Electronic Supplementary Material (ESI) for ChemComm.

Utilizing Iron(III)-Chelation Masking Strategy to Prepare Mono- and Bis-Functionalized Aerobactin Analogues for Targeting Pathogenic Bacteria

Yu-Hin Ho[†a], Sheng-Yang Ho[†a], Cheng-Chih Hsu[a], Jiun-Jie Shie[b] and Tsung-Shing Andrew Wang [a,*]

[a] Department of Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei, 10617, Taiwan.

[b] Institute of Chemistry, Academia Sinica, No. 128, Sec. 2, Academia Rd., Nankang, Taipei 11529, Taiwan.

* Email: wangts@ntu.edu.tw

[†] These authors contributed equally to the work.

Supporting Information

Table of contents

1. General methods

Most of chemicals and solvents were purchased from Alfa Aesar, Acros, Thermo Fisher Scientific, ApexBio and AK scientific. All reagents were reagent grade and used without further purification unless mentioned otherwise. Anhydrous solvents were dried on solvent purification system from LC Technology Solutions Inc. Reaction were monitored by thin-layer chromatography (TLC) on 0.25 mm Merck silica gel 60 F_{254} glass plates, where the compounds were visualized by UV lamp or visualizing agents. Merck Geduran® silica gel 60 (0.040-0.063 mm particle size) was used for all flash column chromatography.

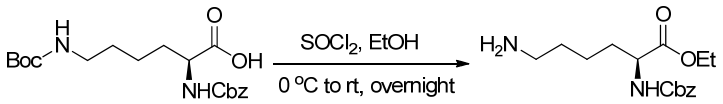
NMR spectra were recorded on a Varian Mercury 400 (1H: 400 MHz, 13C: 100 MHz), Bruker AVIII 400, Bruker DPX 400MHz NMR (1H: 400 MHz, 13C: 100 MHz), or Bruker AVIII 500 (1H: 500 MHz, 13C: 125 MHz) using the residual non-deuterated solvent as reference. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million (ppm) and hertz (Hz) respectively. Chemical shift (δ) was recorded in parts per million (ppm). The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Specific optical rotation data were measured on a Jasco P-2000 Digital Polarimeter.

Analytical high performance liquid chromatography (HPLC) were performed by using Agilent 1260 Infinity Quaternary LC system Agilent eclipse plus C18 column (3.5 µm pore size, 4.6 x 100 mm). Semi-preparative HPLC was performed on a Dr. Maisch Reprosil 300 reverse-phase C18 column (5 µm pore size, 10 x 250 mm) or YMC-Triart reverse-phase C18 column (5 µm pore size, 10 x 250 mm). All the HPLC mobile phases were prepared with HPLC grade solvent or Milli-Q water, and filtered through a 0.22 µm filter before use.

All LC/MS data were obtained by using Agilent 1260 infinity Quaternary LC system coupled with a Bruker MicrOTOF-QII mass spectrometer with Atlantis dC18 reverse-phase C18 column (5 µm pore size, 4.6 x 250 mm). Tandem MS data were collected with the Bruker MicrOTOF-QII mass spectrometer using CID energy from 25 eV to 27 eV.

2. Synthesis and characterization of compounds

Compound 3:

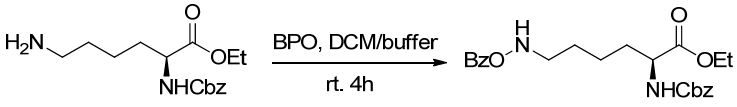


2 **3**

Compound **2** was prepared as previously reported.[1] $SOCl_2$ (3 ml) was added to cold EtOH (30 ml). The resulting solution was vigorously stirred at 0 °C for 30 min. Then **2** (1.08 g, 2.84 mmol, 1 equiv.) in EtOH (20 ml) was added to the mixture dropwise and stirred at 0 °C for additional 2 h until TLC showed no starting material remained. After the removal of ice bath, the resulting solution was allowed to warm to room temperature and stirred overnight. After TLC confirmed that no intermediate remained, the solution was concentrated to dryness under reduced pressure and the crude product **3** was used without further purification.

HRMS (ESI): $[M+H]^+$ *m/z* calcd 309.1809, found 309.1811

Compound 4:[2]



3 **4**

86% for 2 steps

The crude product **3** from last step was diluted with DCM (50 ml) and sodium bicarbonate buffer (50 ml, $NaHCO_3$/NaOH, pH 10). The biphasic solution was cooled to 0°C, dibenzoyl peroxide (BPO, 75 %, 1.4 g, 4.26 mmol, 1.5 equiv.) was added to the mixture. After stirred at 0°C for 2h, the reaction mixture stirred vigorously for further 4h at room temperature. The layers were separated and the aqueous layer was extracted with DCM several times. The combined organic layers were washed with 10% Na_2SO_3, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (DCM to DCM:EtOAc 9:1) to give **4** (1.22 g, 86 %) as a colorless oil.

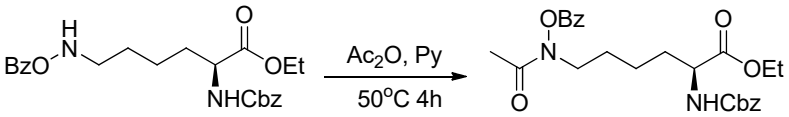
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (2H, d), 7.52 (1H, t), 7.40 (2H, *J* = 7.7 Hz, t), 7.33-7.23 (5H, m), 5.46 (1H, s), 5.06 (2H, s), 4.33 (1H, *J* = 7.9, 5.0 Hz, dd), 4.14 (2H, *J* = 7.1 Hz, q), 3.07 (2H, *J* = 7.0 Hz, t), 1.84 -1.32 (6H, m), 1.21 (3H, *J* = 7.1 Hz, t).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.27, 166.73, 155.83, 136.22, 133.23, 129.21, 128.42, 128.39, 128.29, 128.02, 127.95, 66.82, 61.33, 53.71, 52.06, 32.40, 26.76, 22.65, 14.06.

HRMS (ESI): $[M+Na]^+$ *m/z* calcd 451.1840, found 451.1839

Specific optical rotation: $[\alpha]_D^{24}$ = +15.95 (c = 1.0, $CHCl_3$)

Compound 5:



4 **5** >99%

To a stirred solution of **4** (0.43 g, 1 mmol, 1 equiv.) in DCM (30 ml), Ac_2O (1 ml) and one drop of pyridine were added. Then the mixture was stirred at 50 °C for 4 h. After TLC showed the complete conversion of starting material, the solution was concentrated under reduced pressure. The crude product was purified by flash column chromatography (DCM:EtOAc 9:1) to give **5** (0.47 g, 99 %) as a colorless oil which solidified in several days.

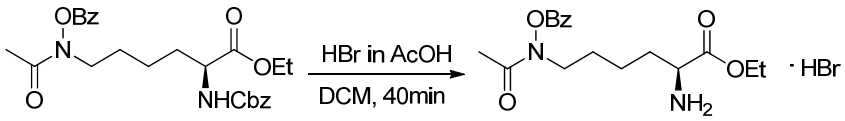
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-7.98 (2H, *J* = 7.8 Hz, d), 7.63 (1H, *J* = 7.8 Hz, t), 7.48 (2H, *J* = 7.8 Hz, t), 7.28 (4H, m), 5.39 (2H, *J* = 8.0 Hz, d), 5.05 (2H, s), 4.30 (1H, *J* = 7.7, 5.2 Hz, dd), 4.14 (2H, *J* = 7.1 Hz, q), 3.85-3.65(2H, m), 2.00 (3H, s), 1.82 (1H, m), 1.63 (3H, m), 1.48-1.26 (2H, m), 1.21 (3H, *J* = 7.1 Hz, t).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.55, 164.70, 156.15, 136.51, 134.78, 130.23, 129.17, 128.72, 128.36, 128.29, 126.87, 77.53, 67.13, 61.70, 53.96, 47.78, 32.37, 26.80, 22.51, 20.58, 14.38.

HRMS (ESI): $[M+H]^+$ *m/z* calcd 471.2126, found 471.2156

Specific optical rotation: $[\alpha]_D^{24}$ = +9.25 (c = 1.2, $CHCl_3$)

Compound 6:[3]

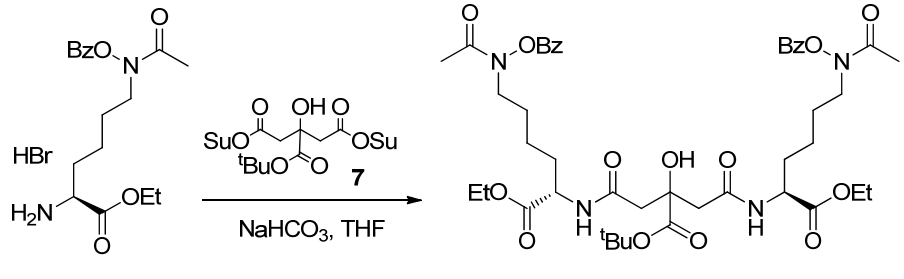


5 **6**

To a stirred solution of **5** (0.13 g, 0.27 mmol) in DCM (15 ml) was added 33% HBr in AcOH (15 ml). After vigorously stir for 40 min, solvent was removed under reduced pressure to give a yellowish solid. Due to the rapid rearrangement, **6** need to be prepared right before use.

HRMS (ESI): $[M+H]^+$ *m/z* calcd 337.1758, found 337.1708

Compound 8:



6 **8**
60%

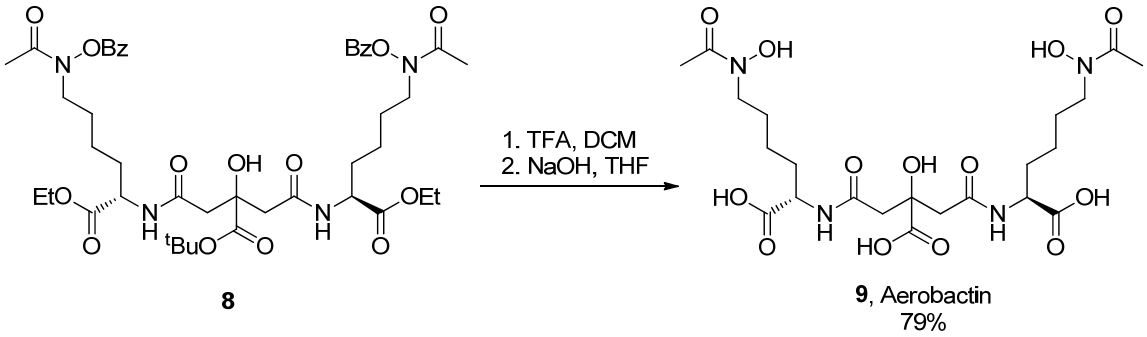
Compound **7** was prepared from citric acid as previously reported[4]. To a stirred solution of **7** (31 mg, 0.125 mmol, 1 equiv.) and HOSu (34 mg, 0.3 mmol, 2.4 equiv.) in THF was added DCC (77 mg, 0.37 mmol, 3 equiv.). A white precipitate was formed after 15 min. After 30 min, H_2O (1 ml) was added and the mixture was stirred for further 10 min. Followed by the addition of $NaHCO_3$ (1 g, 0.012 mol, 100 equiv.), freshly prepared **6** was diluted with THF and added into the solution. The final reaction mixture was stirred overnight at room temperature, then filtered, concentrated under reduced pressure, dilute with EtOAc, washed with water and filtered again to remove any residual DCU. The filtrate was collected and dried with $MgSO_4$ and concentrated again. The crude product was finally purified by flash column chromatography (Hexane:EtOAc 1:1 to 1:2 then DCM:MeOH 20:1 to 10:1) to give **8** (66 mg, 60 %) as a colorless oil. The 2D ^{1}H-^{1}HCOSY spectrum was also measured for structure characterization.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (4 H, *J* = 8.5, 1.5 Hz, dt), 7.68-7.62 (2 H, m), 7.50 (4 H, m), 7.38 (2 H, *J* = 27.3, 8.0 Hz, dd), 4.48 (2 H, m), 4.14 (4 H, m), 3.85-3.69 (4 H, m), 2.78 (1 H, *J* = 14.4 Hz, d), 2.68 (2 H, *J* = 2.1 Hz, d), 2.57 (1 H, *J*=14.4 Hz, d) 2.02 (6 H, s), 1.70 – 1.57 (6 H, m), 1.45 (12 H, m), 1.23 (6 H, m).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.47, 173.09, 171.34, 171.23, 169.46, 164.47, 134.56, 130.00, 128.97, 128.95, 128.56, 126.69, 126.63, 82.54, 74.29, 61.78, 61.69, 52.27, 52.07, 52.00, 47.57, 44.37, 41.85, 31.34, 31.16, 27.82, 27.80, 26.53, 22.73, 22.68, 20.36, 14.09.

