Trihydroxamate Siderophore-Fluoroquinolone Conjugates are Selective Sideromycin Antibiotics that Target *Staphylococcus aureus*

Supporting Information

Timothy A. Wencewicz[a], Timothy E. Long[a,b], Ute Möllmann[c], and Marvin J. Miller[a*]

[a]*Department of Chemistry and Biochemistry, 251 Nieuwland Science Hall, University of Notre Dame, Notre Dame, IN 46556, USA*

[b]Current address: *Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, USA*

[c]*Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Beutenbergstrasse 11a, 07745 Jena, Germany*

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*To whom correspondence should be addressed. M.J.M: phone, (574) 631-7571; fax, (574) 631-6652; email, mmiller1@nd.edu.

I. Table of All Bacterial Strains From This Work

Table S1. Origins and markers of bacterial strains used in this work.

Strain	Marker	Origin/Reference
Gram-positive bacteria		
Bacillus subtilis ATCC 6633	wild type	American Type Culture Collection
Enterococcus faecalis ATCC 49532	wild type	American Type Culture Collection
Enterococcus faecium NCTC 7171	clinical isolate	National Collection of Type Cultures
Micrococcus luteus ATCC 10240	wild type	American Type Culture Collection
Mycobacterium vaccae IMET 10670	wild type	Hans Knöll Institute, Jena, Germany
Staphylococcus aureus SG511	wild type	Hans Knöll Institute, Jena, Germany
Staphylococcus epidermidis ATCC 14990	wild type	American Type Culture Collection
Gram-negative bacteria		
Acinetobacter baumanii ATCC 17961	wild type	American Type Culture Collection
Enterobacter aerogenes ATCC 35029	wild type quality control strain	American Type Culture Collection
Escherichia coli ATCC 25922	wild type MIC standard strain	American Type Culture Collection
Escherichia coli DC0	wild type	Richmond et al. **1976**[1]
Escherichia coli DC2	antibiotic susceptible penetration mutant	Richmond et al. **1976**[1]
Klebsiella pneumonia ATCC 700603	Produces β-lactamase SHV-18 MIC standard strain	American Type Culture Collection
Klebsiella pneumonia ATCC 8308	wild type	American Type Culture Collection
Pseudomonas aeruginosa ATCC 27853	wild type MIC standard strain	American Type Culture Collection
Pseudomonas aeruginosa KW799/WT	wild Type	Zimmermann **1980**[2]
Pseudomonas aeruginosa KW799/61	antibiotic susceptible penetration mutant	Zimmermann **1980**[2]
Salmonella typhimurium ATCC 13311	wild type	American Type Culture Collection
Salmonella typhimurium enb7	ent-, mutant dependent on siderophores for growth on low-Fe media	J. B. Nielands, Univ. of Cal. Berkeley, USA

II. Table of Antibiotic Susceptibility Testing in the Agar Diffusion Assay

Table S2. Diameter of growth inhibition zone (mm) in the agar diffusion antibiotic susceptibility assay.[a,b,c]

	Test Compound																			
	1a	**2a**	**3a**	**3a-Fe**	**1b**		**2b**		**3b**		**1c**		**2c**		**3c**		Lor[d]		Cipro[e]	
	2 mM	2 mM	2 mM	2 mM	1 mM		1 mM		1 mM		0.5 mM		0.5 mM		0.5 mM		0.1 mM		5 μg/mL	
Test Organism	-Fe[f]	-Fe	-Fe	-Fe	+Fe[g]	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
S. aureus SG511	0	0	0	0	27.8 (7)	27.5 (6)	21.2 (9)	22.8 (4)	20.5 (3)	21.5 (3)	14.7 (2)	18.6 (3)	11.6 (7)	17.9 (4)	26.5 (6)	29.5 (2)	25.8 (6)	25.7 (3)	23.3 (1)	23.4 (3)
S. epidermidis ATCC 14990	0	0	0	0	27	27	nt[h]	nt	15	17	18.5	21	nt	12	12	19	22	21.5	27	27
E. faecalis ATCC 49532	0	0	0	0	0	0	nt	nt	0	0	12*	13*	nt	12*	0	0	0	0	15	16
M. luteus ATCC 10240	0	0	0	0	37	42	nt	nt	11*	22	0	0	nt	0	0	0	39	40	0	0
B. subtilis ATCC 6633	0	0	0	0	23	25	nt	nt	12	17	22	22	nt	17	12	15	34	34	34	34.5
M. vaccae IMET 10670	0	0	0	0	nt	nt	nt	nt	nt	nt	nt	25	nt	0	nt	17*	nt	nt	nt	38
A. baumanii ATCC 17961	0	0	0	0	0	0	12.4 (5)	15.9 (5)	0	10.5 (1)	0	0	0	0	0	0	0[i]	0[i]	21.4 (2)	21.1 (3)
E. coli DC0	0	0	20*	0	13*	13*	nt	nt	0	13*	0	12*	nt	nt	0	12*	21	21	21	21
E. coli DC2	0	0	19*	0	18	20	nt	nt	11*	15*	13	15*	nt	nt	0	14*	20	20	26	26
P. aeruginosa 799/WT	0	0	0	0	0	10*	nt	nt	0	0	0	10*	nt	0	0	10*	0	0	25	26
P. aeruginosa 799/61	0	0	0	0	18*	18*	nt	nt	19*	23*	15*	15*	nt	0	0	11*	15*	14*	28	29
K. pneumonia ATCC 8308	0	0	0	0	13*	12*	nt	nt	0	11*	15*	15*	nt	nt	12*	14*	21.5	22	24.5	26
S. typhimurium ATCC 13311	0	0	0	0	21	20	nt	nt	13	14	22	24	nt	nt	19	20	23.5	23	32	33
S. typhimurium enb7	0	0	0	0	14	18	nt	nt	12*	17*	15.5	17	nt	16	14	23.5	22	22	29	30

[a]Exactly 50 μL of each compound solution (dissolved in 10:1 MeOH:DMSO at concentrations provided in table) were added to 9 mm wells in agar media (MHII) inoculated with ~5x10^3 CFU/mL. Diameters of growth inhibition zones were measured (mm) with an electronic caliper after incubation at 37 ºC for 24 h.[3] [b]Compounds with a standard deviation (shown in parentheses for the last significant digit) were tested in triplicate; otherwise, compounds were tested in a single trial. [c]Data in this table was used to generate the bar graphs shown in Figures 6 and 7 in the main text. [d]Lor: Lorabid® was used as a standard at 0.1 mM in H_2O or at concentration indicated for specific strains. [e]Cipro: ciprofloxacin was used as a standard at 5 μg/mL in H_2O. [f]-Fe: Mueller-Hinton agar No. 2 + 100 μM 2,2'-bipyridine. [g]+Fe: Mueller-Hinton agar No. 2 + 100 μM $FeCl_3$. [h]nt: not tested. [i]Lorabid® used at 1.0 mM. *Indiacteas a partially unclear inhibition zone.

III. Table of Data for the Siderophore Competition Agar Diffusion Assay

Table S3. Diameter of growth inhibition in the agar diffusion antibiotic susceptibility assay.[a,b,c]

		Test Organism	
		Staphylococcus aureus SG511	*Acinetobacter baumanii* ATCC 17961
Compound	**Con.**[d]	MHII – Fe[e]	MHII – Fe
1b + **1a**	1 mM	25.9(4)	0
1b + **DFO-B**	1 mM	26.8(9)	nt[f]
2b + **2a**	1 mM	22.9(7)	12.2(2)
2b + **DFO-B**	1 mM	24.1(5)	nt
3b + **3a**	1 mM	22.5(8)	12.5(4)
3b + **3a-Fe**	1 mM	21.1(6)	12.2(3)
3b + **DFO-B**	1 mM	19.6(5)	nt
1c + **1a**	0.5 mM	15.3(5)	nt
1c + **DFO-B**	0.5 mM	16.4(2)	nt
2c + **2a**	0.5 mM	13.3(2)	nt
2c + **DFO-B**	0.5 mM	11.7(2)	nt
3c + **3a**	0.5 mM	23.1(5)	nt
3c + **3a-Fe**	0.5 mM	21.6(9)	nt
3c + **DFO-B**	0.5 mM	0	nt
3c + **FO-B**	0.5 mM	0	nt

[a]Exactly 50 μL of each compound solution (dissolved in 10:1 MeOH:DMSO at concentrations provided in table) were added to 9 mm wells in agar media (MHII) inoculated with ~$5x10^3$ CFU/mL. Diameters of growth inhibition zones were measured (mm) with an electronic caliper after incubation at 37 ºC for 24 h.[3] [b]Compounds with a standard deviation shown in parentheses were tested in triplicate; otherwise, compounds were tested in a single trial. [c]Data in this table was used to generate the bar graphs shown in Figures 6 and 7 in the main text. [d]Con.: all compound mixtures were tested as 1:1 molar mixtures both at the concentrations indicated. [e]MHII–Fe: Mueller-Hinton agar No. 2 + 100 μM 2,2'-bipyridine. [f]nt: not tested. *Indiacteas a partially unclear inhibition zone.

IV. Experimental Procedures and Compound Characterization Data

N-Boc-*O*-PNB-Lorabid® (**7**) was a gift from Eli Lilly and Company. Compounds **4-6**, **3a**, and **3a-Fe** were synthesized by previously reported methods.[4,5] The experimental procedures for the syntheses of compounds **3a**, **3a-Fe**, **3b**, **3c**, **8**, **10**, **15**, and **16** are provided in the main text.

Monohydroxamate siderophore (1a). bis-*O*-Benzyl-monohydroxamate **4** (105.0 mg, 0.26 mmol) was dissolved in 5 mL of MeOH in an HCl-washed, 10 mL round bottom flask sealed under argon. The flask was charged with 10% Pd-C (15.0 mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (1.5:1 CH_3CN:H_2O; $FeCl_3$ stain) and after 6 h there was no remaining starting material (**4**). The flask was flushed with argon and the mixture was diluted with MeOH (10 mL), vacuum filtered through celite, and concentrated under reduced pressure. This gave the desired product (**1a**) in 88% yield as a tan semi-solid (50.6 mg, 0.23 mmol) with no need for purification. ^{1}H-NMR (600 MHz, CD_3OD) δ 3.60 (t, *J* = 6.9 Hz, 2 H), 3.55 (t, *J* = 6.6 Hz, 2 H), 2.75 (t, *J* = 6.9 Hz, 2 H), 2.56 (t, *J* = 6.9 Hz, 2 H), 1.64 (quin, *J* = 7.3 Hz, 2 H), 1.59–1.52 (m, 2 H), 1.40–1.33 (m, 2 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 177.0, 174.5, 62.9, 49.1, 33.4, 29.9, 28.6, 27.6, 24.1; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_9H_{18}NO_5$: 220.1179, found 220.1172.

Bishydroxamate siderophore (2a). tris-*O*-Benzyl-monohydroxamate **5** (90.6 mg, 0.13 mmol) was dissolved in 8 mL of MeOH in an HCl-washed, 25 mL round bottom flask sealed under argon. The flask was charged with 10% Pd-C (19.0 mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (1.5:1 CH_3CN:H_2O; $FeCl_3$ stain) and after 4.5 h there was no remaining starting material (**5**). The flask was flushed with argon and the mixture was diluted with MeOH (10 mL), vacuum filtered through celite, and concentrated under reduced pressure. The crude product was recrystallized from MeOH/Et_2O at -20 ºC to give the desired product (**2a**) in 90% yield as a white powder (49.2 mg, 0.12 mmol). Mp 81–83 ºC; ^{1}H-NMR (600 MHz, CD_3OD) δ 3.64–3.57 (m, 4 H), 3.55 (t, *J* = 6.5 Hz, 2 H), 3.16 (t, *J* = 6.6 Hz, 2 H), 2.80–2.72 (m, 4 H), 2.56 (t, *J* = 6.6 Hz, 2 H), 2.45 (t, *J* = 7.0 Hz, 2 H), 1.68–1.60 (m, 4 H), 1.59–1.48 (m, 4 H), 1.43–1.28 (m, 4 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 177.0, 175.1, 174.6, 62.9, 49.0, 40.4, 33.4, 31.6, 30.1, 30.0, 29.1, 28.6, 27.7, 27.5, 25.0, 24.1; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{18}H_{34}N_3O_8$: 420.2340, found 420.2354.

bis-*O*-Benzyl-monohydroxamate-*O*-PNB-Lorabid® conjugate (11). *O*-PNB-Lorabid® TFA salt (**8**; 87.5 mg, 0.15 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2 and *i*Pr_2EtN (0.1 mL, 0.57 mmol) was added slowly until the solution was basic (pH paper). Bis-*O*-Benzyl-monohydroxamate **4** (59.0 mg, 0.15 mmol), DMAP (5.0 mg, 0.04 mmol), and EDC-HCl (60.0 mg, 0.31 mmol) were then added, respectively. After 18 h at rt, TLC (6% MeOH in CH_2Cl_2; $FeCl_3$ stain) showed no remaining starting material (**4**). The mixture was diluted with CH_2Cl_2 (10 mL), washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography (0.5 x 5 in silica gel; 3% MeOH in CH_2Cl_2) to give the desired product (**11**) in 73% yield as a colorless, viscous oil (93.3 mg, 0.11 mmol). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.41 (d, *J* = 8.2 Hz, 1 H), 8.19 (d, *J* = 8.2 Hz, 2 H), 7.59 (d, *J* = 8.5 Hz, 2 H), 7.42–7.22 (m, 15 H), 6.57 (br s, 1 H), 5.50 (d, *J* = 6.2 Hz, 1 H), 5.43–5.38 (m, 2 H), 5.26 (d, *J* = 13.5 Hz, 1 H), 4.82 (d, *J* = 10.3 Hz, 1 H), 4.71 (d, *J* = 10.0 Hz, 1 H), 4.41 (s, 2 H), 3.89 (dd, *J* = 7.6, 4.1 Hz, 2

H), 3.47–3.39 (m, 2 H), 3.35–3.28 (m, 1 H), 2.93–2.81 (m, 2 H), 2.67–2.52 (m, 2 H), 2.32 (t, J = 5.1 Hz, 2 H), 1.95–1.88 (m, 1 H), 1.68–1.46 (m, 5 H), 1.34–1.28 (m, 2 H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 173.8, 172.2, 171.0, 170.9, 164.8, 160.0, 147.7, 142.2, 138.5, 136.7, 133.9, 130.7, 129.6, 129.3, 129.1, 128.8, 128.5, 128.2, 127.9, 127.8, 127.5, 127.3, 127.1, 127.0, 123.7, 123.6, 123.2, 73.3, 72.7, 70.1, 66.2, 59.5, 59.2, 58.5, 57.8, 53.0, 52.6, 44.6, 32.0, 30.9, 30.4, 29.3, 29.2, 29.1, 28.1, 27.4, 26.5, 26.4, 23.2, 21.4, 21.3; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{46}H_{49}ClN_5O_{10}$: 866.3162, found 866.3193.

