

Dipeptide-Based Metabolic Labeling of Bacterial Cells for Endogenous Antibody Recruitment

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Supporting Information

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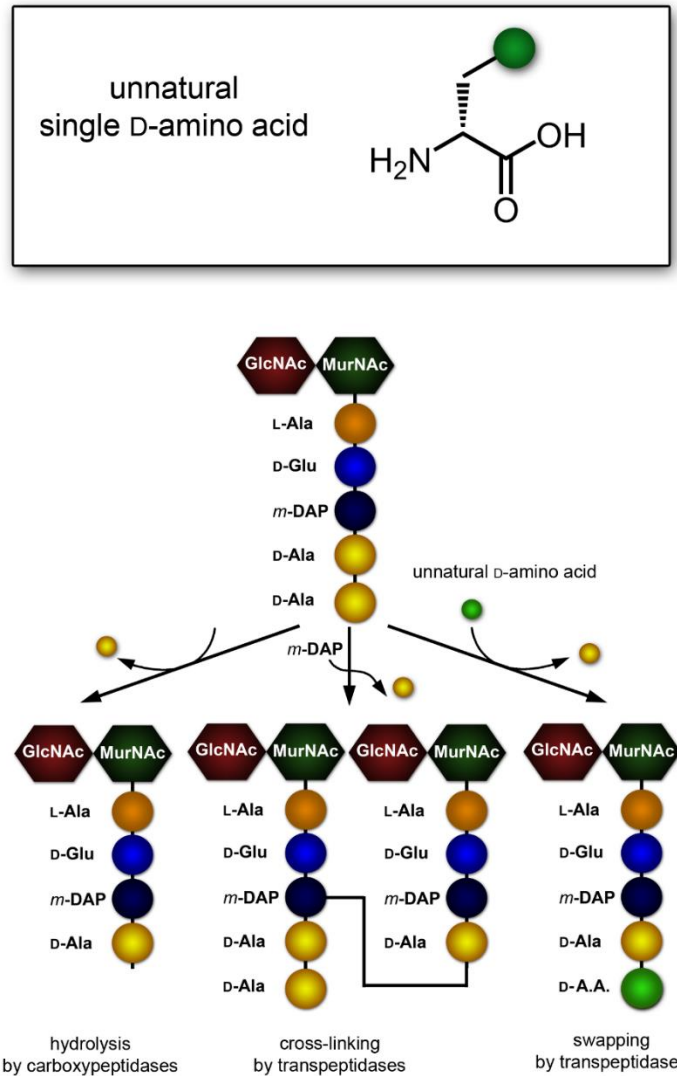


Figure S1. Schematic of peptidoglycan processing by PBPs. Terminal D-Ala residues are hydrolyzed at the C-terminus of the stem peptide by carboxypeptidase (left). Adjacent oligopeptides are crosslinked by transpeptidase enzymes (middle). Exogenous D-amino acids are integrated into the stem peptide through transpeptidase mediated “swapping” of the terminal D-Ala (right). GlcNAc = N-acetylglucosamine, MurNAc = N-acetylmuramic acid, *m*-Dap = meso-diaminopimelic acid.

