Electronic Supporting Information for:

Discovery of Nicoyamycin A, an Inhibitor of Uropathogenic *Escherichia coli* Growth in Low Iron Environments

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Supporting Information Contents

Figures

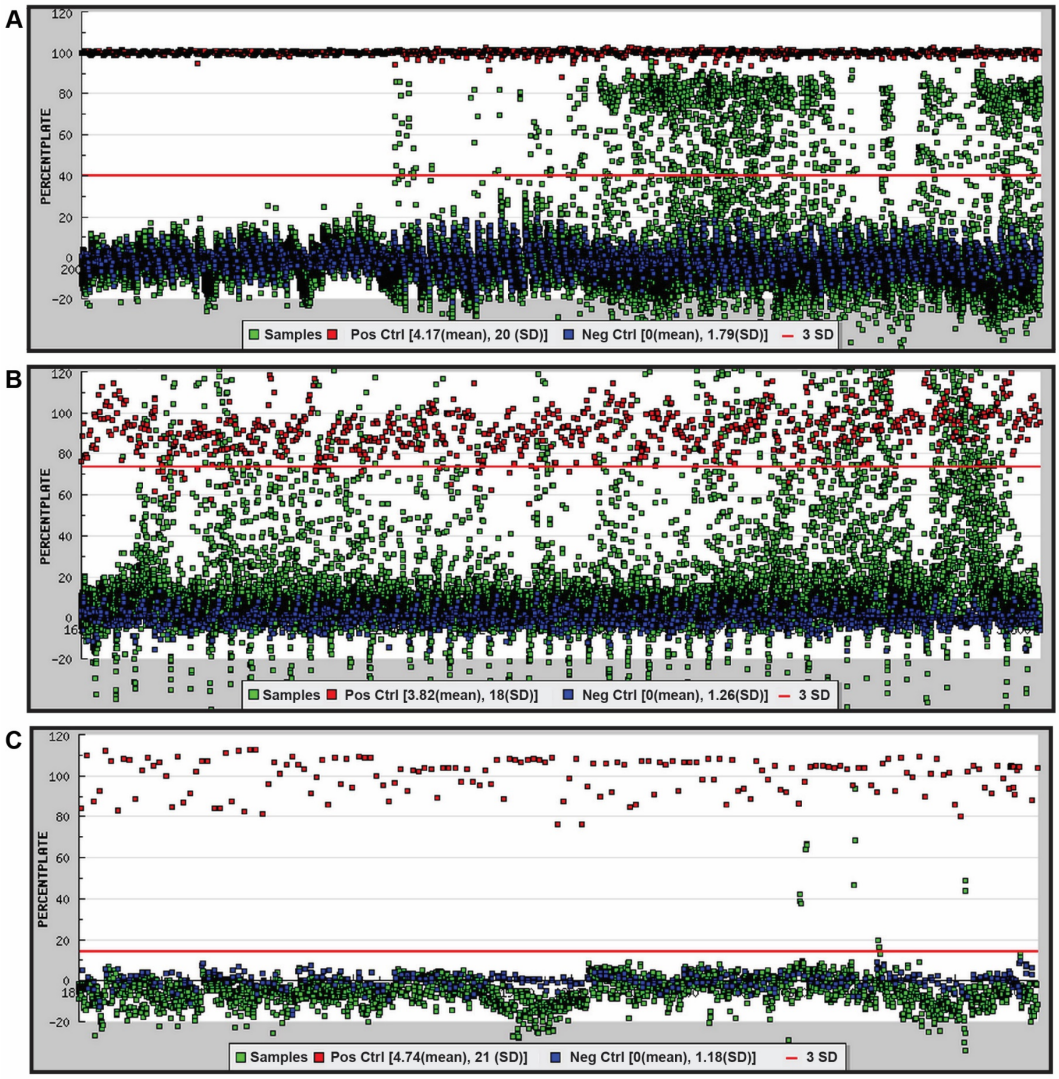

Figure 1. High-throughput screen for natural product inhibitors of UPEC growth in iron-restricted environments. (a) Primary screen: 98, 384-well plates containing natural product extracts (NPEs) were screened for wells that inhibited the growth of wildtype UPEC in low iron medium (MOPS-Fe). Each dot represents a single well reaction on the x-axis and the percent plate (y-axis) represents the reduction of bacterial growth in each well as measured by the absorbance at 600 nm (OD_{600}), relative to the plate average. Blue dots represent negative control (DMSO vehicle) wells and red dots represent positive control (200 mM 2,2'-dipyridyl) wells. The red line represents three standard deviations from the negative control. Setting the hit cut-off at ≥3 SD from the plate controls resulted in the identification of 2,803 hits that were prioritized for further screening. (b) Counter screen: The first 27 plates were excluded from the counter screen due to their low activity. The remaining 71 plates were assayed for iron chelating properties using the chromazural S (CAS) assay. 10 µM EDTA was used as a positive control (red dots) and the vehicle DMSO was used as a negative control (blue dots). The y-axis represents the relative iron chelation measured at 630 nm relative to the plate average. The red line represents three standard deviations from the negative control. NPEs that had iron chelation scores ≥3 SD were eliminated as hits. This

further reduced the number of hits to 995 NPEs. (c) Secondary screen: The 995 hits that passed the primary and counter screens were assayed for growth inhibition properties under iron-replete conditions. Wildtype UPEC was grown in MOPS medium with 40 μM $FeSO_4$ and growth inhibition relative to the plate average was recorded (y-axis). 10 μg/mL ciprofloxacin was used as a positive control (red dots) and the vehicle DMSO was used as a negative control (blue dots). Setting the hit cut-off at <3 SD (red line) from the plate negative controls narrowed the hit list to 989 NPEs. After the counter and secondary screens were completed, the list of hits was further narrowed to only include those NPEs that inhibited UPEC growth by ≥90%. This resulted in the identification of 204 hits (0.55% final hit rate).

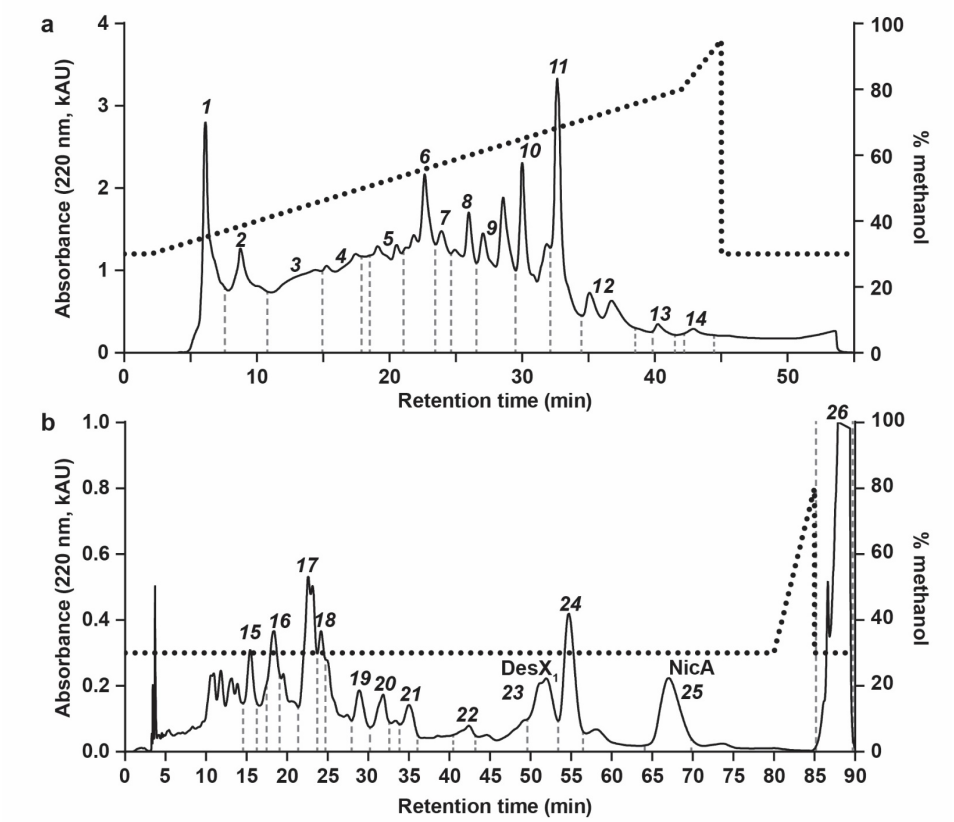

Figure 2. Representative HPLC chromatograms. (a) The 75% v/v water/methanol preparative C18 fraction (ESI Fig. 3) was purified using a reverse phase (RP) C18 column and water/methanol gradient on a Shimadzu HPLC. (b) HPLC fraction 9 was purified using an RP phenyl-hexyl column and an isocratic method (30% v/v methanol/water in water) on a Shimadzu HPLC. The absorbance at 220 nm in kilo absorbance units (kAU) is plotted on the left y-axis (solid black line). The percent volume of methanol is shown on the right y-axis (dotted black line). Collected fractions are indicated by dashed gray lines and the corresponding fraction number is indicated in italics. All HPLC fractions were tested for bioactivity (select fractions shown in ESI Fig. 4).

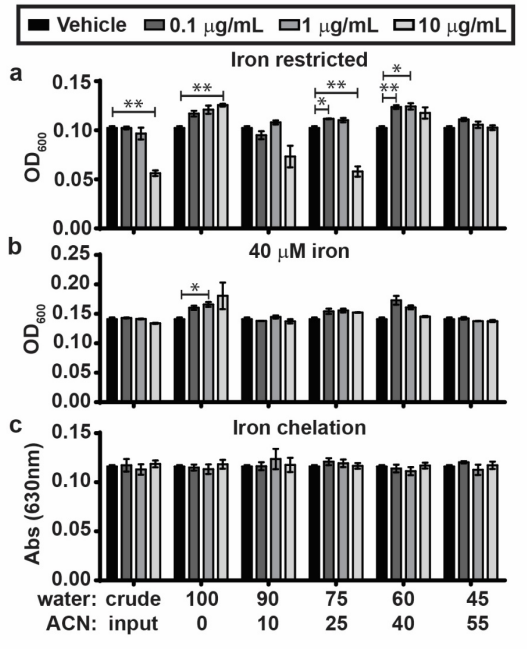

Figure 3. The antibacterial activity of strain *S. nicoyae* elutes in 75% v/v water/methanol. A 10 L culture of *S. nicoyae* was fermented for 4 days and the crude natural product extract was separated using a preparative C18 column and a water/acetonitrile (ACN) gradient. The resulting dried fractions were tested for bioactivity using the assays employed in the high-throughput screen (HTS). UPEC was cultured in (a) MOPS-Fe or (b) MOPS-Fe supplemented with 40 μM $FeSO_4$ for 18 h. Growth was quantified by measuring OD_{600}. (c) Iron chelation was quantified using the chrome azural S (CAS) assay. C18 fractions were incubated in CAS reagent and a decrease in absorbance at 630 nm represents iron chelation. The shading of the gray bars indicates the final concentration of the C18 fraction. Fractions were prepared at 100x concentration in DMSO. Shown is an average of 3 replicates. Error bars represent one standard deviation from the mean. Significance was calculated using an unpaired t-test in Prism and determined using the Holm-Sidak method with alpha = 0.05. Each row was analyzed individually without assuming a consistent standard deviation where *, $p < 0.05$; **, $p < 0.01$.

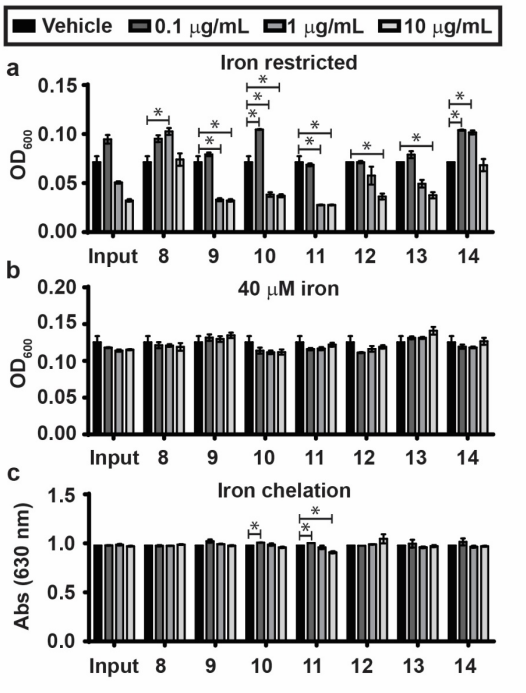

Figure 4. Antibacterial activity of the 75% v/v water/methanol fraction elutes in the gradient HPLC fractions 9-13. UPEC was cultured for 18 h in (a) MOPS-Fe or (b) MOPS-Fe supplemented with 40 μM $FeSO_4$ and the indicated gradient HPLC fraction (x-axis) (ESI Fig. 2A). Growth was quantified by measuring OD_{600}. (c) Iron chelation was quantified using the chrome azural S (CAS) assay. HPLC fractions were incubated in CAS reagent and a decrease in absorbance at 630 nm represents iron chelation. The shading of the gray bars indicates the final concentration of the HPLC fraction. Shown is an average of 3 replicates. Error bars represent one standard deviation from the mean. Significance was calculated using an unpaired t-test in Prism and determined using the Holm-Sidak method with alpha = 0.05. Each row was analyzed individually without assuming a consistent standard deviation where *, $p < 0.05$. Only active fractions are depicted.