HRMS (ESI): $[M+Na]^+$ *m/z* calcd 907.3953, found 907.3984

Compound 9:



8 **9**, Aerobactin 79%

To a stirred solution of **8** (50 mg, 0.056 mmol) in DCM (10 ml) was added TFA (5 ml). Monitored by TLC, after 1 h the reaction was completed and then concentrated under reduced pressure. The mixture was diluted with THF (10 ml), then was added 0.25 M NaOH (5 ml) and stirred. Monitored by TLC, after 40 min the reaction was completed and then 10 % HCl was added to bring pH to 2. THF was removed by reduced pressure and the resulting solution was lyophilized. The mixture was purified by semi-preparative HPLC (Dr. Maisch Reprosil 300 reverse-phase C18 column, A: 0.1% formic acid in H_2O, B: 0.1% formic acid in acetonitrile, 0%-25% B for 40 min) to give **9** as a white solid (25 mg, 79 %). The large-scale purification can also be achieved by gel filtration (LH-20, 20% EtOH). The 2D ^{1}H-^{1}HCOSY spectrum was also measured for structure characterization. NMR characterization is consistent with the literature.[5]

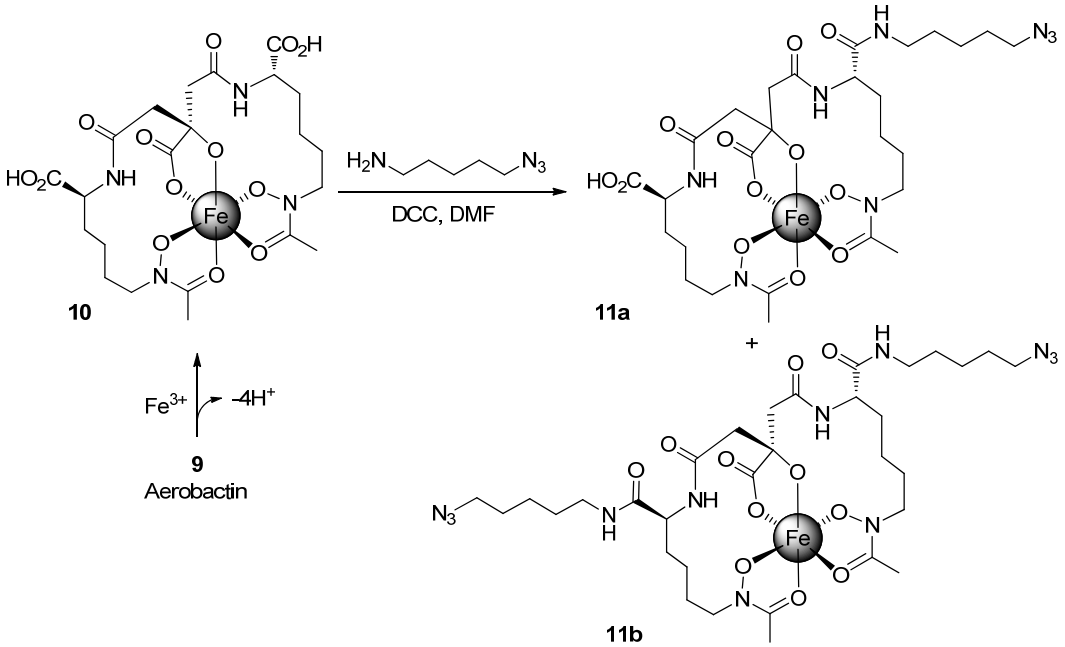
^{1}H NMR (400 MHz, D_2O) δ 4.37-4.21 (2 H, m), 3.56 (4 H, m), 2.95 (2 H, J = 14.9, 7.1 Hz, dd), 2.81 (2 H, J = 14.9, 7.1 Hz, dd), 2.05 (6 H, ss), 1.71 (8 H, m), 1.45-1.18 (4 H, m).

^{13}C NMR (100 MHz, D_2O) δ 176.46, 175.79, 173.67, 171.33, 171.26, 73.95, 52.70, 52.64, 51.29, 47.47, 44.10, 43.95, 30.04, 25.94, 25.21, 21.97, 19.26.

HRMS (ESI): $[M+H]^+$ *m/z* calcd 565.2351, found 565.2352

HRMS (ESI): $[M-4H+Fe(III)+2H]^+$ *m/z* calcd 618.1467, found 618.1464 (minor peak)

Compound 11:



5-azidopentylamine was prepared as previously reported.[6] To a stirred solution of aerobactin **9** (5mg, 0.009 mmol, 1 equiv.) in DMF (5 ml) was added $FeCl_3$ (10% aq. with few HCl, 2 equiv.). After stirred 30min, 5-azido pentylamine (11.5 mg, 0.09 mmol, 10 equiv. for mono- and 46mg, 0.36 mmol, 40 equiv. for bis-) was added and HCl (aq. 10% w/v) was added to adjust pH to 8. Then DCC (0.18 g, 0.9 mmol, 100 equiv.) was added. For mono-C5NO Aero **11a**, the reaction was quenched with water after 8h. For bis-C5NO Aero **11b**, water was added after 24 h. The DCU precipitate was removed by filtration and then the filtrate was lyophilized. After lyophilization, 20 % EtOH water solution was added to re-dissolve the solid, then filtered again to remove residue DCU, and purified by semi-preparative HPLC (YMC-Triart reverse-phase C18 column, A: 0.1% formic acid in H_2O, B: 0.1% formic acid in acetonitrile, 0%-55% B for 50 min) to give **11a** (0.96 mg, 15% determined by UV absorption), **11b** (2.22mg, 30% determined by UV

absorption) and to recover **10** (2.19 mg, 40%, UV absorption, can be reused as the starting material). LC/MS analysis was also provided to show a typical case in supporting figure (Figure S1). Purity check was performed on an Agilent eclipse plus C18 column using a gradient of 0%-80% ACN in water supplemented with 0.1% TFA. (Figure S1)

Compound 11a

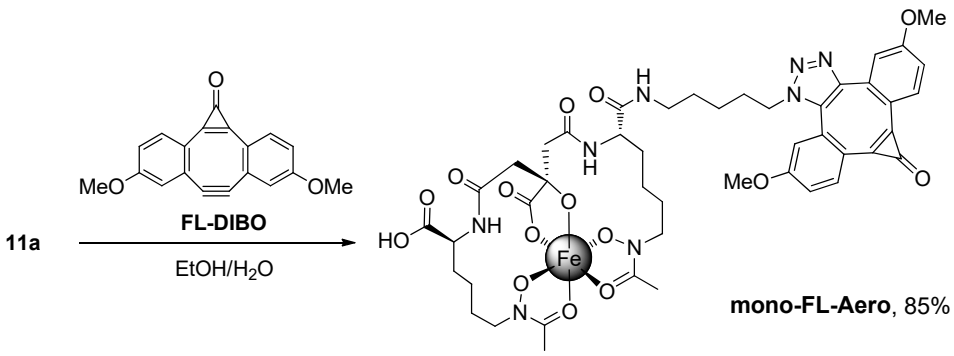
HRMS (ESI): $[M^-+2H]^+$ *m/z* calcd 728.2423, found 728.2396

Compound 11b

HRMS (ESI): $[M^-+2H]^+$ *m/z* calcd 838.3380, found 838.3294

(Note：the formation of ferric aerobactin complex will lose four protons and acquire one iron(III) to give a final one negatively-charged species (M^-), a phenomenon that was commonly found in the following preparations.)

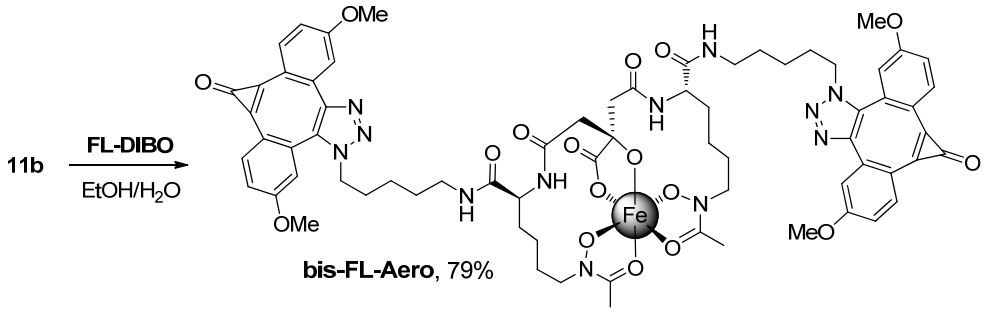
mono-FL-Aero:



FL-DIBO was prepared as previously reported.[7] To a solution of **11a** (0.5 mg, 0.68 nmol, 1 equiv.) in H_2O was added **FL-DIBO** (0.6 mg, 2.04 nmol, 3 equiv.) in EtOH (1 ml). Then the mixture was incubated at 37 °C for 4 d and monitored by LC-MS. After its completion, the reaction mixture was concentrated by reduced pressure and purified by semi-preparative HPLC (YMC-Triart reverse-phase C18 column, A: 0.1% formic acid in H_2O, B: 0.1% formic acid in acetonitrile, 0%-100% B for 60 min) to give **mono-FL-Aero** (0.59 mg, 85%, determined by HPLC). Purity check was performed on an Agilent eclipse plus C18 column using a gradient of 0%-80% ACN in water supplemented with 0.1% TFA. (Figure S2)

HRMS (ESI): $[M^-+2H]^+$ *m/z* calcd 1016.3210, found 1016.3100

bis-FL-Aero:

