsipeptides. glc and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. glc is labeled in red, Dab is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

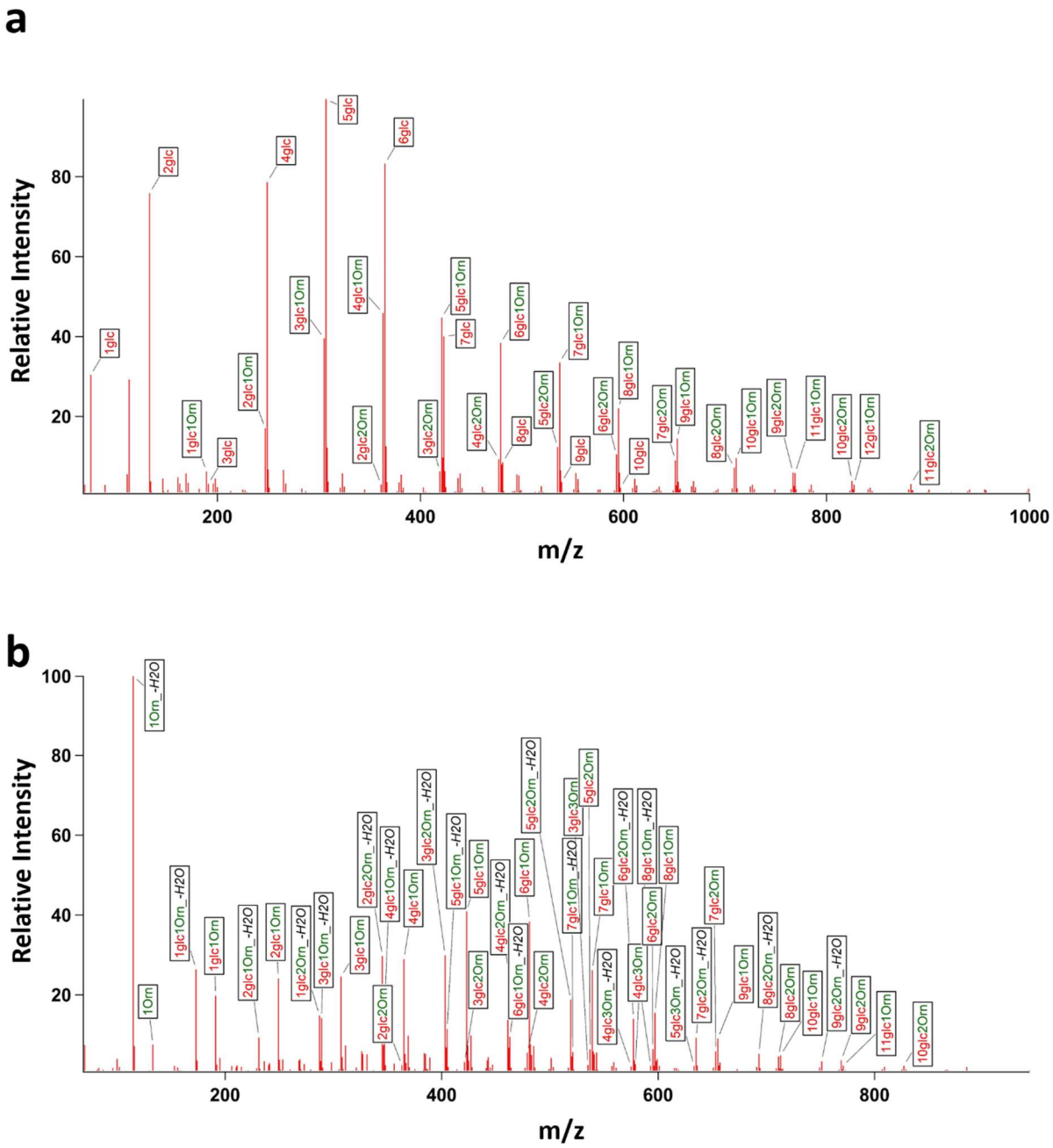


Figure S4. ESI-MS of a dry-down reaction of glc and Orn supports the formation of depsipeptides. glc and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (a) or positive mode ESI-MS (b), indicating a variety of depsipeptides. glc is labeled in red, Orn is labeled in green. Labeled species correspond to [M−H]- ions (a) or [M+H]+ ions (b).

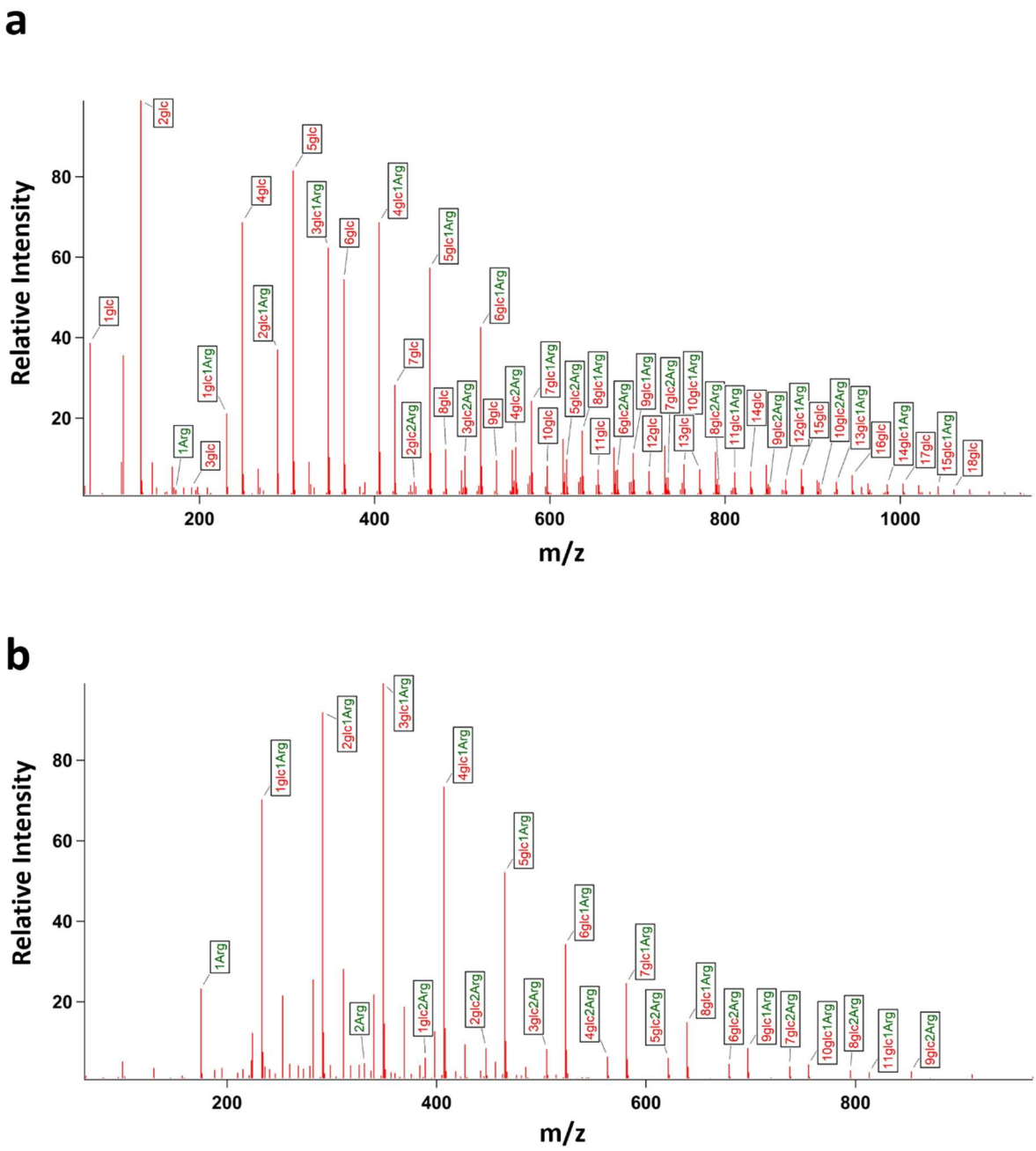


Figure S5. ESI-MS of a dry-down reaction of glc and Arg supports the formation of depsipeptides. glc and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. glc is labeled in red, Arg is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

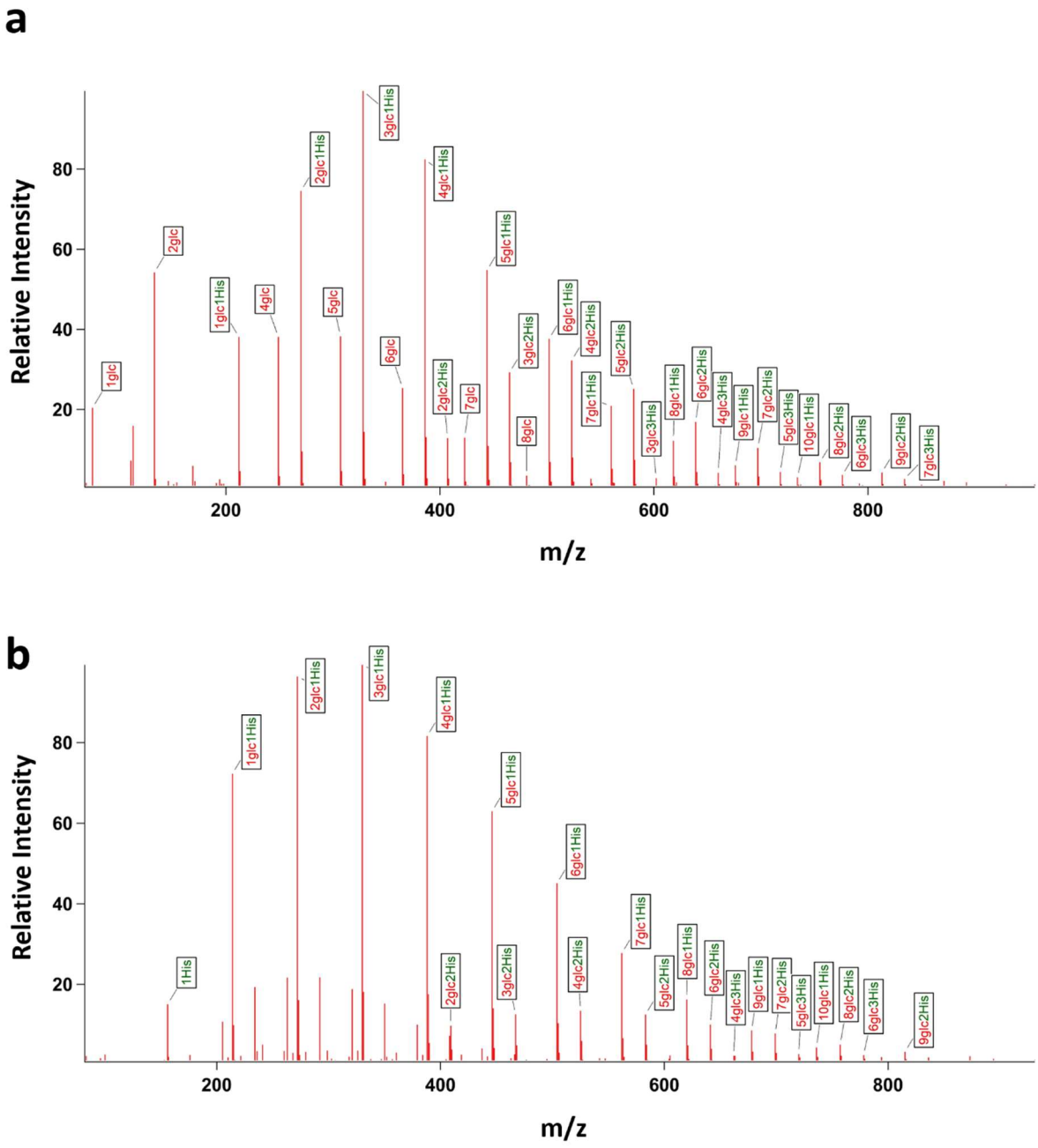


Figure S6. ESI-MS of a dry-down reaction of glc and His supports the formation of depsipeptides. glc and His were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. glc is labeled in red, His is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

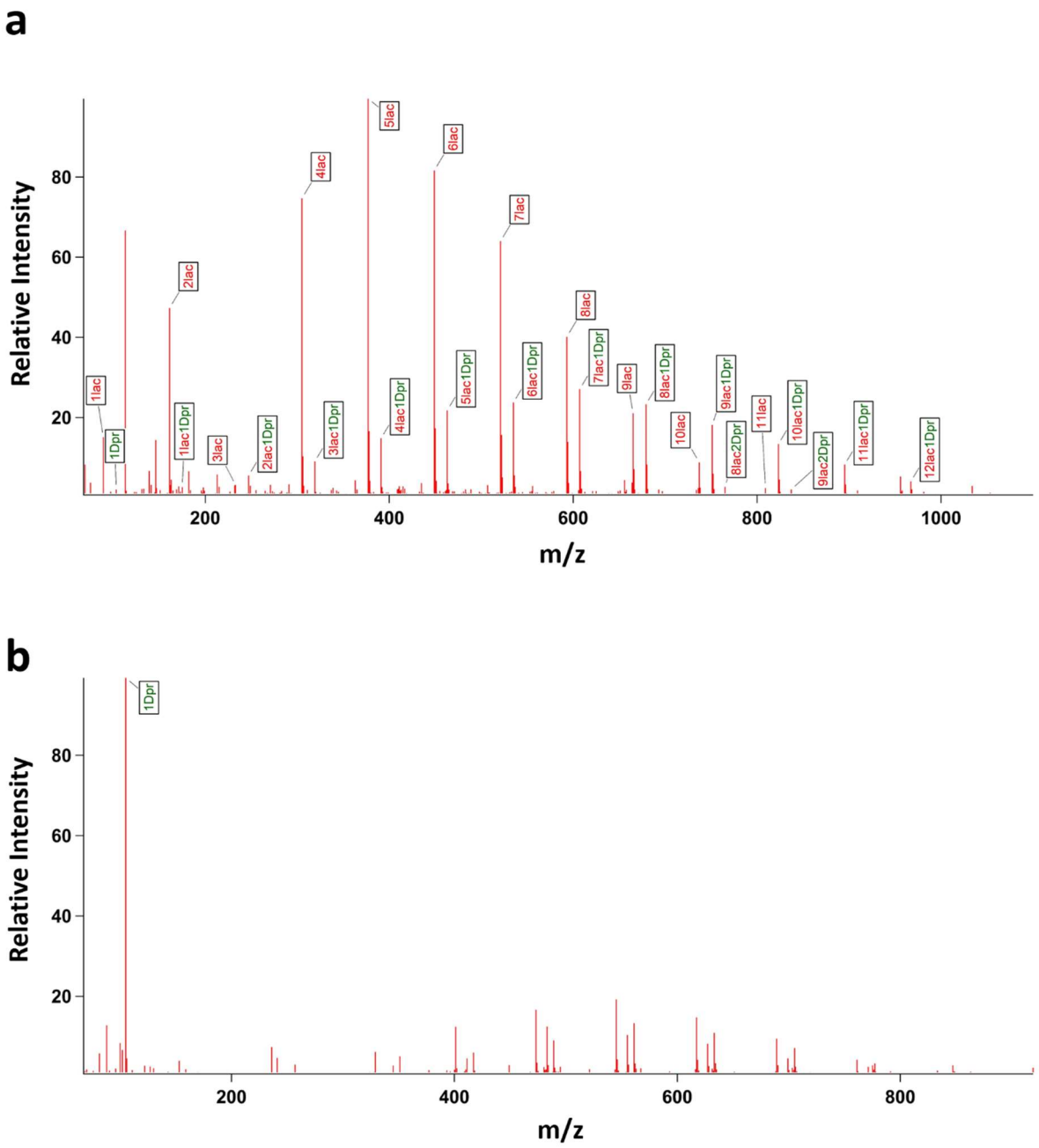


Figure S7. ESI-MS of a dry-down reaction of lac and Dpr supports the formation of depsipeptides. lac and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, Dpr is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

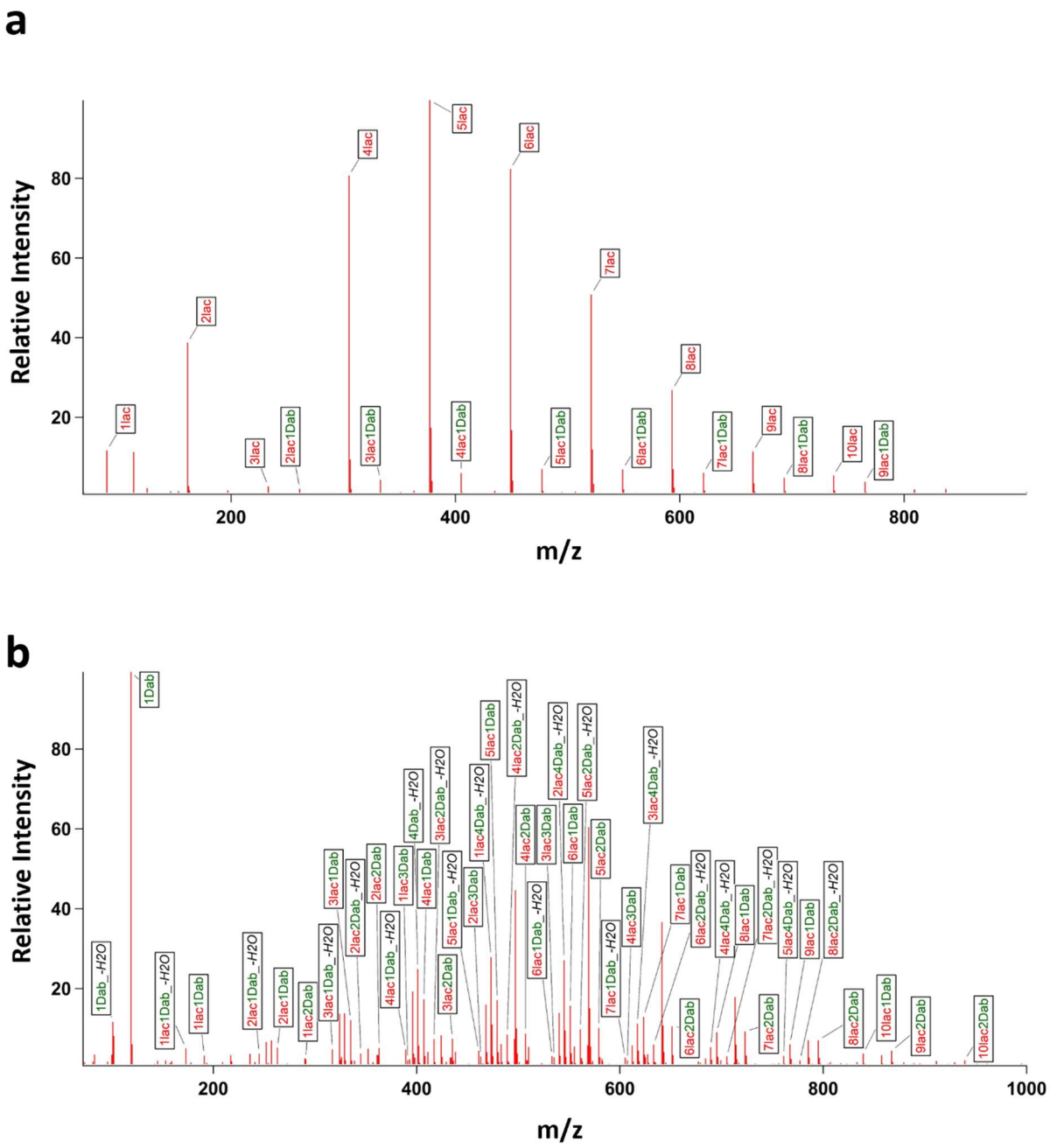


Figure S8. ESI-MS of a dry-down reaction of lac and Dab supports the formation of depsipeptides. lac and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, Dab is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

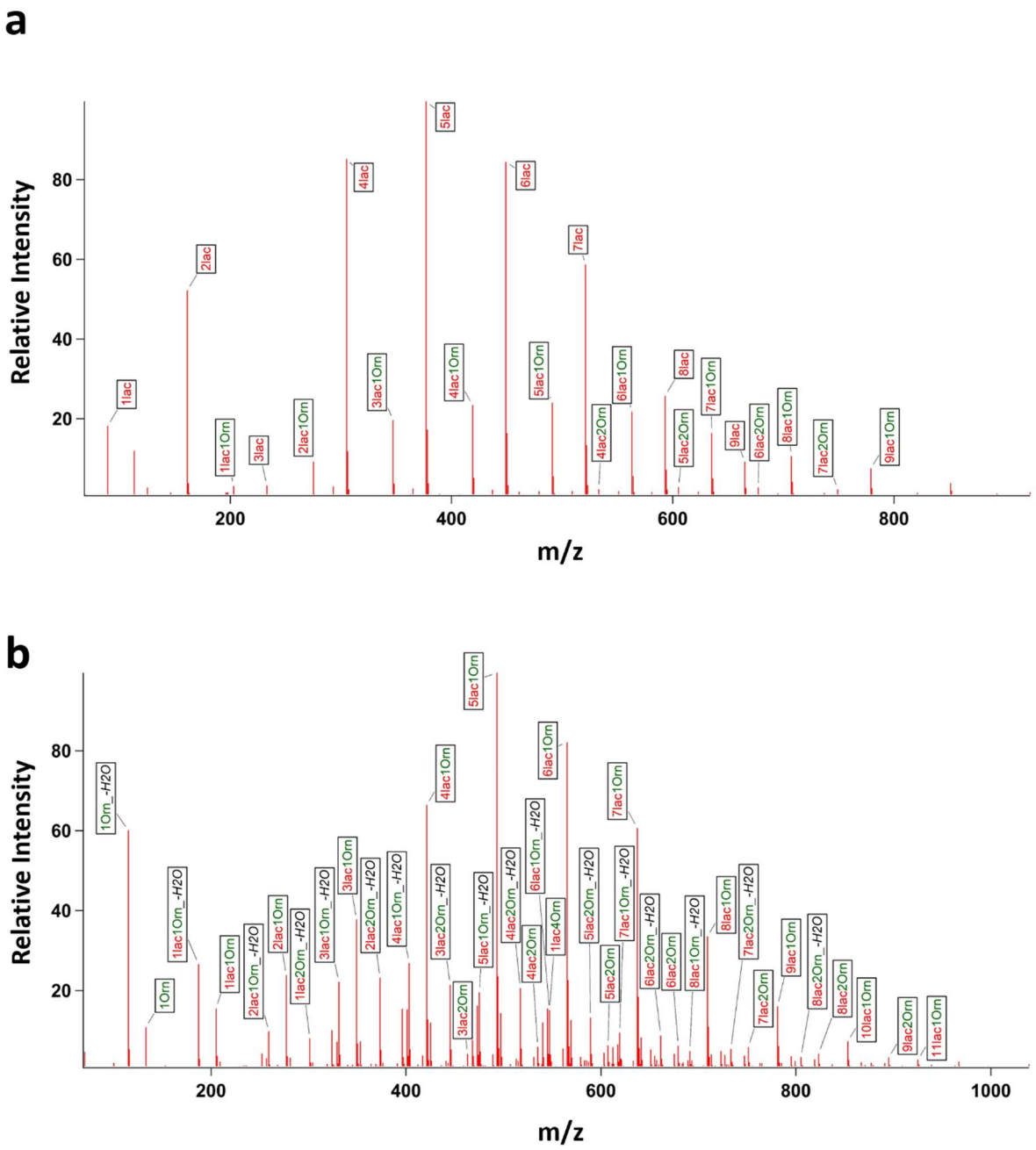


Figure S9. ESI-MS of a dry-down reaction of lac and Orn supports the formation of depsipeptides. lac and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, Orn is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

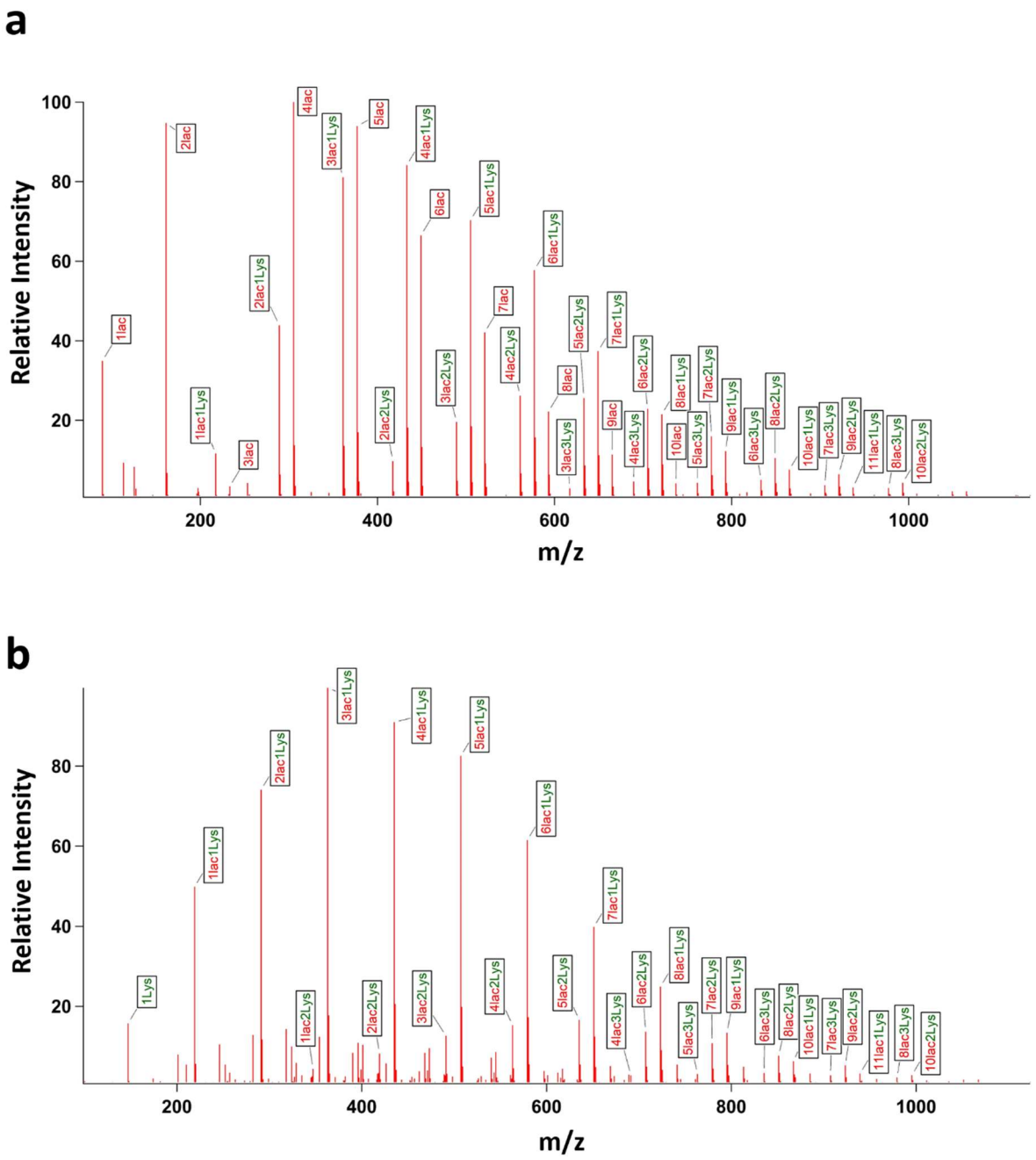


Figure S10. ESI-MS of a dry-down reaction of lac and Lys supports the formation of depsipeptides. lac and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, Lys is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

