Electronic Supplementary Material (ESI) for ChemComm.
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Materials and Methods

Materials. All peptide related reagents (resin, coupling reagent, deprotection reagent, amino acids, and cleavage reagents) were purchased from ChemImpex. D-allylglycine was purchased directly from ChemImpex. Cy5-Methyl Tetrazine and Cy5-TCO were purchased from Click Chemistry Tools. 5-norbornene-2-carboxylic acid was purchased from Alfa Aesar. All other reagents were purchased from Sigma and were used without further purification. The bacterial strain used for the experiments was *Staphylococcus aureus* (*S. aureus*) SCO1.

Bacterial Peptidoglycan Labeling. Lysogeny broth (LB) containing 3 mM D-allylglycine, D-Dap-NB-OH, D-Dap-NB-NH_2, or D-Lys-NB-OH were prepared. *S. aureus* was inoculated (1:100) in the corresponding medias and allowed to grow overnight at 37 ^oC with shaking in a 96-well plate. The bacteria were harvested at 1,000*g* for 3 min. and washed three times with original culture volume with 1x phosphate buffered saline (PBS) followed by fixation with 2% formaldehyde in 1x PBS for 30 min. at room temperature. The formaldehyde was removed with three washes of 1x PBS. The bacteria were then suspended in half the volume of the original culture with 20 μM Cy5-Methyl Tetrazine in 1x PBS. The bacteria were shaken for 15 hr at 37 ^oC, harvested at 1,000*g* for 3 min., and washed three times with 1x PBS. Fluorescence of the samples were then analyzed using a BDFacs Canto II flow cytometer for cells were analyzed using a BDFacs Canto II flow cytometer (BD Biosciences, San Jose, CA) equipped with a 633nm HeNe laser (L1) and a 660/20 band-pass filter (FL5). A minimum of 10,000 events were counted for each data set. The data was analyzed using the FACSDiva version 6.1.1 software. Fluorescent imaging of the D-Dap-NB-NH_2 labeled bacteria was analyzed on a glass slide using a Cy5 HYQ (Nikon 96324; Exc.590-650/Em.663-738) filter.

Tetrazine-Cy5 Live-Cell Peptidoglycan Labeling. LB medium containing 3 mM D-Dap-NB-NH_2 was prepared. *S. aureus* was inoculated (1:100) in the corresponding medium and allowed to grow overnight at 37 ^oC with shaking in a 96-well plate. The bacteria were harvested at 1,000*g* for 3min. and washed three times with original culture volume with 1x PBS. The cells were then immediately suspended in half the volume of the original culture with 20 μM Cy5-Methyl Tetrazine in 1x PBS. A portion of the cells were taken at 30, 60, 120, 180, 240, and 360 min. At each interval, the collected cells were washed three times with 1x PBS followed by immediate fixation with 2% formaldehyde in 1x PBS for 30 min at room temperature. The formaldehyde was removed with three washes of 1x PBS. Samples were then analyzed using a BDFacs Canto II flow cytometer using the previously stated parameters.

TCO-Cy5 Live-Cell Peptidoglycan Labeling. LB medium containing 3 mM D-Dap-Tet-NH_2 was prepared. *S. aureus* was inoculated (1:100) in the corresponding medium and allowed to grow overnight at 37 ^oC with shaking in a 96-well plate. The bacteria were harvested at 1,000*g* for 3 min. and washed three times with original culture volume with 1x PBS. The cells were then immediately suspended in half the volume of the original culture with 50 μM TCO-Cy5 in 1x PBS. A portion of the cells were taken at 5, 15, 30, 45, 60, 120, 180, 240, 300, and 360 min. At each interval, the collected cells were washed three times with 1x PBS followed by immediate fixation with 2% formaldehyde in 1x PBS for 30 min at room temperature. The formaldehyde was removed with three washes of 1x PBS. Samples were then analyzed using a BDFacs Canto II flow cytometer using the previously stated parameters.

***S. aureus* viability.** LB medium containing 3 mM D-Dap-Tet-NH_2 or D-Dap-NB-NH_2 were prepared. *S. aureus* was inoculated (1:100) in the corresponding medium and allowed to grow overnight at 37 °C with shaking in a 96-well plate. 100 uL of the bacterial growth was diluted in 900 uL of LB and the optical density at 600 nm was measured.

Stereospecificity of incorporation. LB medium containing 3 mM D-Dap-NB-NH_2 or L-Dap-NB-NH_2 was prepared. *S. aureus* was inoculated (1:100) in the corresponding medium and allowed to grow overnight at 37 °C with shaking in a 96-well plate. The bacteria were harvested at 1,000*g* for 3 min. and washed three times with original culture volume with 1x phosphate buffered saline (PBS) followed by fixation with 2% formaldehyde in 1x PBS for 30 min. at room temperature. The formaldehyde was removed with three washes of 1x PBS. The bacteria were then suspended in half the volume of the original culture with 20 μM Cy5-Methyl Tetrazine in 1x PBS. The bacteria were shaken for 15 hr at 37 °C, harvested at 1,000*g* for 3 min., and washed three times with 1x PBS. Samples were then analyzed using a BDFacs Canto II flow cytometer using the previously stated parameters.

Incorporation of D-amino acids via competition assay. LB medium containing 500 μM D-allylglycine, D-Dap-NB-OH, D-Dap-NB-NH_2, or D-Lys-NB-OH were prepared. To each sample, D-lysine-nitrobenzoxadiazole (D-Lys-NBD-OH) was added for a final concentration of 100 μM. *S. aureus* was inoculated (1:100) in the corresponding medias and allowed to grow overnight at 37 °C with shaking in a 96-well plate. The bacteria were harvested at 1,000*g* for 3 min. and washed three times with original culture volume with 1x phosphate buffered saline (PBS) followed by fixation with 2% formaldehyde in 1x PBS for 30 min. at room temperature. The formaldehyde was removed with three washes of 1x PBS. Samples were then analyzed using a BDFacs Canto II flow cytometer using a 488nm argon laser (L1) and a 530/30 band-pass filter (FL1). A minimum of 10, 000 events were counted for each data set.

Peptidoglycan Isolation. *S. aureus* SCO1 bacteria (50 mL) were grown at 37 °C OD_{600} 0.6 in LB medium, at which point the medium was replaced with LB medium supplemented with 3 mM of D-Dap-NB-NH_2. The cells were allowed to incubate at 37 °C overnight in this medium before being harvested and washed with 1x phosphate buffer saline (PBS) (3 × 50 mL each). The cells were then resuspended in 1x PBS and boiled for 7 min and then centrifuged at 14,000*g* for 8 min at 4 °C. Cells were then placed in 25 mL of 5% (w/v) sodium dodecyl sulfate (SDS) and boiled for 25 min followed by centrifugation at 14,000*g* for 8 min at 4 °C. Following centrifugation, cells were boiled again in 25 mL of 4% (w/v) SDS for 15 min followed by centrifugation using same parameters as before. Cells were then washed 5 times with 60 °C DI water to remove all SDS. After washing, cells were incubated in 6 mL of 50 mM Tris HCl and 2 mg mL^{-1} Proteinase K for 1 h at 60 °C, and then washed 3 times with DI water. The cell wall pellet was then resuspended and digested with 250 μg/mL lysozyme in 25 mM sodium phosphate buffer pH 5.6 for 15 h at 37 °C. The digestion was then ceased by boiling for 3 min. The sample was then centrifuged at 14,000*g* for 8 min, the supernatant was retained and concentrated *in vacuo*. The labeled peptidoglycan was purified using PerkinElmer Series 200 HPLC. The purified D-Dap-NB-NH_2 labeled peptidoglycan was analyzed using a Bruker Microflex MALDI-TOF MS.

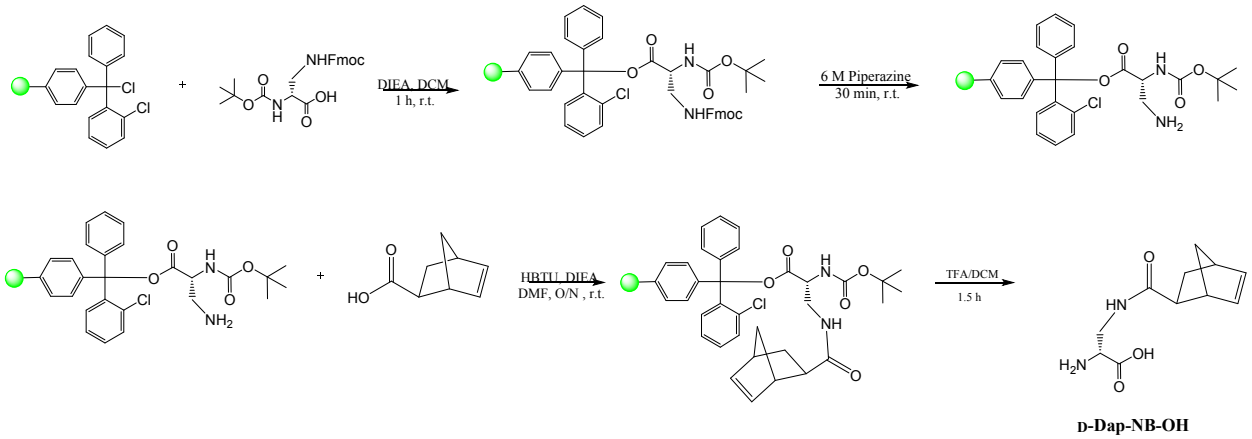
Reaction Analyses by Analytical RP-HPLC. The specified amino acid derivatives were analyzed using a PerkinElmer Series 200 reverse phase HPLC on a Phenomenex C4 column with an eluent

consisting of solvent A (H_2O /0.01% TFA) and solvent B (CH_3CN /0.01% TFA) with a 60 minute gradient consisting of 5 to 100 % B, a flow rate of 3 mL/min and monitoring at 220 nm. (λ_{220}). The standard solution was 3,6-Diphenyl-1,2,4,5-tetrazine (200 µM) and internal standard benzophenone (400 µM) in MeOH. 50 mM D-allylglycine, D-Dap-NB-OH, D-Dap-NB-NH_2, or D-Lys-NB-OH in MeOH were reacted with 3,6-Diphenyl-1,2,4,5-tetrazine (200 µM) and internal standard benzophenone (400 µM) in MeOH. The reactions were stirred for 6 hr at 37 °C and then analyzed by RP-HPLC.