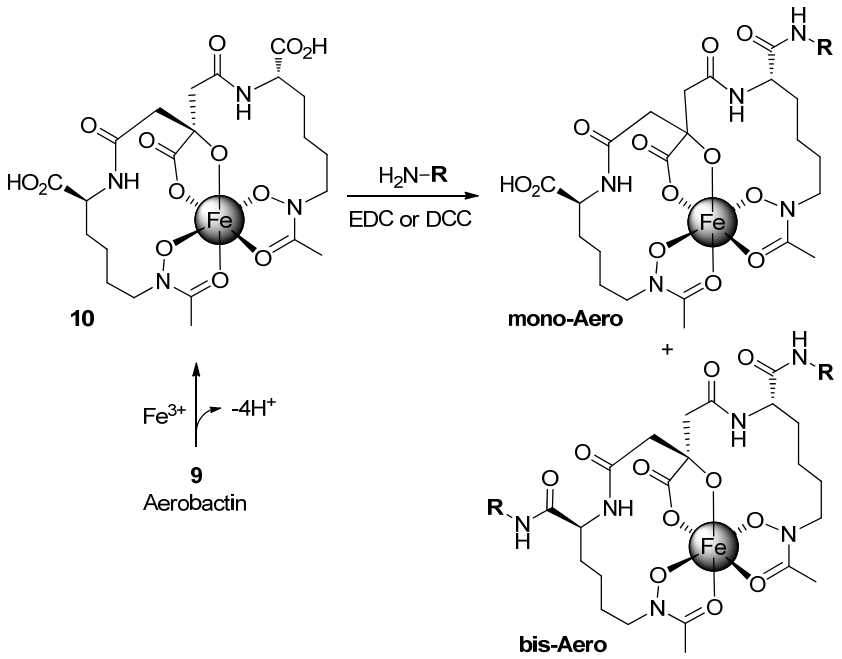


To a solution of **11b** (0.5 mg, 0.59 nmol, 1 equiv.) in H_2O was added **FL-DIBO** (1.4 mg, 4.7 nmol,8 equiv.) in EtOH (1 ml). Then the mixture was incubated at 37 ^oC for 7 d and monitored by LC-MS. After its completion, the reaction mixture was concentrated by reduced pressure and purified by semi-preparative HPLC (YMC-Triart reverse-phase C18 column, A: 0.1% formic acid in H_2O, B: 0.1% formic acid in acetonitrile, 0%-80% B for 50 min) to give **bis-FL-Aero** (0.66 mg, 79%, determined by HPLC). Purity check was performed on an Agilent eclipse plus C18 column using a gradient of 0%-80% ACN in water supplemented with 0.1% TFA. (Figure S3)

HRMS (ESI): $[M^-+3H]^{2+}$ *m/z* calcd 707.7513, found 707.7544

HRMS (ESI): $[M^-+2H]^+$ *m/z* calcd 1414.4954, found 1414.4821

3. Investigation of iron(III)-chelation masking amide coupling of Aerobactin:



To a stirred solution of aerobactin **9** (1 mg, 0.002 mmol, 1 equiv.) in DMF was added $FeCl_3$ (10% aq. with few HCl, 2 equiv.). After stirred 30min, amine containing linker (10 equiv.) was added and HCl (aq. 10% w/v) was added to adjust pH to 8. Then EDC or DCC (100 equiv.) was added. For mono-substituted aerobactin (a), the reaction was quenched with water after 8h. For bis-substituted aerobactin (b), water was added after 24 h. The reaction mixtures were filtered to remove precipitates before subjected to LC/MS analysis. (Table S1)

LC/MS analysis showed similar situation of various substrates that bis-substituted aerobactin accumulated faster than mono-substituted one like Figure S4. We anticipated that the first amide formation might be the rate determined step of the reaction. A variety of versatile linkers containing amino group were proven to be available substrates in our iron(III)-templated amide coupling system without generating tri-substituted product which could be observed in insufficient $FeCl_3$ condition. To be more specific, in the case of linkers containing polyethylene glycol spacer, EDC was better coupling reagent and the more linkers attached, the more hydrophilic product we got. While in other cases, DCC could achieve higher conversion alongside with the ease of removing corresponding byproduct DCU.

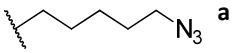
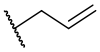
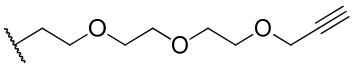
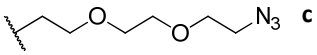
amine containing linker, RNH_2, R =	product	HRMS calcd. $[M^-+2H]^+$ (*m/z*)	HRMS Found $[M^-+2H]^+$ (*m/z*)
 **a**	**mono, 11a**	728.2423	728.2396
	bis, 11a	838.3380	838.3294
	**mono**	657.1945	657.1915
	bis	696.2412	696.2419
	**mono**	655.1783	655.1714
	bis	692.2099	692.1417
 **b**	**mono**	787.2570	787.2589
	bis	956.3673	956.3658
 **c**	**mono**	774.2478	774.2464
	bis	930.3489	930.3486

Table S1. Substrate scope of iron(III)-templated amide coupling of aerobactin. **a**[6], **b**[8] and **c**[9] was prepared as previously reported.

4. General procedure of photolysis:

To a photochemical reactor equipped with 419 nm light tubes (8W x 6) was placed the reaction tube with 100 μg/mL of aerobactin and its analogues (**10**, **11a** and **11b**) in water. Each reaction last for 8 h before subjected to LC/MS analysis directly. The reaction was performed under open air, and the cooling fan was sufficient to keep the temperature under 40 ^oC. (Figure S5, S6, 1)

5. Methods in bacterial experiments

5-1. Bacterial strains and media:

Klebsiella pneumoniae 1084 (*K. pneumonia*, KP) was given from Dr. Yi-Chyi Lai at Chung Shan Medical University. *Escherichia coli* CFT073 was given by Dr. Ching-Hao Teng at National Cheng Kung University. *Escherichia coli* O157:H7 ATCC 43895, *Bacilus subtilis* ATCC 23857 (*B. subtilis*, BA), *Salmonella enterica* ATCC14028 (*S. enterica*, SE), *Vibrio cholerae* ATCC 14035 (*V. cholerae*, VC), *Staphylococcus epidermidis* ATCC 14990 (*S. epidermidis*, SE) were purchased from Bioresource Collection and Research Center (Taiwan). Frozen Stocks of other bacteria strains were prepared from single colony in 25% glycerol/75% LB.

Media were prepared as described. LB medium: tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L. MM9 minimal medium:[10] 0.1M Tris buffer pH = 7.4, KH_2PO_4 0.03g/L, NaCl 0.5g/L, NH_4Cl 1g/L, 1M $MgSO_4$ 2ml/L, 20% aq. Glucose 20ml/L, 1M $CaCl_2$ 100 µL/L

5-2. Bacteria imaging experiments:

Each frozen bacteria stock was inoculated into 2 mL LB medium and allowed to grow in an orbital shaking incubator at 220 rpm, 37^{o}C overnight. The overnight culture was diluted 1:1000 (v/v) into 2 mL of MM9 minimal medium containing 1µM of $FeCl_3$ and incubated at 220 rpm, 37 ^{o}C for 16 hours. **mono-FL-Aero** or **bis-FL-Aero** was prepared as a 1 mM stock in water. The concentration of the **mono-FL-Aero** and **bis-FL-Aero** stock solution was estimated by UV at 360 nm.[7]

Before addition of either probes, 1 mL of bacteria culture was spun down and then re-suspended to corresponding amount of fresh minimal medium to adjust OD_{600} into 1. **mono-FL-Aero** and **bis-FL-Aero** stock solution was added into 50 µL of bacteria culture to a final concentration of 20 µM and the culture was incubated at 220 rpm, 37 ^{o}C for 3 hours. The bacteria culture was then spun down, removed of supernatant, and re-suspended in 50 µL PBS. The spin down-resuspend procedure was repeated 3 times. 0.3 µL of bacteria solution was then loaded onto an imaging slide and covered with cover slip. Agarose gel pads for bacteria immobilization cannot be used since it will strongly reduce fluorescence signal of this probe.

Imaging was performed on Axio Observer Z1 (Zeiss) with 63x oil immersion lens. Fluorescent signal was monitored by using AF405 channel (excitation 335-383 nm, emission 420-470 nm).

For iron supplement experiment, overnight culture was diluted 1:1000 (v/v) into 2 mL of MM9 medium supplemented with 100µM $FeCl_3$, incubated at 220 rpm, 37^{o}C for 16 hours, and proceeded on to the **mono-FL-Aero** and **bis-FL-Aero** treatment as described above.

6. Supporting figures

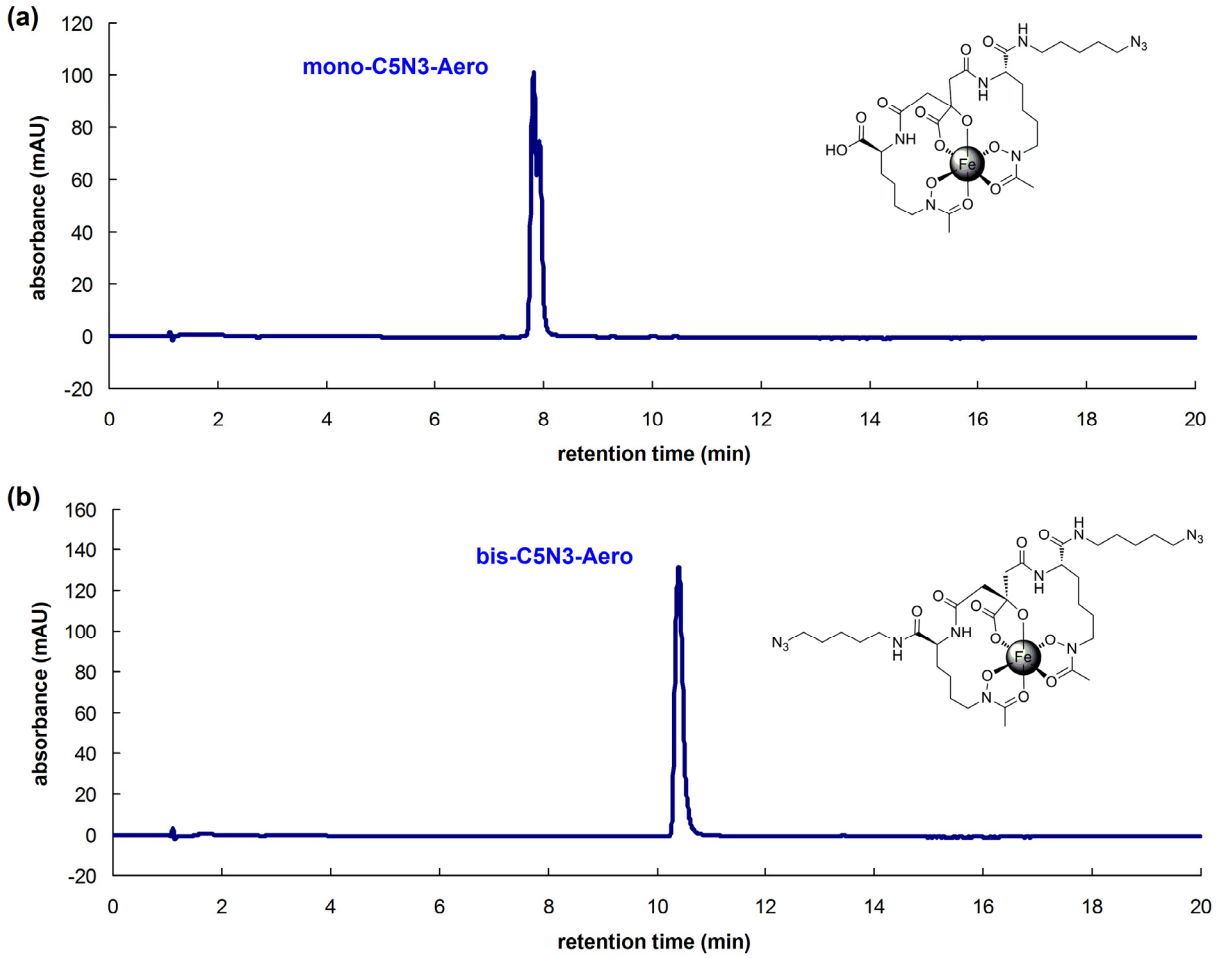


Figure S1. HPLC purity check of mono- and bis-substituted aerobactin analogues at 210 nm. All purities were >90%. (a) mono-C5N3 Aero **11a**. (b) bis-C5N3 Aero **11b**.