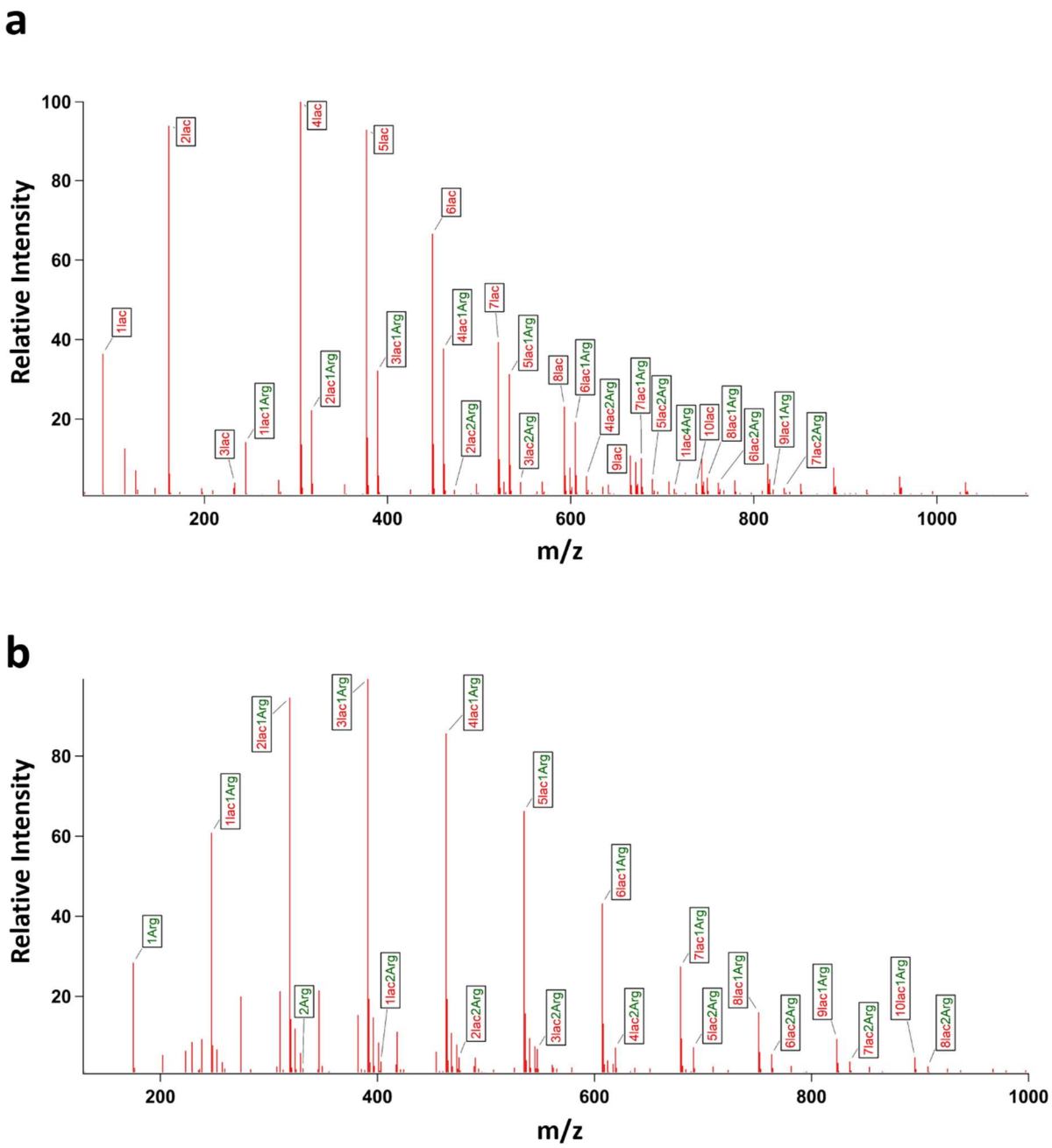


Figure S11. ESI-MS of a dry-down reaction of lac and Arg supports the formation of depsipeptides. lac and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, Arg is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

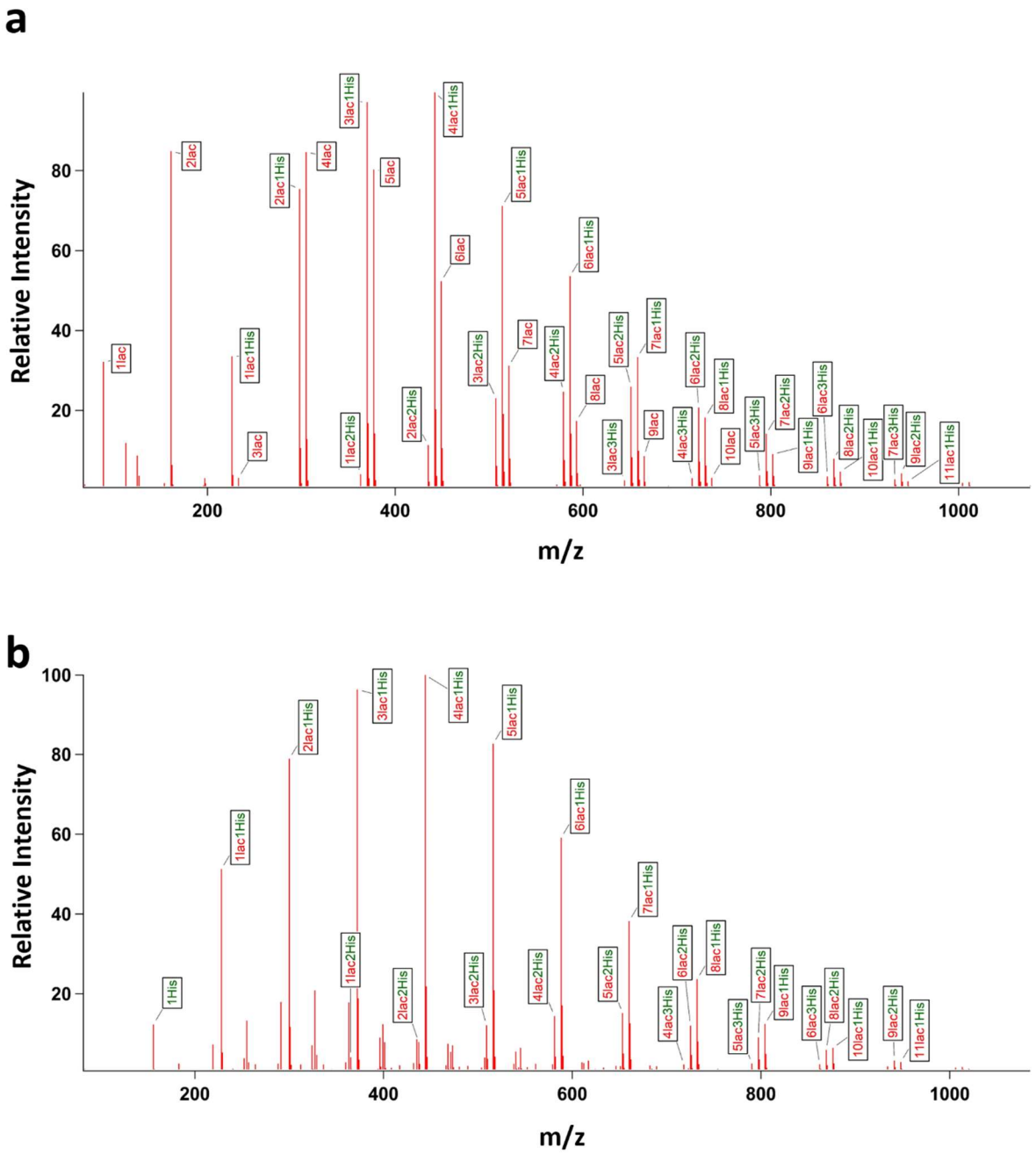


Figure S12. ESI-MS of a dry-down reaction of lac and His supports the formation of depsipeptides. lac and His were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of depsipeptides. lac is labeled in red, His is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

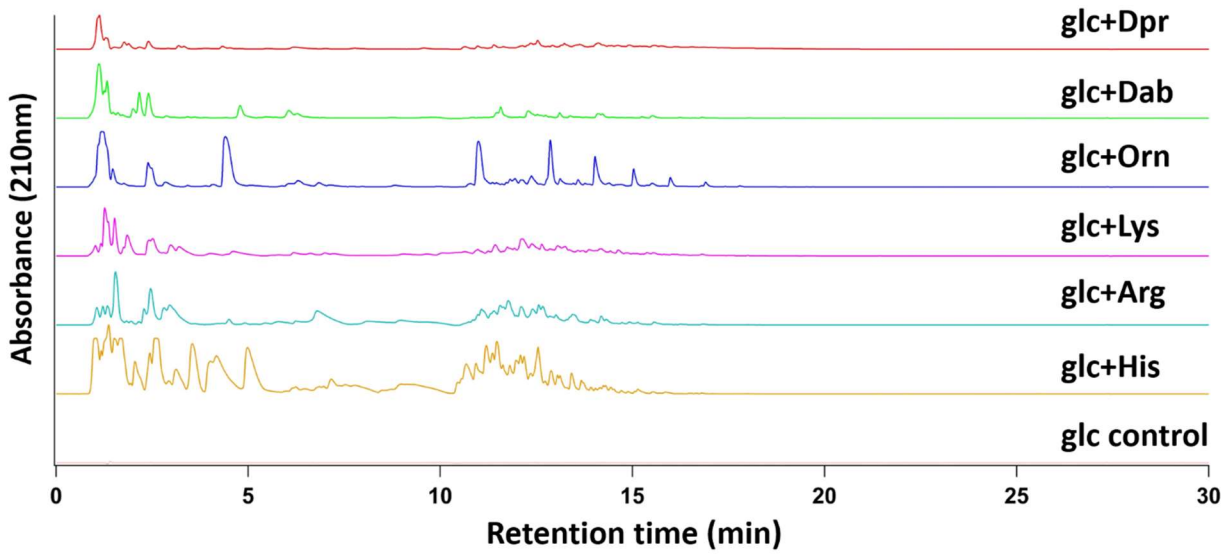


Figure S13. C18-HPLC analysis verifies the formation of depsipeptides following dry-down reactions of glc and cationic amino acids. A mixture of glc and various amino acids were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by hydrophobicity-based separation using C18-HPLC. The presence of various peaks verifies the complexity and the abundance of many different products following the dry-down reactions.

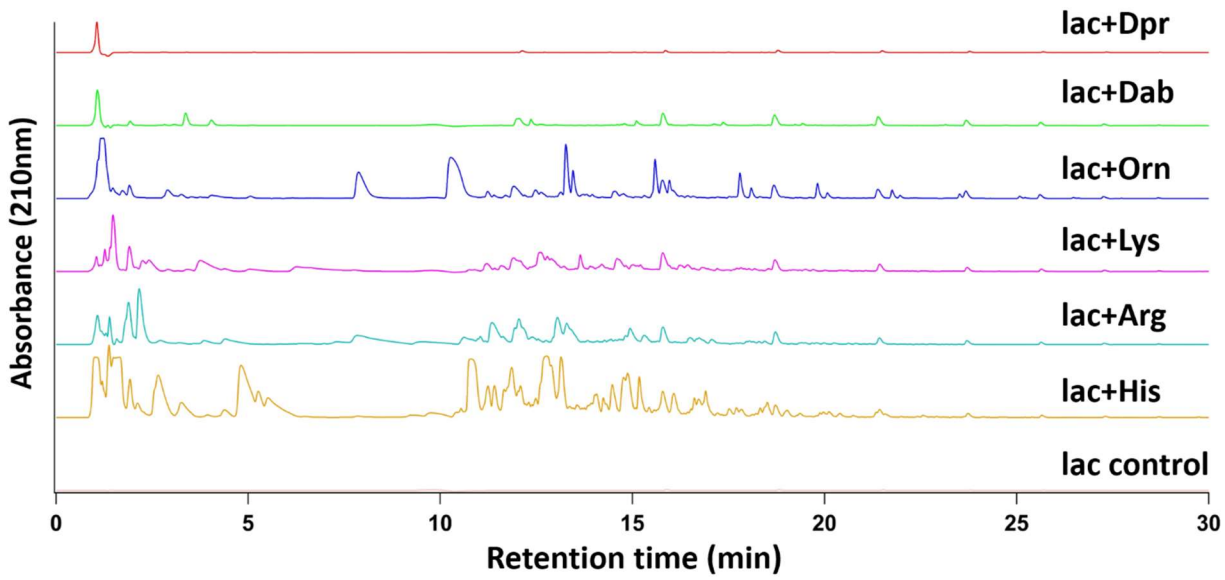


Figure S14. C18-HPLC analysis verifies the formation of depsipeptides following dry-down reactions of lac cationic amino acids. A mixture of lac and various amino acids were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by hydrophobicity-based separation using C18-HPLC. The presence of various peaks verifies the complexity and the abundance of many different products following the dry-down reactions.

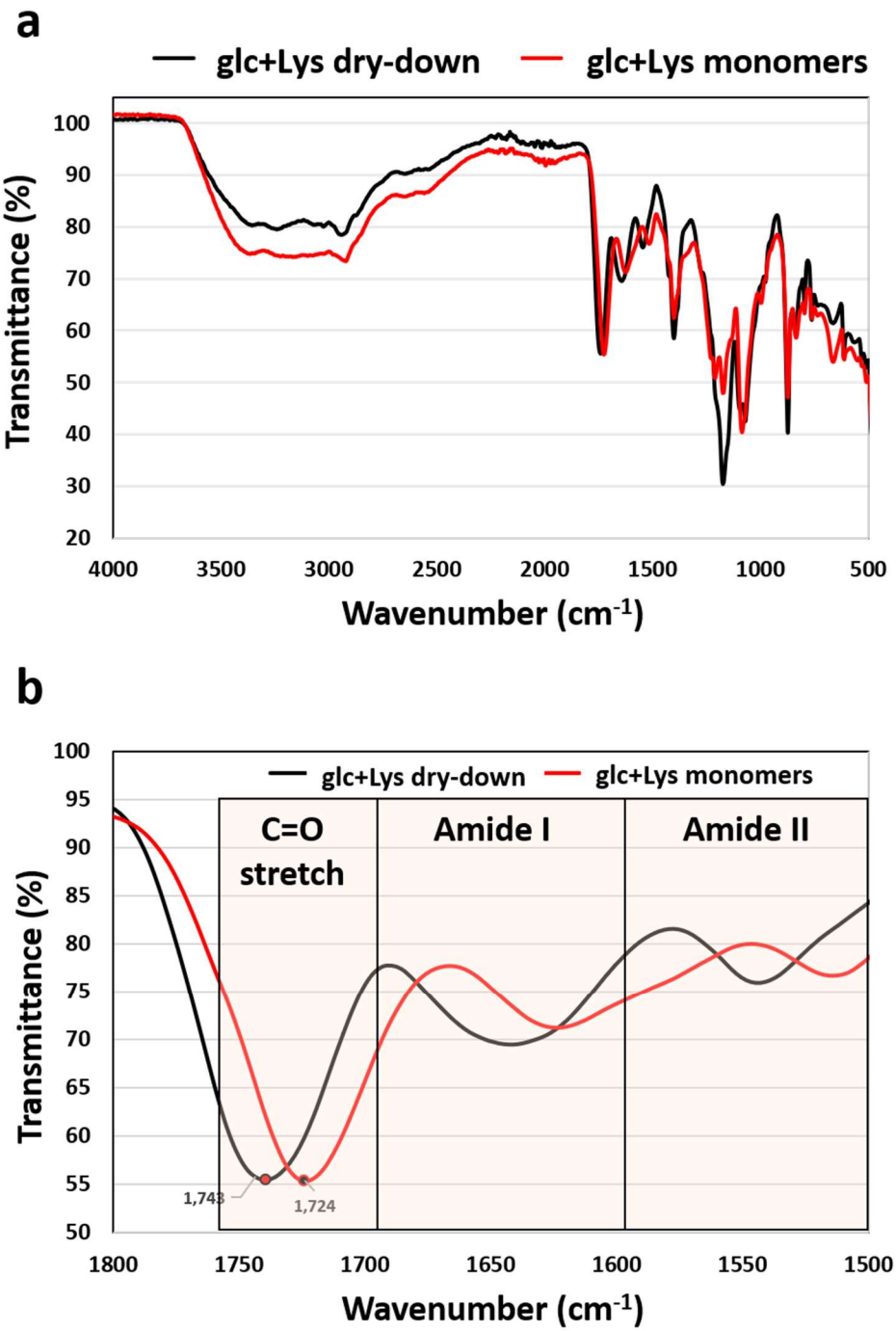


Figure S15. Fourier Transform Infrared Spectroscopy (FTIR) shows shifts in the C=O band and in the amide regions upon dry-down of glc and Lys, thus supportive of depsipeptide formation. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR (**a-b**). The C=O band shifts from a free acid (1724 cm^{-1}) to an ester (1743 cm^{-1}) upon drying.[1] Shifts are also evident in the amide regions (Amide I and Amide II) upon dry-down of the starting mixture.

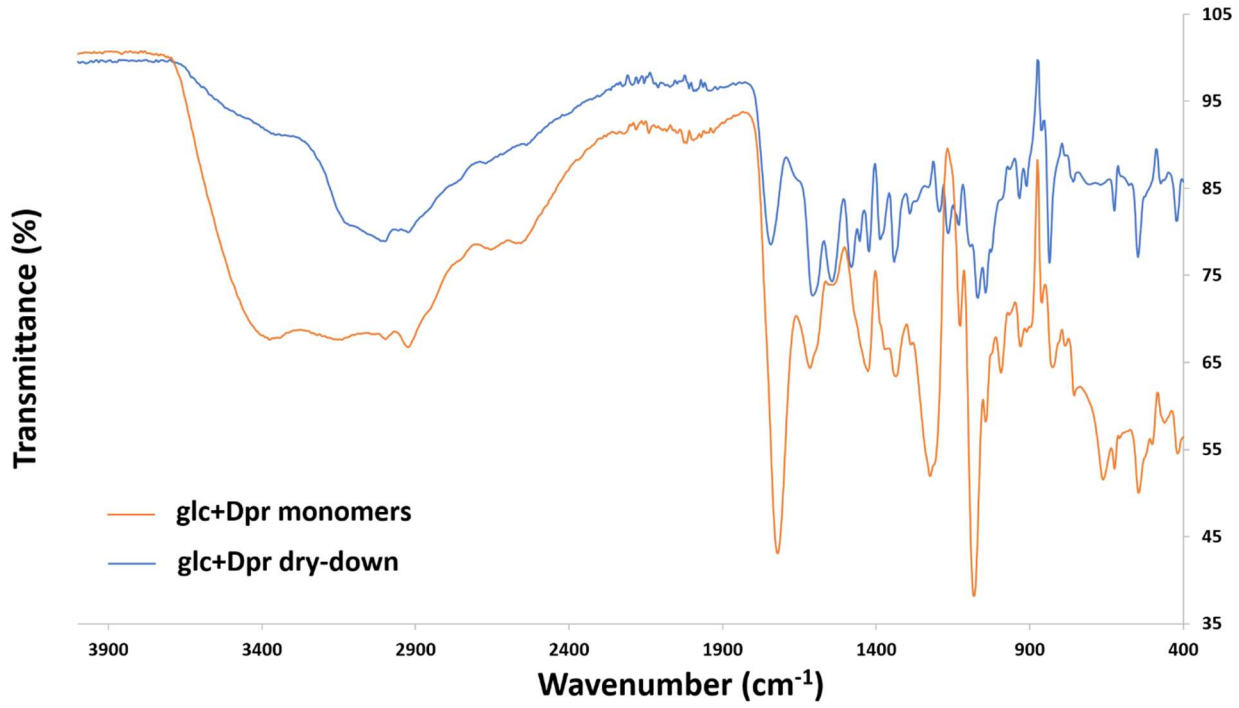


Figure S16. FTIR spectra changes upon dry-down of glc and Dpr support the formation of depsipeptides. glc and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

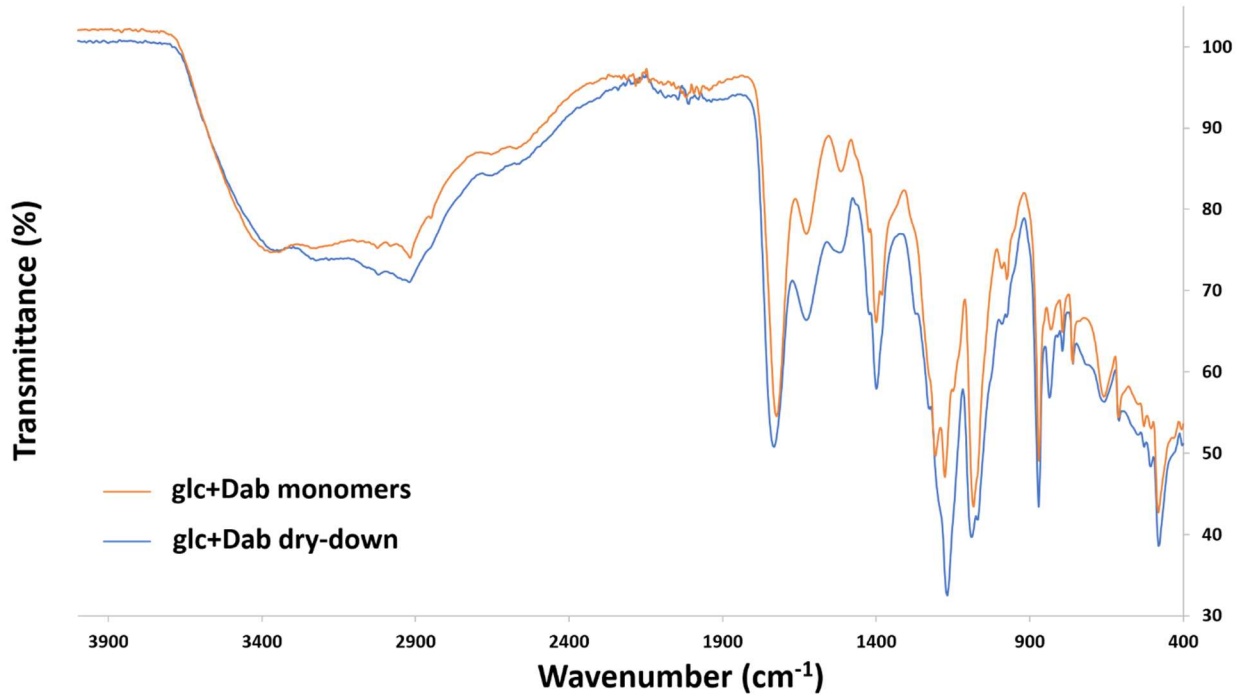


Figure S17. FTIR spectra changes upon dry-down of glc and Dab support the formation of depsipeptides. glc and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

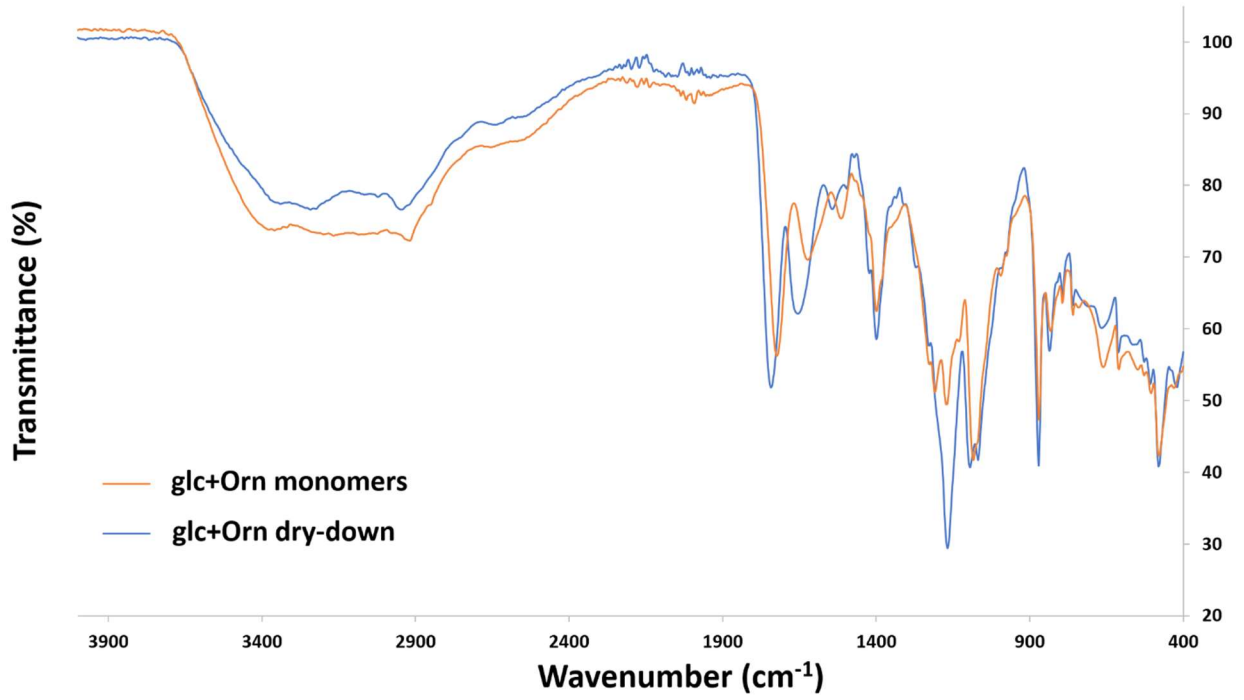


Figure S18. FTIR spectra changes upon dry-down of glc and Orn support the formation of depsipeptides. glc and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

