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Authors: Kazuo Nagasawa, Ph.D.; Chao Wang; Mana Oki; Toru Nishikawa; Daisuke Harada; Mari Yotsu-Yamashita

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Total Synthesis of 11-Saxitoxinethanoic Acid and Evaluation of its Inhibitory Activity on Voltage-gated Sodium Channels

Chao Wang,^[a] Mana Oki,^[a] Toru Nishikawa,^[a] Daisuke Harada,^[a] Mari Yotsu-Yamashita^[b] and Kazuo Nagasawa*^[a]

Abstract: 11-Saxitoxinethanoic acid (SEA) is a member of the saxitoxin family of paralytic shellfish poisons, but unusually contains a C-C bond at the C11 position. Herein, we reported a total synthesis of SEA. The key to our synthesis lies in a Mukaiyama-aldol condensation reaction of silyl enol ether with glyoxylate in the presence of an anhydrous fluoride reagent, $[\text{Bu}_4\text{N}][\text{Ph}_3\text{SnF}_2]$, which directly constructs the crucial C-C bond at the C11 position in SEA. The Na_vCh -inhibitory activity of SEA and its derivatives was evaluated by means of cell-based assay. SEA showed an IC_{50} value of 47 ± 12 nM, being approximately twice as potent as decarbamoyl-STX (dcSTX).

Saxitoxin (**1**, STX, Figure 1), which was first isolated as a paralytic shellfish poison,^[1] is an inhibitor of voltage-gated sodium channels (Na_vCh).^[2] So far, more than 50 analogs have been discovered,^[3] and they have attracted considerable interest from synthetic chemists.^[1c,d,4] Among the STX analogs, only zetekitoxin AB (**6**, ZTX)^[5] and 11-saxitoxinethanoic acid (**7**, SEA)^[6] contain a C-C bond at the C11 position. It is extremely difficult to understand how this C-C bond arises, in terms of proposed biosynthetic pathways for STXs.^[7]

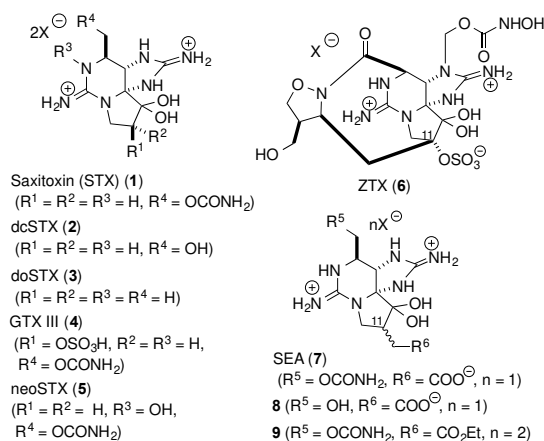
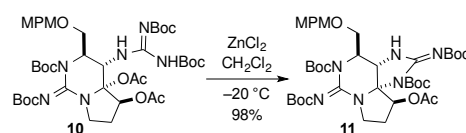


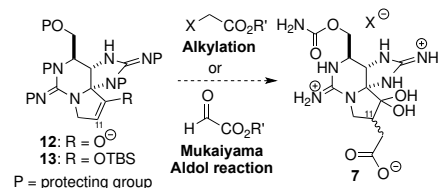
Figure 1 Structures of saxitoxin (**1**) and its derivatives **2-5**, zetekitoxin AB (**6**, ZTX), 11-saxitoxinethanoic acid (**7**, SEA) and its derivatives **8** and **9**.

We are interested in developing subtype-selective Na_vCh inhibitors, and in this work we focused on the synthesis of SEA (**7**) and its analogs as candidate Na_vCh modulators.^[8] SEA (**7**) was originally isolated from xanthid crab *Atergatis floridus* in 1995,^[6] and it contains a saxitoxin core with an acetic acid group at the C11 position; the natural product is a 9:1 mixture of stereoisomers. The toxicity of SEA (**7**) was reported as 830 mouse units per μmol on i.p. injection into mice, which corresponds to approximately one-third of the toxicity of STX (**1**).^[3,6] In this communication, we describe the synthesis of SEA (**7**)^[9] and its derivatives **8** and **9**. The Na_vCh -inhibitory activity of these new STX derivatives in cell-based assay is also reported.



Scheme 1. Synthesis of fully protected saxitoxinol **11** in our group.

We have recently developed a synthesis of fully protected saxitoxinol **11**^[4g] by utilizing neighboring acyl group-assisted construction of the 5-membered cyclic guanidine structure in STXs under mild conditions (Scheme 1). Compound **11** is a key intermediate for the synthesis of STX and its derivatives, which have highly polar nature due to the two guanidine groups. We have employed **11** in syntheses of dcSTX (**2**), GTX III (**4**), and artificial STX derivatives.^[4g,8b] Here, we envisaged application of **11** to the synthesis of SEA (**7**) through C-C bond formation at the C11 position.



Scheme 2 Synthetic strategies to SEA (**7**).

Regarding synthetic approaches to SEA (**7**), there are two possibilities to construct the C-C bond at the C11 position (Scheme 2), i.e., direct alkylation of the enolate **12** with halides in the presence of base, and Mukaiyama-aldol reaction of the silyl enol ether **13** with glyoxylate.

We initially investigated the alkylation strategy. However, the conversions were extremely low, and only trace amounts of the corresponding alkylation product were obtained.

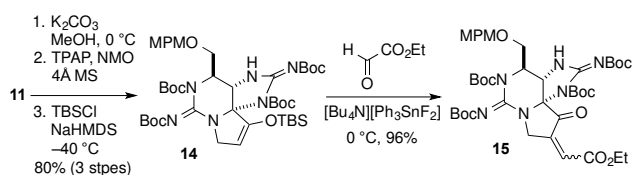
Then, the Mukaiyama aldol reaction was investigated for construction of the C-C bond at the C11 position. The silyl enol ether **14** was synthesized from the fully protected saxitoxinol **11** in three steps, i.e., (i) hydrolysis of acetate with

[a] C. Wang, M. Oki, T. Nishikawa, Prof. Dr. K. Nagasawa
Department of Biotechnology and Life Science
Faculty of Technology, Tokyo University of Agriculture and
Technology
2-24-16 Nakamachi, Koganei, Tokyo 184-8588 (Japan)
E-mail: knaga@cc.tuat.ac.jp

[b] Prof. Dr. M. Yotsu-Yamashita
Graduate School of Agricultural Science
Tohoku University
1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, (Japan)

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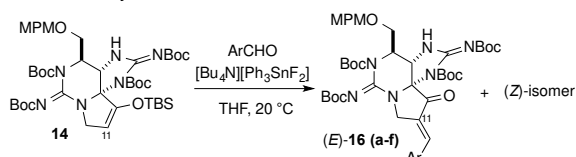
potassiumcarbonate, (ii) oxidation of the resulting alcohol by means of Ley oxidation,^[10] and (iii) silyl enol ether formation from the resulting ketone with TBSCl in the presence of NaHMDS (Scheme 3). With the silyl enol ether **14** in hand, the Mukaiyama aldol reaction was investigated with ethyl glyoxylate under various conditions. At first, Lewis acid-promoted conditions with TiCl₄ or BF₃·Et₂O were tested,^[11] but substrate **14** decomposed to generate mostly Boc-protected compounds, and no aldol reaction products were obtained. Next, fluoride anion-promoted reaction conditions were investigated. In the case of Bu₄NF,^[12] no reaction occurred. On the other hand, in the case of [Bu₄N][Ph₃SnF₂],^[13] which is an anhydrous fluoride anion reagent, the condensation product **15** was obtained in 96% yield as a mixture of stereoisomers of the double bond (5:1).



Scheme 3 Mukaiyama aldol reaction of **14** with ethyl glyoxylate.

Since the Mukaiyama aldol condensation reaction appears to be a powerful tool for constructing the C-C bond at C11 in STXs, the scope of the reaction was investigated. Thus, various aromatic aldehydes were subjected to reaction with **14**, and the corresponding aldol condensation adducts (**16a-f**) were obtained in 45–80% yields (Table 1).

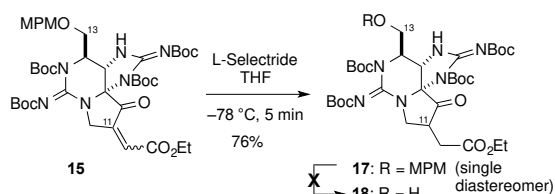
Table 1. Substrate scope of the Mukaiyama aldol condensation reaction of **14** with aromatic aldehydes.



entry	Ar	time(h)	product	<i>E/Z</i> ^b	yield(%)
1	4-Me-C ₆ H ₄	48	16a	>10:1	45
2	C ₆ H ₅	24	16b	>10:1	60
3	3-F-C ₆ H ₄	24	16c	>10:1	63
4	4-Cl-C ₆ H ₄	24	16d	>10:1	65
5 ^a	4-NO ₂ -C ₆ H ₄	2	16e	6:1	80
6 ^a	2-furyl	2	16f	>10:1	80

^aReaction was carried out at 0 °C. ^bRatios at C11 were determined by ¹H NMR.^[14]

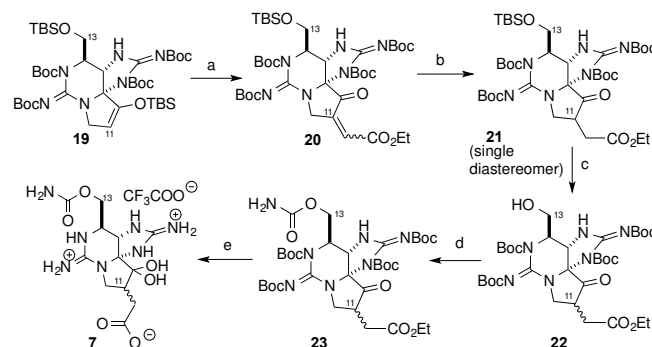
After construction of the crucial C-C bond at the C11 position of SEA (**7**), the double bond in **15** had to be reduced, followed by deprotection of the MPM group at the C13 position. For the reduction, after extensive investigation,^[15] L-selectride was found to be quite effective, giving **17** in 76% yield as a single diastereomer (Scheme 4).^[17] Unfortunately, subsequent depro-



Scheme 4 Investigation of the reduction at C11 and deprotection of the MPM group at C13.

tection of MPM group at the C13 failed under all the conditions we investigated, e.g., NBS-Et₃N,^[49] DDQ or CAN.^[18] Finally, we decided to change the protecting group from MPM to TBS ether at an earlier stage.

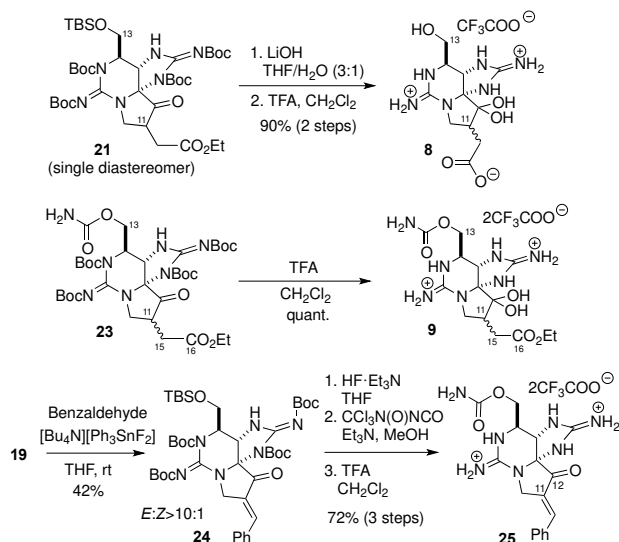
Thus, silyl enol ether **19** with TBS ether at the C13 position was synthesized by following a similar procedure to that used for **14**,^[19] and the Mukaiyama aldol condensation reaction with ethyl glyoxylate was examined in the presence of [Bu₄N][Ph₃SnF₂]. Under these conditions, aldol condensation adduct **20** was obtained in 85% yield. Interestingly, the TBS ether at C13 remained intact under these conditions (Scheme 5). After reduction of the double bond with L-selectride, deprotection of the silyl ether in **21** at C13 took place smoothly on treatment with HF·Et₃N complex to give **22** in 87% yield.^[20] The resulting hydroxyl group was further converted to a carbamoyl group by reaction with trichloroisocyanate followed by hydrolysis with triethylamine in methanol to give **23** in 68% yield. Finally, 11-saxitoxinethanoic acid (**7**) was obtained by further hydrolysis of the ethyl ester with lithium hydroxide and deprotection of all four Boc groups with TFA, in 90% yield.^[21] The stereochemistry at C11 of synthetic **7** was found to be a mixture of ca. 9:1 ratio, which is identical with that of the natural product reported by Onoue.^[6]



Scheme 5 Synthesis of SEA (**7**). Reagents and conditions: a) ethyl glyoxylate, [Bu₄N][Ph₃SnF₂], THF, 0 °C, 85%; b) L-Selectride, THF, -78 °C, 76%; c) HF·Et₃N, THF/Et₃N (5:1), rt, 87%; d) CCl₃C(O)NCO, CH₂Cl₂, 0 °C, then Et₃N, MeOH, rt, 68%; e) LiOH, THF/H₂O (3:1), 0 °C; then, TFA, CH₂Cl₂, rt, 90%. L-Selectride = lithium tri-sec-butyl(hydrido)borate(1-), TFA = trifluoroacetic acid.

The inhibitory activities towards Na_vCh of SEA (**7**) and its synthetic derivatives decarbamoyl-11-saxitoxinethanoic acid (**8**, dcSEA), 11-saxitoxinethanoic ethyl ester (**9**, SEE), which were obtained from **21** and **23**, respectively (Scheme 6), were evaluated in a cell-based assay. In addition, 11-benzyliden-saxitoxin (**25**, 11-benzylidenSTX), synthesized from **19** via **24** (Scheme 6), was also tested.^[22] Specifically, the Na_vCh-inhibitory activity of ligands was evaluated in terms of cytotoxicity to mouse neuroblastoma Neuro-2a cells, which express Na_vCh.^[23] In this cell-based assay, Neuro-2a is treated with a sodium channel activator, veratridine, in the presence of ouabain, an inhibitor of Na⁺/K⁺ ATPase. This blocks sodium ion efflux, and decreases the cell viability. Na_vCh inhibition by tetrodotoxin (TTX), STX and related compounds antagonizes this effect, and rescues the cells in a dose-dependent manner.^[23e,8] The inhibitory activities of **7**, **8**, **9** and **25** were calculated from the cell viability in the above assay, and the results are summarized in Table 2. SEA (**7**), dcSEA (**8**), SEE (**9**), and 11-benzylidenSTX (**25**) showed a concentration-dependent Na_vCh-inhibitory effect, and their IC₅₀ values were determined to be 47±12 nM, 5.7±3.1 μM, 185±74 nM, and 16±6.9 nM,^[24] respectively (the IC₅₀ values of synthetic dcSTX (**2**)^[49] and TTX, used as controls, were 89±36 nM and 5.0±1.6 nM, respectively). Thus, SEA (**7**) was twice as potent as dcSTX (**2**) on its Na_vCh-

inhibitory activity. In the case of dcSEA (**8**), the inhibitory activity was markedly decreased, and it was approximately hundred times less potent than dcSTX (**2**). SEE (**9**) showed about half weaker inhibitory activity than dcSTX (**2**). Interestingly, 11-benzylidenSTX (**25**) showed the same level inhibitory activity with dcSTX (**2**), although hydrated form of ketone at C12 in STXs is suggested to be important for their Na_vCh -inhibitory activity.^[25] Further structure-activity relationship studies are under way to examine the inhibitory activity of other STX derivatives having a C-C bond at the C11 position.



Scheme 6 Synthesis of dcSEA (**8**), SEE (**9**) and (**25**).

Table 2 Na_vCh -inhibitory activity of SEA (**7**) and its derivatives dcSEA (**8**), SEE (**9**) and 11-benzylidenSTX (**25**) in a cell-based assay with Neuro-2a cells.

Compound	IC ₅₀ (mean ± SD)	n
SEA (7)	47 ± 12 nM	3
dcSEA (8)	5.7 ± 3.1 μM	3
SEE (9)	185 ± 74 nM	4
11-benzylidenSTX (25)	16 ± 6.9 nM ^[24]	5
dcSTX (2) ^[49]	89 ± 36 nM	3
TTX	5.0 ± 1.6 nM	6

In conclusion, a total synthesis of SEA (**7**) has been achieved from the silyl enol ether **19** in 35% overall yield. The synthesis features a Mukaiyama aldol condensation reaction to construct the C-C bond at the C11 position of STX. The derivatives (**8**, **9**, and **25**) were similarly synthesized. In a cell-based assay, SEA (**7**) showed approximately twice more potent Na_vCh -inhibitory activity than dcSTX (**2**), but dcSEA (**8**) showed about hundred times less potent activity, in marked contrast to the relationship between STX (**1**) and dcSTX (**2**).^[26] Interestingly, the inhibitory activity of 11-benzylidenSTX (**25**) was in a similar level with dcSTX (**2**), and interacting mode of **25** with Na_vCh is quite intriguing.

Acknowledgements

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Keywords: 11-saxitoxinethanoic acid, Mukaiyama-aldol condensation, total synthesis, sodium channel inhibitor

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- Stereochemistry of olefin in **16a** was determined by NOESY (see supporting information), and according to **16a**, the stereochemistries for **16b-f** were determined.
- Metal catalysts (Pd/C, Pd(OH)₂/C, PtO₂, RhCl(PPh₃)₃, [Ir(cod)py(PCy₃)]PF₆ and Raney Ni) were tried first under hydrogenation conditions. In the case of Pd/C and Pd(OH)₂/C, the desired product was not obtained and Boc-protected products were generated. In the case of the next three catalysts, the reaction did not proceed at all, and starting material **15** was recovered quantitatively. Raney Ni in MeOH under hydrogenation conditions gave desired **17** in 70% yield, but reproducibility was poor in large-scale reaction. In the case of

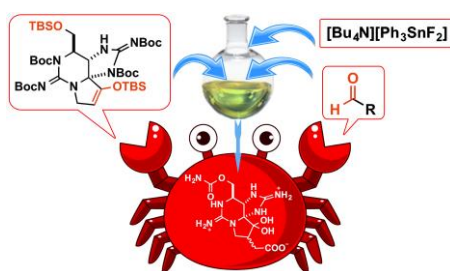
- single electron reduction using activated Mg in dry methanol,^[16] the desired product **17** was obtained in 30% yield.
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- [17] The ¹H NMR spectrum indicated the presence of only one diastereoisomer, but the stereochemistry at C11 in **17** was difficult to determine.
- [18] Standard conditions (DDQ and CAN) did not give **18** and caused deprotection of the Boc groups in **17**.
- [19] For the synthesis of silyl enol ether **19**, see supporting information.
- [20] In the case of Bu₄NF, the substrate **21** decomposed immediately. When HF·Py was tried, the reaction proceeded extremely slowly.
- [21] Intermediates **20**, **21**, **22** and **23** were quite unstable under regular silica gel column purification conditions. After extensive investigation, we found that rapid purification on neutral silica gel gave acceptable results. Since C11 in **22** and **23** epimerized easily, the products were obtained as diastereomeric mixtures. Data for the major diastereomers are presented in supporting information. (For the data collection process, **22** and **23** were purified by preparative thin layer chromatography. During this process, the minor diastereomer decomposed, and we collected only the major diastereomer.)
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Daisuke Harada, Mari Yotsu-Yamashita
and Kazuo Nagasawa*

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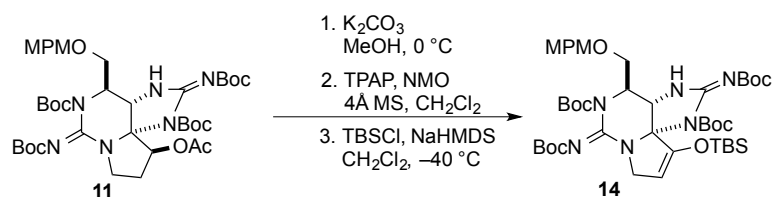
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Precise assignment of 9	S73

1. General

Flash chromatography was performed on Silica gel 60 (spherical, particle size 40~100 μm ; Kanto), Chromatorex NH (particle size 75~150 μm ; Fuji Silysia). Optical rotations were measured on a JASCO P-2200 polarimeter, using the sodium D line. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-ECX 300 or 400. The spectra are referenced internally according to residual solvent signals of CDCl_3 (^1H NMR; $\delta = 7.26$ ppm, ^{13}C NMR; $\delta = 77.16$ ppm), D_2O (^1H NMR; $\delta = 4.80$ ppm). For compounds **7**, **8**, **9** and **25**, 1,4-dioxane was used as internal standard, which is referenced at 3.75 ppm (^1H NMR) and 67.4 ppm (^{13}C NMR), respectively. Data for ^1H NMR are recorded as follows: chemical shift (δ , ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), integration, coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ , ppm). Mass spectra were recorded on a JEOL JMS-T100X spectrometer with ESI-MS mode using methanol or methanol/ H_2O as solvent.

2. Experimental Procedures for S2-S8, 7-9, 14-17, 19-25

Synthesis of silyl enol ether **14**:



To a solution of protected STXol **11** (100 mg, 0.125 mmol) in methanol (2 mL) was added K_2CO_3 (35 mg, 0.25 mmol) at 0 °C. After stirring for 15 min, the reaction was diluted with EtOAc (5 mL) and H_2O (5 mL), and extracted with EtOAc (5 mL) three times. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give alcohol, which was used without further purification.

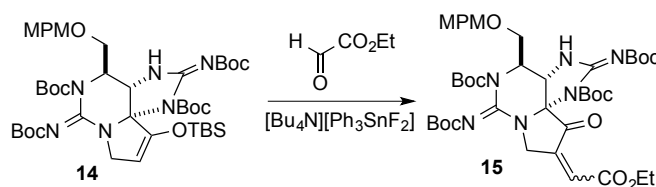
To a solution of the alcohol in CH_2Cl_2 (2 mL) was added NMO (59 mg, 0.5 mmol) and 4Å MS (63 mg) at 0 °C. After stirring for 10 min, TPAP (5 mg, 0.0125 mmol) was added, and the reaction mixture was stirred for another 1 h at room temperature. Then, reaction mixture was diluted with hexane/EtOAc (1:1) (2 mL) and filtered through a pad of neutral silica gel, and then, washed with hexane/EtOAc (1:1) (10 mL). The filtrates were concentrated *in vacuo* to give ketone.

To a solution of the ketone in CH_2Cl_2 (1 mL) was added NaHMDS (0.33 mL, 0.63 mmol) under Ar atmosphere at -40 °C. After stirring for 10 min, TBSCl (75 mg, 0.5 mmol) was added and the reaction mixture was stirred overnight at -40 °C. Then, the reaction was quencher with H_2O (2 mL) and warmed to room temperature and extracted with CH_2Cl_2 (3 mL) three times. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 5:1 to 3:1) to give silyl enol ether **14** (87 mg, 80% three steps).

Spectral data for **14**: $[\alpha]_D^{25} = +19.9$ (*c* 1.4 in $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 4.82 (t, *J* = 4.1 Hz, 1H), 4.58-4.54 (m, 2H), 4.33 (s, 2H), 4.09 (d, *J* = 14.8 Hz, 1H), 3.80 (s, 3H), 3.73-3.69 (m, 1H), 3.64-3.58 (m, 1H), 3.51 (d, *J* = 15.1 Hz, 1H), 1.51 (s, 9H), 1.45 (s, 18H), 1.44 (s,

9H), 0.87 (s, 9H), 0.18 (s, 9H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.7, 159.1, 151.0, 150.7, 149.1, 146.5, 146.2, 129.9, 129.7, 113.4, 98.5, 84.9, 83.9, 82.8, 81.2, 79.2, 77.4, 73.2, 70.9, 58.8, 55.2, 52.4, 28.2, 28.1, 28.0, 27.8, 25.3, 17.8, -4.7, -5.2; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{43}\text{H}_{68}\text{N}_6\text{O}_{11}\text{SiNa}$ 895.4613 found 895.4579.

Synthesis of Mukaiyama aldol condensation adduct **15**:

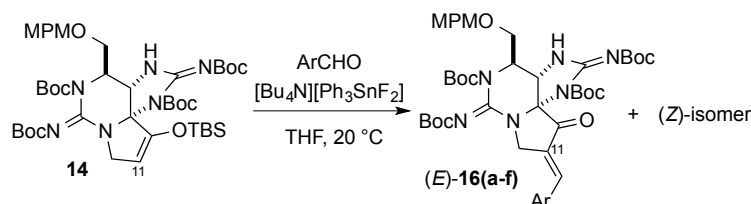


To a solution of silyl enol ether **14** (87 mg, 0.10 mmol) in THF (4 mL) was added ethyl glyoxylate (80 μL) at 0 $^\circ\text{C}$. After stirring for 10 min, $[\text{Bu}_4\text{N}][\text{Ph}_3\text{SnF}_2]$ (67 mg, 0.11 mmol) was added and the reaction was stirred for another 2 hrs at the same temperature. Then, the reaction was quenched with sat. NH_4Cl aq (3 mL), and the solution was extracted with EtOAc (5 mL) three times. The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 3:1) to give **15** (81 mg, 96%).

Spectral data for **15**: $[\alpha]_D^{25} = +24.6$ (*c* 2.1 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 9.53 (s, 1H), 7.02 (d, $J = 8.6$ Hz, 2H), 6.76 (d, $J = 8.6$ Hz, 1H), 6.40 (t, $J = 2.4$ Hz, 0.75H), 6.34-6.28 (m, 0.15H), 4.92 (d, $J = 2.4$ Hz, 1H), 4.72-4.44 (m, 3H), 4.27-4.10 (m, 4H), 3.82 (d, $J = 10.3$ Hz, 1H), 3.78 (s, 3H), 3.67-3.57 (m, 1H), 1.53 (s, 9H), 1.45 (s, 18H), 1.34 (s, 9H), 1.25 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 197.7, 164.5, 159.5, 151.2, 151.1, 149.1, 145.9, 143.9, 130.0, 128.8, 121.0, 113.9, 88.3, 83.7, 81.9, 79.9, 78.2, 77.4, 73.54, 73.45, 71.7, 61.5, 57.8, 55.4, 55.2, 29.8, 28.3, 28.0, 27.9, 27.8, 14.3; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{41}\text{H}_{58}\text{N}_6\text{O}_{13}\text{Na}$ 865.3960 found 865.3959.

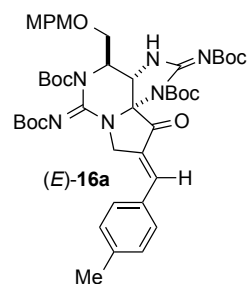
Synthesis of Mukaiyama aldol condensation adduct **16a-f**

General procedure for the Mukaiyama aldol condensation:

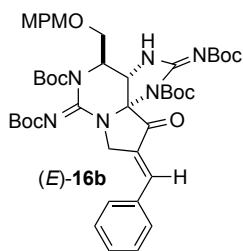


To a solution of silyl enol ether **14** (1 equiv) in THF was added aldehyde (5 equiv) at 0 °C. After stirring for several minutes, $[\text{Bu}_4\text{N}][\text{Ph}_3\text{SnF}_2]$ (1.05 equiv) was added and the reaction was stirred at the same temperature. Upon completion of the reaction (2-48 h), the reaction was quenched with sat. NH_4Cl aq., and the solution was extracted with EtOAc three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified with preparative thin layer chromatography (hexane/EtOAc; 1:1).