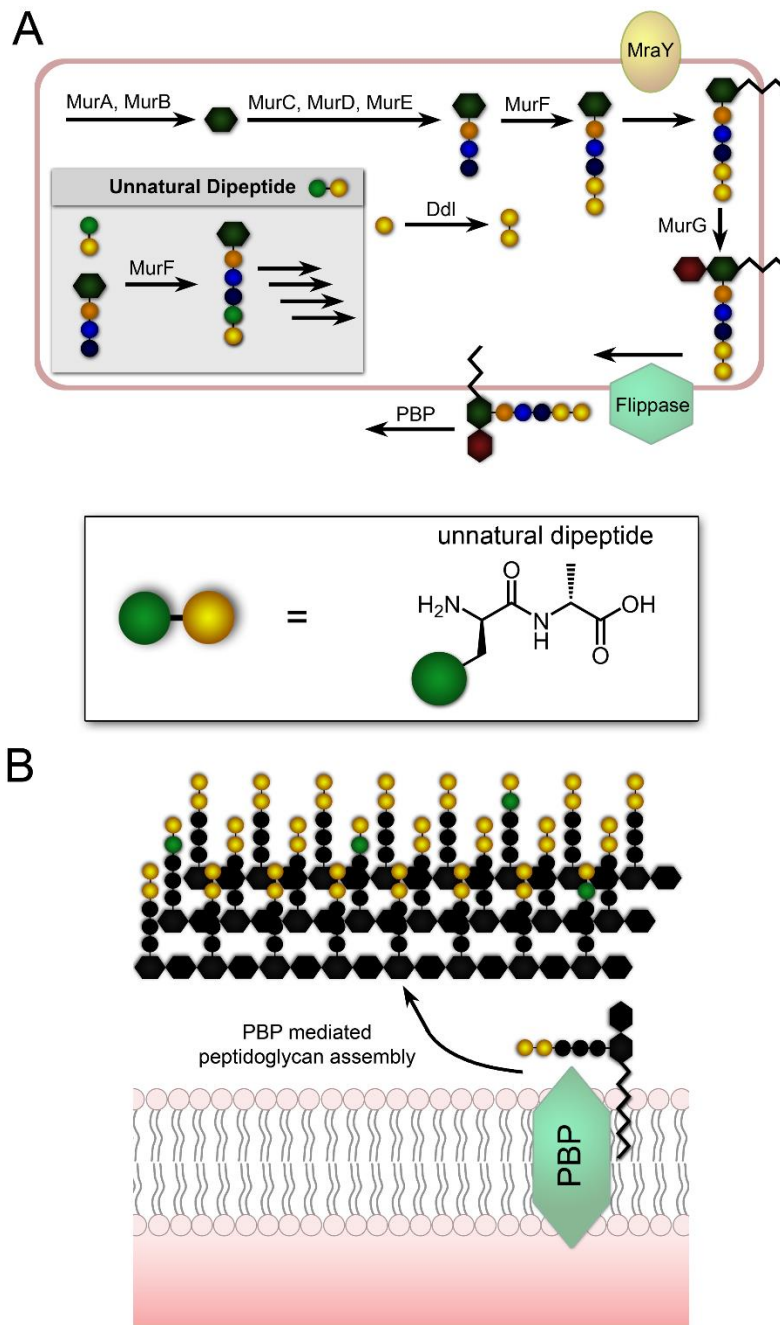


Figure S2. (A) Intracellular MurF promiscuity enables ligation of unnatural exogenous dipeptides into the growing stem peptide during peptidoglycan biosynthesis. (B) Unnatural dipeptide cell wall analogs are integrated into the growing peptidoglycan. Incorporation of chemically reactive handles facilitates site-selective modification of bacterial cell surfaces.

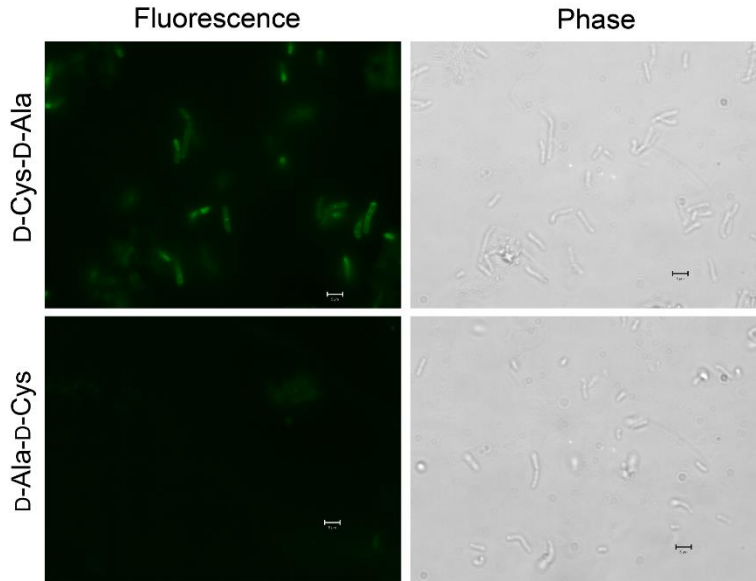


Figure S3. Phase and epifluorescent microscopy images. *B. subtilis* cells (wild type) were incubated overnight with 1 mM of either D-Cys-D-Ala or D-Ala-D-Cys. Subsequently, cells were treated with 50 μ M of FL0 for 30 min and visualized using fluorescence microscopy. Scale bars = 5 μ m.

Materials. Reagents and Bacterial strains. Amino acids were purchased from Chem-Impex. Antibody reagents were purchased from Life-Technologies. All other organic reagents were purchased from Fisher Scientific and used without further purification. *B. subtilis* Δ *dacA* and *B. subtilis* NCIB3610 were the strains of bacteria used for experiments.

General Synthesis of Sulfhydryl Dipeptides (solid phase). A 25ml synthetic flask was charged with 200 mg (0.275 mmol) of 2-Chlorotrityl Chloride Resin. The resin was initially washed with dry CH_2Cl_2 (3 x 5 ml) and dry DMF (3 x 5ml). Initial loading was performed by the addition of Fmoc-protected amino acids (1 equiv, 0.275 mmol) in dry CH_2Cl_2 (5 ml) to the reaction flask with DIEA (4 equiv, 1.1 mmol) and the flask was agitated for 2 hours at room temperature. The resin was consequently washed with DMF, CH_2Cl_2 , MeOH, CH_2Cl_2 and DMF (3 x5 ml each). The Fmoc-protecting group was removed with a solution of 6 M piperazine/100 mM HOBt in DMF (10 ml). The flask was agitated for 25 min and the deprotection solution was drained. The resin was washed with DMF, CH_2Cl_2 , MeOH, CH_2Cl_2 , and DMF (2 x 5 ml each). The addition of the second amino acid was performed by adding Fmoc-protected amino acids (4 equiv, 1.1 mmol), HBTU (4 equiv, 1.1 mmol), DIEA (8 equiv, 2.2 mmol) in DMF and agitating for two hours. Dipeptides were subsequently Fmoc-deprotected, washed as stated before and then cleaved using a TFA cocktail solution (82.5% TFA, 2.5% 1,2 ethanedithiol (EDT), 5% thioanisole, 5% H_2O , 5% phenol). The resulting solutions were concentrated *in vacuo* to remove the TFA. The residue was triturated in cold diethyl ether and the precipitate was collected by centrifugation. Peptides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC) using a Phenomenex C8 prep column with an eluent consisting of solvent A (H_2O / 0.1% TFA) and solvent B (MeOH / 0.1% TFA) using a 60 min gradient transitioning from 5% B to 100% B. The purity of the peptides were verified by analytical RP-HPLC using a Phenomenex C4 column with an eluent consisting of solvent A (H_2O /0.01% TFA) and solvent B (CH_3CN / 0.01% TFA) with a 30 min gradient transitioning from 5% B to 100% B at a flow rate of 3 ml/min and monitored at 220nm and molecular weight was confirmed using electrospray ionization mass spectrometry.