Monohydroxamate-Lorabid® conjugate (1b). bis-*O*-Benzyl-monohydroxamate-Lorabid® conjugate **11** (84.0 mg, 0.10 mmol) and concentrated HCl (42.1 μL, 30.0 mmol) were dissolved in 1.4 mL of DMF:H_2O (95:5) in an HCl-washed, 10 mL round bottom flask sealed under argon. The flask was charged with 10% Pd-C (29.4 mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (2.5:1 H_2O:CH_3CN; $FeCl_3$ stain) and after 41 h there was no remaining starting material (**11**). The flask was flushed with argon and the mixture was diluted with MeOH, vacuum filtered through celite, and concentrated using high vaccum rotary evaporation (~1 mm Hg). The crude product was purified by preparative HPLC using a 150 x 20 mm YMC-Pack Proc C18 column fit with a guard column, 0.1% TFA in H_2O (A) and 0.1% TFA in CH_3CN (B) as mobile phases, and a flow rate of 15 mL/min. A gradient was formed from 30%-60% of B over 4 min where the desired compound (**1b**) elutes at 3 min. Pure fractions were lyophilized and the obtained solid was recrystallized from MeOH/Et_2O to give the desired product (**1b**) in 38% yield as a tan solid (20.2 mg, 0.04 mmol). Mp 110–112 ºC (color change), 190–195 ºC (dec.); ^{1}H-NMR (600 MHz, CD_3OD) δ 7.44–7.31 (m, 5 H), 5.44–5.42 (m, 1 H), 5.37 (d, J = 4.7 Hz, 1 H), 3.88 (dt, J = 11.5, 4.4 Hz, 1 H), 3.82 (ddd, J = 14.0, 7.3, 7.1 Hz, 1 H), 3.60–3.52 (m, 4 H), 3.30–3.25 (m, 1 H), 3.19–3.14 (m, 2 H), 2.86–2.82 (m, 2 H), 2.76 (t, J = 7.2 Hz, 2 H), 2.71–2.61 (m, 2 H), 2.60–2.51 (m, 3 H), 2.47 (t, J = 7.2 Hz, 2 H), 2.40 (dt, J = 14.9, 5.5 Hz, 1 H), 1.77–1.71 (m, 1 H), 1.67–1.47 (m, 9 H), 1.39–1.27 (m, 4 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 175.6, 175.1, 174.7, 174.6, 173.9, 166.3, 138.3, 130.0, 129.6, 129.4, 62.9, 59.9, 59.6, 54.1, 48.7, 40.5, 33.4, 32.5, 31.7, 31.3, 29.9, 29.0, 28.9, 27.6, 27.2, 24.8, 24.1, 23.1; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{25}H_{32}ClN_4O_8$: 551.1903, found 551.1889.

bis-*O*-Benzyl-monohydroxamate-*O*-benzylciprofloxacin conjugate (12). *O*-Benzyl-ciprofloxacin hydrochloride salt (**10**) was free-based using Amberlite IR400(OH^-) resin in $CHCl_3$ for 4 h. The resulting *O*-benzyl-ciprofloxacin amine (53.1 mg, 0.13 mmol), bis-*O*-Benzyl-monohydroxamate **4** (50.5 mg, 0.13 mmol), *i*Pr_2EtN (0.05 mL, 0.29 mmol), DMAP (4.0 mg, 0.03 mmol), and EDC-HCl (36.2 mg, 0.189 mmol) were dissolved in 5 mL of anhydrous CH_2Cl_2, respectively. After 5 h at rt, TLC (6% MeOH in CH_2Cl_2; $FeCl_3$ stain) showed no remaining starting material **4**. The mixture was diluted with CH_2Cl_2 (25 mL), washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography (0.75 x 4 in silica gel; 3% MeOH in CH_2Cl_2) to give the desired product (**12**) in 92% yield as a clear, yellow oil (93.3 mg, 0.12 mmol). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.54 (s, 1 H), 8.09–8.06 (m, 1 H), 7.52 (d, J = 7.6 Hz, 2 H), 7.43–7.24 (m, 14 H), 5.40 (s, 2 H), 4.93 (s, 2 H), 4.48 (s, 2 H), 3.87–3.84 (m, 2 H), 3.77–3.73 (m, 2 H), 3.66 (t, J = 6.6 Hz, 2 H), 3.46 (t, J = 6.5 Hz, 2 H), 3.43–3.38 (m, 1 H), 3.31–3.27 (m, 2 H), 3.24–3.20 (m, 2 H), 2.89–2.83 (m, 2 H), 2.70 (t, J = 6.3 Hz, 2 H), 1.71–1.60 (m, 4 H), 1.42–1.36 (m, 2 H), 1.30 (q, J = 6.7 Hz, 2 H), 1.14–1.10 (m, 2 H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 173.5, 173.0, 170.5, 165.5, 153.3 (d, J = 248.5 Hz), 148.4, 144.1 (d,

J = 10.7 Hz), 138.5, 137.9, 136.4, 129.1, 128.8, 128.7, 128.5, 128.3, 128.0, 127.9, 127.6, 127.5, 123.5 (d, $J_{C\text{-}F}$ = 7.3 Hz), 113.5 (d, $J_{C\text{-}F}$ = 23.6 Hz), 110.2, 105.1, 76.3, 72.8, 70.1, 66.4, 50.2, 49.7, 45.5, 45.2, 41.5, 34.5, 29.4, 27.5, 27.3, 26.7, 23.4, 8.2; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{47}H_{52}FN_4O_7$: 803.3815, found 803.3825.

Monohydroxamate-ciprofloxacin conjugate (1c). bis-*O*-Benzyl-monohydroxamate-*O*-benzylciprofloxacin conjugate (**12**; 75.0 mg, 0.09 mmol) was dissolved in 8 mL of MeOH in an HCl-washed, 25 mL rb flask sealed under argon. The flask was charged with 10% Pd-C (15.0 mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (1.5:1 CH_3CN:H_2O; $FeCl_3$ stain) and after 12 h there was no remaining starting material (**12**). The flask was flushed with argon and the mixture was diluted with MeOH, vacuum filtered through celite, and concentrated under reduced pressure. The resulting solid was dissolved in a minimal amount of MeOH and precipitated by addition of cold Et_2O. After trituration with Et_2O the desired product (**1c**) was obtained in 70% yield as a light tan solid (35.0 mg, 0.065 mmol). Mp 180–185 ºC (dec.); ^{1}H-NMR (600 MHz, CD_3OD) δ 8.71 (s, 1 H), 7.84 (d, J = 13.2 Hz, 1 H), 7.55 (br s, 1 H), 3.88–3.78 (m, 4 H), 3.74 (br s, 1 H), 3.61 (t, J = 6.9 Hz, 2 H), 3.55 (t, J = 6.5 Hz, 2 H), 3.47–3.40 (m, 2 H), 3.40–3.33 (m, 2 H), 2.83 (t, J = 5.9 Hz, 2 H), 2.73 (t, J = 6.2 Hz, 2 H), 1.65 (dt, J = 14.4, 7.2 Hz, 2 H), 1.59–1.53 (m, 2 H), 1.43–1.34 (m, 4 H), 1.24–1.18 (m, 2 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 178.2, 174.7, 173.2, 170.0, 155.1 (d, $J_{C\text{-}F}$ = 249.6 Hz), 149.3, 146.8 (d, $J_{C\text{-}F}$ = 10.7 Hz), 140.8, 121.3, 112.7 (d, $J_{C\text{-}F}$ = 23.0 Hz), 107.5, 62.9, 51.2, 50.7, 46.6, 42.9, 37.0, 33.4, 28.7, 28.6, 27.7, 24.1, 8.7; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{26}H_{34}FN_4O_7$: 533.2406, found 533.2415.

tris-*O*-Benzyl-bishydroxamate-*O*-PNB-Lorabid® conjugate (13). *O*-PNB-Lorabid® TFA salt (**8**; 87.9 mg, 0.15 mmol) was dissolved in 15 mL of anhydrous CH_2Cl_2 and iPr$_2$EtN (0.13 mL, 0.75 mmol) was added slowly until the solution was basic (pH paper). Tris-*O*-Benzyl-bishydroxamate **5** (103.6 mg, 0.15 mmol), DMAP (5.0 mg, 0.04 mmol), and EDC-HCl (65.0 mg, 0.34 mmol) were then added, respectively. After 16 h at rt, TLC (6% MeOH in CH_2Cl_2; $FeCl_3$ stain) showed no remaining starting material (**5**). The mixture was diluted with CH_2Cl_2 (10 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), 10% aqueous citric acid (10 mL), and brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography (1 x 4 in silica gel; 3% MeOH in CH_2Cl_2) to give the desired product (**13**) in 66% yield as a colorless, viscous oil (114.7 mg, 0.10 mmol). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.58 (d, J = 7.3 Hz, 1 H), 8.22 (d, J = 8.5 Hz, 2 H), 7.72 (d, J = 6.7 Hz, 1 H), 7.61 (d, J = 8.5 Hz, 2 H), 7.45–7.21 (m, 20 H), 6.58 (br s, 1 H), 5.60 (d, J = 6.5 Hz, 1 H), 5.46–5.39 (m, 2 H), 5.30 (d, J = 13.2 Hz, 1 H), 4.88–4.79 (m, 3 H), 4.76 (d, J = 10.0 Hz, 1 H), 4.47 (s, 2 H), 4.04–3.96 (m, 1 H), 3.90 (dd, J = 11.0, 4.5 Hz, 1 H), 3.41 (t, J = 6.6 Hz, 2 H), 3.40–3.29 (m, 2 H), 3.22 (dt, J = 14.0, 5.0 Hz, 1 H), 2.96–2.80 (m, 3 H), 2.77–2.68 (m, 1 H), 2.68–2.37 (m, 6 H), 1.95–1.88 (m, 1 H), 1.82–1.75 (m, 2 H), 1.74–1.45 (m, 6 H), 1.44–1.33 (m, 3 H), 1.31–1.20 (m, 3 H), 1.19–1.09 (m, 1 H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 174.4, 173.9, 172.5, 171.9, 171.5, 165.3, 160.0, 147.8, 142.0, 138.5, 136.7, 134.2, 134.0, 131.0, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127.8, 127.6, 127.5, 123.7, 76.3, 76.1, 72.8, 70.1, 66.2, 58.9, 57.9, 52.9, 45.4, 43.5, 39.2, 32.0, 30.4, 30.1, 29.3, 27.8, 27.6, 27.5, 26.5, 25.6, 23.3, 23.2, 21.9, 21.3; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{62}H_{71}ClN_7O_{13}$: 1156.4793, found 1156.4778.