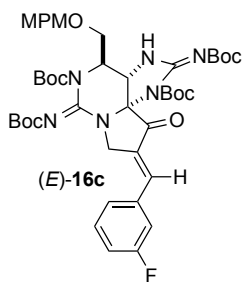
Spectral data for (*E*)-**16a-f**:



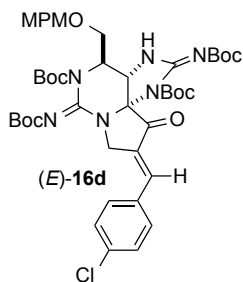
(*E*)-**16a**: $[\alpha]_D^{25} = +13.2$ (*c* 0.6 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 9.58(s, 1H), 7.39 (s, 1H), 7.22-7.16 (m, 4H), 6.91 (d, $J = 8.2$ Hz, 2H), 6.45 (d, $J = 8.6$ Hz, 2H), 4.90 (t, $J = 2.8$ Hz, 1H), 4.69 (s, 1H), 4.64 (d, $J = 17.4$ Hz, 1H), 4.31 (d, $J = 17.4$ Hz, 1H), 4.11 (s, 2H), 3.84-3.78 (m, 1H), 3.62 (dd, $J = 3.6, 10.1$ Hz, 1H), 3.60 (s, 3H), 2.41 (s, 1H), 1.56 (s, 9H), 1.47 (s, 18H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 197.2, 159.7, 159.1, 152.0, 151.5, 151.1, 149.2, 146.2, 141.8, 134.7, 131.5, 131.0, 130.0, 129.8, 129.0, 128.5, 113.6, 87.2, 83.6, 81.7, 79.9, 78.6, 77.8, 77.4, 73.7, 73.6, 71.8, 58.6, 54.9, 52.1, 29.8, 28.4, 28.1, 28.0, 27.9, 21.8; HRMS (ESI, $\text{M}+\text{Na}$)⁺ calcd for $\text{C}_{45}\text{H}_{60}\text{N}_6\text{O}_{11}\text{Na}$ 883.4217 found 883.4175.



(E)-16b: $[\alpha]_D^{25} = +16.8$ (*c* 1.7 in CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 9.58 (s, 1H), 7.44-7.38 (m, 4H), 7.31-7.27 (m, 2H), 6.91 (d, $J = 8.6$ Hz, 2H), 6.46 (d, $J = 8.6$ Hz, 2H), 4.91 (t, $J = 3.0$, 1H), 4.71-4.62 (m, 2H, overlap), 4.35 (d, $J = 15.5$ Hz, 1H), 4.11 (s, 2H), 3.86-3.79 (m, 1H), 3.66-3.57 (m, 4H, overlap), 1.58 (s, 9H), 1.46 (s, 18 H), 1.27 (s, 9H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 197.2, 159.6, 159.0, 151.9, 151.4, 151.1, 149.2, 146.2, 134.5, 133.6, 131.3, 130.9, 129.7, 129.5, 129.2, 128.9, 113.6, 87.3, 83.6, 81.7, 79.9, 78.5, 77.4, 73.7, 73.5, 71.8, 58.5, 55.0, 52.1, 28.3, 28.1, 28.0, 27.8; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{44}\text{H}_{58}\text{N}_6\text{O}_{11}\text{Na}$ 869.4061 found 869.4048.

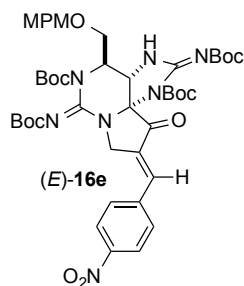


(E)-16c: $[\alpha]_D^{25} = +17.9$ (*c* 1.0 in CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 9.57 (s, 1H), 7.37 (dd, $J = 7.9, 14.1$ Hz, 1H), 7.30 (s, 1H), 7.16-7.07 (m, 1H), 7.04 (d, $J = 7.6$ Hz, 1H), 6.91 (d, $J = 8.3$ Hz, 2H), 6.45 (d, $J = 8.3$ Hz, 2H), 4.91 (s, 1H), 4.69 (s, 1H), 4.61 (d, $J = 15.5$ Hz, 1H), 4.28 (d, $J = 15.5$ Hz, 1H), 4.14 (d, $J = 11.2$ Hz, 1H), 4.09 (d, $J = 11.0$ Hz, 1H), 3.86 (d, $J = 8.3$ Hz, 1H), 3.69-3.58 (m, 4H), 1.57 (s, 9H), 1.47 (s, 18H), 1.26 (s, 9H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 197.3, 164.6, 159.6, 159.1, 151.8, 151.4, 151.1, 149.2, 146.2, 135.6, 135.5, 132.9, 130.7, 129.8, 128.9, 127.0, 118.0, 117.8, 117.6, 117.4, 113.5, 87.4, 83.7, 81.8, 80.0, 78.4, 78.0, 77.4, 73.8, 73.7, 72.2, 58.5, 55.0, 51.9, 28.4, 28.1, 28.0, 27.9; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{44}\text{H}_{57}\text{N}_6\text{O}_{11}\text{FNa}$ 887.3967 found 887.3966.

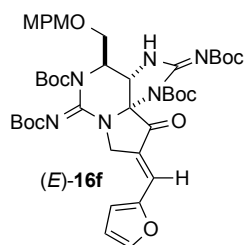


(E)-16d: $[\alpha]_D^{25} = +21.0$ (*c* 1.1 in CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 9.57 (s, 1H), 7.36 (d, $J = 8.6$ Hz, 2H), 7.29 (s, 1H), 7.15 (d, $J = 8.3$ Hz, 2H), 6.90 (d, $J = 8.6$ Hz, 2H), 6.40 (d, $J = 8.3$ Hz, 2H), 4.91 (t, $J = 2.4$ Hz, 1H), 4.69 (s, 1H), 4.58 (dd, $J = 2.1, 15.8$ Hz, 1H), 4.22 (dd, $J = 2.1, 15.8$ Hz, 1H), 4.11 (dd, $J = 11.0, 14.4$ Hz, 2H), 3.88 (dd, $J = 2.1, 10.3$ Hz, 1H), 3.66 (dd, $J = 3.5, 10.0$ Hz, 1H), 3.59 (s, 3H), 1.57 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.24 (s, 9H); $^{13}\text{C NMR}$ (75

MHz, CDCl₃) δ 197.2, 159.6, 159.1, 151.9, 151.4, 151.1, 149.2, 146.2, 137.2, 132.9, 132.4, 132.0, 129.8, 129.5, 128.9, 113.5, 87.3, 83.7, 81.8, 80.0, 78.4, 78.0, 77.4, 73.8, 72.4, 58.6, 54.9, 51.9, 28.3, 28.1, 28.0, 27.8; HRMS (ESI, M+Na)⁺ calcd for C₄₄H₅₇N₆O₁₁ClNa 903.3672 found 903.3691.

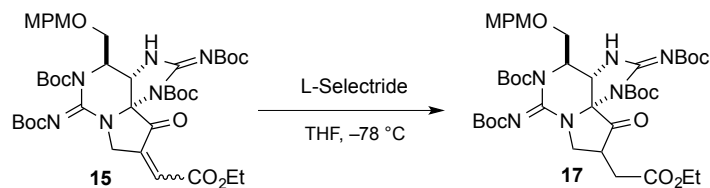


(E)-16e: $[\alpha]_D^{25} = +32.7$ (c 0.8 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 9.54 (s, 1H), 8.20 (d, *J* = 8.6 Hz, 2H), 7.35-7.28 (m, 3H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.31 (d, *J* = 8.6 Hz, 2H), 4.92 (s, 1H), 4.69 (s, 1H), 4.66-4.57 (m, 1H), 4.30-4.20 (m, 1H), 4.14 (d, *J* = 11.0 Hz, 1H), 4.07 (d, *J* = 10.7 Hz, 1H), 3.91 (d, *J* = 9.3 Hz, 1H), 3.75-3.65 (m, 2H), 3.52 (s, 3H), 1.57 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.24 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 197.2, 159.5, 159.0, 151.8, 151.2, 151.0, 149.2, 148.4, 146.1, 139.2, 133.1, 131.6, 130.9, 129.8, 128.9, 124.2, 113.4, 87.5, 83.9, 81.9, 80.2, 78.2, 77.4, 73.9, 72.8, 58.6, 54.8, 51.9, 28.3, 28.0, 27.9, 27.8; HRMS (ESI, M+Na)⁺ calcd for C₄₄H₅₇N₇O₁₃Na 914.3912 found 914.3900.



(E)-16f: $[\alpha]_D^{25} = +27.5$ (c 1.3 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 9.58 (s, 1H), 7.57 (s, 1H), 7.11 (s, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.68 (d, *J* = 3.5 Hz, 1H), 6.54 (d, *J* = 8.6 Hz, 2H), 6.52-6.49 (m, 1H), 4.90 (t, *J* = 2.8 Hz, 1H), 4.68 (s, 1H), 4.61 (d, *J* = 16.8 Hz, 1H), 4.33 (d, *J* = 16.8 Hz, 1H), 3.83 (dd, *J* = 2.4, 10.0 Hz, 1H), 3.70-3.60 (m, 4H, overlap), 1.55 (s, 9H), 1.46 (s, 9H), 1.45 (s, 9H), 1.26 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 196.5, 159.7, 159.1, 151.8, 151.5, 151.1, 150.8, 149.2, 147.1, 146.2, 129.8, 129.1, 127.0, 119.7, 119.0, 113.4, 113.0, 87.2, 83.5, 81.7, 79.8, 79.0, 78.0, 77.4, 73.6, 71.9, 58.5, 55.0, 52.1, 28.3, 28.1, 27.9, 27.8; HRMS (ESI, M+Na)⁺ calcd for C₄₂H₅₆N₆O₁₂Na 859.3854 found 859.3899.

Synthesis of 17:



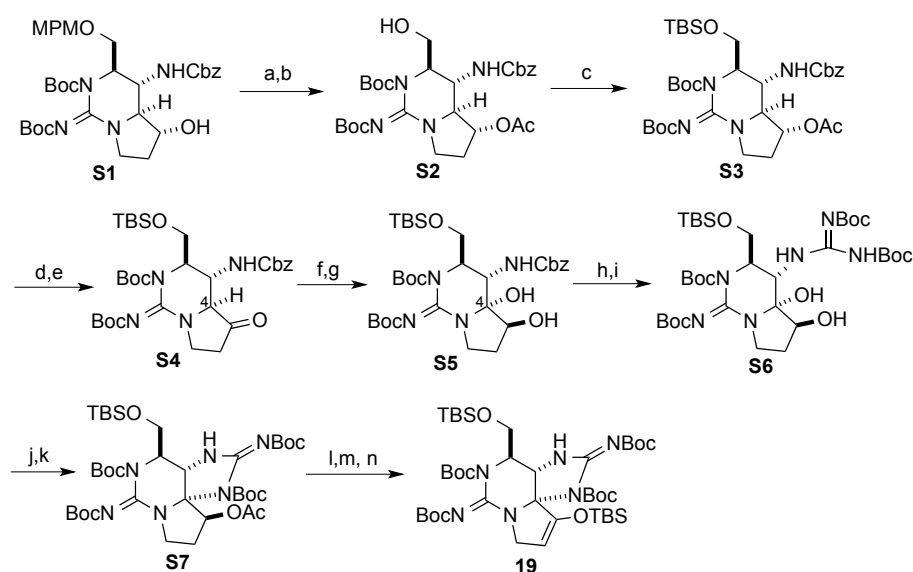
To a solution of **15** (20 mg, 0.024 mmol) in THF (1 mL) was added L-selectride (71 μL , 0.071 mmol) at $-78\text{ }^\circ\text{C}$. After stirring for 5 min, the reaction was quenched with sat. NH_4Cl aq (1 mL), and the solution was extracted with EtOAc (2 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified immediately by neutral silica gel column chromatography (hexane/EtOAc; 2:1) to give **17** (15.4 mg, 76%).

Spectral data for **17**: $[\alpha]_D^{25} = +13.3$ (*c* 2.6 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.29 (s, 1H), 7.19 (d, $J = 8.7$ Hz, 2H), 6.83 (d, $J = 8.7$ Hz, 2H), 4.86 (t, $J = 4.1$ Hz, 1H), 4.54 (s, 1H), 4.39 (dd, $J = 11, 17.4$ Hz, 2H), 4.17-4.09 (m, 2H), 3.98 (t, $J = 10.1$ Hz, 1H), 3.78 (s, 3H), 3.75-3.63 (m, 2H), 3.49 (t, $J = 9.2$ Hz, 1H), 2.78 (dd, $J = 4.6, 17.0$ Hz, 1H), 2.72-2.62 (m, 1H), 2.38 (dd, $J = 8.7, 13.3$ Hz, 1H), 1.52 (s, 9H), 1.50 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 208.7, 171.4, 159.6, 151.2, 148.6, 144.0, 130.2, 129.1, 114.0, 87.7, 83.4, 82.0, 79.5, 78.6, 77.4, 73.4, 71.8, 70.9, 60.9, 58.2, 55.3, 51.6, 42.5, 33.3, 28.3, 28.0, 14.3; HRMS (ESI, $\text{M}+\text{Na}$)⁺ calcd for $\text{C}_{41}\text{H}_{60}\text{N}_6\text{O}_{13}\text{Na}$ 867.4116 found 867.4090.

Synthesis of silyl enol ether 19:^[1]

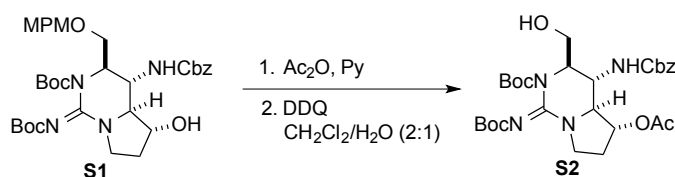
The route was commenced with the known compound **S1** (Scheme S1), acylation of the hydroxyl group with acetic anhydride followed by removal of the MPM group with DDQ afforded **S2** and further protection with TBS gave **S3**. Hydrolysis of the acetate with potassium carbonate followed by Swern oxidation gave ketone **S4**. With the ketone **S4** in hand, oxidation at the C-4 position was carried out with IBX followed by reduction with NaBH_4 generated diol **S5**. Remove the Cbz group with

$\text{Pd}(\text{OH})_2$ in MeOH then the second guanidine group was introduced by treatment with bis(Boc)-2-methyl-2-thiopeudourea in presence of Mercury(II) chloride to give **S6**. Esterification of the diol with acetic anhydride and cyclization in presence of ZnCl_2 yielded the fully protected saxitoxinol **S7** with TBS protecting group at the C13 position. With intermediate **S7** in hand, following the same reaction conditions abovementioned (Scheme 3), silyl enol ether **19** with TBS protecting group at the C13 position was obtained.



Scheme S2. Synthesis of silyl enol ether **19** with TBS protecting group at the C13 position. Reagents and conditions: a) Ac_2O (10 equiv), pyridine (20 equiv), rt, 10 h; b) DDQ (10 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (2:1), rt, overnight, 79% (2 steps); c) TBSOTf (2 equiv), 2,6-lutidine (4 equiv), CH_2Cl_2 , rt, 20 min, 87%; d) K_2CO_3 (2 equiv), MeOH, 0 °C, 20 min; e) $(\text{COCl})_2$ (3.5 equiv), DMSO (4.2 equiv), CH_2Cl_2 , -78 °C, 1 h, then Et_3N (10 equiv), -78 °C, 10 min; f) IBX (1.1 equiv), DMSO, 50 °C, 1 h; g) NaBH_4 (0.5 equiv), MeOH, 0 °C, 20 min, 68% (4 steps); h) $\text{Pd}(\text{OH})_2$ (20% wt), MeOH, rt, 2.5 h; i) $\text{NBoc}=\text{C}(\text{SMe})\text{NHBoc}$ (1 equiv), HgCl_2 (1 equiv), Et_3N (3 equiv), DMF, rt, 1 h, 94% (2 steps); j) Ac_2O (10 equiv), DMAP (0.1 equiv), pyridine (20 equiv), rt, 3 h; k) ZnCl_2 (1.5 equiv), CH_2Cl_2 , -20 °C, 5 h, 80% (2 steps); l) K_2CO_3 (2 equiv), MeOH, 0 °C, 20 min; m) NMO (4.0 equiv), 4 Å MS (500 mg/mmol), CH_2Cl_2 , 0 °C, 10 min; then, TPAP (0.1 equiv), rt, 1 h; n) NaHMDS (5 equiv), TBSCl (4 equiv), CH_2Cl_2 , -40 °C, overnight, 85% (3 steps). DDQ = 2,3-dichloro-5,6-dicyano-p-benzoquinone, TBSOTf = tert-butyldimethylsilyl trifluoromethanesulfonate, DMSO = dimethyl sulfoxide, IBX = 2-iodoxybenzoic acid, DMAP = 4-(dimethylamino)pyridine, NMO = *N*-methylmorpholine-*N*-oxide, TPAP = tetrapropylammonium perruthenate.

Synthesis of S2

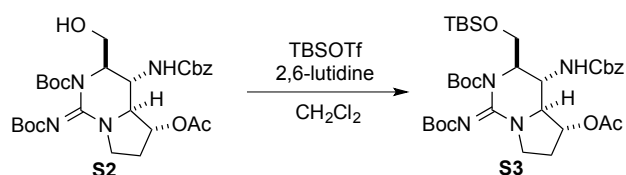


To a solution of alcohol **S1** (7.6 g, 11.38 mmol) in pyridine (10 mL) was added acetic anhydride (5 mL) at room temperature. After stirring for 3 hrs, the reaction mixture was concentrated *in vacuo* to give ester.

To a solution of ester in a mixture solvent ($\text{CH}_2\text{Cl}_2 = 130 \text{ mL}$, $\text{H}_2\text{O} = 65 \text{ mL}$) was added DDQ (25.85 g, 113.8 mmol) at 0 °C. After stirring overnight at room temperature, the reaction was quenched with sat. NaHCO_3 aq (75 mL), and the solution was extracted with CH_2Cl_2 (100 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc; 2:1 to 1:1) to give **S2** (5.2 g, 79% two steps).

Spectral data for **S2**: $[\alpha]_D^{25} = +117.9$ (c 1.0 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.37-7.32 (m, 5H), 5.37 (br, 1H), 5.29 (d, $J = 8.7 \text{ Hz}$, 1H), 5.22 (d, $J = 3.2 \text{ Hz}$, 1H), 5.17 (d, $J = 9.2 \text{ Hz}$, 1H), 5.10 (d, $J = 9.0 \text{ Hz}$, 1H), 3.99-3.80 (m, 4H), 3.66-3.55 (m, 3H), 2.33-2.21 (m, 1H), 2.10-2.04 (m, 4H), 1.49 (s, 9H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.9, 162.4, 156.1, 150.6, 136.1, 128.6, 128.3, 128.1, 83.3, 80.0, 77.4, 75.6, 67.3, 64.5, 61.7, 60.8, 52.7, 46.4, 28.8, 28.3, 28.2, 21.1; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_9\text{Na}$ 599.2693 found 599.2644.

Synthesis of S3

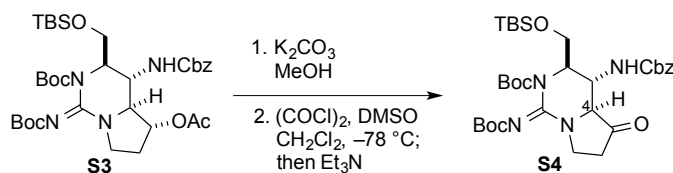


To a solution of alcohol **S2** (5.2 g, 9.02 mmol) in CH_2Cl_2 (55 mL) was added 2,6-lutidine (4.20 mL, 36.08 mmol) at 0 °C, then TBSOTf (4.14 mL, 18.04 mmol) was added and the reaction mixture was stirred at rt for 1 h. After that, the reaction

was quenched with sat. NaHCO₃ aq (30 mL), and the solution was extracted with CH₂Cl₂ (50 mL) three times. The combined organic layers was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc; 5:1 to 2:1) to give **S3** (5.42 g, 87%).

Spectral data for **S3**: [α]_D²⁵ = +58.0 (*c* 2.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.36-7.30 (m, 5H), 5.30 (d, *J* = 5.8 Hz, 1H), 5.21 (d, *J* = 3.9 Hz, 1H), 5.14 (d, *J* = 9.3 Hz, 1H), 5.10 (d, *J* = 9.3 Hz, 1H), 4.12 (s, 1H), 4.09-3.99 (br, 1H), 3.91-3.82 (m, 2H), 3.75-3.70 (m, 1H), 3.66-3.59 (m, 1H), 3.43 (d, *J* = 11.5 Hz, 1H), 2.24-2.11 (m, 1H), 2.10-2.01 (m, 4H), 1.47 (s, 18H), 0.86 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 159.0, 156.0, 151.6, 151.1, 136.2, 128.5, 128.2, 128.0, 82.6, 78.1, 77.4, 75.9, 67.0, 64.7, 64.3, 61.0, 53.7, 45.8, 29.1, 28.6, 28.2, 25.9, 21.1, 18.4, -5.3, -5.8; HRMS (ESI, M+Na)⁺ calcd for C₃₄H₅₅N₄O₉SiNa 691.3738 found 691.3780.

Synthesis of S4

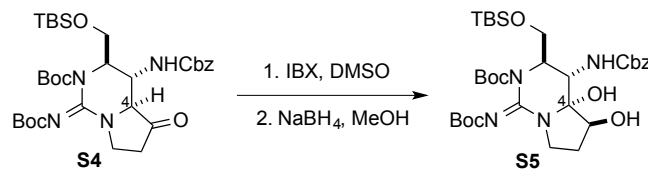


To a solution of **S3** (5.42g, 7.84 mmol) in methanol (50 mL) was added K₂CO₃ (2.17 g, 15.69 mmol) at 0 °C under N₂ atmosphere. After stirring for 15 min, the reaction was quenched with water (40 mL) and extracted with EtOAc (50 mL) three times. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to give crude alcohol.

To a solution of DMSO (2.36 mL, 32.95 mmol) in CH₂Cl₂ (150 mL) was slowly added oxalylchloride (2.40 mL, 27.46 mmol) at -78 °C under N₂ atmosphere. The reaction mixture was stirred for 30 min, and then crude alcohol was added as a solution in CH₂Cl₂ (30 mL) under N₂ atmosphere. After stirring for 1 h, Et₃N (10.93 mL, 78.45 mmol) was added to the reaction mixture. After stirring for another 5 min, the reaction was rapidly quenched with water (20 mL), warmed to room temperature and extracted with CH₂Cl₂ (50 mL) three times. The combined organic layer was

dried over MgSO_4 , filtered and concentrated *in vacuo* to give ketone **S4**.

Synthesis of **S5**

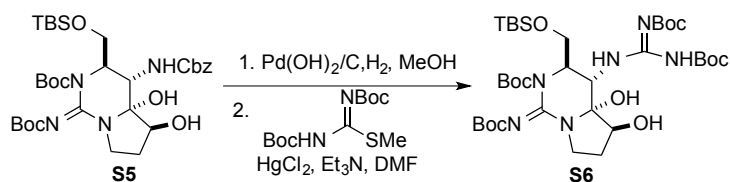


To a solution of ketone **S4** in DMSO (65 mL) and H_2O (0.24 mL) was added IBX (2.43 g, 8.63 mmol) at room temperature. Then the reaction mixture was warmed to 50 °C and stirred for 1 h. After that, the reaction was quenched with 10% $\text{Na}_2\text{S}_2\text{O}_3$ aq (30 mL) and saturated NaHCO_3 aq (30 mL). Then, the solution was diluted with EtOAc (50 mL). The organic layer was washed with water (100 mL) three times. The organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo* to give a diol.

To a solution of diol in methanol (45 mL) was added NaBH_4 (0.15g, 3.92 mmol) at 0 °C. After stirring for 15 min, the reaction was quenched with water (60 mL), and the solution was extracted with EtOAc (100 mL) three times. The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc; 4:1 to 1:1) to give diol **S5** (3.56g, 68% four steps).

Spectral data for **S5**: $[\alpha]_D^{25} = +41.8$ (*c* 1.9 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.40-7.33 (m, 5H), 5.30 (d, *J* = 9.0 Hz, 1H), 5.16 (d, *J* = 9.0 Hz, 1H), 5.09 (d, *J* = 9.2 Hz, 1H), 4.95 (s, 1H), 4.56 (dd, *J* = 6.0, 9.2 Hz, 1H), 2.17-2.05 (m, 1H), 2.00-1.95 (m, 1H), 1.47 (s, 9H), 1.46 (s, 9H), 0.85 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 158.8, 157.3, 151.7, 149.1, 135.7, 128.7, 128.5, 128.3, 91.3, 83.0, 78.8, 77.4, 67.8, 64.3, 58.9, 52.4, 46.4, 29.1, 28.4, 28.3, 25.8, 18.1, -5.4, -5.6; HRMS (ESI, $\text{M}+\text{Na}$)⁺ calcd for $\text{C}_{32}\text{H}_{52}\text{N}_4\text{O}_9\text{SiNa}$ 687.3401 found 687.3438.

Synthesis of **S6**

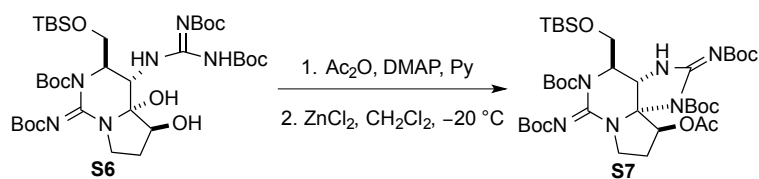


To a solution of diol **S5** (3.56 g, 5.36 mmol) in methanol (45 mL) was added 20% Pd(OH)₂ (0.71 g). The suspension was vigorously stirred under H₂ atmosphere (balloon) at room temperature for 3 hrs and then was filtered through a pad of Celite. The filtrates were concentrated *in vacuo* to give amine.

To a solution of the amine, Et₃N (2.24 mL, 16.08 mmol) and bis(Boc)-2-methyl-2-thiopseudourea (1.92 g, 5.36 mmol) in DMF (30 mL) was added HgCl₂ (1.46 g, 5.36 mmol) at room temperature under N₂ atmosphere. After stirring for 1 h, the reaction mixture was diluted with EtOAc (45 mL) and filtered through a pad of Celite. The filtrate was washed with water (50 mL) and brine (50 mL) twice. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to give yellow oil. The crude mixture was purified by chromatorex NH gel column chromatography (hexane/EtOAc; 4:1 to 1:1) to give bis-guanidine **S6** (3.89 g, 94% two steps).

Spectral data for **S6**: $[\alpha]_D^{25} = +27.7$ (*c* 1.9 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 11.40 (s, 1H), 8.74 (d, *J* = 13.2 Hz, 1H), 6.90 (s, 1H), 4.88 (dd, *J* = 5.5, 8.7 Hz, 1H), 4.21-4.16 (m, 1H), 4.00-3.90 (m, 3H), 3.70-3.62 (m, 2H), 2.30 (s, 1H), 2.18-1.99 (m, 2H), 1.51 (m, 18H), 1.48 (s, 9H), 1.45 (s, 9H), 0.83 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 162.3, 158.8, 155.8, 152.8, 151.5, 148.7, 91.5, 83.9, 83.1, 79.8, 78.9, 77.4, 76.2, 64.6, 59.0, 52.6, 46.5, 29.0, 28.4, 28.21, 28.17, 28.1, 25.7, 18.1, -5.4, -5.6; HRMS (ESI, M+Na)⁺ calcd for C₃₅H₆₄N₆O₁₁SiNa 795.4300 found 795.4312.

Synthesis of S7

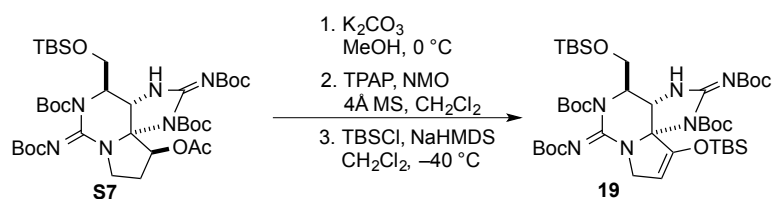


To a solution of bis-guanidine **S6** (3.89 g, 5.03 mmol) in pyridine (16 mL) was added catalytic amount of DMAP (62 mg, 0.05 mmol) and acetic anhydride (8 mL) at room temperature. After stirring for 1 h, the reaction mixture was concentrated *in vacuo* to give diester.

To a solution of diester in CH₂Cl₂ (60 mL) was added ZnCl₂ (1.03 g, 7.55 mmol) under Ar atmosphere at -20 °C. After stirring for 3 hrs, the reaction mixture was quenched with saturated NaHCO₃ (30 mL) and extracted with CH₂Cl₂ (50 mL) three times. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by Chromatorex NH column chromatography (hexane/EtOAc; 10:1 to 6:1) to give protected STXol **S7** (3.20 g, 80% two steps).