General Synthesis of Fluorophore and Hapten Dipeptides (solid phase). A 25ml synthetic flask was charged with 200 mg (0.275 mmol) of 2-Chlorotrityl Chloride Resin. The resin was initially washed with dry CH_2Cl_2 (3 x 5 ml) and dry DMF (3 x 5ml). Initial loading was performed by the adding a Fmoc-alanine protected amino acid (1 equiv, 0.275 mmol) in dry CH_2Cl_2 (5 ml) to the reaction flask with DIEA (4 equiv, 1.1 mmol) and agitating the flask for 2 hours at room temperature. The resin was consequently washed with DMF, CH_2Cl_2 , MeOH, CH_2Cl_2 and DMF (3 x5 ml each). The Fmoc protecting group was removed with a solution of 6 M piperazine/100 mM HOBt in DMF (10 ml). The flask was agitated for 25 min and the deprotection solution was drained. The resin was washed with DMF, CH_2Cl_2 , MeOH, CH_2Cl_2 , and DMF (2 x 5 ml each). For dipeptides modified with NBD or DNP, either a N^α -Boc- N^ϵ -Fmoc-D-Lysine or a N^α -Boc- N^ϵ -Fmoc-D-2,3 diaminopropionic acid was added to the resin.

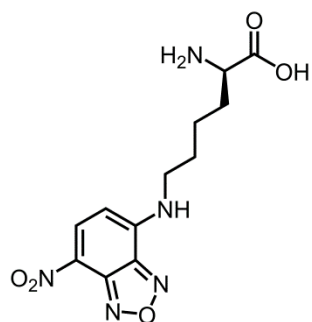
Upon addition, the Fmoc-protecting group was removed as stated before. Following washing of the resin, either 4-Chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Chloride) (5 equiv, 1.38 mmol) and DIEA (7.5 equiv, 2.06 mmol) in DMF (10 ml) or 1-fluoro-2,4 dinitrobenze (DNFB) (5 equiv, 1.38mmol) and DIEA (7.5 equiv, 2.06 mmol) in DMF (10 ml) was added to the flask and was agitated for 1 h at room temperature protected from light was added to the flask and agitated for 1 h at room temperature protected from light for NBD-conjugated dipeptides. The resin was drained and subsequently washed with DMF, CH₂Cl₂, MeOH, CH₂Cl₂, DMF (3 x 5 ml each). For cleavage of the dipeptides and amino acid compounds not containing a cysteine residue from the resin, a trifluoroacetic acid (TFA) cocktail solution (95%, 2.5% triisopropylsilane (TIPS), 2.5 % H₂O) was added to the resin and agitated for 1 h at room temperature protected from light. The resulting solutions were concentrated *in vacuo* to remove the TFA. The residue was triturated in cold diethyl ether and the precipitate was collected by centrifugation. Peptides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC) using a Phenomenex C8 prep column with an eluent consisting of solvent A (H₂O / 0.1% TFA) and solvent B (MeOH / 0.1% TFA) using a 60 min gradient transitioning from 5% B to 100% B. The purity of the peptides were verified by analytical RP-HPLC using a Phenomenex C4 column with an eluent consisting of solvent A (H₂O /0.01% TFA) and solvent B (CH₃CN / 0.01% TFA) with a 30 min gradient transitioning from 5% B to 100% B at a flow rate of 3 ml/min and monitored at 220nm and molecular weight was confirmed using electrospray ionization mass spectrometry.

General Synthesis for Fluorescent-Maleimide-Linkers and Antigen-Maleimide-Linkers (solid phase). A 25ml synthetic flask was charged with 250 mg (0.12 mmol) of Rink Amide Resin. The resin was initially washed with DMF (3 x 5 ml) and deprotected with 6 M piperazine/100 mM HOBT. The flask was agitated for 25 min and the deprotection solution was drained. The resin was washed with DMF, CH₂Cl₂, MeOH, CH₂Cl₂, and DMF (2 x 5 ml each). Initial loading was performed by the addition of N^α-Fmoc-N^ε-methyltrityl-L-Lysine(3 equiv, 0.36 mmol), HBTU (3 equiv, 0.36 mmol), and DIEA(6 equiv, 0.72 mmol) in DMF to the reaction flask and was agitated for 2 hours at room temperature. Addition of Fmoc-protected amino acids was carried out using the afore mentioned protocol to produce linker molecules constituted of Gly-Gly-Ser amino acid repeating units of differing length. Once the desired linker length was achieved, the Fmoc-protecting group of the terminal glycine was then removed with 6M piperazine/100 mM HOBT solution by agitating the flask for 25 minutes. The protecting solution was then drained and the resin was washed with DMF, CH₂Cl₂, MeOH, CH₂Cl₂, and DMF (2 x 5 ml each). Subsequently, NBD-Chloride or DNFB, (5 equiv, 0.60 mmol) with DIEA (7.5 equiv 0.90 mmol) in DMF was added to the resin and agitated for 1 hour protected from light to conjugate the either the NBD fluorophore or DNP antigen. Following addition of the fluorophore or hapten, the solution was drained and washed as stated before. The N^ε-methyltrityl protecting group of the lysine was then deprotected by adding a 10mls of a TFA cocktail solution (1% TFA, 2% TIPS in CH₂Cl₂) to the resin and agitating for 10 mins protected from light. The