Compound Synthesis and Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX500 (500MHz) NMR spectrometer. ^{1}H NMR spectra are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons.

Scheme S1. Synthesis of D-Dap-NB-OH.



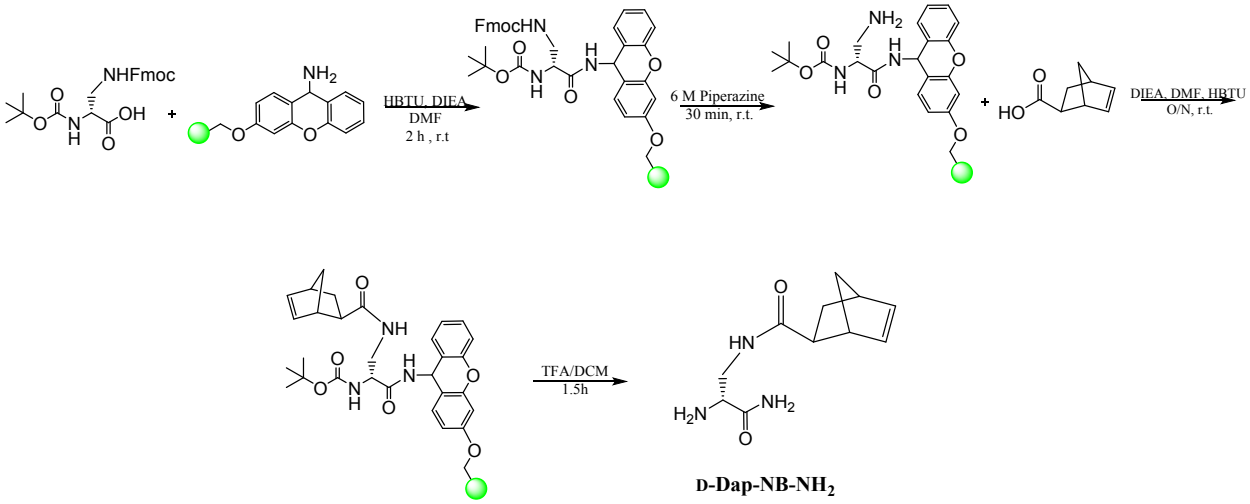
D-Dap-NB-OH

A 25 mL synthetic flask charged with 2-Chlorotrityl Resin (500 mg, 0.72 mmol) was initially washed with CH_2Cl_2 (3 x 10 mL) and DMF (3 x 10 mL). To the resin was added N^{α}-Boc-N^{β}-Fmoc-D-2,3-diaminopropionic acid (3 eq, 914 mg, 2.15 mmol), HBTU (3 eq, 813 mg, 2.15 mmol), and DIEA (6 eq, 0.750 mL, 4.29 mmol) in DMF (10 mL) and agitated for 2 h at room temperature. The resin was washed with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, and DMF (3 x 10 mL each). The Fmoc protecting group was removed with 6 M piperazine/100 mM HOBt in DMF (15 ml) for 30 min at room temperature. The resin was washed and a solution of 5-norbornene-2-carboxylic acid (5 eq, 493 mg, 3.57 mmol), HBTU (5 eq, 1.36 g, 3.57 mmol) and DIEA (10 eq, 1.25 mL, 7.18 mmol) in DMF (10 mL) was added to the resin and agitated overnight at room temperature, afterwards the resin was washed as before. A solution of TFA/DCM (1:1, 25 mL) was added to the resin and agitated for 1 h at room temperature. The resin was filtered and the resulting solution was concentrated *in vacuo*. The residue was trituated with cold diethyl ether to yield **D-Dap-NB-OH**.

^{1}H NMR (500 MHz, CD_3OD) : δ 6.15 (m, 1H), 5.90 (m 1H), 4.08 (m, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 3.33 (m, 1H), 3.15 (m, 1H), 2.95 (m, 1H), 2.90 (m , 1H), 1.85 (m, 1H), 1.40 (m, 1H), 1.35 (m, 1H).

^{13}C NMR (500 MHz, CD_3OD) : δ 178.02, 168.96, 137.57, 131.46, 53.72, 49.72, 46.14, 44.03, 42.98, 38.97, 29.07.
MS (ESI) [M+H^+]: 225.2 (calculated) ; 225.4 (found)

Scheme S2. Synthesis of D-Dap-NB-NH_2.

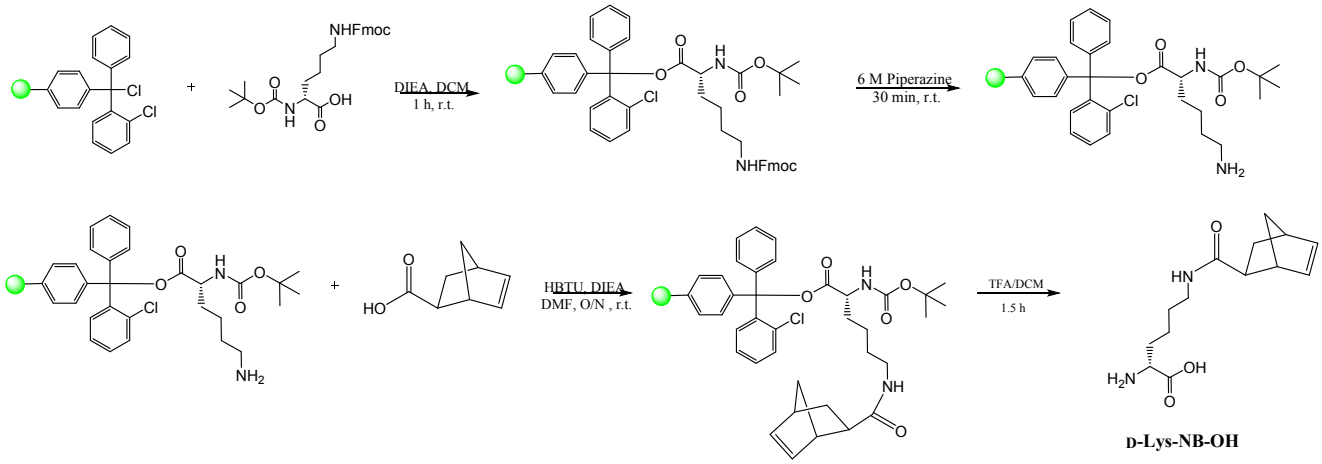


D-Dap-NB-NH_2

A 25 mL synthetic flask was charged with Sieber Amide Resin (1.00 g, 0.41 mmol) and washed with CH_2Cl_2 (3 x 10 mL) and DMF (3 x 10 mL). N^{α}-Boc-N^{β}-Fmoc-D-2,3-diaminopropionic acid (3 eq, 523 mg, 1.23 mmol), HBTU (3 eq, 466 mg, 1.23 mmol), and DIEA (6 eq, 0.430 mL, 2.47 mmol) in DMF (15 mL) were added to the reaction flask and agitated for 2 h at room temperature. The resin was washed with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, and DMF (3 x 10 mL each). The Fmoc protecting group was removed with 6 M piperazine/100 mM HOBt in DMF (15 ml) for 30 min at room temperature, then the resin was washed as before. 5-norbornene-2-carboxylic acid (5 eq, 283 mg, 2.05 mmol), HBTU (5 eq, 776 mg, 2.05 mmol) and DIEA (10 eq, 0.70 mL, 4.10 mmol) in DMF (10 mL) was added to the resin and agitated overnight at room temperature, afterwards the resin was washed as before. A solution of TFA/DCM (1:1, 25 mL) was added to the resin and agitated for 1 h at room temperature. The resin was filtered and the resulting solution was concentrated *in vacuo*. The residue was trituated with cold diethyl ether to yield **D-Dap-NB-NH_2**.