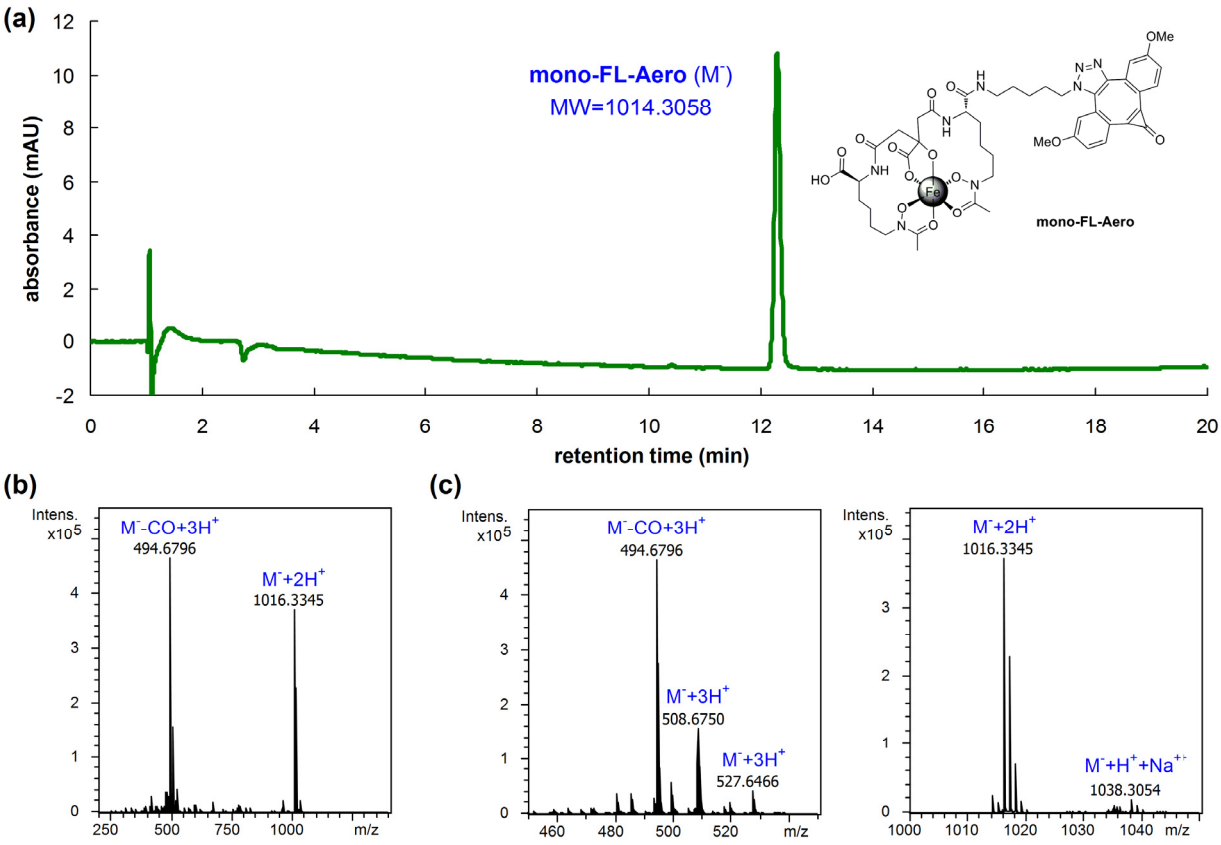


Figure S2. Characterization and identification of **mono-FL-Aero** (a) HPLC purity check of **mono-FL-Aero** at 210 nm with purity >90 %. (b) HRMS of **mono-FL-Aero** and (c) HRMS of **mono-FL-Aero** in the selected *m/z* range.

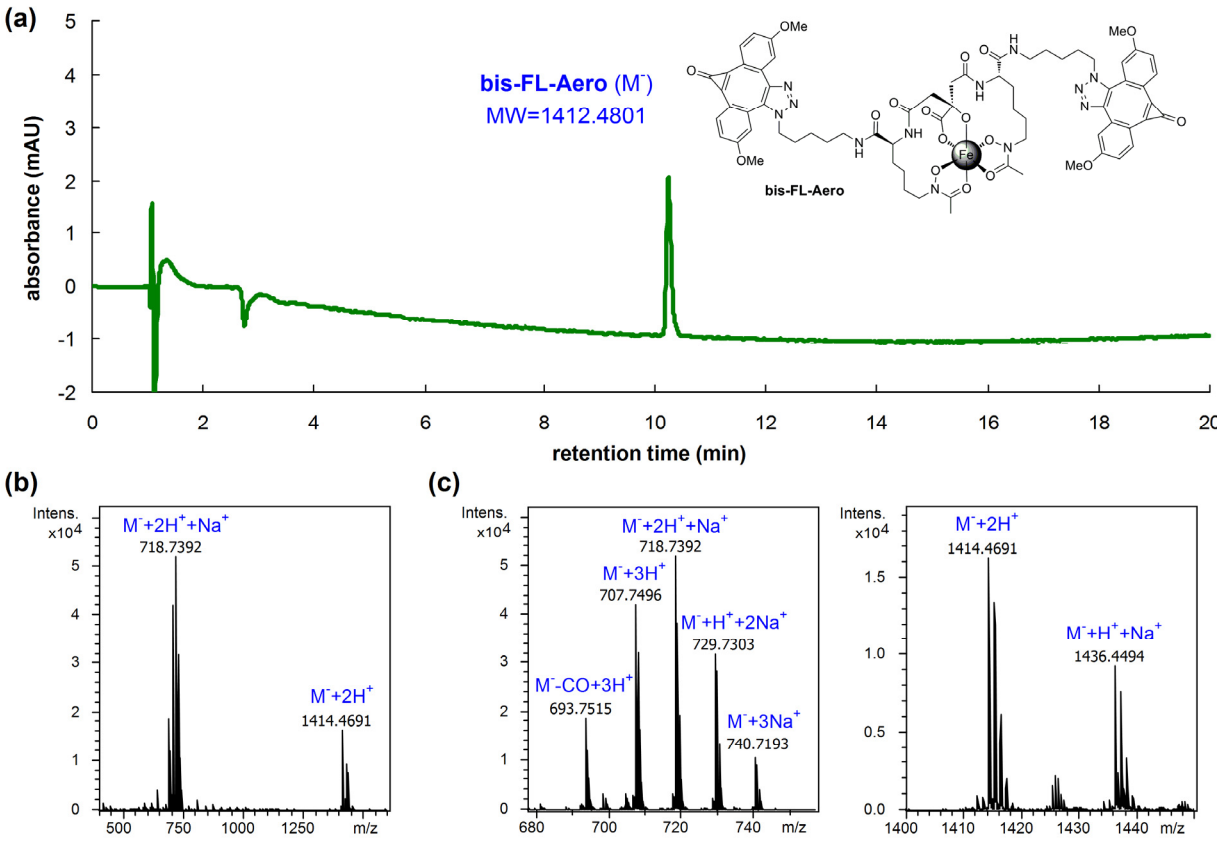


Figure S3. Characterization and identification of **bis-FL-Aero** (a) HPLC purity check of **bis-FL-Aero** at 210 nm with purity >90 %. (b) HRMS of **bis-FL-Aero** and (c) HRMS of **bis-FL-Aero** in the selected *m/z* range.

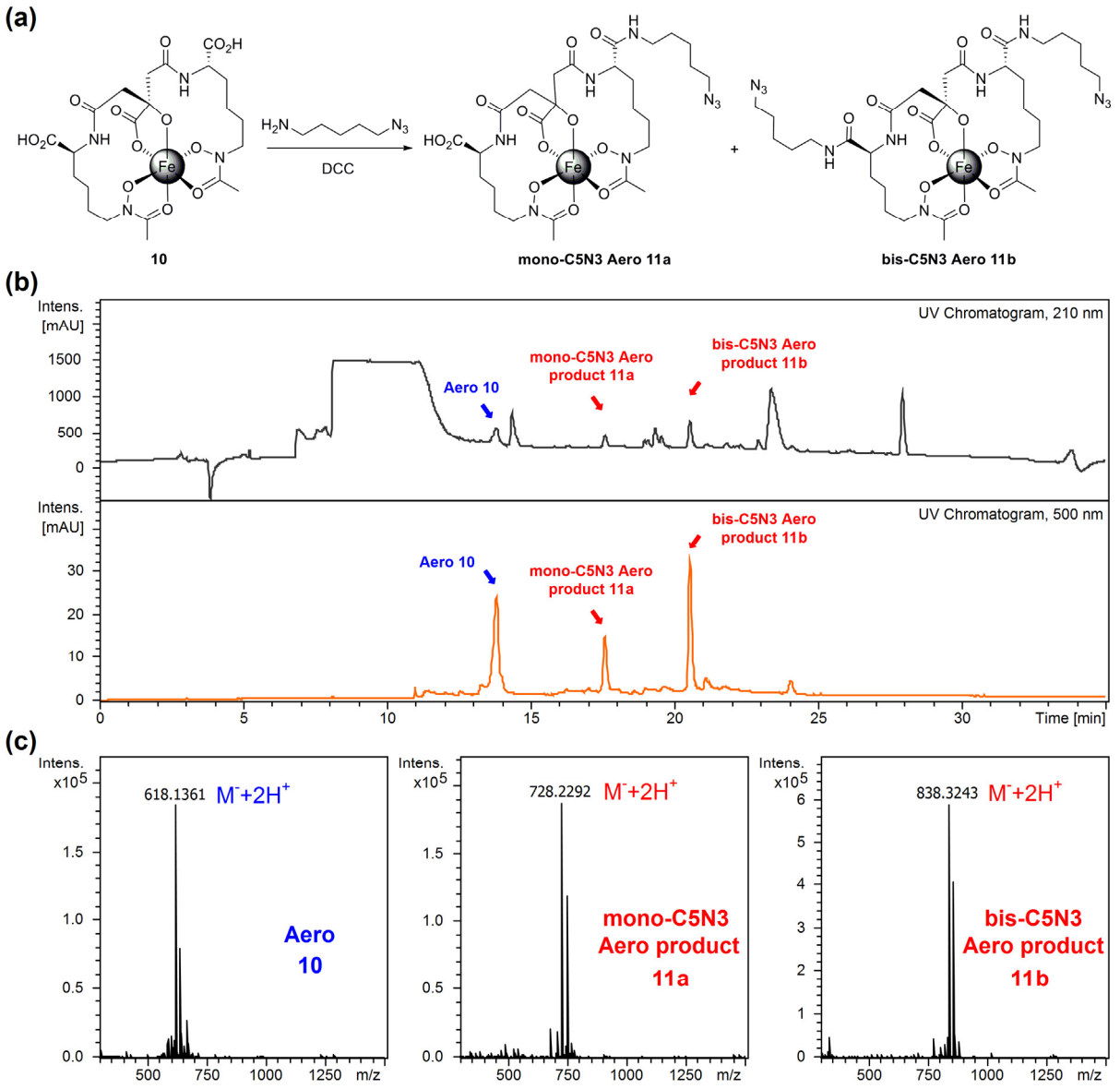


Figure S4. LC/MS analysis of iron(III)-chelation masking amide coupling reaction. LC chromatograms and MS spectra are shown separately. (a) Schematic of iron(III)-chelation masking amide coupling reaction (b) HPLC chromatograms of reaction at 210 nm and 500 nm. (c) MS spectra of corresponding peaks of interest: starting material, Aero **10**, and two products, mono-C5N3 Aero **11a** and bis-C5N3 Aero **11b** are shown.