Spectral data for **S7**: $[\alpha]_D^{25} = +45.2$ (*c* 1.9 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 9.49 (br, 1H), 5.90 (t, *J* = 9.2 Hz, 1H), 4.96 (s, 1H), 4.64 (dd, *J* = 5.5, 10.1 Hz, 1H), 3.90 (dd, *J* = 5.5, 10.5 Hz, 1H), 3.84-3.77 (m, 1H), 3.64 (t, *J* = 10.1 Hz, 1H), 3.40 (t, *J* = 10.1 Hz, 1H), 2.42-2.35 (m, 1H), 2.08 (s, 3H), 1.89-1.78 (m, 1H), 1.58 (s, 9H), 1.48 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H), 0.91 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.2, 159.2, 151.1, 150.9, 149.3, 149.2, 146.3, 86.5, 83.7, 83.0, 81.5, 79.5, 77.4, 76.7, 66.6, 62.5, 60.1, 46.7, 29.0, 28.4, 28.2, 28.1, 27.8, 26.0, 20.8, 18.4, -5.2, -5.3; HRMS (ESI, M+Na)⁺ calcd for C₃₇H₆₄N₆O₁₁SiNa 819.4300 found 819.4311.

Synthesis of silyl enol ether 19



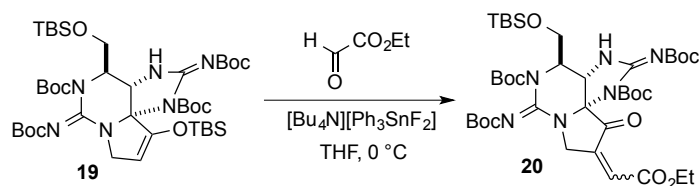
To a solution of protected STXol **S7** (0.95 g, 1.19 mmol) in methanol (12 mL) was added K_2CO_3 (0.33 g, 2.38 mmol) at 0 °C. After stirring for 15 min, the reaction was diluted with EtOAc (10 mL) and H_2O (10 mL), and extracted with EtOAc (15 mL) three times. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude alcohol.

To a solution of crude alcohol in CH_2Cl_2 (12 mL) was added NMO (0.56 g, 4.76 mmol) and 4Å MS (0.60 g) at 0 °C. After stirring for 10 min, TPAP (42 mg, 0.119 mmol) was added, and the reaction mixture was stirred for another 1 h at room temperature. Then, reaction mixture was diluted with hexane/EtOAc (2:1) (12 mL) and filtered through a pad of neutral silica gel, and then, washed with hexane/EtOAc (2:1) (50 mL). The filtrates were concentrated *in vacuo* to give ketone.

To a solution of ketone in CH_2Cl_2 (8 mL) was added NaHMDS (3.14 mL, 5.97 mmol) under Ar atmosphere at -40 °C. After stirring for 10 min, TBSCl (0.72 g, 4.77 mmol) was added and the reaction mixture was stirred overnight at -40 °C. Then, the reaction was quenched with H_2O (10 mL) and warmed to room temperature and extracted with CH_2Cl_2 (10 mL) three times. The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 8:1 to 3:1) to give silyl enol ether **19** (0.88 g, 85% three steps).

Spectral data for **19**: $[\alpha]_D^{25} = +13.6$ (*c* 1.2 in $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 4.80 (s, 1H), 4.72-4.68 (m, 2H), 4.35 (d, *J* = 18.8 Hz, 1H), 3.92-3.84 (m, 2H), 3.37 (t, *J* = 12.4 Hz, 1H), 1.50 (s, 9H), 1.48 (s, 9H), 1.45 (s, 18H), 0.88 (s, 9H), 0.86 (s, 9H), 0.21 (s, 6H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 151.1, 150.0, 149.1, 147.3, 146.4, 97.7, 85.1, 83.5, 82.9, 81.2, 79.4, 77.4, 69.0, 62.4, 60.1, 52.1, 28.19, 28.15, 28.0, 27.8, 26.0, 25.3, 18.4, 17.8, -4.7, -4.9, -5.2, -5.6; HRMS (ESI, $M+Na$)⁺ calcd for $C_{41}H_{75}N_6O_{10}Si_2Na$ 867.5083 found 867.5045.

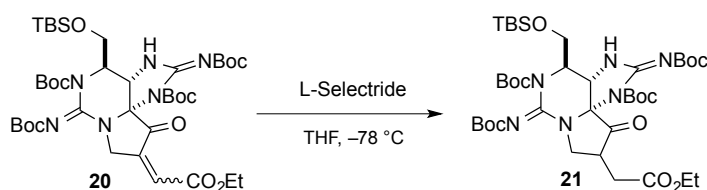
Synthesis of 20



To a solution of silyl enl ether **19** (0.23 g, 0.27 mmol) in THF (10 mL) was added ethyl glyoxylate (210 μ L) at 0 °C. After stirring for several minutes, $[\text{Bu}_4\text{N}][\text{Ph}_3\text{SnF}_2]$ (0.177 g, 0.28 mmol) was added and the reaction was stirred for another 2 hrs at the same temperature. Then, the reaction was quenched with sat. NH_4Cl aq (5 mL), and the solution was extracted with EtOAc (10 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 3:1) to give **20** (0.19 g, 85%).

Spectral data for **20**: $[\alpha]_D^{25} = +11.3$ (*c* 0.7 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 9.50 (s, 1H), 6.70 (t, *J* = 2.4 Hz, 0.75 H), 6.62-6.57 (m, 0.15 H), 4.86-4.78 (m, 4H), 4.33-4.25 (m, 2H), 3.89 (dd, *J* = 5.2, 10.7 Hz, 1H), 3.44 (dd, *J* = 7.6, 10.6 Hz, 1H), 1.51 (s, 9H), 1.47 (s, 9H), 1.45 (s, 9H), 1.39 (s, 9H), 1.31 (t, *J* = 7.2 Hz, 3H), 0.79 (s, 9H), -0.03 (s, 3H), -0.10 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 196.9, 164.6, 159.3151.0, 149.1, 145.8, 143.3, 122.9, 88.5, 83.8, 81.9, 80.1, 77.8, 77.4, 72.2, 63.2, 61.9, 59.0, 51.4, 28.3, 28.0, 27.9, 27.8, 26.0, 18.6, 14.4, -5.4, -5.6; HRMS (ESI, $\text{M}+\text{Na}^+$) calcd for $\text{C}_{39}\text{H}_{64}\text{N}_6\text{O}_{12}\text{SiNa}$ 859.4249 found 859.4262.

Synthesis of 21

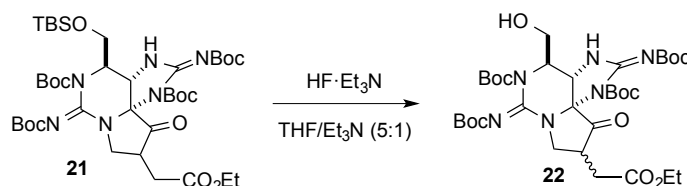


To a solution of **20** (190 mg, 0.23 mmol) in THF (8 mL) was added L-selectride (0.68 mL, 0.68 mmol) at -78 °C. After stirring for 5 min, the reaction was quenched

with sat. NH_4Cl aq (10 mL), and the solution was extracted with EtOAc (10 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified immediately by neutral silica gel column chromatography (hexane/EtOAc; 3:1) to give **21** (145 mg, 76%).

Spectral data for **21**: $[\alpha]_D^{25} = +5.2$ (*c* 1.3 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.30 (s, 1H), 4.35 (dd, *J* = 5.9, 8.3 Hz, 1H), 4.75 (s, 1H), 4.30 (dd, *J* = 8.9, 10.3 Hz, 1H), 4.21-4.13 (m, 2H), 3.95 (dd, *J* = 5.8, 10.6 Hz, 1H), 3.66 (dd, *J* = 8.9, 11.0 Hz, 1H), 3.50 (t, *J* = 9.5 Hz, 1H), 3.04-2.94 (m, 2H), 2.60 (dd, *J* = 10.3, 17.9 Hz, 1H), 1.52 (s, 9H), 1.49 (s, 9H), 1.47 (s, 9H), 1.44 (s, 9H), 1.28 (t, *J* = 6.9 Hz, 3H), 0.84 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (75MHz, CDCl_3) δ 207.6, 171.5, 159.3, 151.2, 150.0, 148.8, 148.5, 143.8, 87.8, 83.6, 81.9, 79.7, 78.0, 77.4, 69.9, 62.5, 61.1, 59.0, 51.3, 42.9, 33.4, 29.7, 28.2, 27.9, 25.9, 18.3, 14.3, -5.4; HRMS (ESI, $\text{M}+\text{Na}^+$) calcd for $\text{C}_{39}\text{H}_{66}\text{N}_6\text{O}_{12}\text{SiNa}$ 861.4406 found 861.4400.

Synthesis of **22**

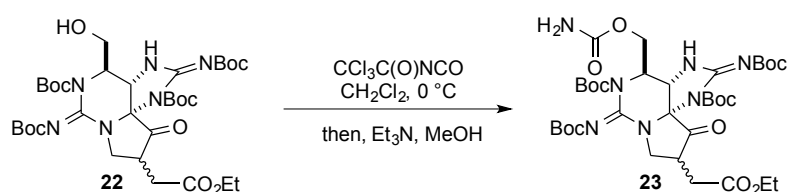


To a solution of **21** (145 mg, 0.17 mmol) in a mixture solvent (THF = 4 mL, Et₃N = 0.8 mL) was added HF·Et₃N (150 μL) at 0 °C. After stirring for 5 hrs at room temperature, the reaction was quenched with sat. NaHCO_3 aq (5 mL), and the solution was extracted with EtOAc (5 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 2:1 to 1:1) to give **22** as diastereomers mixture (109 mg, 87%). Major diastereomer was obtained by further purified with preparative thin layer chromatography (hexane/EtOAc; 1:1).

Spectral data for **22**: $[\alpha]_D^{25} = -1.4$ (*c* 0.8 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.29 (s, 1H), 4.92 (d, *J* = 3.5 Hz, 1H), 4.89 (d, *J* = 3.8 Hz, 1H), 4.41 (dd, *J* = 9.3,

11.0 Hz, 1H), 4.21-4.14 (m, 2H), 3.69-3.57 (m, 2H), 3.44 (t, $J = 11.3$ Hz, 1H), 3.03-2.92 (m, 2H), 2.58 (dd, $J = 10.0, 17.5$ Hz, 1H), 1.52 (s, 9H), 1.50 (s, 9H), 1.46 (s, 9H), 1.45 (s, 9H), 1.28 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 207.5, 171.2, 161.2, 151.1, 150.3, 148.8, 148.4, 143.9, 88.3, 83.6, 82.1, 81.0, 78.0, 77.4, 70.8, 61.5, 61.2, 60.3, 50.8, 42.7, 33.2, 28.0, 27.9, 27.84, 27.78, 14.2; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{33}\text{H}_{52}\text{N}_6\text{O}_{12}\text{Na}$ 747.3541 found 747.3551.

Synthesis of **23**

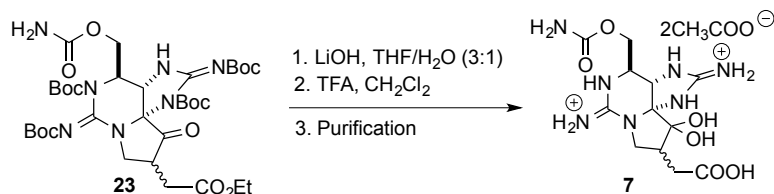


To a solution of **22** (109 mg, 0.15 mmol) in CH_2Cl_2 (6 mL) was added $\text{CCl}_3\text{C}(\text{O})\text{NCO}$ (90 μL , 0.75 mmol). After stirring for 15 min, Et_3N (0.48 mL) and MeOH (2 mL) were added, and the reaction temperature was increased to room temperature. After stirring for another 5 hrs, the resulting mixture was concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 1:1) to give **23** as diastereomers mixture (78 mg, 68%). Major diastereomer was obtained by further purified with preparative thin layer chromatography (hexane/EtOAc; 1:2).

Spectral data for **23**: $[\alpha]_D^{25} = -58.0$ (c 1.1 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.31 (s, 1H), 4.93 (dd, $J = 5.5, 9.3$ Hz, 1H), 4.49 (s, 1H), 4.42-4.30 (m, 2H, overlap), 4.17 (dd, $J = 7.2, 14.4$ Hz, 2H), 4.15-4.08 (m, 1H), 3.69 (dd, $J = 9.3, 10.3$ Hz, 1H), 3.38-3.29 (m, 1H), 3.03 (dd, $J = 3.8, 17.5$ Hz, 1H), 2.59 (dd, $J = 10, 17.5$ Hz, 1H), 1.52 (s, 9H), 1.49 (s, 9H), 1.48 (s, 9H), 1.44 (s, 9H), 1.27 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): 207.6, 171.8, 159.3, 156.1, 150.9, 149.5, 148.8, 148.5, 144.4, 88.1, 83.9, 82.1, 80.0, 78.1, 77.4, 70.3, 62.9, 61.2, 57.2, 51.3, 42.6, 33.3, 28.3, 28.0, 27.9, 14.4; HRMS (ESI, $\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{34}\text{H}_{53}\text{N}_7\text{O}_{13}\text{Na}$ 790.3599 found 790.3606.

Synthesis of 7, 8, 9

Synthesis of 11-saxitoxinethanoic acid (7)

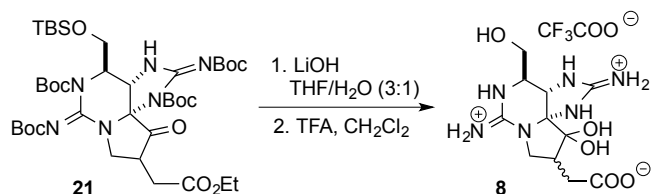


To a solution of **23** (78 mg, 0.10 mmol) in a mixture solvent (THF = 2 mL, H₂O = 0.6 mL) was added LiOH·H₂O (21 mg, 0.51 mmol) at 0 °C. After stirring for 1.5 h, the reaction was quenched with 1.2 N HCl aq until the pH reached 1, and the solution was extracted with EtOAc (3 mL) three times. The combined organic layers was dried over MgSO₄, filtered and concentrated *in vacuo* to give crude carboxylic acid.

To a solution of crude carboxylic acid in CH₂Cl₂ (1 mL) was added TFA (0.5 mL) at room temperature. After stirring for 1 h, the reaction was concentrated *in vacuo*. The residue was dissolved in milli-Q (2 mL), and filtered through Millex filter unit (Millipore, 0.45 μm). The filtrate was lyophilized and the residue was purified by reverse phase HPLC (CH₃CN: 0.1% CH₃COOH aq. = 15: 85, Shiseido Cancel Pak AQ C18 column, Rt= 8.5 min, 214 nm UV detection) to give SEA (**7**) as diacetate salt.

Spectral data for **7**: $[\alpha]_{\text{D}}^{25} = +15$ (*c* 0.1 in MeOH); ¹H NMR (300 MHz, D₂O, after 12 h), δ 4.74 (s, 1H), 4.25 (dd, *J* = 9.3, 11.7 Hz, 1H), 4.02 (dd, *J* = 5.5, 11.7 Hz, 1H), 3.90 (d, *J* = 10 Hz, 1H) 3.80 (dd, *J* = 5.2, 8.9 Hz, 1H), 3.18 (t, *J* = 10.0 Hz, 1H), 2.68 (d, *J* = 16.5 Hz, 1H), 2.49 (d, *J* = 16.1 Hz, 1H), 1.90 (s, 6H); ¹³C NMR (100 MHz, D₂O; 1,4-dioxan (67.4 ppm) was used as internal standard) δ 182.2, 181.0, 159.3, 158.3, 156.1, 99.3, 83.6, 63.6, 57.5, 53.5, 48.2, 39.9, 34.1, 24.0; HRMS (ESI, M+H)⁺ calcd for C₁₂H₂₀N₇O₆ 358.1475 found 358.1482.

Synthesis of **8**

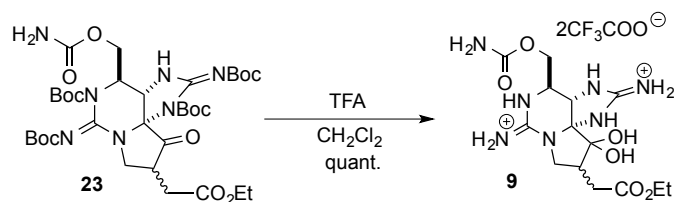


To a solution of **21** (22 mg, 0.0256 mmol) in a mixture solvent (THF = 0.6 mL, H₂O = 0.2 mL) was added LiOH·H₂O (6 mg, 0.128 mmol) at 0 °C. After stirring for 2 h, the reaction was quenched with 1.2 N HCl aq until the pH reached 1, and the solution was extracted with EtOAc (2 mL) three times. The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo* to give crude carboxylic acid.

To a solution of crude carboxylic acid in CH₂Cl₂ (1 mL) was added TFA (0.5 mL) at room temperature. After stirring for 1 h, the reaction was concentrated *in vacuo*. The residue was dissolved in milli-Q (2 mL), and filtered through Millex filter unit (Millipore, 0.45 μm). The filtrate was lyophilized to give **8** as an analytically pure material (7 mg, 90%).

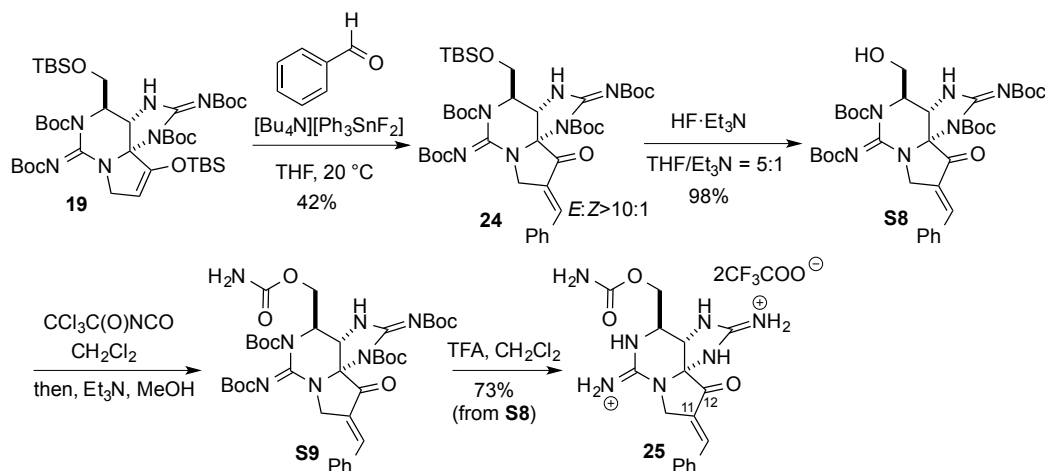
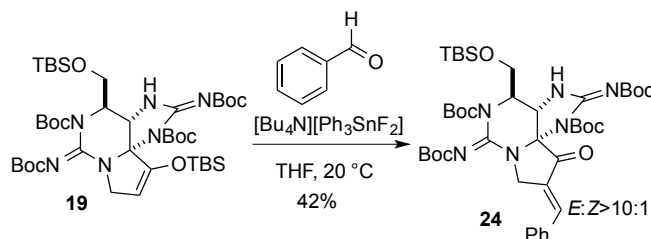
Spectral data for **8**: $[\alpha]_{\text{D}}^{25} = +48.3$ (*c* 0.4 in MeOH); ¹H NMR (300 MHz, D₂O, after 12 h in D₂O) δ 4.72 (s, 1H), 3.98 (d, *J* = 10 Hz, 1H), 3.71-3.55 (m, 3H), 3.19 (d, *J* = 10 Hz, 1H), 2.80 (d, *J* = 16.8 Hz, 1H), 2.57 (d, *J* = 17.1 Hz); ¹³C NMR (100 MHz, D₂O; 1,4-dioxan (67.4 ppm) was used as internal standard) δ 177.4, 158.1, 156.1, 99.6, 83.1, 61.7, 57.2, 56.0, 48.3, 31.7; HRMS (ESI, M+H)⁺ calcd for C₁₁H₁₈N₆O₅ 315.1417 found 315.1430.

Synthesis of **9**



To a solution of **23** (11.8 mg, 0.015 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.5 mL) at room temperature. After stirring for 1 h, the reaction was concentrated *in vacuo*. The residue was dissolved in milli-Q (2 mL), and filtered through Millex filter unit (Millipore, 0.45 μm). The filtrate was lyophilized to give **9** as an analytically pure material (6 mg, quant.).

Spectral data for **9**: $[\alpha]_{\text{D}}^{25} = +49.5$ (*c* 0.6 in MeOH); ^1H NMR (300 MHz, D_2O , after 12 h in D_2O) δ 4.75 (d, $J = 1.1$ Hz, 1H), 4.25 (dd, $J = 9.6, 11.7$ Hz, 1H), 4.17 (dd, $J = 7.2, 14.4$ Hz, 2H), 4.05-3.96 (m, 2H), 3.80 (dd, $J = 5.2, 10.3$ Hz, 1H), 3.25 (d, $J = 10.3$ Hz, 1H), 2.83 (d, $J = 16.5$ Hz, 1H), 2.62 (d, $J = 16.8$ Hz, 1H), 1.25 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, D_2O , 1,4-dioxan (67.4 ppm) was used as internal standard) δ 174.0, 158.3, 157.2, 155.1, 98.4, 82.1, 62.5, 62.0, 56.5, 52.5, 47.3, 38.5, 30.7, 13.1; HRMS (ESI, $\text{M}+\text{H}^+$) calcd for $\text{C}_{14}\text{H}_{24}\text{N}_7\text{O}_6$ 386.1788 found 386.1766.

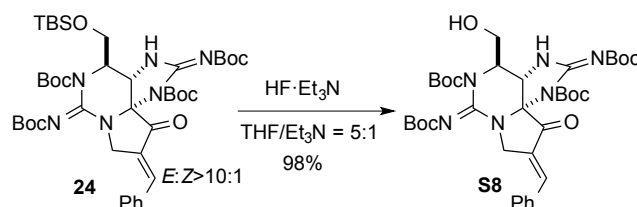
Synthesis of **25**Scheme S2. Synthesis of **25**Synthesis of **24**

To a solution of silyl enol ether **19** (140mg, 0.162 mmol) in THF (3 mL) was added benzaldehyde (164 μ L, 1.62 mmol) at 0 °C. After stirring for several minutes, $[\text{Bu}_4\text{N}][\text{Ph}_3\text{SnF}_2]$ (107 mg, 0.17 mmol) was added. After stirring for 24 hrs at room temperature, the reaction was quenched with sat. NH_4Cl aq (5 mL), and the solution was extracted with EtOAc (10 mL) three times. The combined organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 4:1 to 1:1) to give **24** (57 mg, 42%).

Spectral data for **24**: $[\alpha]_D^{25} = -10.4$ (*c* 5 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 9.57 (s, 1H), 7.58 (s, 1H), 7.49 (s, 5H), 4.81- 4.90 (m, 3H), 4.65 (d, $J = 15.57$ Hz, 1H), 3.85 (dd, $J = 5.0, 10.6$ Hz, 1H), 3.38 (t, $J = 10.1, 8.7$ Hz, 1H), 1.52 (s, 9H), 1.48 (s, 9H), 1.46 (s, 9H), 1.35 (s, 9H), 0.75 (s, 9H), -0.09 (s, 3H), -0.19 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 196.4, 159.4, 151.4, 151.1, 150.9, 149.2, 146.1, 136.2, 133.6,

131.4, 131.3, 129.5, 129.0, 87.5, 83.8, 81.7, 80.2, 78.0, 77.4, 71.9, 62.9, 59.4, 51.1, 28.3, 28.1, 28.0, 25.9, 18.5, -5.4, -5.6; HRMS (ESI, $M+Na$)⁺ calcd for $C_{42}H_{64}N_6O_{10}SiNa$ 863.4350 found 863.4385.

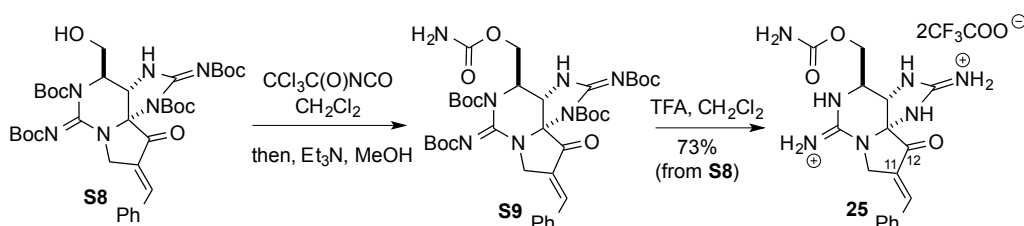
Synthesis of **S8**



To a solution of **24** (8 mg, 0.009 mmol) in a mixture solvent (THF = 0.5 mL, Et_3N = 0.1 mL) was added $HF \cdot Et_3N$ (40 μL) at 0 °C. After stirring for 5 h at room temperature, the reaction was quenched with sat. $NaHCO_3$ aq (1 mL), and the solution was extracted with EtOAc (3 mL) three times. The combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (hexane/EtOAc; 2:1 to 1:1) to give **S8** (6.8 mg, 98%).

Spectral data for **S8**: $[\alpha]_D^{25} = -20.9$ (*c* 2.3 in $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.62 (s, 1H), 7.50 (s, 5H), 4.92- 4.88 (m, 2H), 4.75 (d, $J = 16.0$ Hz, 1H), 4.33 (s, 1H), 3.53 (dd, $J = 3.7, 11.9$ Hz, 1H), 3.16 (dd, $J = 11.9$ Hz, 1H), 1.53 (s, 9H), 1.47 (s, 18H), 1.35 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.1, 161.4, 151.3, 151.2, 150.1, 149.1, 146.3, 137.3, 133.2, 131.8, 131.5, 129.6, 127.8, 87.9, 84.1, 82.0, 81.8, 77.9, 77.4, 72.7, 61.6, 60.9, 50.8, 28.11, 28.1, 27.9; HRMS (ESI, $M+Na$)⁺ calcd for $C_{36}H_{50}N_6O_{10}Na$ 749.3486 found 749.3473.

Synthesis of **25**



To a solution of **S8** (7 mg, 0.0096 mmol) in CH₂Cl₂ (0.5 mL) was added CCl₃C(O)NCO (5.7 μL, 0.048 mmol). After stirring for 15 min, Et₃N (48 μL) and MeOH (0.2 mL) were added, and the reaction temperature was increased to room temperature. After stirring for another 5 hrs, the resulting mixture was concentrated *in vacuo* to give **S9** (5.4 mg), which was used without further purification.