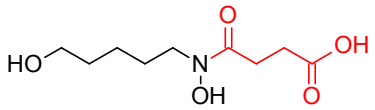
Bishydroxamate-Lorabid® conjugate (2b). tris-*O*-Benzyl-bishydroxamate-Lorabid® conjugate **13** (103.0 mg, 0.09 mmol) and concentrated HCl (38.6 μL, 27.0 mmol) were dissolved in 1.3 mL of DMF:H_2O (95:5) in an HCl-washed, 10 mL round bottom flask sealed under argon. The flask was charged with 10% Pd-C (27.0 mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (2.5:1 H_2O:CH_3CN; $FeCl_3$ stain) and after 24 h there was no remaining starting material (**13**). The flask was flushed with argon and the mixture was diluted with MeOH, vacuum filtered through celite, and concentrated using high vaccum rotary evaporation (~1 mm Hg). The crude product was purified by preparative HPLC using a 150 x 20 mm YMC-Pack Proc C18 column fit with a guard column, 0.1% TFA in H_2O (A) and 0.1% TFA in CH_3CN (B) as mobile phases, and a flow rate of 15 mL/min. A gradient was formed from 30%-60% of B over 4 min. Fractions containing pure compound **2b** were lyophilized and the obtained solid was recrystallized from MeOH/Et_2O to give the desired product (**2b**) in 42% yield as an off-white solid (26.0 mg, 0.035 mmol). Mp 96–98 ºC (color change), 160–165 ºC (dec.); ^{1}H-NMR (600 MHz, CD_3OD) δ 7.44–7.31 (m, 5 H), 5.44–5.42 (m, 1 H), 5.37 (d, *J* = 4.7 Hz, 1 H), 3.88 (dt, *J* = 11.5, 4.4 Hz, 1 H), 3.82 (ddd, *J* = 14.0, 7.3, 7.1 Hz, 1 H), 3.60–3.52 (m, 4 H), 3.30–3.25 (m, 1 H), 3.19–3.14 (m, 2 H), 2.86–2.82 (m, 2 H), 2.76 (t, *J* = 7.2 Hz, 2 H), 2.71–2.61 (m, 2 H), 2.60–2.51 (m, 3 H), 2.47 (t, *J* = 7.2 Hz, 2 H), 2.40 (dt, *J* = 14.9, 5.5 Hz, 1 H), 1.77–1.71 (m, 1 H), 1.67–1.47 (m, 9 H), 1.39–1.27 (m, 4 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 175.6, 175.0, 174.7, 174.6, 173.9, 166.3, 138.3, 130.0, 129.6, 129.4, 62.9, 59.9, 59.6, 54.1, 48.7, 40.5, 33.4, 32.5, 31.7, 31.3, 29.9, 29.0, 28.9, 27.6, 27.2, 24.8, 24.1, 23.1; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{34}H_{48}ClN_6O_{11}$: 751.3064, found 751.3051.

tris-*O*-Benzyl-bishydroxamate-*O*-benzylciprofloxacin conjugate (14). *O*-Benzyl-ciprofloxacin hydrochloride salt (**10**; 70.0 mg, 0.15 mmol), tris-*O*-benzyl-bishydroxamate **5** (96.0 mg, 0.14 mmol), *i*Pr_2EtN (0.1 mL, 0.57 mmol), DMAP (4.3 mg, 0.035 mmol), and EDC-HCl (53.3 mg, 0.28 mmol) were dissolved in 10 mL of anhydrous CH_2Cl_2, respectively. After 23 h at rt, TLC (6% MeOH in CH_2Cl_2; $FeCl_3$ stain) showed no remaining starting material **5**. The mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography (1 x 4 in silica gel; 3% MeOH in CH_2Cl_2) to give the desired product (**14**) in 66% yield as a clear, colorless oil (101.0 mg, 0.09 mmol). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.52 (s, 1 H), 8.09 - 8.05 (m, 1 H), 7.51 (d, *J* = 7.0 Hz, 2 H), 7.42 - 7.23 (m, 19 H), 6.25 (br s, 1 H), 5.38 (s, 2 H), 4.90 (s, 2 H), 4.83 (s, 2 H), 4.46 (s, 2 H), 3.86–3.81 (m, 2 H), 3.76–3.72 (m, 2 H), 3.69–3.63 (m, 2 H), 3.63–3.56 (m, 2 H), 3.42 (t, *J* = 6.6 Hz, 2 H), 3.41–3.36 (m, 1 H), 3.31–3.26 (m, 2 H), 3.24–3.17 (m, 4 H), 2.85–2.80 (m, 2 H), 2.80–2.74 (m, 2 H), 2.69 (t, *J* = 6.0 Hz, 2 H), 2.45 (t, *J* = 6.6 Hz, 2 H), 1.68–1.56 (m, 6 H), 1.51–1.45 (m, 2 H), 1.38–1.24 (m, 6 H), 1.11–1.07 (m, 2 H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 173.7, 173.0, 172.2, 170.4, 165.5, 153.3 (d, J_{C-F} = 248.5 Hz), 148.4, 144.1 (d, J_{C-F} = 10.7 Hz), 138.5, 137.9, 136.4, 134.5, 129.5, 129.2, 129.1, 128.9, 128.7, 128.6, 128.5, 128.3, 127.9, 127.8, 127.6, 127.5, 123.5 (d, J_{C-F} = 6.7 Hz), 113.5 (d, J_{C-F} = 23.0 Hz), 110.2, 105.1, 76.3, 76.2, 72.8, 70.1, 66.4, 50.3, 50.2, 49.6, 49.5, 45.5, 45.2, 45.0, 41.5, 39.3, 34.5, 30.7, 29.3, 28.9, 28.2, 27.3, 27.2, 26.7, 26.4, 23.8, 23.4, 8.1; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{63}H_{74}FN_6O_{10}$: 1093.5445, found 1093.5457.

Bishydroxamate-ciprofloxacin conjugate (2c). tris-*O*-Benzyl-bishydroxamate-*O*-benzylciprofloxacin conjugate (**14**; 95.0 mg, 0.09 mmol) was dissolved in 8 mL of MeOH in an HCl-washed, 25 mL rb flask sealed under argon. The flask was charged with 10% Pd-C (15.0

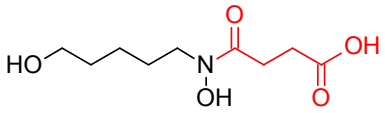
mg) and exposed to a balloon of hydrogen gas (~1 atm). Reaction progress was monitored by RP-C18 TLC (1.5:1 CH_3CN:H_2O; $FeCl_3$ stain) and after 16 h there was no remaining starting material (**14**). The flask was flushed with argon and the mixture was diluted with MeOH, vacuum filtered through celite, and concentrated under reduced pressure. This gave the desired product (**2c**) in 86% yield as a light yellow solid (54.7 mg, 0.075 mmol) with no need for purification. Mp 77–79 ºC (color change), 143–150 ºC (dec.); ^{1}H-NMR (600 MHz, CD_3OD) δ 8.73 (s, 1 H), 7.85 (d, J = 13.2 Hz, 1 H), 7.57 (br s, 1 H), 3.88–3.79 (m, 4 H), 3.75 (br s, 1 H), 3.61 (t, J = 6.9 Hz, 2 H), 3.58 (t, J = 6.9 Hz, 2 H), 3.54 (t, J = 6.5 Hz, 2 H), 3.48–3.42 (m, 2 H), 3.40–3.35 (m, 2 H), 3.17 (t, J = 6.7 Hz, 2 H), 2.87–2.80 (m, 2 H), 2.78–2.69 (m, 4 H), 2.45 (t, J = 7.0 Hz, 2 H), 1.69–1.59 (m, 4 H), 1.58–1.49 (m, 4 H), 1.44–1.39 (m, 2 H), 1.38–1.30 (m, 4 H), 1.25–1.20 (m, 2 H); ^{13}C-NMR (150 MHz, CD_3OD) δ 178.2, 174.9, 174.6, 174.4, 173.1, 169.6, 155.0 (d, $J_{C\text{-}F}$ = 249.6 Hz), 149.3, 146.8 (d, $J_{C\text{-}F}$ = 9.5 Hz), 140.7, 120.9 (d, $J_{C\text{-}F}$ = 8.4 Hz), 112.5 (d, $J_{C\text{-}F}$ = 23.0 Hz), 107.4, 62.8, 51.0, 50.6, 46.4, 42.7, 40.3, 36.9, 33.3, 31.5, 29.9, 28.9, 28.5, 28.4, 27.5, 27.3, 24.9, 24.0, 8.6; HRMS-ESI (m/z): $[M+H]^+$ calcd. for $C_{35}H_{50}FN_6O_{10}$: 733.3567, found 733.3562.

V. Copies of ^{1}H-NMR and ^{13}C-NMR Spectra



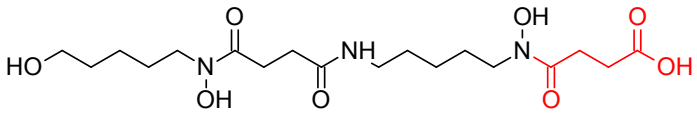
1a

^{1}H-NMR (600 MHz, CD_3OD)



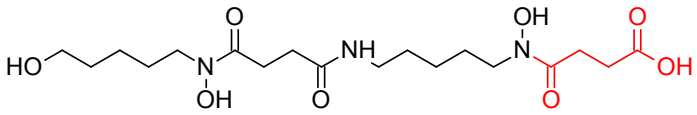
1a

^{13}C-NMR (150 MHz, CD_3OD)



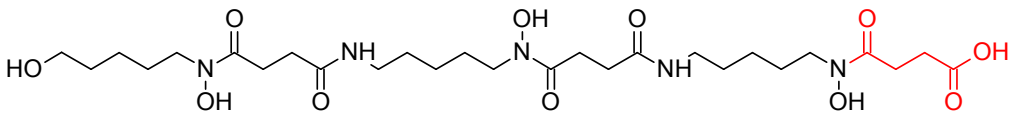
2a

^{1}H-NMR (600 MHz, CD_3OD)



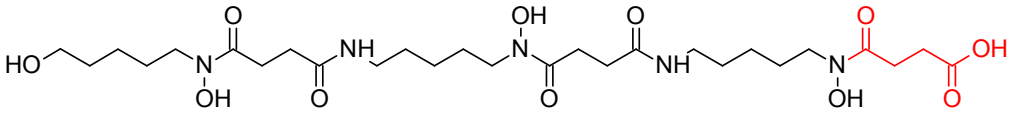
2a

^{13}C-NMR (150 MHz, CD_3OD)



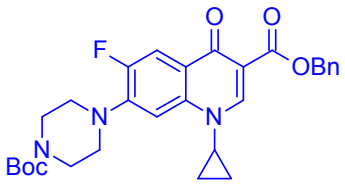
3a

^{1}H-NMR (600 MHz, DMSO-d_6)

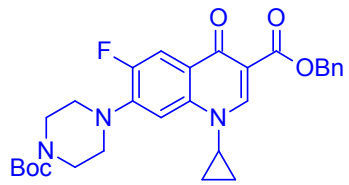


3a

^{13}C-NMR (150 MHz, DMSO-d_6)

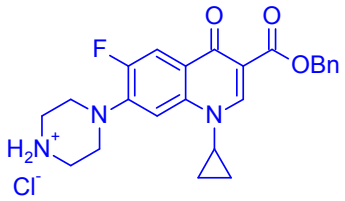


N-Boc-*O*-benzyl-ciprofloxacin
^{1}H-NMR (600 MHz, $CDCl_3$)



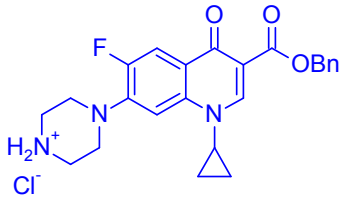
N-Boc-*O*-benzyl-ciprofloxacin

^{13}C-NMR (150 MHz, $CDCl_3$)



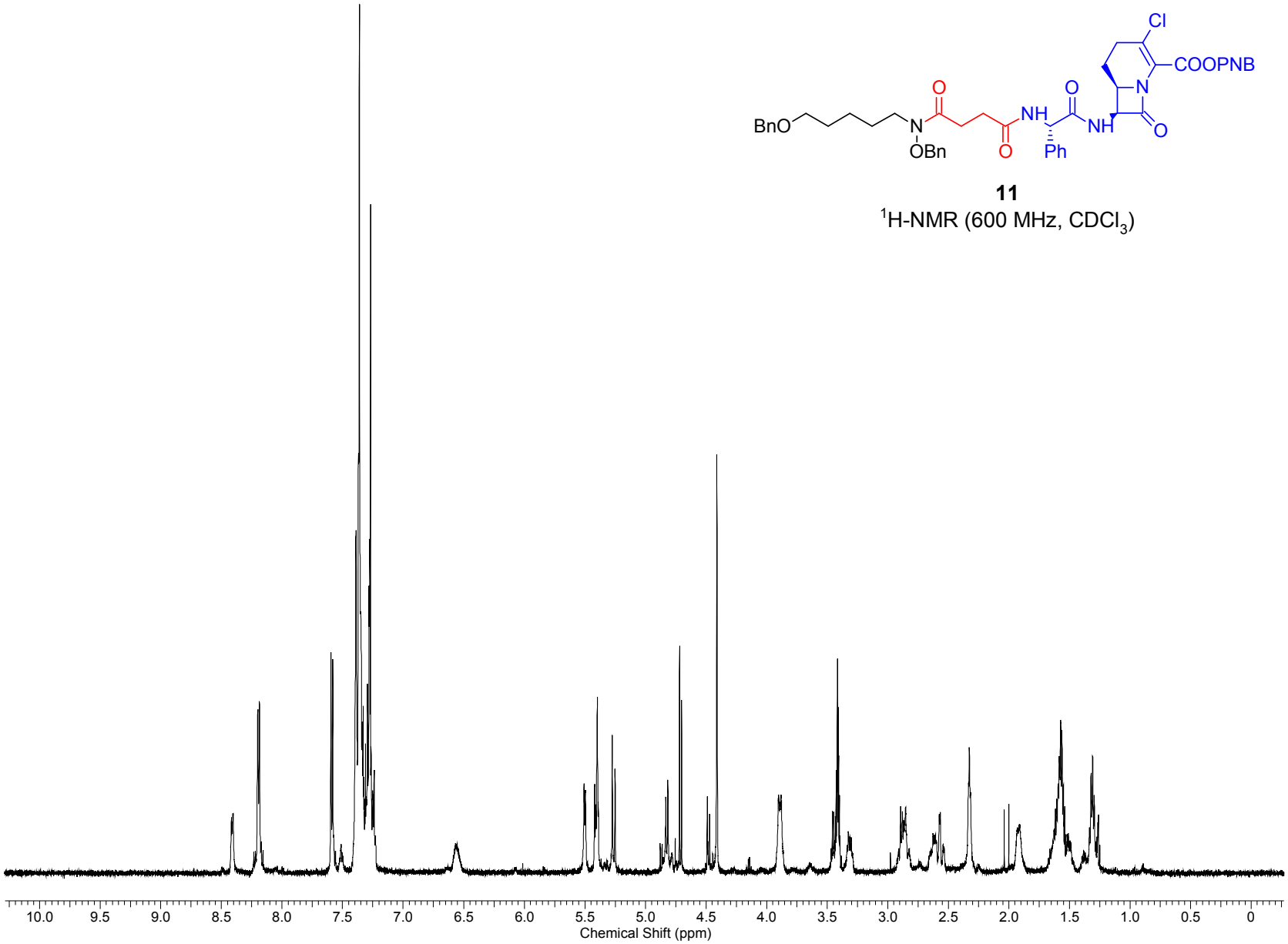
10

^{1}H-NMR (600 MHz, DMSO-d_6)