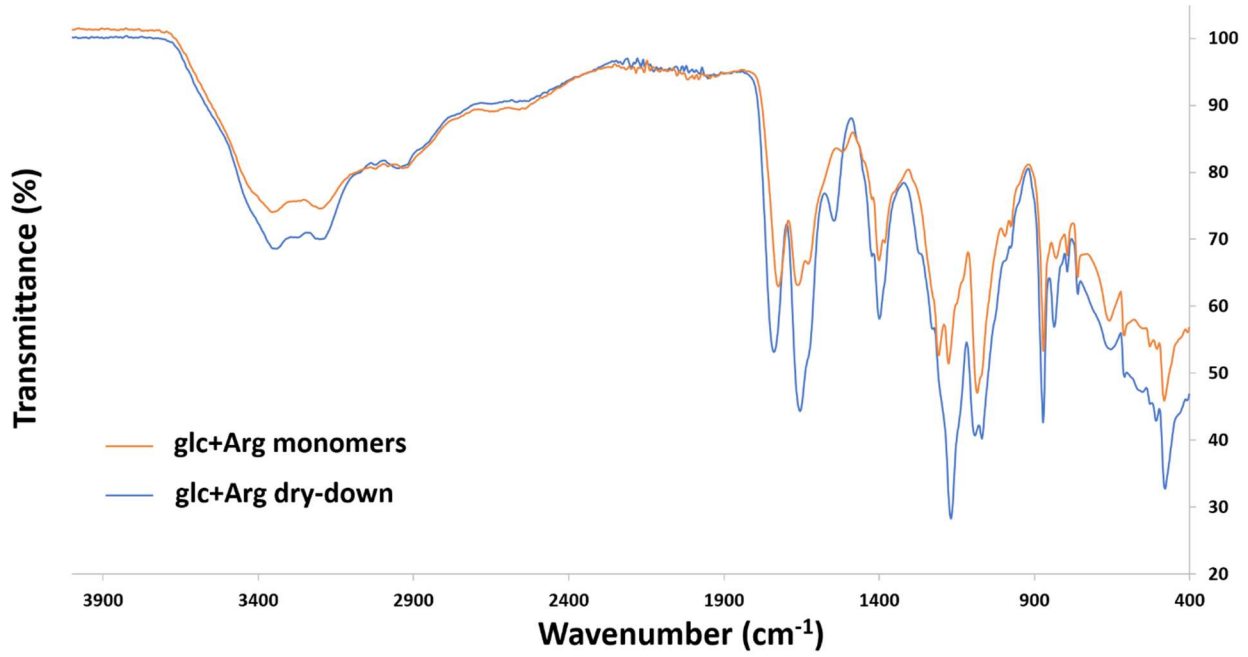


Figure S19. FTIR spectra changes upon dry-down of glc and Arg support the formation of depsipeptides. glc and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

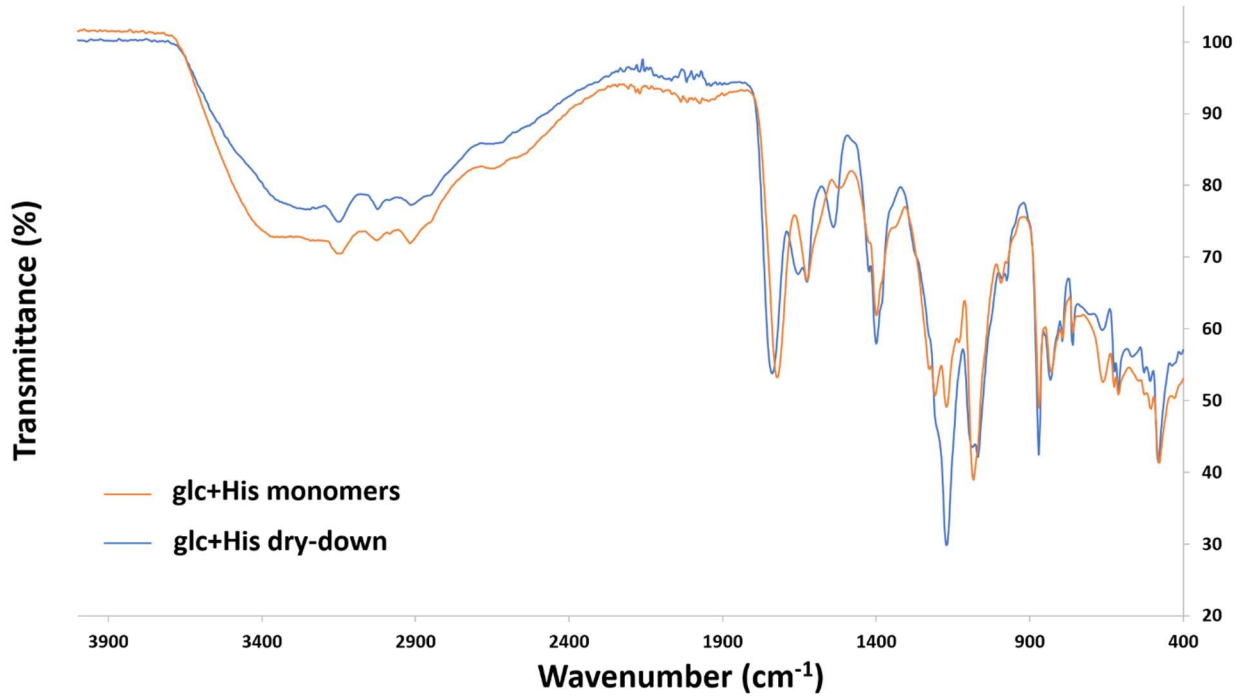


Figure S20. FTIR spectra changes upon dry-down of glc and His support the formation of depsipeptides. glc and His were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

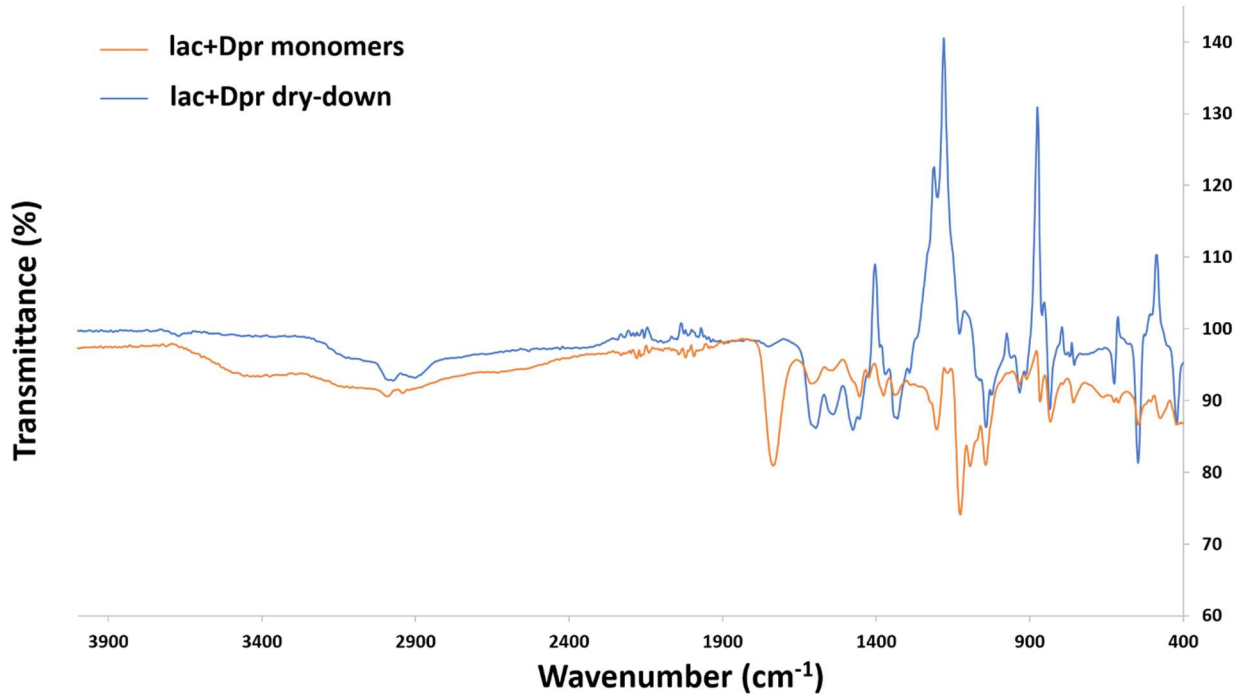


Figure S21. FTIR spectra changes upon dry-down of lac and Dpr support the formation of depsipeptides. lac and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

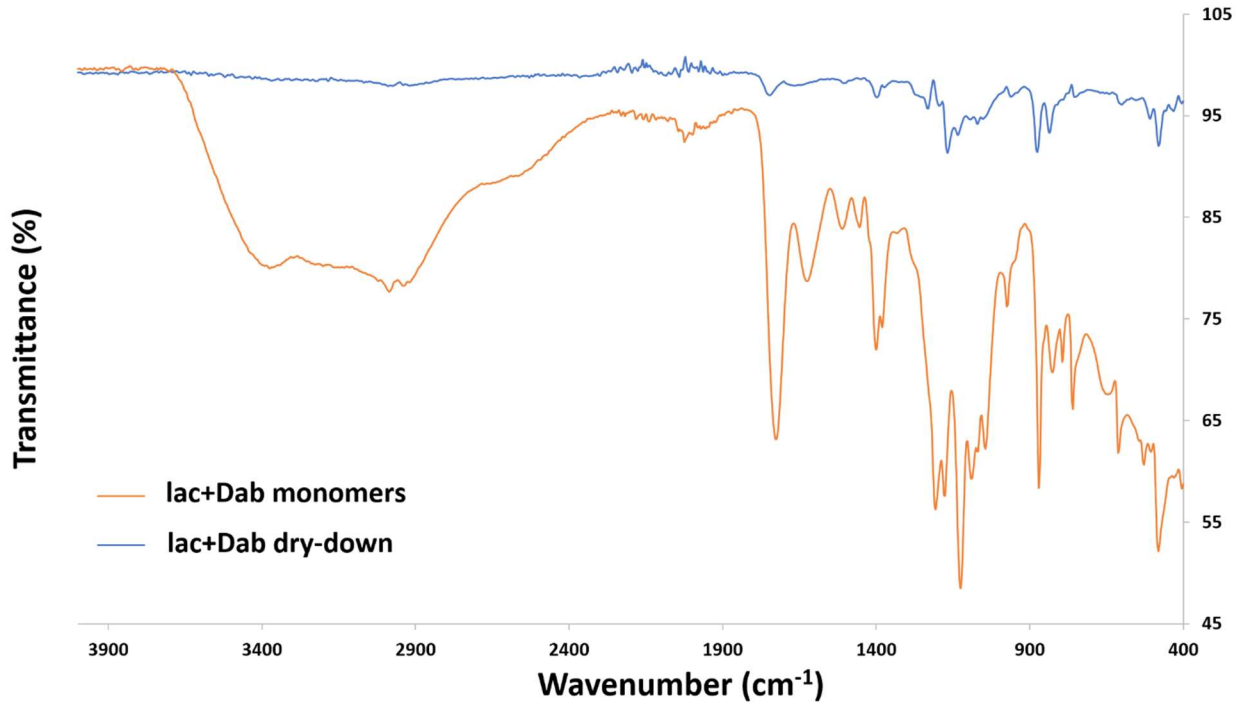


Figure S22. FTIR spectra changes upon dry-down of lac and Dab support the formation of depsipeptides. lac and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

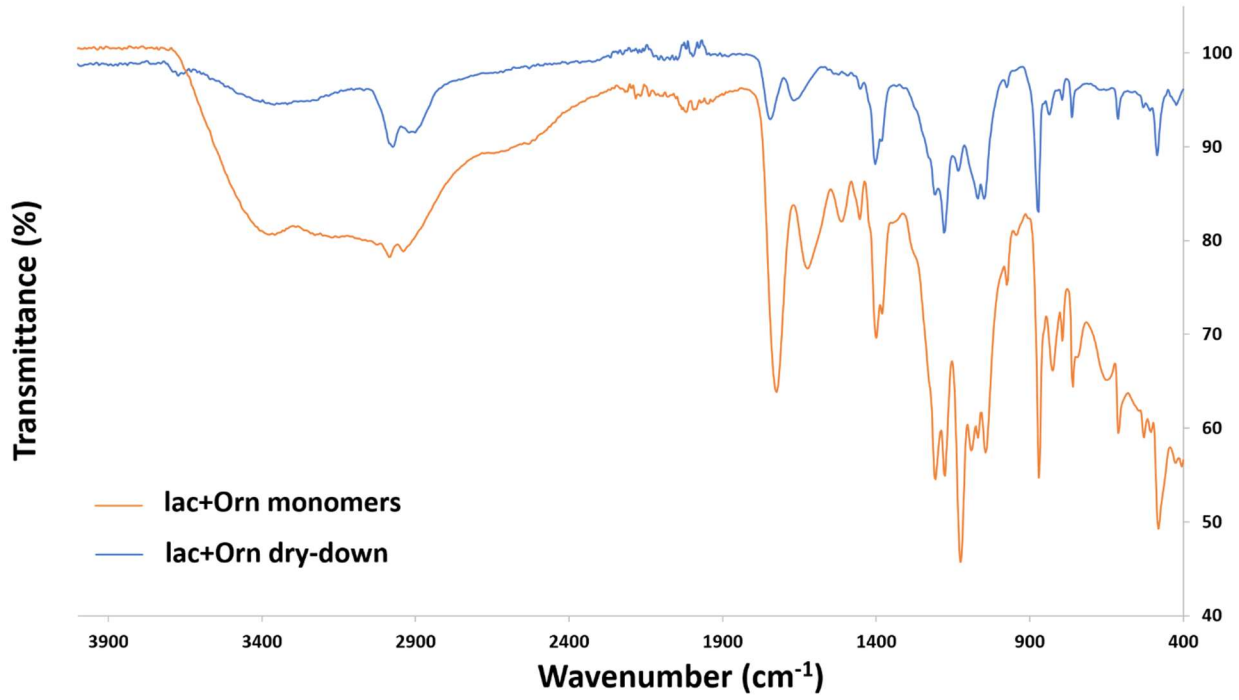


Figure S23. FTIR spectra changes upon dry-down of lac and Orn support the formation of depsipeptides. lac and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

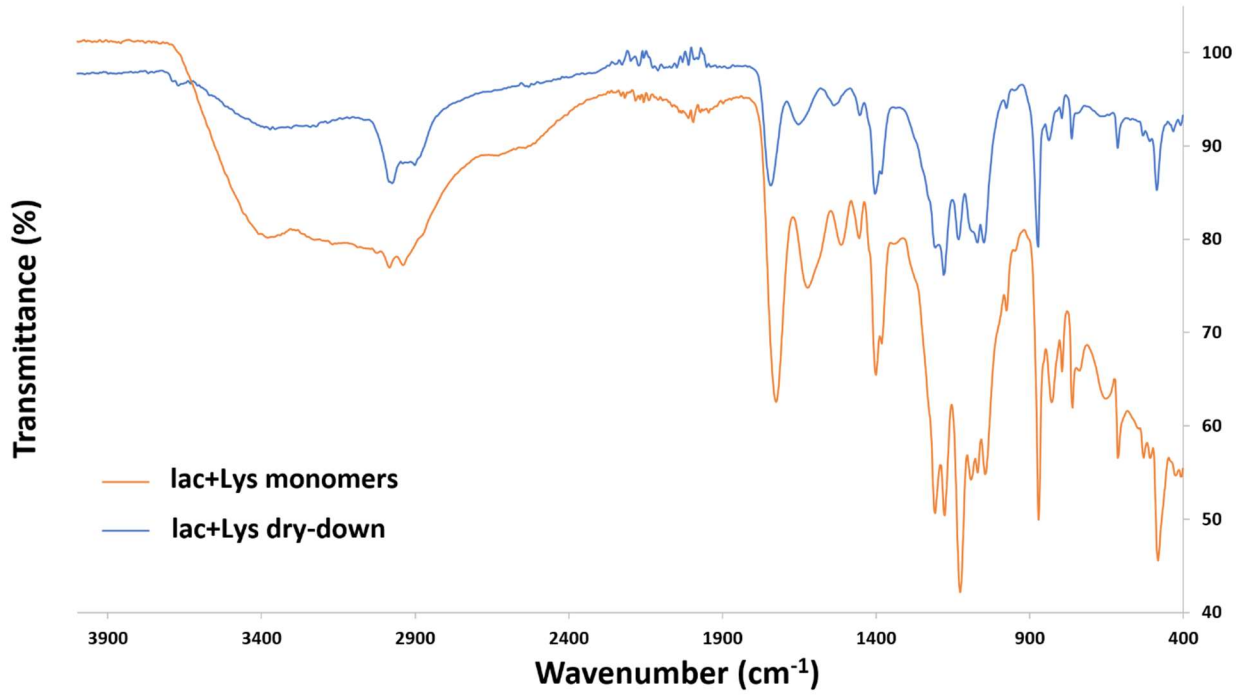


Figure S24. FTIR spectra changes upon dry-down of lac and Lys support the formation of depsipeptides. lac and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

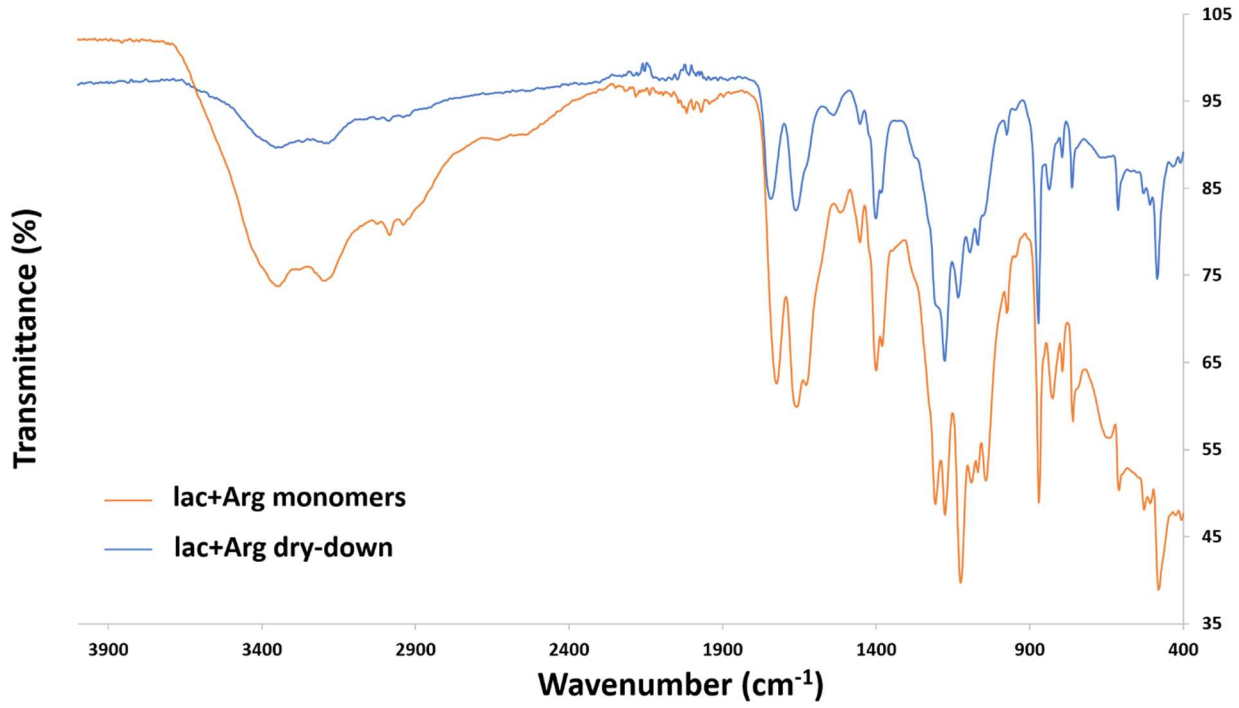


Figure S25. FTIR spectra changes upon dry-down of lac and Arg support the formation of depsipeptides. lac and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

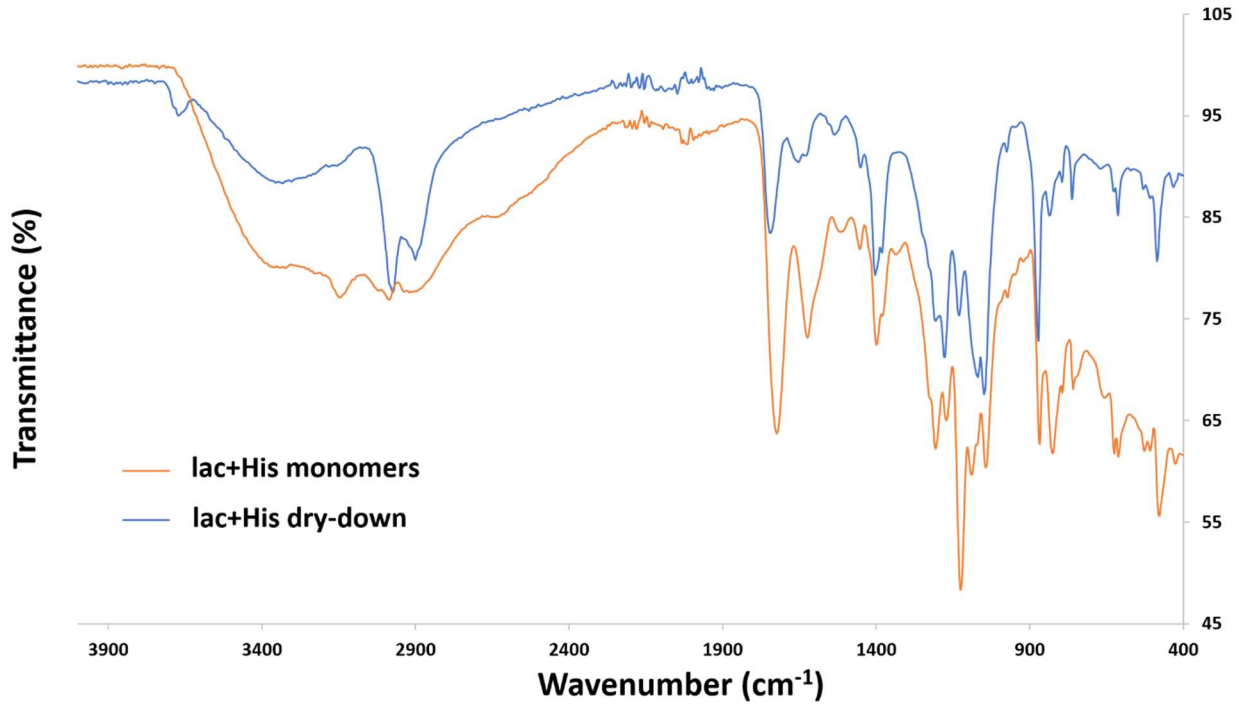


Figure S26. FTIR spectra changes upon dry-down of lac and His support the formation of depsipeptides. lac and His were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by FTIR.

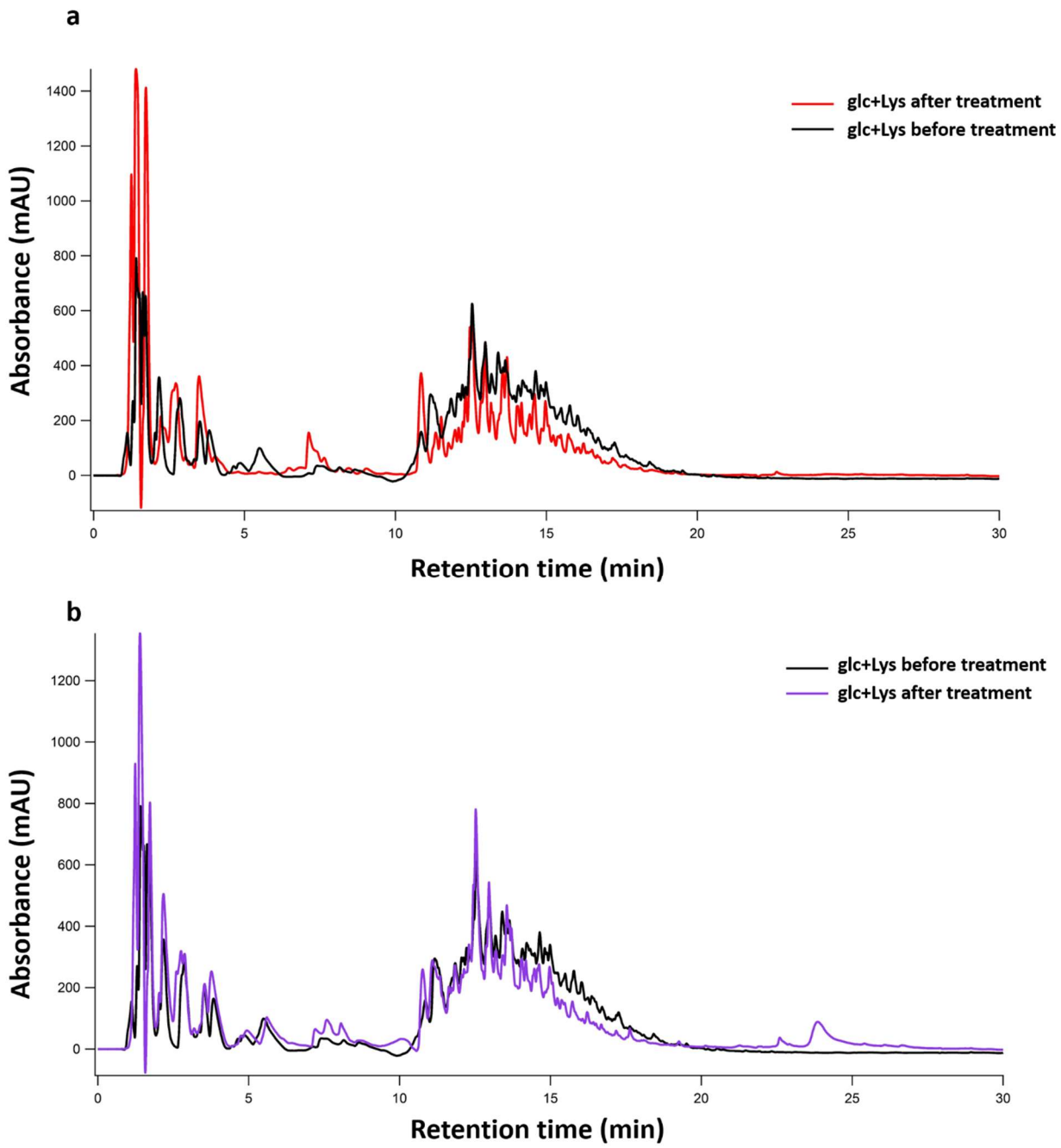


Figure S27. Degradation via hydrolytic enzymes confirms the presence of amide and ester bonds following a dry-down of a mixture of glc acid and Lys. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were subjected to incubation with the trypsin peptidase (**a**) or with an esterase from porcine liver (**b**) for 19 hr at 37˚C. Samples were analyzed by C18-HPLC column.