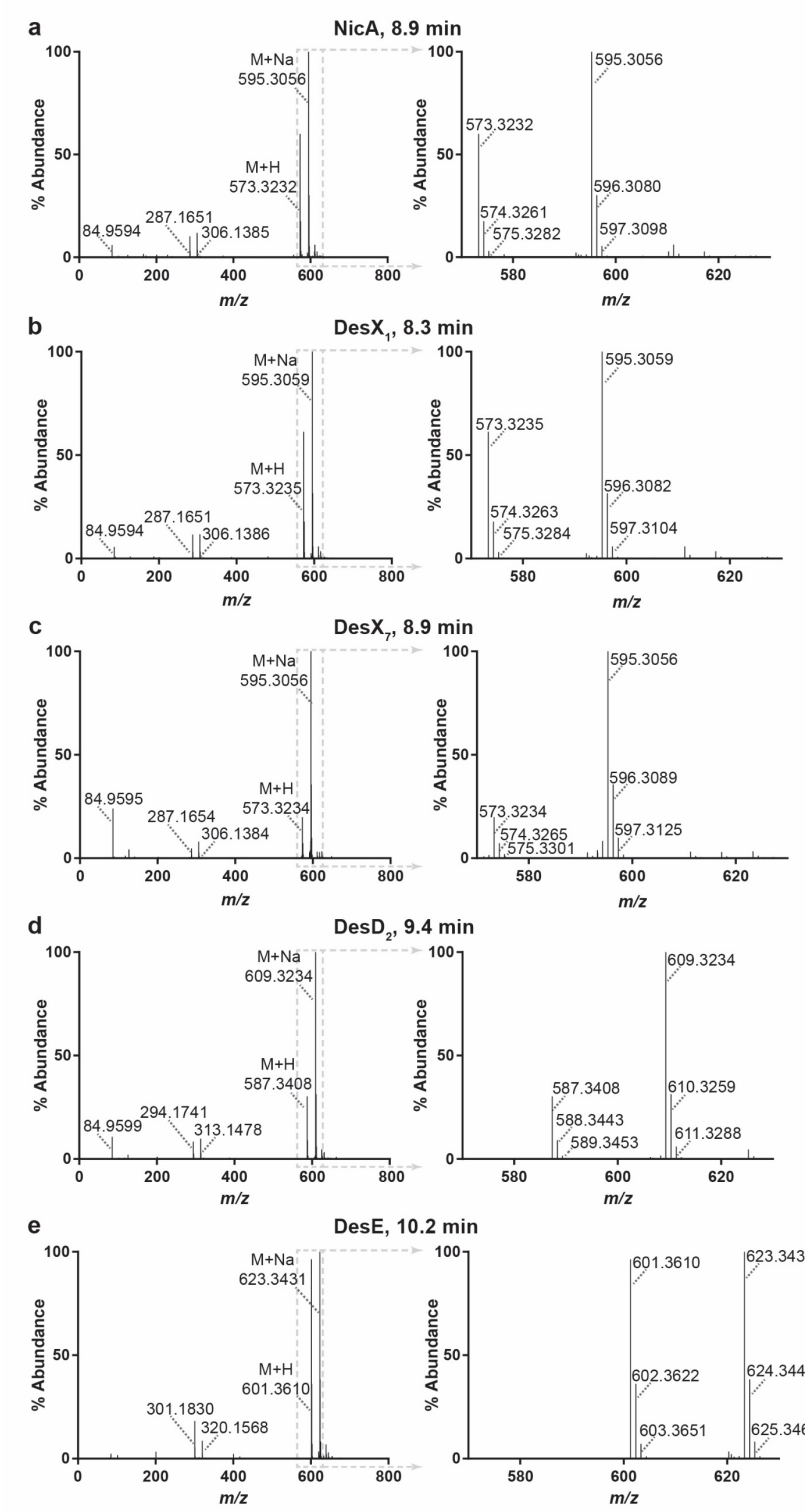

Figure 5. High-resolution ESI-MS spectra of NicA and cyclic desferrioxamines. LC-HRESI-MS profiles of five pure molecules purified from *S. nicoyae*. 4 mL of 0.5 mg/mL of each molecule was injected onto a C18 column and separated using a 10% to 60% v/v methanol/water gradient with 0.1% v/v formic acid over 12 min. The retention time (r.t.) of each molecule is reported in the title next to the molecule name, where (a) NicA, (b) $DesX_1$, (c) $DesX_7$, (d) $DesD_2$, and (e) DesE. On the left is the full *m/z* scan at the reported r.t. and on the right is an inset of 570-630 *m/z* (emphasized by the gray box in the left full scan).

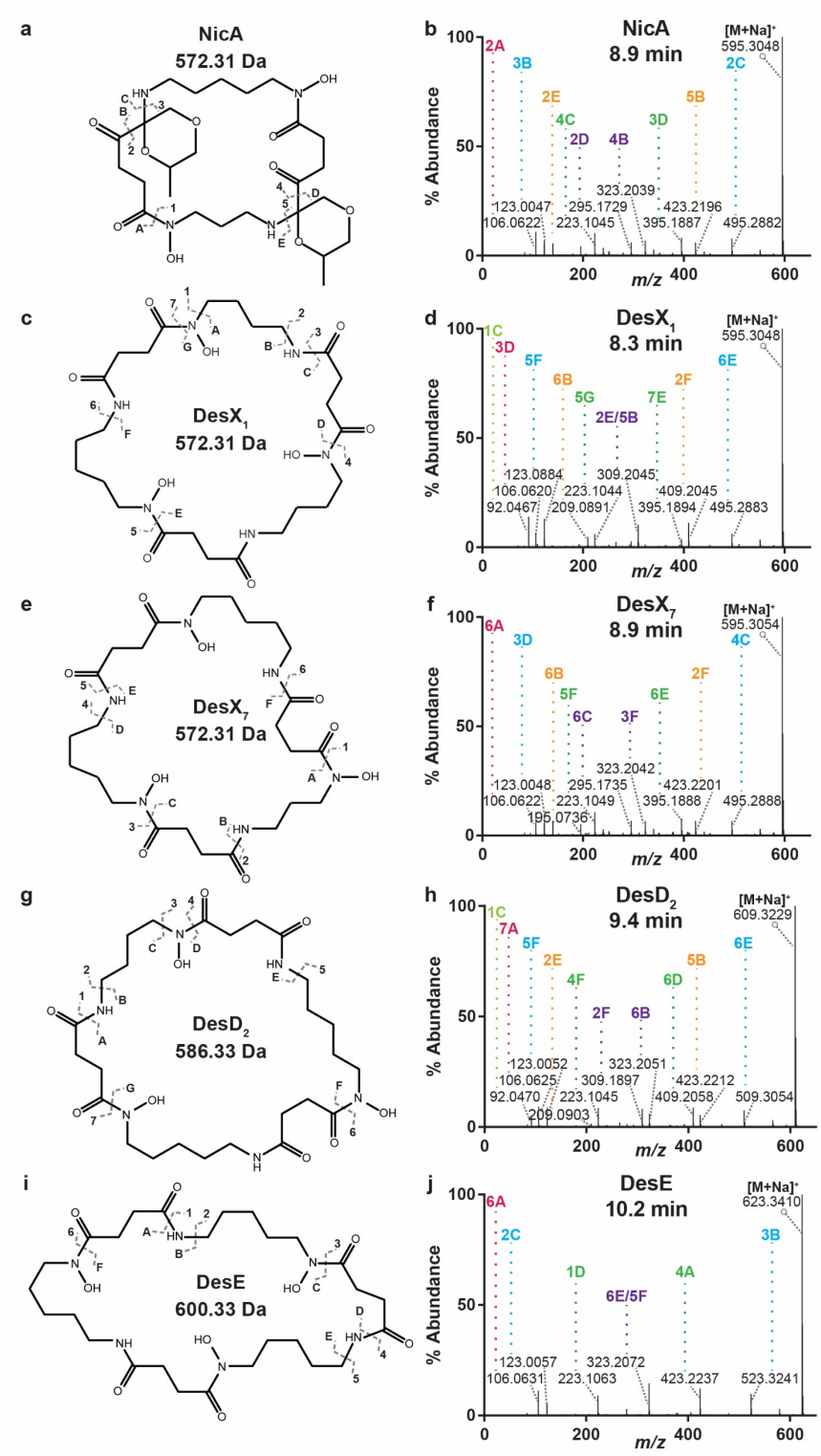

Figure 6. High-resolution ESI-MS/MS spectra of NicA and cyclic desferrioxamines. LC-HRESI-MS/MS profiles of five pure molecules (a,c,e,g,i) purified from *S. nicoyae*. (b,d,f,h,j) In each MS/MS panel, the parent ion is denoted with an open circle and fragment ions are identified with dotted gray lines. Fragment ions (right) are matched to the predicted molecule fragment (left) with the corresponding number and letter. Most ions correspond to two halves of the molecule and these are identified with matching colors.

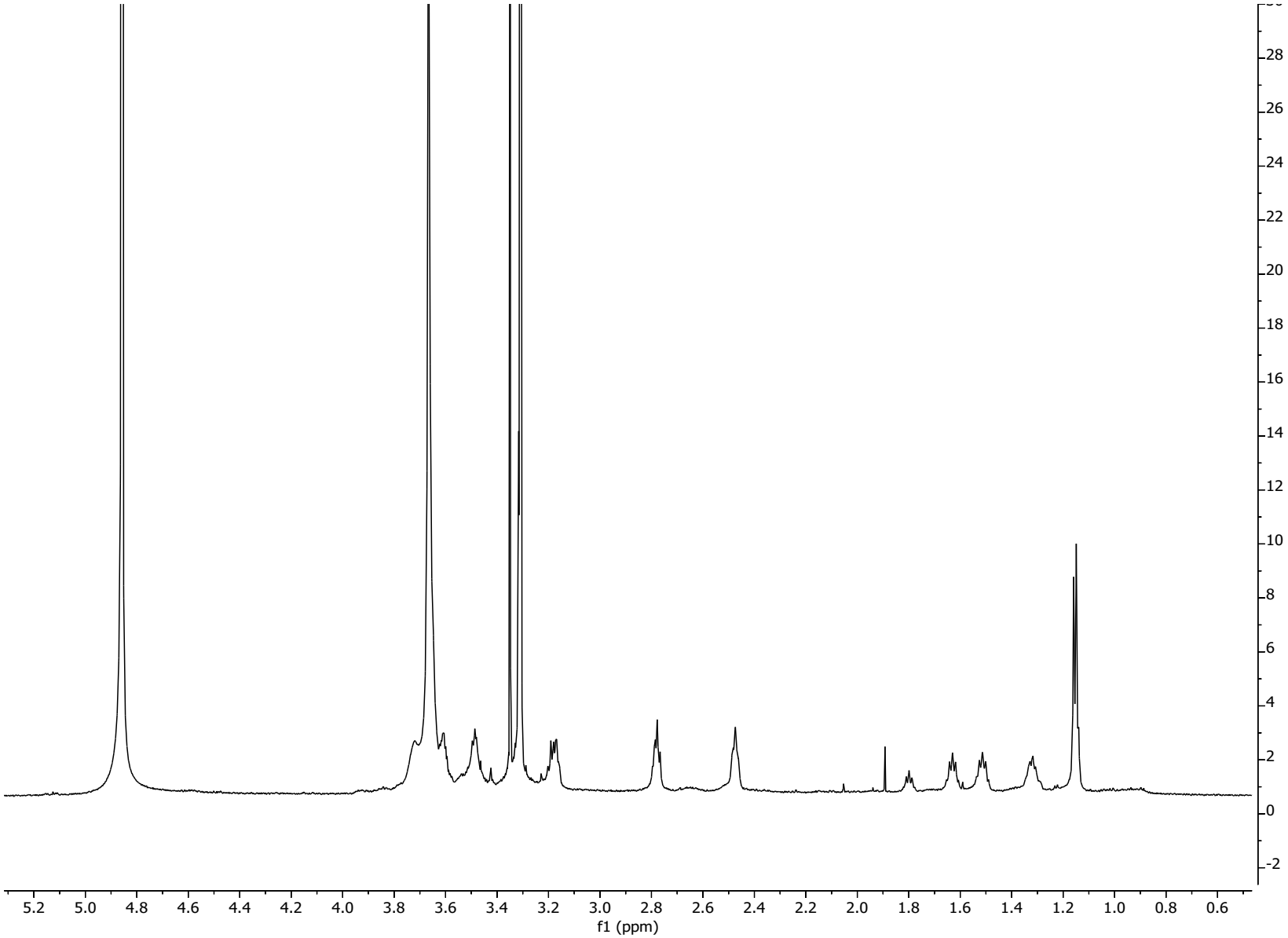

Figure 7. ^{1}H NMR spectrum of nicoyamycin A recorded at 600 MHz in CD_3OD.