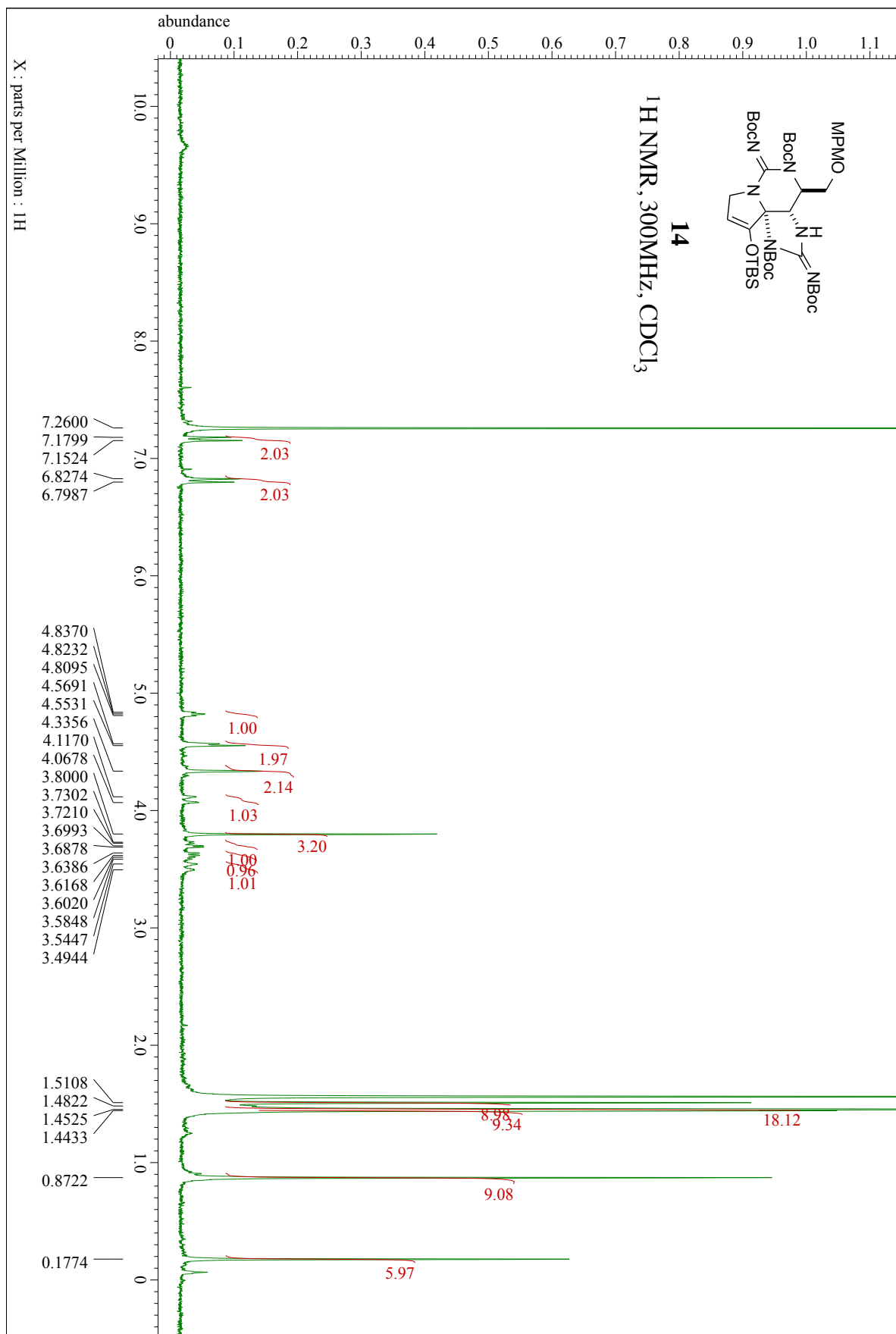
To a solution of **S9** (5.4 mg) in CH₂Cl₂ (0.5 mL) was added TFA (0.25 mL) at room temperature. After stirring for 1 h, the reaction was concentrated *in vacuo*. The residue was dissolved in milli-Q (1 mL), washed with CH₂Cl₂ (5 mL) and filtered through Millex filter unit (Millipore, 0.45 μm). The filtrate was lyophilized to give **25** as an analytically pure material (2.6 mg, 73% 2 steps).

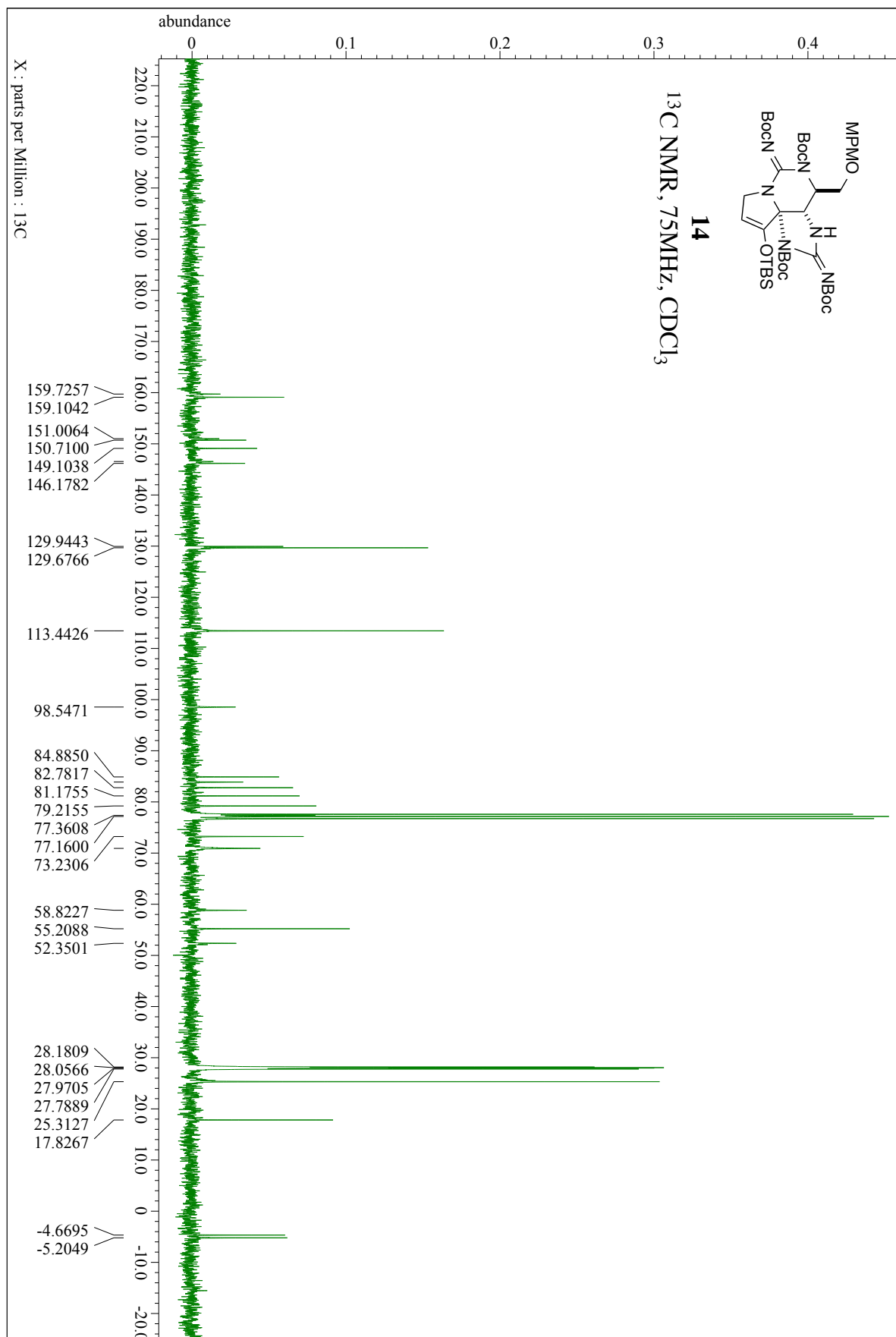
Spectral data for **25**: $[\alpha]_D^{25} = -36.4$ (*c* 0.2 in MeOH); ¹H NMR (300 MHz, D₂O) δ 7.92 (s, 1H), 7.63-7.57 (m, 5H), 4.96 (s, 1H), 4.85 (s, 1H), 4.75 (s, 1H), 4.26 (dd, *J* = 4.5, 12.4 Hz, 1H), 4.16 (dd, *J* = 3.8, 12.4 Hz, 1H), 3.96 (t, *J* = 3.8, 4.2 Hz, 1H); ¹³C NMR (100 MHz, D₂O; 1,4-dioxan (67.4 ppm) was used as internal standard) δ 194.1, 158.6, 158.1, 157.0, 142.6, 133.6, 133.4, 132.7, 130.3, 124.7, 76.4, 65.9, 61.9, 53.0, 48.8; HRMS (ESI, M+H)⁺ calcd for C₁₇H₂₀N₇O₃ 370.1627 found 370.1660.

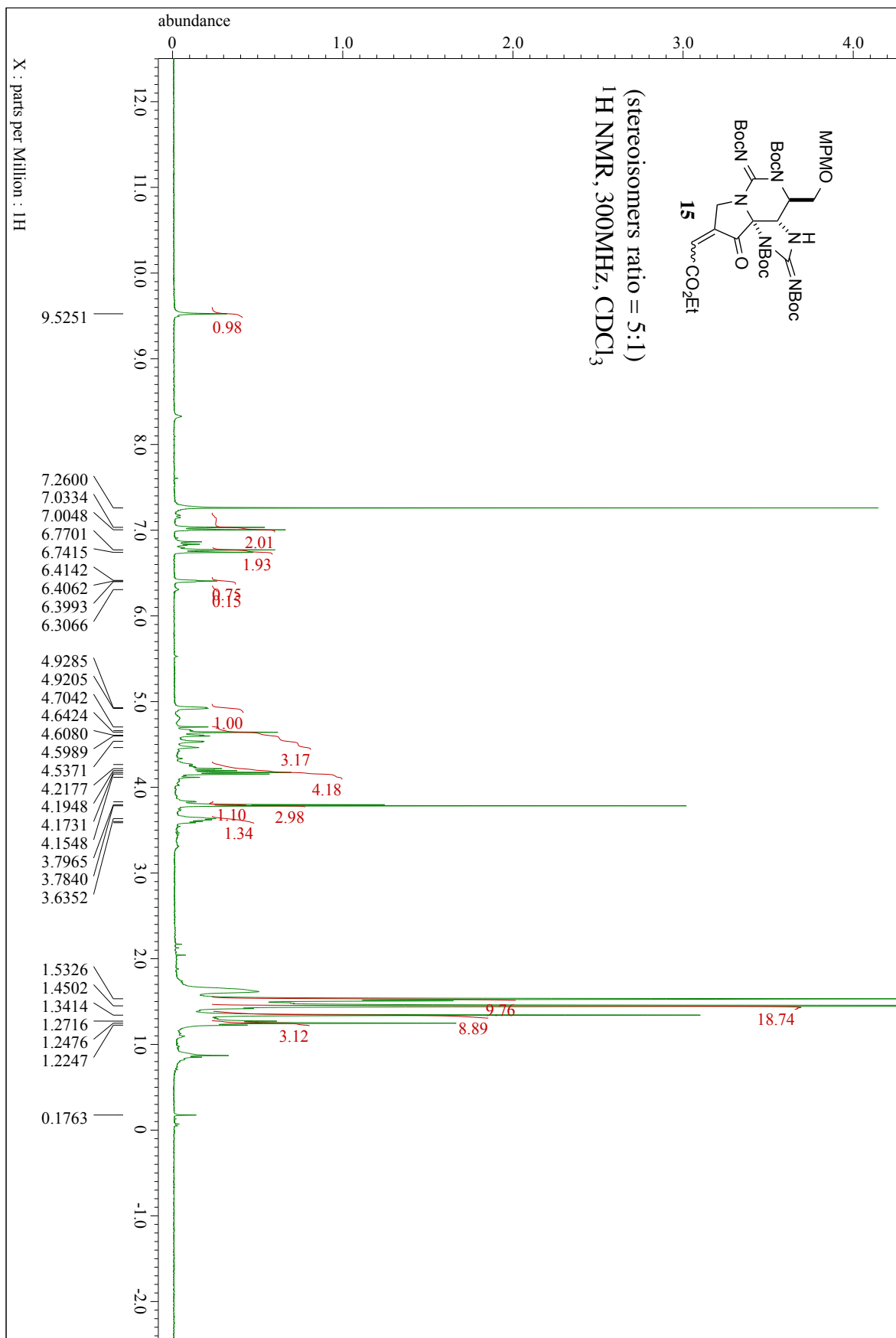
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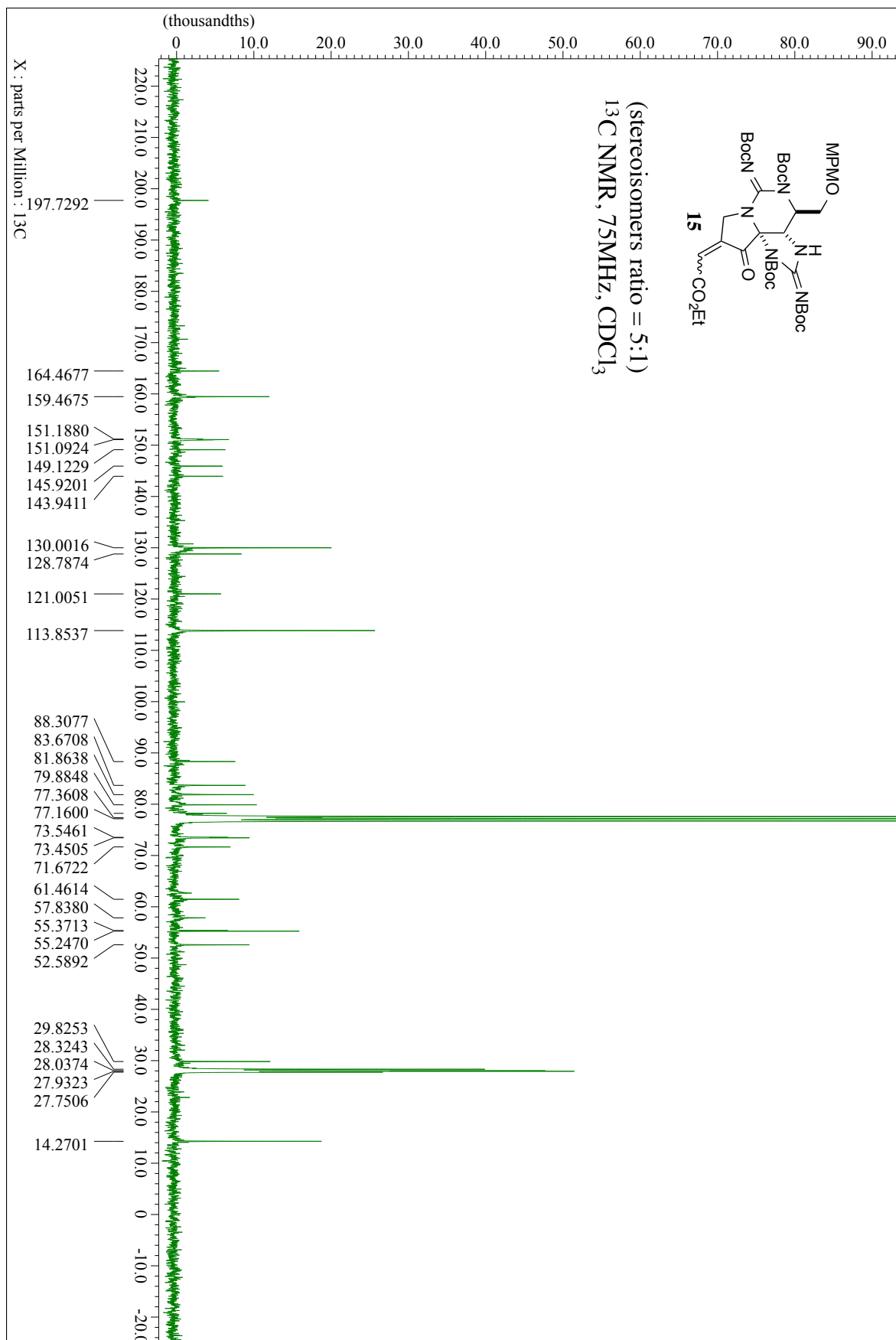
[1] O. Iwamoto, K. Nagasawa, *Org. Lett.* **2010**, *12*, 2150–2153.

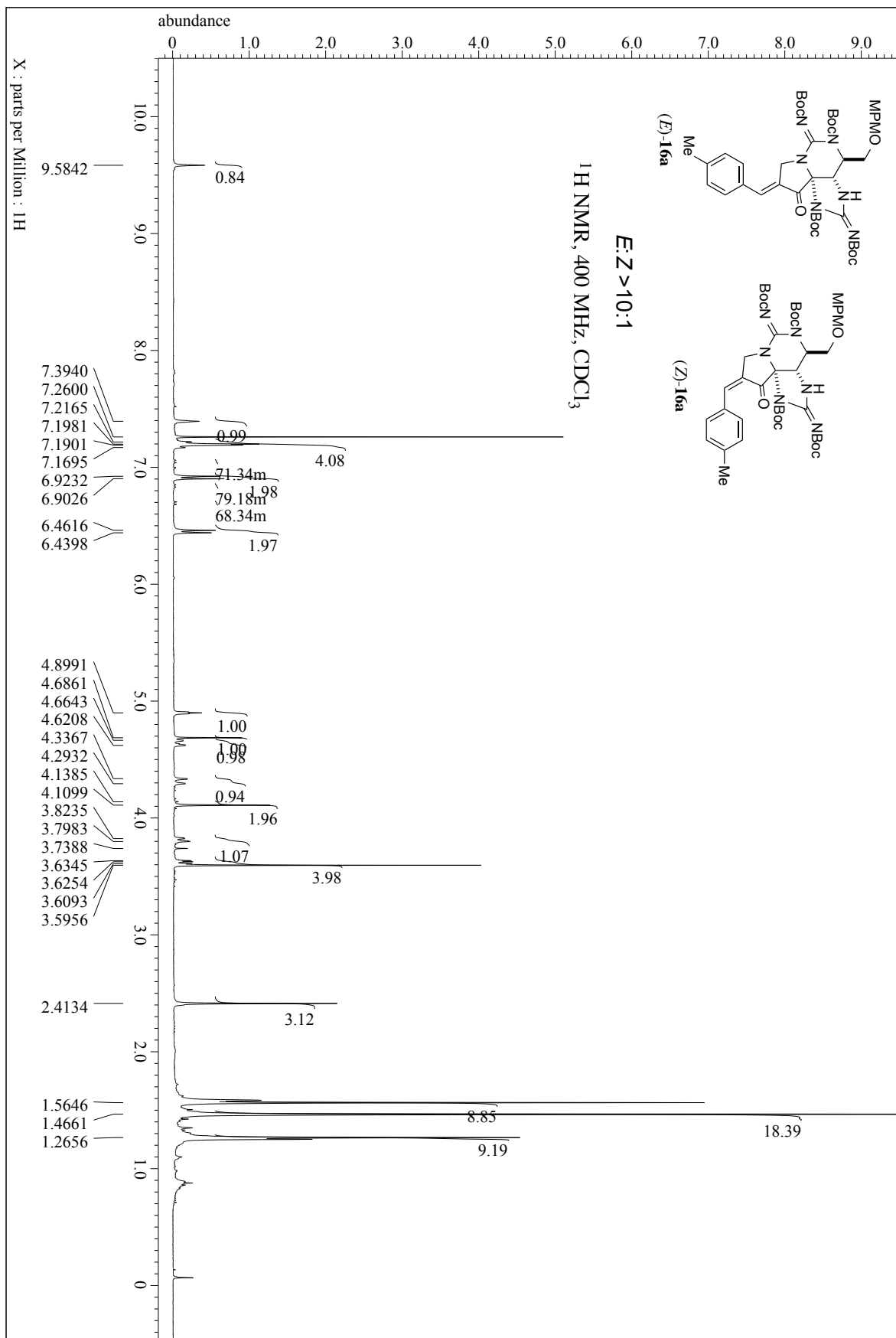
3. Copies of NMR spectra of intermediates for S2-S8, 7-9, 14-17, 19-25