solution was drained and this procedure was repeated four additional times. The solution was then drained and washed as previously stated. Upon MTT deprotection, 3-maleimidopropionic acid (3 equiv, 0.36 mmol), HBTU (0.36 equiv, 0.36 mmol), and DIEA (6 equiv, 0.72 mmol) in DMF was added to the resin. The peptides were cleaved by adding a trifluoroacetic acid (TFA) cocktail solution (95%, TFA, 2.5% TIPS, and 2.5 % H₂O) to the resin and the mixture was agitated for 1 h at room temperature protected from light. The resulting solution was concentrated *in vacuo* to remove the TFA. The residue was triturated in cold diethyl ether and the precipitate was collected by centrifugation. Peptides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC) using a Phenomenex C8 prep column with an eluent consisting of solvent A (H₂O / 0.1% TFA) and solvent B (MeOH / 0.1% TFA) using a 60 min gradient transitioning from 5% B to 100% B. The purity of the peptides were verified by analytical RP-HPLC using a Phenomenex C4 column with an eluent consisting of solvent A (H₂O /0.01% TFA) and solvent B (CH₃CN / 0.01% TFA) with a 30 min gradient transitioning from 5% B to 100% B at a flow rate of 3 ml/min and monitored at 220nm and molecular weight was confirmed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

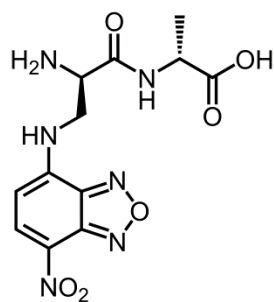
Compound Characterization

Compound 1:



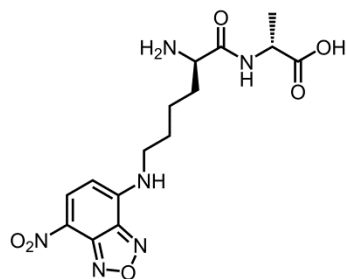
MS (ESI) $[M+H]^+$: 310.3 (calculated), 310.3 (found)

Compound 2:



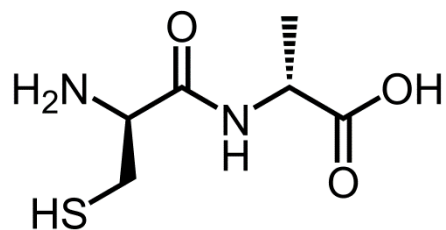
MS (ESI) $[M+H]^+$: 339.3(calculated), 339.4 (found)

Compound 3:



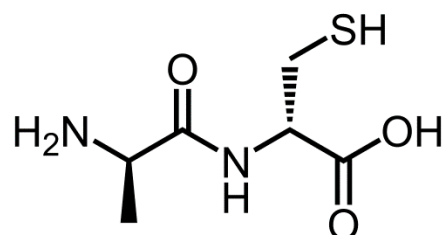
MS (ESI) $[M+H]^+$: 381.4(calculated), 381.2 (found)

Compound 4



MS (ESI) [M+H⁺]: 193.3 (calculated), 193.3 (found)

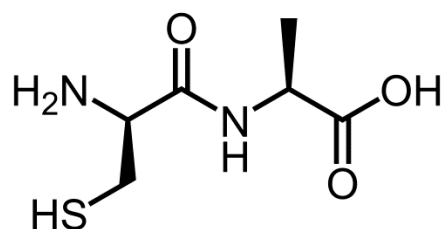
Compound 5



MS (ESI) [M+H⁺]: 193.3 (calculated), 193.3 (found)

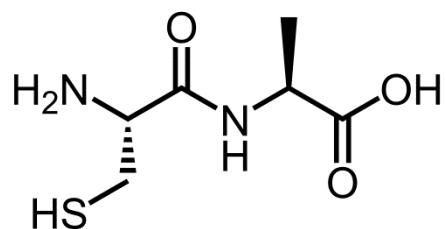
Compound 6: Purchased from ChemImpex and used without any further synthesis or purification.

Compound 7



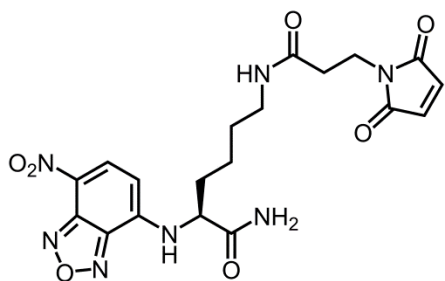
MS (ESI) [M+H⁺]: 193.3 (calculated), 193.3 (found)

Compound 8



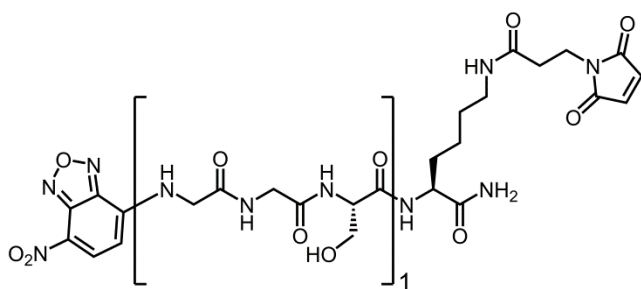
MS (ESI) [M+H⁺]: 193.3 (calculated), 193.3 (found)