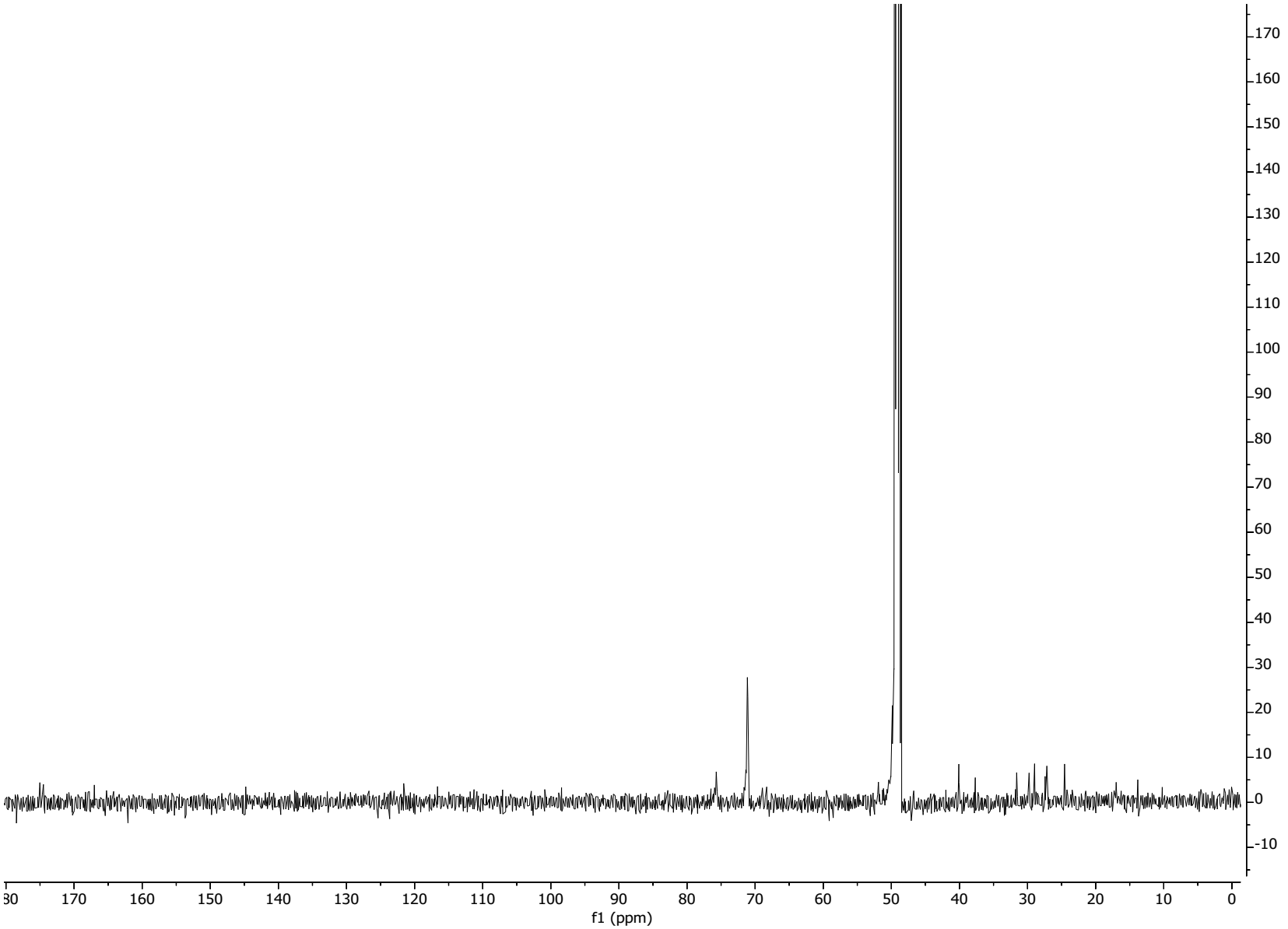

Figure 8. ^{13}C NMR spectrum of nicoyamycin A recorded at 175 MHz in CD_3OD.

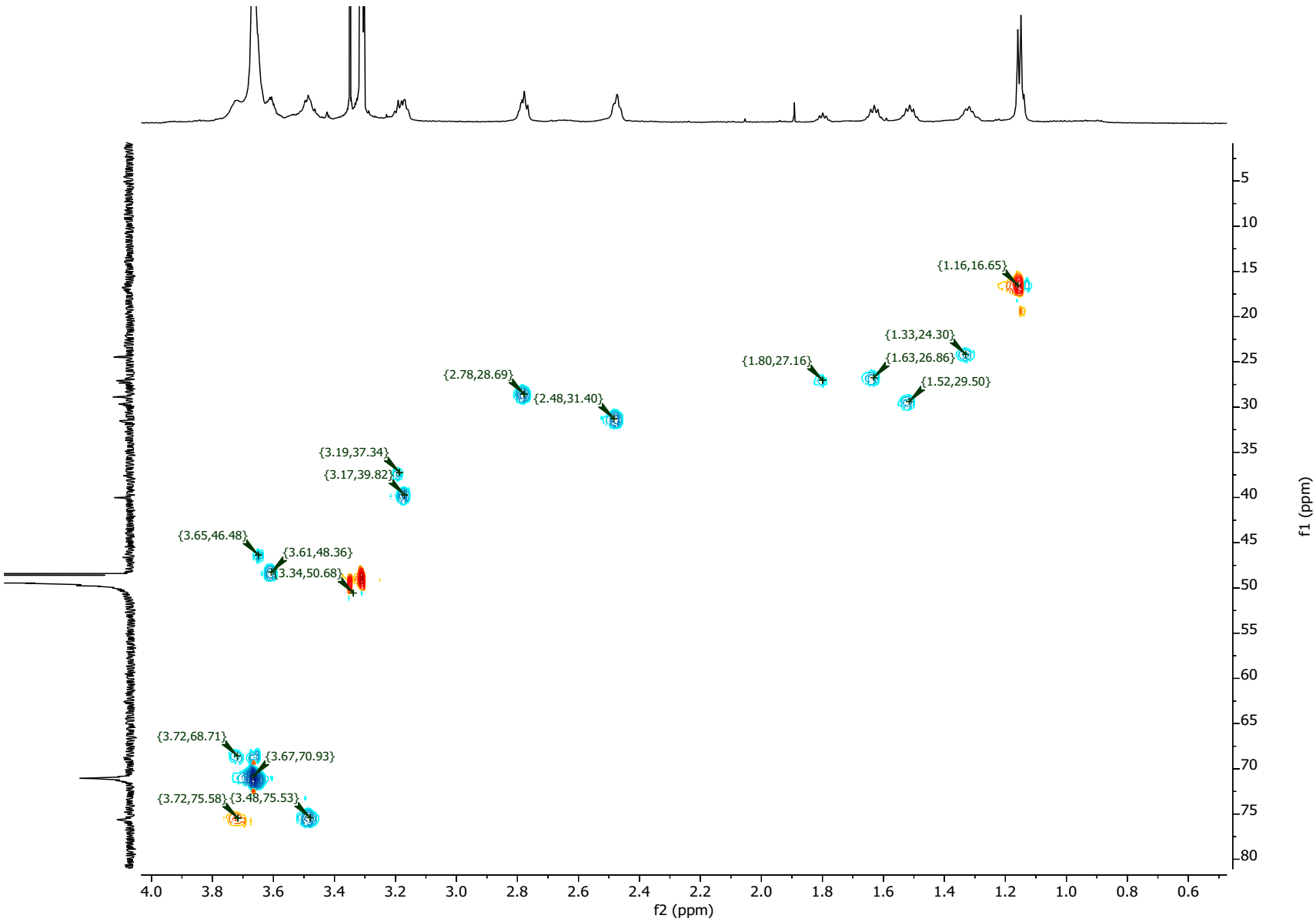

Figure 9. gHSQCAD NMR spectrum of nicoyamycin A recorded at 600 MHz in CD_3OD.

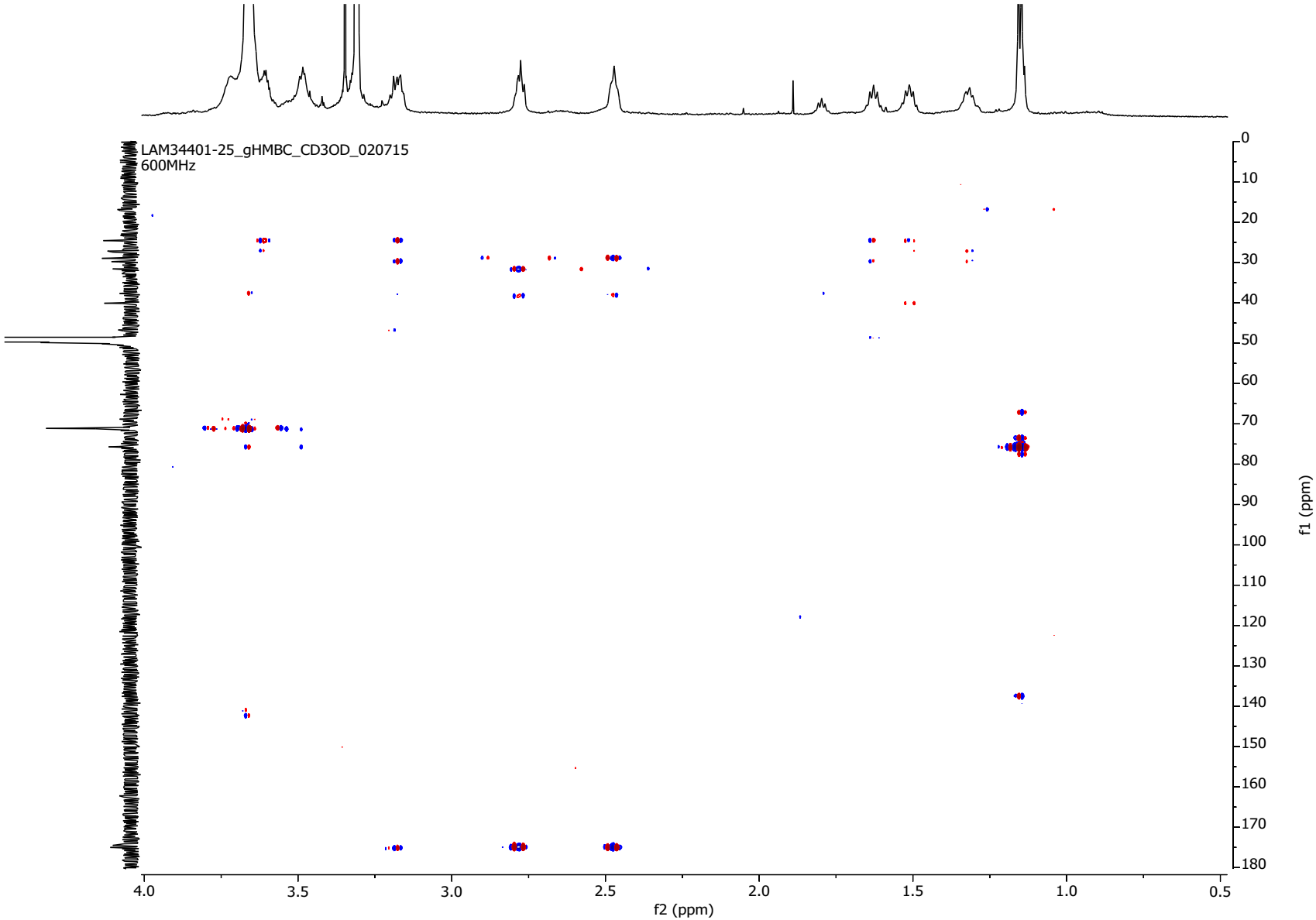

Figure 10. gHMBC NMR spectrum of nicoyamycin A recorded at 600 MHz in CD_3OD.

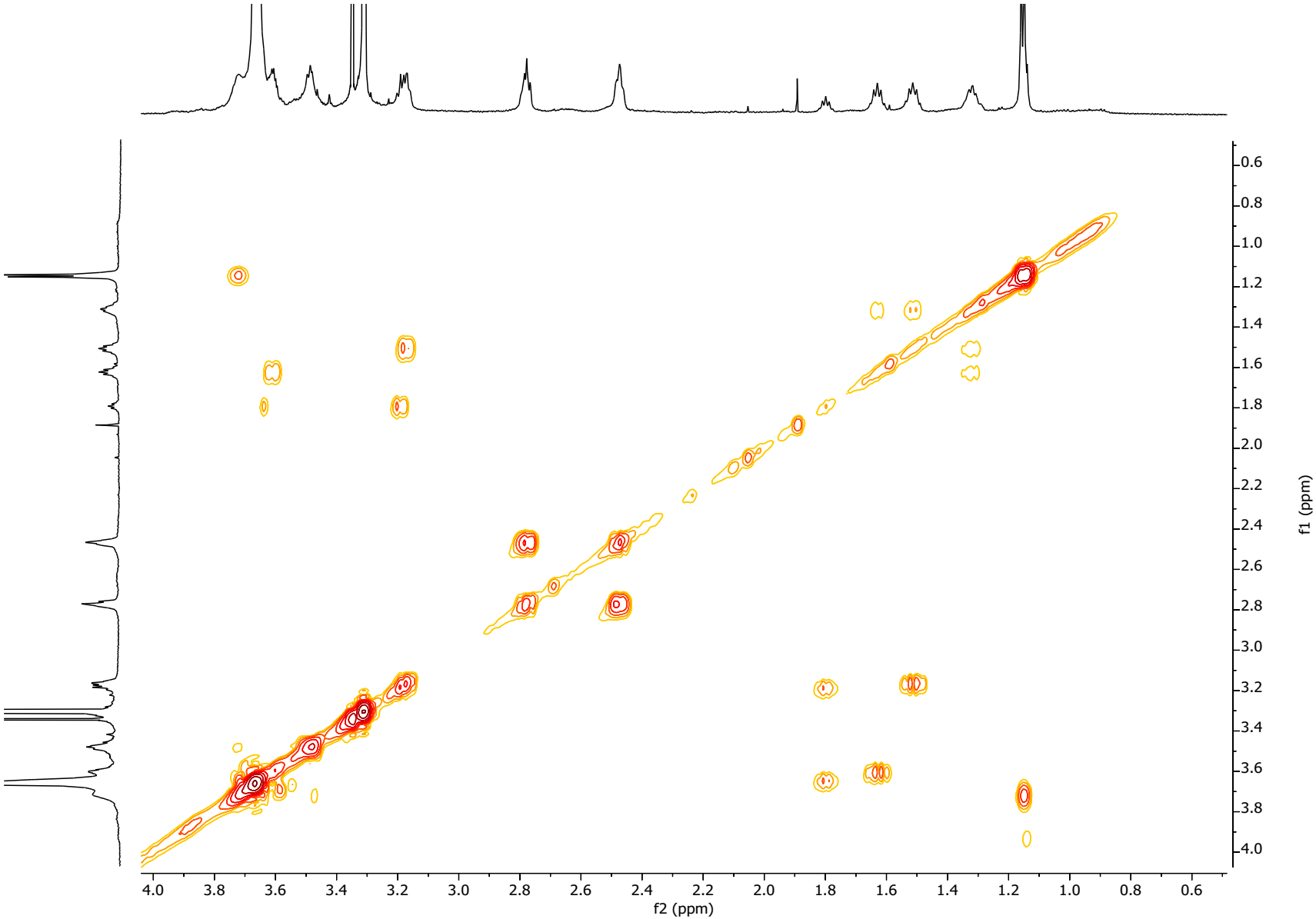

Figure 11. gCOSY NMR spectrum of nicoyamycin A recorded at 600 MHz in CD_3OD.