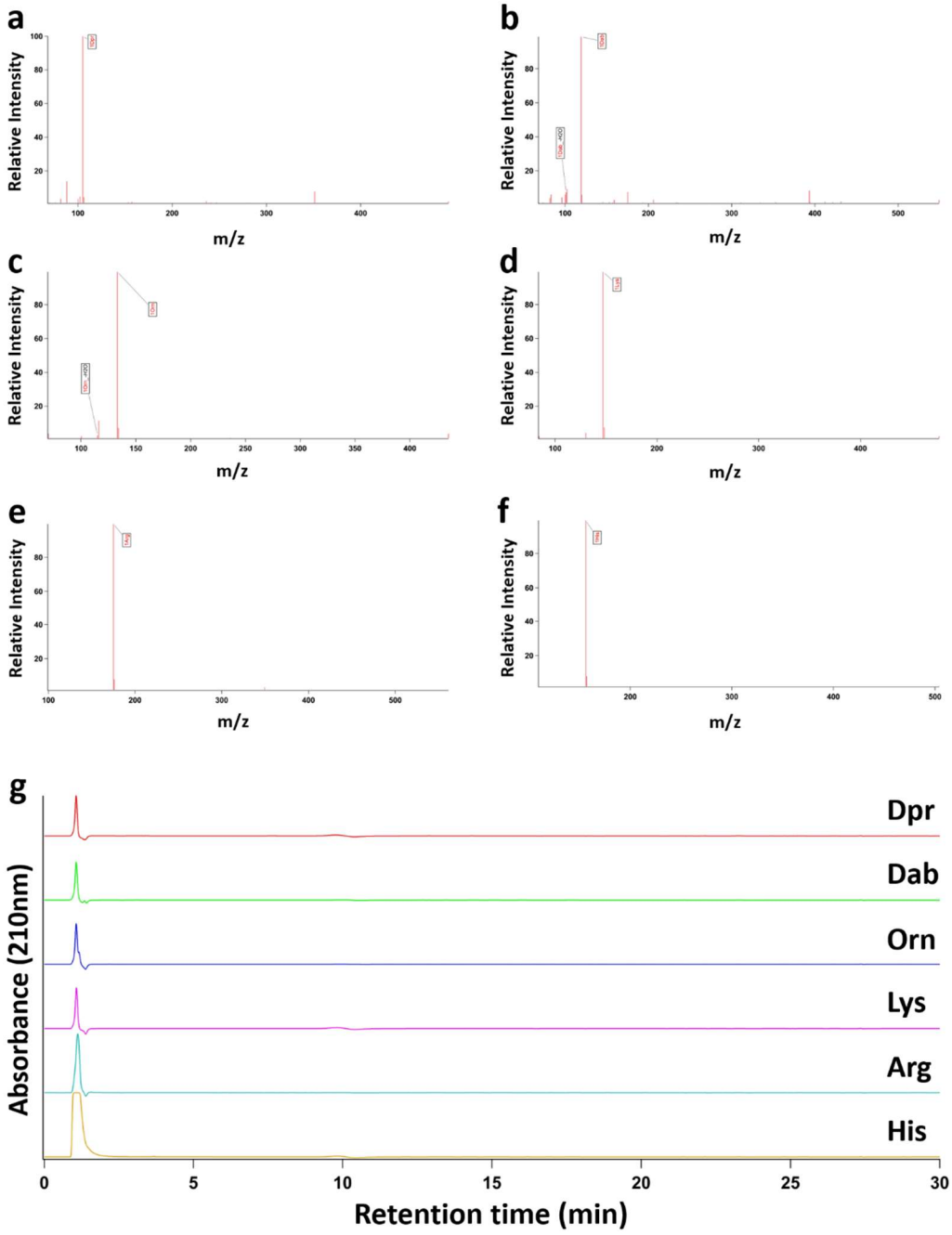


Figure S28. ESI-MS and C18-HPLC analysis of control dry-down reactions of the amino acids in the absence of hydroxy acids confirm the absence of peptides. Dpr (**a**), Dab (**b**), Orn (**c**), Lys (**d**), Arg (**e**) or His (**f**) were dried at 85˚C for seven days and analyzed by positive-mode ESI-MS. Labeled species correspond to $[M+H]^+$ ions. (**g**) C18-HPLC analysis verifies that no peptides formed upon dry-down reactions of the amino acids in the absence of hydroxy acids, and only the amino acid monomer peaks are present.

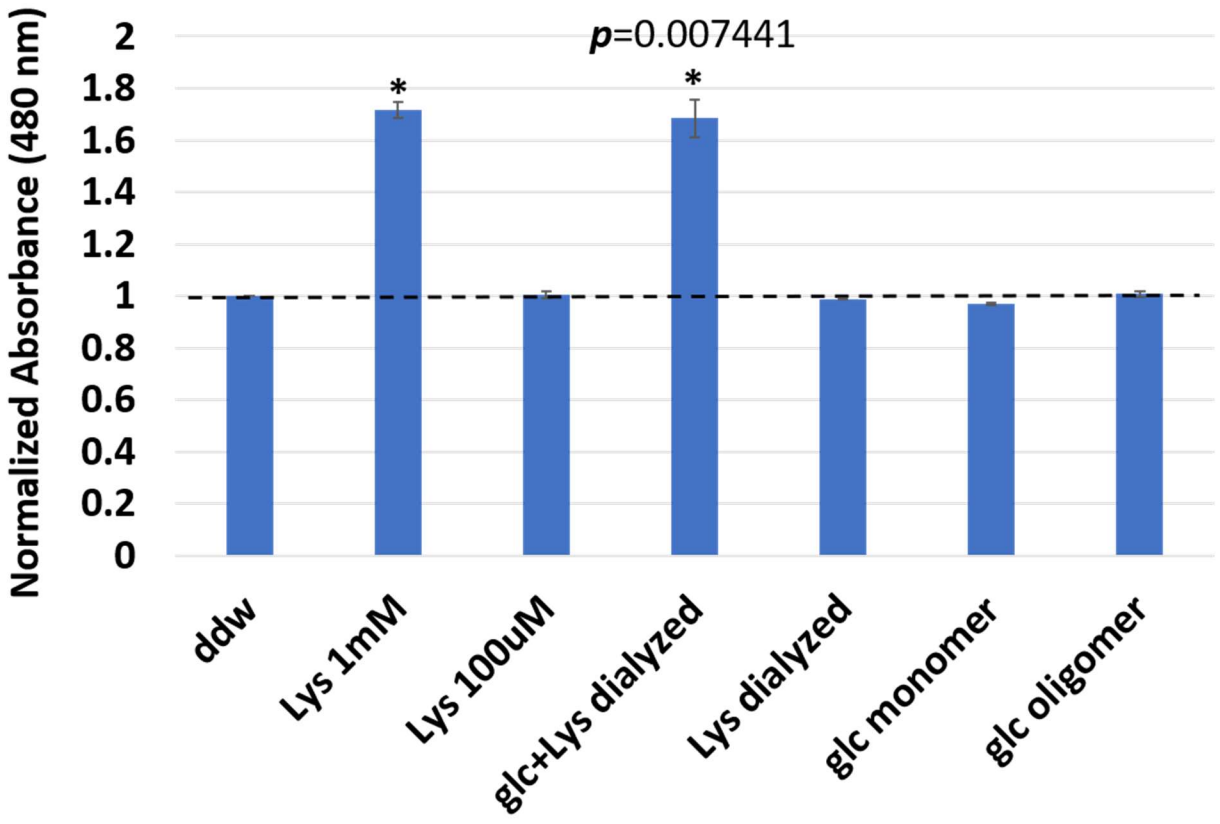


Figure S29. Ninhydrin assay verifies the cationic properties of depsipeptides in dry-down reactions of glc acid and Lys. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were dialyzed using a cutoff of 0.5-1.0 kDa. As a negative control, similar protocol was followed for a solution of Lys monomer. For ninhydrin assay, dialyzed samples as well as negative controls (water, fresh or dried glc) and positive controls (Lys dilutions) were tested in triplicates. Free amines were detected measuring the absorbance at 480 nm. Standard errors are indicated, and the p-value was calculated using a student t-test.

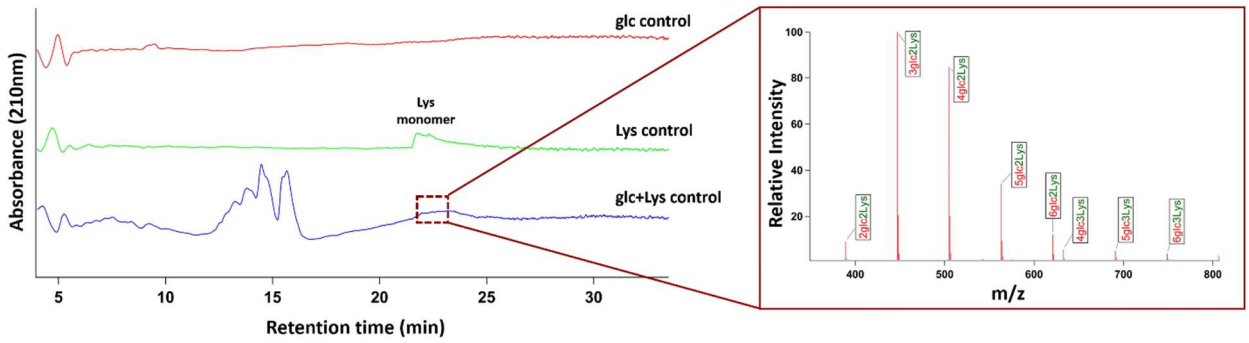


Figure S30. Hydrophilic interaction chromatography (HILIC) LC–MS verifies the cationic properties of depsipeptides in dry-down reactions involving glc and Lys. A mixture of glc and Lys and their control reactions (glc or Lys) were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by hydrophilic interaction chromatography (HILIC) using LCMS. As indicated by the retained depsipeptides peaks and the negative-mode ESI-MS, dry-down of glc with Lys results in a variety of hydrophilic depsipeptides.

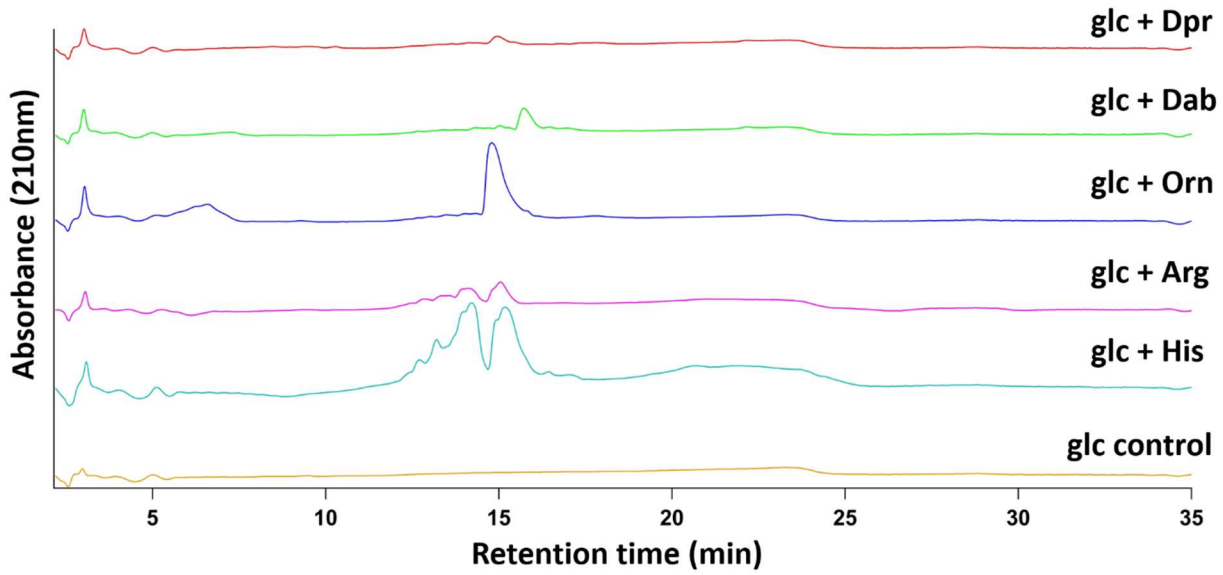


Figure S31. Hydrophilic interaction chromatography (HILIC) LC–MS verifies the cationic properties of depsipeptides in dry-down reactions involving glc and cationic amino acids. A mixture of glc and various amino acids were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by hydrophilic interaction chromatography (HILIC) using LCMS. The depsipeptides that retained on the column exhibit hydrophilic properties.

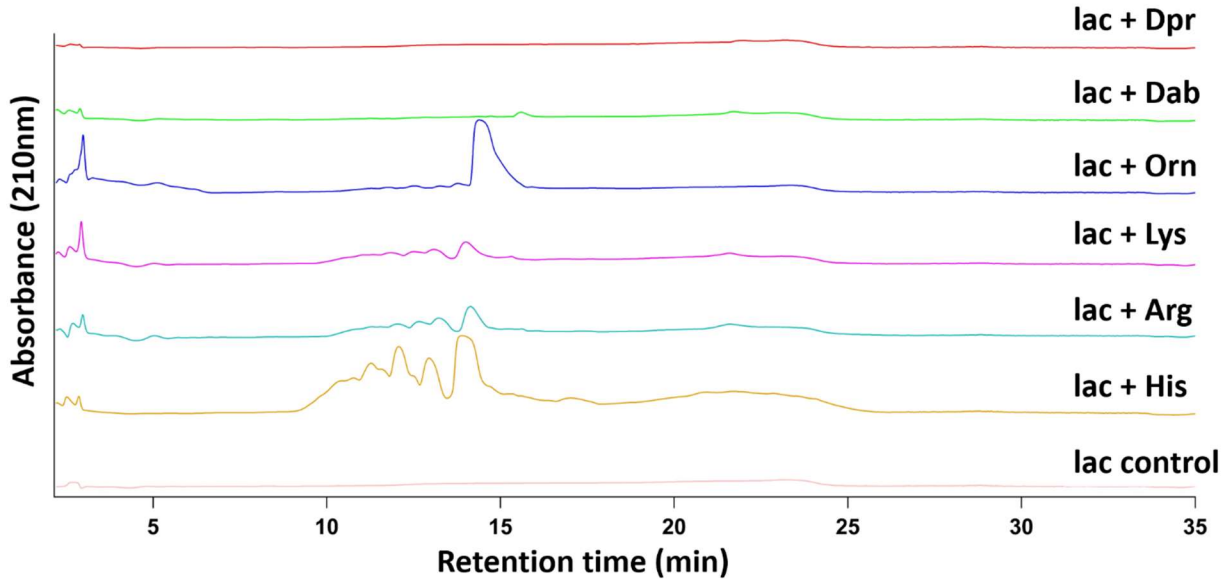


Figure S32. Hydrophilic interaction chromatography (HILIC) LC–MS verifies the cationic properties of depsipeptides in dry-down reactions involving lac and cationic amino acids. A mixture of lac and various amino acids were dried at 85 ˚C for seven days and the resulting depsipeptides were analyzed by hydrophilic interaction chromatography (HILIC) using LCMS. The depsipeptides that retained on the column exhibit hydrophilic properties.

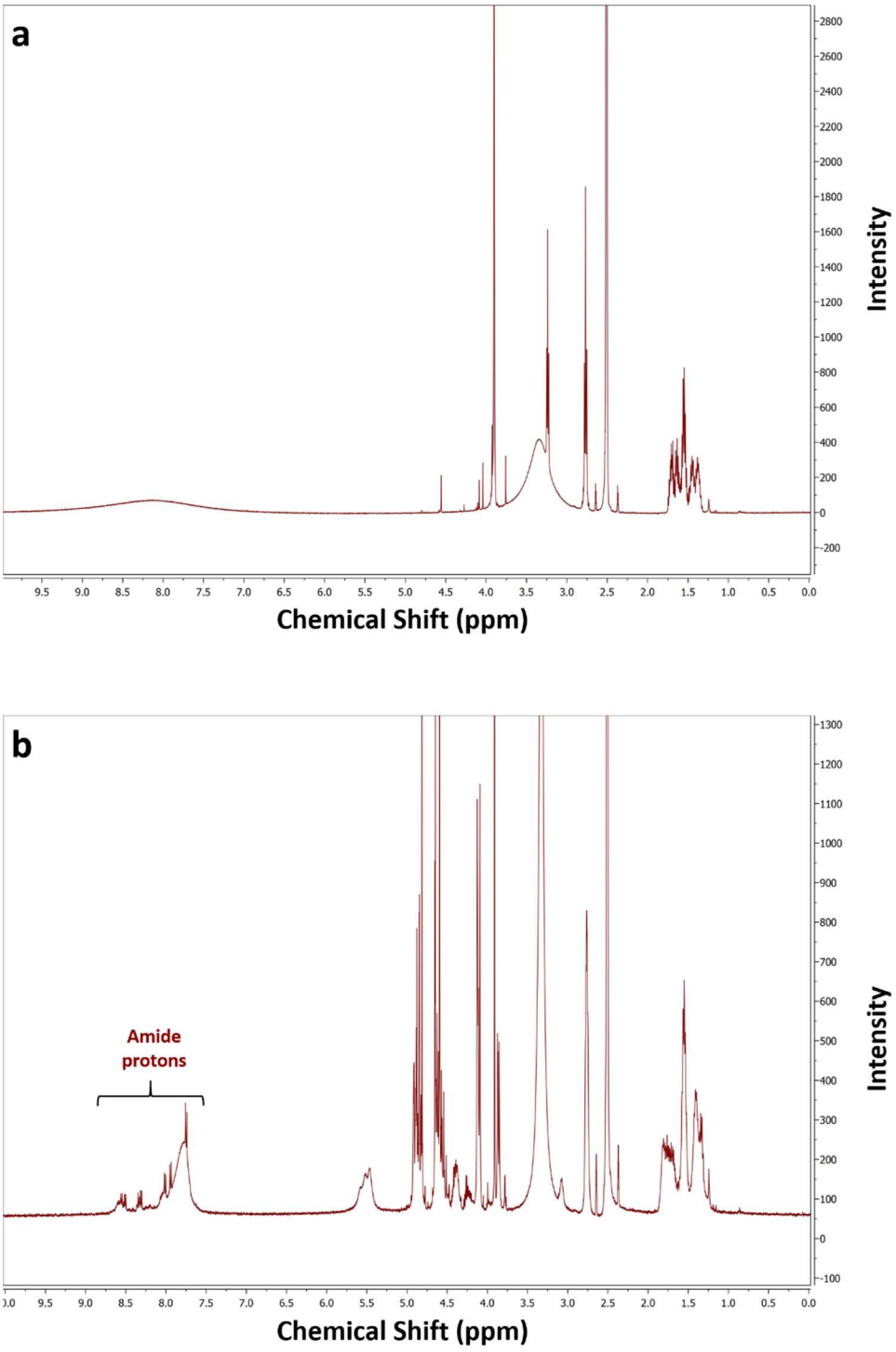


Figure S33. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Lys. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

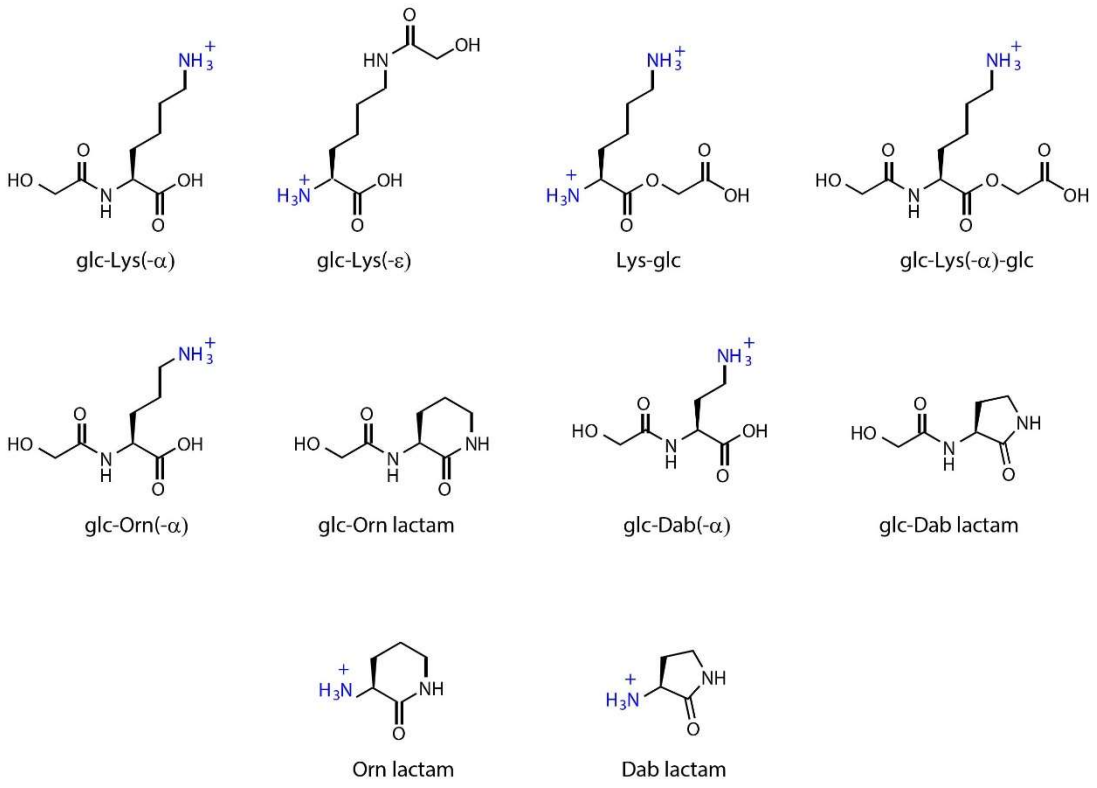


Figure S34. Chemically synthesized standards that were used for assignments of ^{1}H-NMR resonances in this study.

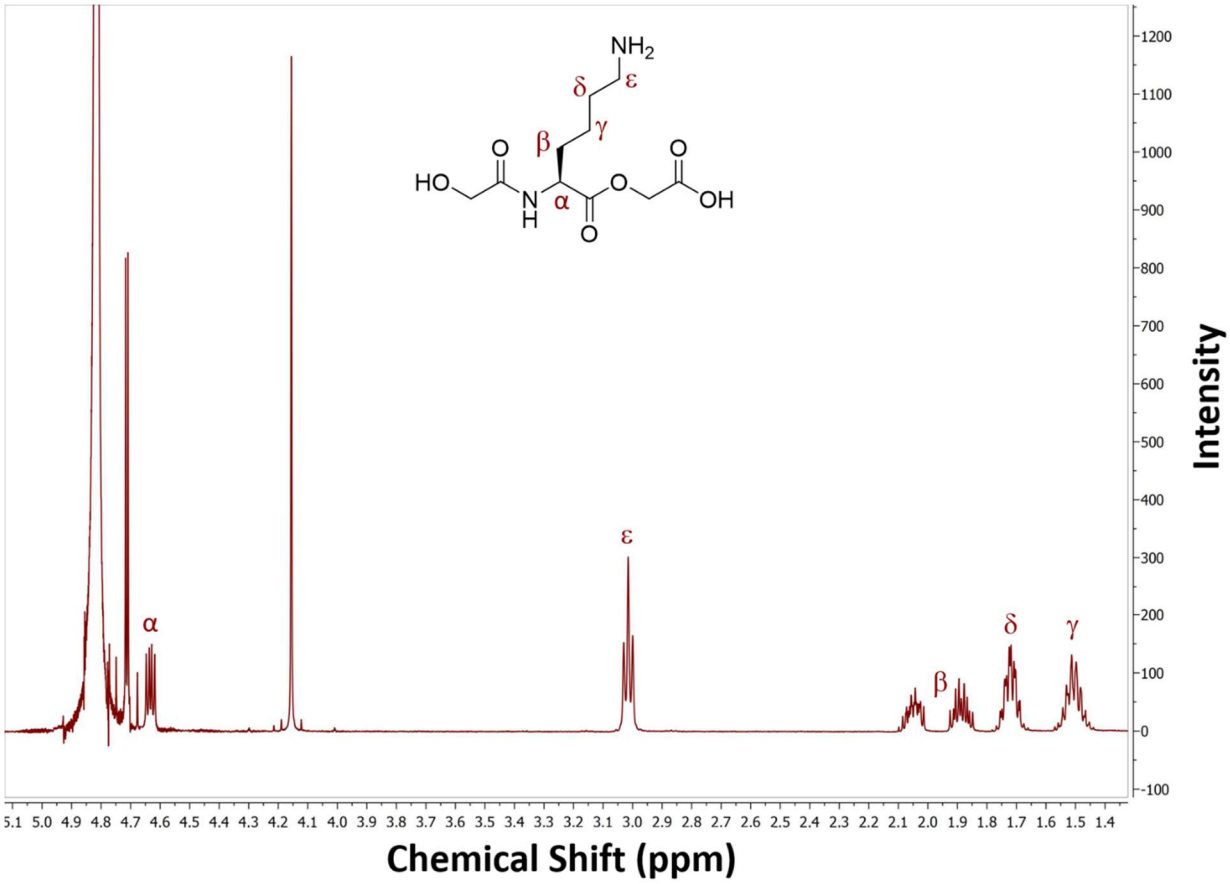


Figure S35. ^{1}H NMR of the glc-Lys(-α)-glc standard in D2O. ^{1}H NMR spectra of a depsipeptide standard, in which Lys is both α-amidated with glc and esterified with another glc.