Compound FL0



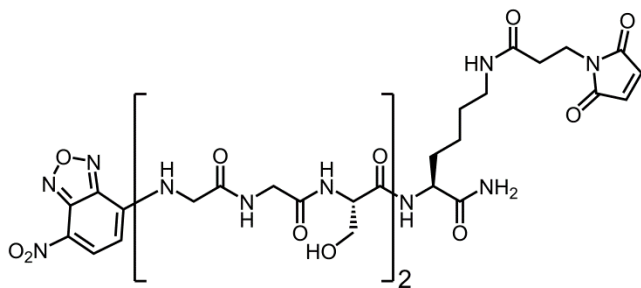
MS (ESI) [M+H⁺]: 460.2 (calculated), 460.2 (found)

Compound FL1



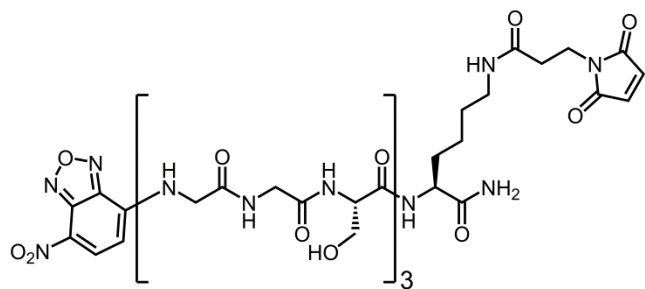
MALDI-TOF [M+Na⁺]: 683.6 (calculated), 683.5 (found)

Compound FL2



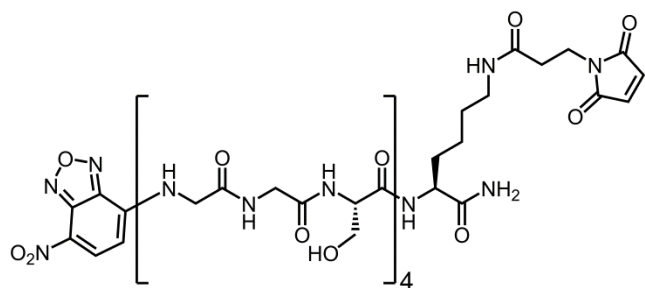
MALDI-TOF [M+Na⁺]: 886.8 (calculated), 886.5 (found)

Compound FL3



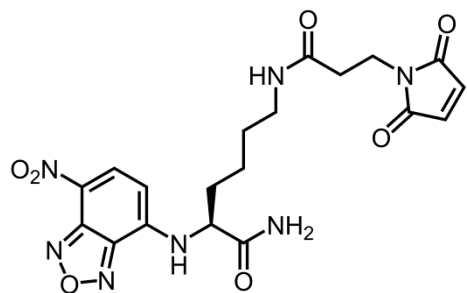
MALDI-TOF $[M+Na^+]$: 1086.0 (calculated), 1086.4 (found)

Compound FL4



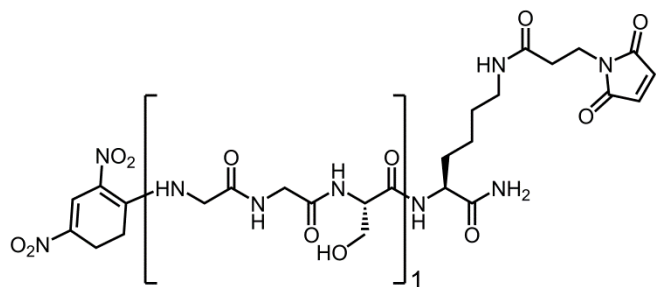
MALDI-TOF $[M+Na^+]$: 1287.2 (calculated), 1287.1 (found)

Compound DL0



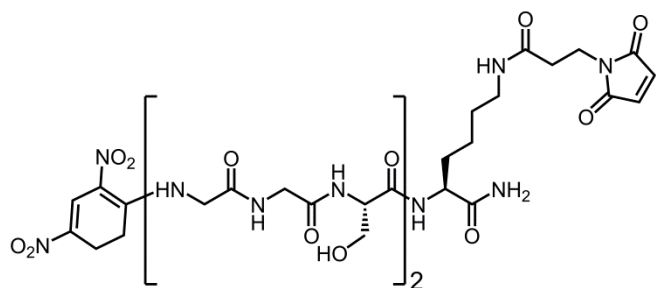
MS (ESI) $[M+H^+]$: 463.4 (calculated), 463.2 (found)

Compound DL1



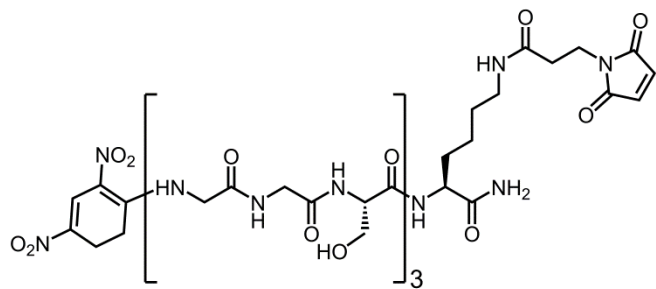
MALDI-TOF [$M+Na^+$]: 686.6 (calculated), 686.7 (found)

Compound DL2



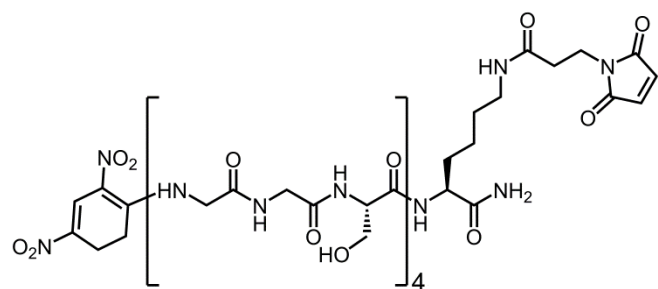
MALDI-TOF [$M+H^+$]: 887.8 (calculated), 887.7 (found)