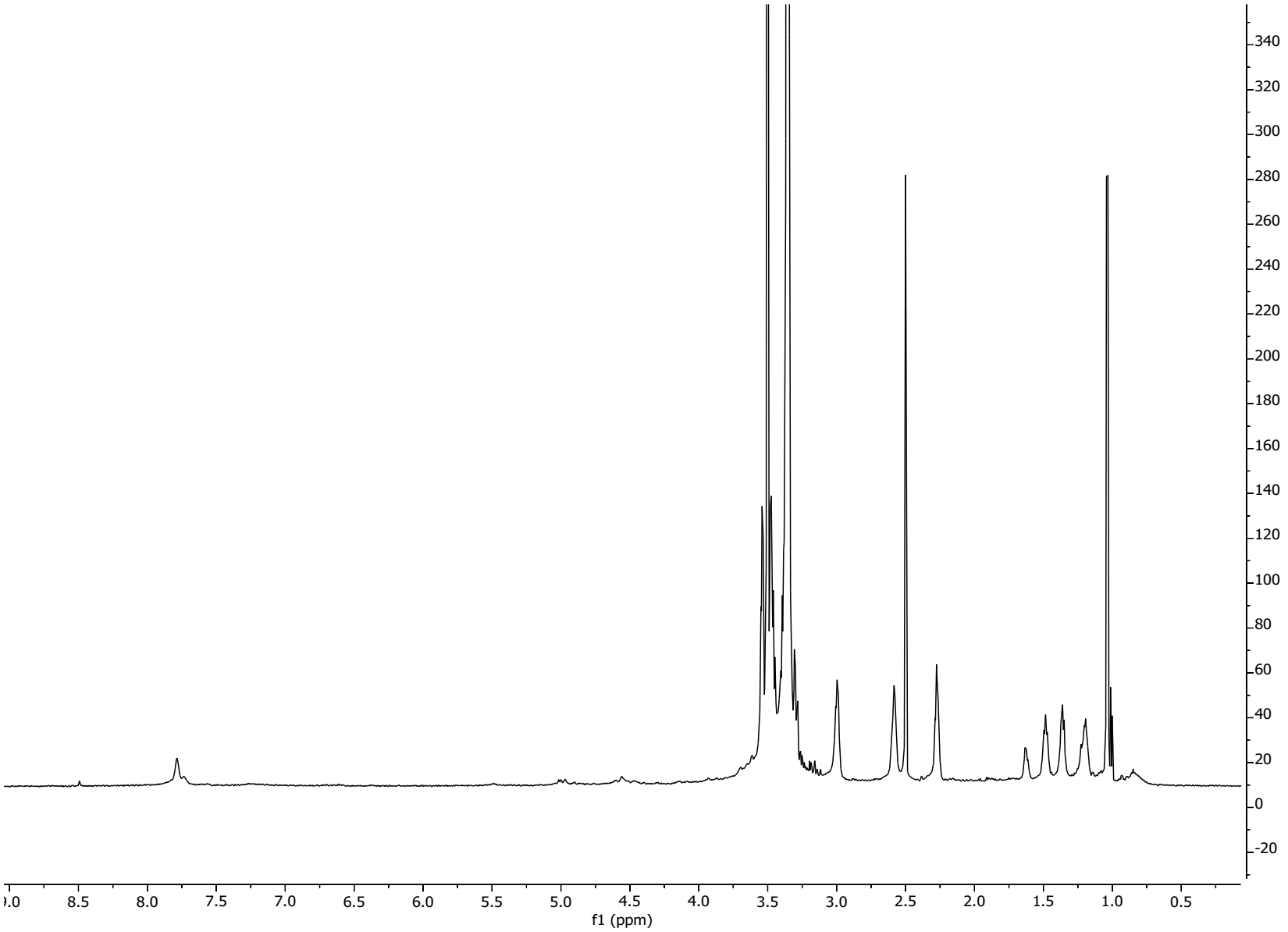

Figure 12. ^{1}H NMR spectrum of nicoyamycin A recorded at 600 MHz in DMSO-d_6.

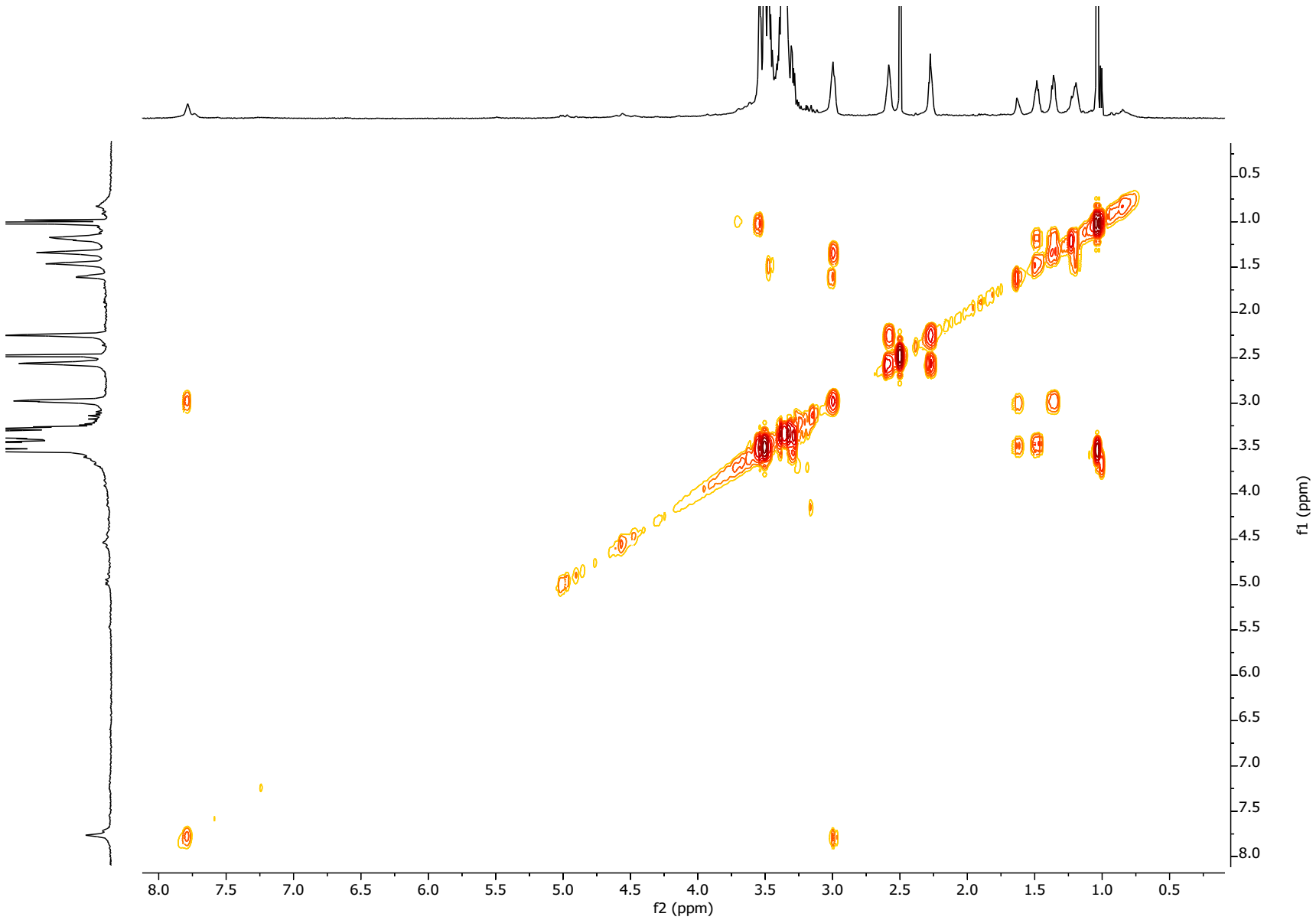

Figure 13. gCOSY NMR spectrum of nicoyamycin A recorded at 600 MHz in DMSO-d_6.

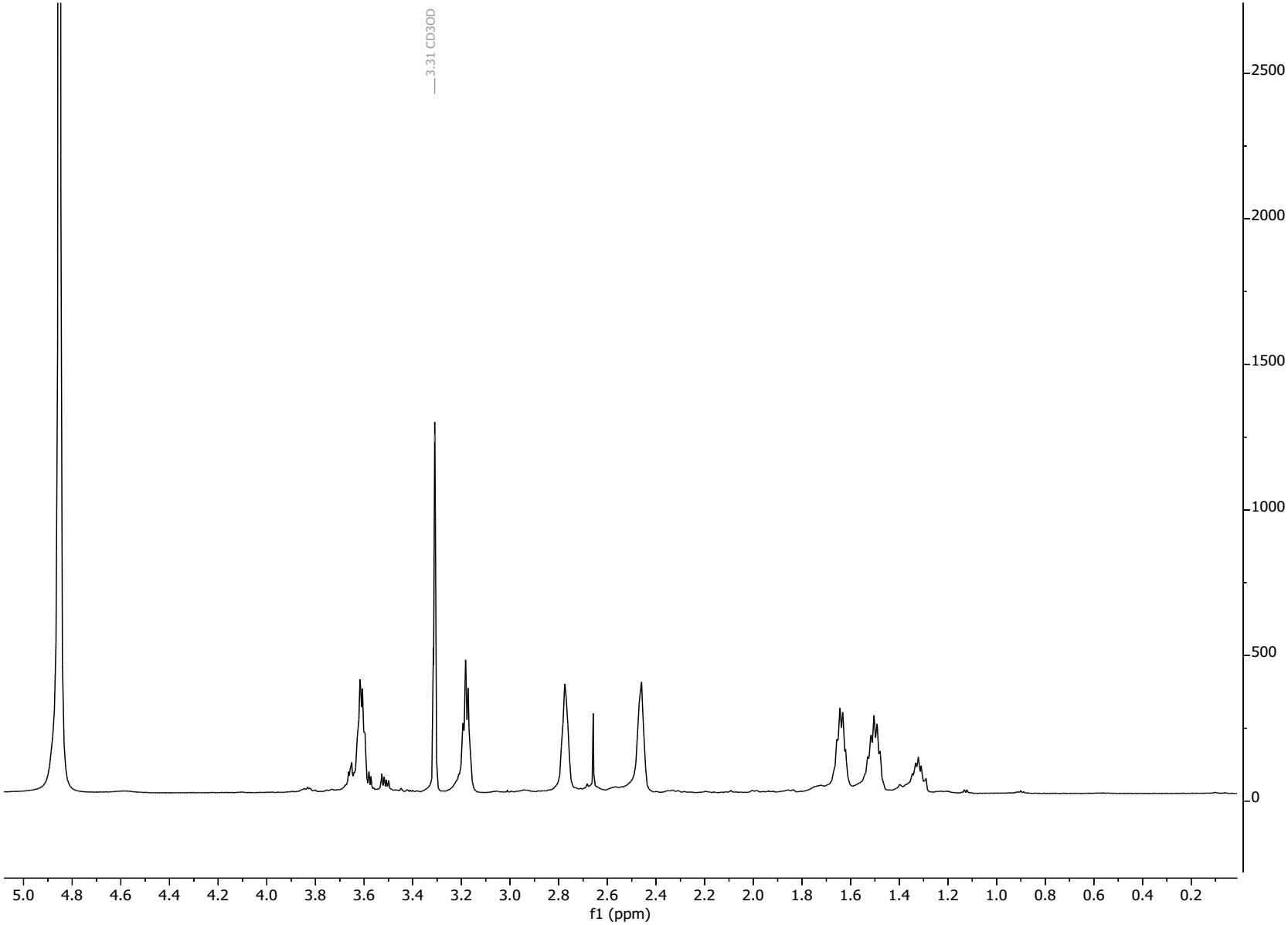

Figure 14. ^{1}H NMR spectrum of desferrioxamine X_1 recorded at 600 MHz in CD_3OD.