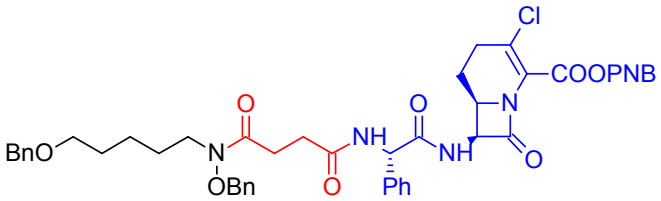
10

^{13}C-NMR (150 MHz, DMSO-d_6)



11

^{1}H-NMR (600 MHz, $CDCl_3$)



11

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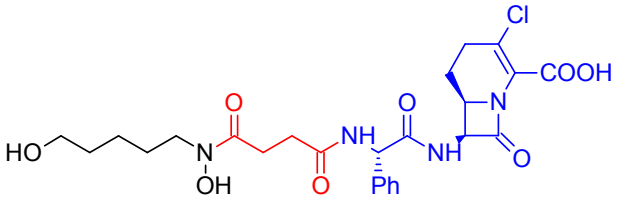
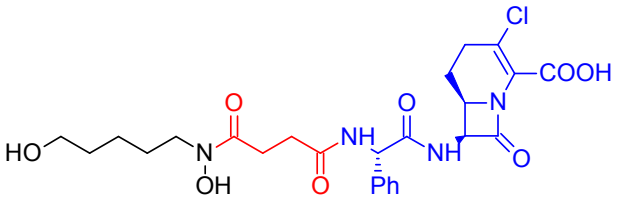


1b

^{1}H-NMR (600 MHz, CD_3OD)



1b

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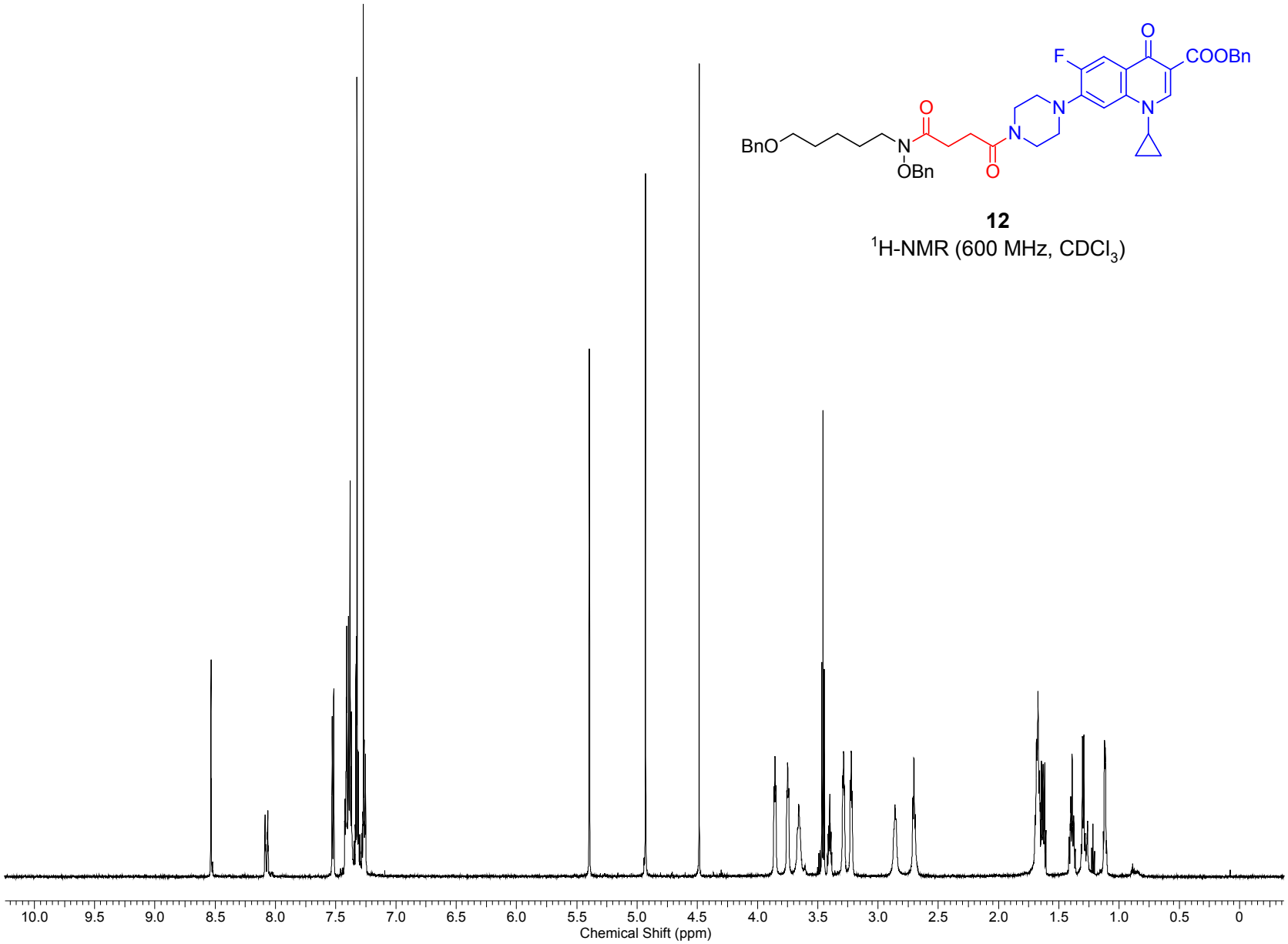

12

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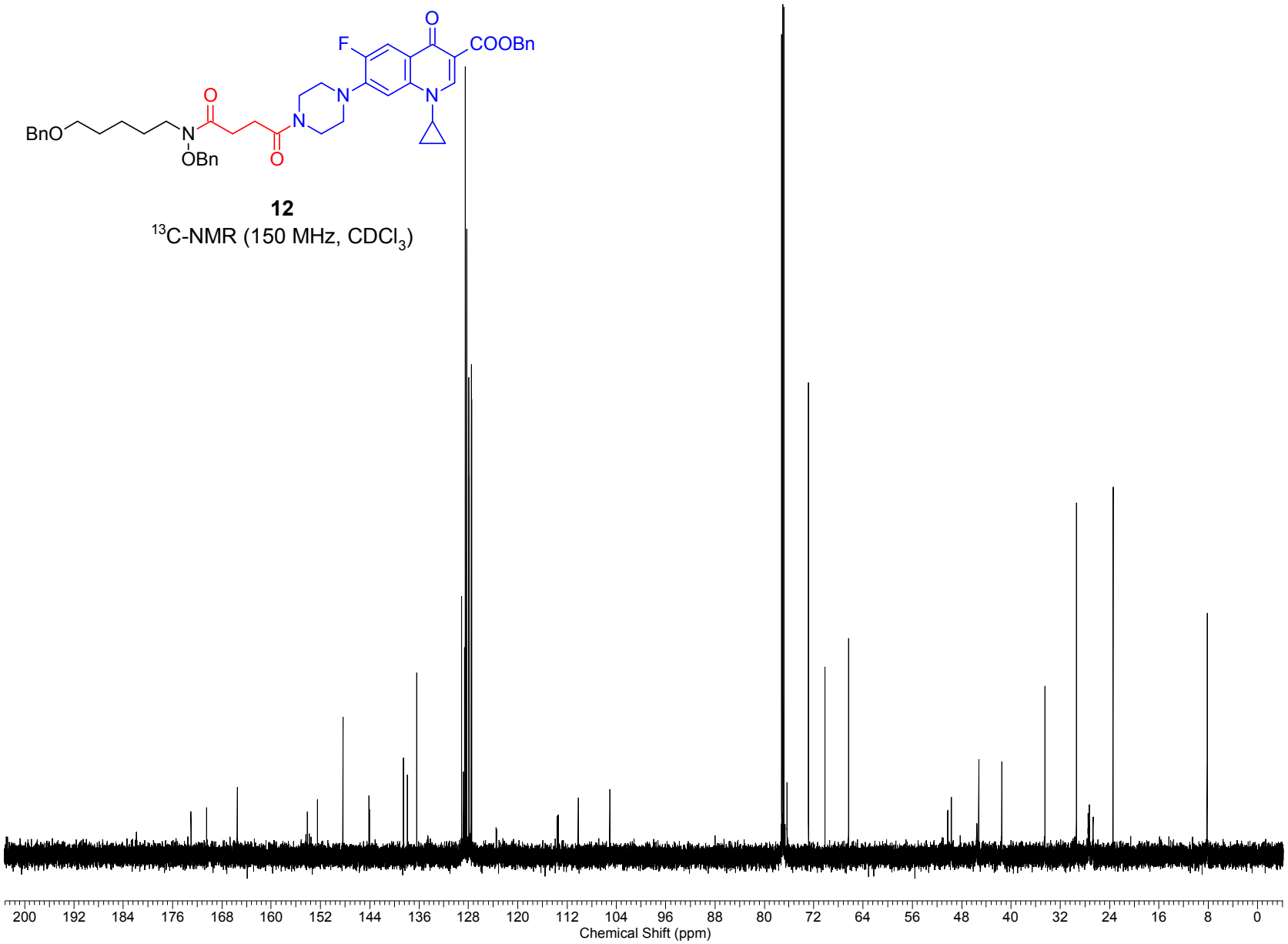

12

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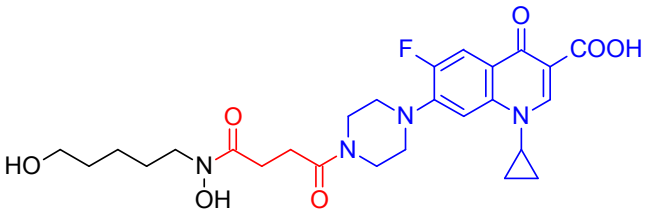
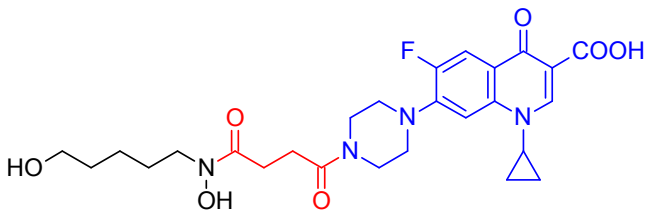