^{1}H NMR (500 MHz, CD_3OD) : δ 6.13 (m, 1H), 5.92 (m 1H), 4.02 (m, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 3.33 (m, 1H), 3.15 (m, 1H), 2.95 (m, 1H), 2.90 (m , 1H), 1.85 (m, 1H), 1.40 (m, 1H), 1.35 (m, 1H).
^{13}C NMR (500 MHz, CD_3OD) : δ 177.70, 168.71, 137.30, 131.68, 53.72, 49.30, 46.56, 43.82, 42.77, 40.66, 28.65.
MS (ESI) [M+H^+]: 224.2 (calculated) ; 224.4 (found)

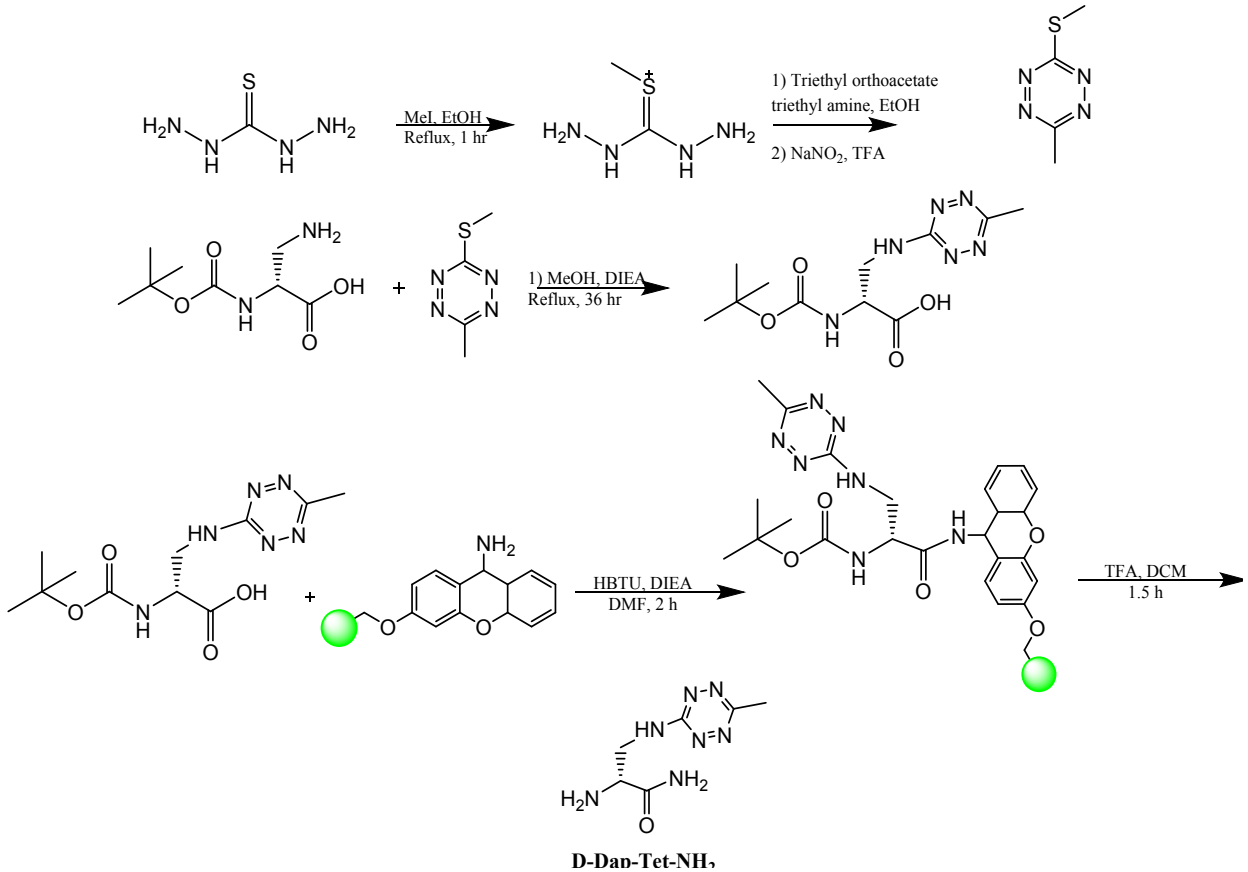
Scheme S3. Synthesis of D-Lys-NB-OH.



A 25 mL synthetic flask charged with 2-Chlorotrityl Resin (500 mg, 0.72 mmol) was initially washed with CH_2Cl_2 (3 x 10 mL) and DMF (3 x 10 mL). To the resin was added N^{α}-Boc-N^{ε}-Fmoc-D-lysine (3 eq, 1.00 g, 2.15 mmol), HBTU (3 eq, 813 mg, 2.15 mmol), and DIEA (6 eq, 0.750 mL, 4.29 mmol) in DMF (10 mL) and agitated for 2 h at room temperature. The resin was washed with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, and DMF (3 x 10 mL each). The Fmoc protecting group was removed with 6 M piperazine/100 mM HOBt in DMF (15 ml) for 30 min at room temperature. The resin was washed and a solution of 5-norbornene-2-carboxylic acid (5 eq, 493 mg, 3.57 mmol), HBTU (5 eq, 1.36 g, 3.57 mmol) and DIEA (10 eq, 1.25 mL, 7.18 mmol) in DMF (10 mL) was added to the resin and agitated overnight at room temperature, afterwards the resin was washed as before. A solution of TFA/DCM (1:1, 25 mL) was added to the resin and agitated for 1 h at room temperature. The resin was filtered and the resulting solution was concentrated *in vacuo*. The residue was trituated with cold diethyl ether to yield **D-Lys-NB-OH**.

^{1}H NMR (500 MHz, CD_3OD) : δ 6.18 (m, 1H), 5.89 (m 1H), 3.91 (m, 1H), 3.49 (m, 2H), 3.20 (m, 1H), 2.85 (m, 1H), 1.90 (m, 1H), 1.85 (m, 2H), 1.55 (m , 2H), 1.45 (m, 2H), 1.35 (m, 2H), 1.22 (m, 2H).
^{13}C NMR (500 MHz, CD_3OD) : δ 175.91, 170.64, 137.57, 131.88, 52.67, 49.51, 46.14, 44.24, 42.56, 38.55, 29.95, 28.65, 28.35, 22.33.
MS (ESI) [$M+H^+$]: 267.3 (calculated) ; 267.1 (found)

Scheme S4. Synthesis of D-Dap-Tet-NH_2.



Methyl-thiocarbohydrazide

Thiocarbohydrazide (4.98 g, 46.9 mmol) was dissolved in 200 mL of absolute ethanol and brought to reflux. MeI (3.2 mL, 1.1 eq) in 20 mL of absolute ethanol was added dropwise over 15 minutes and then refluxed for 1 hr with stirring. The solution was then filtered hot using a C type filter crucible and the filtrate was cooled to room temperature over 12 hr. The solution was decanted and the solid product was dried *in vacuo* to obtain methyl-thiocarbohydrazide.

Methyl-thiomethyl-tetrazine

Methyl-thiocarbohydrazide (6.10 g, 50.4 mmol) was dissolved in 150 mL absolute ethanol. Triethyl orthoacetate (10.1 mL, 1.1 eq.) was added, then after five minutes triethyl amine (7.0 mL, 1.0 eq.) was added. The reaction mixture was refluxed for 30 minutes to form an orange colored solution. $NaNO_2$ (3.43 g, 1.0 eq.) and TFA (1.87 mL, 1.0 eq) were added and the solution was refluxed for an additional 30 minutes. Hexane (150 mL) was added and the solution was cooled to room temperature followed by the addition of water (300 mL). The mixture was extracted with diethyl ether (3 x 100 mL). The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to ~5-10 mL of product. The oil was purified by silica gel column with EtOAc:Hexane to obtain methyl-thiomethyl-tetrazine.

Boc-D-Dap-Tet-OH

N^{α}-Boc-D-2,3-diaminopropionic acid (1.0032 g, 4.92 mmol) and methyl-thiomethyl-tetrazine (980 mg, 6.88 mmol) were dissolved in 20 mL of dry methanol and refluxed for 36 hr. The solution was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was dissolved in 50 mL EtOAc. The organic mixture was washed with 0.5 M NaOH (100 mL). The aqueous layer was then washed with EtOAc (3 x 50 mL). The aqueous layer was then acified with 1 M HCl to pH 5. The aqueous layer was then washed with EtOAc (3 x 50 mL). The organic layers were combined, dried with $MgSO_4$, and concentrated *in vacuo*. The product was purified by silica gel column with DCM:MeOH to obtain Boc-D-Dap-Tet-OH as a purple oil.

D-Dap-Tet-NH_2

A 25 mL synthetic flask was charged with Sieber Amide Resin (1.00 g, 0.41 mmol) and washed with CH_2Cl_2 (3 x 10 mL) and DMF (3 x 10 mL). Boc-D-Dap-Tet-OH (356 mg, 1.19 mmol), HBTU (451 mg, 1.19 mmol), and DIEA (0.42 mL, 2.41 mmol) in DMF (15 mL) were added to the reaction flask and agitated for 2 hr at room temperature. The resin was washed with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, and DMF (3 x 10 mL each). A solution of TFA/DCM (1:1, 25 mL) was added to the resin and agitated for 1 h at room temperature. The resin was filtered and the resulting solution was concentrated *in vacuo*. The residue was trituated with cold diethyl ether to yield **D-Dap-Tet-NH_2**.

^{1}H NMR (500 MHz, CD_3OD) : δ 4.10 (m, 1H), 2.15 (s, 3H), 1.30 (m, 2H),
^{13}C NMR (500 MHz, CD_3OD) : δ 169.59, 160.16, 159.88, 57.09, 53.93, 22.96.
MS (ESI) [$M+H^+$]: 198.1 (calculated) ; 198.0 (found)

Supporting Figures.