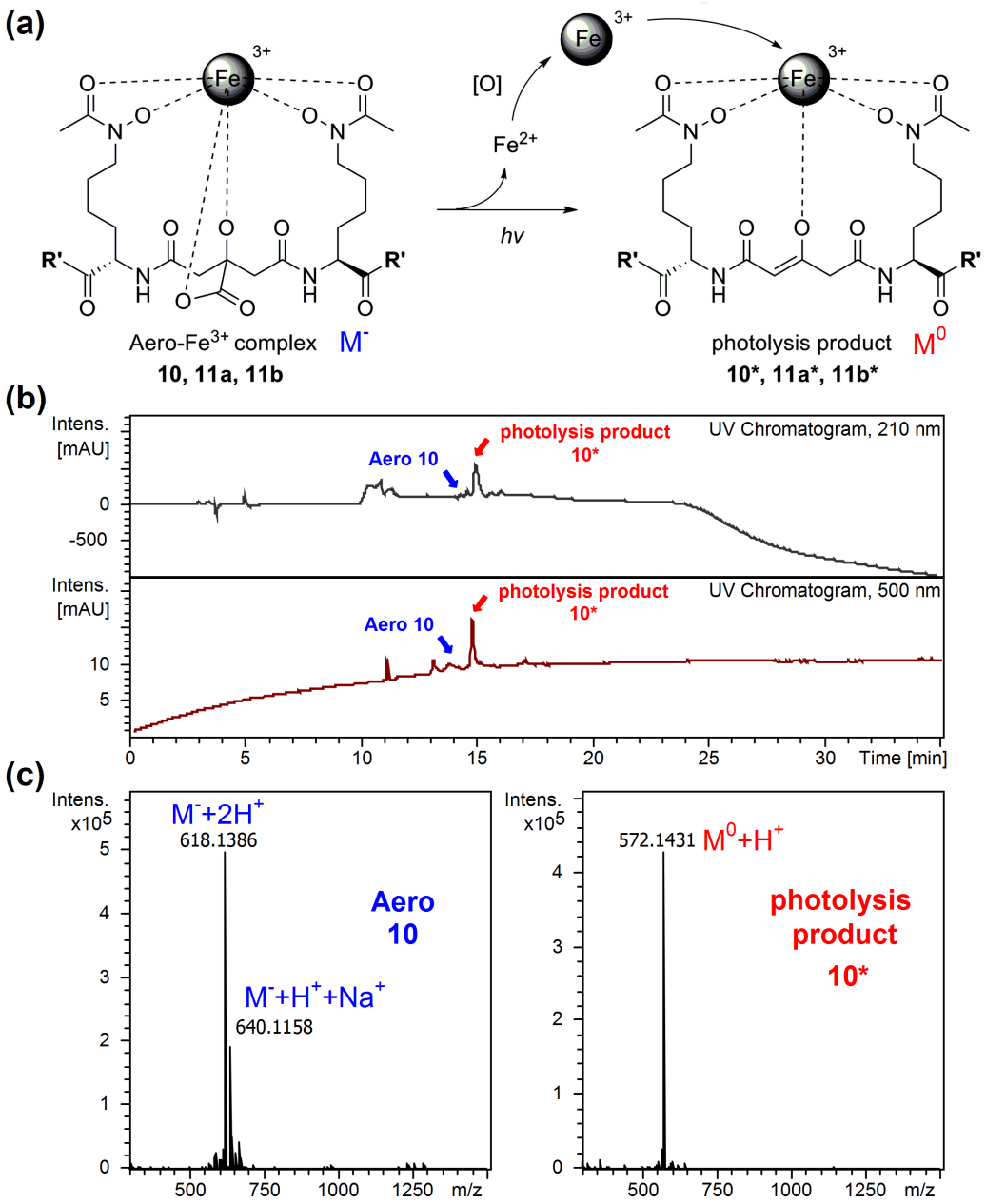


Figure S5. Photolysis of ferric aerobactin and its analogues. (a) The general scheme of photolysis reaction on the α-hydroxyl carboxylate moiety in the presence of ferric iron is shown. (b) HPLC chromatograms of photolysis of ferric aerobactin at 210 nm and 500 nm. (c) MS spectra of ferric aerobactin **10** and photolysis product **10***.

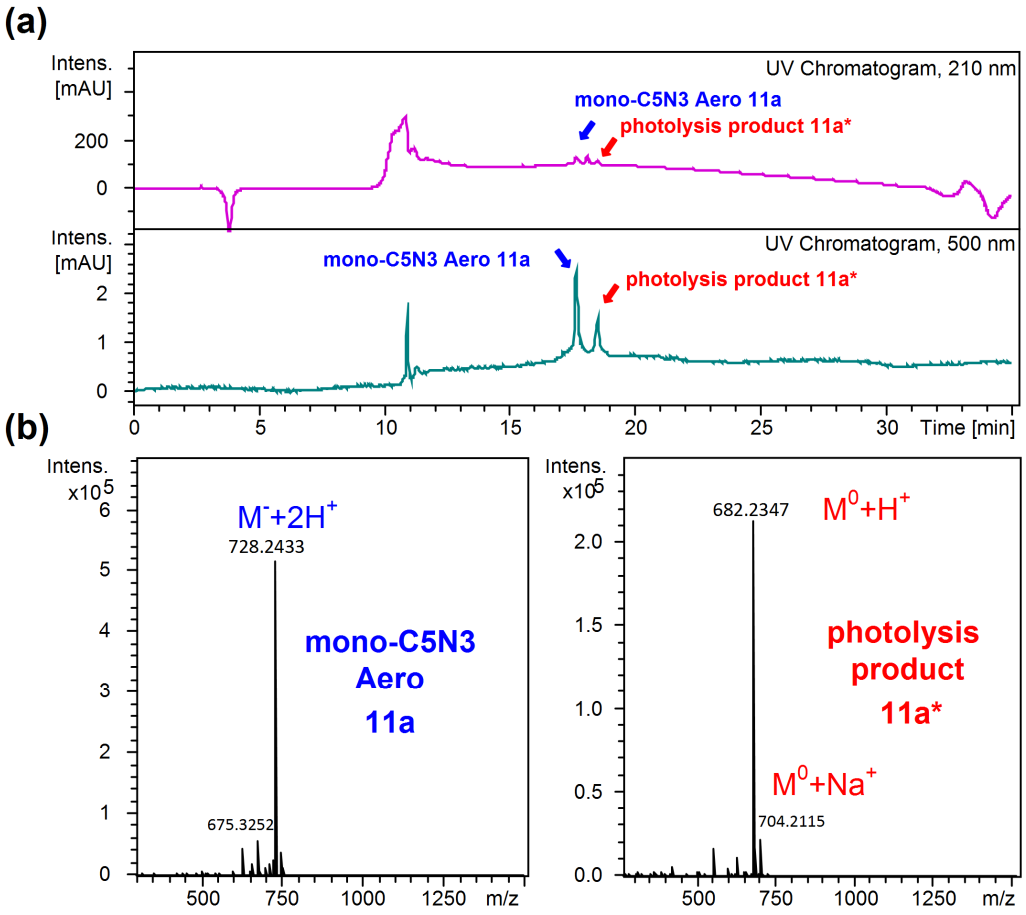


Figure S6. Photolysis of mono-C5N3 Aero **11a**. (a) HPLC chromatograms of photolysis after 8 hour of reaction (210 nm and 500 nm). (b) MS spectra of mono-C5N3 Aero **11a** and its photolysis product **11a***.

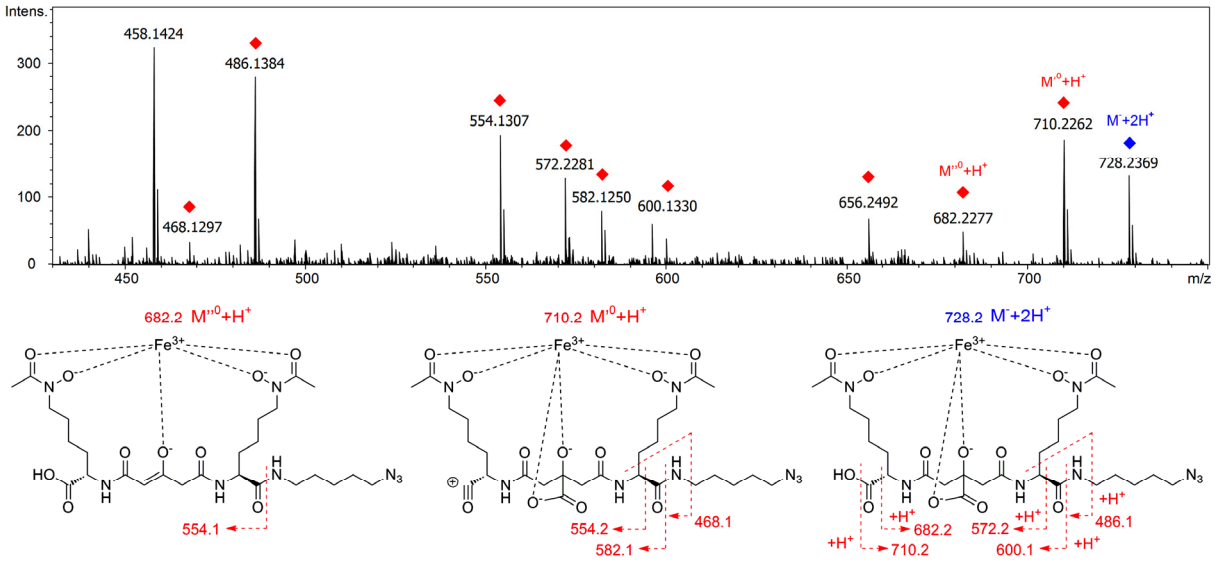


Figure S7. Tandem MS spectrum and the fragmentation pattern of mono-C5N3 Aero **11a**.

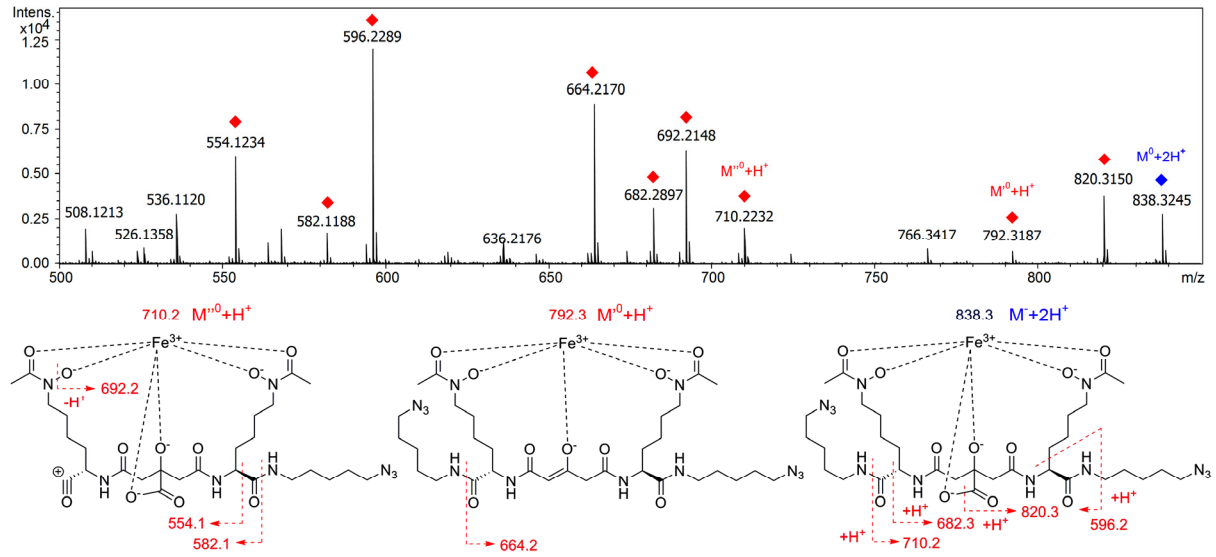


Figure S8. Tandem MS spectrum the fragmentation pattern of bis-C5N3 Aero **11b**.