Compound DL3



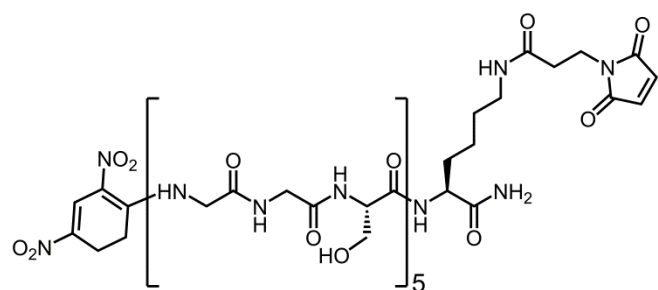
MALDI-TOF [$M+Na^+$]: 1089.0 (calculated), 1088.4 (found)

Compound DL4



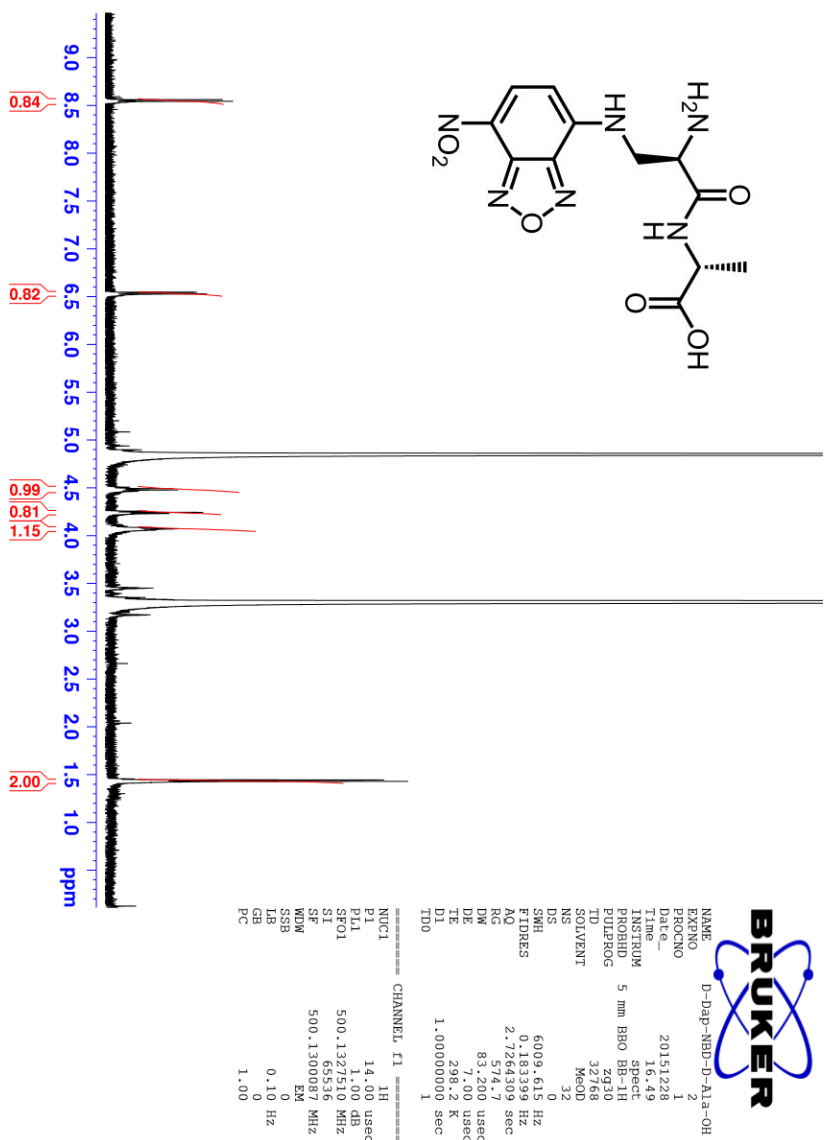
MALDI-TOF $[M+Na^+]$: 1290.1(calculated), 1290.3(found)

Compound DL5



MALDI-TOF $[M+Na^+]$: 1491.3 (calculated), 1489.0 (found)