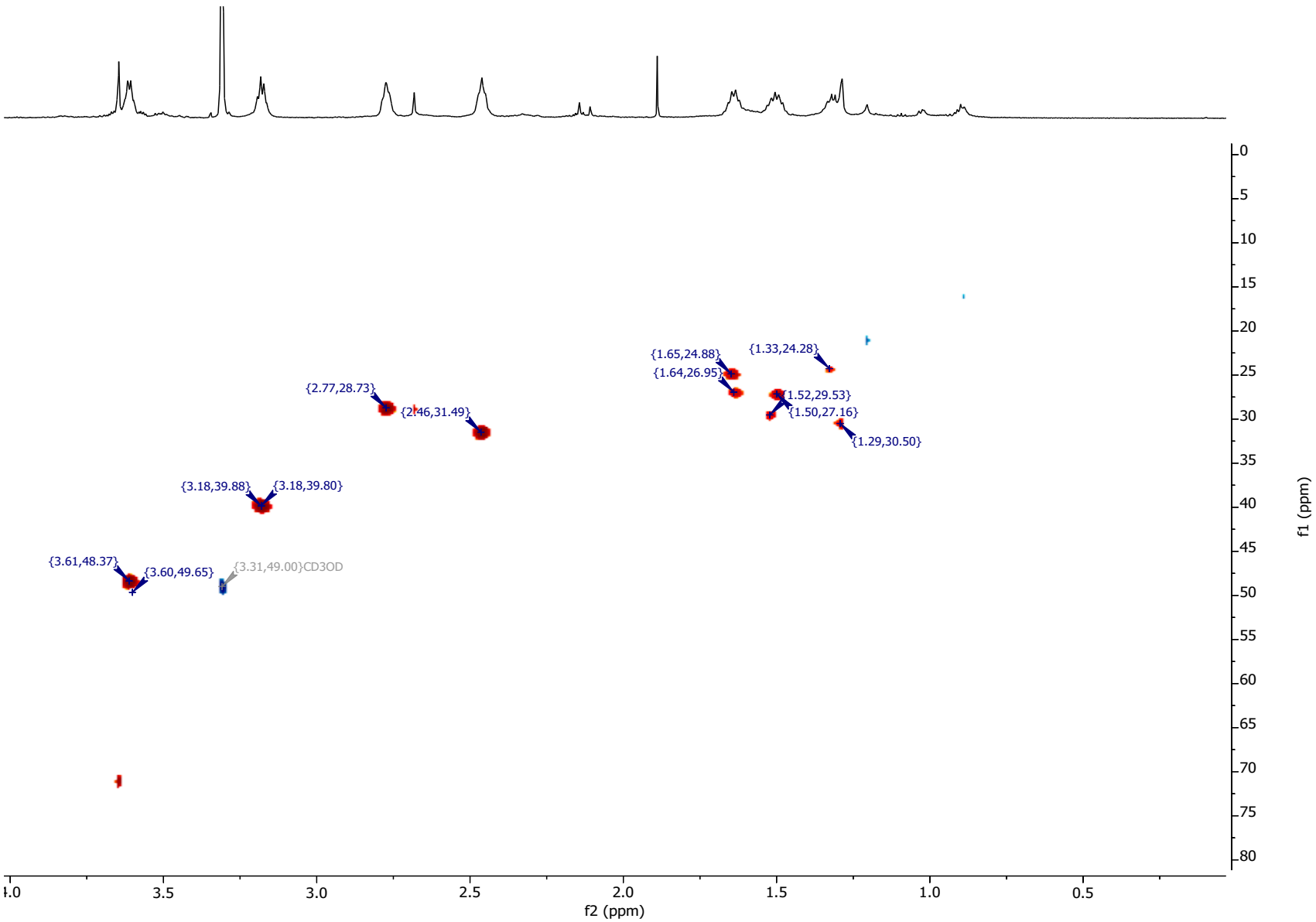

Figure 15. gHSQCAD NMR spectrum of desferrioxamine X_1 recorded at 600 MHz in CD_3OD.

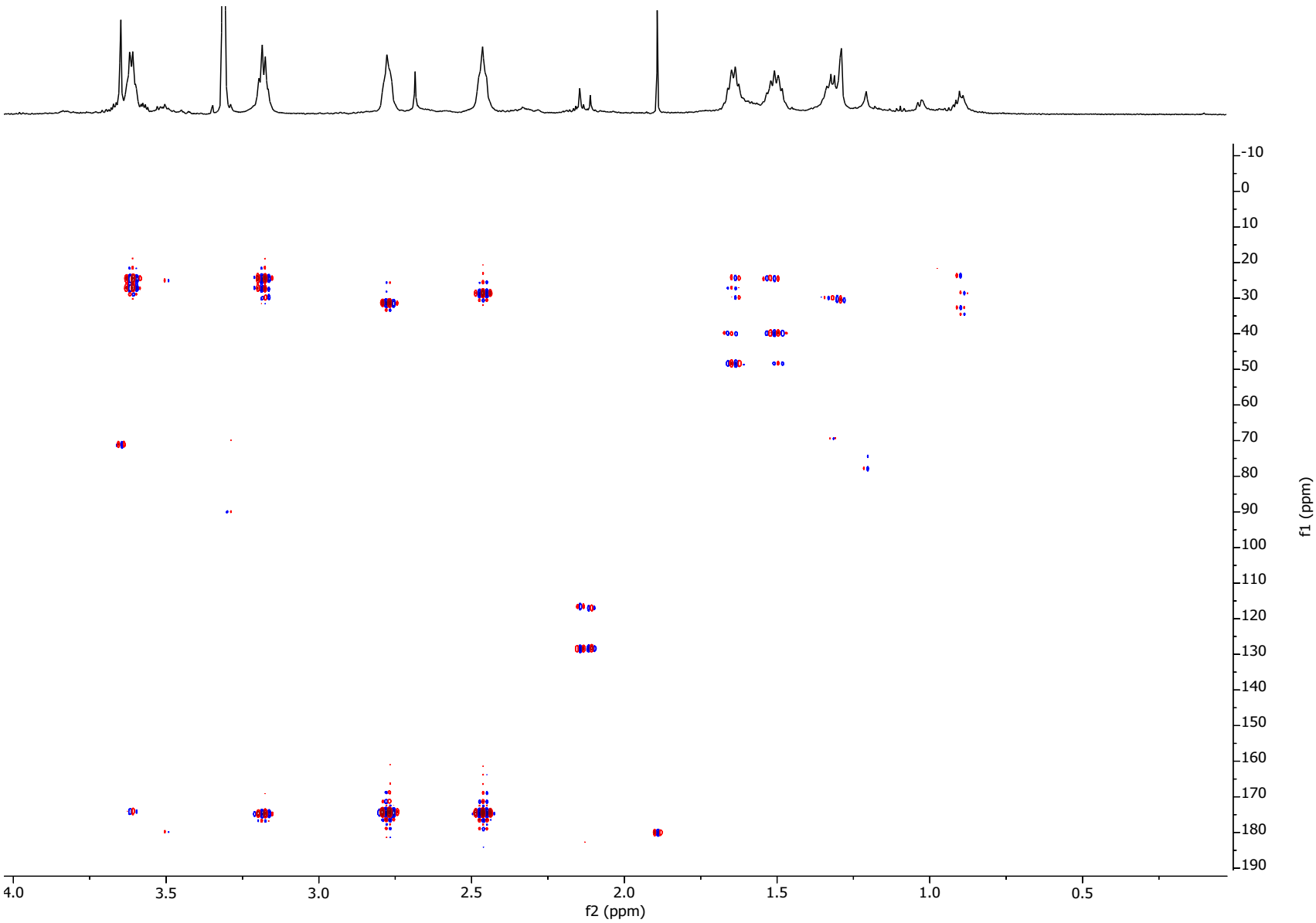

Figure 16. gHMBC NMR spectrum of desferrioxamine X_1 recorded at 600 MHz in CD_3OD.

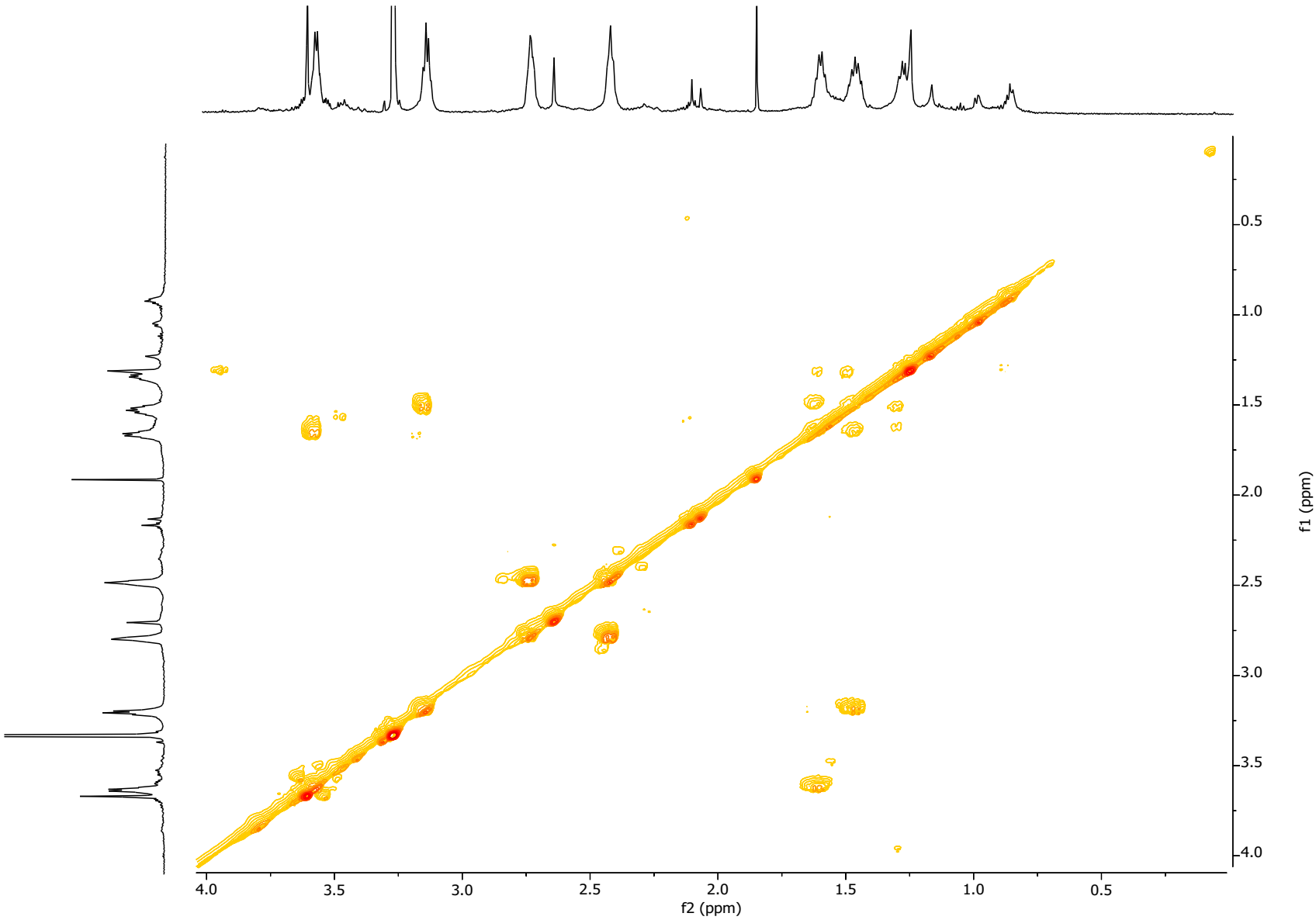

Figure 17. gCOSY NMR spectrum of desferrioxamine X_1 recorded at 600 MHz in CD_3OD.

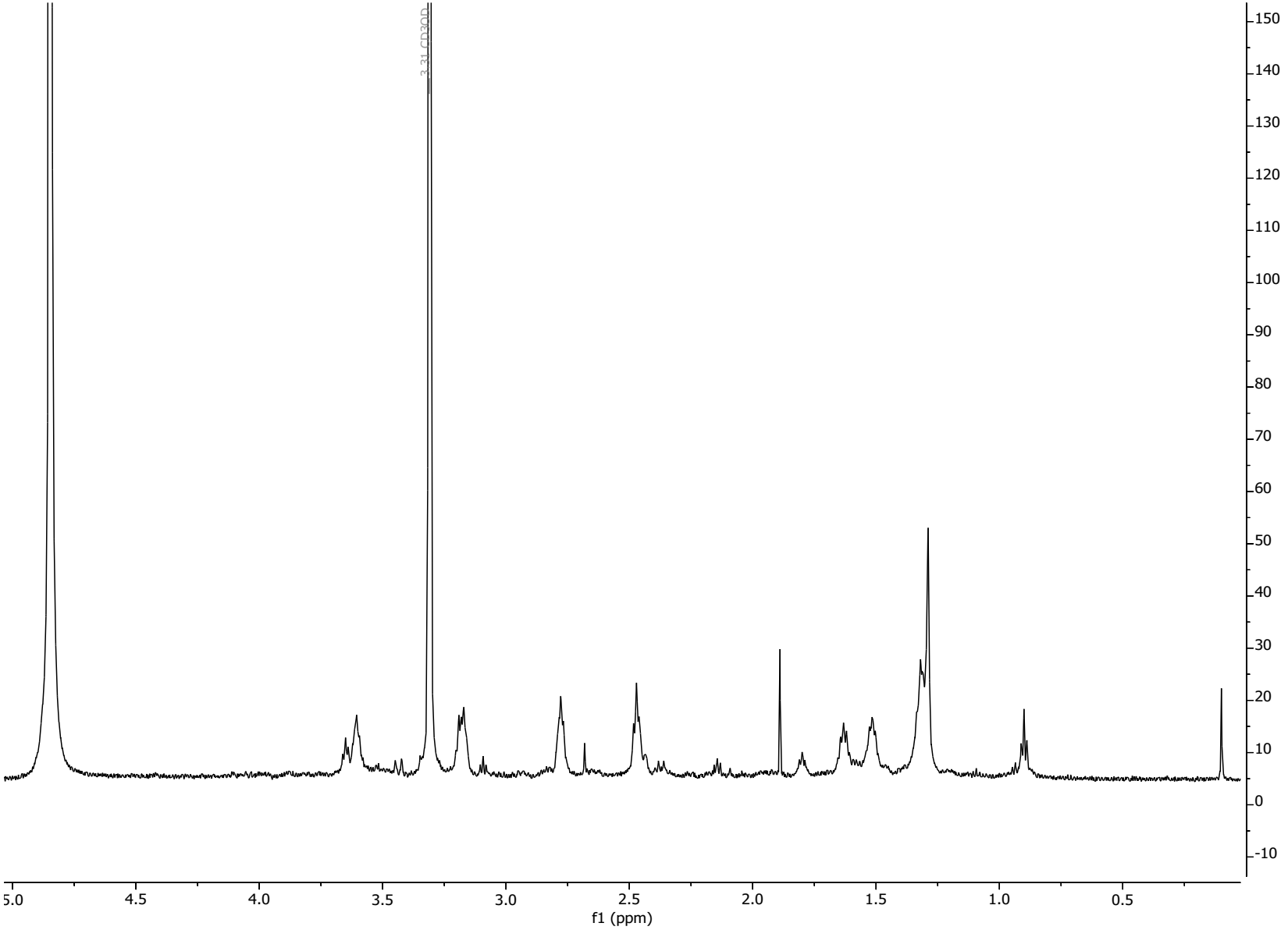

Figure 18. ^{1}H NMR spectrum of desferrioxamine X_7 recorded at 600 MHz in CD_3OD.