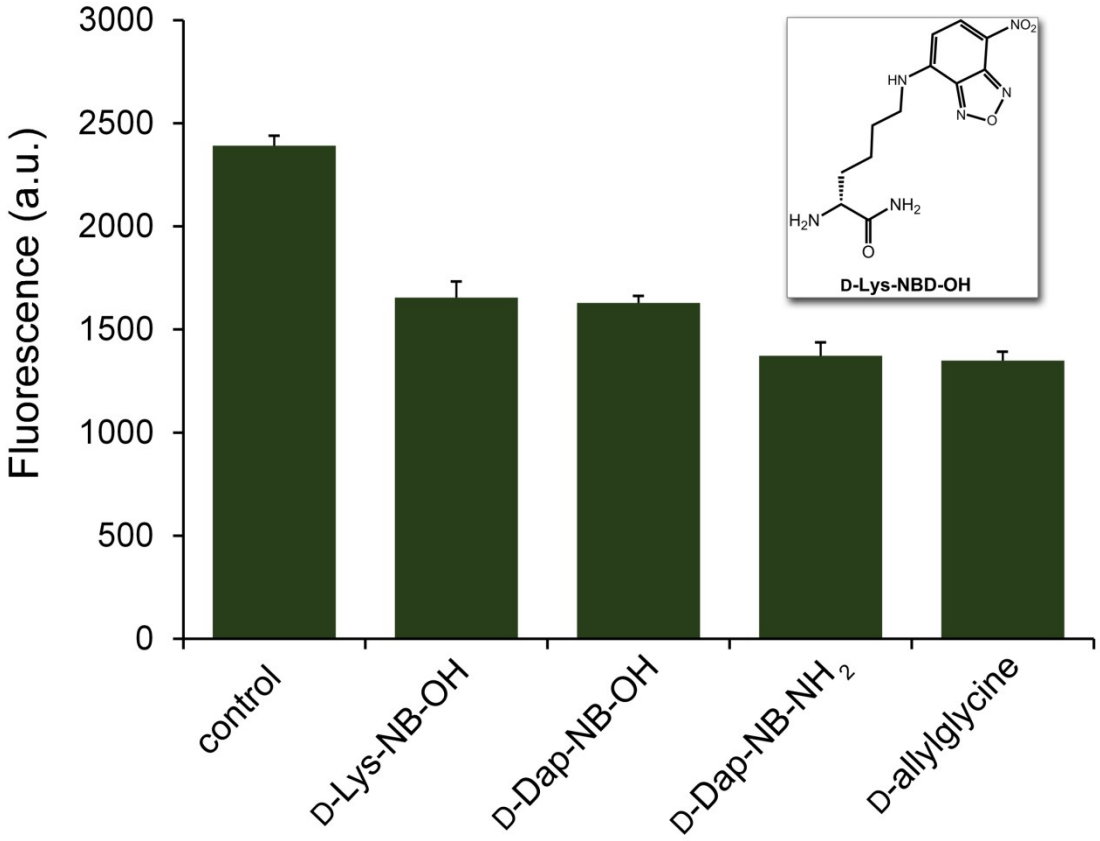


Figure S1. Incorporation of D-amino acids via competition assay. Flow cytometry analysis of *S. aureus* co-incubated overnight in the presence of 500 μM of D-allylglycine, D-Dap-NB-OH, D-Dap-NB-NH_2, or D-Lys-NB-OH and 100 μM D-Lys-NBD-OH. Data are represented as mean + SD (n=3). *Inset*: Chemical structure of D-Lys-NBD-OH.

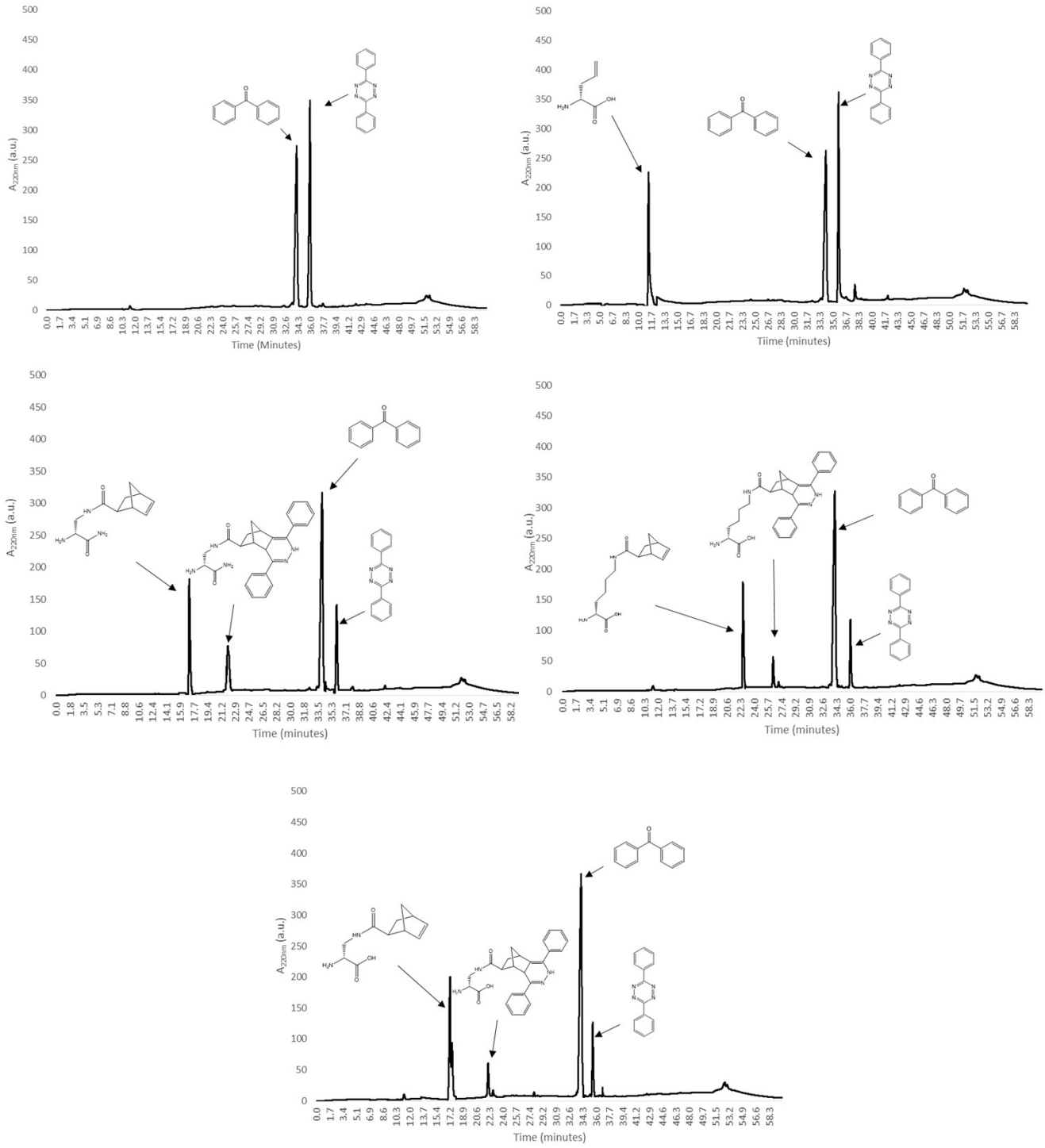


Figure S2. Reaction Analyses by Analytical RP-HPLC. Aliquots of D-allylglycine, D-Dap-NB-OH, D-Dap-NB-NH_2, or D-Lys-NB-OH in MeOH were reacted with 3,6-Diphenyl-1,2,4,5-tetrazine (200 μM) and internal standard benzophenone (400 μM) in MeOH. The reactions were allowed to incubate for 6 h at 37 °C and then analyzed by RP-HPLC.

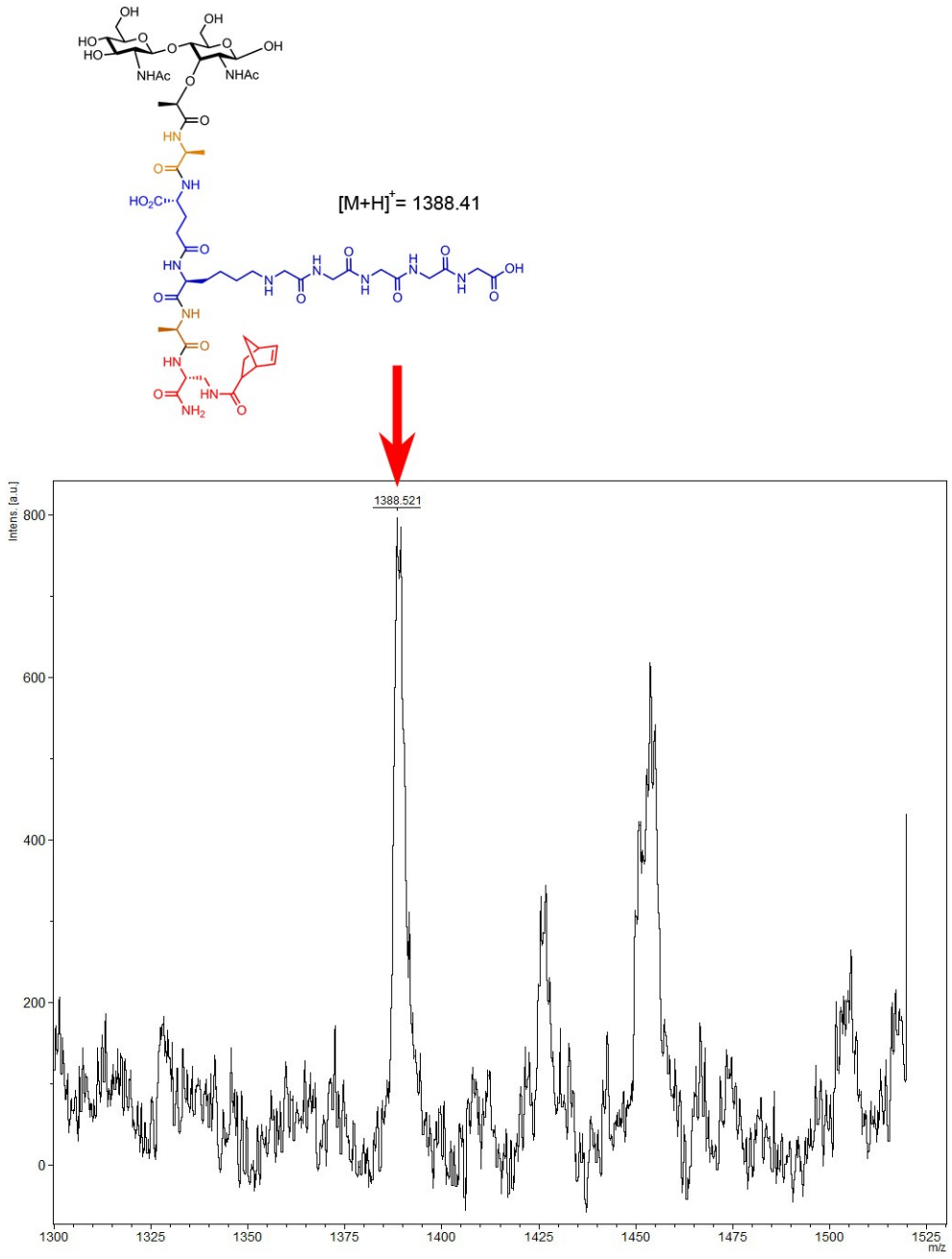


Figure S3. Peptidoglycan Isolation. (A) MALDI-TOF analysis of peptidoglycan isolated from *S. aureus* cells labeled with D-Dap-NB-NH_2 following RP-HPLC separation. (B) Structure of expected peptidoglycan repeating unit with the inclusion of D-Dap-NB-NH_2 and the expected molecular weight.