7. References

1. (a) S. Wiejak, E. Masiukiewicz and B. Rzeszotarska, *Chem. Pharm. Bull. (Tokyo)*, 1999, **47**, 1489-1490; (b) O. Hideo, I. Ken and J. Yoshiko, *Bull. Chem. Soc. Jpn.*, 1964, **37**, 1471-1477.

2. Q. X. Wang, J. King and O. Phanstiel IV, *J. Org. Chem.*, 1997, **62**, 8104-8108.

3. K. A. Fennell and M. J. Miller, *Org. Lett.*, 2007, **9**, 1683-1685.

4. H. Guo, S. A. Naser, G. Ghobrial and O. Phanstiel IV, *J. Med. Chem.*, 2002, **45**, 2056-2063.

5. (a) F. Gibson and D. Magrath, *Biochim. Biophys. Acta*, 1969, **192**, 175-184; (b) M. G. Haygood, P. D. Holt and A. Butler, *Limnol. Oceanogr.*, 1993, **38**, 1091-1097.

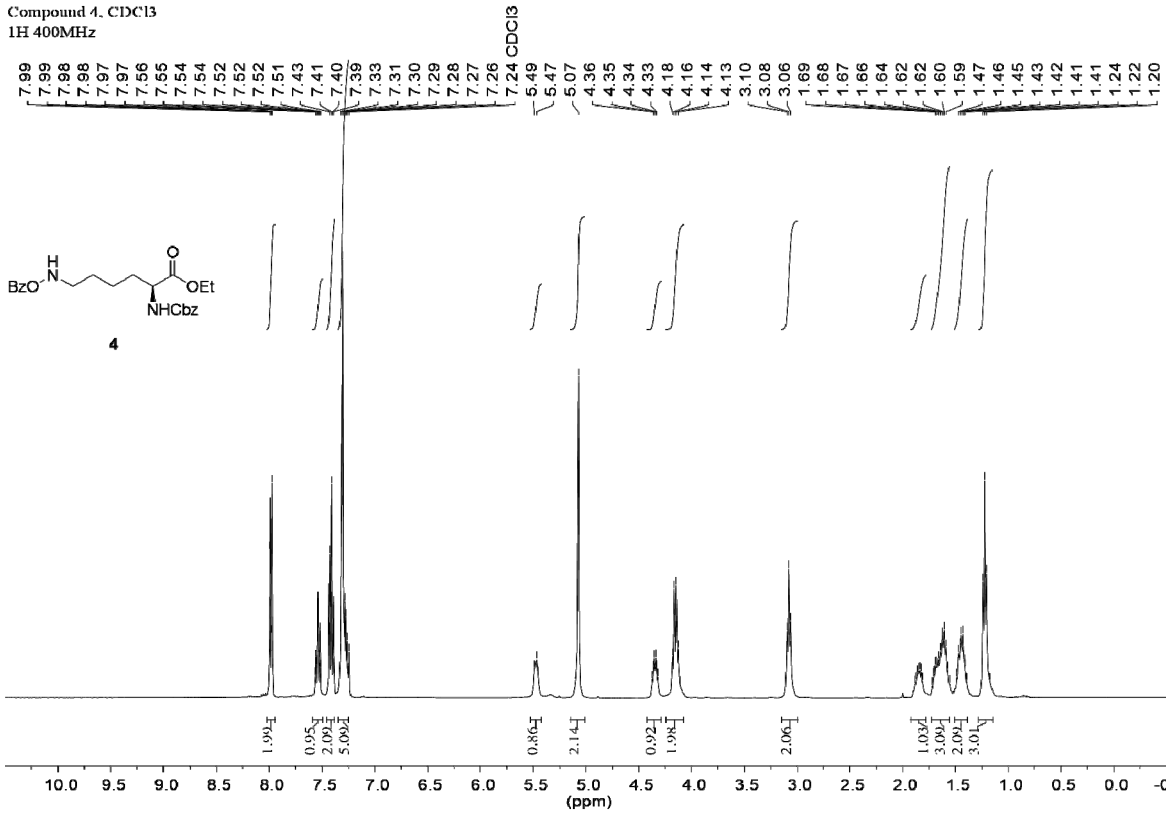
6. R. Srinivasan, L. P. Tan, H. Wu, P.-Y. Yang, K. A. Kalesh and S. Q. Yao, *Org. Biomol. Chem.*, 2009, **7**, 1821-1828.

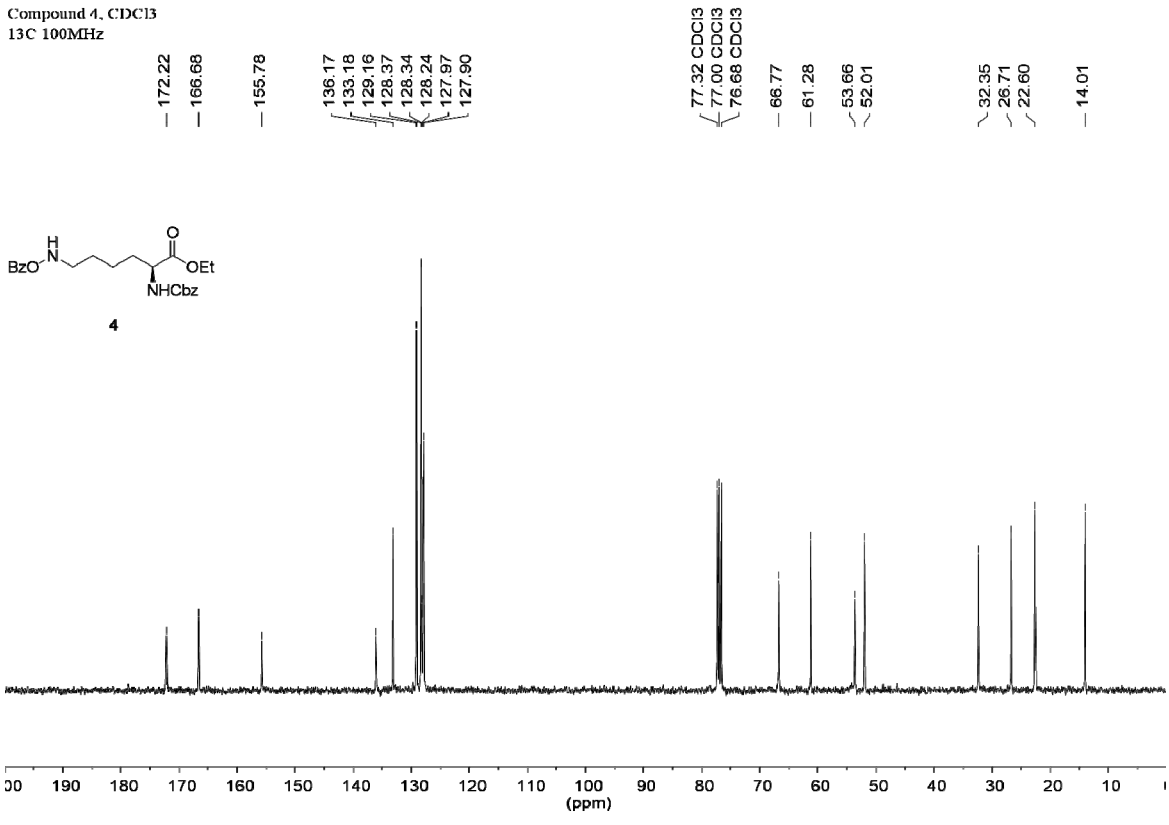
7. F. D. R. Friscourt, C. J. Fahrni and G.-J. Boons, *J. Am. Chem. Soc.*, 2012, **134**, 18809-18815.

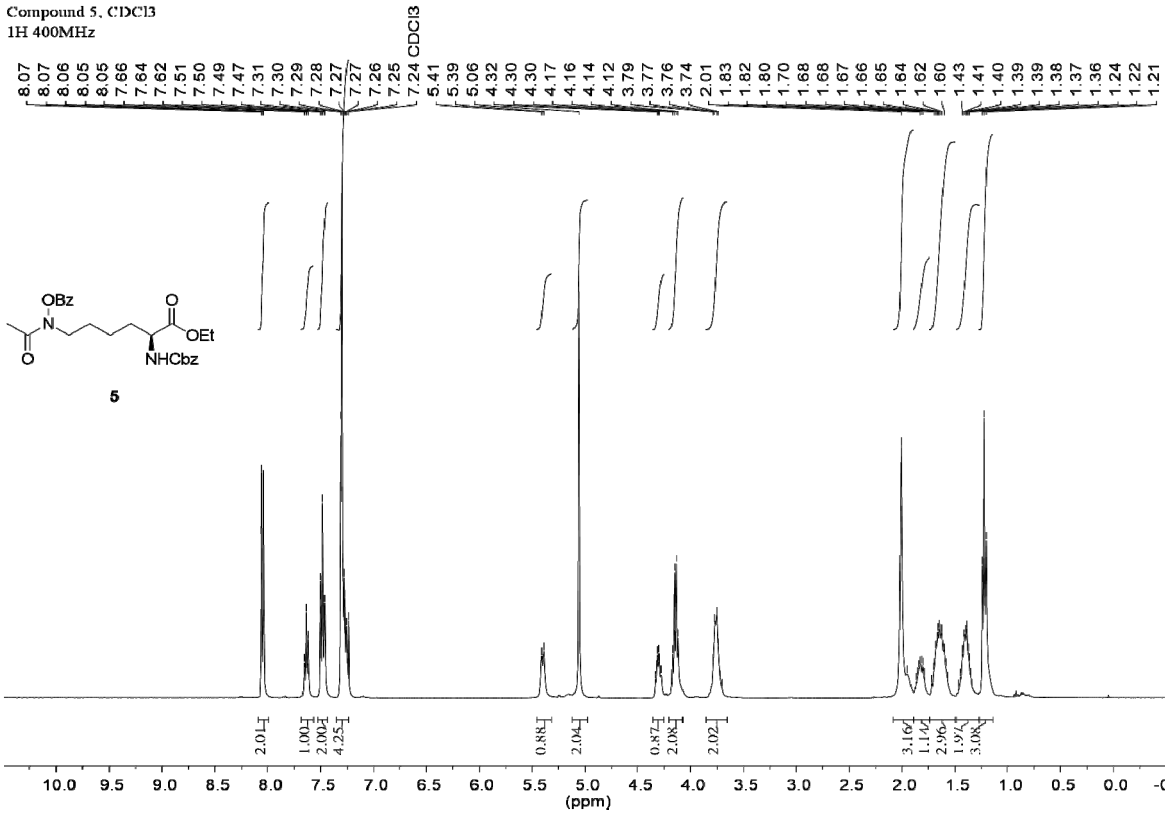
8. A. Natarajan, W. Du, C.-Y. Xiong, G. L. DeNardo, S. J. DeNardo and J. Gervay-Hague, *Chem. Commun.*, 2007, 695-697.