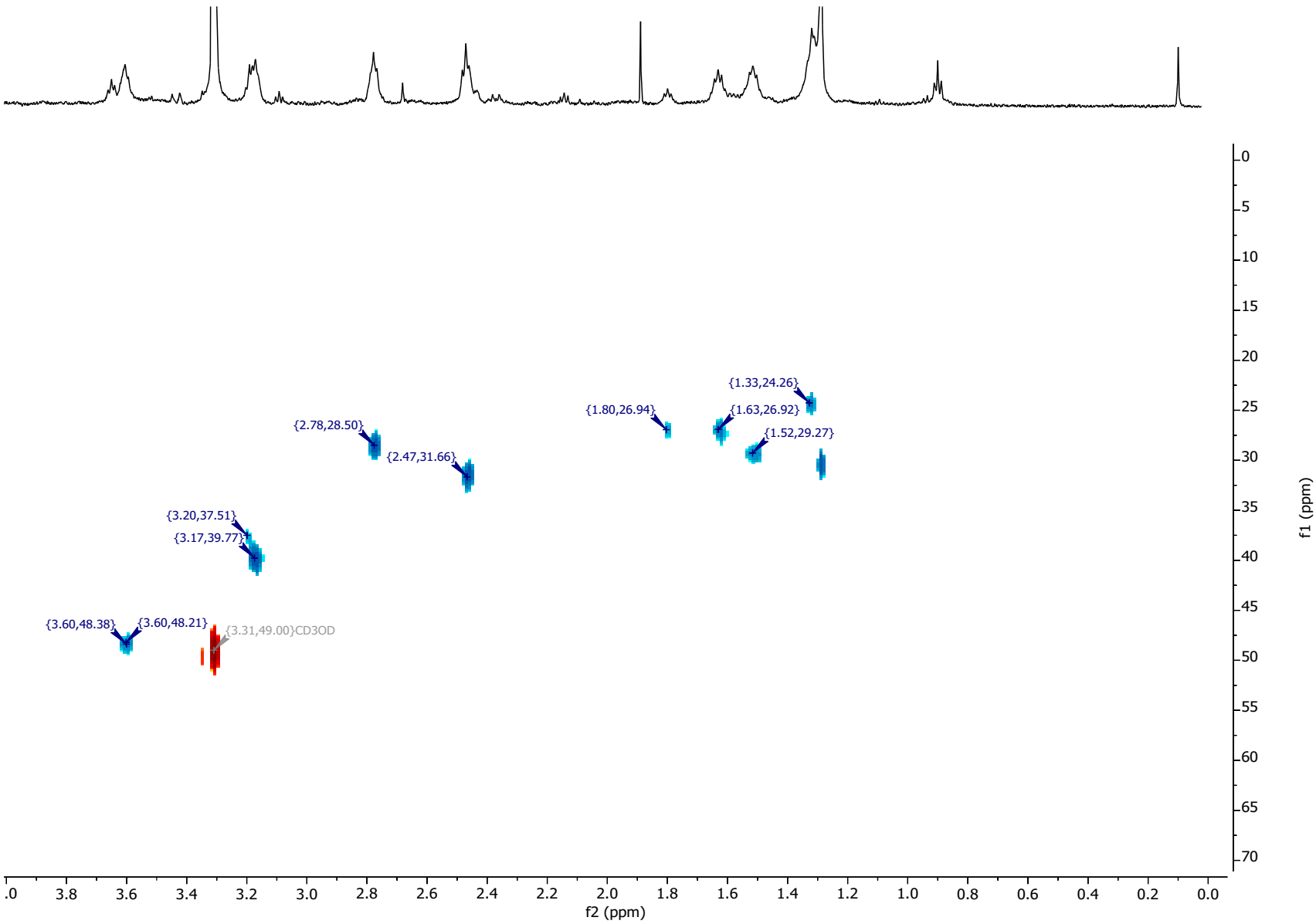

Figure 19. gHSQCAD NMR spectrum of desferrioxamine X_7 recorded at 600 MHz in CD_3OD.

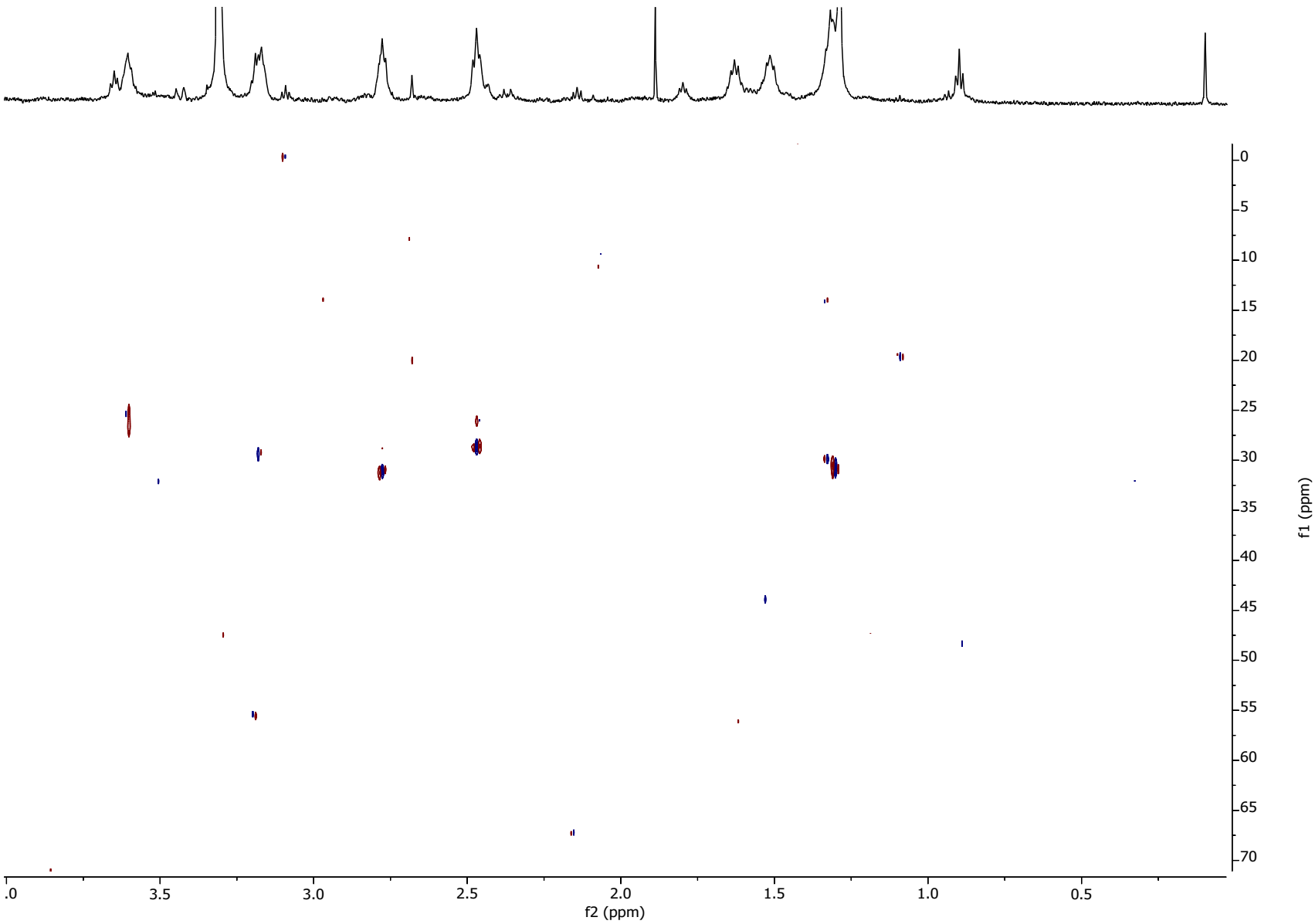

Figure 20. gHMBC NMR spectrum of desferrioxamine X_7 recorded at 600 MHz in CD_3OD.

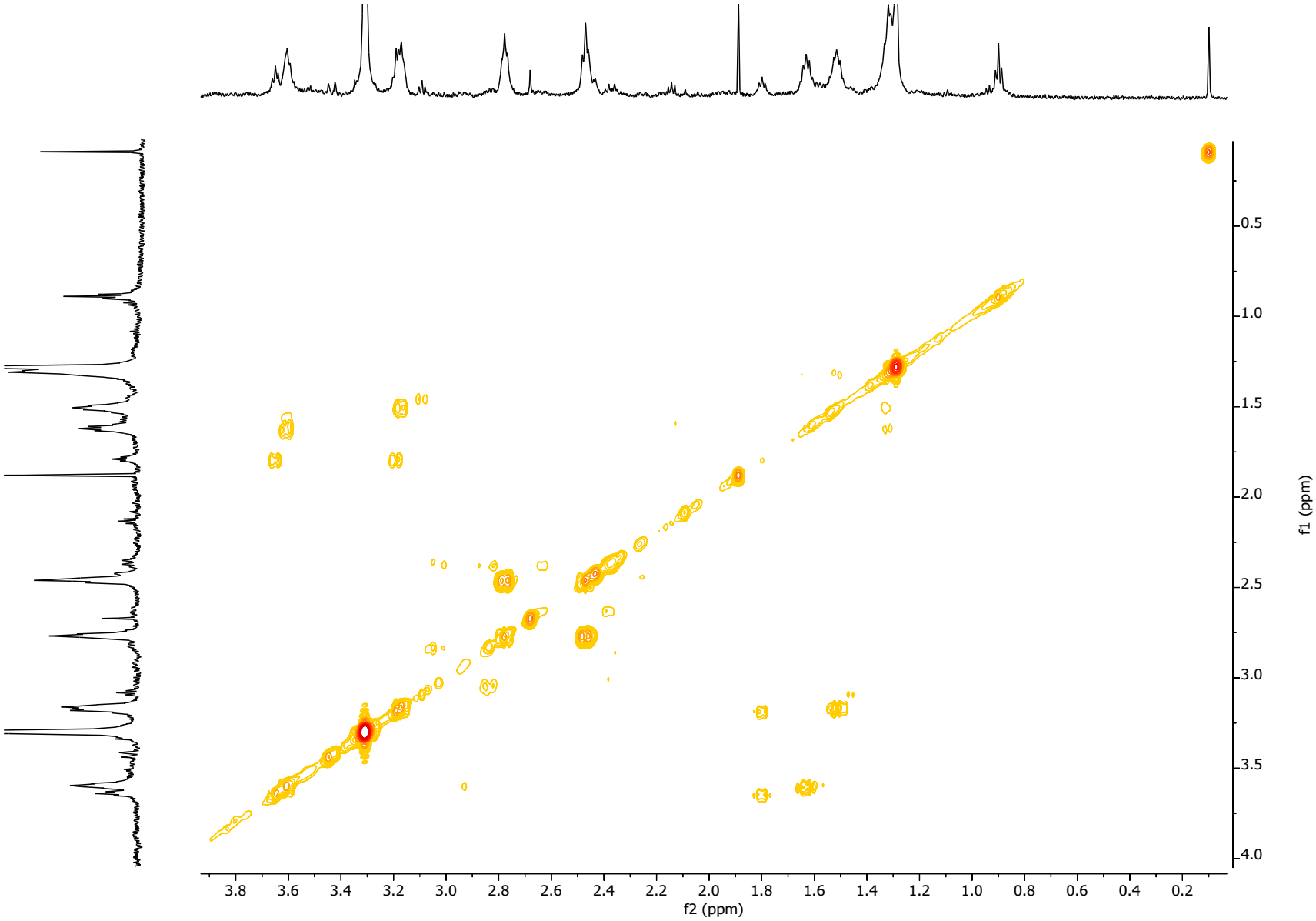

Figure 21. gCOSY NMR spectrum of desferrioxamine X_7 recorded at 600 MHz in CD_3OD.