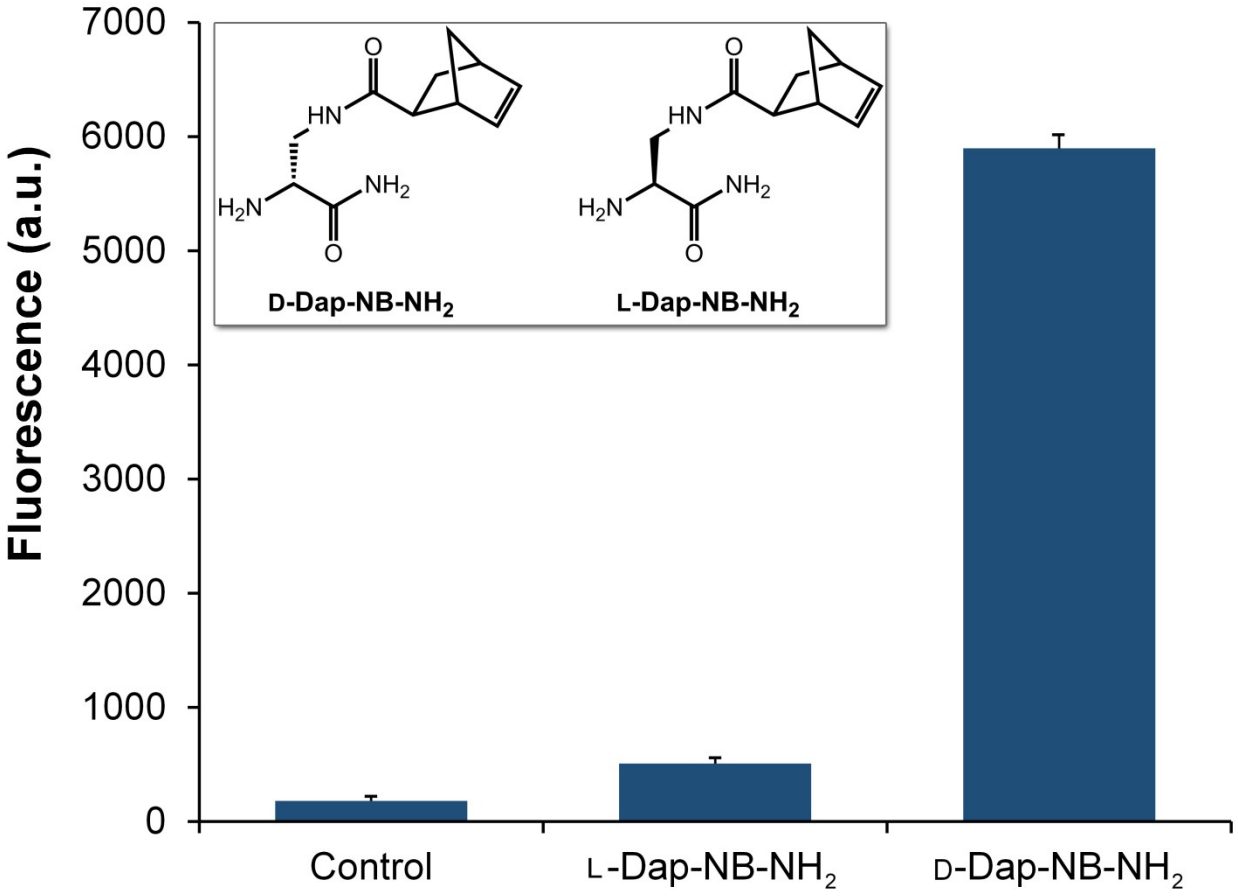


Figure S4. Stereospecificity of incorporation. Flow cytometry analysis of *S. aureus* incubated overnight in the presence of 3 mM of L-Dap-NB-NH_2 and D-Dap-NB-NH_2. Data are represented as mean + SD (n=3). *Inset*: Chemical structures of L-Dap-NB-NH_2 and D-Dap-NB-NH_2. (B)