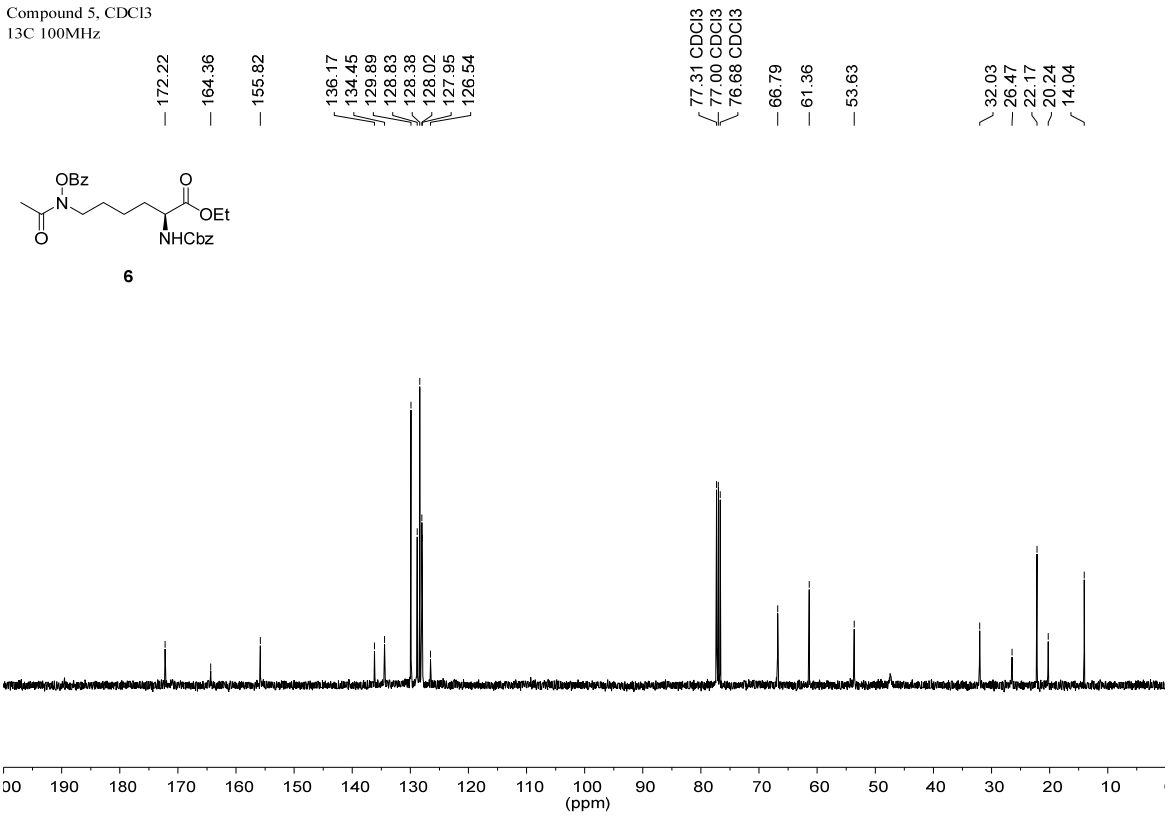
9. E. Klein, S. DeBonis, B. Thiede, D. A. Skoufias, F. Kozielski and L. Lebeau, *Bioorg. Med. Chem.*, 2007, **15**, 6474-6488.

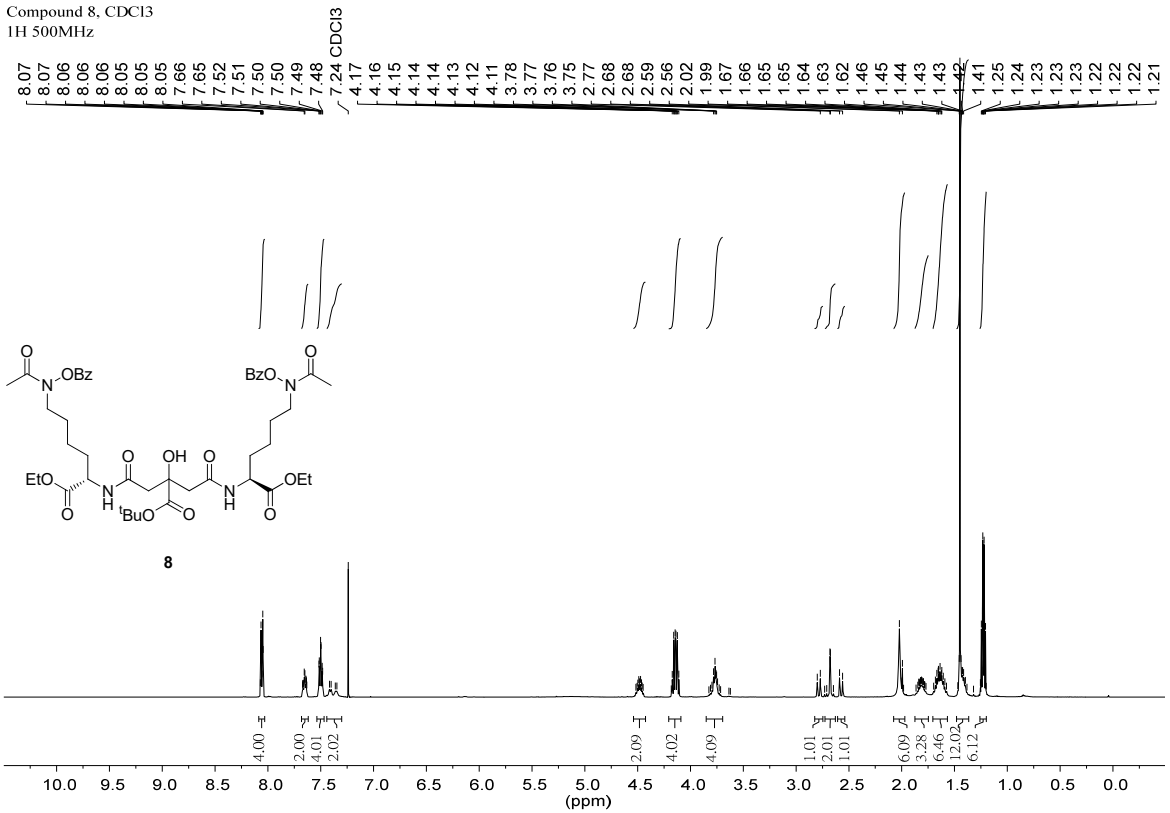
10. B. Schwyn and J. Neilands, *Anal. Biochem.*, 1987, **160**, 47-56.

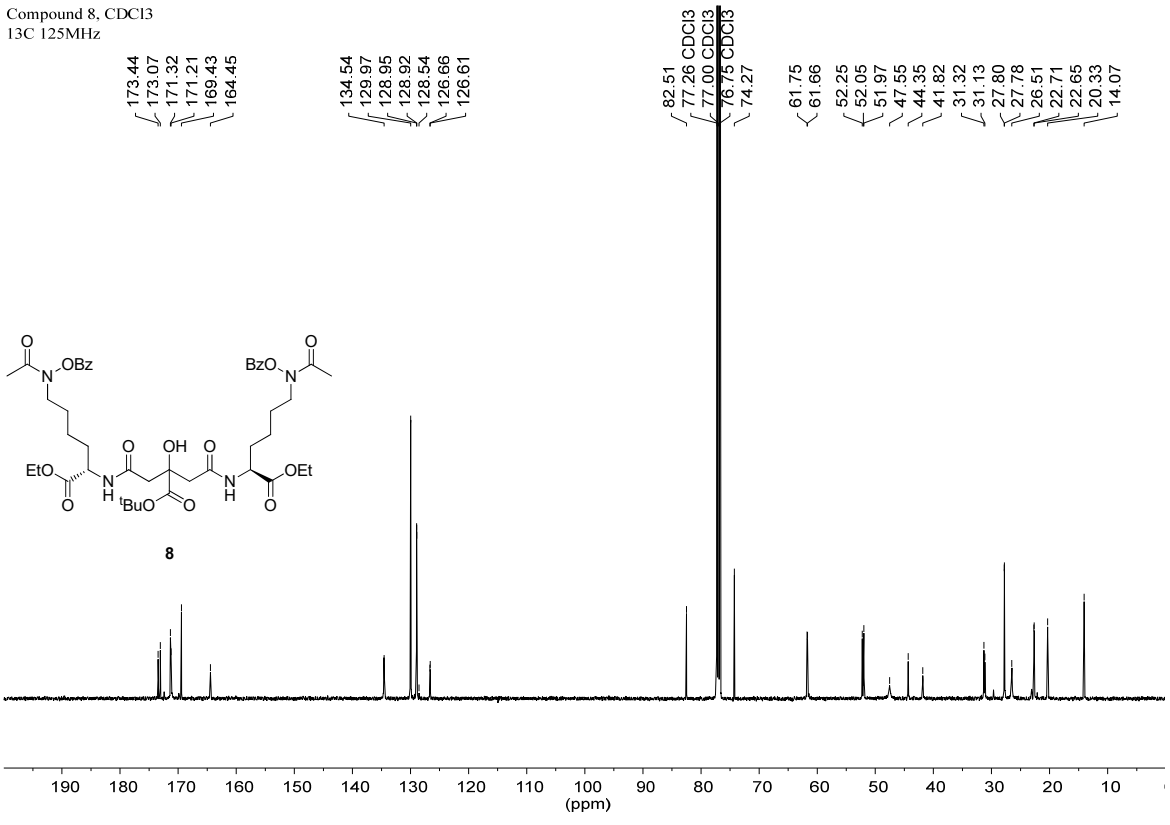

Compound 4, CDCl3
1H 400MHz
7.24 CDCl3
BzO
OEt
NHCbz
4
(ppm)

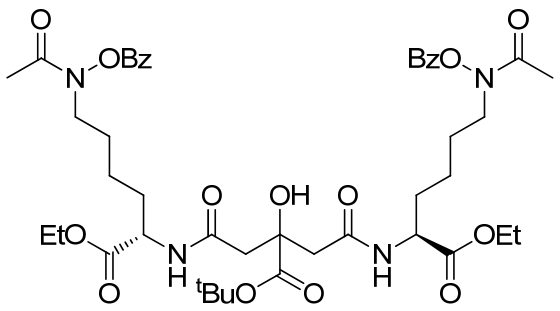

Compound 4, CDCl3
13C 100MHz
172.22
166.68
155.78
136.17
133.18
129.16
128.37
128.34
128.24
127.97
127.90
77.32 CDCl3
77.00 CDCl3
76.68 CDCl3
66.77
61.28
53.66
52.01
32.35
26.71
22.60
14.01
BzO
OEt
NHCbz
4
(ppm)


Compound 5, CDCl3
1H 400MHz
CDCl3
OBz
O
N
OEt
NHCbz
5
(ppm)