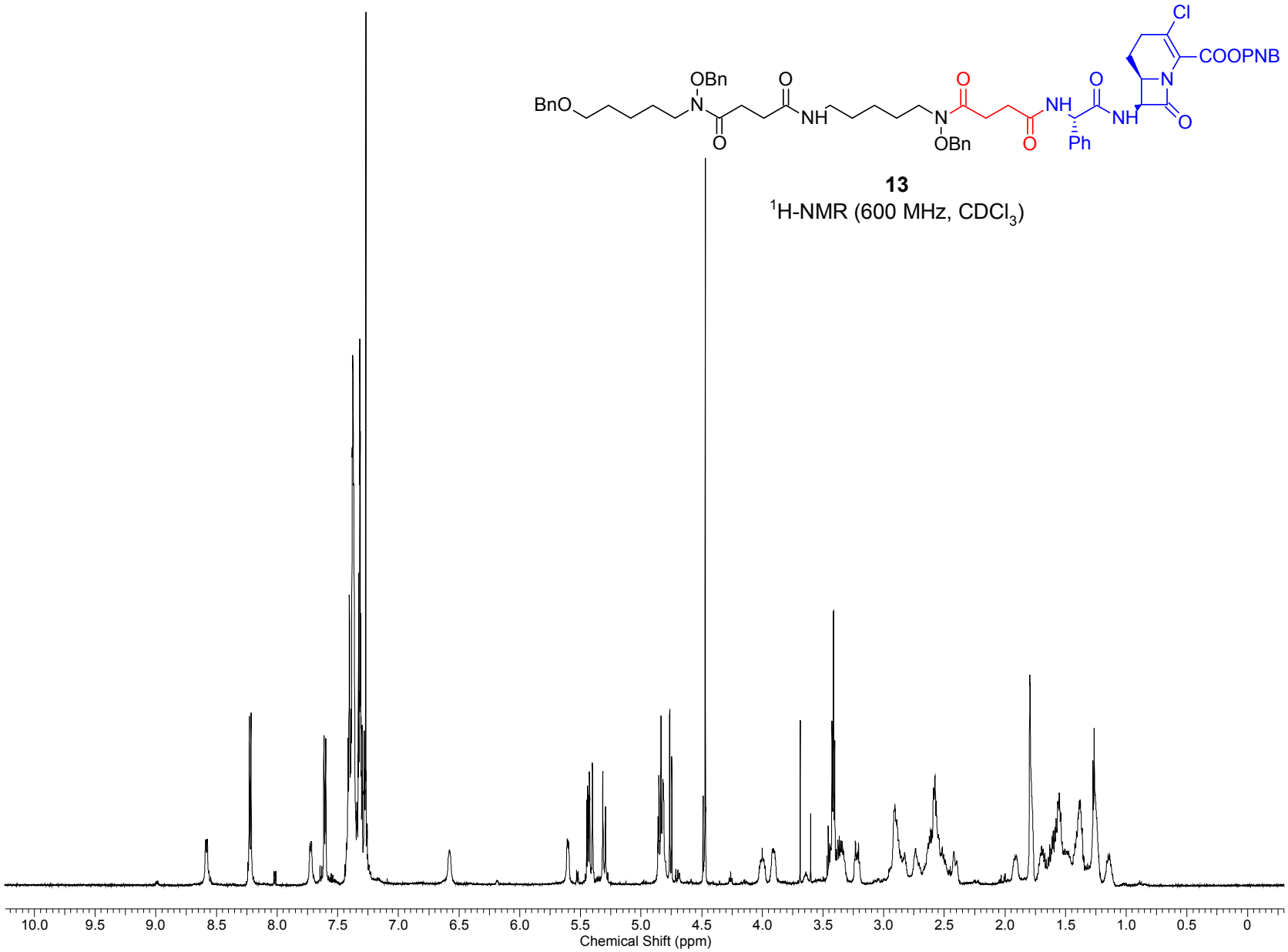
1c

^{1}H-NMR (600 MHz, CD_3OD)



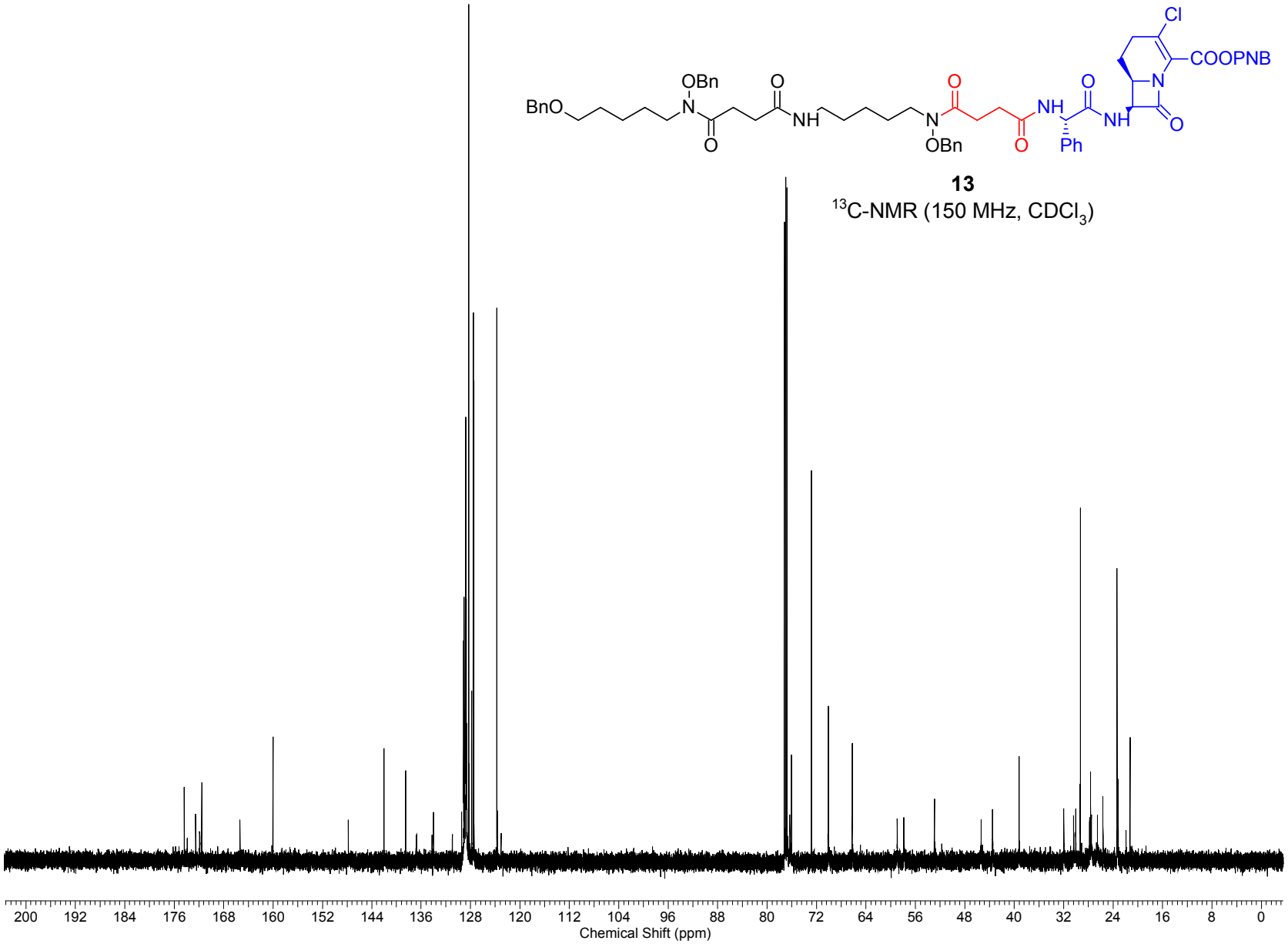
1c

^{13}C-NMR (150 MHz, CD_3OD)



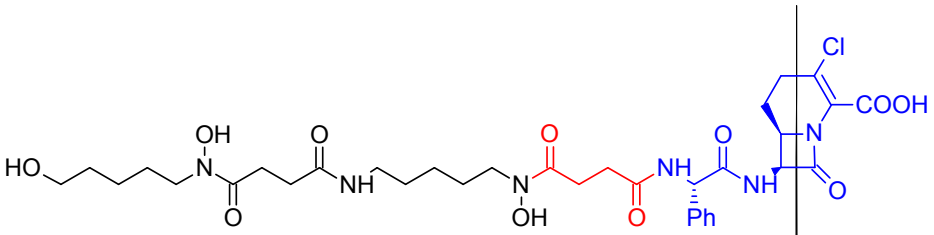
13

^{1}H-NMR (600 MHz, $CDCl_3$)



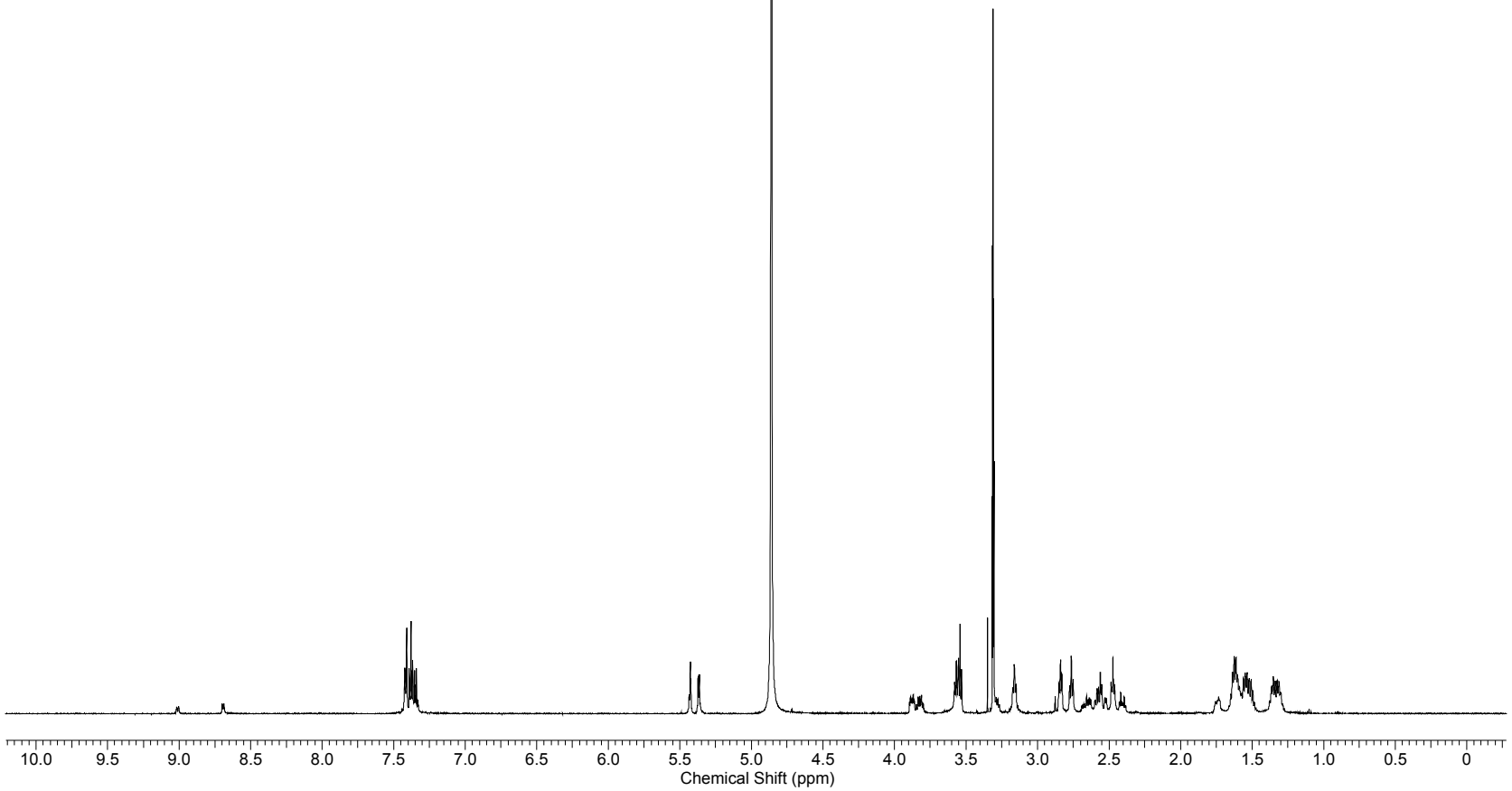
13

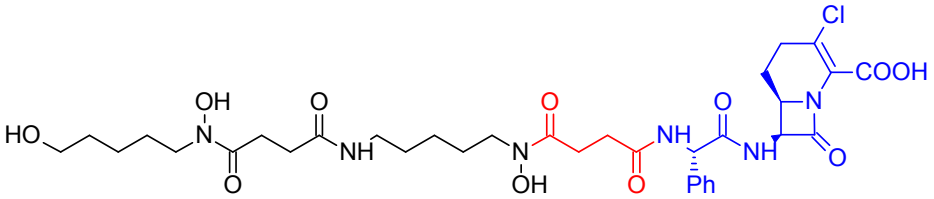
^{13}C-NMR (150 MHz, $CDCl_3$)