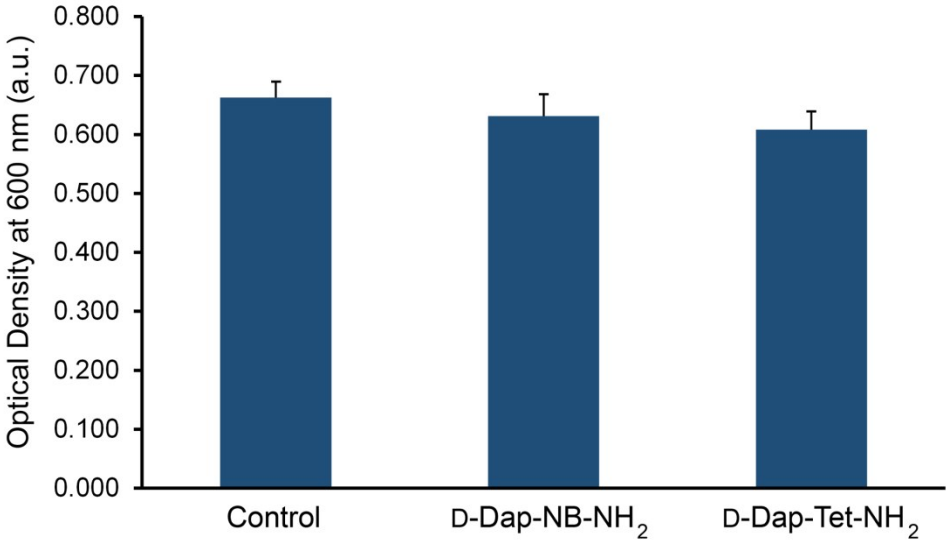
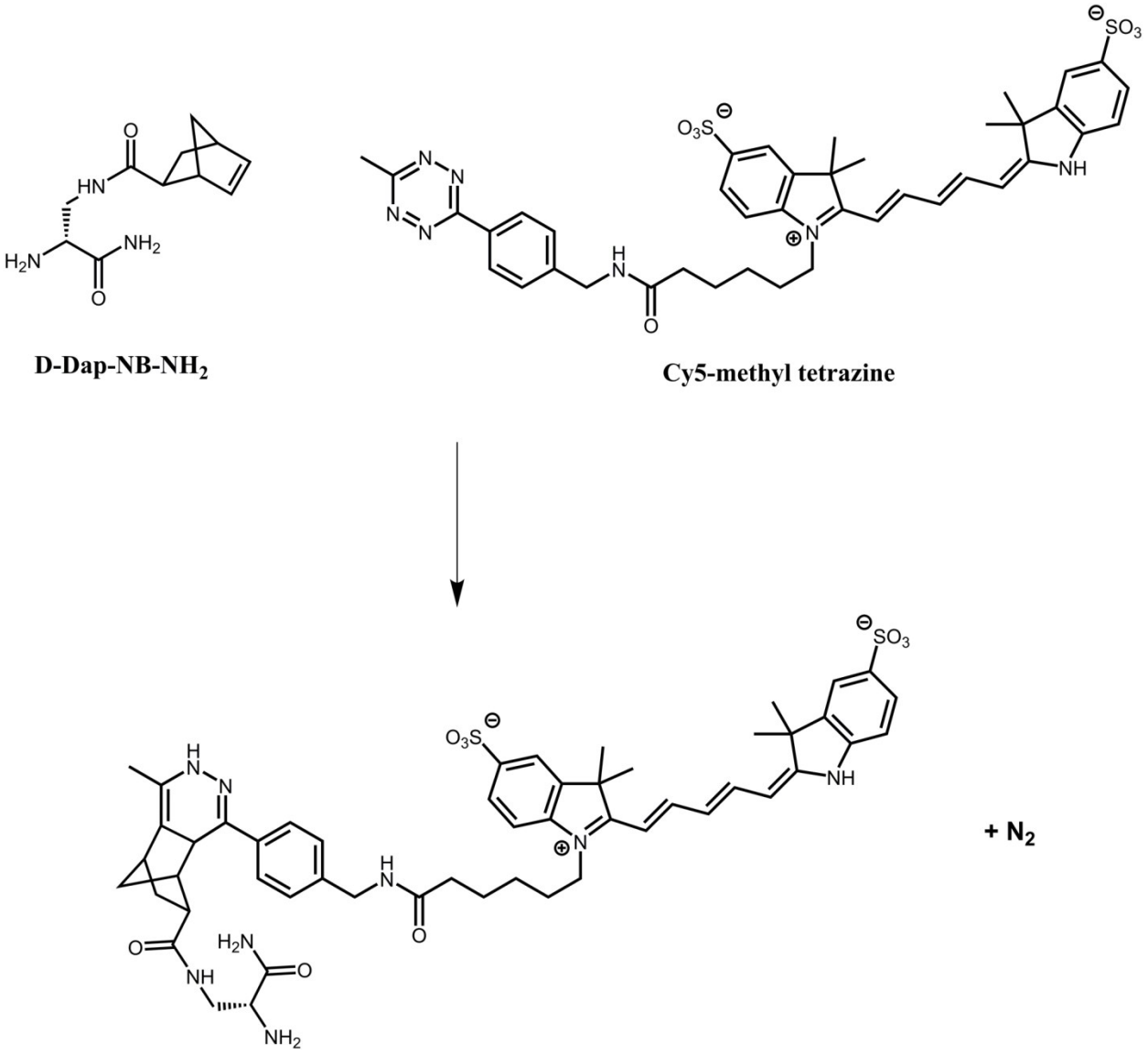
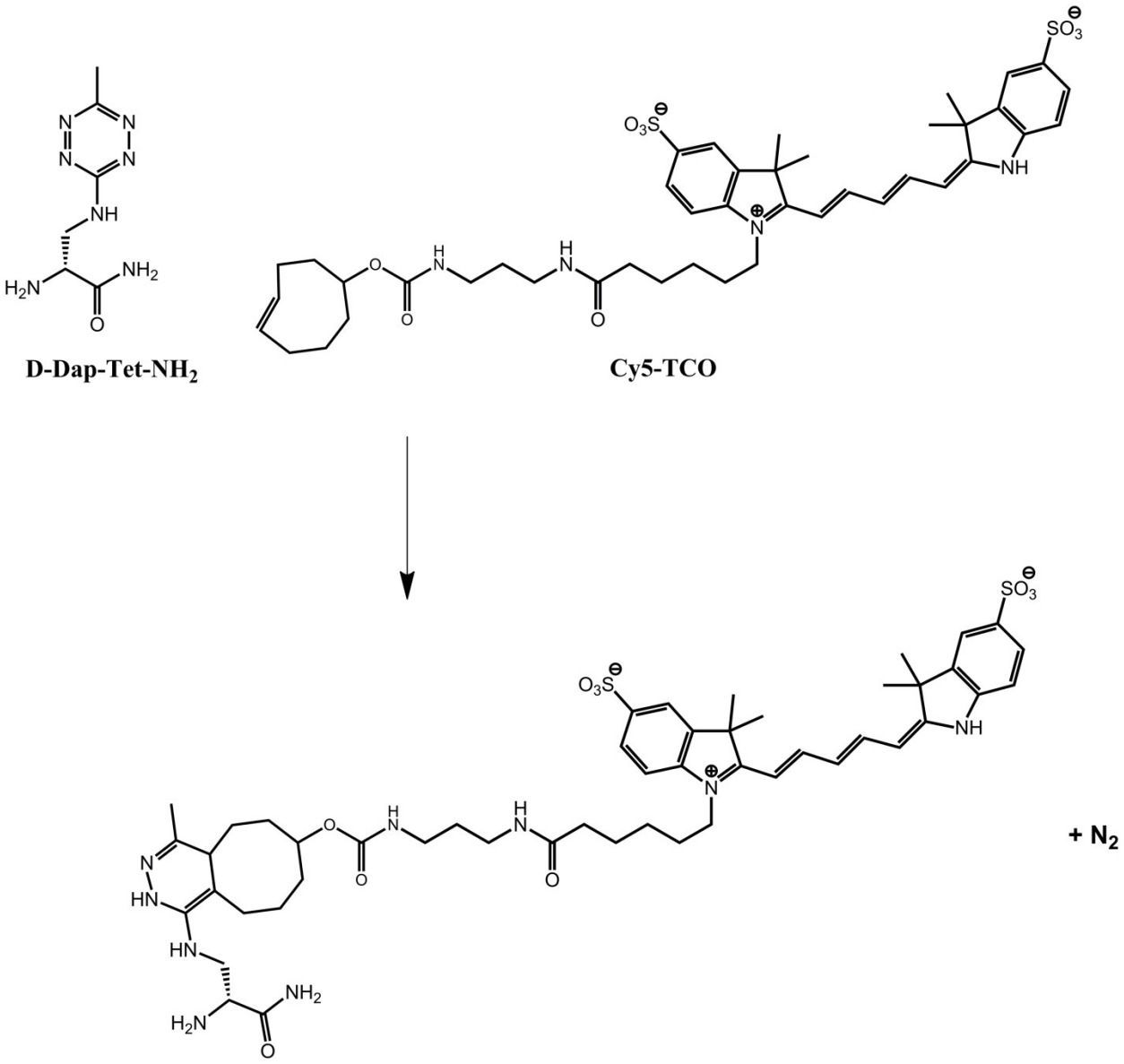


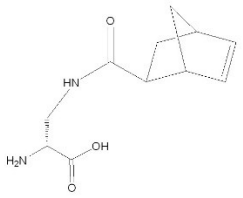
Figure S5. *S. aureus* viability. *S. aureus* were incubated overnight in the presence of 3 mM of unnatural D-amino acid or in LB alone. The next morning the optical density at 600 nm was measured for each condition. Data are represented as mean + SD (n=3).

Schematic diagram showing the reaction between the norbornene group and tetrazine conjugated to the fluorophore.

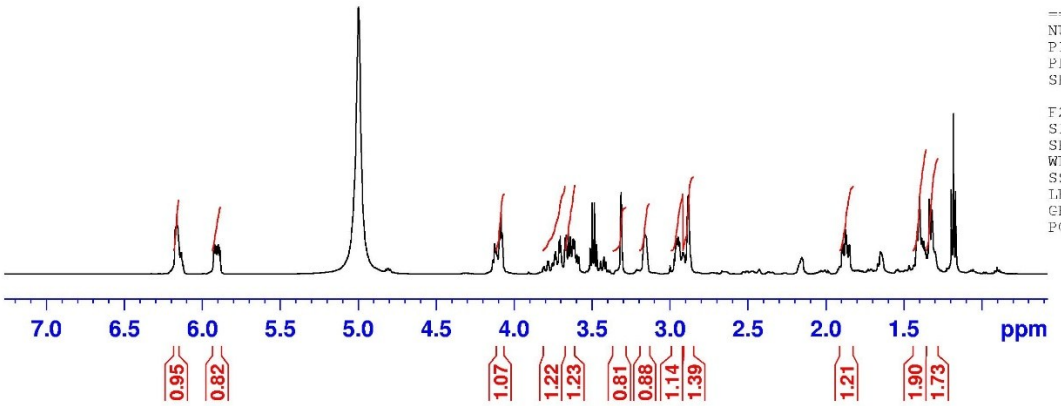


Schematic diagram showing the reaction between the tetrazine group and *trans*-cyclooctene conjugated to the fluorophore.





D-Dap-NB-OH

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PROCNO                      1

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TD                  32768
SOLVENT              MeOD
NS                     24
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FIDRES          0.183399 Hz
AQ              2.7264309 sec
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DE                  7.00 usec
TE                 295.7 K
D1            1.00000000 sec
TD0                     1

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PL1                 1.00 dB
SFO1         500.1327510 MHz

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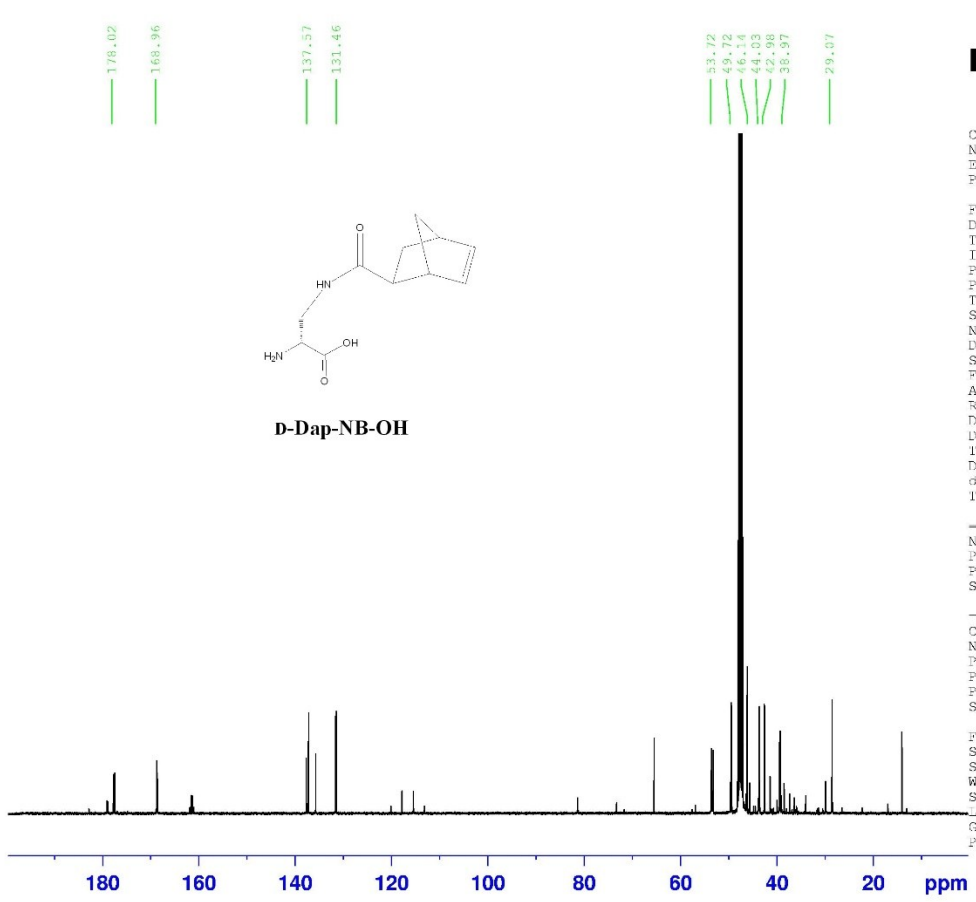