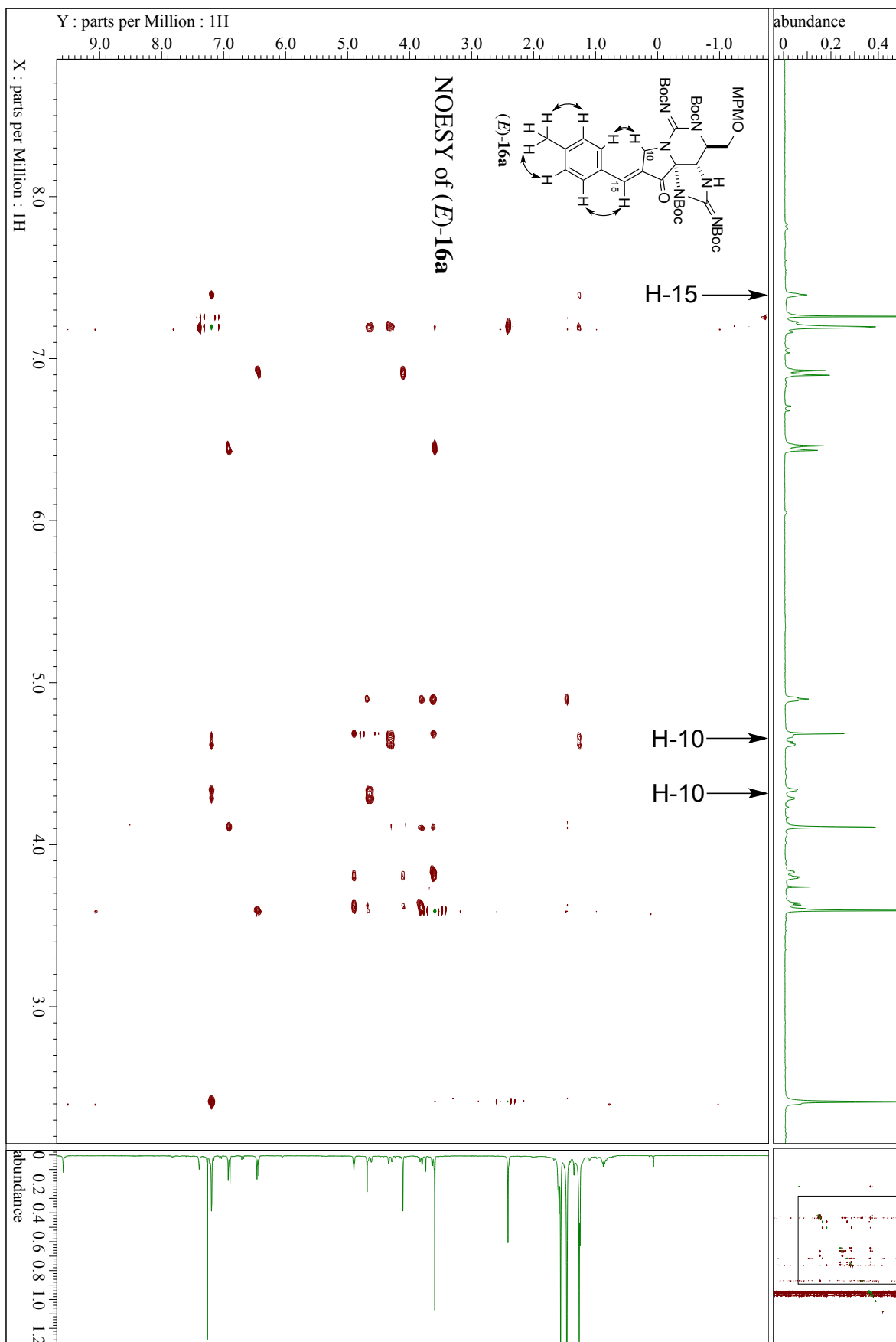


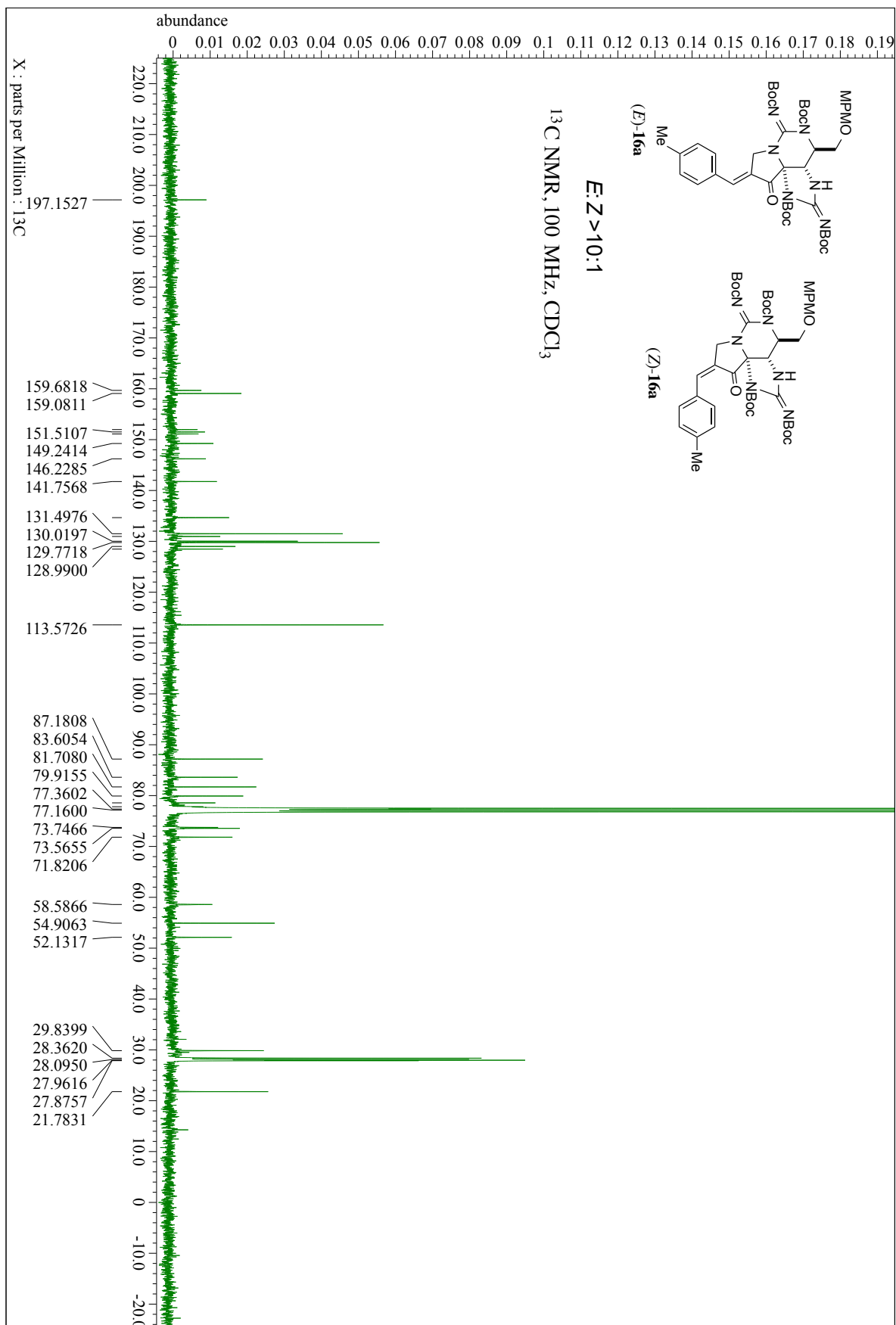


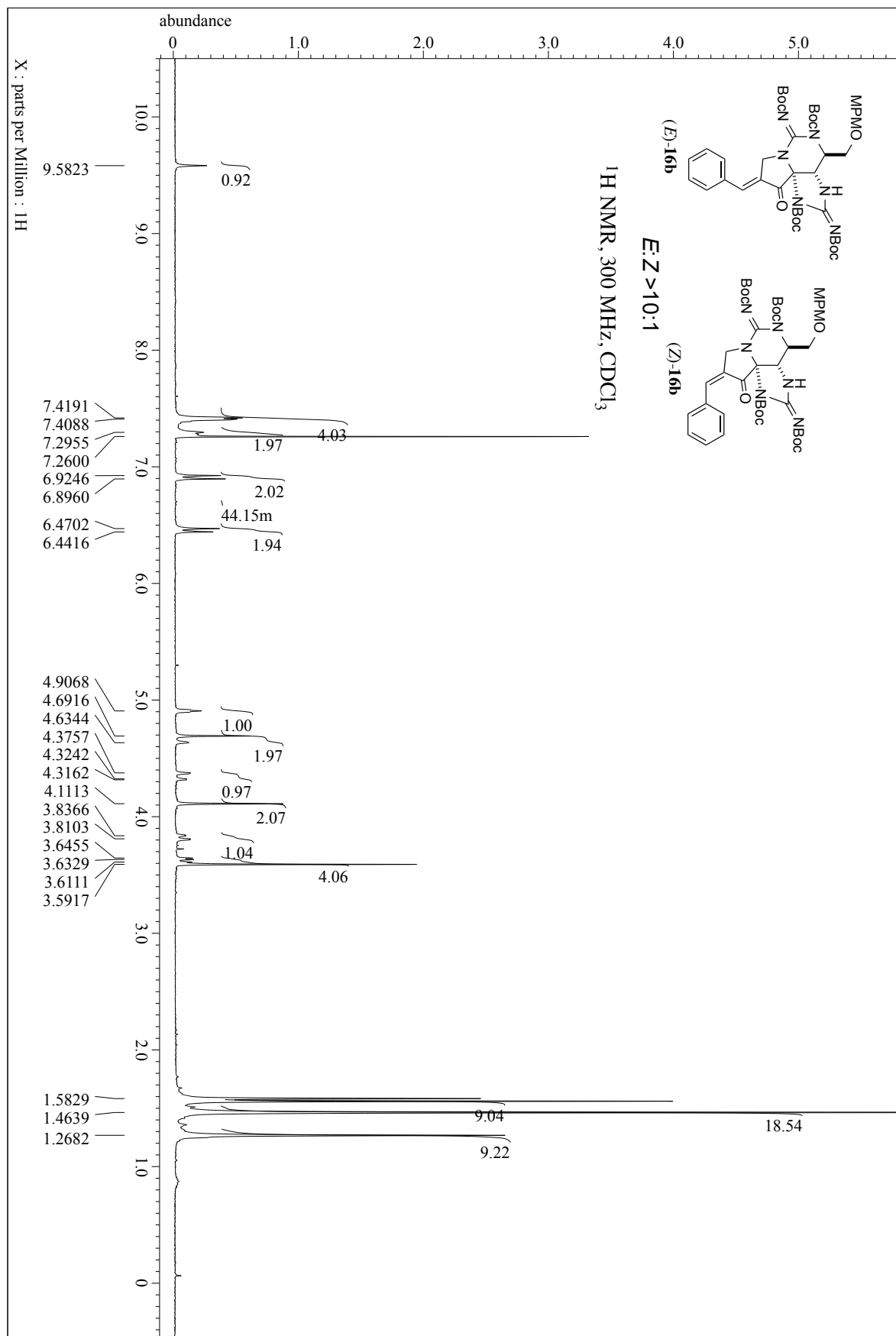


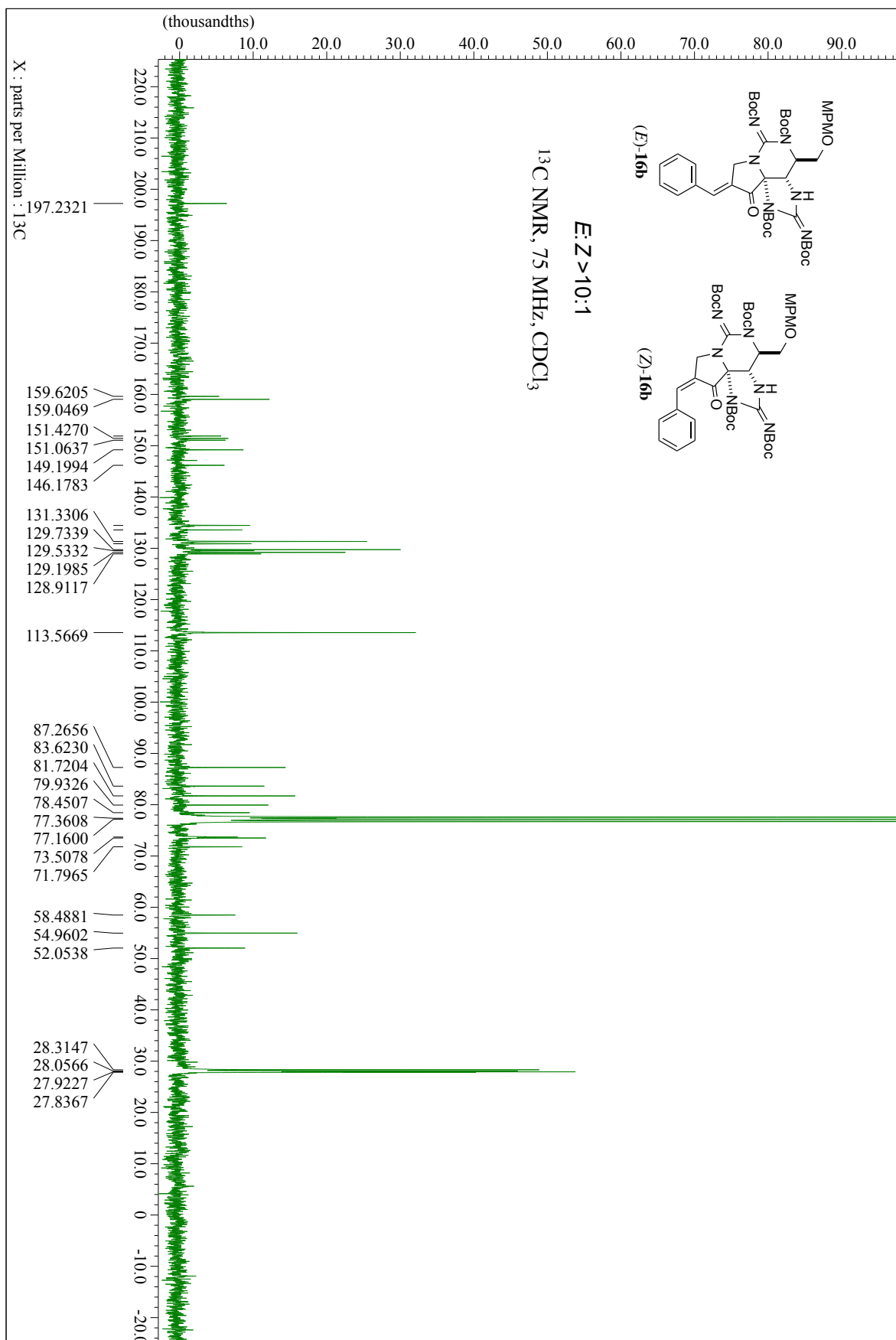


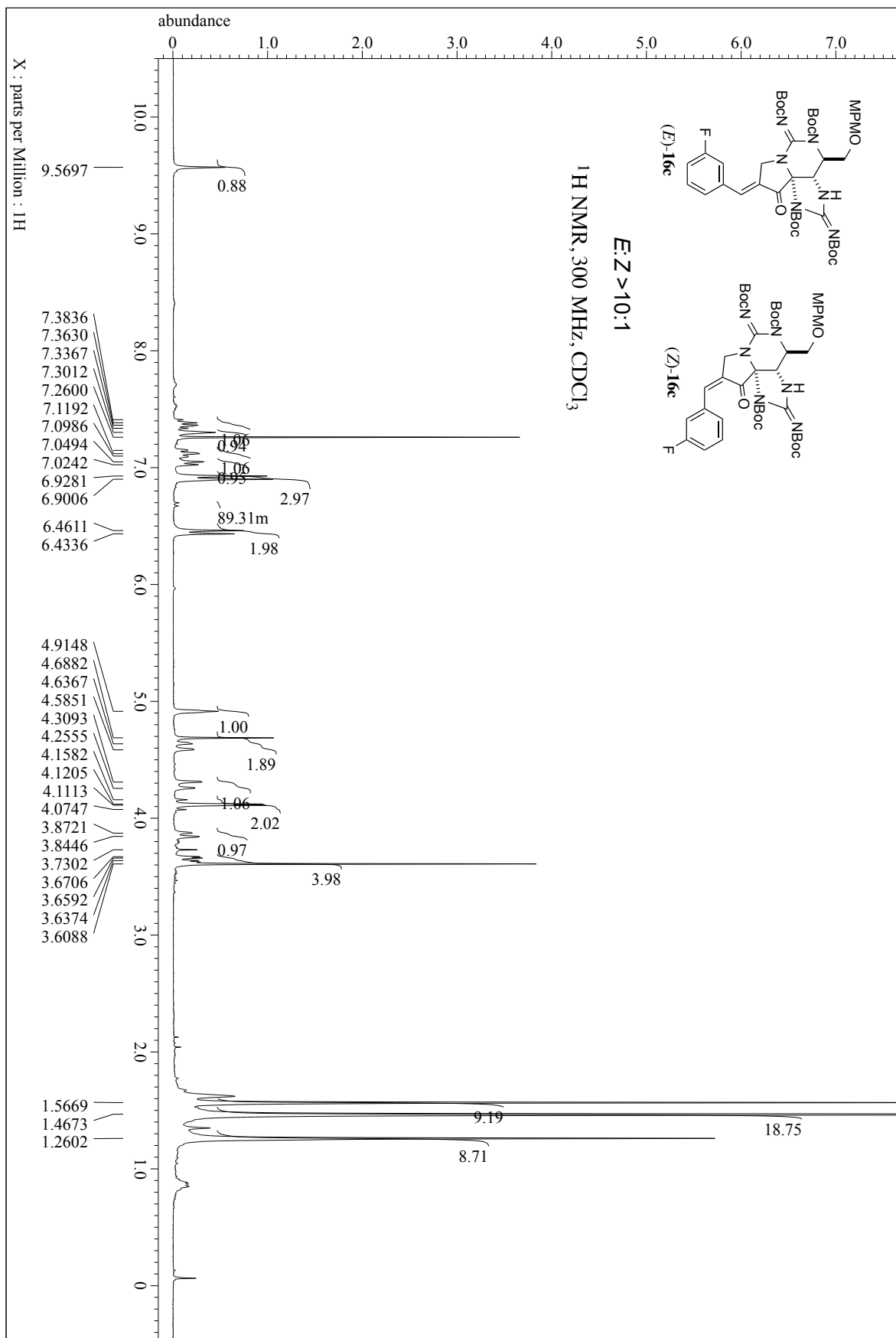


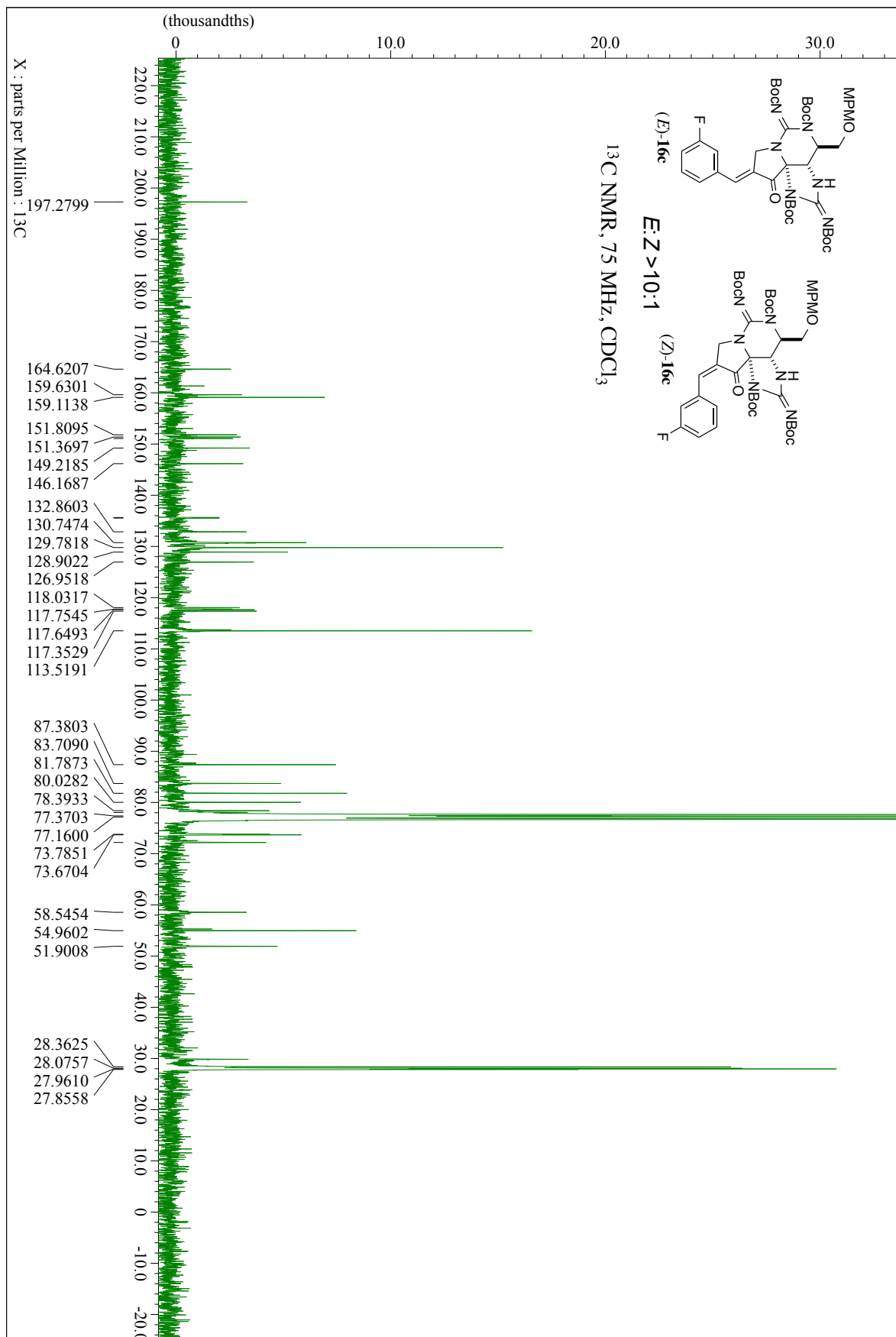


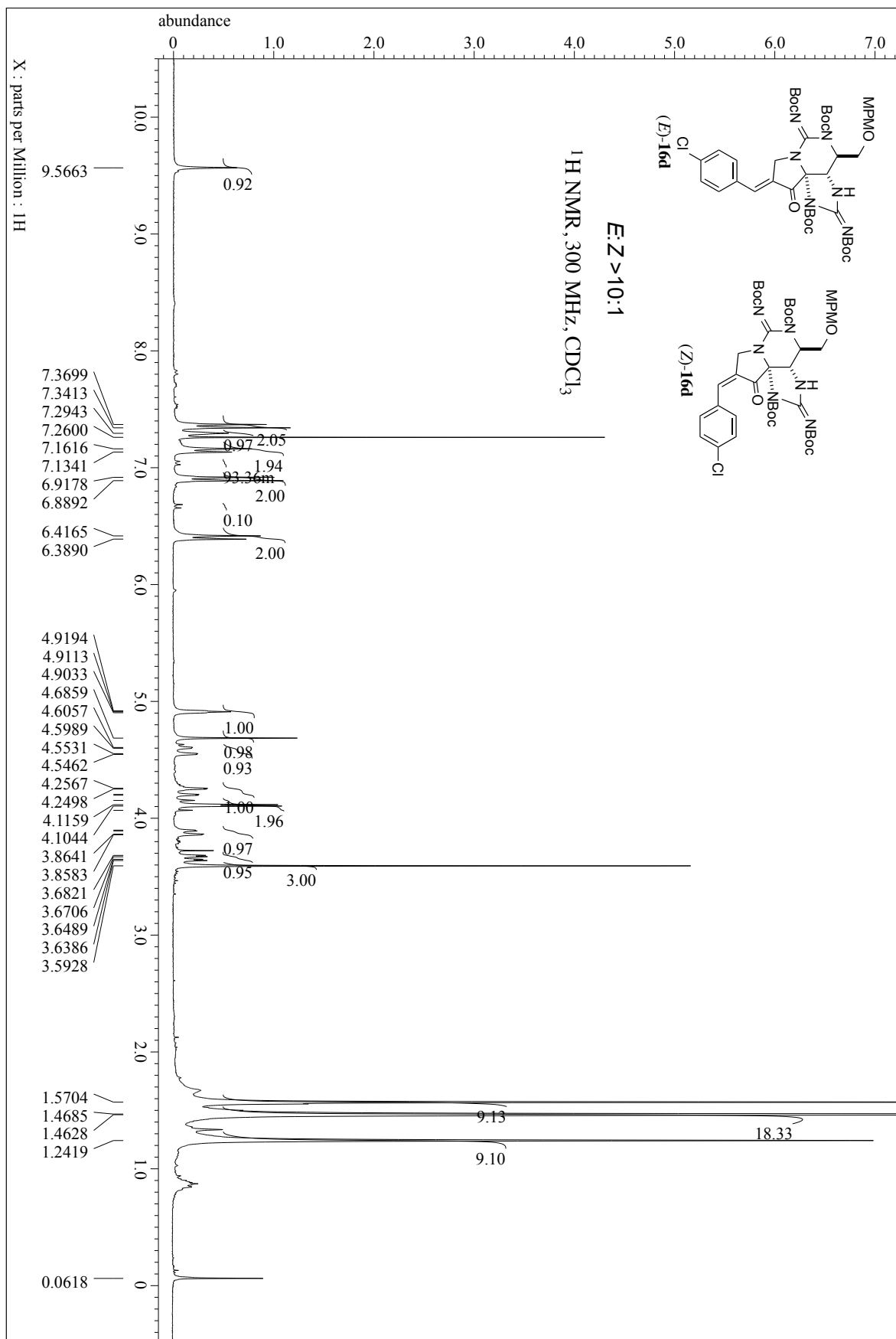


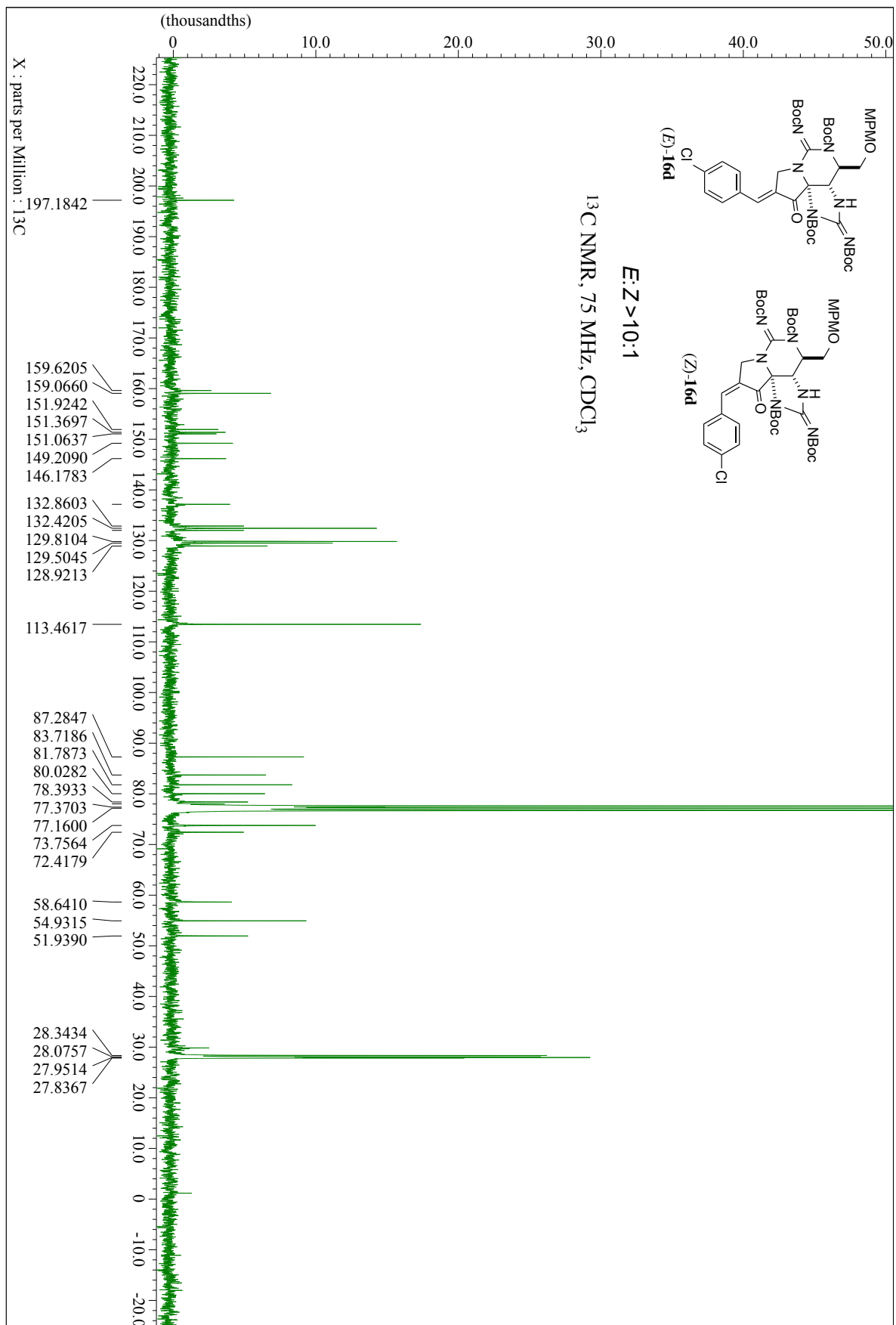


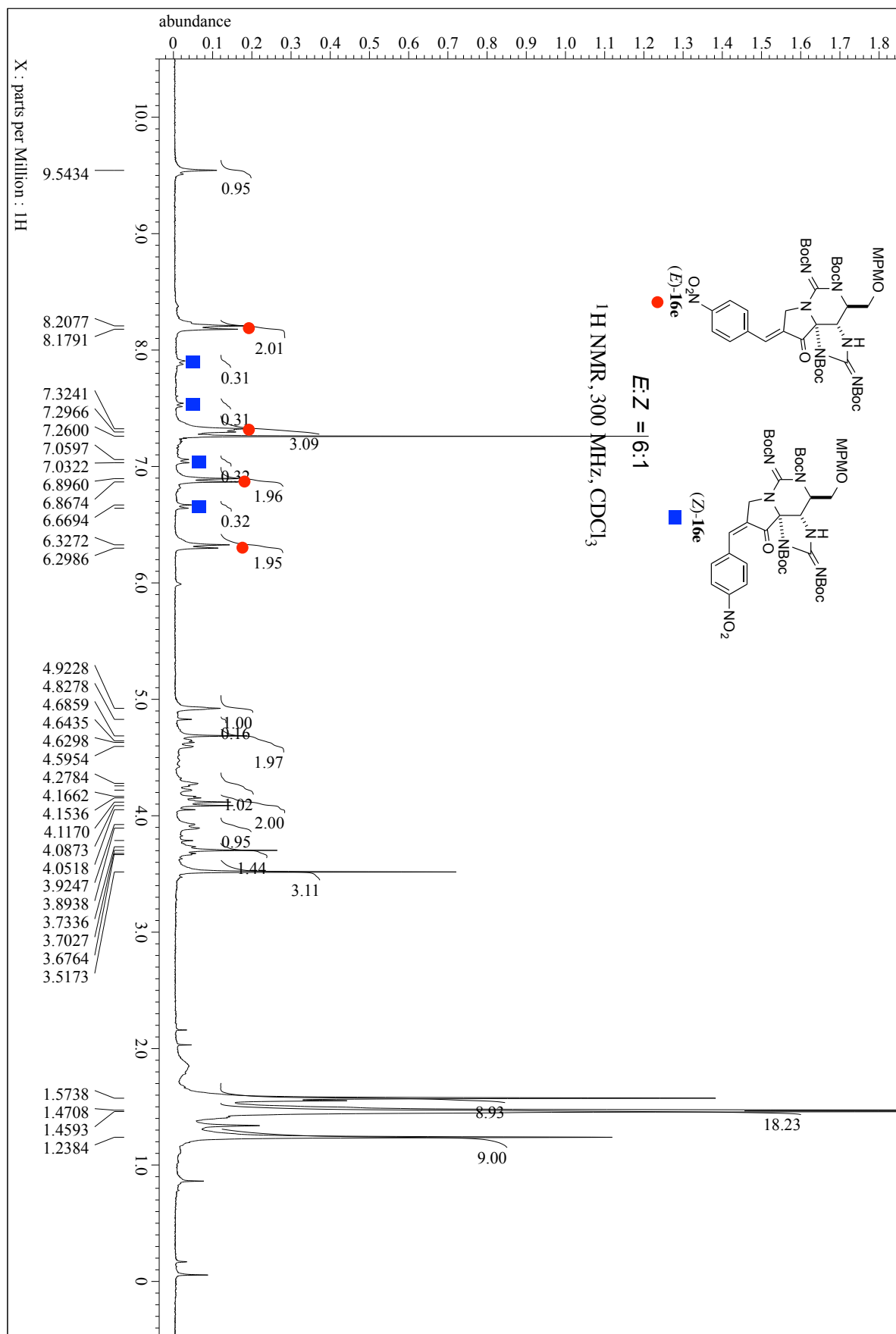