2b

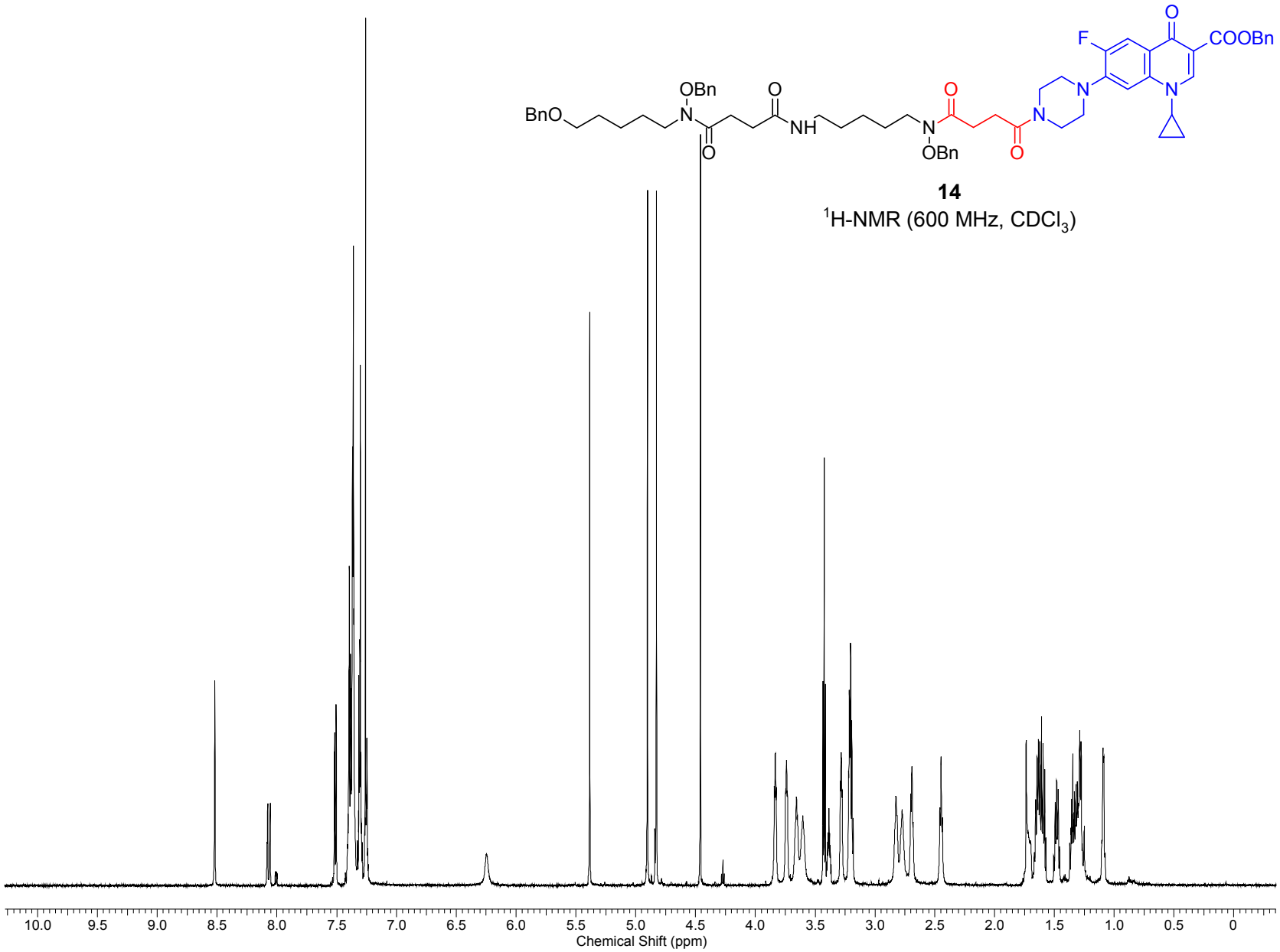
^{1}H-NMR (600 MHz, CD_3OD)





2b

^{13}C-NMR (150 MHz, CD_3OD)



14

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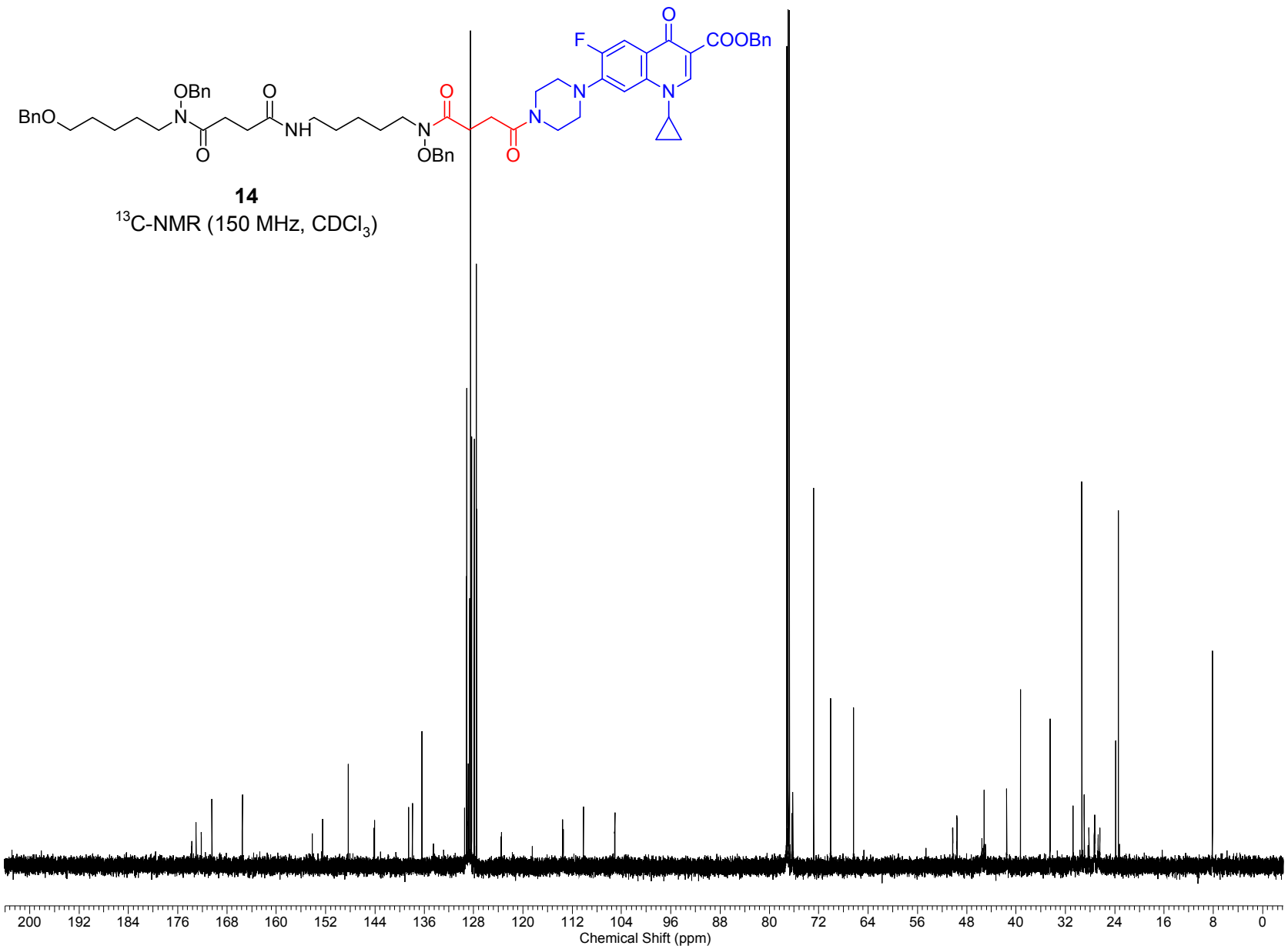
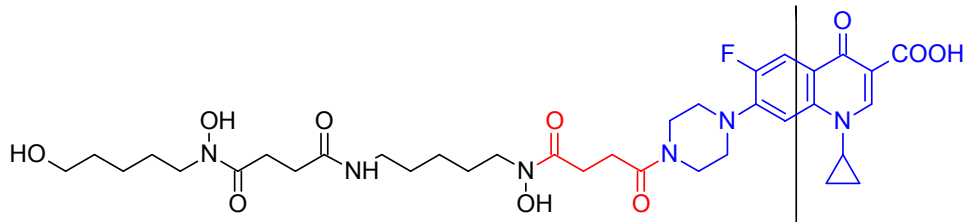


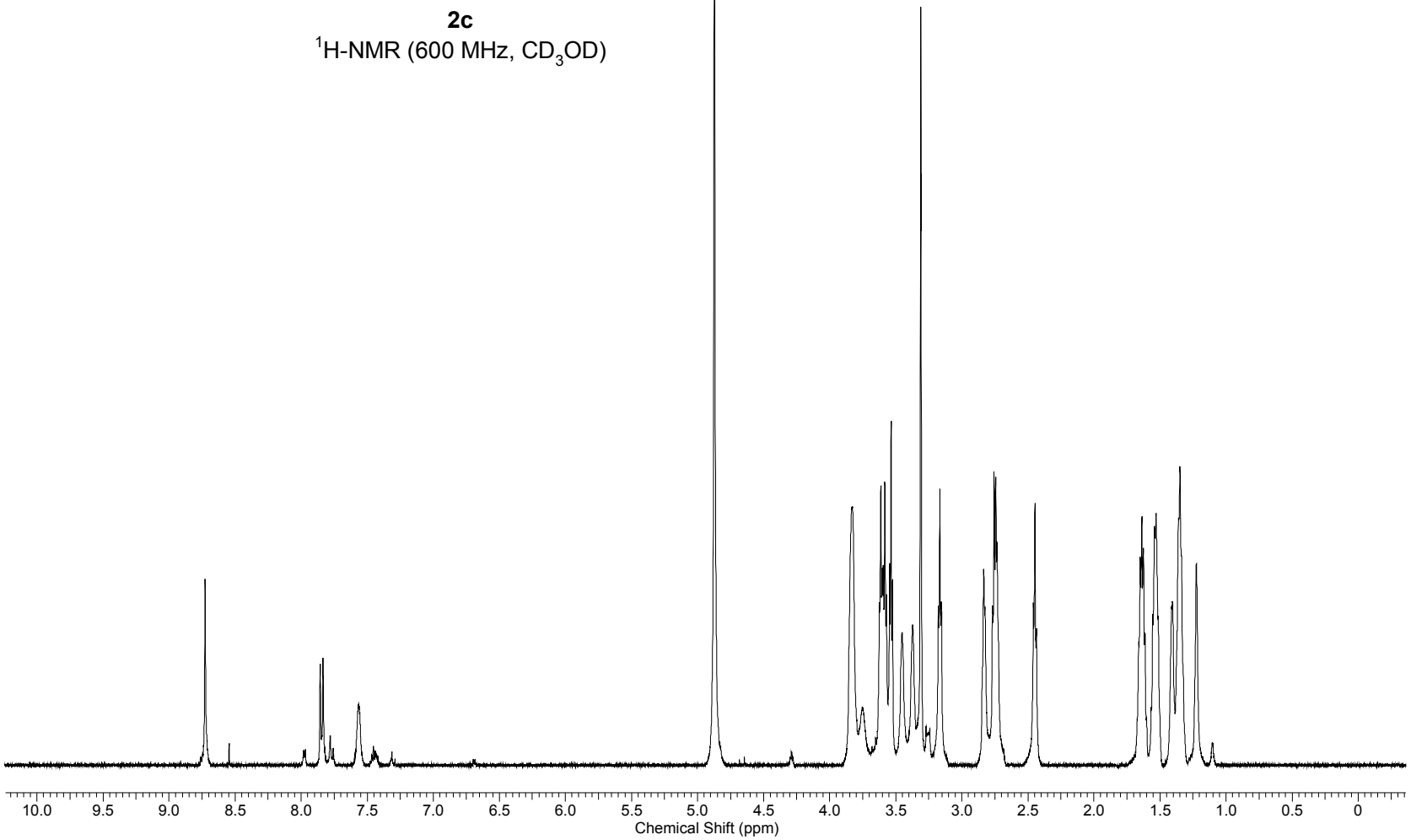
14

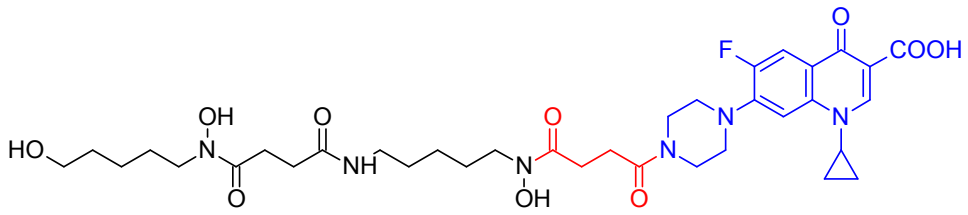
^{13}C-NMR (150 MHz, $CDCl_3$)



2c

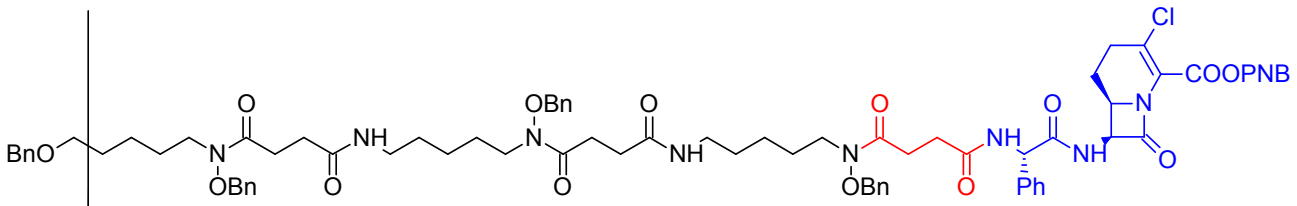
^{1}H-NMR (600 MHz, CD_3OD)