D-Dap-NB-OH

BRUKER

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D1             2.00000000 sec
d11            0.03000000 sec
D0                       1

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PL1                   1.00 dB
SFO1         125.7703648 MHz

-------- CHANNEL f2 --------
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PL2                   1.00 dB
PL12                 17.50 dB
SFO2         500.1322150 MHz

F2 - Processing parameters
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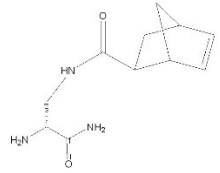
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```



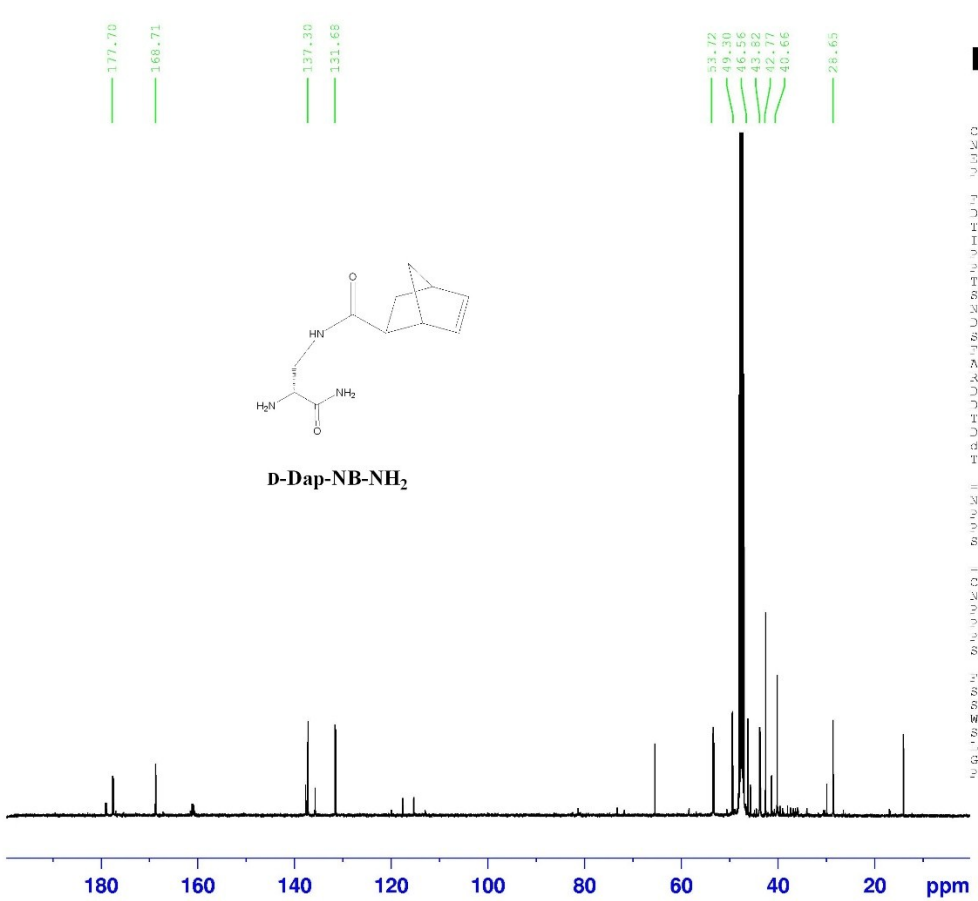
D-Dap-NB-NH_2


```
Current Data Parameters
NAME      D-Dap-Amide Norbornene
EXPNO                          1
PROCNO                         1

F2 - Acquisition Parameters
Date_              20150206
Time                   18.01
INSTRUM                spect
PROBHD    5 mm BBO BB-1H
PULPROG                zg30
TD                    32768
SOLVENT                MeOD
NS                       32
DS                        0
SWH                6009.615 Hz
FIDRES             0.183399 Hz
AQ                 2.7264309 sec
RG                      128
DW                   83.200 usec
DE                    7.00 usec
TE                   296.7 K
D1                 1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                     14.00 usec
PL1                     1.00 dB
SFO1           500.1327510 MHz

F2 - Processing parameters
SI                        65536
SF             500.1300087 MHz
WDW                       EM
SSB                        0
LB                      0.10 Hz
GB                         0
PC                      1.00
```



D-Dap-NB-NH$_2$

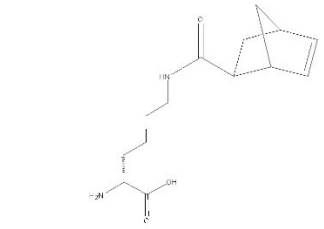
```
Current Data Parameters
NAME      D-Dap-Amide Norbornene
EXPNO                          3
PROCNO                         1

F2 - Acquisition Parameters
Date_               20150207
Time                    8.28
INSTRUM                 spect
PROBHD    5 mm BBO BB-1H
PULPROG              zgdc30
TD                      32768
SOLVENT                 MeOD
NS                      19000
DS                          0
SWH                 25062.656 Hz
FIDRES              0.764852 Hz
AQ                  0.6537915 sec
RG                       4096
DW                   19.950 usec
DE                    7.00 usec
TE                    297.5 K
D1                2.00000000 sec
d11               0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                       8.60 usec
PL1                      1.00 dB
SFO1              125.7703648 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  100.00 usec
PL2                      1.00 dB
PL12                    17.50 dB
SFO2              500.1322150 MHz

F2 - Processing parameters
SI                      32768
SF                125.7577890 MHz
WDW                        EM
SSB                         0
LB                       1.00 Hz
GB                          0
PC                       1.40
```



D-Lys-NB-OH

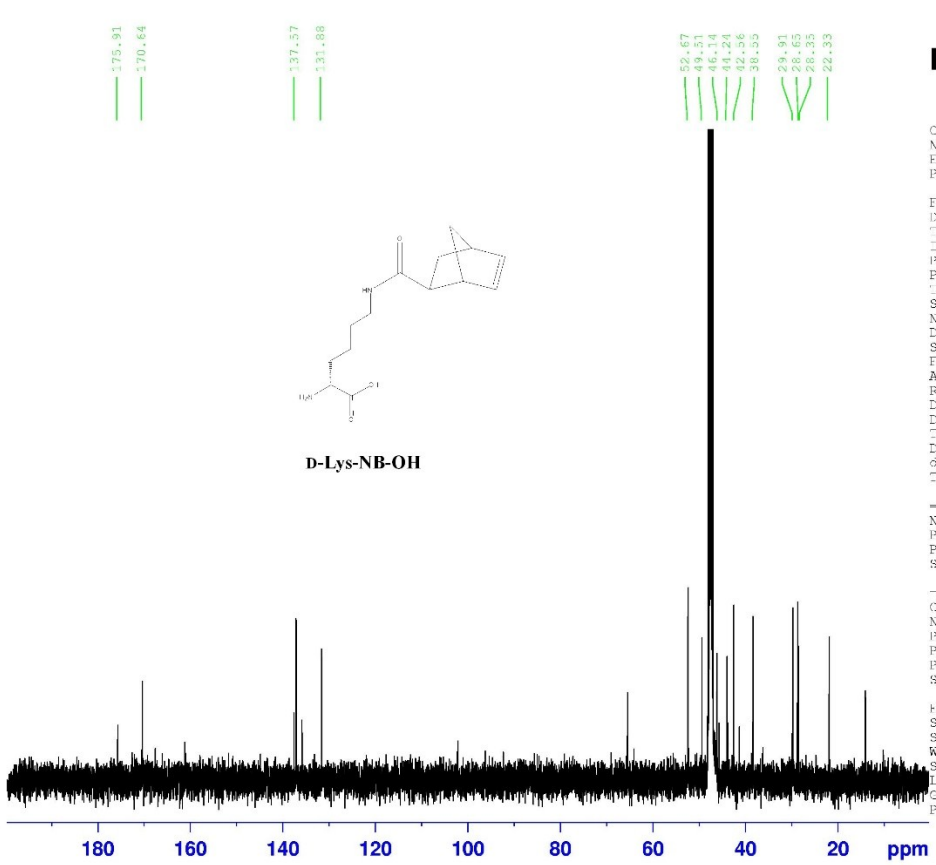
BRUKER

```
Current Data Parameters
NAME       D-Lys-OH Norbornene
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20150222
Time                     13.44
INSTRUM                  spect
PROBHD    5 mm BBO BB-1H
PULPROG                  zg30
TD                      32768
SOLVENT                  MeOD
NS                         32
DS                          0
SWH                  6009.615 Hz
FIDRES               0.183399 Hz
AQ                   2.7264309 sec
RG                       322.5
DW                     83.200 usec
DE                      7.00 usec
TE                      297.2 K
D1                   1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                        1H
P1                       14.00 usec
PL1                       1.00 dB
SFO1              500.1327510 MHz

F2 - Processing parameters
SI                        65536
SF                500.1300087 MHz
WDW                          EM
SSB                           0
LB                         0.10 Hz
GB                            0
PC                         1.00
```