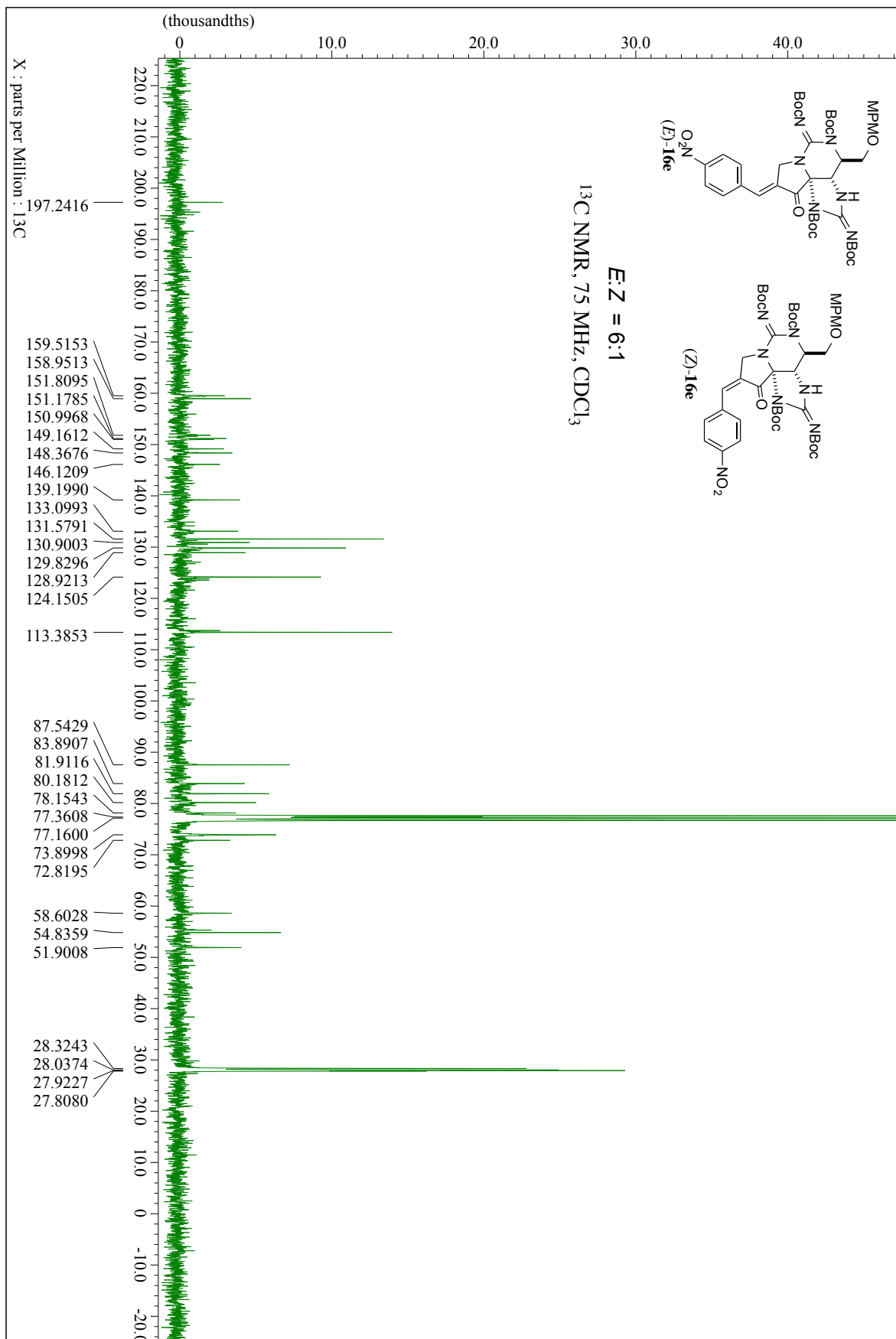


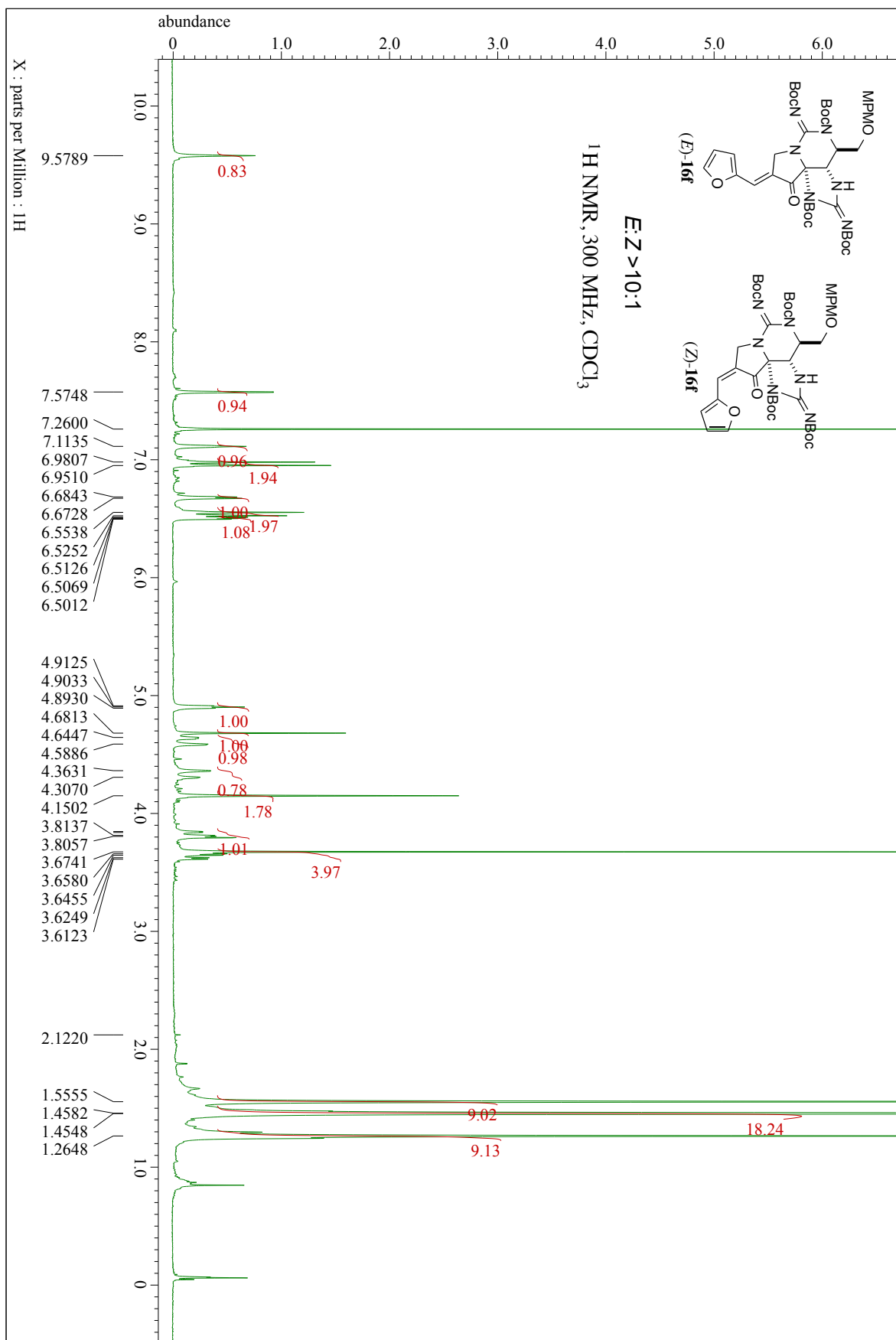


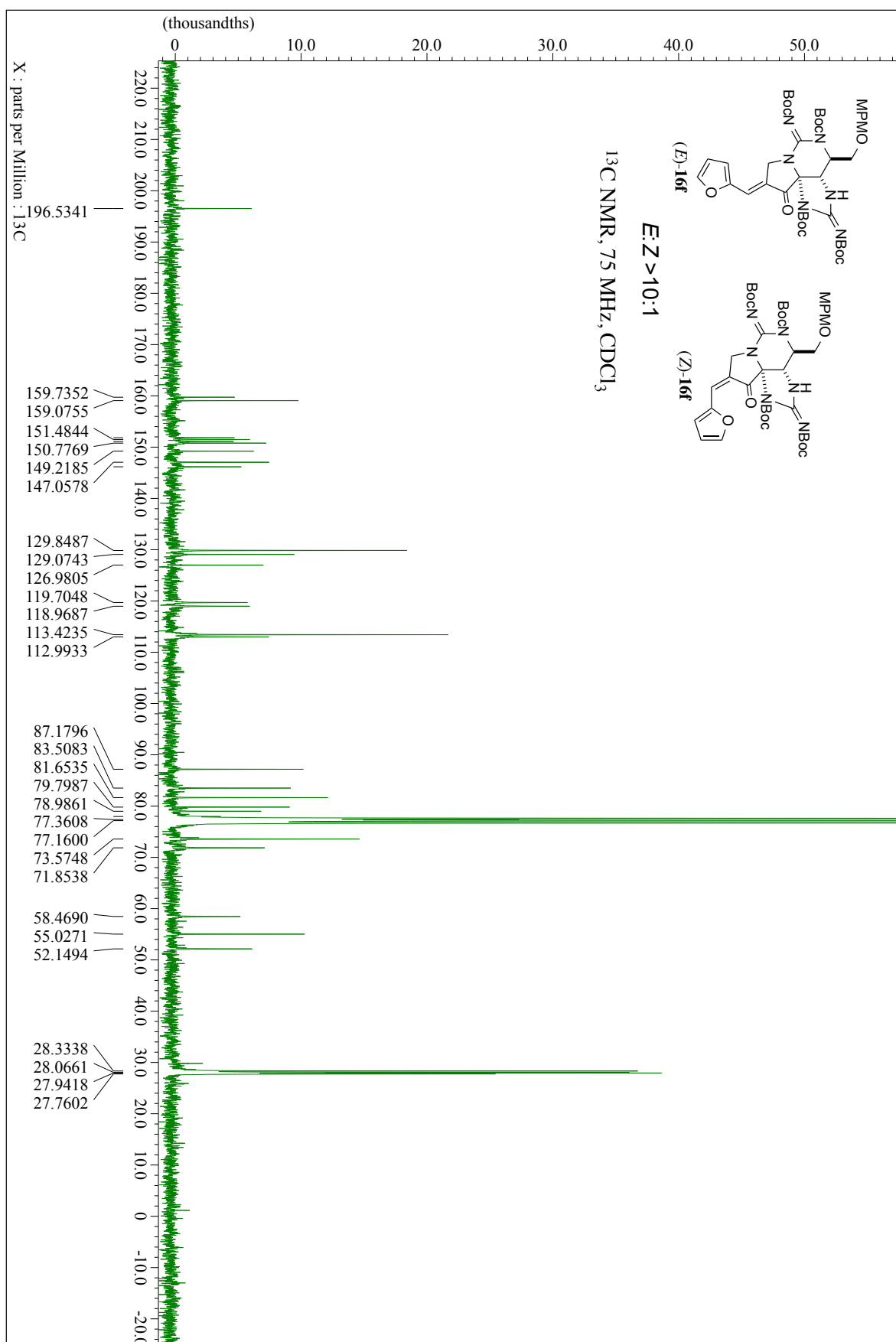


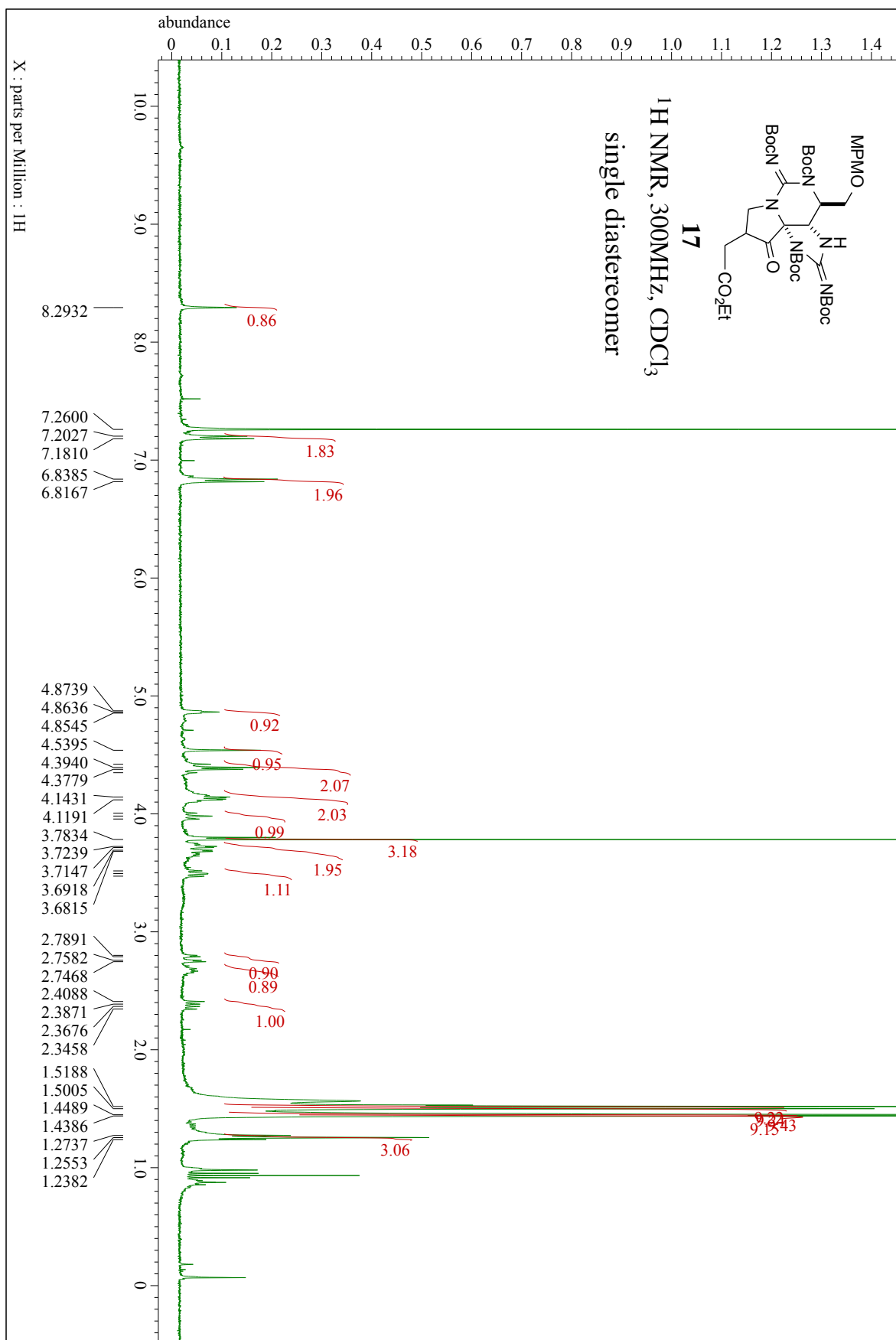


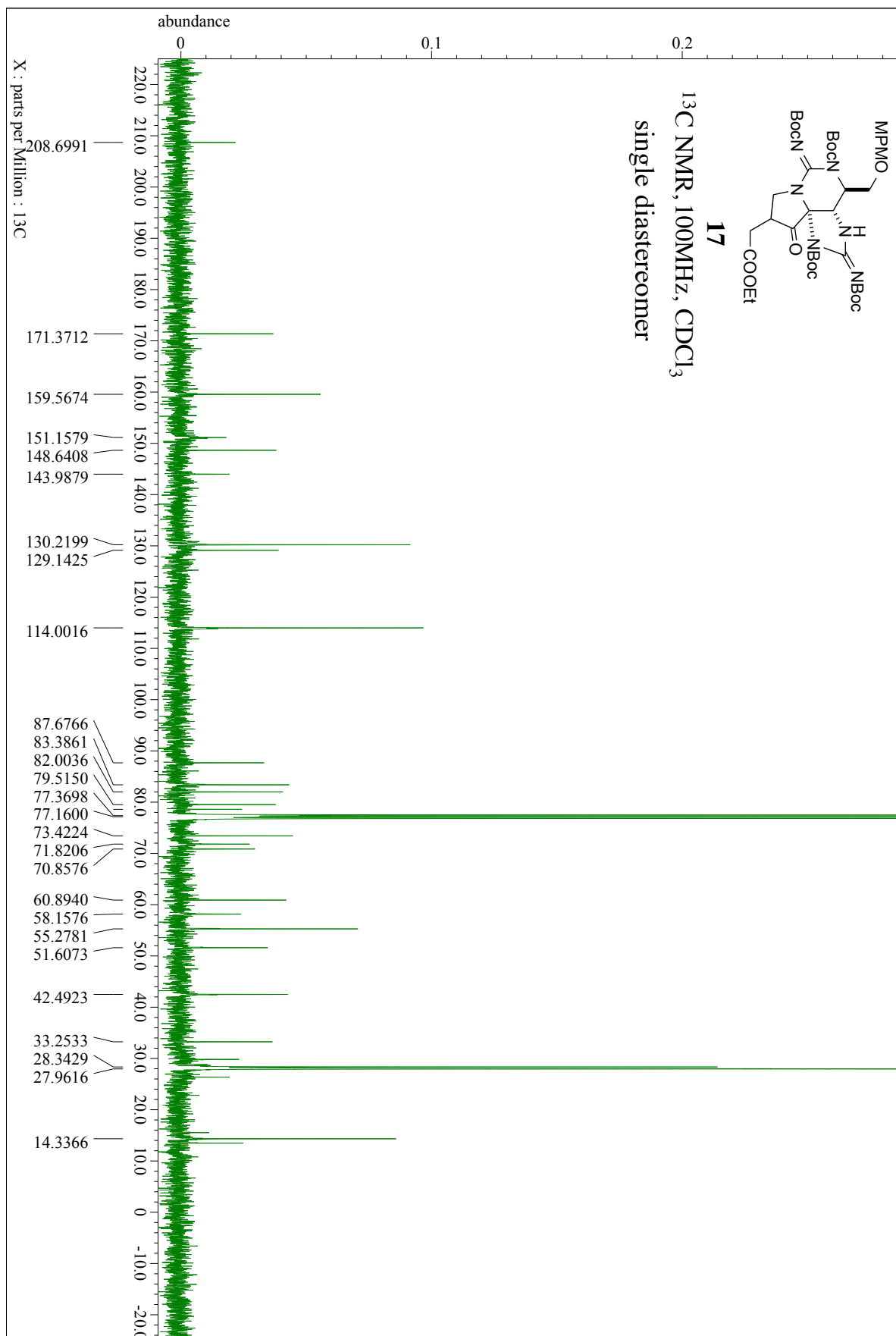


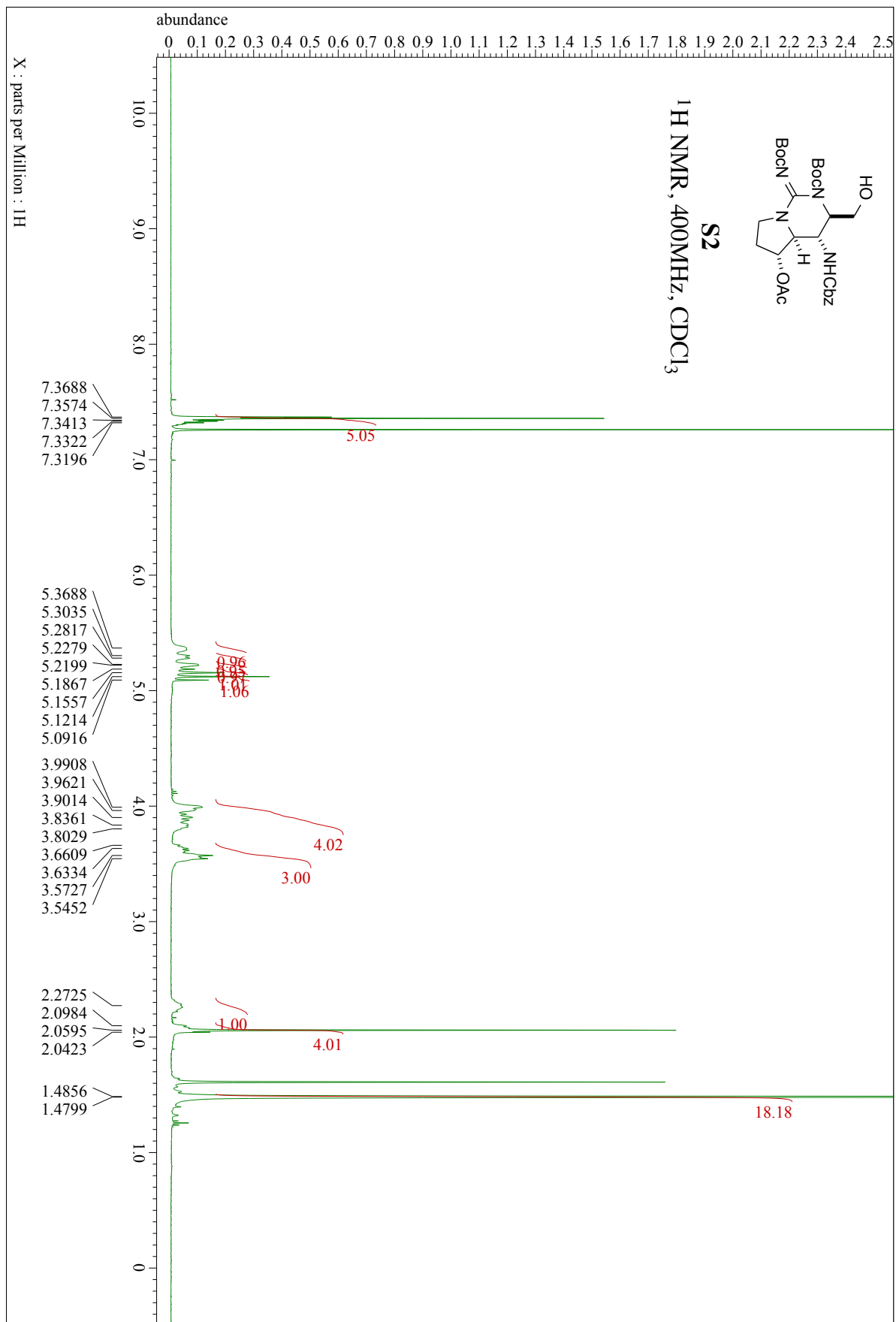


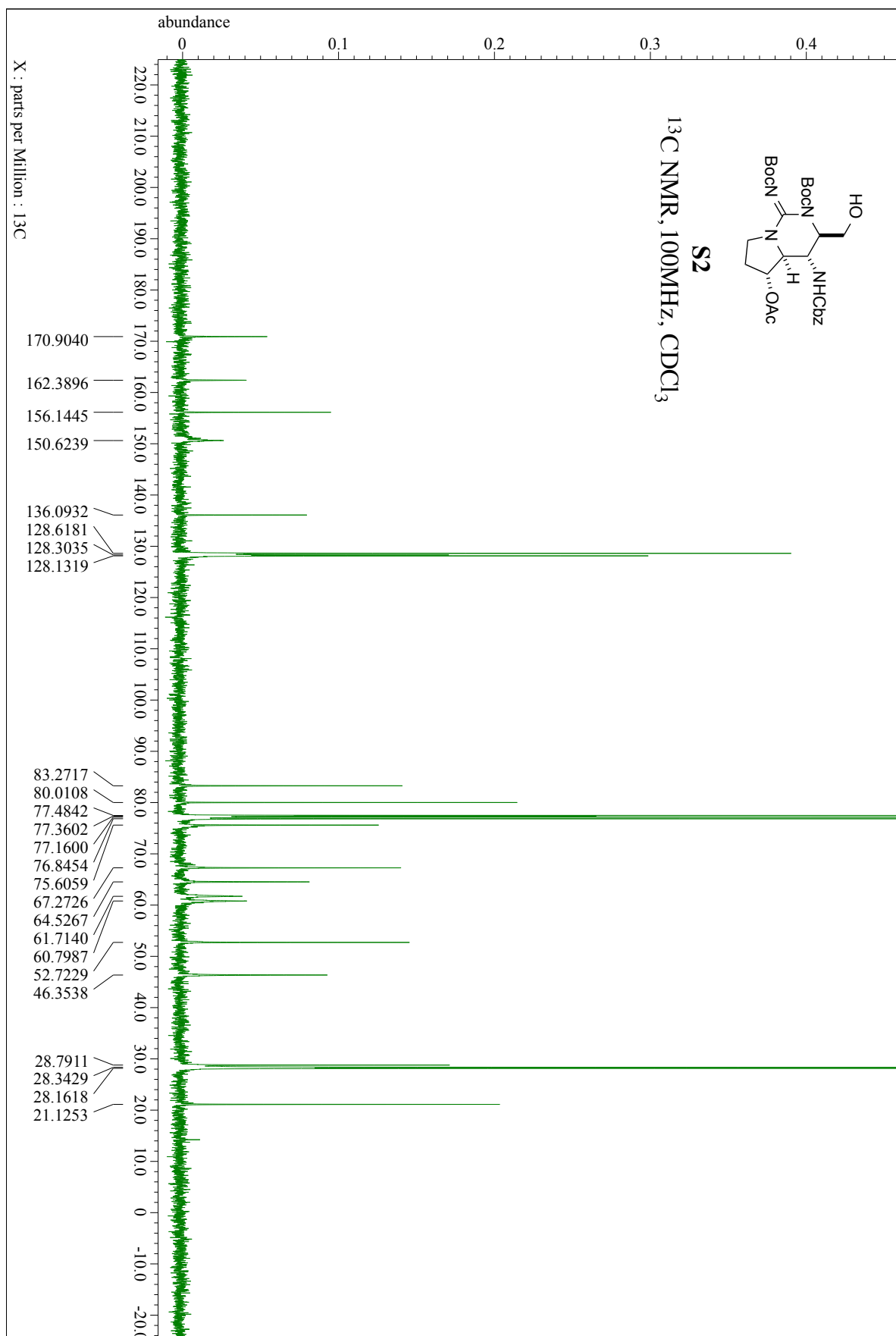


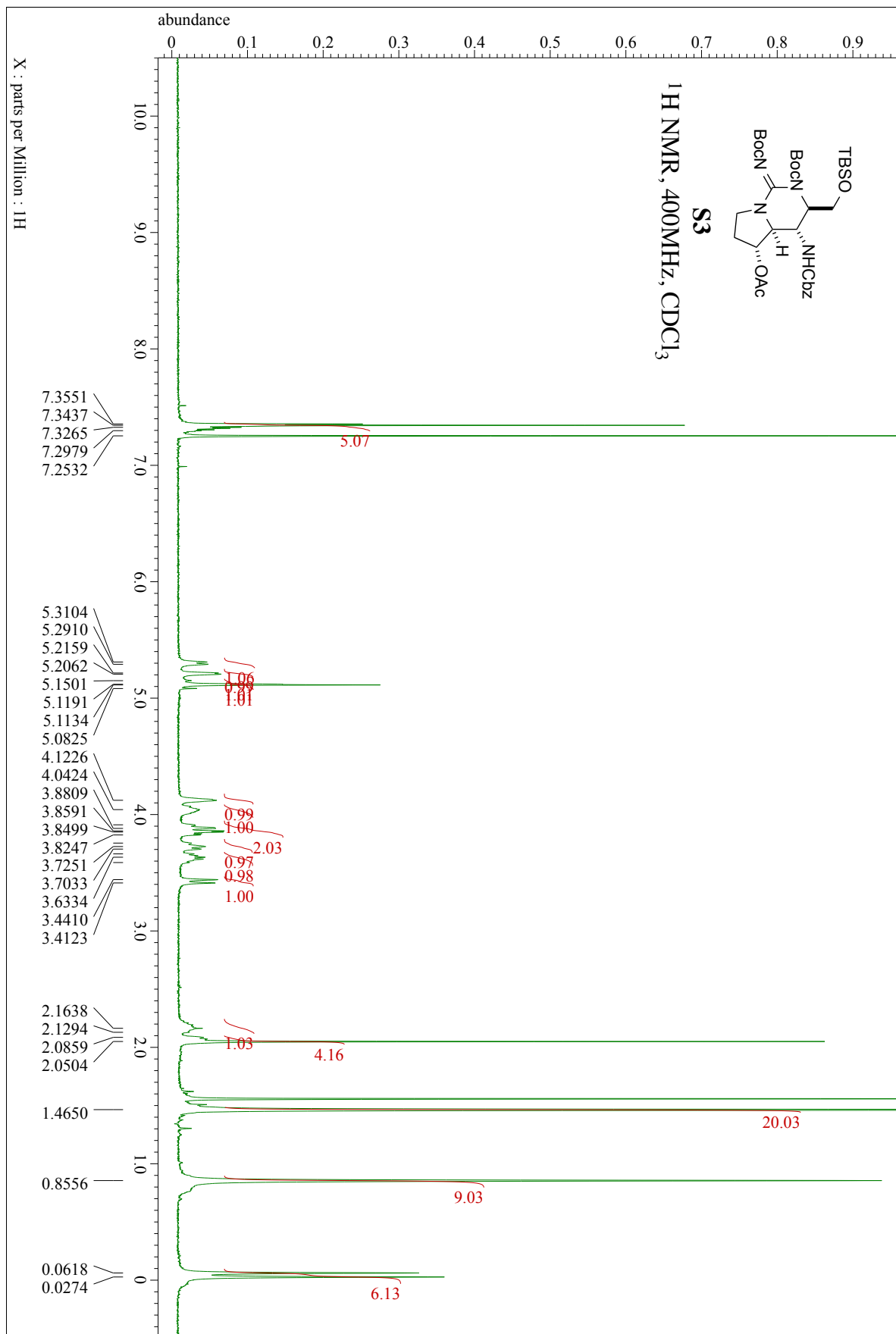


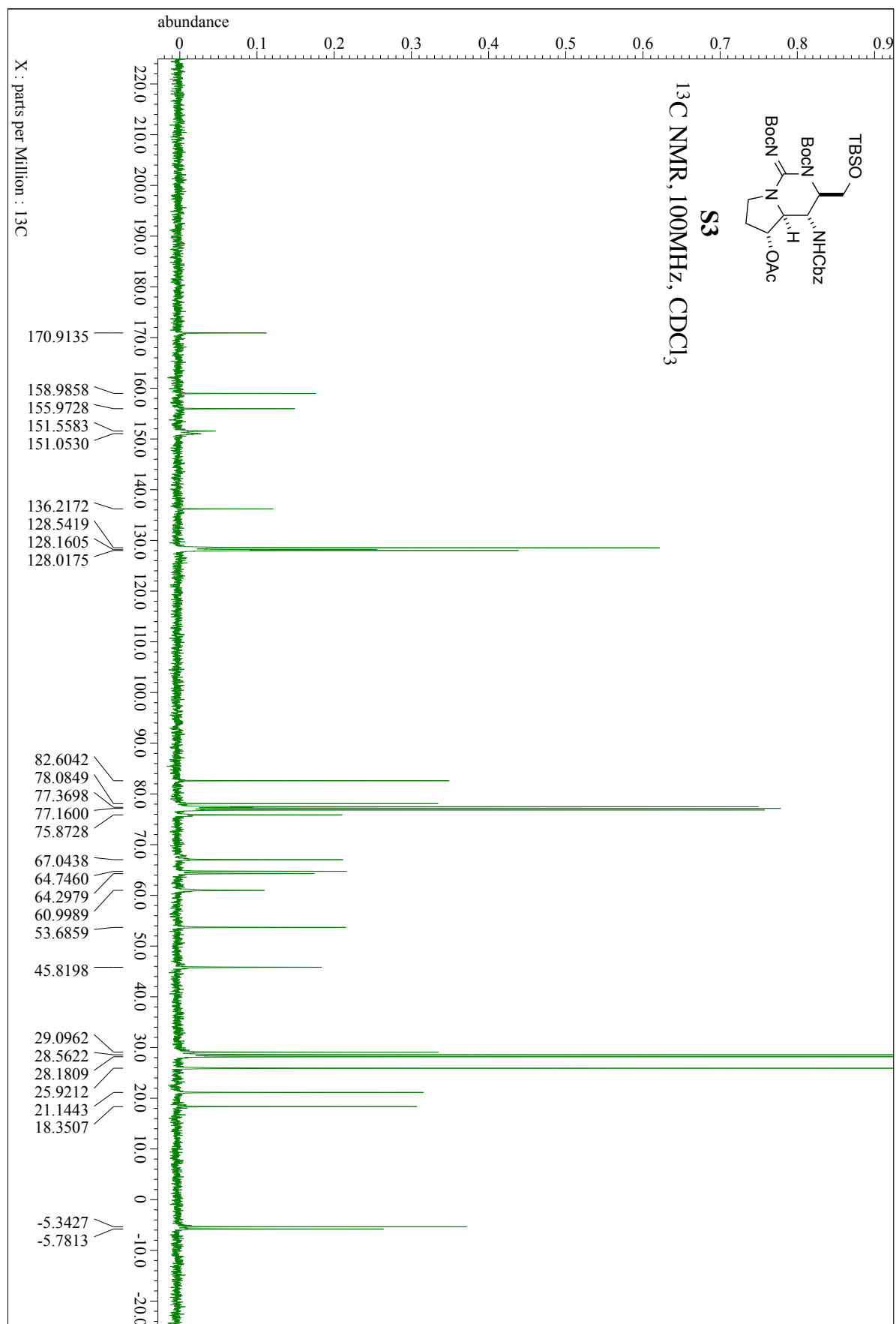