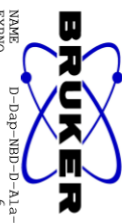
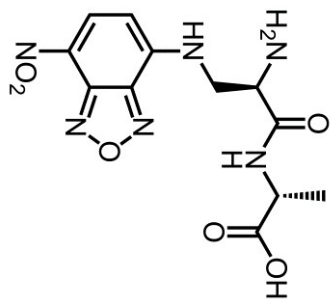
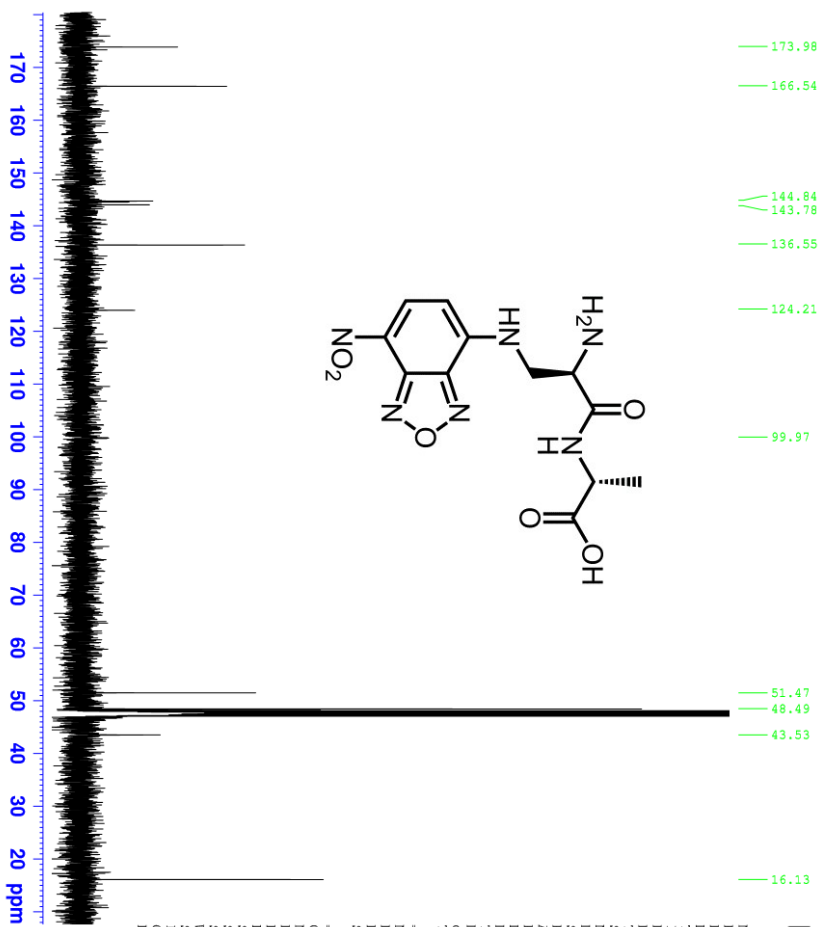
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 FWHM 0.18320 Hz
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 RG 574.7
 DE 83.200 usec
 TE 298.2 K
 D1 1.00000000 sec
 TD0 1

CHANNEL f1
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 P1 14.00 usec
 PL1 1.00 dB
 SFO1 500.1327510 MHz
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 EX
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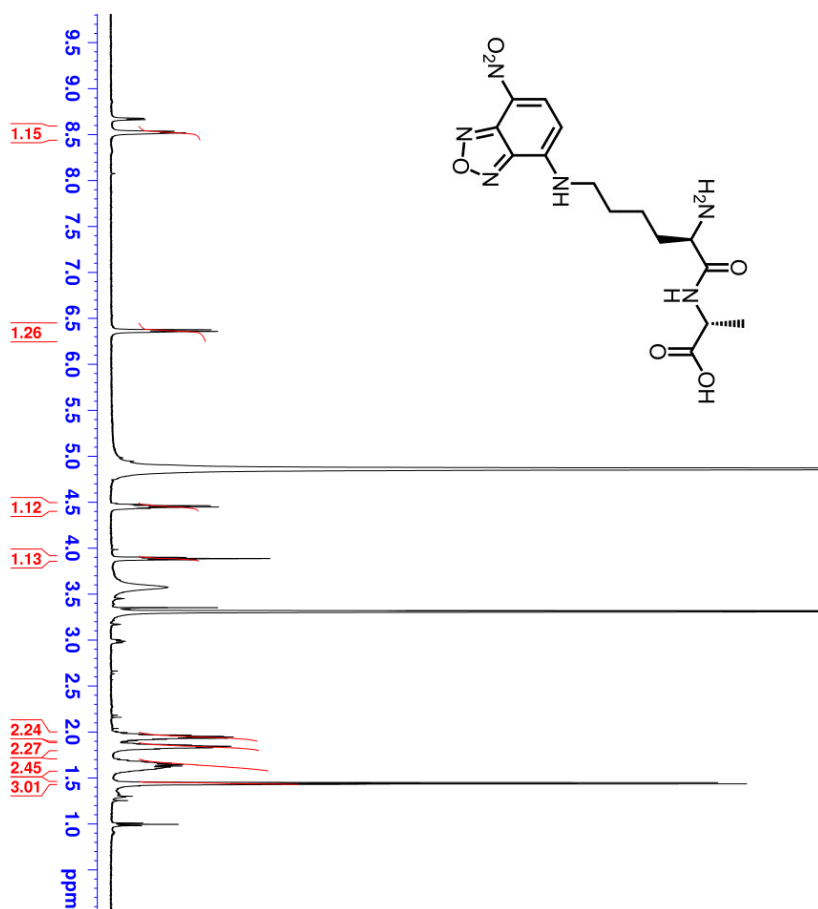


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 SWH 0.754852 Hz
 FIDRES 0.6537915 sec
 AQ 19.950
 FWH 19.950 usec
 DE 7.00 usec
 TE 296.9 K
 D1 2.0000000 sec
 d11 0.03000000 sec
 TD0 1

===== CHANNEL f1 =====
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 P1 8.60 usec
 PL1 1.00 dB
 SFO1 125.7703648 MHz

===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 100.00 usec
 F1Z 17.00 dB
 F1Z2 17.00 dB
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Compound 3: 1H-NMR