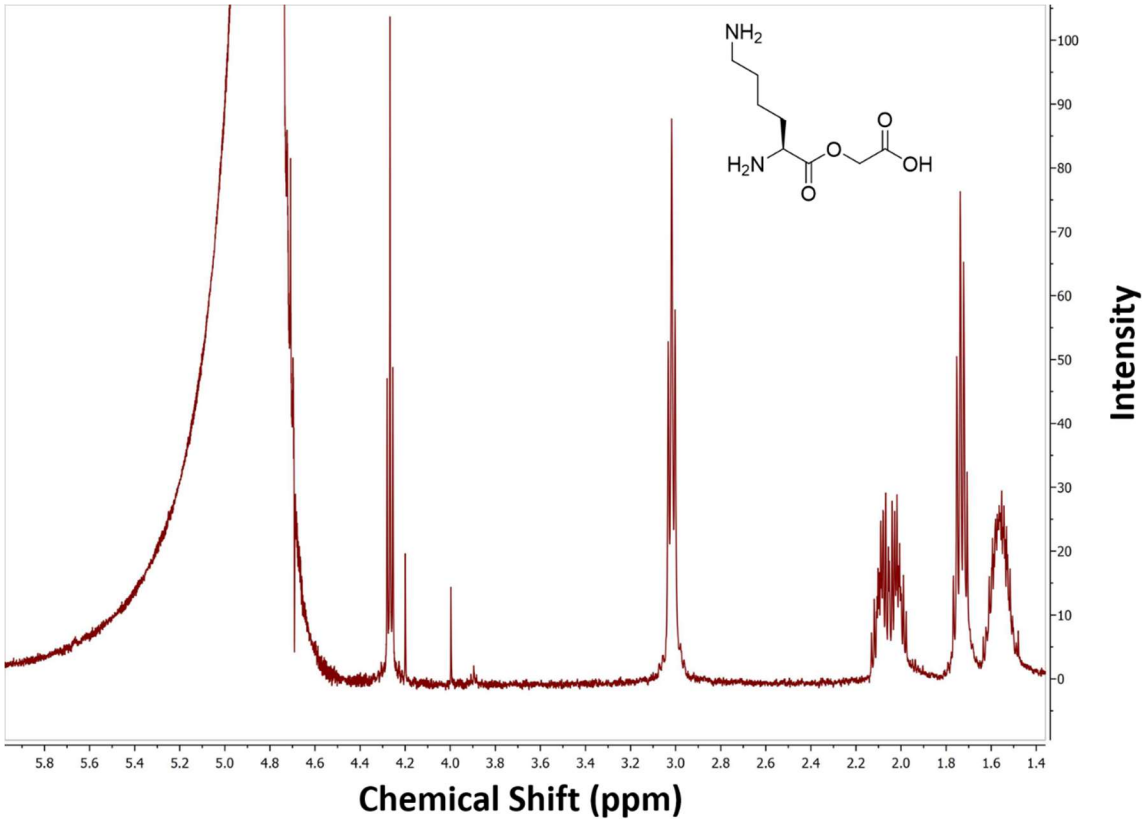


Figure S36. ^{1}H NMR of the esterified Lys standard Lys-glc in D2O. ^{1}H NMR spectra of a depsipeptide standard, in which Lys is esterified with glc. This product is not seen in the ^{1}H NMR spectrum upon dry-down of glc and Lys.

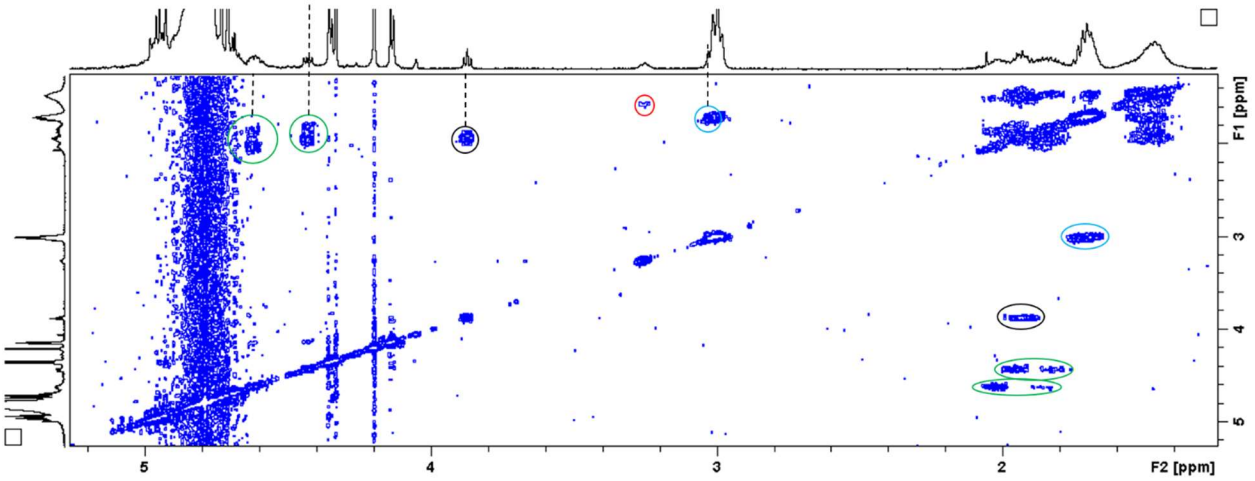


Figure S37. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and Lys. glc and Lys were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. The α-proton correlates to the β protons and in free Lys has a chemical shift of 3.86 ppm. The chemical shift of the α-proton shifts down-field upon α-amidation with glc to 4.43 ppm or to ~4.62 upon both α-amidation and esterification on its carboxylic side. The two ε protons correlate with the up-field δ protons and have a chemical shift of 3.01 ppm when not amidated (free). The ε protons shift down-field to 3.27 ppm upon ε-amidation. Cross peaks of interest are circled: black - correlation between the α-proton and β protons of Lys that is not α-amidated; green - correlation between α-amidated protons and their corresponding β protons; turquoise - correlation between ε-protons and δ protons of Lys that are not ε -amidated; red - correlation between ε-amidated protons and their corresponding δ protons.

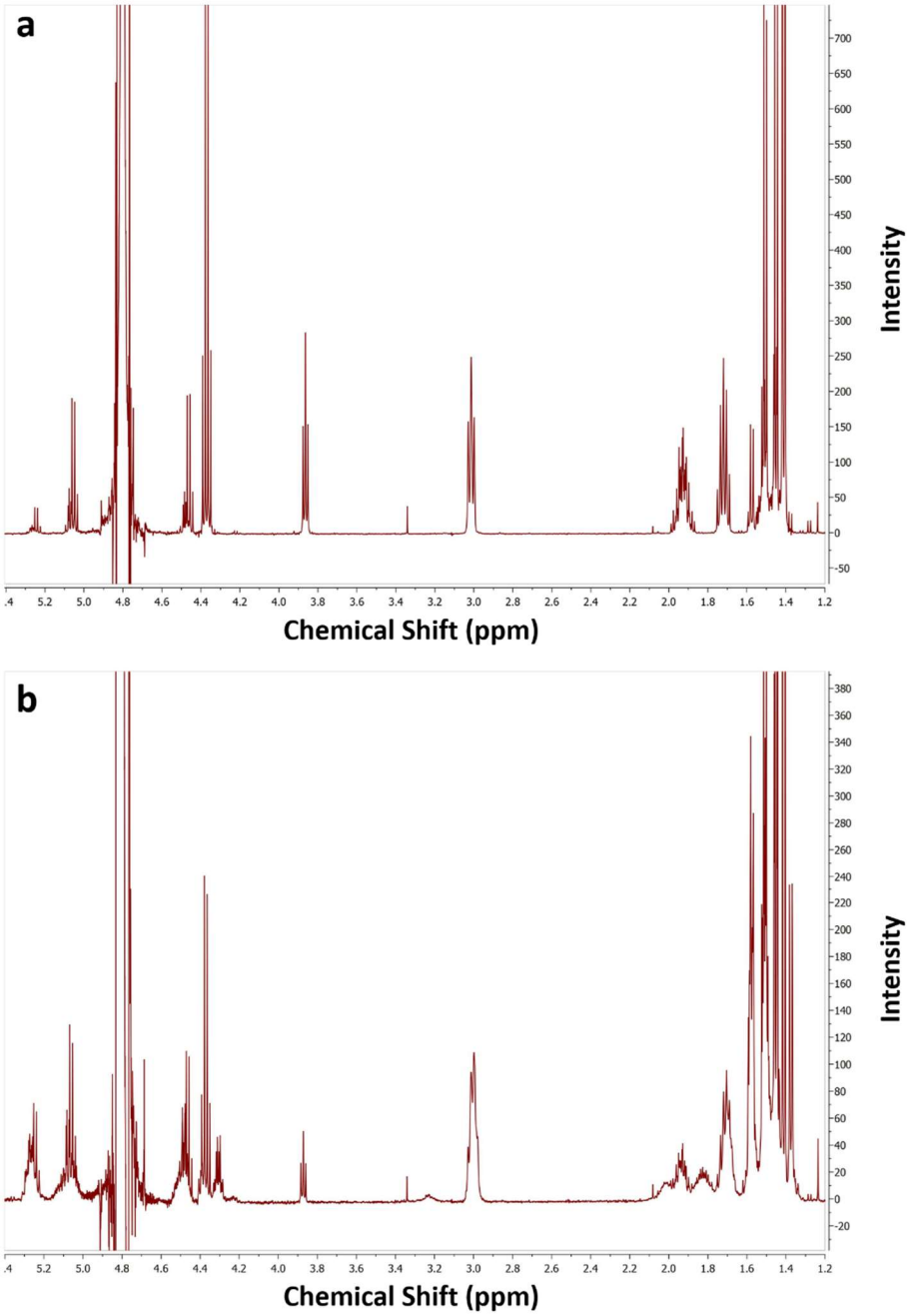


Figure S38. ^{1}H NMR spectrum supports the formation of cationic depsipeptides upon dry-down reactions of lac and Lys. ^{1}H NMR spectrum of a mixture of lac and Lys in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

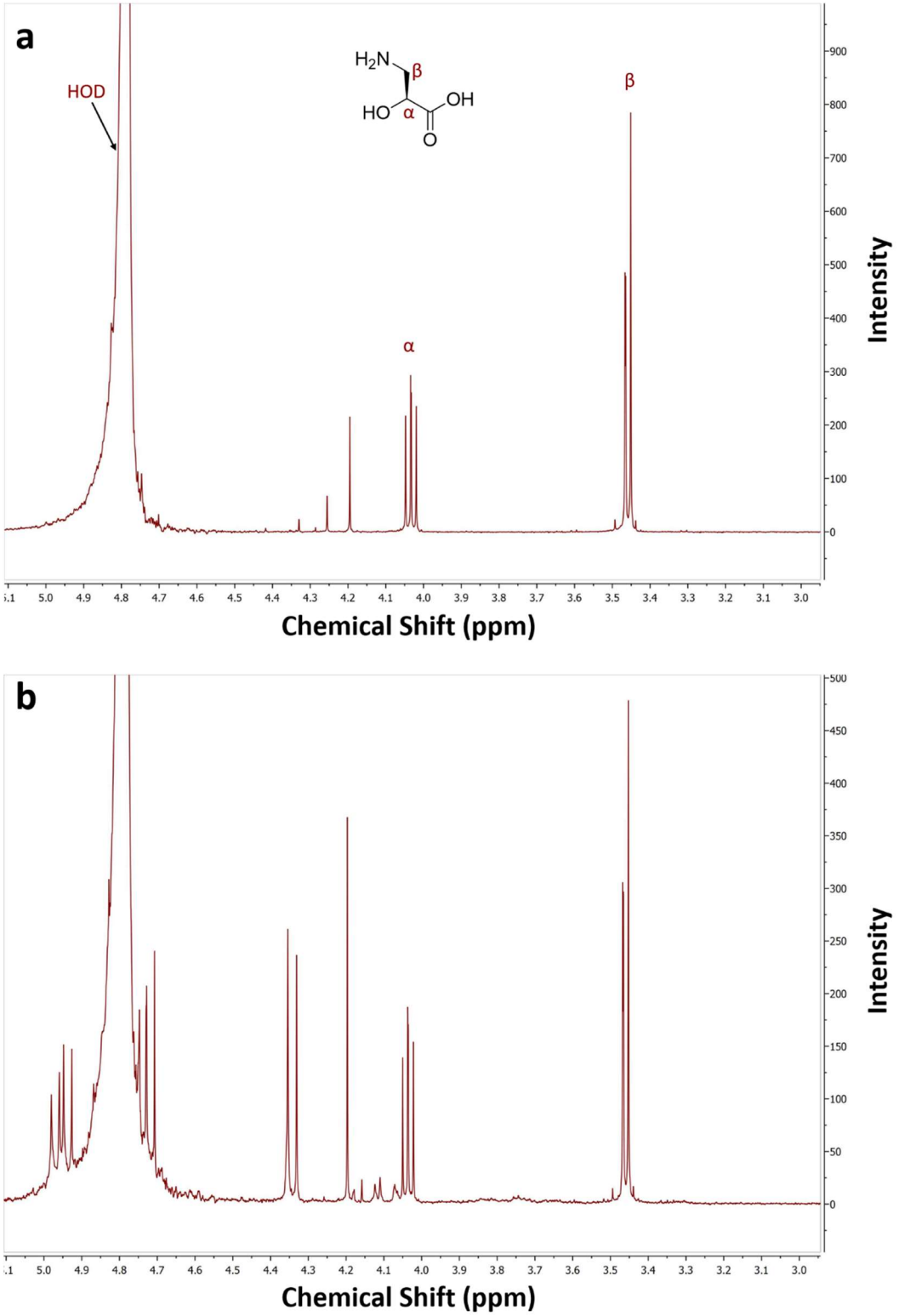


Figure S39. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Dpr. ^{1}H NMR spectrum of a mixture of glc and Dpr in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

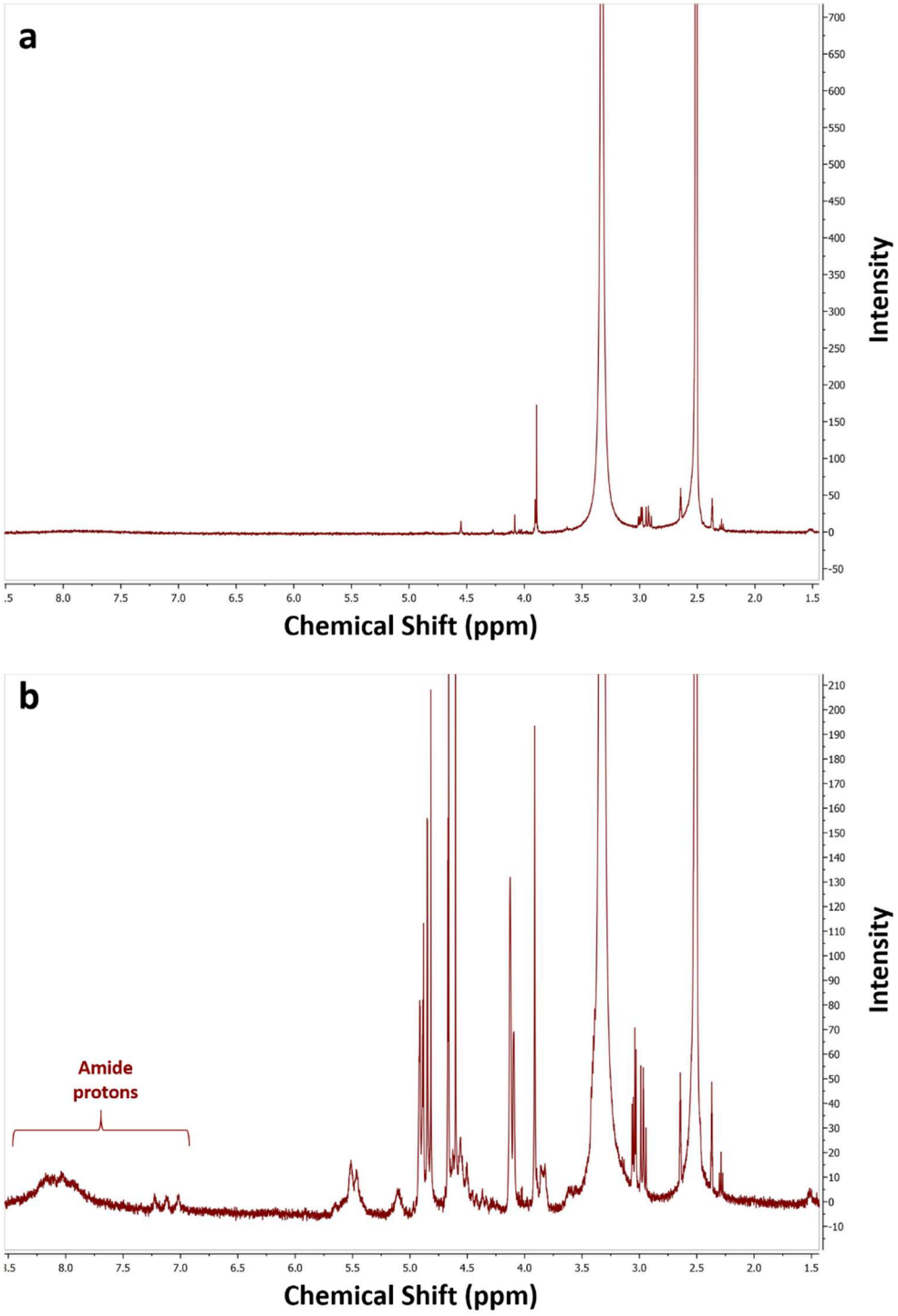


Figure S40. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Dpr. glc and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

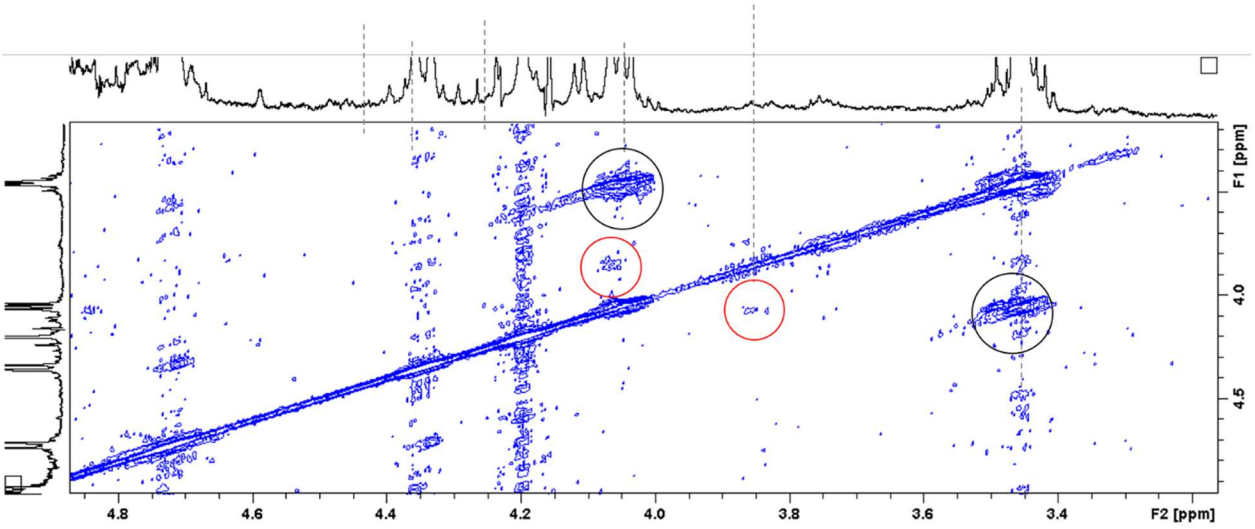


Figure S41. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and Dpr. glc and Dpr were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. Cross peaks of interest are circled: black - correlation between the α-proton and β protons in free Dpr; red- correlation between presumably β-amidated protons and their corresponding α protons.

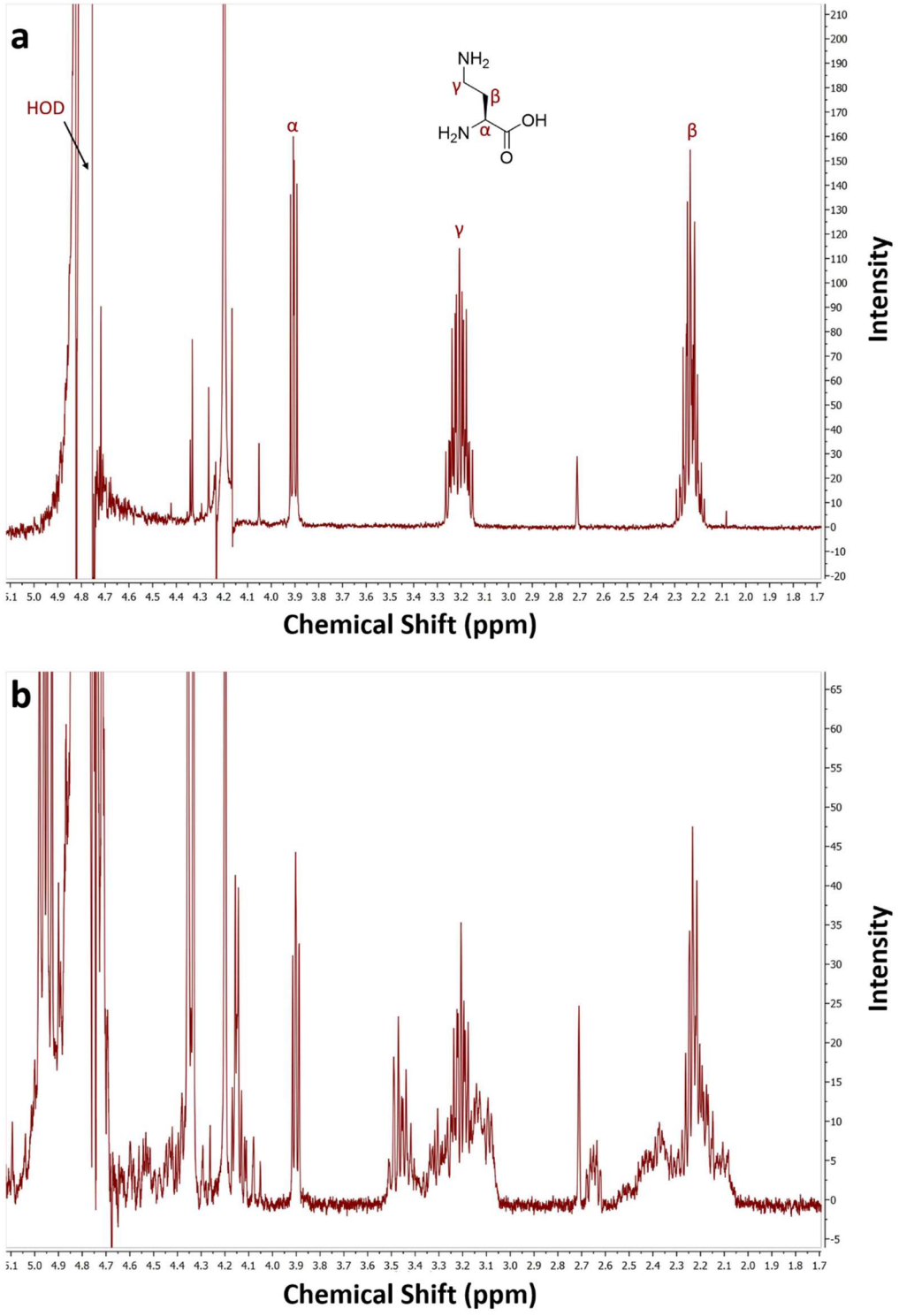


Figure S42. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Dab. ^{1}H NMR spectrum of a mixture of glc and Dab in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

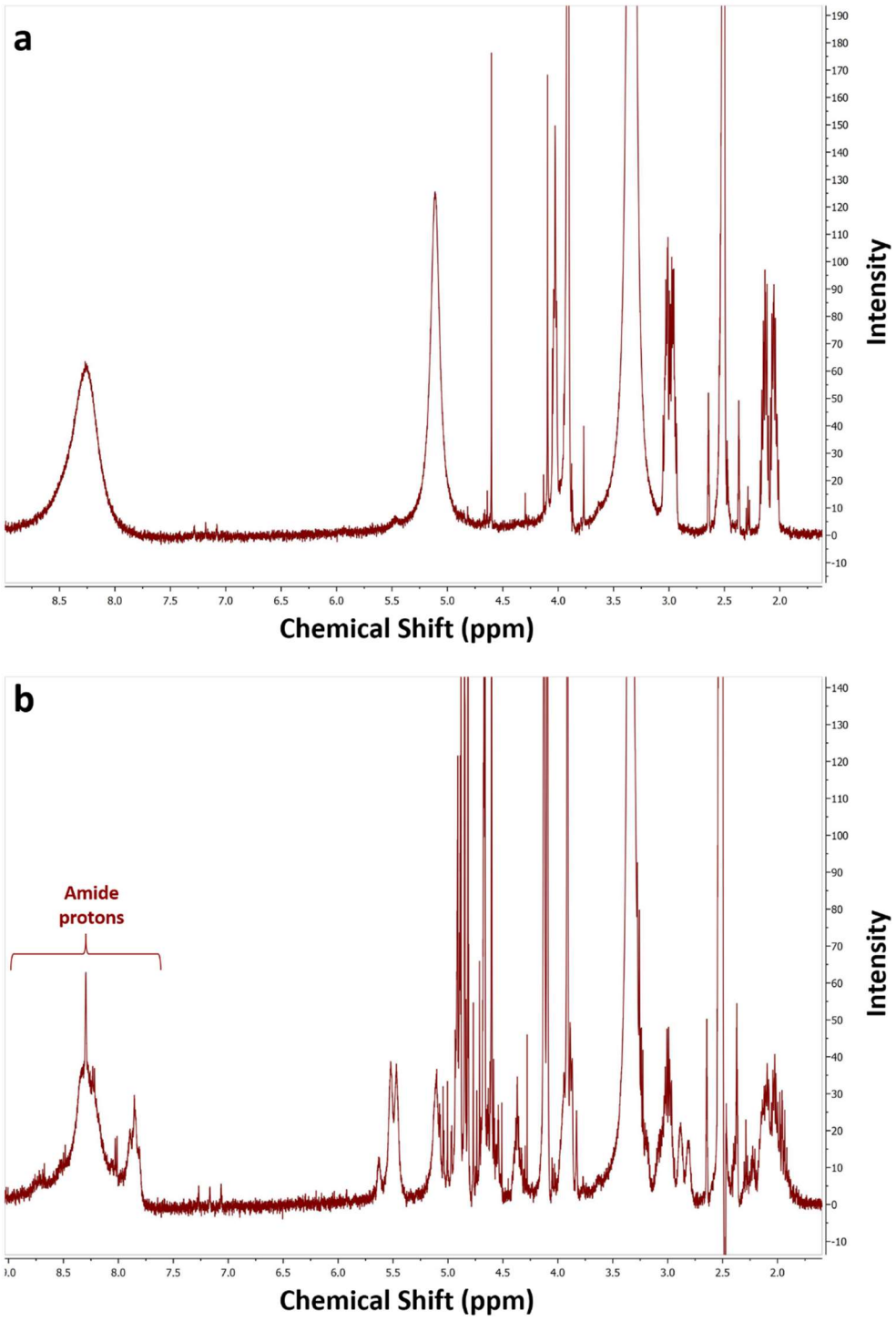


Figure S43. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Dab. glc and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

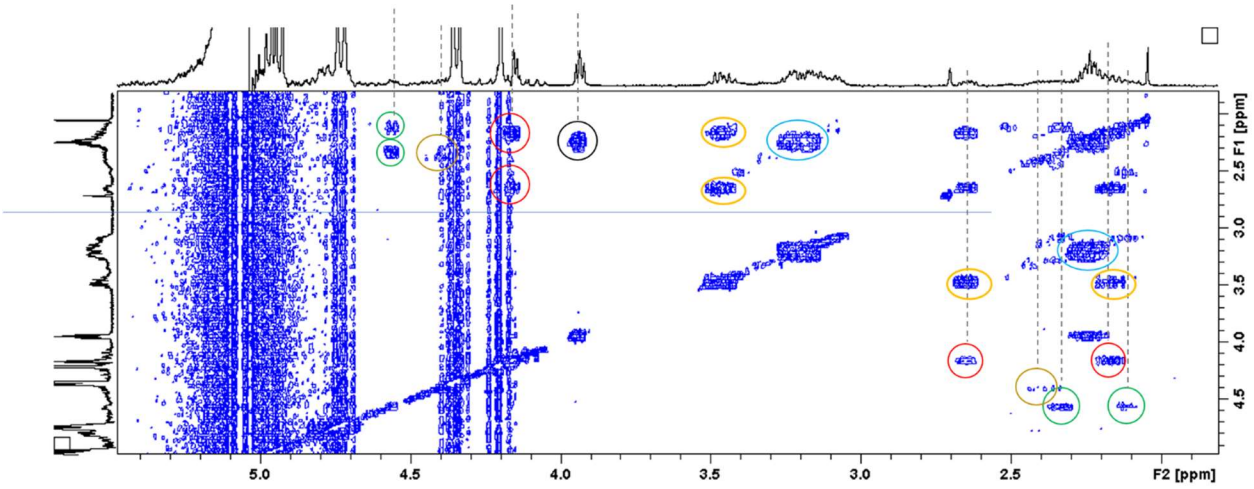


Figure S44. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and Dab. glc and Dab were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. Cross peaks of interest are circled: black - correlation between the α-proton and β protons of Dab that is not α-amidated; green/brown - correlations between α-amidated protons and their corresponding β protons; turquoise - correlation between γ-protons and β protons of Dab that are not γ-amidated; red - correlation between the α-protons of Dab lactam monomer and their corresponding β protons; yellow - correlation between γ-amidated protons and their corresponding β protons.