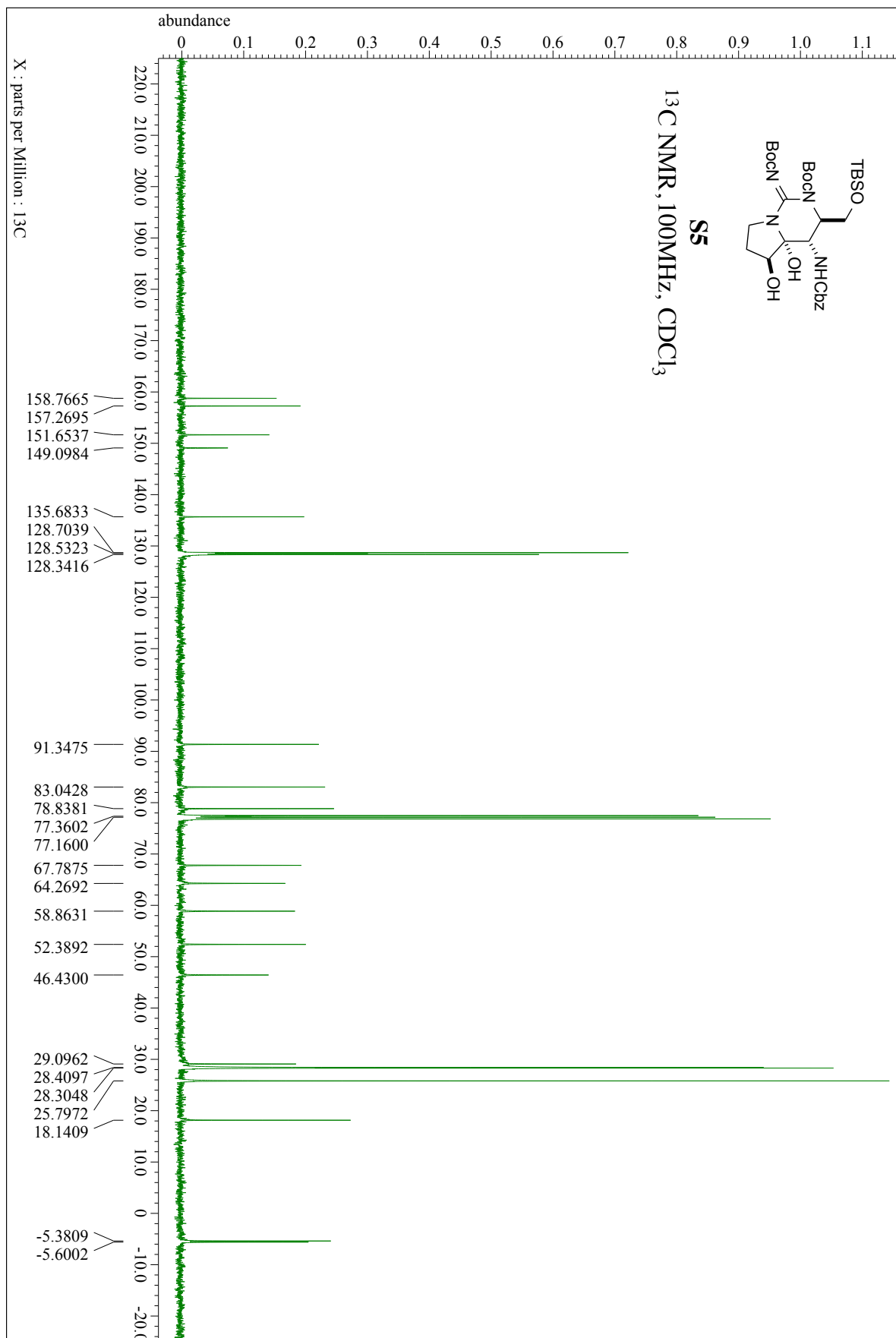


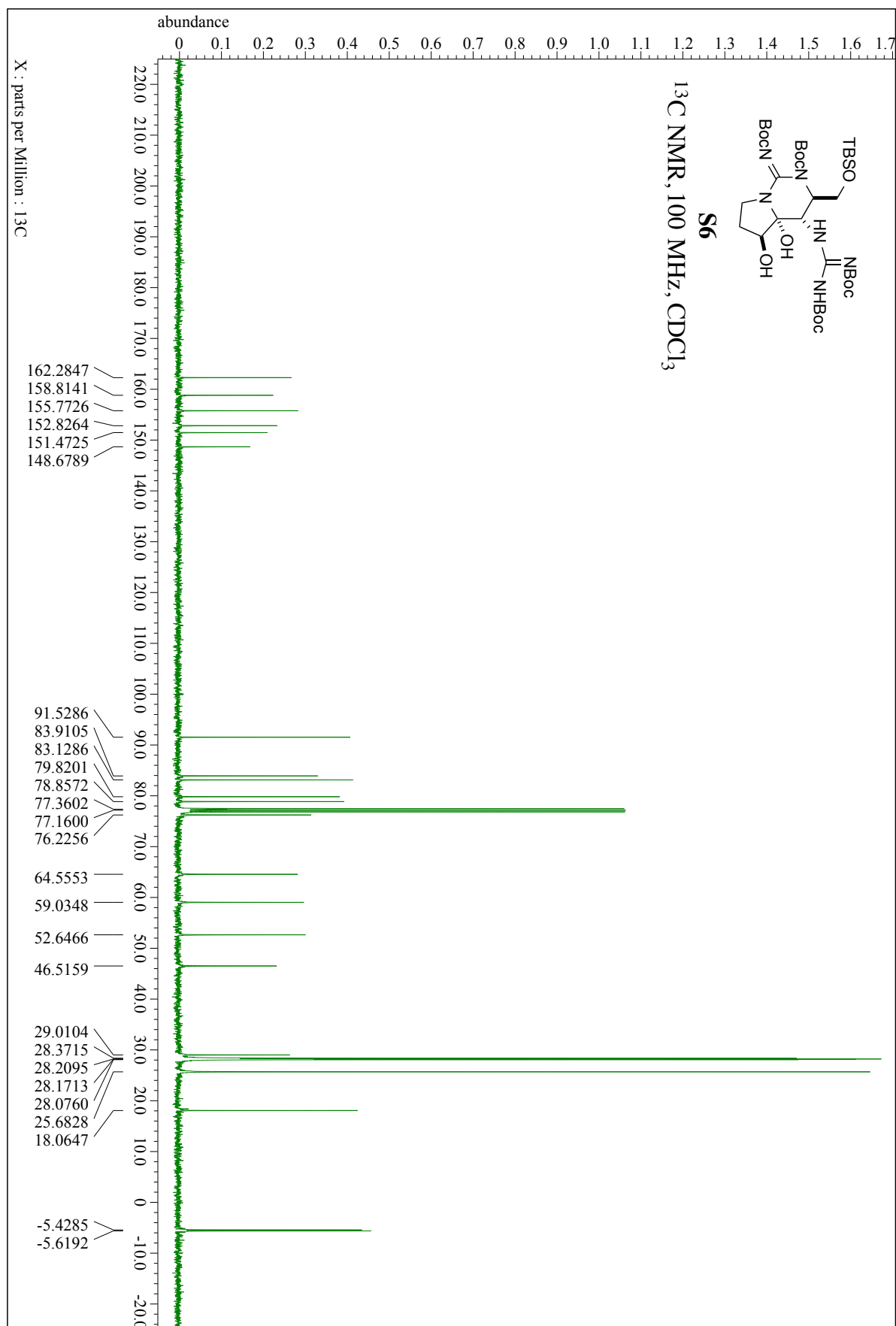


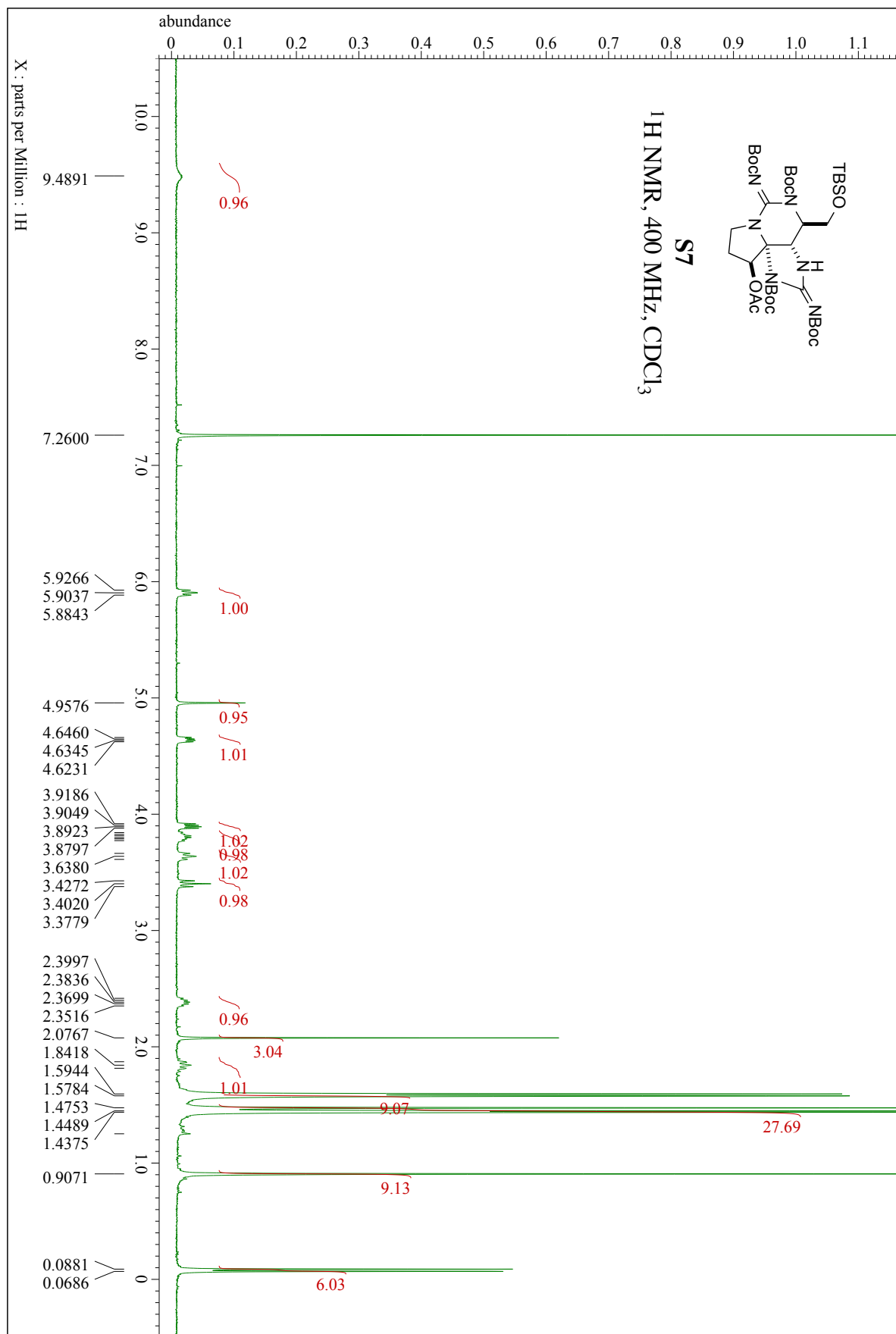


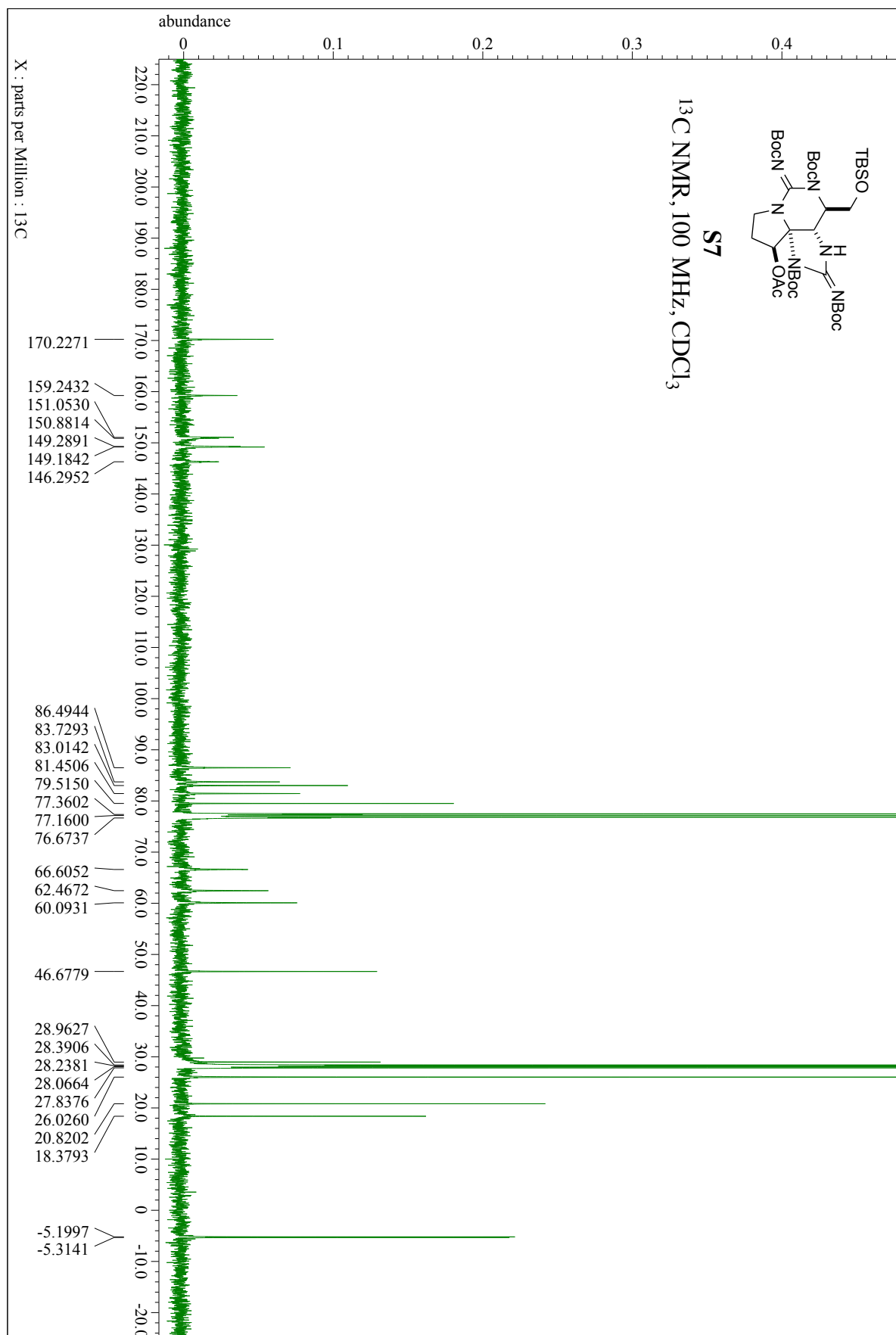


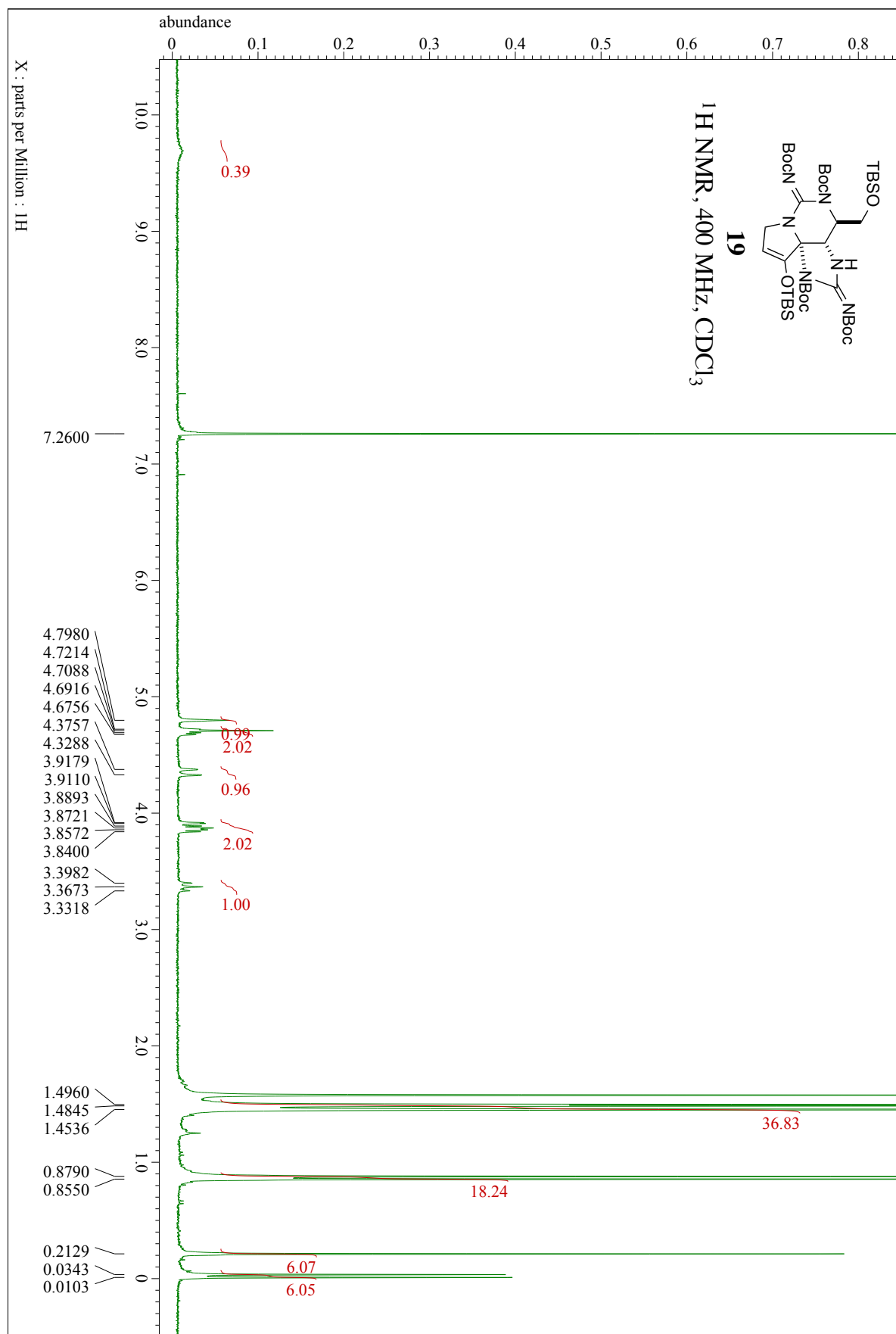


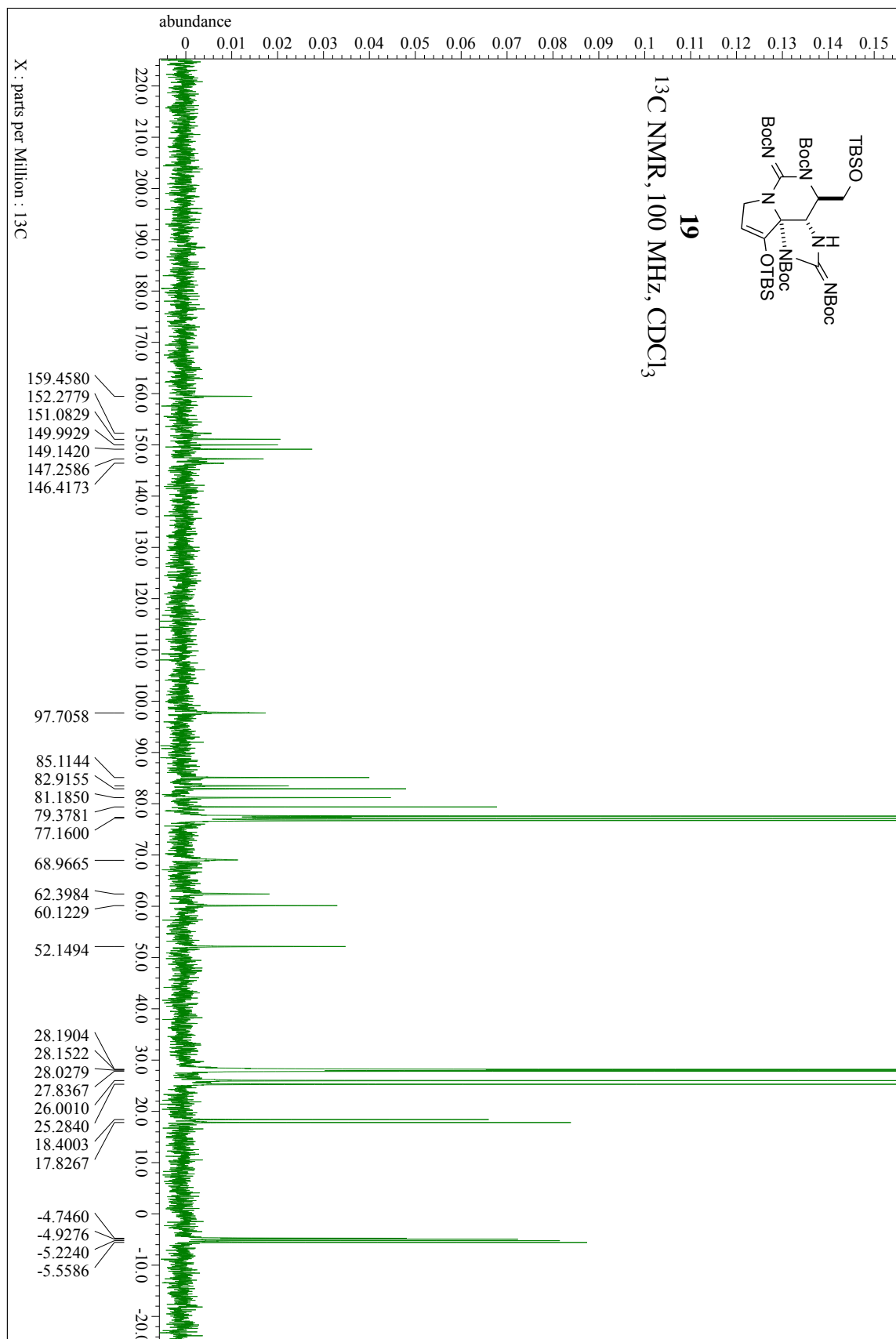


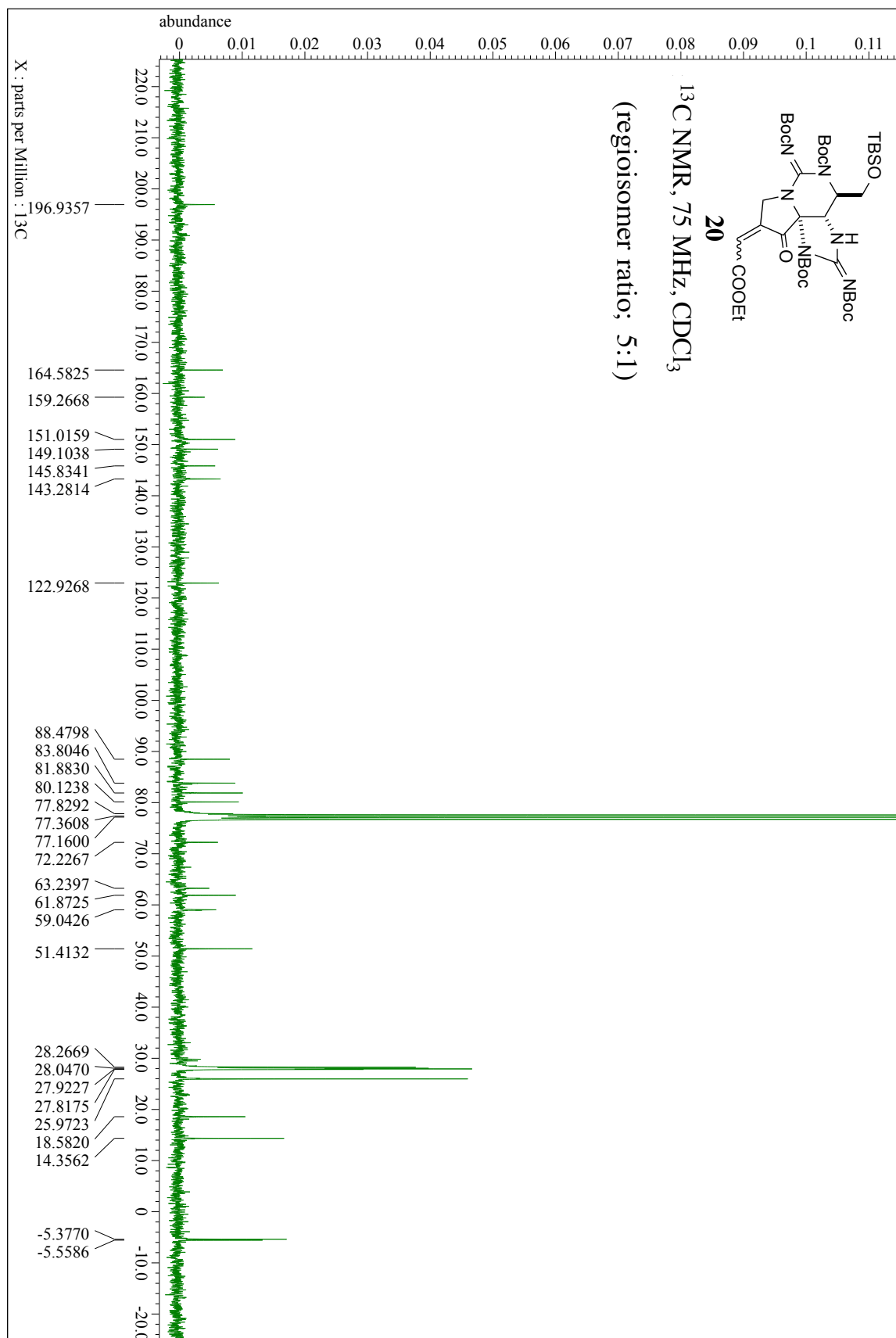


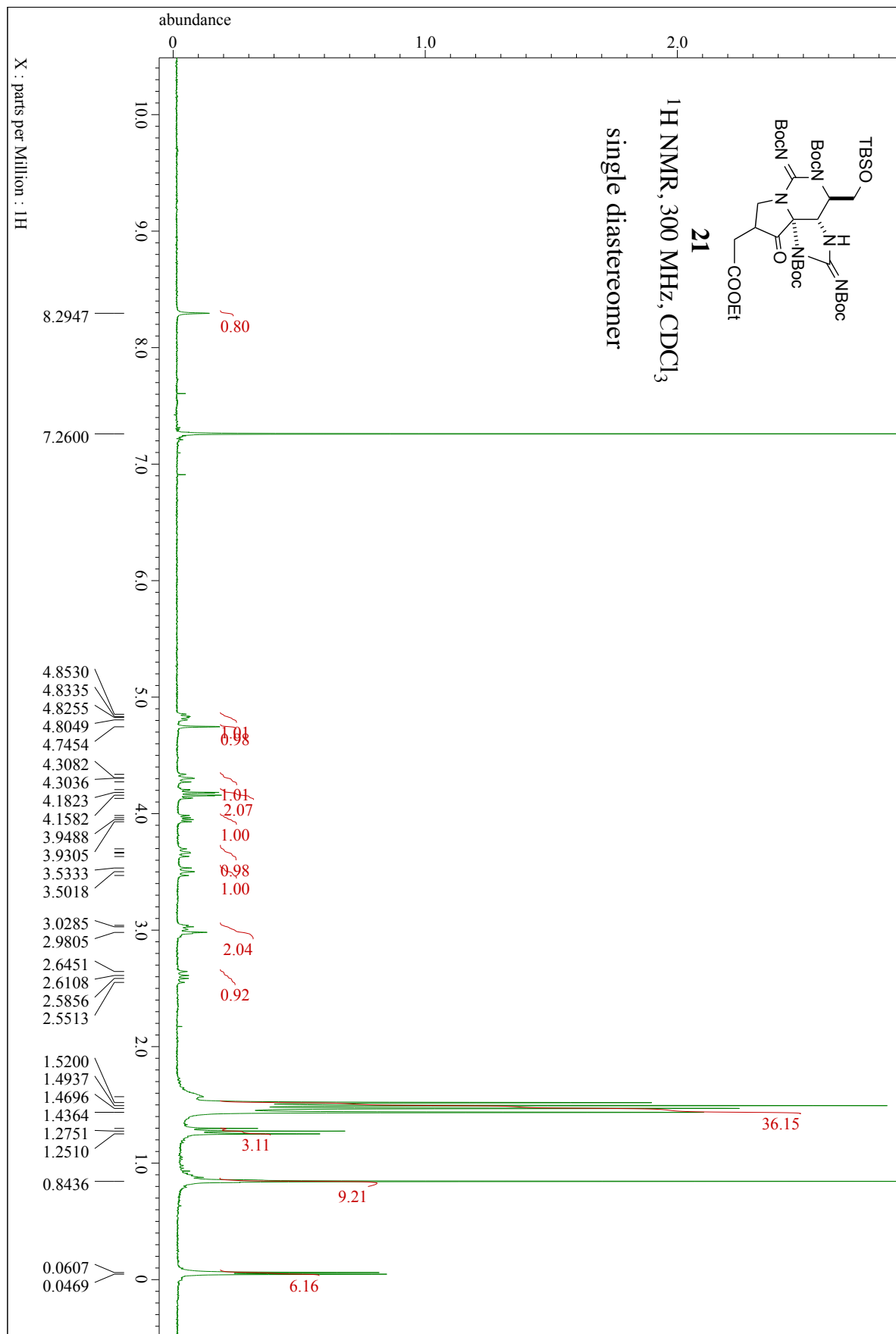


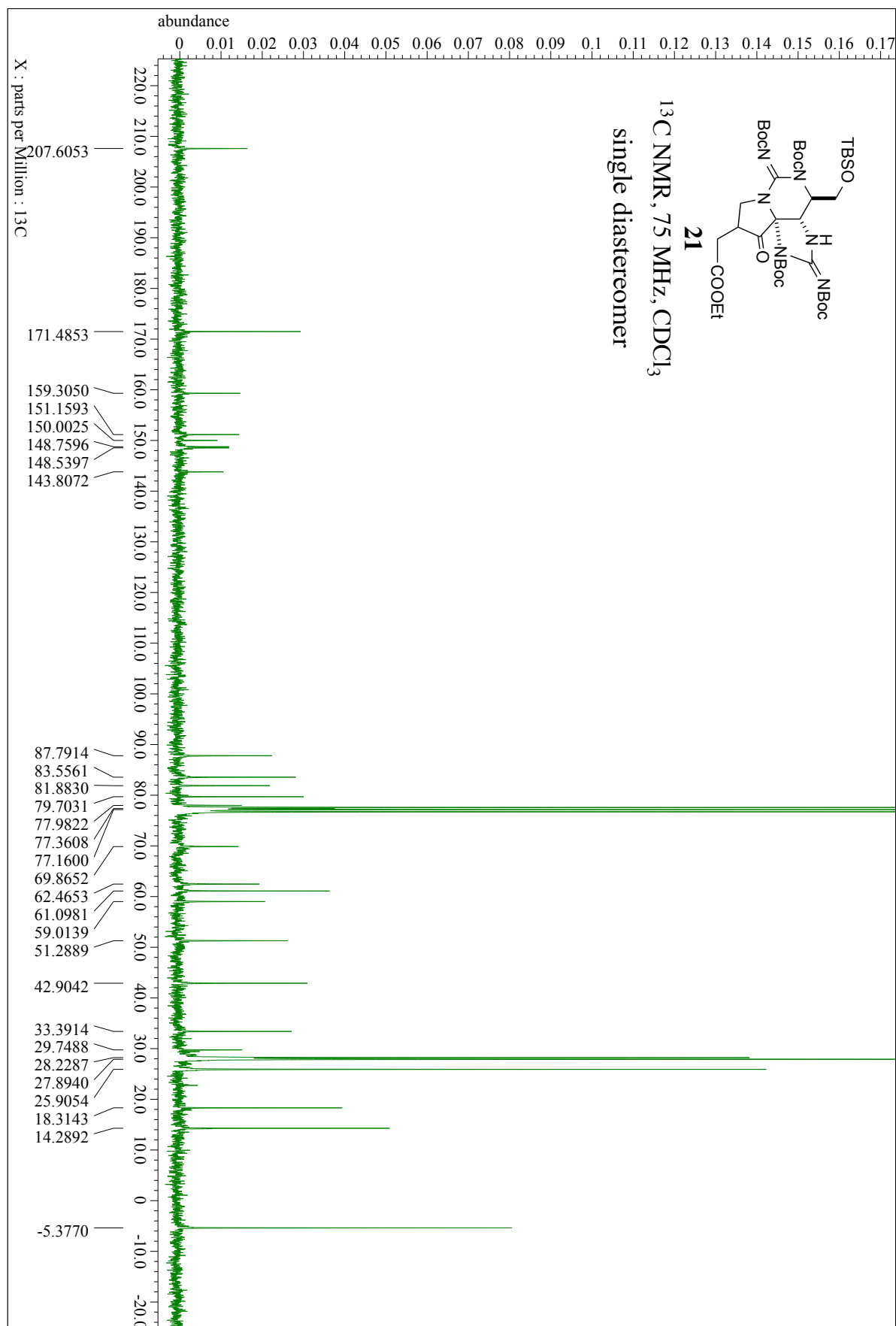