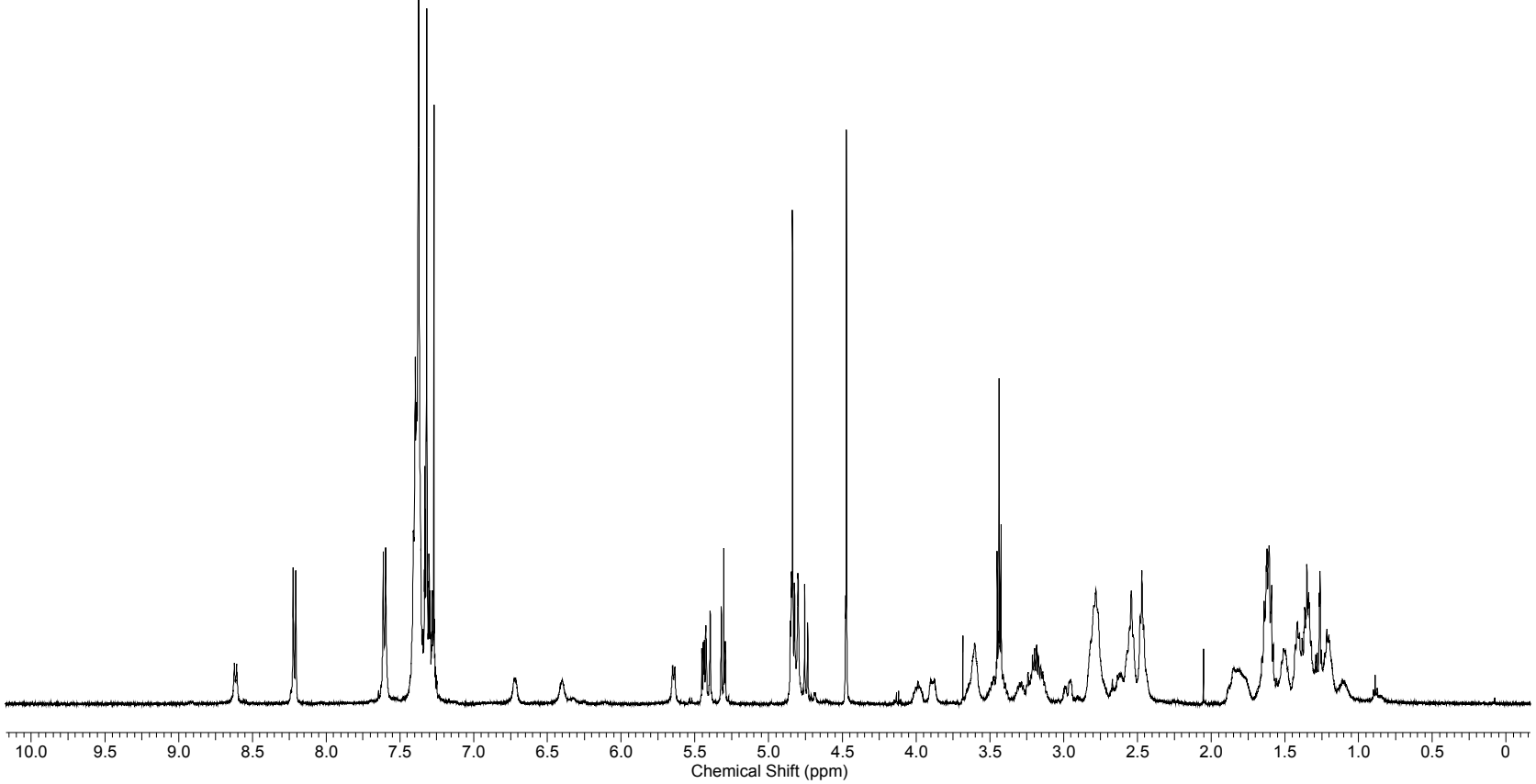
2c

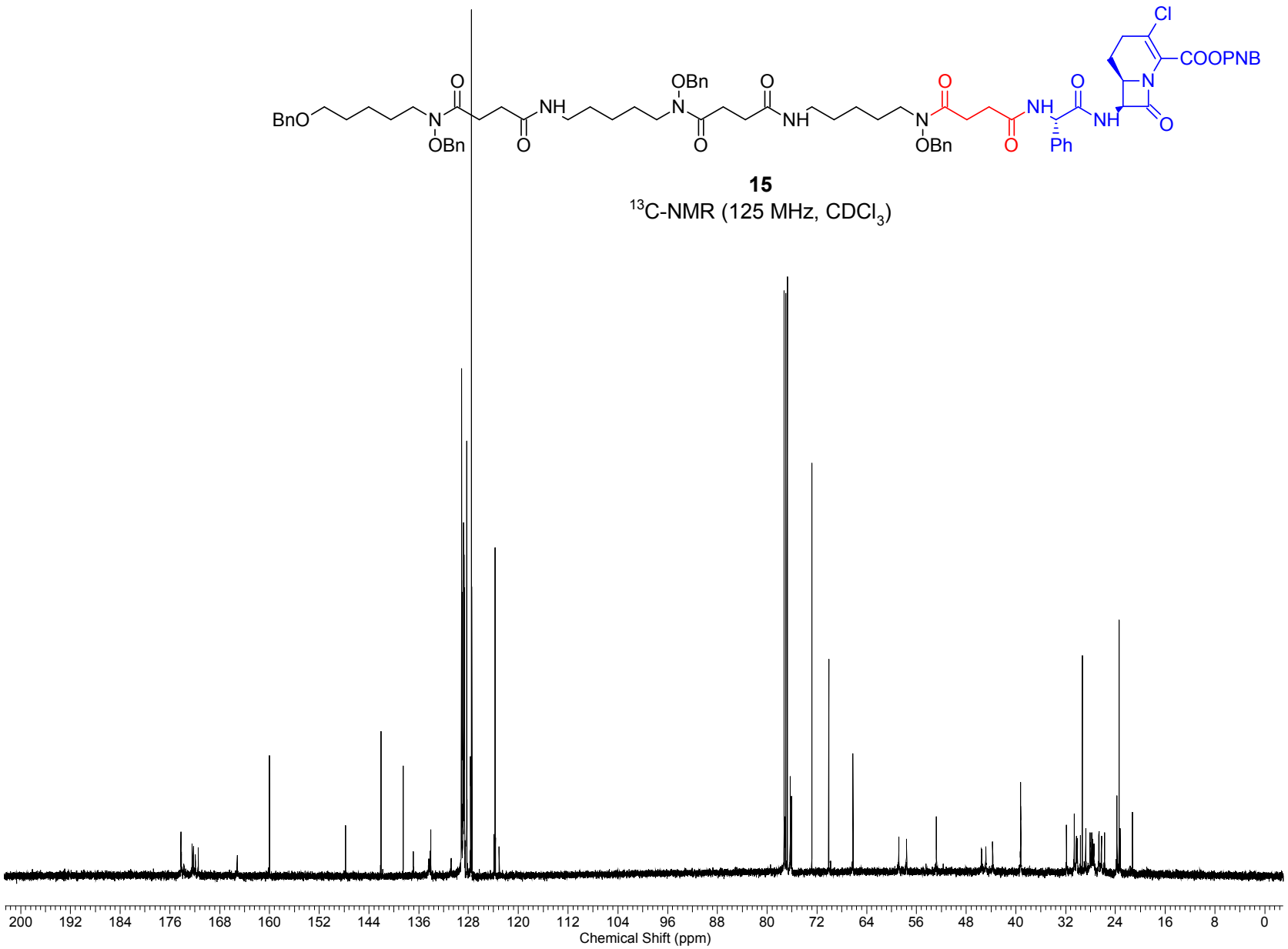
^{13}C-NMR (150 MHz, CD_3OD)



15

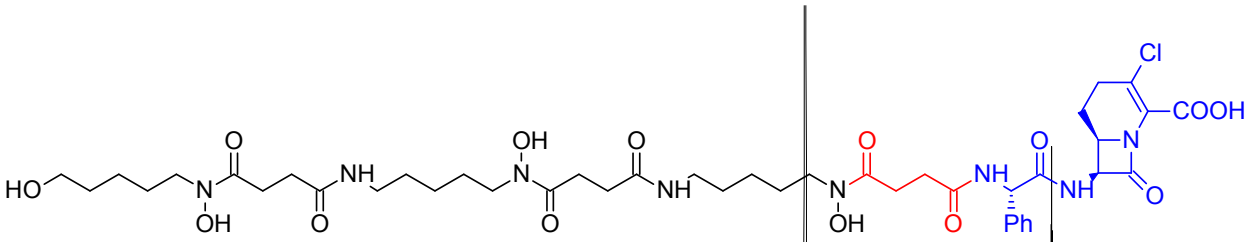
^{1}H-NMR (500 MHz, $CDCl_3$)





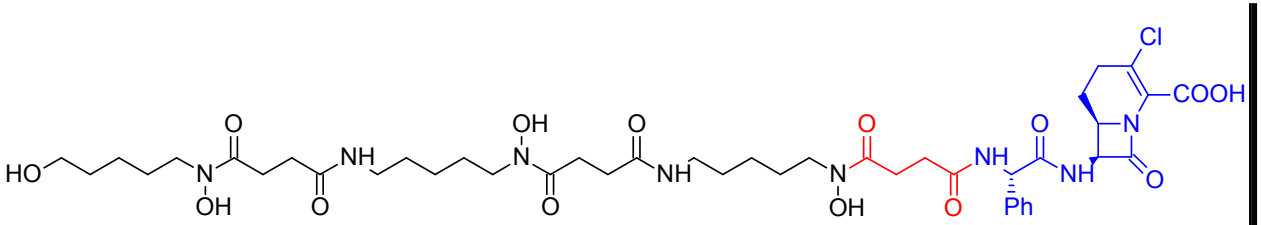
15

^{13}C-NMR (125 MHz, $CDCl_3$)