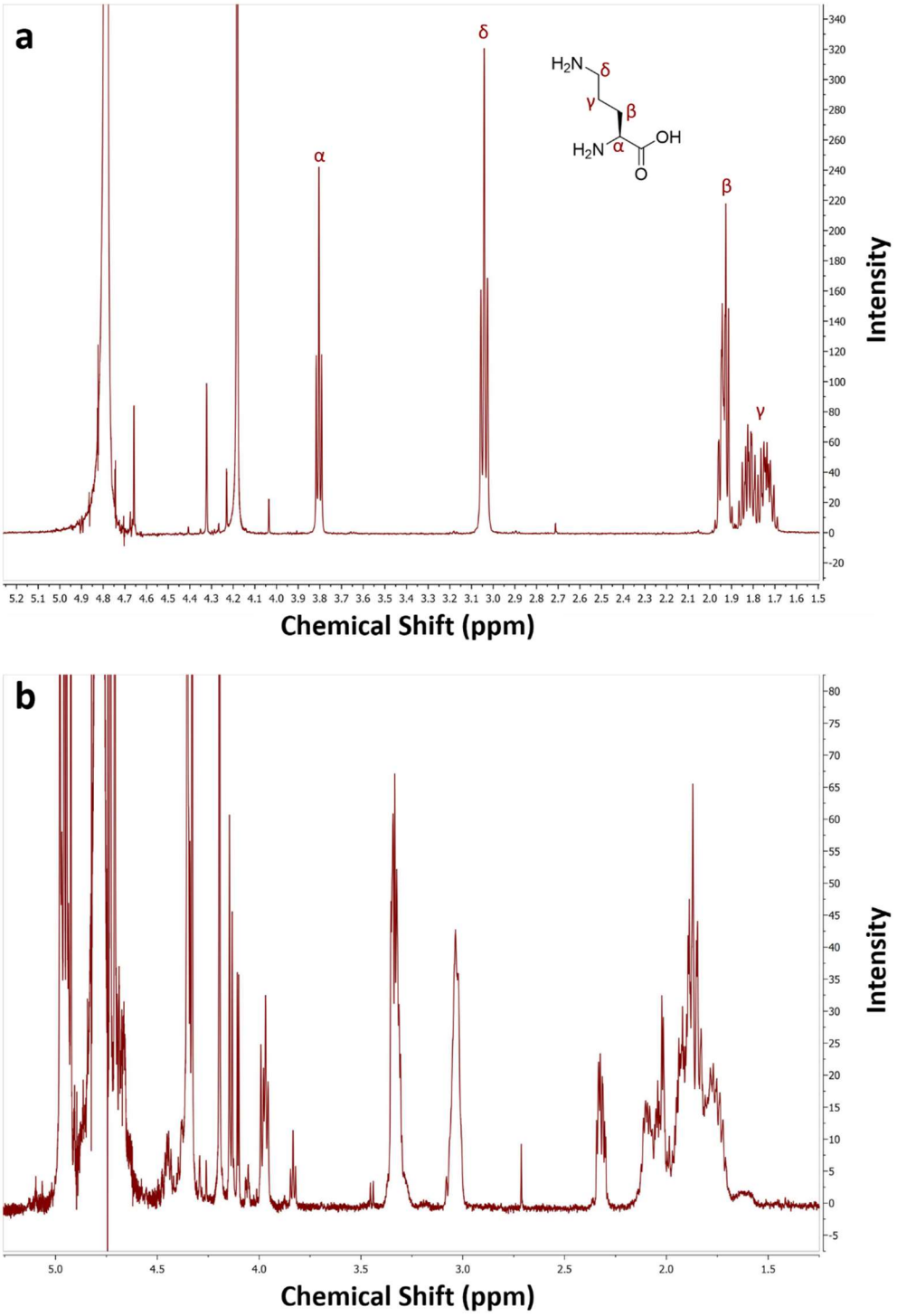


Figure S45. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Orn. ^{1}H NMR spectrum of a mixture of glc and Orn in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

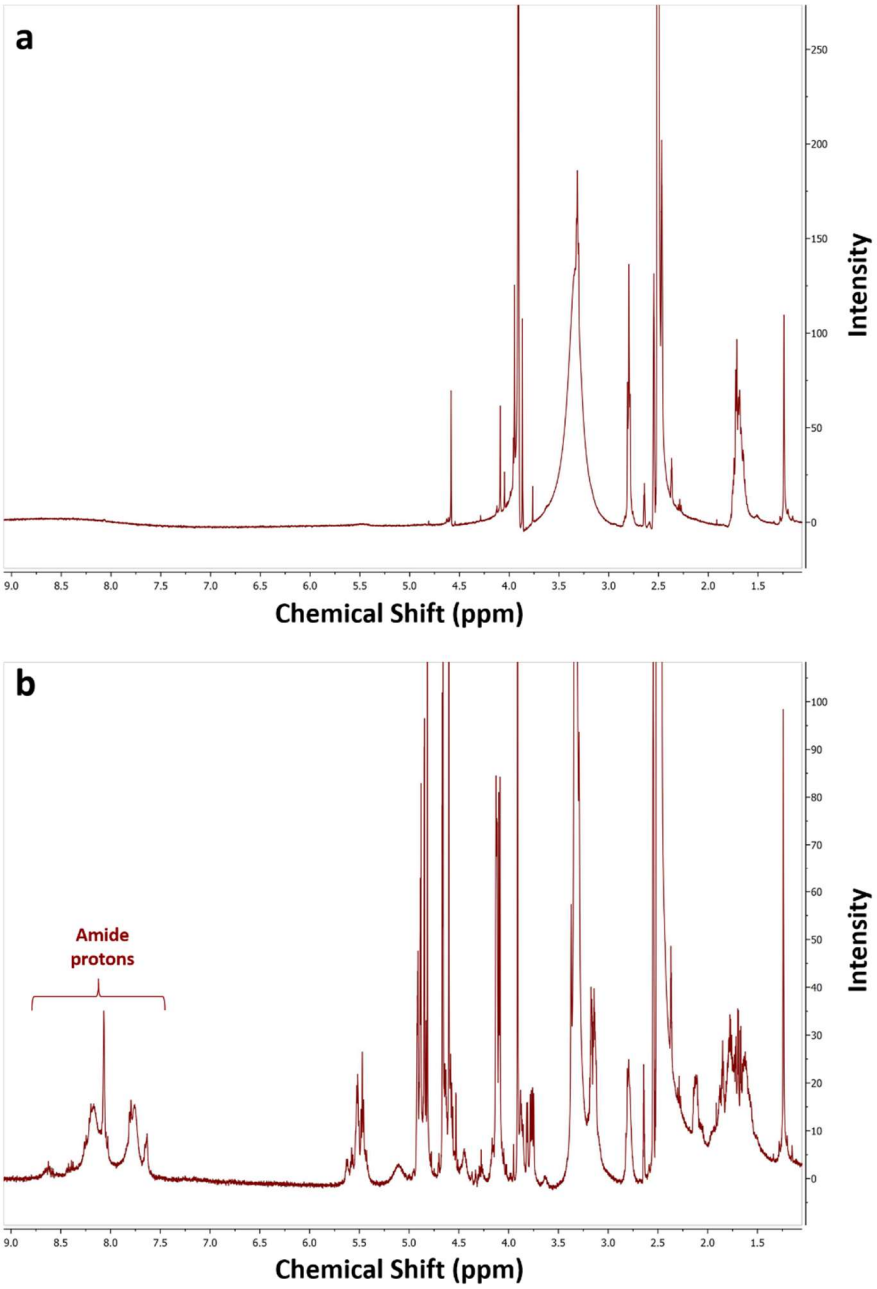


Figure S46. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Orn. glc and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

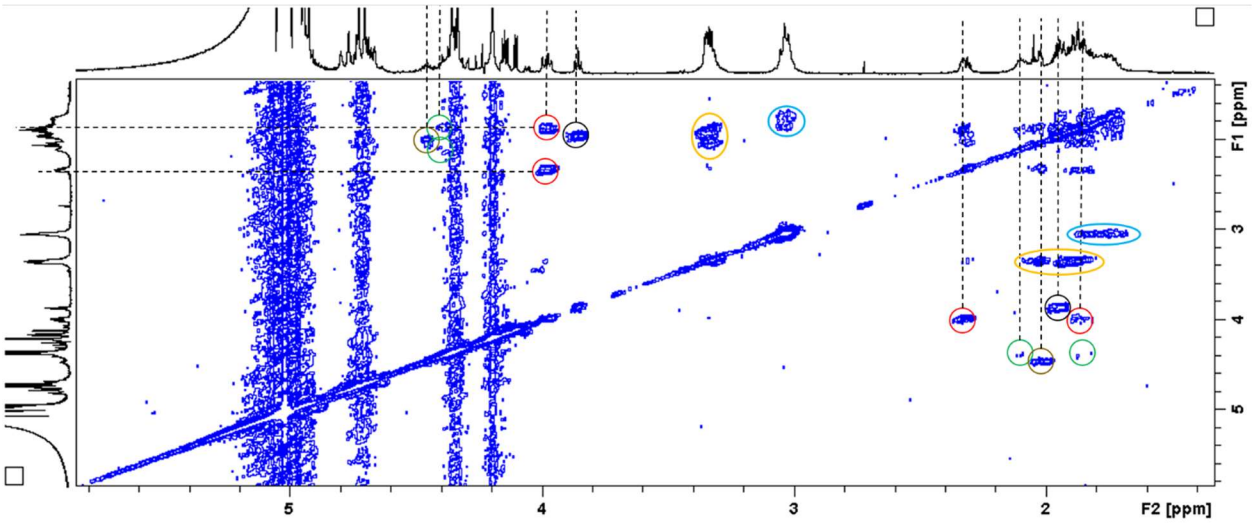


Figure S47. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and Orn. glc and Orn were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. Cross peaks of interest are circled: black – correlation between the α-proton and β protons of Orn that is not α-amidated; green/brown - correlations between α-amidated protons and their corresponding β protons; turquoise - correlation between δ-protons and γ protons of Orn that are not δ-amidated; red - correlation between the α-protons of Orn lactam monomer and their corresponding β protons; yellow - correlation between δ-amidated protons and their corresponding γ protons.

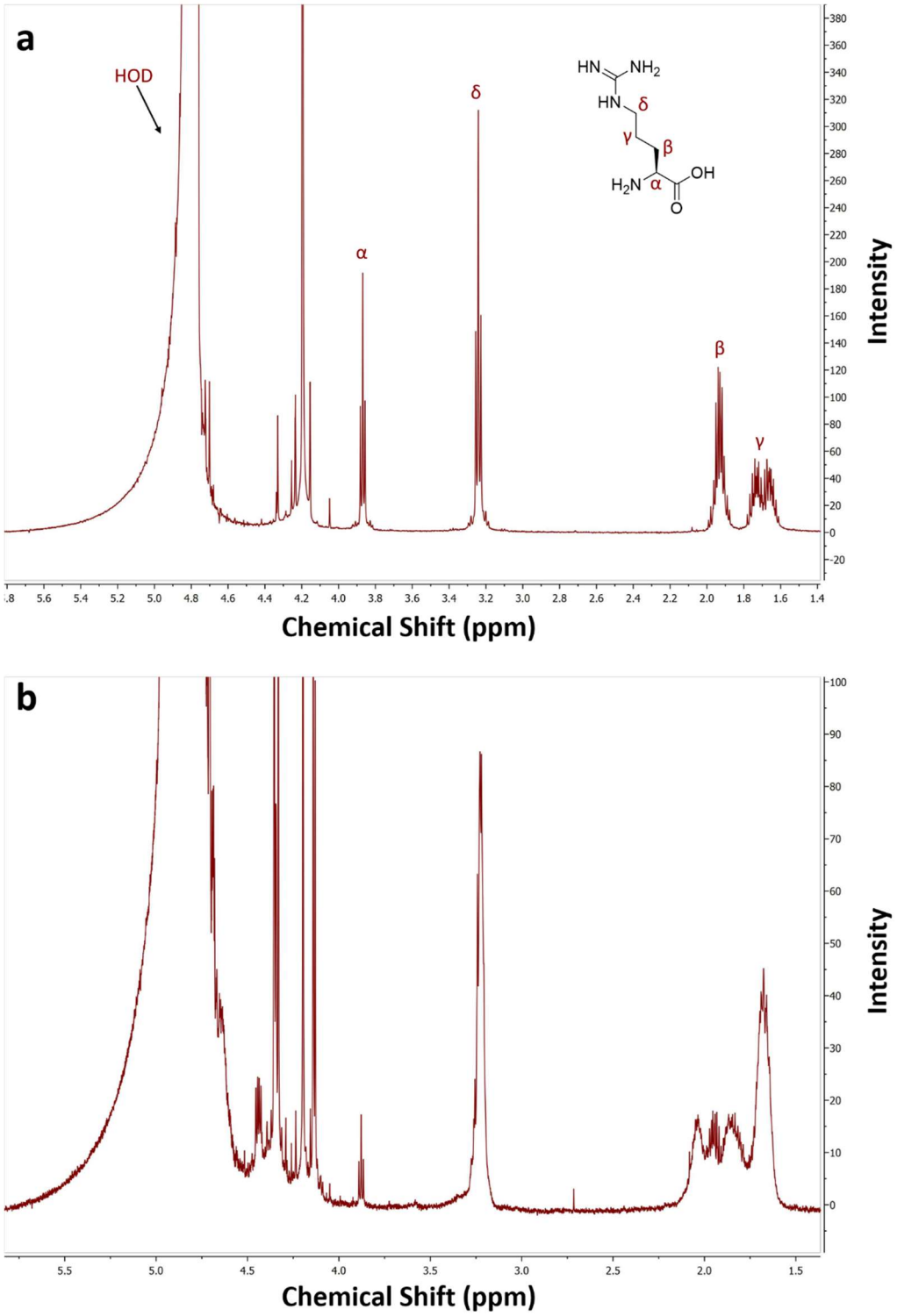


Figure S48. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Arg. ^{1}H NMR spectrum of a mixture of glc and Arg in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

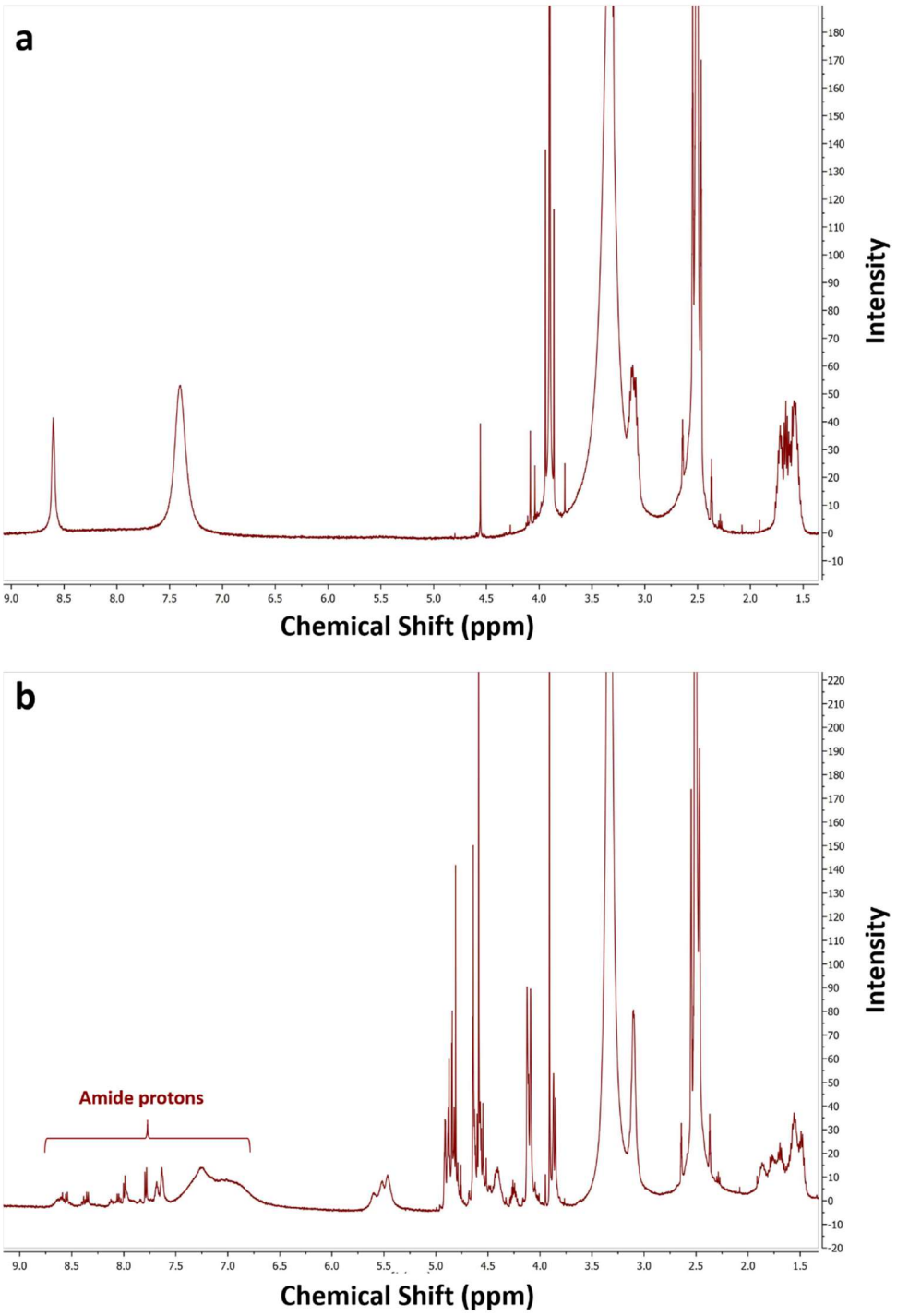


Figure S49. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and Arg. glc and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

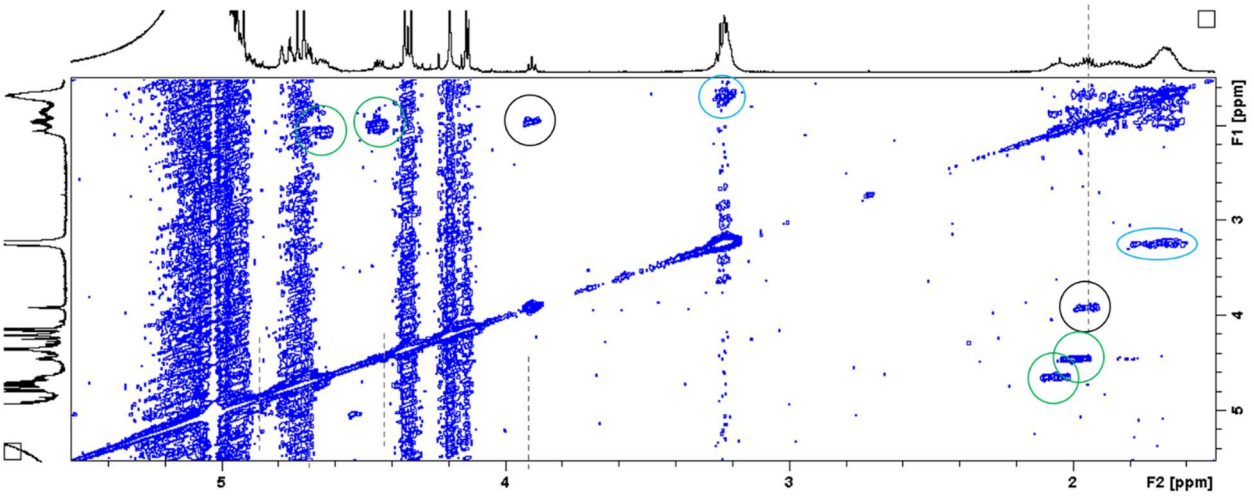


Figure S50. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and Arg. glc and Arg were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. Cross peaks of interest are circled: black - correlation between the α-proton and β protons of Arg that is not α-amidated; green - correlation between α-amidated protons and their corresponding β protons; turquoise - correlation between δ-protons and γ protons of Arg that are not side chain amidated.