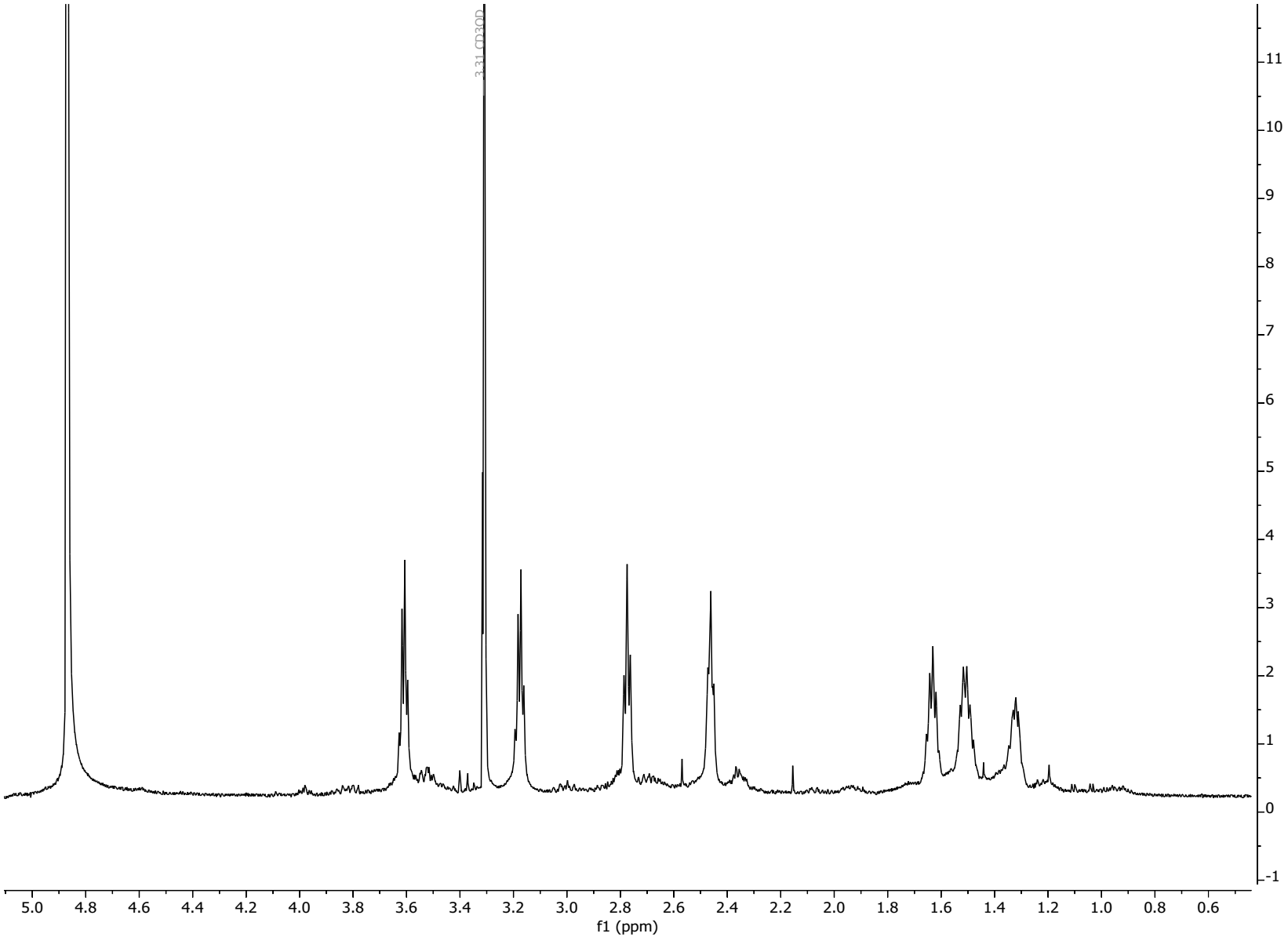

Figure 22. ^{1}H NMR spectrum of desferrioxamine D_2 recorded at 600 MHz in CD_3OD.

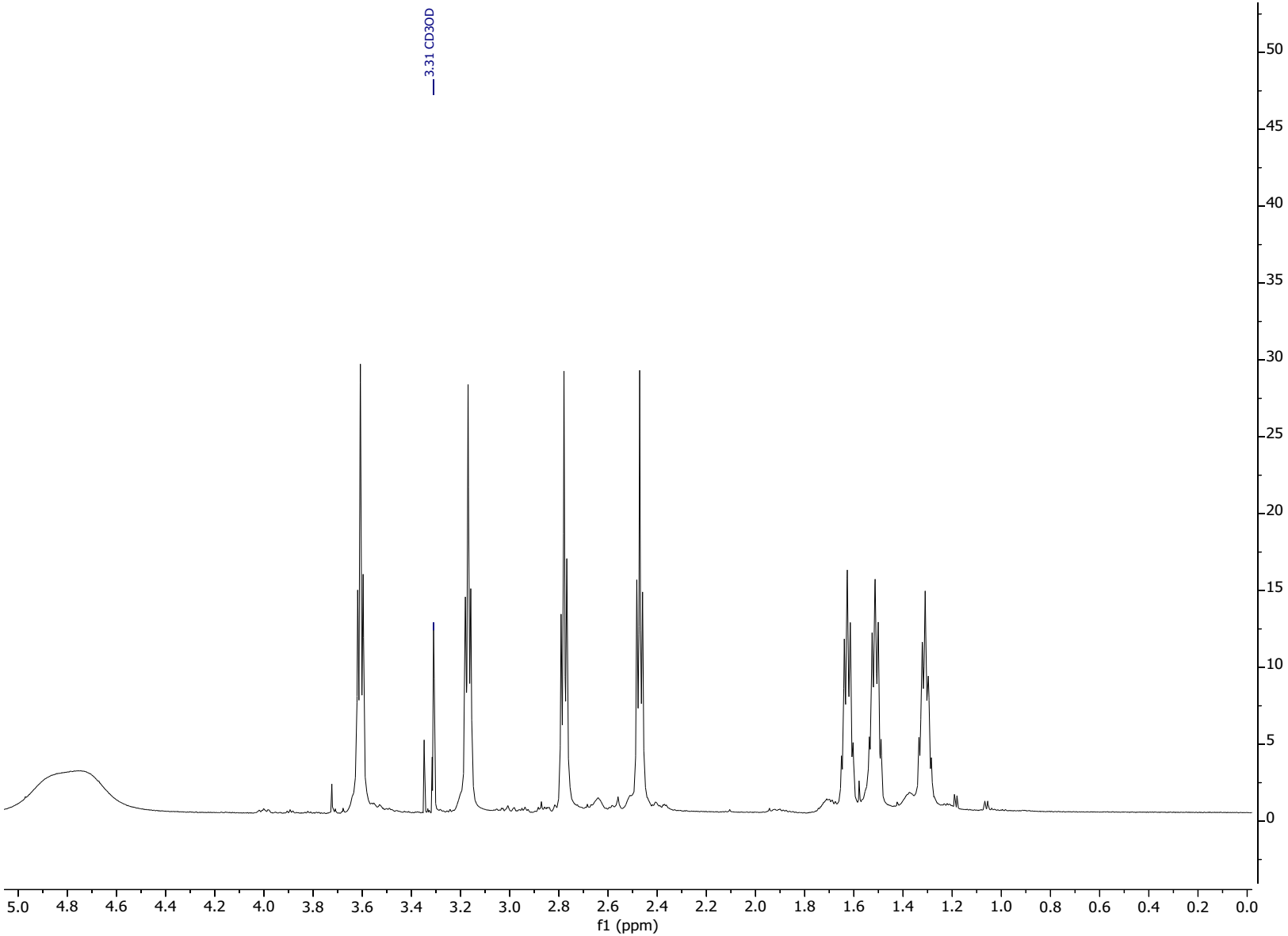

Figure 23. ^{1}H NMR spectrum of desferrioxamine E recorded at 600 MHz in CD_3OD.

Tables

Table 1. NMR spectroscopic data for nicoyamycin A at 600 MHz

	in CD_3OD				in DMSO-d_6			
	δ_C	δ_H, m (*J* in Hz)	COSY	HMBC	δ_C	δ_H, m (*J* in Hz)	COSY	HMBC
1						7.74, brs	2	
2	40.1	3.18, m	3	3	38.2	2.99, quint (6.8, 12.5)	1,3	3
3	29.6	1.52, quint (6.7, 13.3)	2,4	2, 4, 5	28.5	1.36, quint (6.8, 13.9)	2,4	2, 5
4	24.5	1.33, m	3,5	2, 3, 5, 6	23.1	1.19, m	3,5	2, 3, 5, 6
5	27.0	1.63, quint (6.9, 13.7)	4,6	4, 6	25.5	1.48, quint (6.6, 13.7)	4,6	3, 6
6	48.3	3.61, m	5	5	46.7	3.46, m	5	5
7						8.48, brs		
7'						8.48, brs		
8	174.5			9	171.9			9
8'	174.6				172			
9	28.9	2.78, m	10	10	27.5	2.58, m	10	10
9'	28.9	2.78, m	10'		27.5	2.58, m	10'	
10	31.5	2.48, m	9	9	30.1	2.27, m	9	9
10'	31.5	2.48, m	9'		30.1	2.27, m	9'	
11	175.0			10, 18	171.4			10, 18
11'	175.1			10, 2	171.5			10, 2
12	142.5			13	98.9			
12'	137.3			13'; 16'	98.1			
13	71.1	3.67, s			74.2	3.3, dd (4.2, 10.1)		16
						3.38, m		
13'	71.1	3.67, s			74.2	3.3, dd (4.2, 10.1)		16'
						3.38, m		
14	75.5	3.48, m	15	13, 16	70.1	3.47, m	15	15
14'	75.5	3.48, m	15'		70.1	3.47, m	15'	
15	75.6	3.72, m	14,16	14, 16	74.1	3.54, m	14,16	16
15'	75.6	3.72, m	14',16'		74.1	3.54, m	14',16'	
16	16.6	1.16, d (6.2)	15		20.2	1, d (6.3)	15	
16'	16.6	1.16, d (6.2)	15'		17.2	1.03, d (6.3)	15'	13', 14’
17						7.78, brs	18	
18	37.6	3.19, m	19	13, 19, 20	36.2	3, m	17,19	19, 20
19	27.2	1.8, quint (6.6, 13.4)	18,20	20	26.4	1.62, m	18,20	20
20	46.4	3.65, m	19	18	45.3	3.48, m	19	

Table 2. NMR spectroscopic data for desferrioxamine X_1 at 600 MHz

	in CD_3OD			
	δ_C	δ_H, m (J in Hz)	COSY	HMBC
1				
2	39.72	3.20, m	3	3, 4
3	29.53	1.52, m	2, 4	2, 4
4	24.28	1.33, m	3, 5	2, 3, 5, 6
5	26.95	1.64, m	4, 6	3, 4, 6
6	49.65	3.6, m	5	4, 5
7				
8	174.4			6, 9
8'	174.4			9', 16
8"	174.4			9", 16'
9	28.73	2.77, m	10	10
9'	28.73	2.77, m	10'	10'
9"	28.73	2.77, m	10"	10"
10	31.49	2.46, m	9	9
10'	31.49	2.46, m	9'	9'
10"	31.49	2.46, m	9"	9"
11	174.5			10, 13
11'	174.5			10', 13'
11"	174.5			10", 2
12				
12'				
13	39.8	3.18, m	14	14
13'	39.8	3.18, m	14'	14'
14	27.16	1.5, m	13, 15	13, 16
14'	27.16	1.5, m	13', 15'	13', 16'
15	24.88	1.65, m	14,16	16
15'	24.88	1.65, m	14',16'	16'
16	48.37	3.61, m	15	14, 15
16'	48.37	3.61, m	15'	14', 15'
17				

Table 3. NMR spectroscopic data for desferrioxamine X_7 at 600 MHz

	in CD_3OD			
	δ_C	δ_H, m (J in Hz)	COSY	HMBC
1				
1'				
2	39.76	3.17, m	3	3
2'	39.76	3.17, m	3'	3'
3	29.39	1.51, m	2, 4	2, 4
3'	29.39	1.51, m	2', 4'	2', 4'
4	24.32	1.33, m	3, 5	3
4'	24.32	1.33, m	3', 5'	3'
5	27.04	1.63, m	4, 6	3, 4, 6
5'	27.04	1.63, m	4', 6'	3', 4', 6'
6	48.32	3.69, m	5	5
6'	48.32	3.69, m	5'	5'
7				
8	174.3			9
8'	174.3			9'
8"	174.3			9"
9	28.58	2.78, m	10	10
9'	28.58	2.78, m	10'	10'
9"	28.58	2.78, m	10"	10"
10	31.44	2.47, m	9	9
10'	31.44	2.47, m	9'	9'
10"	31.44	2.47, m	9"	9"
11	174.2			10, 13
11'	174.2			10', 2'
11"	174.2			10", 2
12				
13	37.67	3.2, m	14	14
14	26.92	1.8, m	13, 15	
15	48.26	3.62, m	14	14
16				

Materials and Methods

Strains and culture conditions

Uropathogenic *E. coli* strain CFT073 was isolated from the blood and urine of a hospitalized patient diagnosed with acute pyelonephritis in the 1980s and the unmodified wildtype strain was used in all assays.[1] Overnight cultures were incubated in 3 mL of LB medium in aeration tubes shaken at 200 rpm at 37ºC. Growth assays were performed in MOPS minimal medium without iron (MOPS-Fe) or supplemented with 40 μM iron sulfate (MOPS+Fe). 10X MOPS minimal medium without iron was purchased from Teknova. Deionized distilled water was de-metallated by stirring with 1 g/L of Chelex (Bio-Rad) overnight and the Chelex removed by sterile filtration. The de-metallated water was used to prepare 100X K_2HPO_4 (0.132 M) and glucose (20% w/v). 1X MOPS-Fe was prepared by combining 100 mL of 10X MOPS minimal medium without iron with 10 mL of the 100X K_2HPO_4 and glucose stocks and bringing the medium to a total volume of 1 L. The complete MOPS-Fe was sterilized by vacuum filtration.

Streptomyces nicoyae, strain #34401-A3, was isolated from marine sediment collected in Las Baulas National Marine Park in Costa Rica (10º20'46.30"N and 85º52'00.10"E) (collection permit R-CM-INBio-30-2007).[2] Based on 16S sequencing, it is closely related to *Streptomyces albus* J1074 and *Streptomyces* sp. PVA 94-07.[3] *S. nicoyae* was revived from -80ºC storage by streaking it as a lawn on oatmeal agar plates (6% w/v oatmeal, 1.25% w/v agar, 3% w/v NaCl) and cultured at 28ºC for 1-2 weeks until fine white spores covered the plate. Liquid cultures were grown in ISP2 medium (1% w/v malt extract, 0.4% w/v yeast extract, 0.4% w/v dextrose, 3% w/v NaCl) with shaking at 200 rpm and 28ºC.