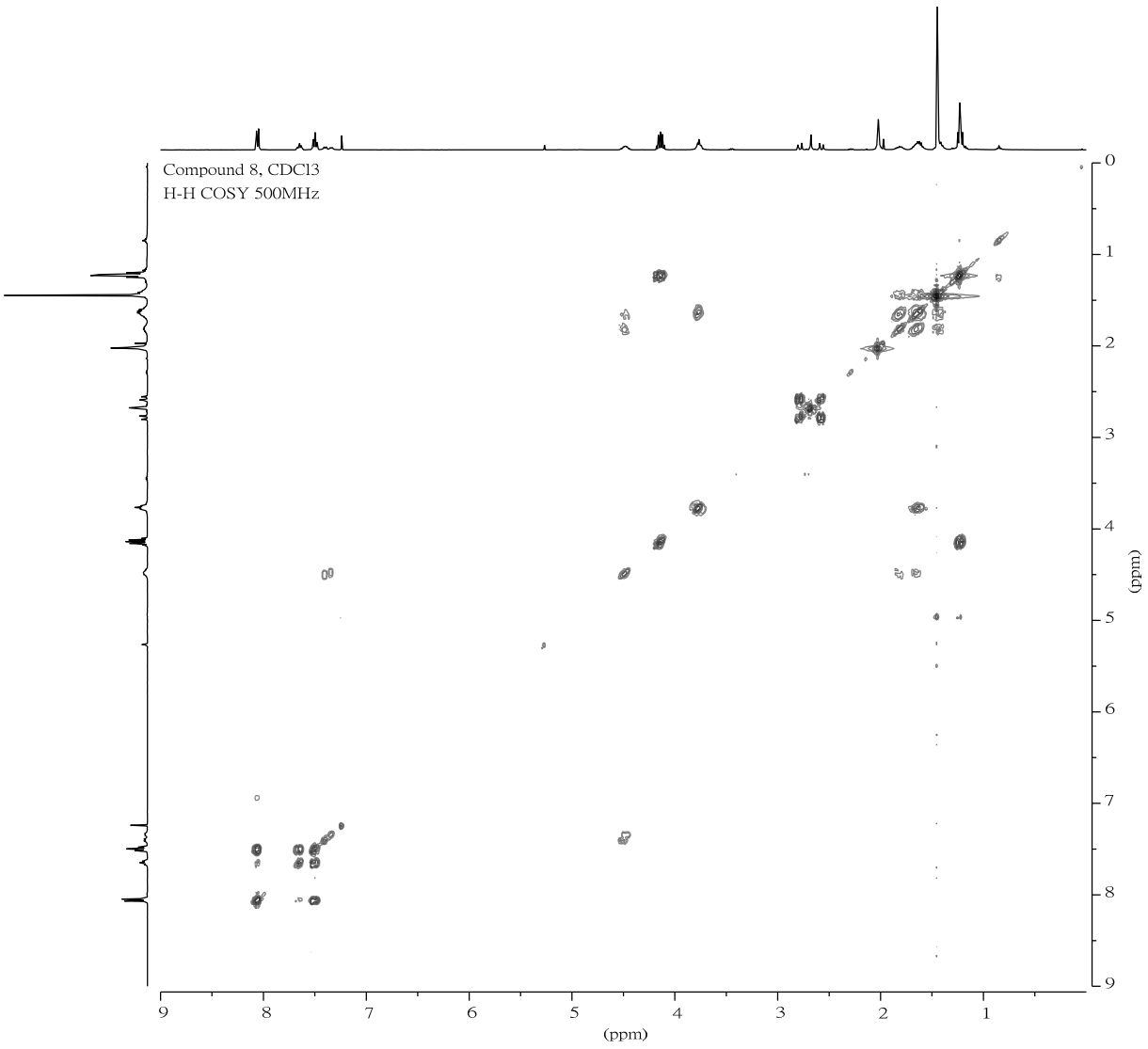

Compound 5, CDCl3
13C 100MHz
77.31 CDCl3
77.00 CDCl3
76.68 CDCl3
OBz
O
N
OEt
NHCbz
6
(ppm)

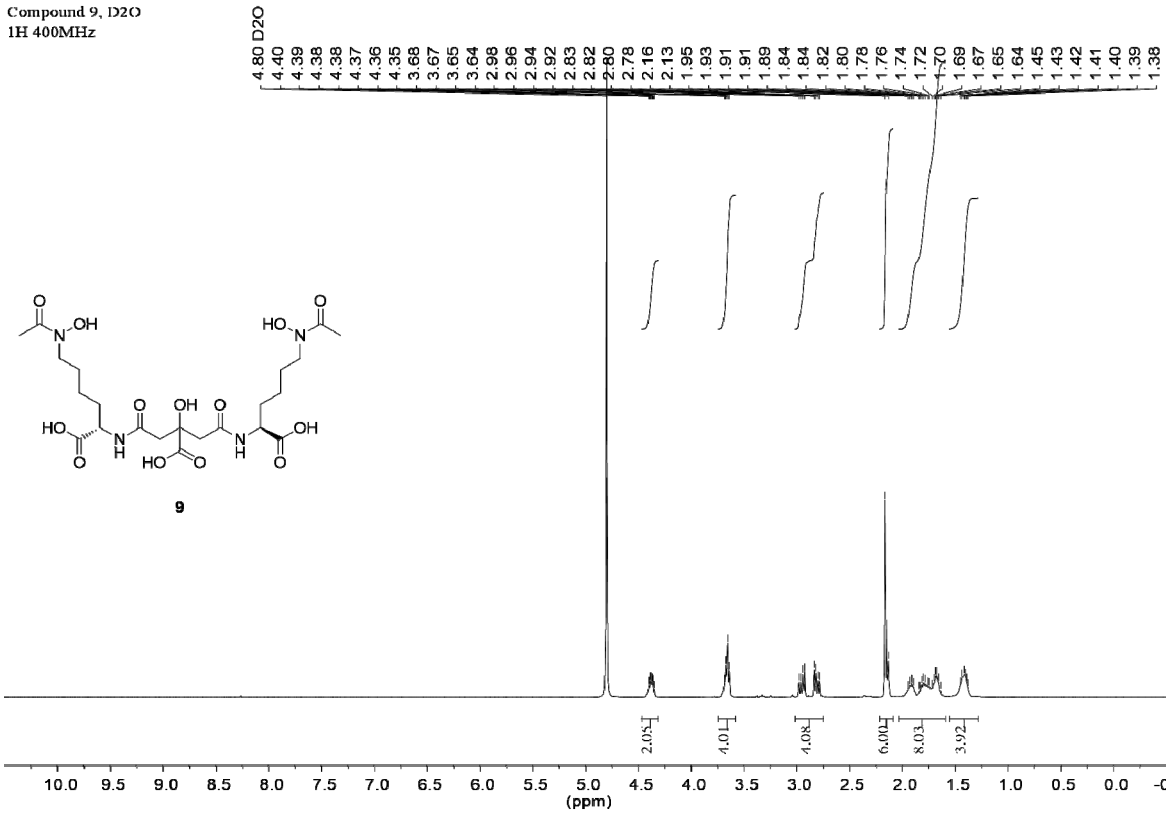

Compound 8, CDCl3
1H 500MHz
7.24 CDCl3
8
(ppm)

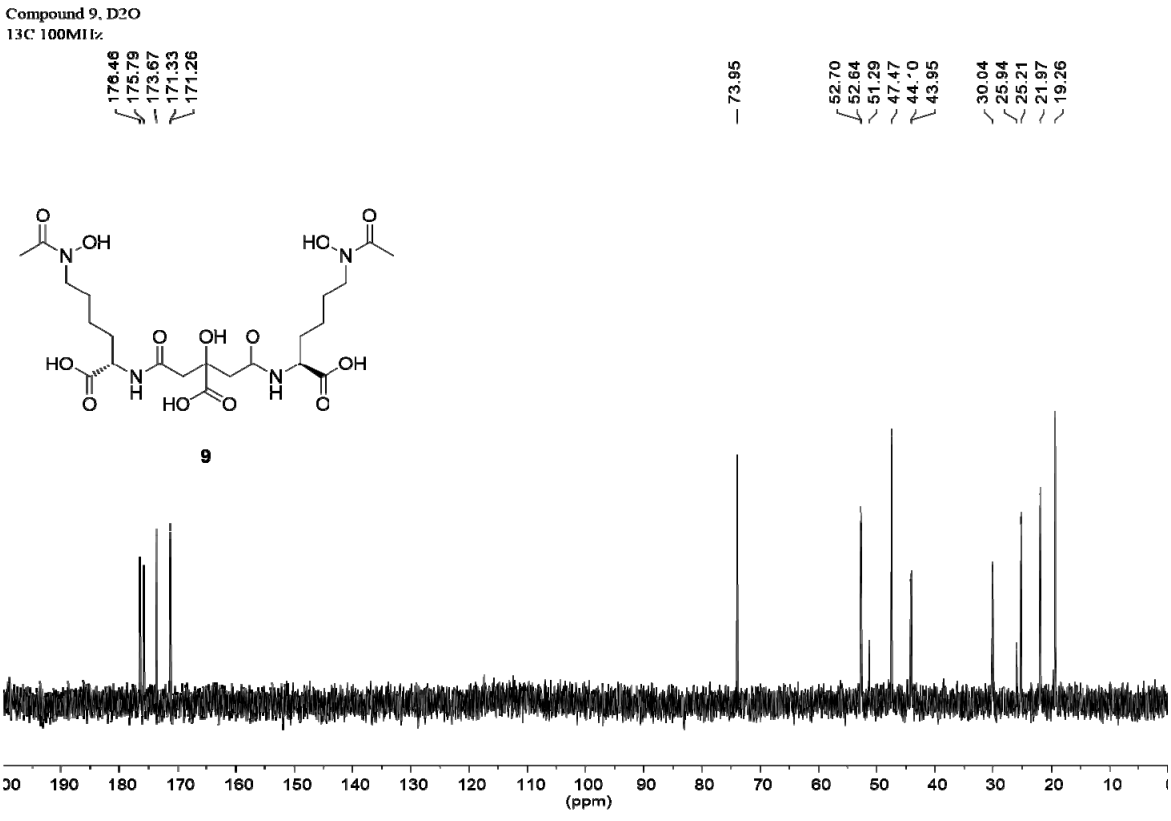

Compound 8, CDCl3
13C 125MHz
77.26 CDCl3
77.00 CDCl3
76.75 CDCl3
8
(ppm)

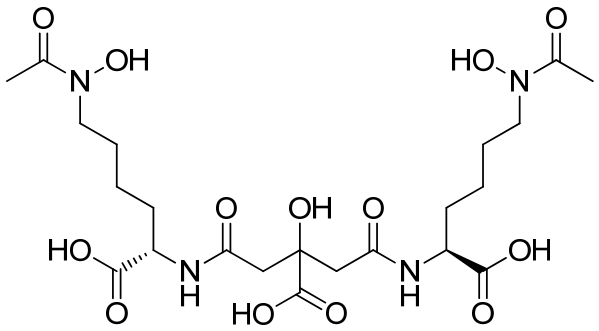

O
N
OBz
BzO
N
O
EtO
O
N
H
O
OH
O
tBuO
O
N
H
O
OEt
O

8


Compound 8, CDCl3
H-H COSY 500MHz
(ppm)
(ppm)


Compound 9, D2O
1H 400MHz
4.80 D2O
9
(ppm)


Compound 9, D2O
13C 100MHz
176.46
175.79
173.67
171.33
171.26
73.95
52.70
52.64
51.29
47.47
44.10
43.95
30.04
25.94
25.21
21.97
19.26
9
(ppm)



9