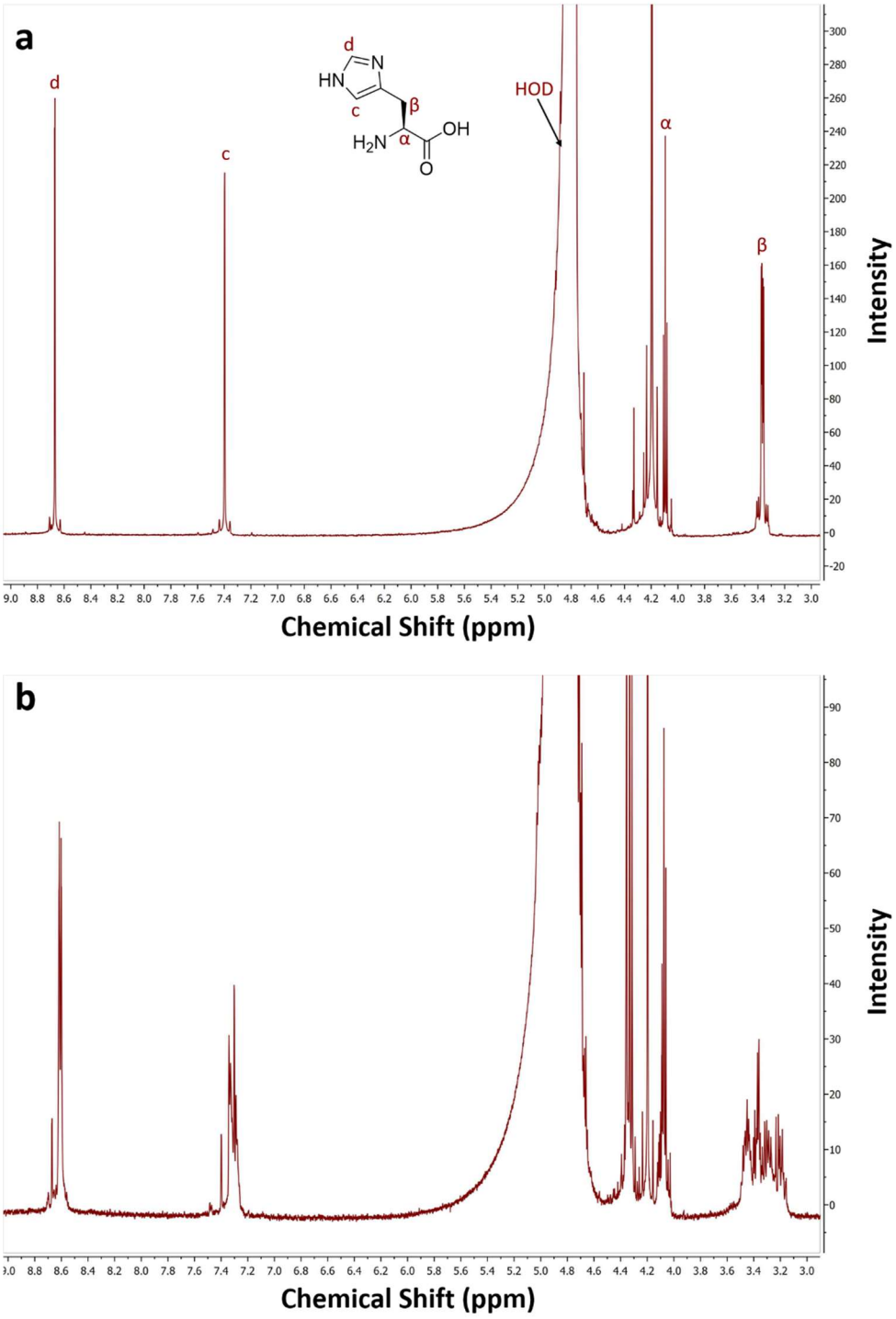


Figure S51. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and His. ^{1}H NMR spectrum of a mixture of glc and His in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

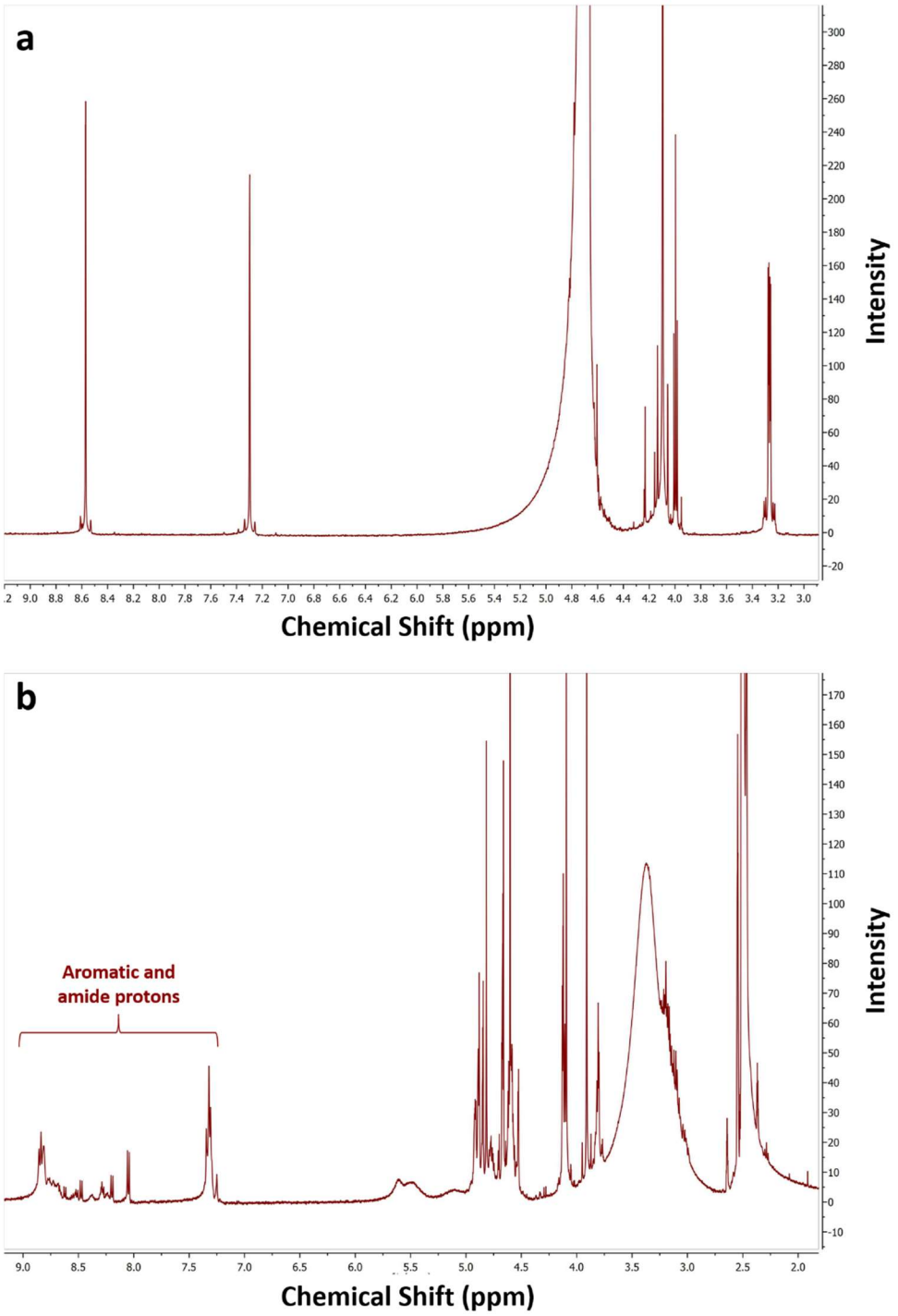


Figure S52. ^{1}H NMR spectrum supports the formation of amide bonds upon dry-down reactions of glc and His. glc and His were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in DMSO-D_6 and analyzed by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture is shown (**a**) before and (**b**) after the dry-down, indicating changes in the amide region upon dry-down.

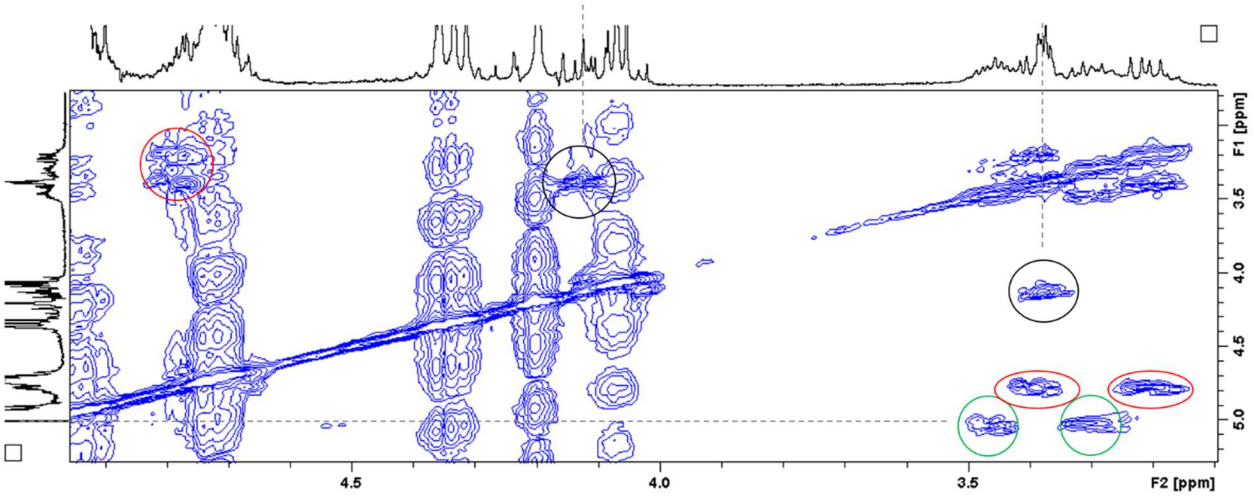


Figure S53. ^{1}H-^{1}H COSY NMR spectra of dry-down reactions of glc and His. glc and His were dried at 85 ˚C for seven days and the resulting depsipeptides were resuspended in D_2O and analyzed by ^{1}H-^{1}H COSY for peak assignments. Cross peaks of interest are circled: black – correlation between the α-proton and β protons of His that is not α-amidated; green/red - correlations between α-amidated protons and their corresponding β protons.

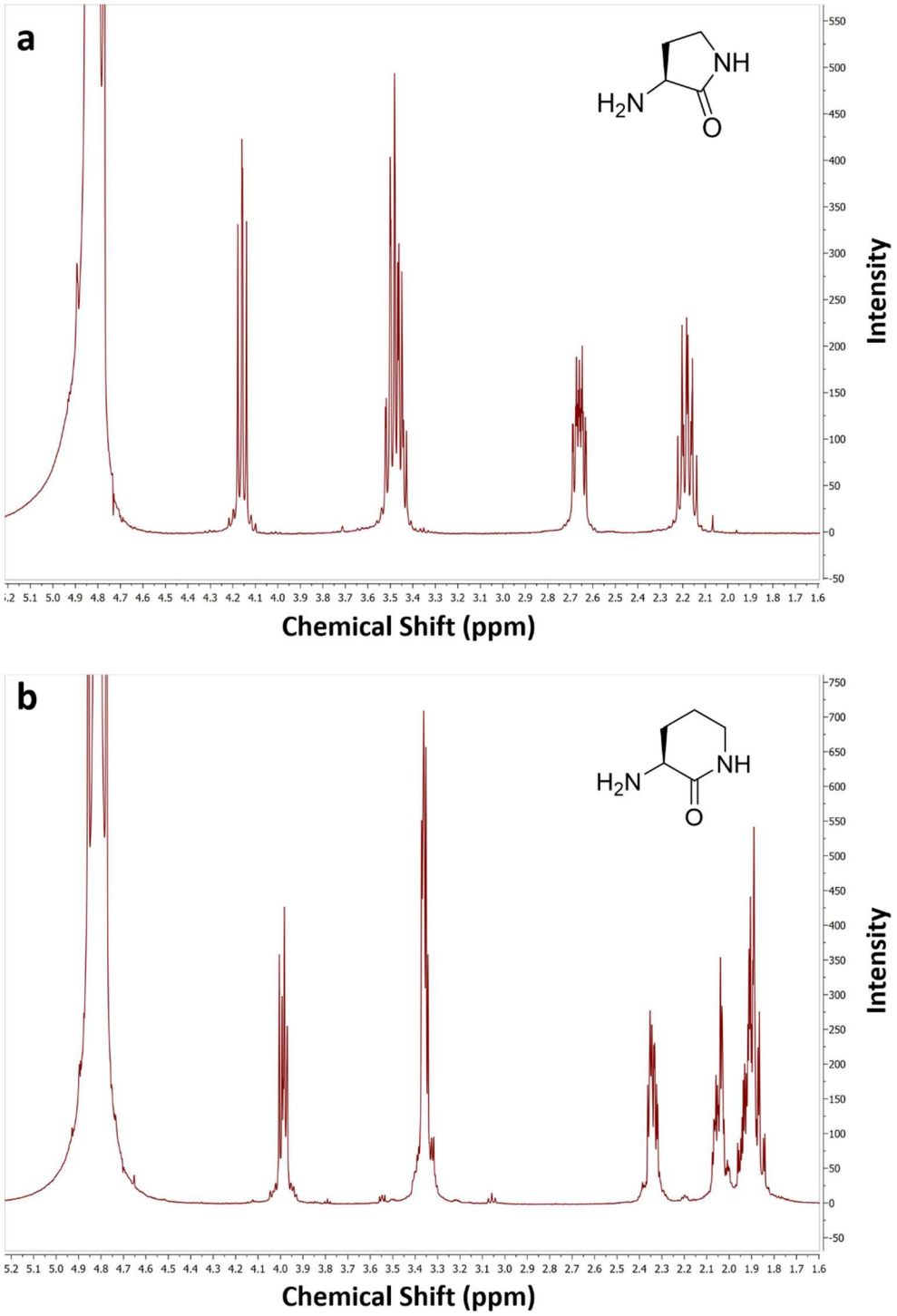


Figure S54. ^{1}H NMR spectrum of cyclic Dab and cyclic Orn standards confirms the formation of lactams upon dry-down reactions involving Dab and Orn. (**a**) Cyclic Dab (3-Aminopyrrolidin-2-one) and (**b**) cyclic Orn (3-Aminopiperidine-2-one) were resuspended in D_2O and analyzed by ^{1}H NMR.

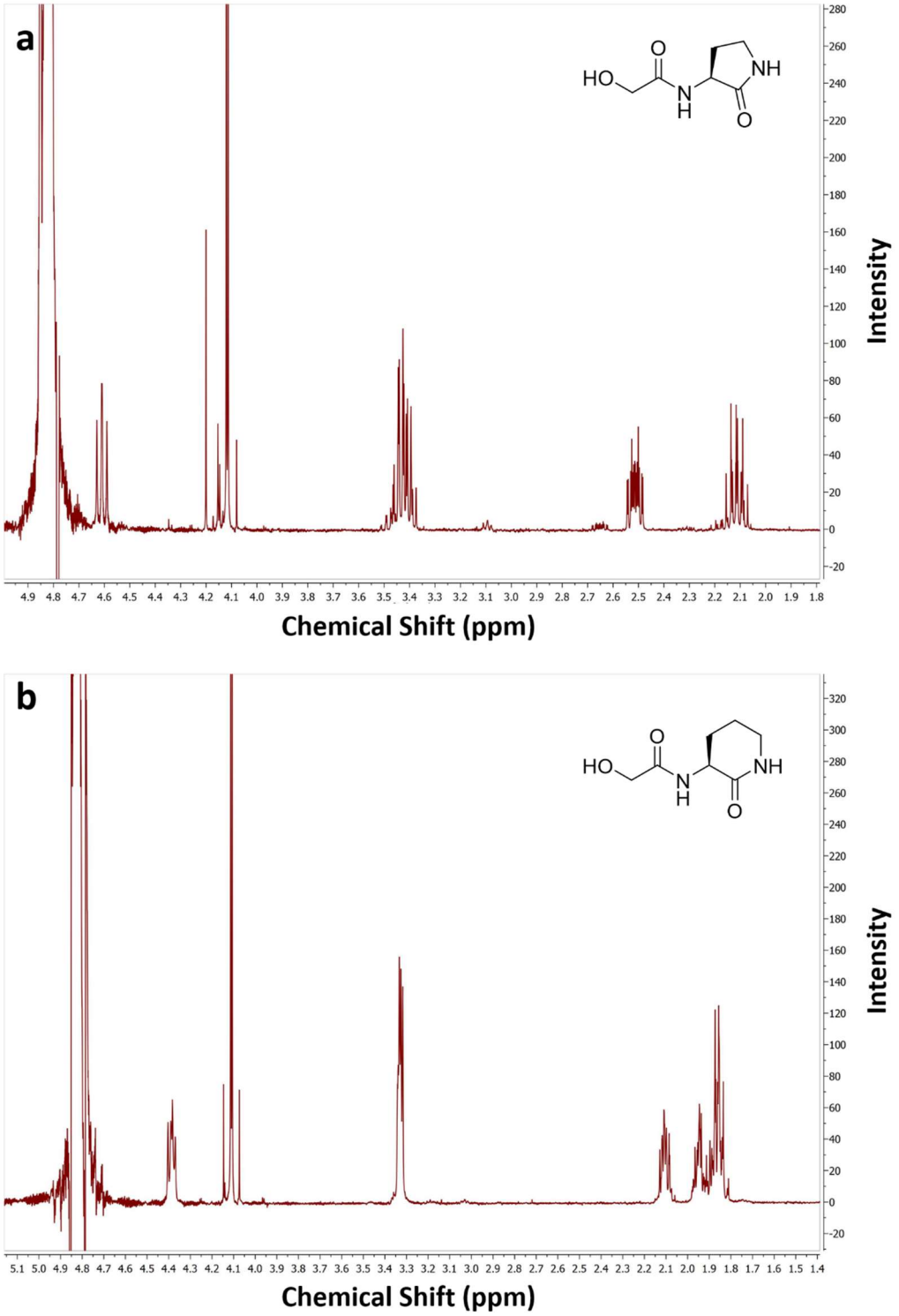


Figure S55. ^{1}H NMR spectrum of glc standards containing a C-terminal lactam. ^{1}H NMR spectrum of glc standards, amidated to a Dab lactam (**a**) or to an Orn lactam (**b**). Standards were resuspended in D_2O and analyzed by ^{1}H NMR.

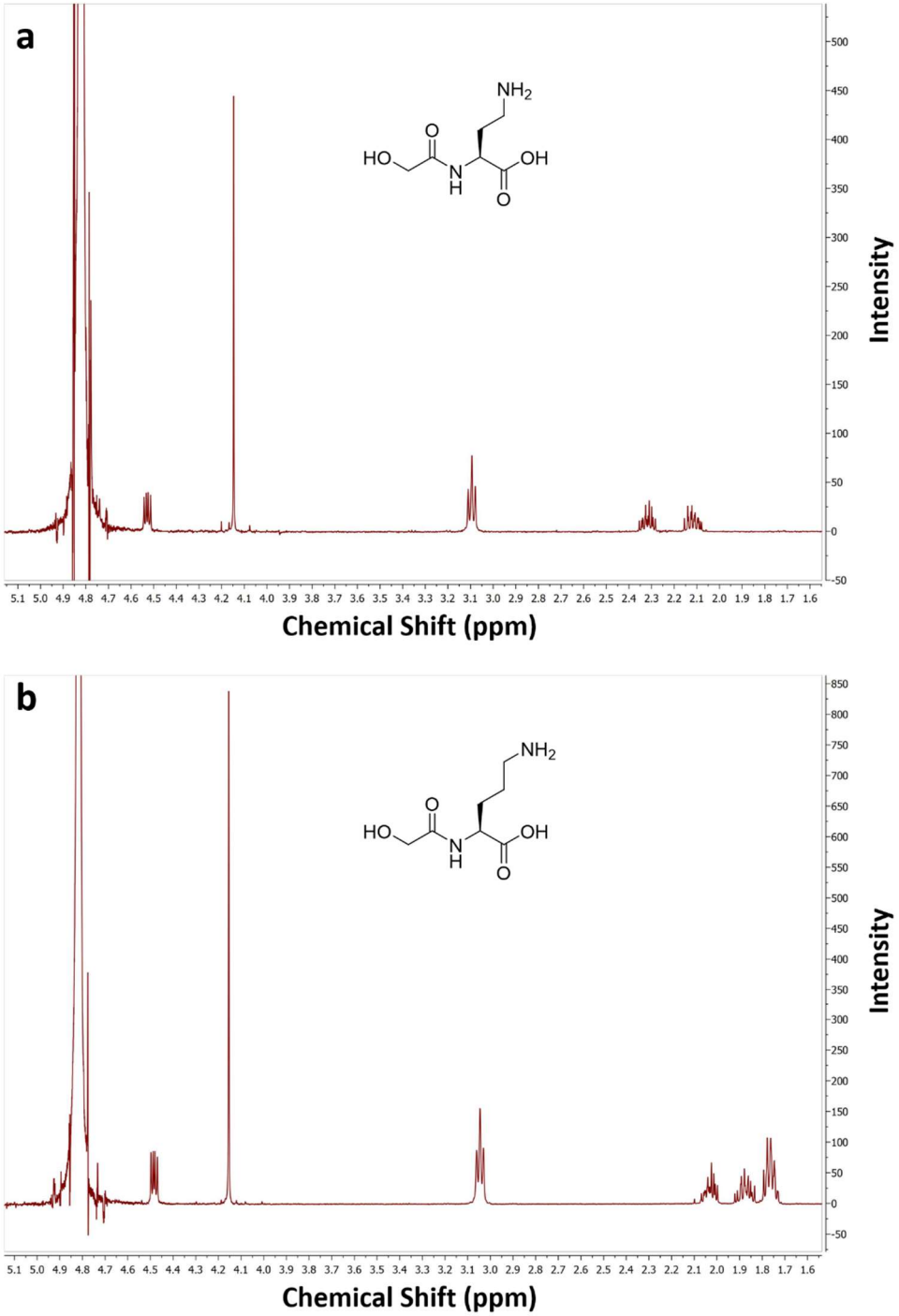


Figure S56. ^{1}H NMR spectrum of glc-Dab and glc-Orn standards. ^{1}H NMR spectrum of glc standards, α-amidated to Dab (**a**) or to Orn (**b**) with free side chains. Standards were resuspended in D_2O and analyzed by ^{1}H NMR.

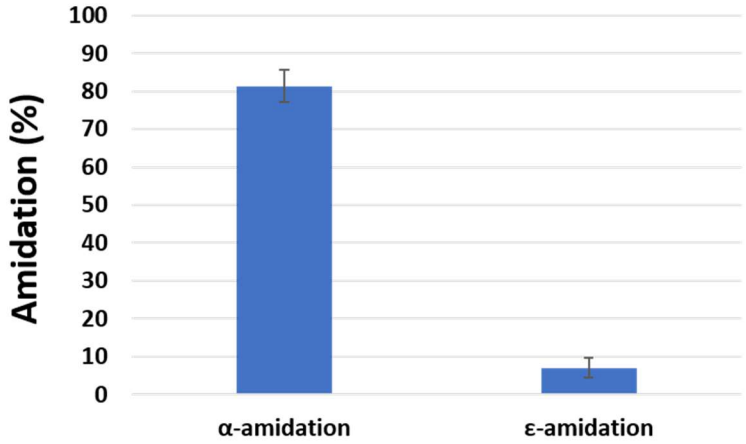


Figure S57. Extent of amidation on the α-amine or on the ε-amine of Lys in five independent replicates of glc+Lys dry-downs verifies reproducibility of the product mixtures. Amidation percentages were quantitated by integration of ^{1}H NMR peaks. Error bars indicate 95% confidence limits.

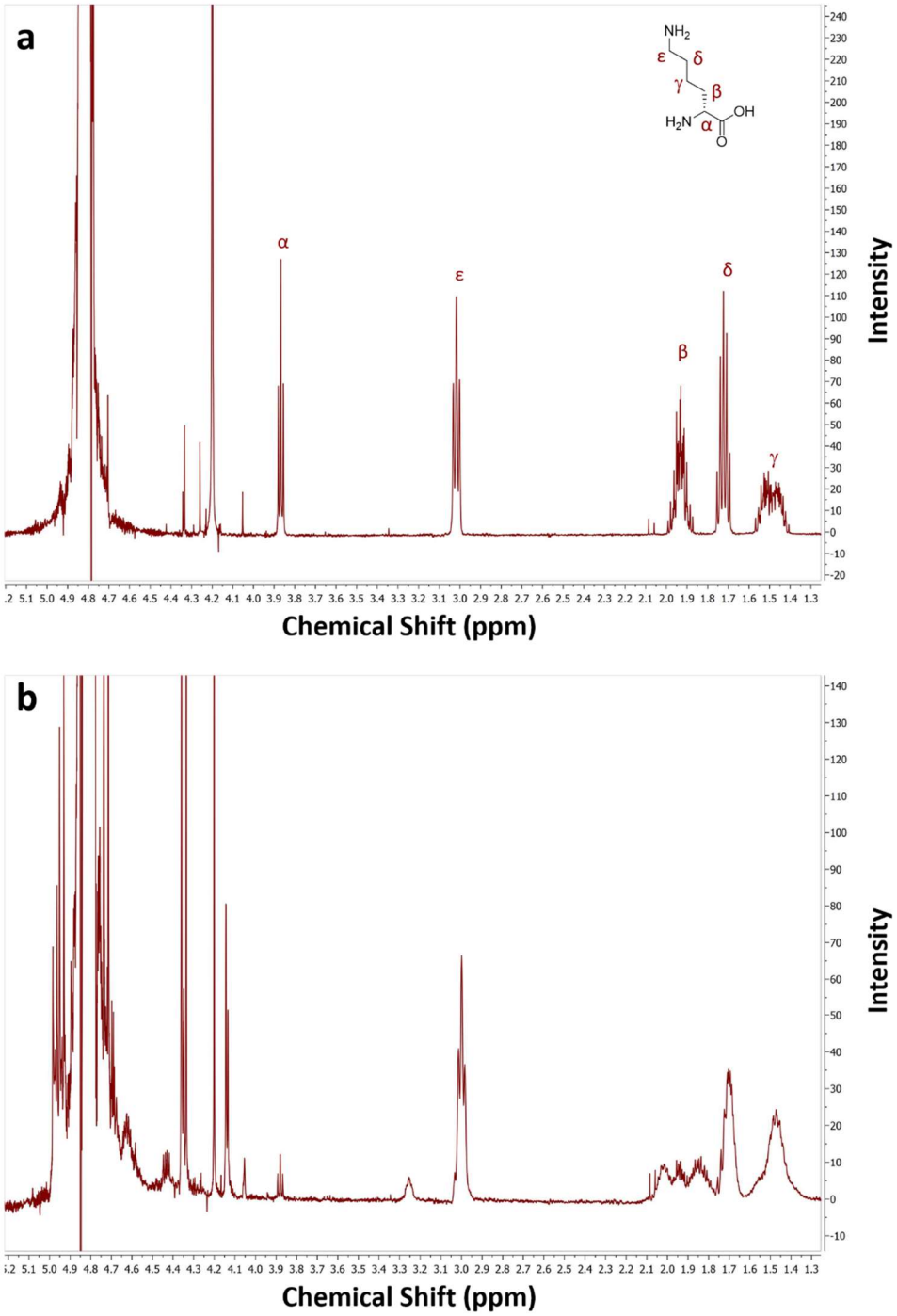


Figure S58. ^{1}H NMR spectrum supports similar oligomeric distribution upon dry-down of glc with D-Lys or L-Lys. ^{1}H NMR spectrum of a mixture of glc and D-Lys in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days shows similar product distribution compared with that observed for a dry-down of glc with L-Lys (Fig. 3D).