High-throughput screen

The natural product extract (NPE) library housed at the University of Michigan Center for Chemical Genomics contained ~33,000 extracts at the time of the screen. The NPEs were isolated from marine *Streptomyces* isolates collect from Costa Rica, Panama, and Papua New Guinea and the preparation of the extracts has been described previously.[2]

The HTS was executed as described previously,[4] except that wildtype CFT073 was used instead of CFT073 *tolC::kan,* and accordingly, no antibiotics were included in the MOPS-Fe medium. 50 nL of NPE was spotted into each well using a Mosquito X1 hit-picking liquid handler (TTP Labtech). The Z'-factor was calculated for each plate and averaged, as previously described.[5] The average plate Z'-factor for the primary screen was 0.75±0.08, enabling outstanding signal-to-noise differentiation.[5] NPEs that inhibited CFT073 growth greater than three standard deviations from the average OD_{600} of the negative control wells were considered hits.

Due to the prevalence of NPEs that chelated iron, a counter screen for iron chelation was run in parallel to the primary screen for NPEs that inhibit UPEC growth in low iron medium. The chrome azurol S (CAS) shuttle assay[6] was used to quantify iron chelation, as described previously in a 384-well format.[4] 50 nL of NPE was spotted into each well using a Mosquito X1 hit-picking liquid handler (TTP Labtech). The NPEs that reduced the absorbance at 630 nm by more than three standard deviations as compared to the negative controls were eliminated as iron chelators. The average plate Z'-factor was 0.52±0.07.[5]

Counter screen

The 995 hits that inhibited UPEC growth in MOPS-Fe, but did not appreciably chelate iron were cherry picked from the library for confirmation in triplicate growth assays. 50 nL were pin tooled into 384 well plates and 40 µl of CFT073 in MOPS-Fe were added to the wells and assessed

for the ability to inhibit bacterial growth. A secondary screen was simultaneously executed to ensure that hits were specifically inhibiting growth in low iron; the 995 hits were inoculated with 40 µl of CFT073 in MOPS-Fe with 40 µM $FeSO_4$ added back to the medium. DMSO was used as a negative control and 10 µg/mL of ciprofloxacin was used as a positive control. The average plate Z'-factor was 0.72±0.07.[5] Six NPEs were eliminated since they inhibited the growth of CFT073 in the presence of iron more than three standard deviations from the OD_{600} of the negative control wells.

The 989 hits were prioritized based on their percent of growth inhibition relative to DMSO-treated CFT073. 204 NPEs inhibited the growth to ≥90% of the vehicle controls. A list of *Streptomyces* isolates that produced the 204 NPEs was compiled and prioritized based on the activity of the NPEs in the HTS, if the strain produced multiple active wells, and what other strains were co-isolated from the sediment. Thirty-eight *Streptomyces* strains were revived on oatmeal agar plates and the NPEs from a 100 mL fermentation screened for bioactivity.

Hit confirmation of S. nicoyae

A sterile inoculating loop was used to scrape the spores from an oatmeal plate and inoculate 3 mL of ISP2 medium (1% w/v malt extract, 0.4% w/v yeast extract, 0.4% w/v dextrose, 3% w/v NaCl) in aeration tubes with shaking at 200 rpm and 28ºC for seven days. 100 mL of ISP2 in a 250 mL baffled flask was inoculated with the 3 mL of starter culture and grown at 200 rpm and 28ºC for four days. The culture was pelleted at 9000 x *g* for 15 min. The flask was rinsed with distilled water and the supernatants were transferred back to the empty flask. Two 2 g satchets of Amberlite XAD-16N resin were first washed with acetone and then water. The two washed

satchets were added to the flask containing the supernatant and shaken at 28ºC and 200 rpm overnight.

The next day the satchets were removed from the supernatant, rinsed with distilled water, and transferred to a clean flask. The resin satchets were covered with ethyl acetate and shaken at 28ºC and 200 rpm for 15 min. The ethyl acetate was transferred to a round bottom flask and the extraction was repeated twice, once with acetone, and finally with methanol. The ethyl acetate, acetone, and methanol extraction were pooled in a round bottom flask and dried under vacuum.

Growth assays

E. coli CFT073 was cultured in triplicate overnight in 3 mL of LB broth in aeration tubes at 37ºC and 200 rpm. The cultures was pelleted at 5000 x *g* for 5 min at 4ºC, the supernatant was discarded and the pellets washed with 3 mL of MOPS-Fe a total of three times. After the final wash, the pellet was resuspended in 3 mL of MOPS-Fe and kept on ice. At the time of use, the OD_{600} was measured and each sample diluted to an OD_{600} of 0.01 in MOPS-Fe or MOPS-Fe supplemented with 40 µM $FeSO_4$.

Each NPE, fraction or pure molecule was dried under nitrogen or vacuum (Biotage V-10 Touch) and then prepared in DMSO at 10 mg/mL for growth and CAS assays. The 10 mg/mL stock was diluted 10 fold in DMSO to make 1, 0.1, and 0.01 mg/mL stocks. 1 µl of each concentration was aliquoted into a 96 well, non-tissue culture treated, flat bottom plate (Falcon) in triplicate. 1 µl of DMSO was used as a negative control, and 100 µg/mL ampicillin and 200 µM 2′,2′-dipyridyl (DIP) were used as positive controls. 100 µl of bacterial suspension was diluted to an OD_{600} of 0.01 and added to each well. The plate was wrapped in plastic wrap to prevent evaporation and incubated without shaking at 37ºC for 18 h. The next day, bacteria were

resuspended by vortexing gently and then the OD_{600} was quantified on a Bio-tek μQuant plate reader.

Chrome azurol-S assay

The CAS shuttle assay was used to quantify iron chelation. Briefly, 100 μl of the CAS shuttle solution (0.6 mM hexadecyltrimethylammonium bromide (HDTMA), 0.015 mM $FeCl_3$, 0.15 mM CAS, 0.75 M hydrochloric acid, 0.5 M anhydrous piperazine, and 4 mM 5-sulfosalicylic acid) was added to 1 μl of the molecule stock solution to be assayed. For NPEs, the stock solutions were 0.01, 0.1, and 1 mg/mL in DMSO; the positive control was 1 mg/mL EDTA; and the negative control was DMSO. The assay was incubated for at least 30 min at room temperature and then the absorbance at 630 nm was quantified on a Bio-tek μQuant plate reader.

Large scale production and isolation

S. nicoyae spores were scraped from an entire oatmeal agar plate and inoculated directly into 50 mL of ISP2 in a baffled flask and incubated at 28ºC and 200 rpm for seven days. 35 mL of stationary phase *S. nicoyae* in ISP2 was used to inoculate 2 L of ISP2 in a 6 L flask with springs and cultured at 28ºC and 200 rpm for six days. The culture was pelleted at 9000 x *g* for 15 min. The springs were removed from the flask and then the flasks were rinsed with distilled water. The supernatants were transferred back to the empty flasks. Two 20 g satchets of Amberlite XAD-16N resin were first washed with acetone and then water. The two washed satchets were then added to the flask containing the supernatant and shaken at 28ºC and 200 rpm overnight.

The next day, the satchets were removed from the supernatant, rinsed with distilled water, and transferred to a clean flask. The resin satchets were covered with ethyl acetate and shaken at

28ºC and 200 rpm for 15 min. The ethyl acetate solution was transferred to a round bottom flask and the extraction was repeated twice, once with acetone, and finally with methanol. The ethyl acetate, acetone, and methanol extraction were pooled in a round bottom flask and dried under vacuum to yield 5.11 g of whole crude extract.

Purification

A C18 column (20 × 2.6 cm, YMC Gel ODS-A, 12 nm, S-150 μm) with a column volume of ~75 mL was washed with 500 mL of 1:1 ethyl acetate:methanol and 500 mL of methanol. It was then equilibrated with 500 mL of deionized water. The 5.11 g of crude extract was resuspended in 15 mL of deionized water, dissolved in a sonicating water bath, and applied to the prepared C18 column. The column was sequentially eluted with 200 mL of a stepwise gradient of water/ACN: 100/0, 90/10, 75/25, 60/40, 45/55, 30/70, 15/85, and 0/100. The eluted fractions were dried under nitrogen overnight.

HPLC Purification

All HPLC was performed with a Shimadzu LC-20AT HPLC system with an FRC-10A fraction collector. Gradient RP-HPLC was performed using Econosil C18 HPLC column with a pore size of 10 μm and dimensions of 250 x 20 mm (P/N 6251). The 75% v/v water/methanol fraction from the preparative C18 purification was prepared at 300 mg/mL in methanol and clarified by centrifugation. The column was equilibrated in 30% v/v methanol/water with a flow rate of 7 mL/min. After injecting the sample, the mobile phase remained 30% v/v methanol/water for 2 min and then increased to 80% v/v methanol/water over 40 min. The mobile phase was then

increased to 95% v/v methanol/water over 3 min and then returned to 30% v/v methanol/water and maintained for 10 min.

Isocratic RP-HPLC was performed using Luna 5 μm phenyl-hexyl HPLC column with a pore size 100 Å and dimensions of 250 x 20 mm (P/N 00G-4257-N0). The fraction 9 from the gradient HPLC purification was prepared at 20 mg/mL in 4:1 methanol:water and clarified by centrifugation. The column was equilibrated in 30% v/v methanol/water with a flow rate of 3.5 mL/min. After injecting the sample, the mobile phase remained 30% methanol for 80 min, then increased to 80% v/v methanol/water over 5 min, and then returned to 30% v/v methanol/water and maintained for 10 min. $DesX_1$ eluted in a mixture at 50 min from this isocratic RP-HPLC method.

Fractions containing $DesX_1$ were pooled and prepared at 10 mg/mL in 30% v/v methanol/water in water for a second round of isocratic RP-HPLC using the same Luna 5 μm phenyl-hexyl HPLC column (P/N 00G-4257-N0). The column was equilibrated in 27% v/v methanol/water in water containing 0.1% v/v formic acid, with a flow rate of 3.5 mL/min. After injecting the sample, the mobile phase remained 27% v/v methanol/water for 90 min; no wash step was required.

Further analysis provided access to $DesD_2$ and DesE, which eluted in the wash of the $DesX_1$ and $DesX_7$ separations using a phenyl-hexyl HPLC column. $DesD_2$ and DesE were purified using a reverse phase C8 column with 40% v/v methanol/water and 0.1% v/v formic acid (r.t. 16 min and 24 min, respectively), and the observed elution order agrees with previous reports.[7] Their respective MS/MS fragmentation pattern and ^{1}H NMR spectra agree with previously published data.[7-9]

Mass spectrometry (MS) analysis

All high-resolution ESI-MS and MS/MS spectra were collected using an Agilent 6545 Q-TOF mass spectrometer equipped with an Agilent 1290 HPLC system and 1260 detector in the Life Sciences Institute at the University of Michigan. Samples were prepared at 0.5 mg/mL in 3:2 methanol:water. 4 µl was injected onto a Luna 5 µm, C18(2) column with a pore size of 100 Å and dimensions of 100 x 2 mm (Phenomenex P/N 00D-4252-B0) equilibrated in 10% v/v methanol/water with 0.1% v/v formic acid and a flow rate of 0.4 mL/min. The column was held at 10% v/v methanol/water for 1 min and then increased to 60% v/v methanol/water for 12 min. The column was held at 60% v/v methanol/water for 1 min and then returned to 10% v/v methanol/water and held for 2 min (16 min total run time). The MS spectra were collected in positive mode from 50-1500 *m/z* and stored in centroid mode. MS/MS spectra were collected in Auto MS/MS mode with a range of 50-1500 *m/z* for MS and 50-700 *m/z* for MS/MS. The fragmentor was set at 125 V and the collision energy was 40 V.

Nuclear Magnetic Resonance (NMR) analysis

All NMR spectra were acquired on a Varian INOVA 600 MHz spectrometer at the NMR Facility in the Life Sciences Institute at the University of Michigan. Molecules were dissolved in either CD_3OD or DMSO-d_6 and placed in a symmetrical NMR tube (Shigemi) matched to the solvent system.

References

1. H. L. Mobley, D. M. Green, A. L. Trifillis, D. E. Johnson, G. R. Chippendale, C. V. Lockatell, B. D. Jones and J. W. Warren, *Infect. Immun.*, 1990, **58**, 1281-1289.
2. N. A. Magarvey, J. M. Keller, V. Bernan, M. Dworkin and D. H. Sherman, *Appl. Environ. Microb.*, 2004, **70**, 7520-7529.
3. A. R. Wattam, J. J. Davis, R. Assaf, S. Boisvert, T. Brettin, C. Bun, N. Conrad, E. M. Dietrich, T. Disz, J. L. Gabbard, S. Gerdes, C. S. Henry, R. W. Kenyon, D. Machi, C. Mao, E. K. Nordberg, G. J. Olsen, D. E. Murphy-Olson, R. Olson, R. Overbeek, B. Parrello, G. D. Pusch, M. Shukla, V. Vonstein, A. Warren, F. Xia, H. Yoo and R. L. Stevens, *Nucleic Acids Res.*, 2017, **45**, D535-d542.
4. A. Yep, T. McQuade, P. Kirchhoff, M. Larsen and H. L. T. Mobley, *mBio*, 2014, **5**, e01089-01013.
5. J.-H. Zhang, T. D. Y. Chung and K. R. Oldenburg, *J. Biomol. Screen.*, 1999, **4**, 67-73.
6. B. Schwyn and J. B. Neilands, *Anal. Biochem.*, 1987, **160**, 47-56.
7. G. J. Feistner, D. C. Stahl and A. H. Gabrik, *Org. Mass Spectrom.*, 1993, **28**, 163-175.
8. H. Maehr, W. Benz, J. Smallheer and H. Williams Thomas, *Z. Naturforsch B*, 1977, **32**, 937.
9. H.-S. Lee, H. J. Shin, K. H. Jang, T. S. Kim, K.-B. Oh and J. Shin, *J. Nat. Prod.*, 2005, **68**, 623-625.