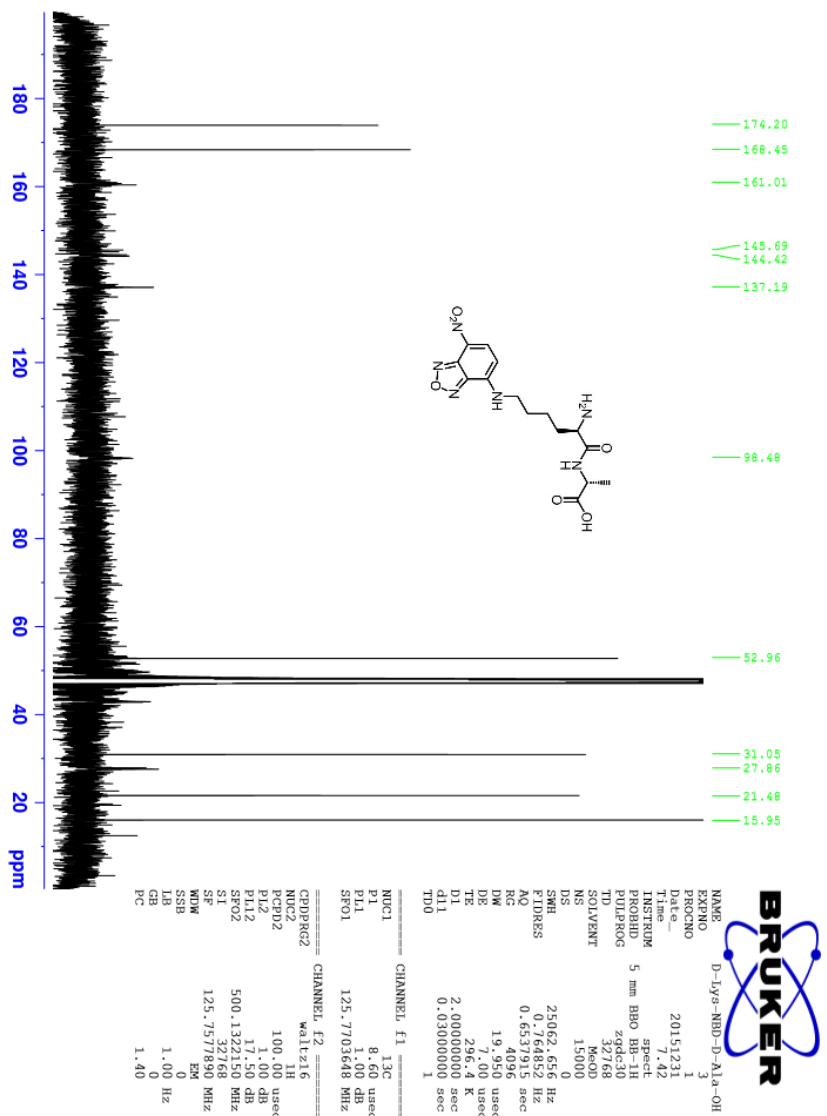


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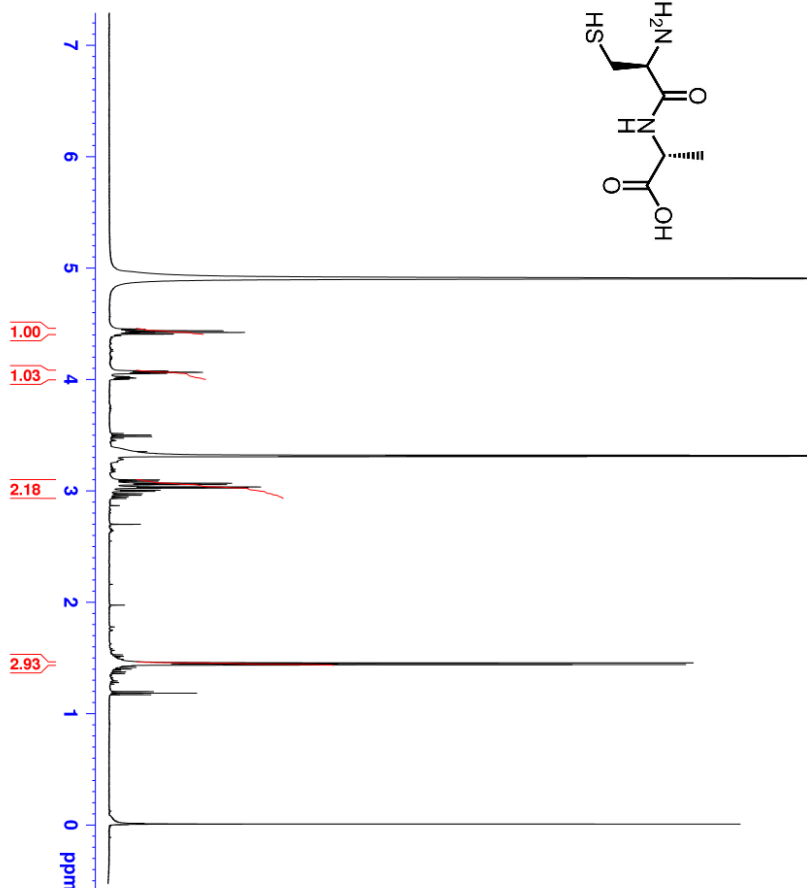
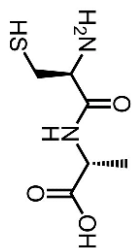
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 FIDRES 0.183399 Hz
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 RG 362
 DW 83.200 usec
 DE 2.00 usec
 TE 300.2 K
 TI 1
 TDO 1.00000001

CHANNEL F1
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 PL1 1.00 dB
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Compound 3: 13C-NMR



Compound 4: 1H-NMR



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 PROCNO 1
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 Time 18.36
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 TD 32768
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 TDO 1

===== CHANNEL f1 =====
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 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

Compound 4: 13C-NMR