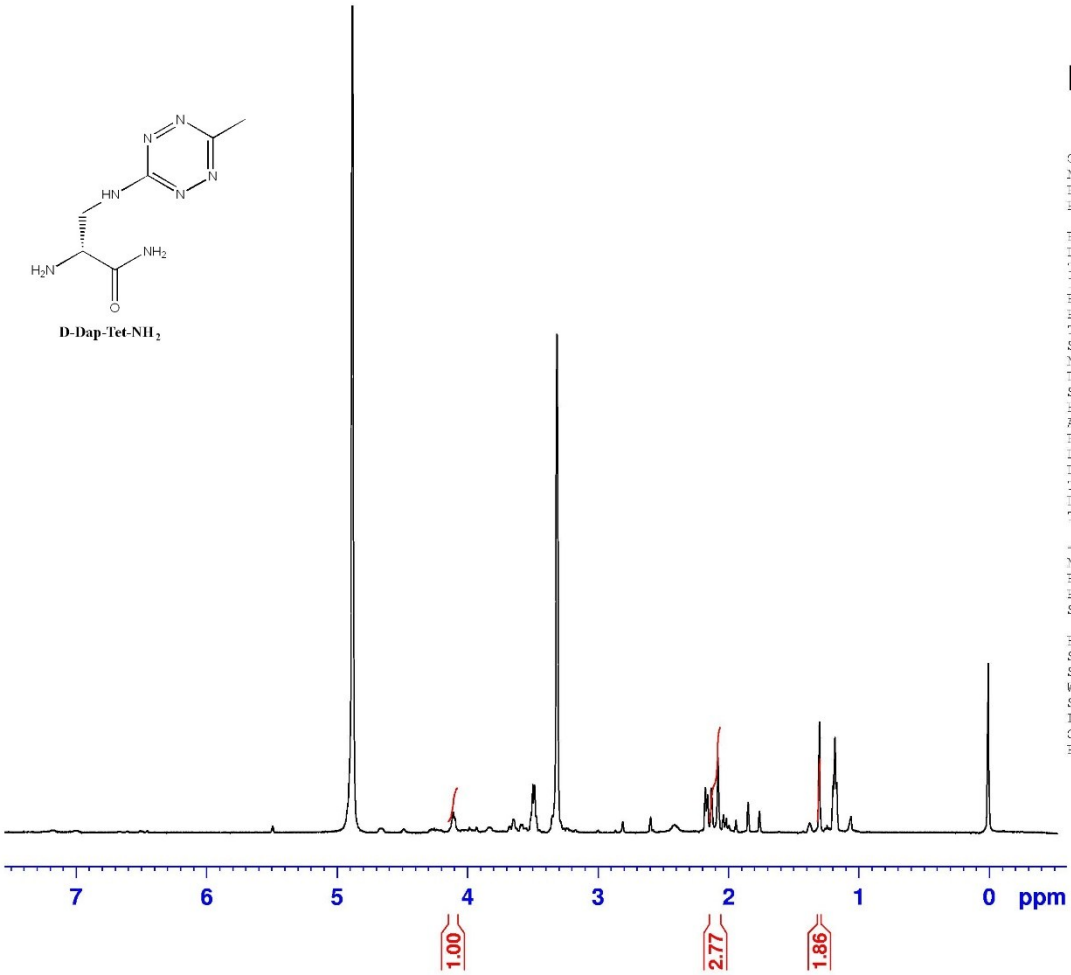

```
Current Data Parameters
NAME      D-Lys-O- Norbornene
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_              20150222
Time                  14.52
INSTRUM                spect
PROBHD   5 mm BBO BB-1H
PULPROG             zgdc30
TD                   32768
SOLVENT               MeOD
NS                    1400
DS                       0
SWH              25062.656 Hz
FIDRES           0.764852 Hz
AQ               0.653715 sec
RG                    4096
DW                19.950 usec
DE                 7.00 usec
TE                298.7 K
D1             2.00000000 sec
d11            0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    13C
P1                   8.60 usec
PL1                  1.00 dB
SFO1          125.7703648 MHz

-------- CHANNEL f2 --------
CPDPRG2             waltz16
NUC2                    1H
PCPD2              100.00 usec
PL2                  1.00 dB
PL12                17.50 dB
SFO2          500.1322150 MHz

F2 - Processing parameters
SI                   32768
SF            125.7577890 MHz
WDW                     EM
SSB                      0
LB                   1.00 Hz
GB                       0
PC                    1.40
```



D-Dap-Tet-NH$_2$

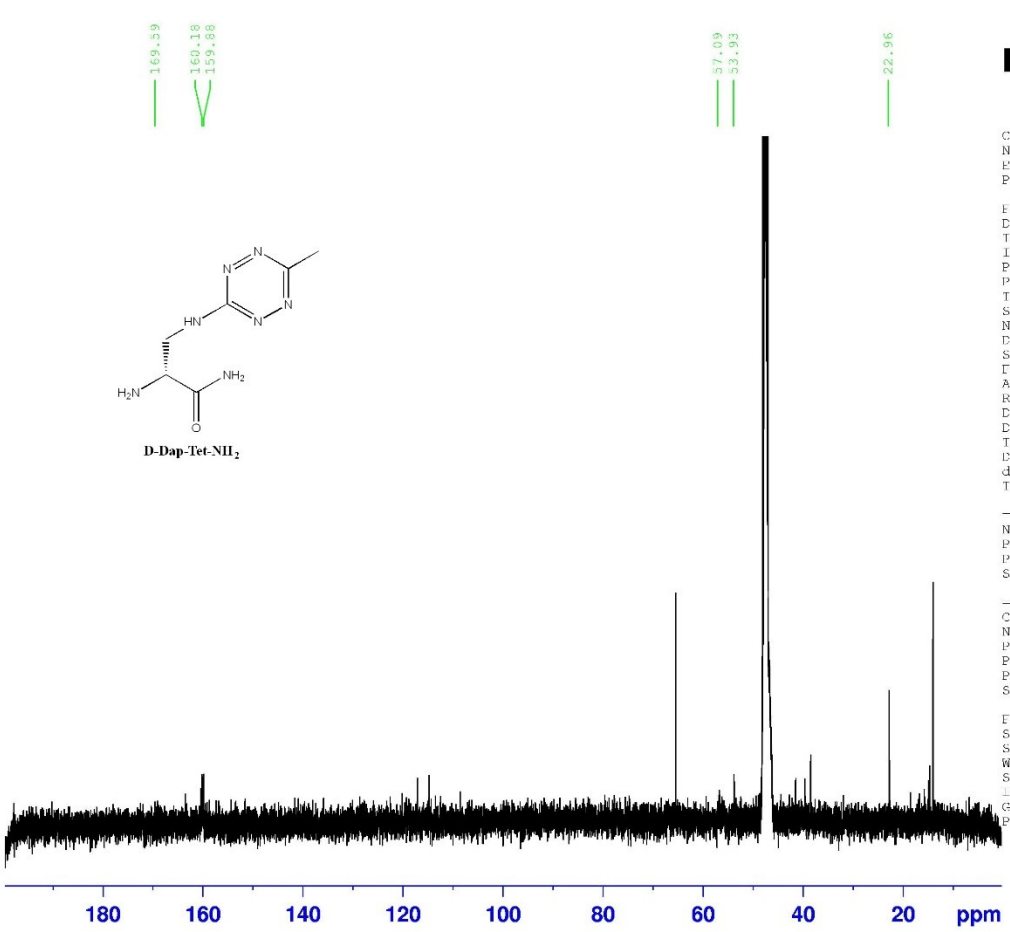
BRUKER

```
Current Data Parameters
NAME      D-Dap-Amide Tetrazine
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_              20150222
Time                   15.15
INSTRUM                spect
PROBHD   5 mm BBO BB-1H
PULPROG                zg30
TD                    32768
SOLVENT                MeOD
NS                       32
DS                        0
SWH                6009.615 Hz
FIDRES             0.183399 Hz
AQ                 2.7264309 sec
RG                      512
DW                   83.200 usec
DE                    7.00 usec
TE                   297.5 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                     14.00 usec
PL1                     1.00 dB
SFO1            500.1327510 MHz

F2 - Processing parameters
SI                        65536
SF              500.1300087 MHz
WDW                        EM
SSB                        0
LB                      0.10 Hz
GB                         0
PC                      1.00
```



BRUKER

```
Current Data Parameters
NAME      D-Dap-Amide Tetrazine
EXPNO                         3
PROCNO                        1

F2 - Acquisition Parameters
Date_              20150223
Time                   7.46
INSTRUM               spect
PROBHD   5 mm BBO BB-1H
PULPROG             zgdc30
TD                   32768
SOLVENT               MeOD
NS                   20000
DS                       0
SWH            25062.656 Hz
FIDRES          0.764852 Hz
AQ              0.6537915 sec
RG                   13004
DW                19.950 usec
DE                 7.00 usec
TE                298.9 K
D1             2.00000000 sec
d11            0.03000000 sec
TD0                      1

-------- CHANNEL f1 --------
NUC1                   13C
P1                  8.60 usec
PL1                 1.00 dB
SFO1         125.7703648 MHz

-------- CHANNEL f2 --------
CPDPRG2           waltz16
NUC2                   1H
PCPD2             100.00 usec
PL2                 1.00 dB
PL12               17.50 dB
SFO2         500.1322150 MHz

F2 - Processing parameters
SI                   32768
SF           125.7577890 MHz
WDW                     EM
SSB                      0
LB                  1.00 Hz
GB                       0
PC                  1.40
```