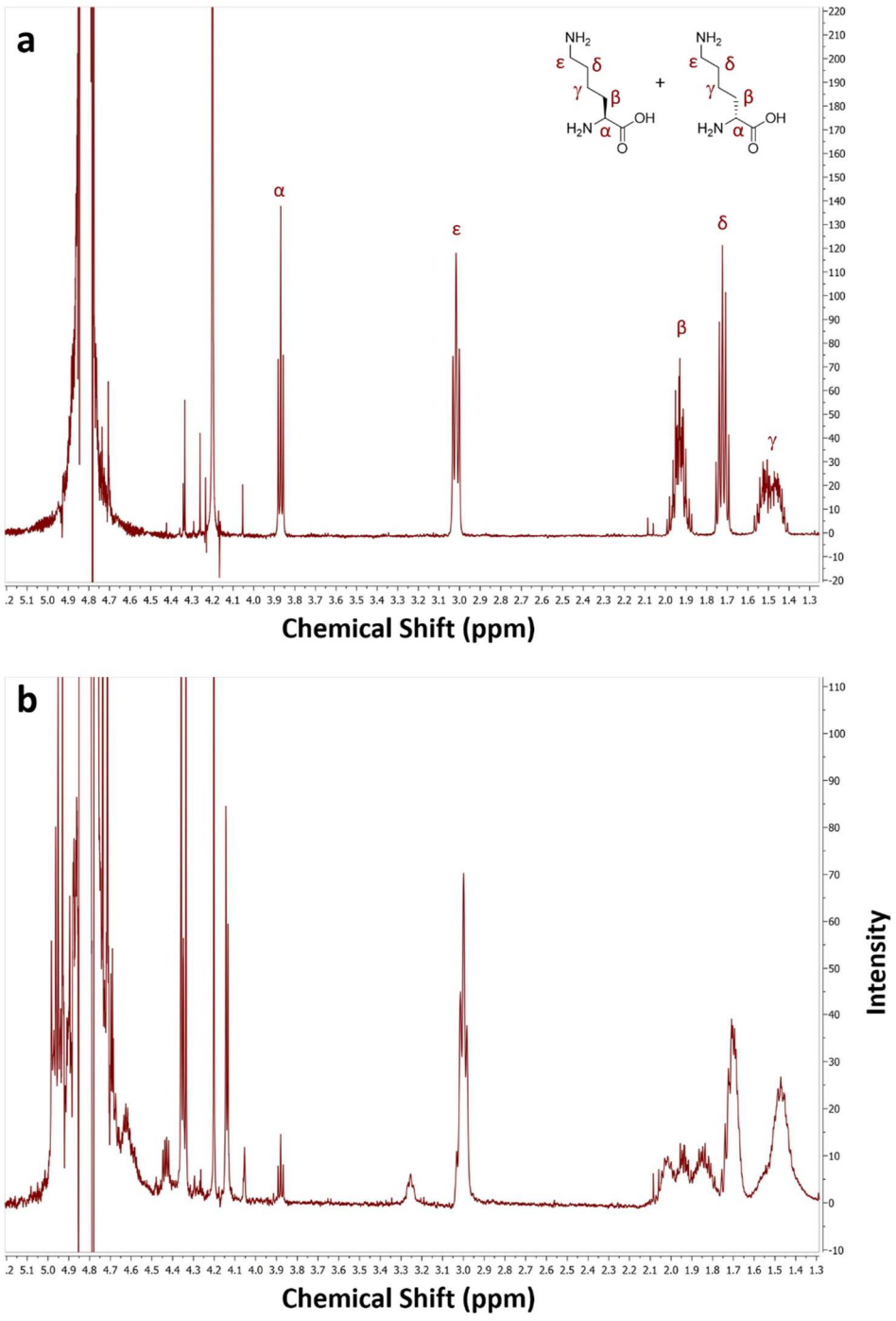


Figure S59. ^{1}H NMR spectrum supports a similar oligomeric distribution upon dry-down of glc with a racemate of Lys as with L-Lys. ^{1}H NMR spectrum of a mixture of glc with a racemic mixture of Lys in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days shows similar product distribution compared with that observed for a dry-down of glc with L-Lys (Fig. 3D).

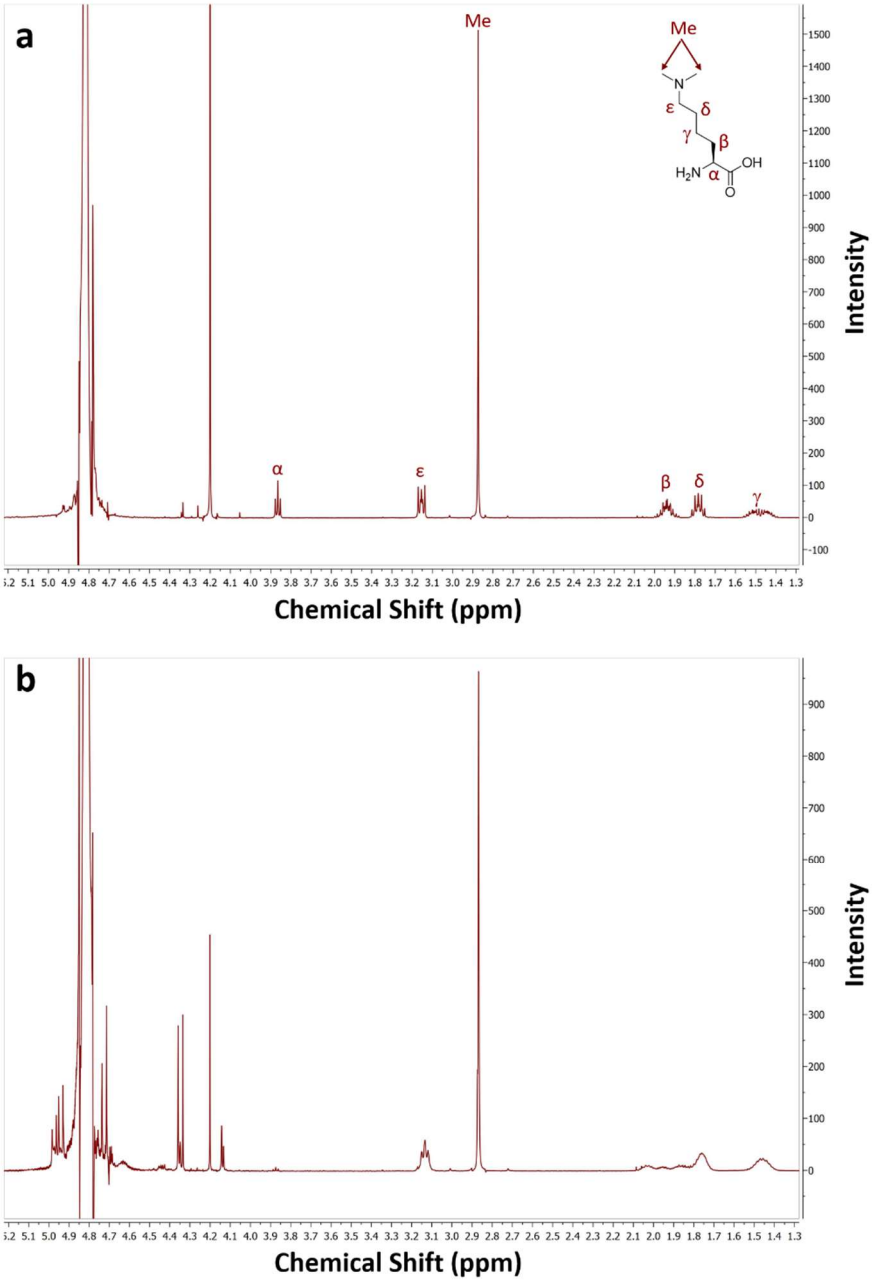


Figure S60. ^{1}H NMR spectrum of a dry-down of glc with an N-ε di-methylated Lys confirms reactivity through the α-amine. ^{1}H NMR spectrum of a mixture of glc with a di-methylated Lys in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days confirms reactivity at the α-amine and the absence of amidation at the ε-amine.

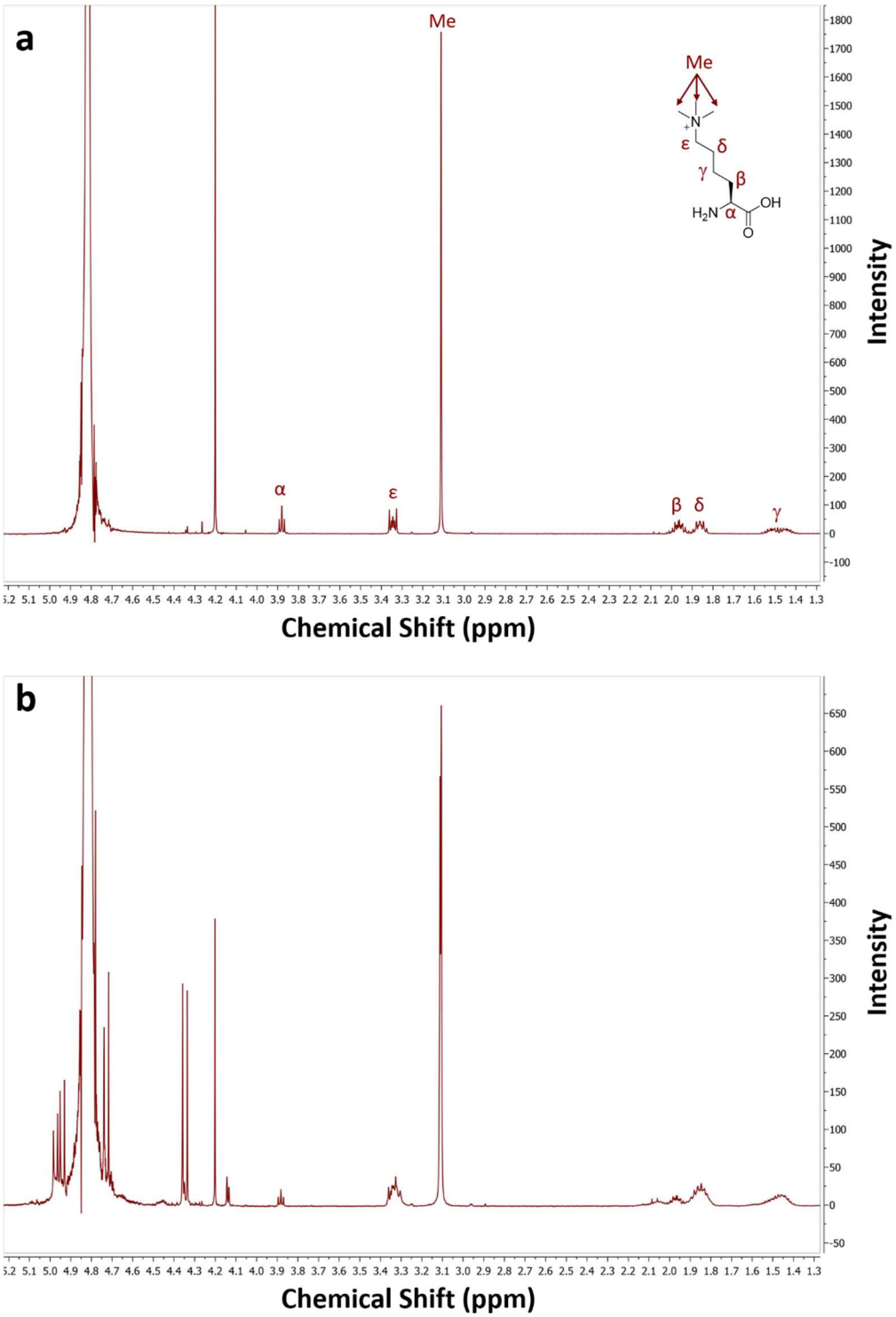


Figure S61. ^{1}H NMR spectrum of a dry-down of glc with an N-ε tri-methylated Lys confirms reactivity through the α-amine. ^{1}H NMR spectrum of a mixture of glc with a tri-methylated Lys in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days confirms reactivity at the α-amine and the absence of amidation at the ε-amine.

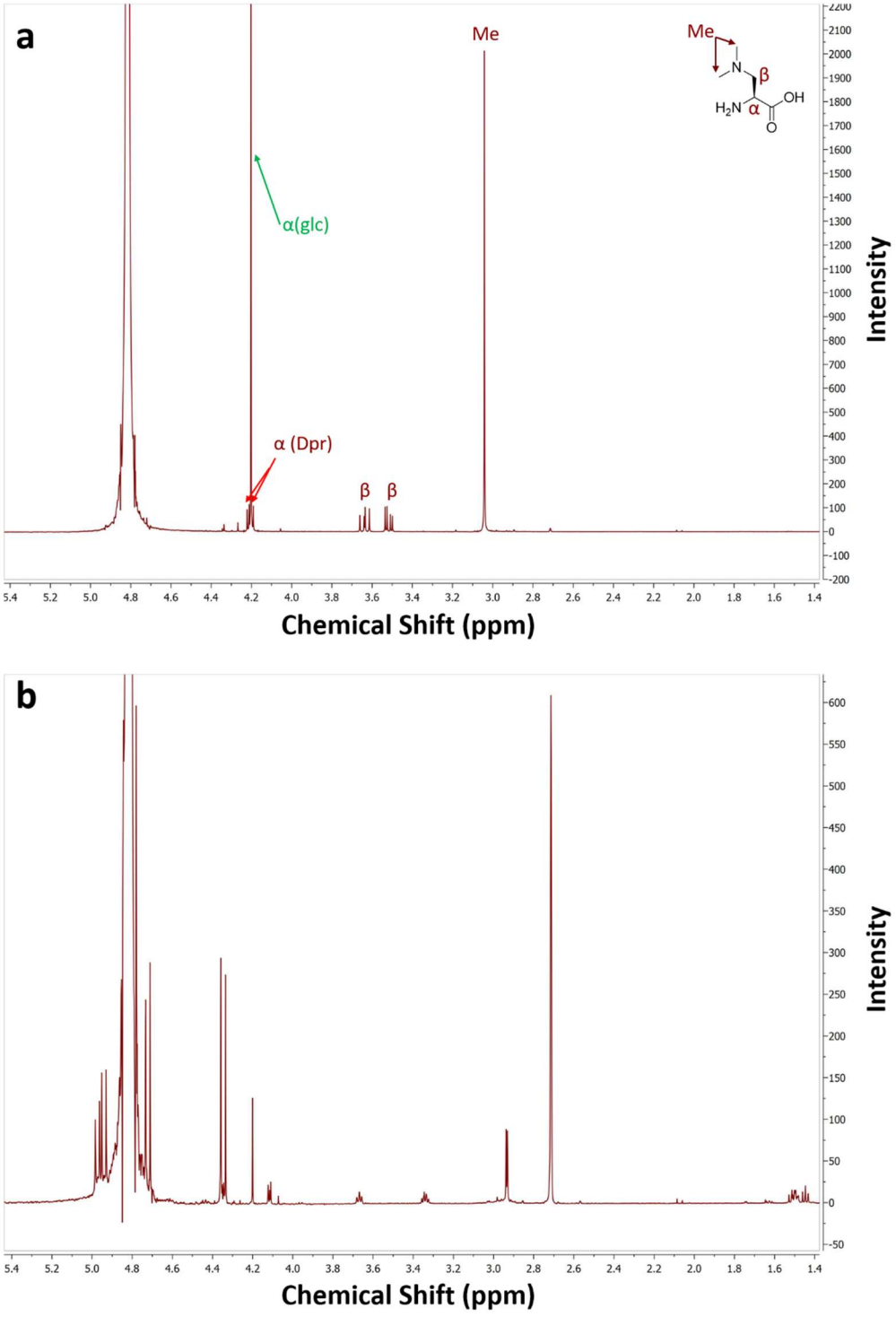


Figure S62. ^{1}H NMR spectrum of a dry-down of glc with a N-β di-methylated Dpr. ^{1}H NMR spectrum of a mixture of glc with a di-methylated Dpr in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days.

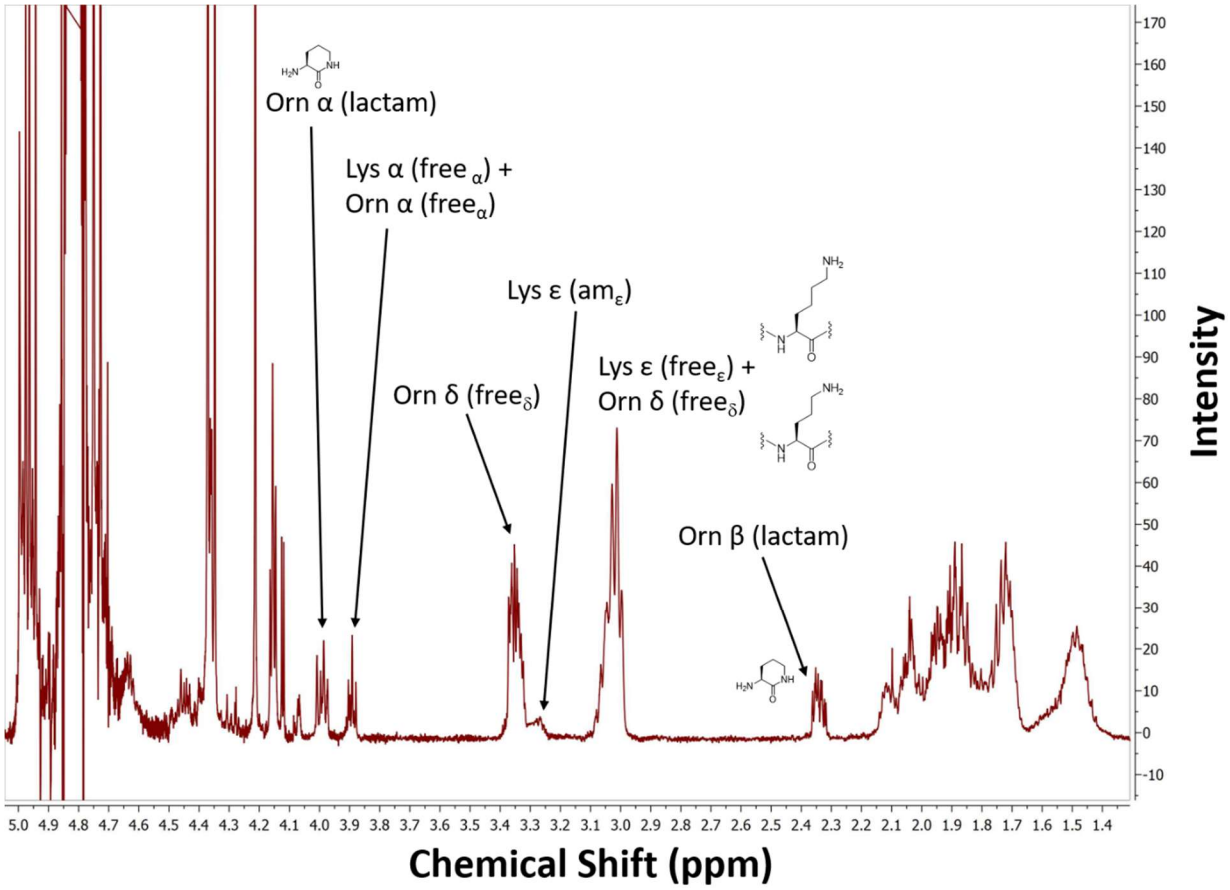


Figure S63. Dry-down reactions chemically select for proteinaceous amino acids. The ^{1}H-NMR spectrum of a dry-down reaction containing glc, Lys, and Orn indicated that Lys maintains free ε-amines upon its polymerization whereas Orn with free side chain amines is extensively excluded from the oligomers. Rather, Orn is observed mainly as the lactam.

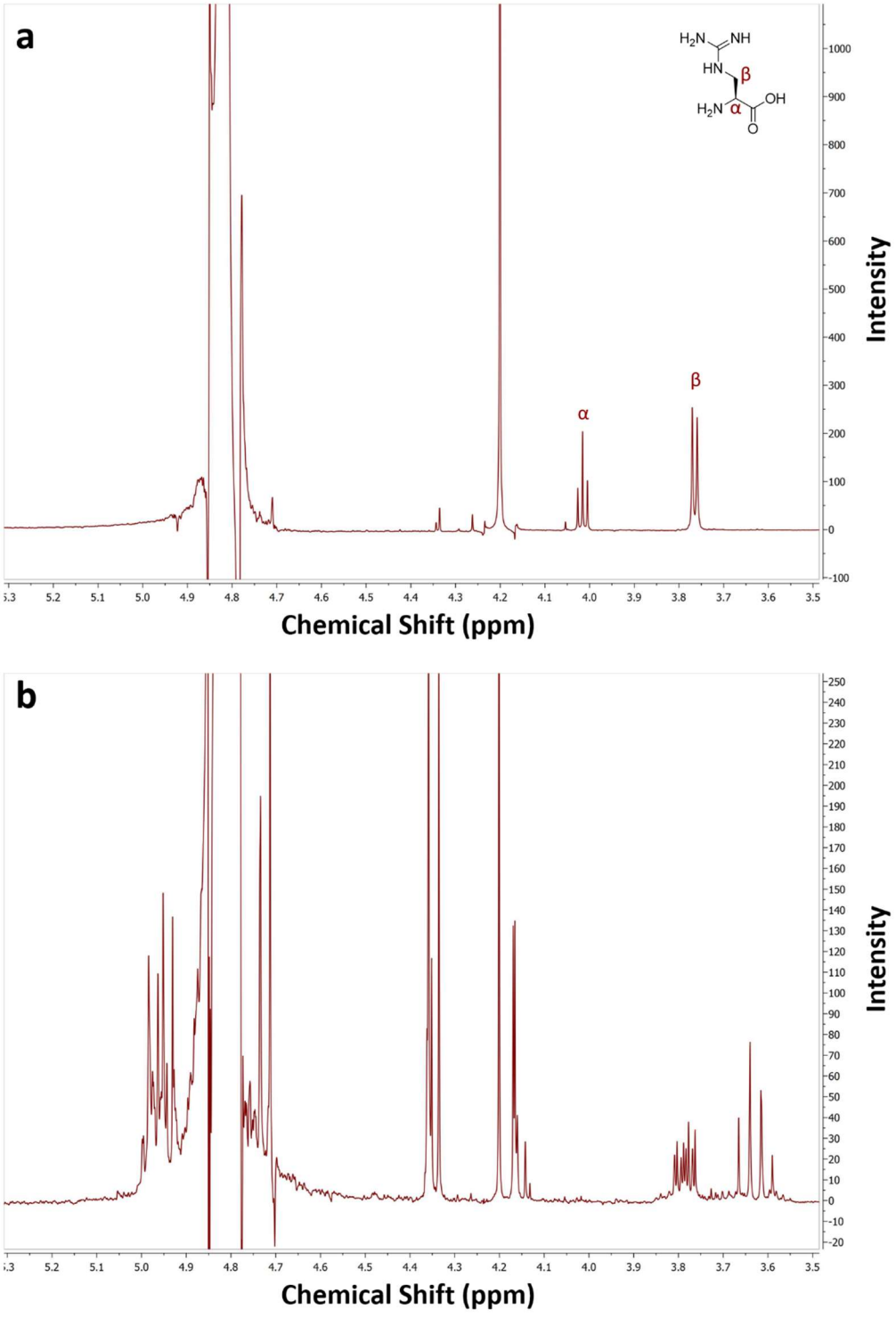


Figure S64. ^{1}H NMR spectrum of a dry-down of glc with L-2-amino-3-guanidinopropionic acid (Agp) resulted in formation of oligomers. ^{1}H NMR spectrum of a mixture of glc with Agp in D_2O (**a**) before and (**b**) after dry-down at 85 ˚C for seven days resulted in the formation of oligomers.

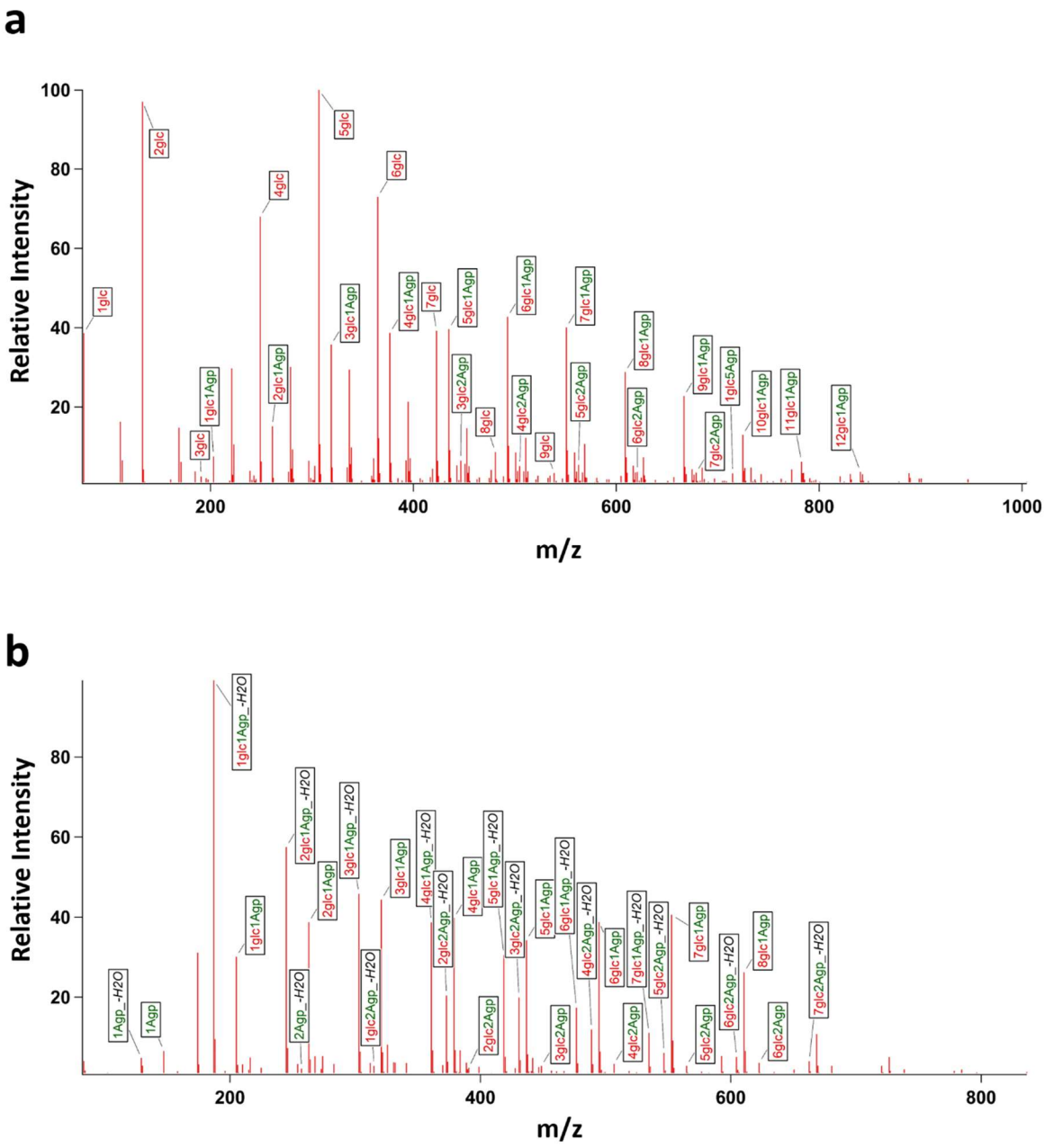


Figure S65. ESI-MS of a dry-down reaction of glc and L-2-amino-3-guanidinopropionic acid (Agp) resulted in cyclized products. glc and Agp were dried at 85 ˚C for seven days and the resulting products were analyzed by negative-mode ESI-MS (**a**) or positive mode ESI-MS (**b**), indicating a variety of oligomers. glc is labeled in red, Agp is labeled in green. Labeled species correspond to $[M-H]^-$ ions (**a**) or $[M+H]^+$ ions (**b**).

III. Supplemental References

1 Pandey, A., Pandey, G. C. & Aswath, P. B. Synthesis of polylactic acid-polyglycolic acid blends using microwave radiation. *J Mech Behav Biomed Mater* **1**, 227-233, doi:10.1016/j.jmbbm.2007.12.001 (2008).