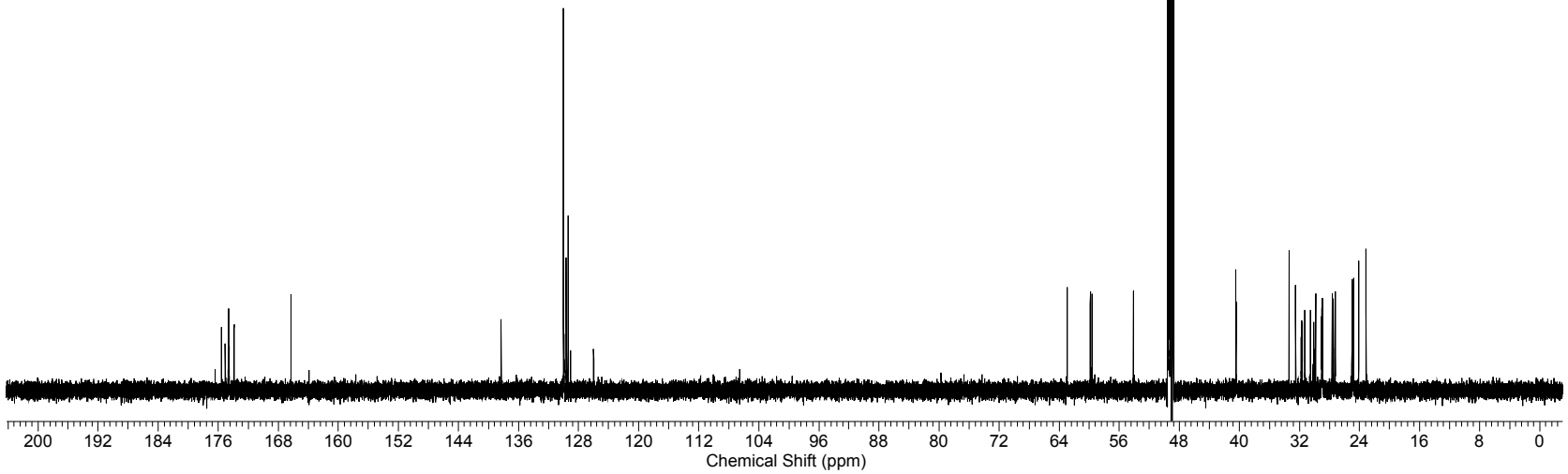
3b

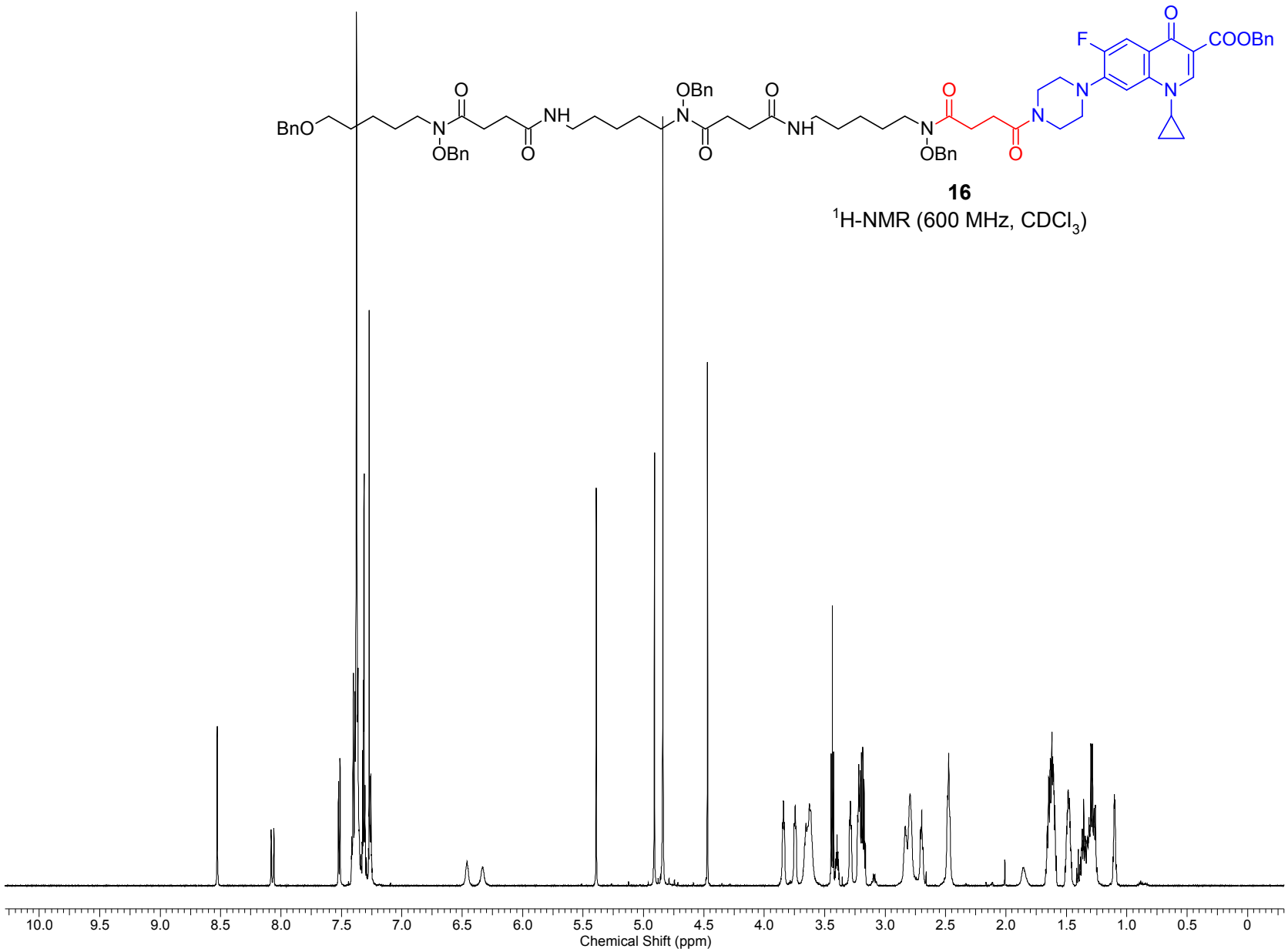
^{1}H-NMR (600 MHz, CD_3OD)



3b

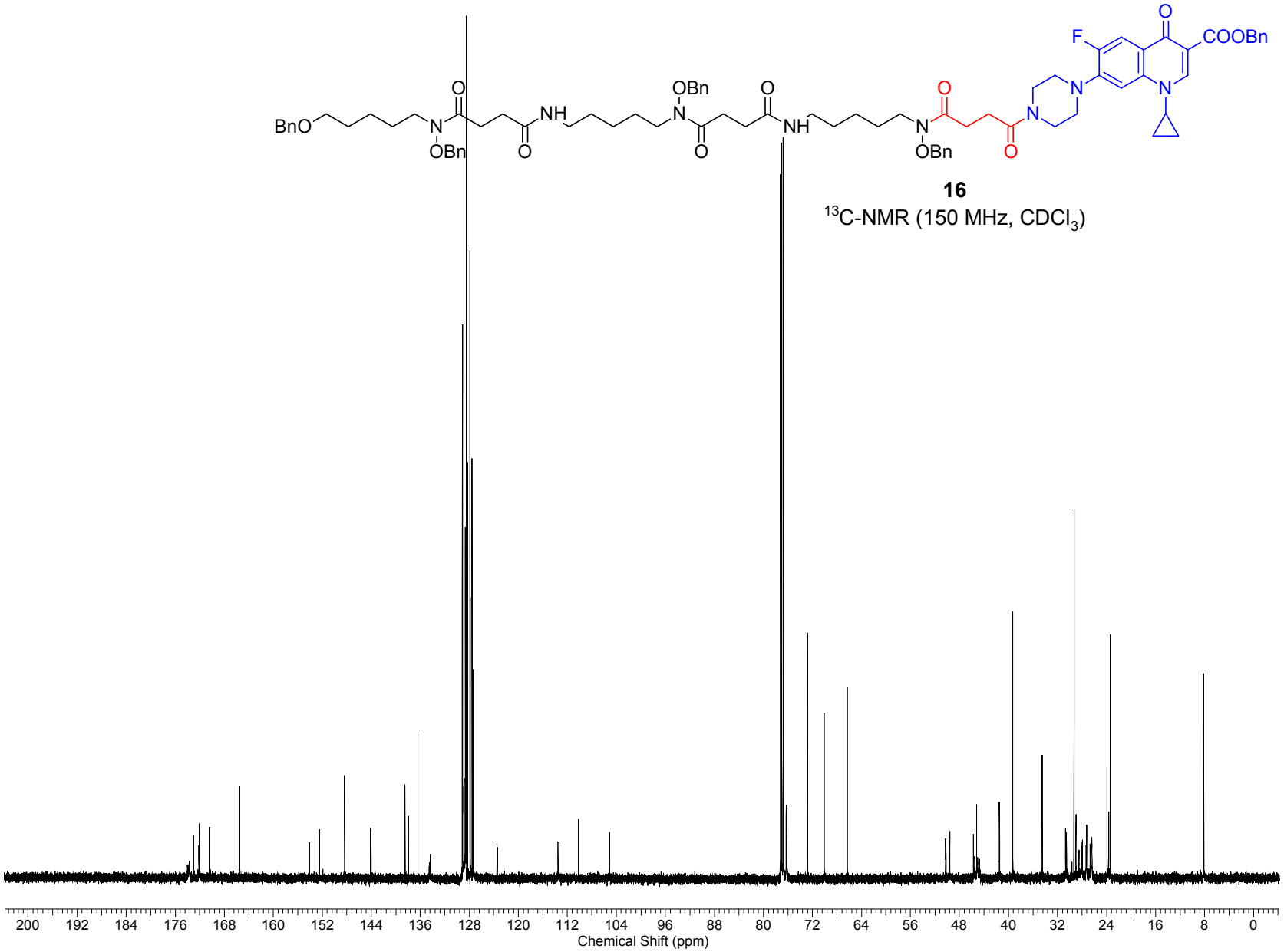
^{13}C-NMR (150 MHz, CD_3OD)





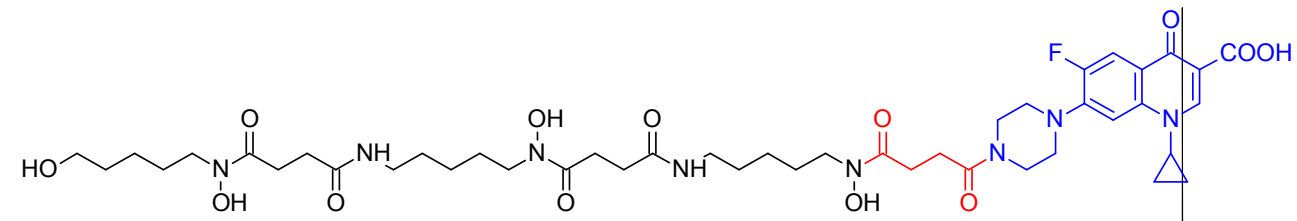
16

^{1}H-NMR (600 MHz, $CDCl_3$)



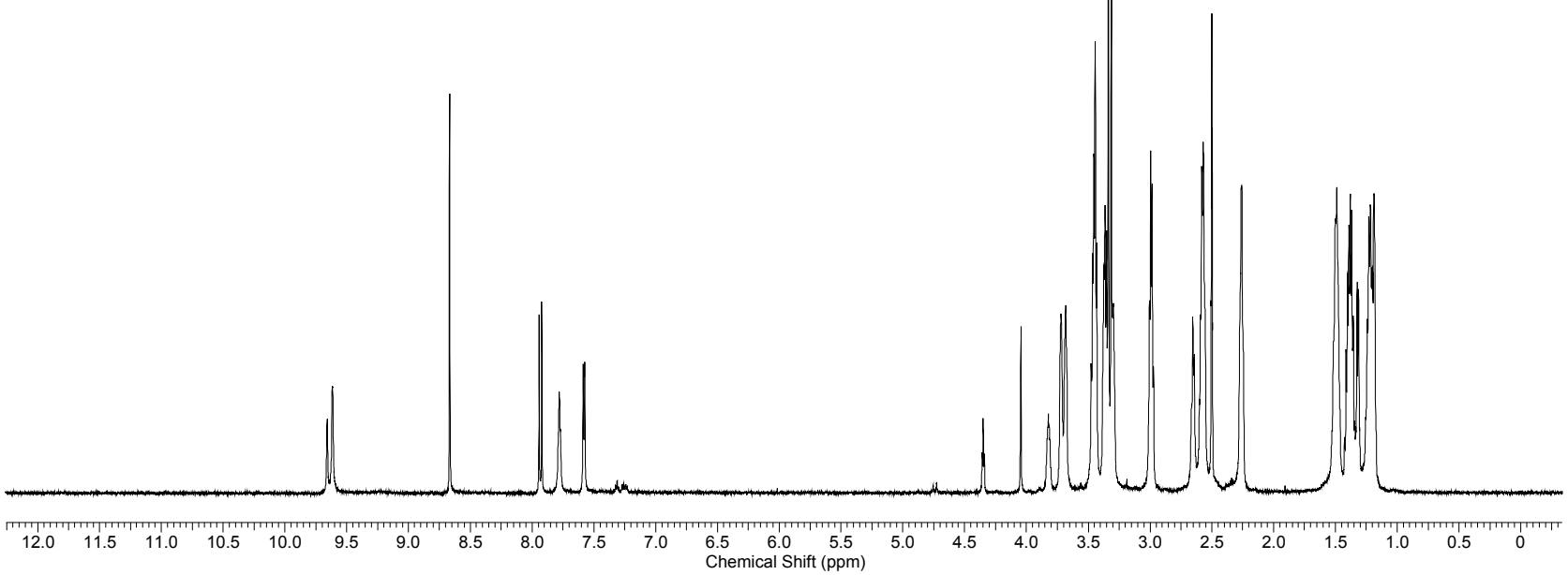
16

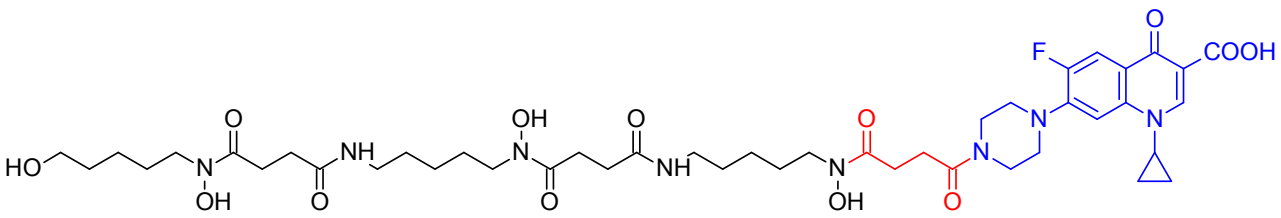
^{13}C-NMR (150 MHz, $CDCl_3$)



3c

^{1}H-NMR (600 MHz, DMSO-d_6)





3c

^{13}C-NMR (150 MHz, DMSO-d_6)

VI. References

1. Richmond, M. H.; Clark, D. C.; Wotton, S. "Indirect method for assessing the penetration of beta-lactamase-nonsusceptible penicillins and cephalosporins in *Escherichia coli*." *Antimicrob. Agents Chemother.* **1976**, *10*, 215-218.

2. Zimmerman, W. "Penetration of β-lactam antibiotics into their target enzymes in *Pseudomonas aeruginosa*: Comparison of a highly sensitive mutant with its parent strain." *Antimicrob. Agents Chemother.* **1980**, *18*, 94-100.

3. An experimental procedure for this assay is provided in the main text. For literature example and general description of the modified Kirby-Bauer agar diffusion assay used in this study, see: (a) Afonin, S.; Glaser, R. W.; Berditchevskaia, M.; Wadhwani, P.; Gührs, K.-H.; Möllmann, U.; Perner, A.; Ulrich, A. S. "4-Fluoro-phenylglycine as a label for ^{19}F-NMR structure analysis of membrane associated peptides." *ChemBioChem* **2003**, *4*, 1151-1163. (b) Wencewicz, T. A.; Yang, B.; Rudloff, J. R.; Oliver, A. G.; Miller, M. J. "N-O Chemistry for antibiotics: Discovery of *N*-alkyl-*N*-(pyridin-2-yl)hydroxylamine scaffolds as selective antibacterial agents using nitroso Diels-Alder and ene chemistry." *J. Med. Chem.* **2011**, *54*, 6843-6858.

4. Roosenberg, J. M., Jr.; Miller, M. J. "Total synthesis of the siderophore danoxamine." *J. Org. Chem.* **2000**, *65*, 4833-4838.

5. Wencewicz, T. A.; Oliver, A. G.; Miller, M. J. "Iron(III)-templated macrolactonization of trihydroxamate siderophores." *Org. Lett.* **2012**, *14*, 4390-4393.