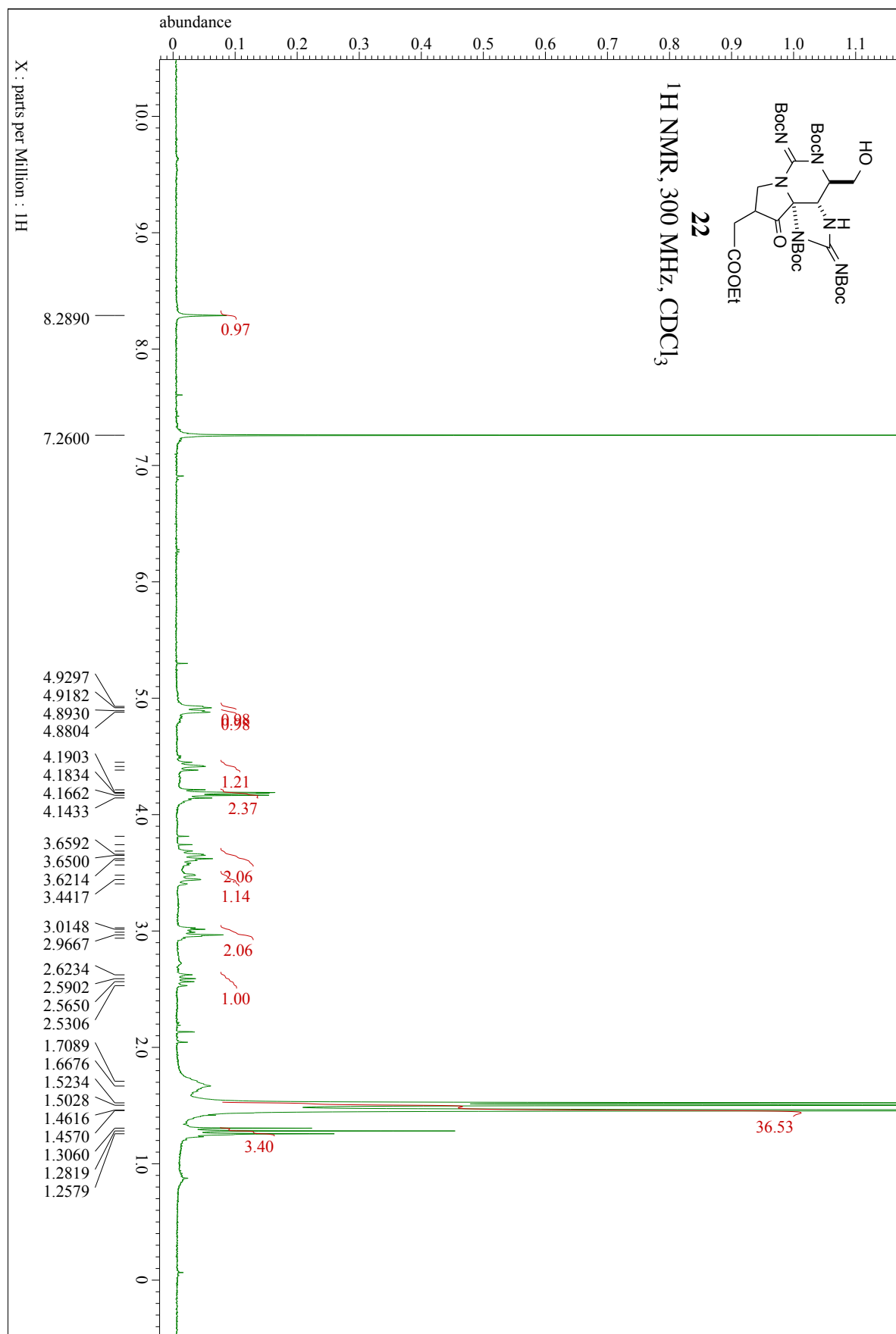


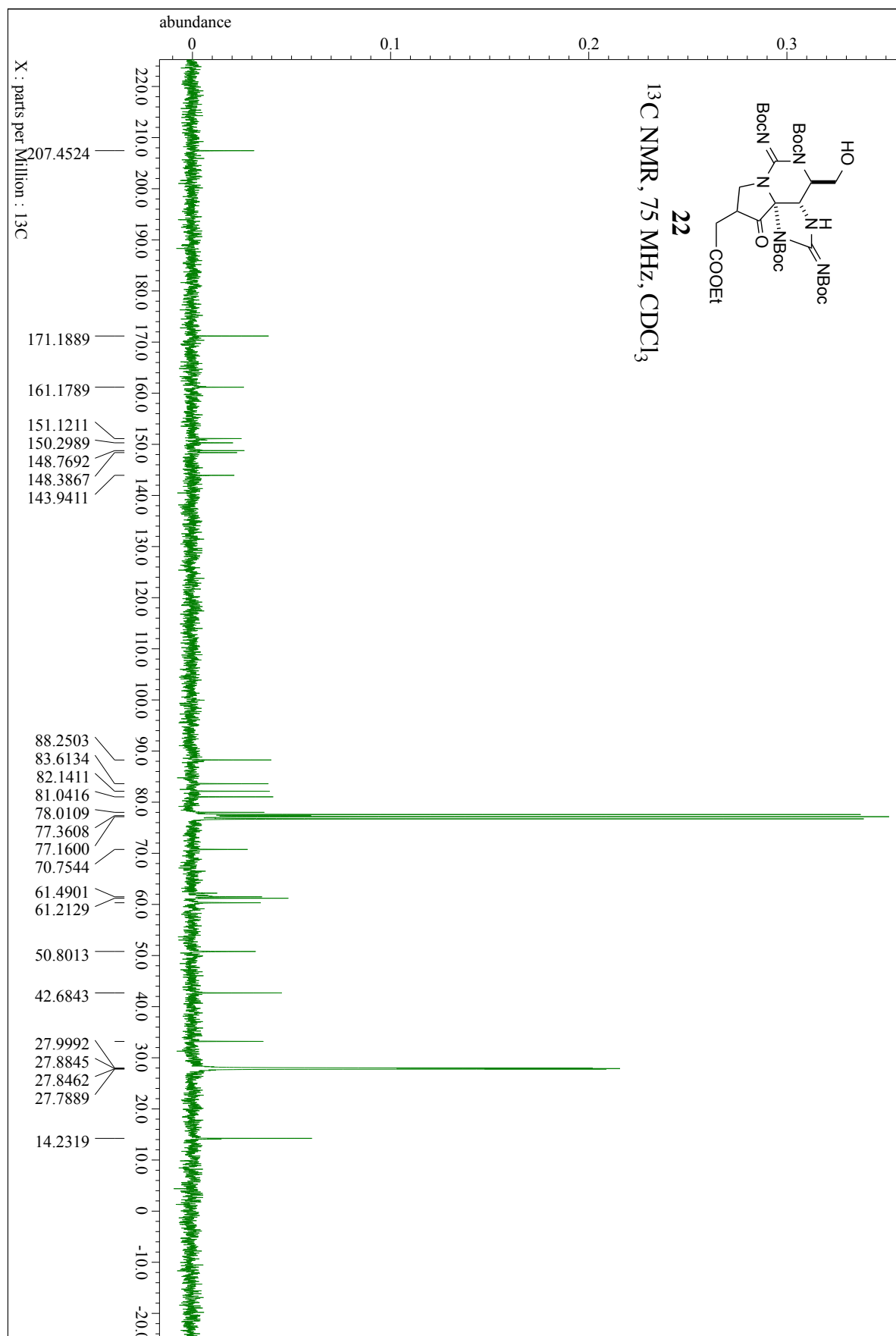


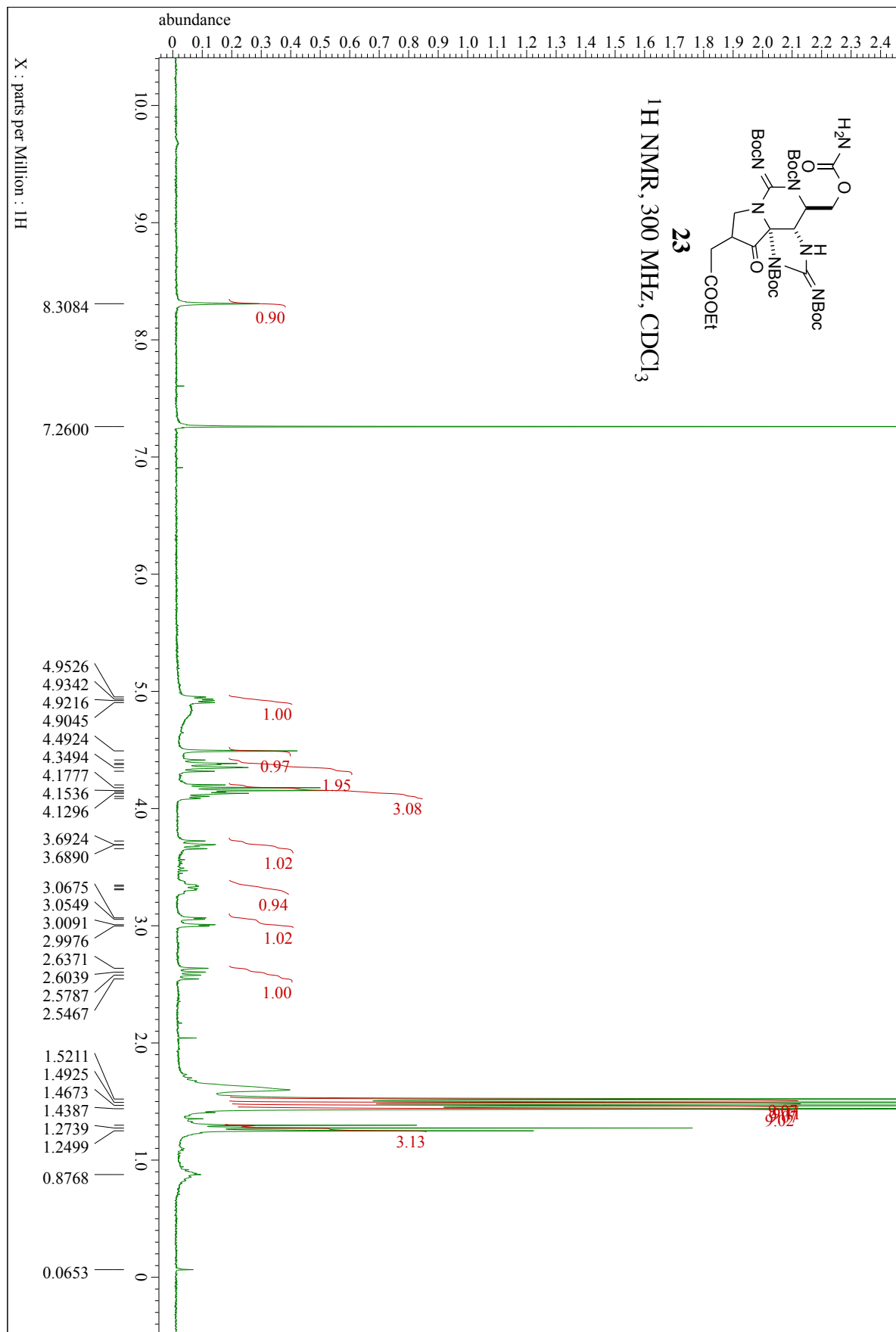


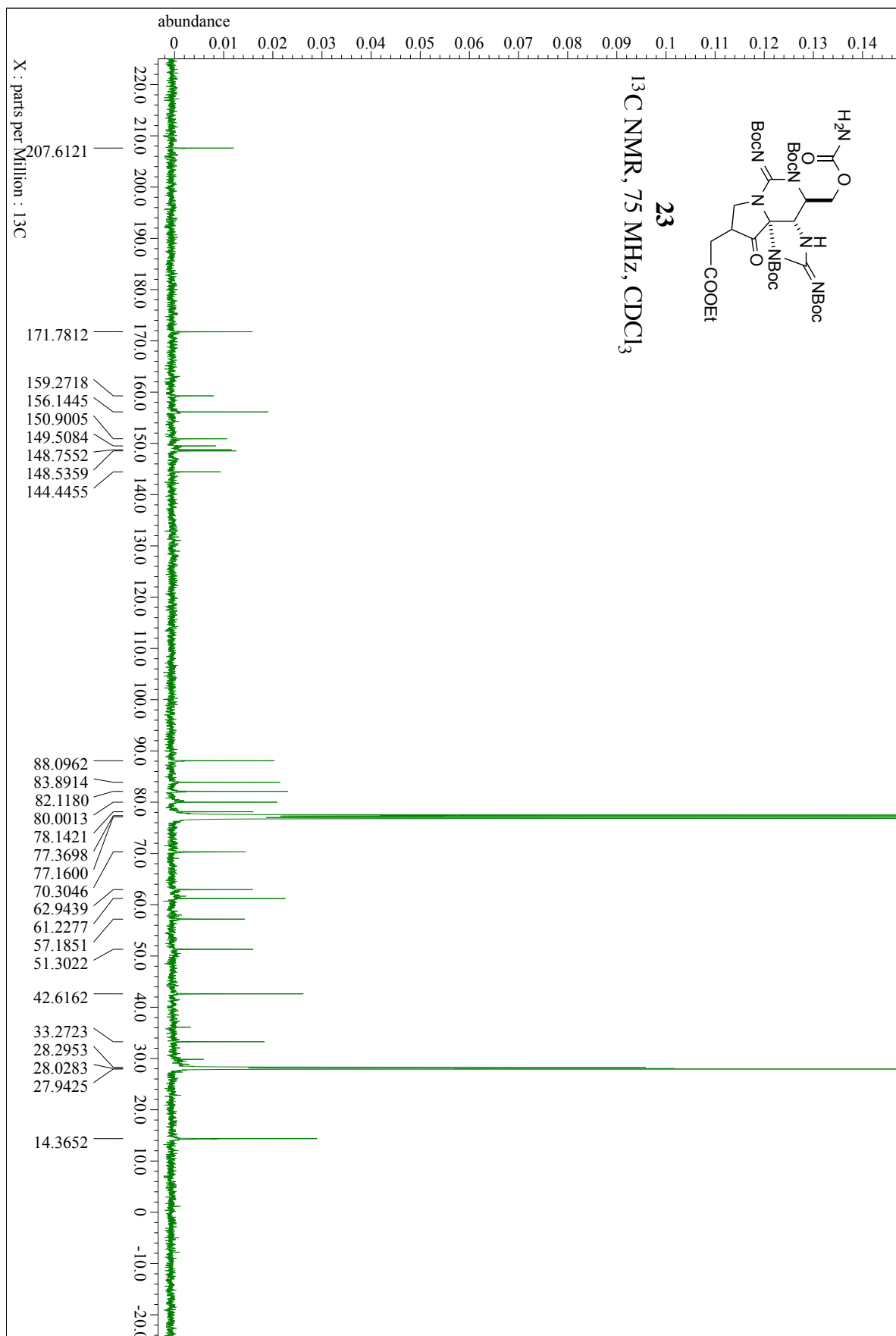


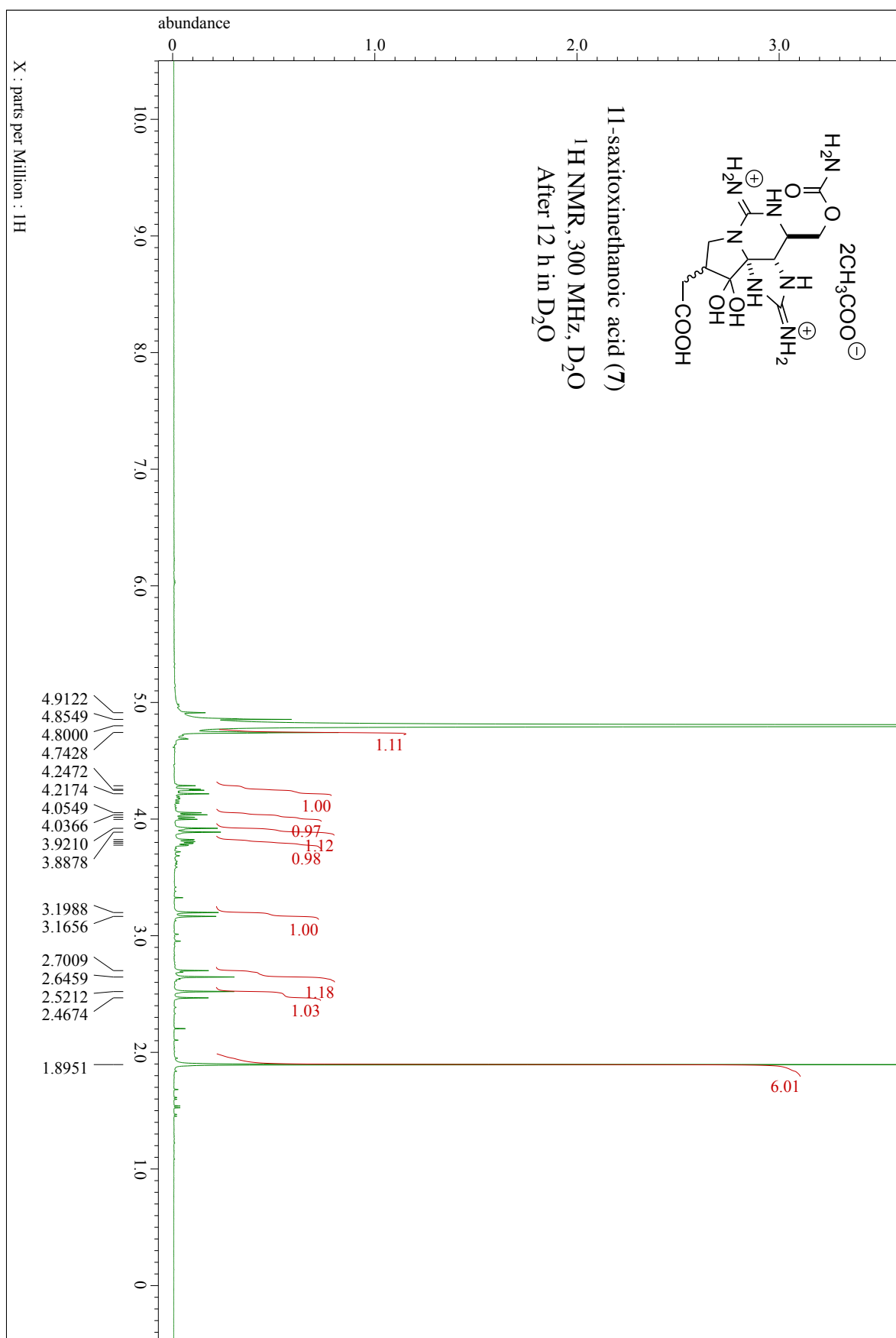




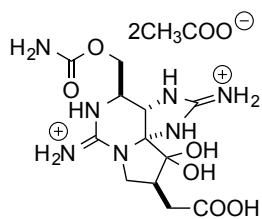
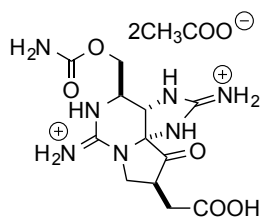




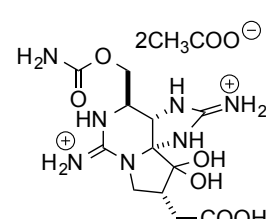
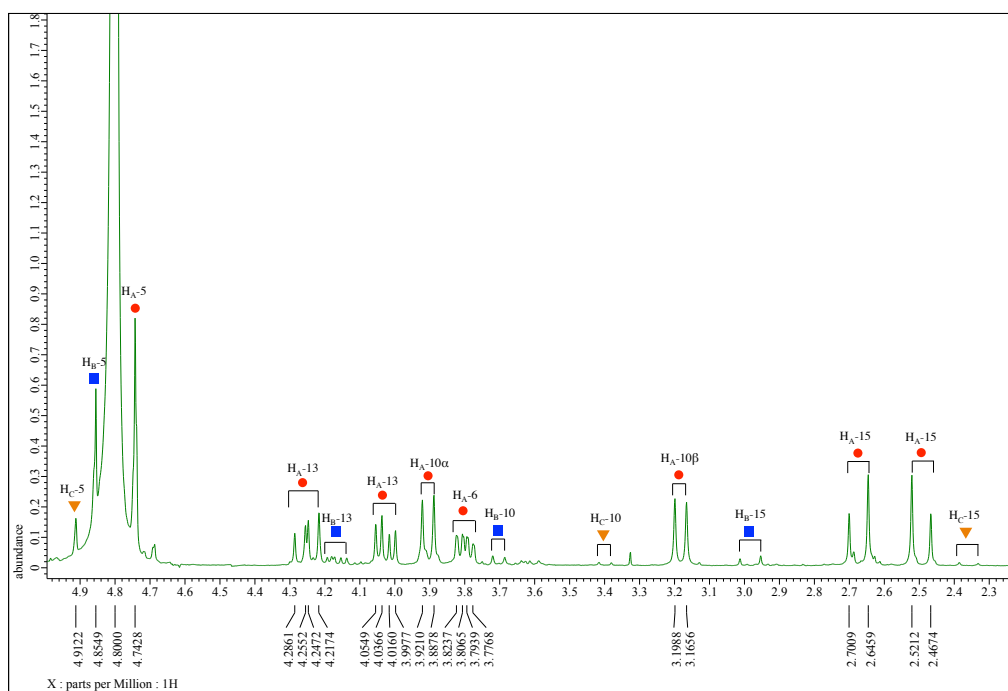


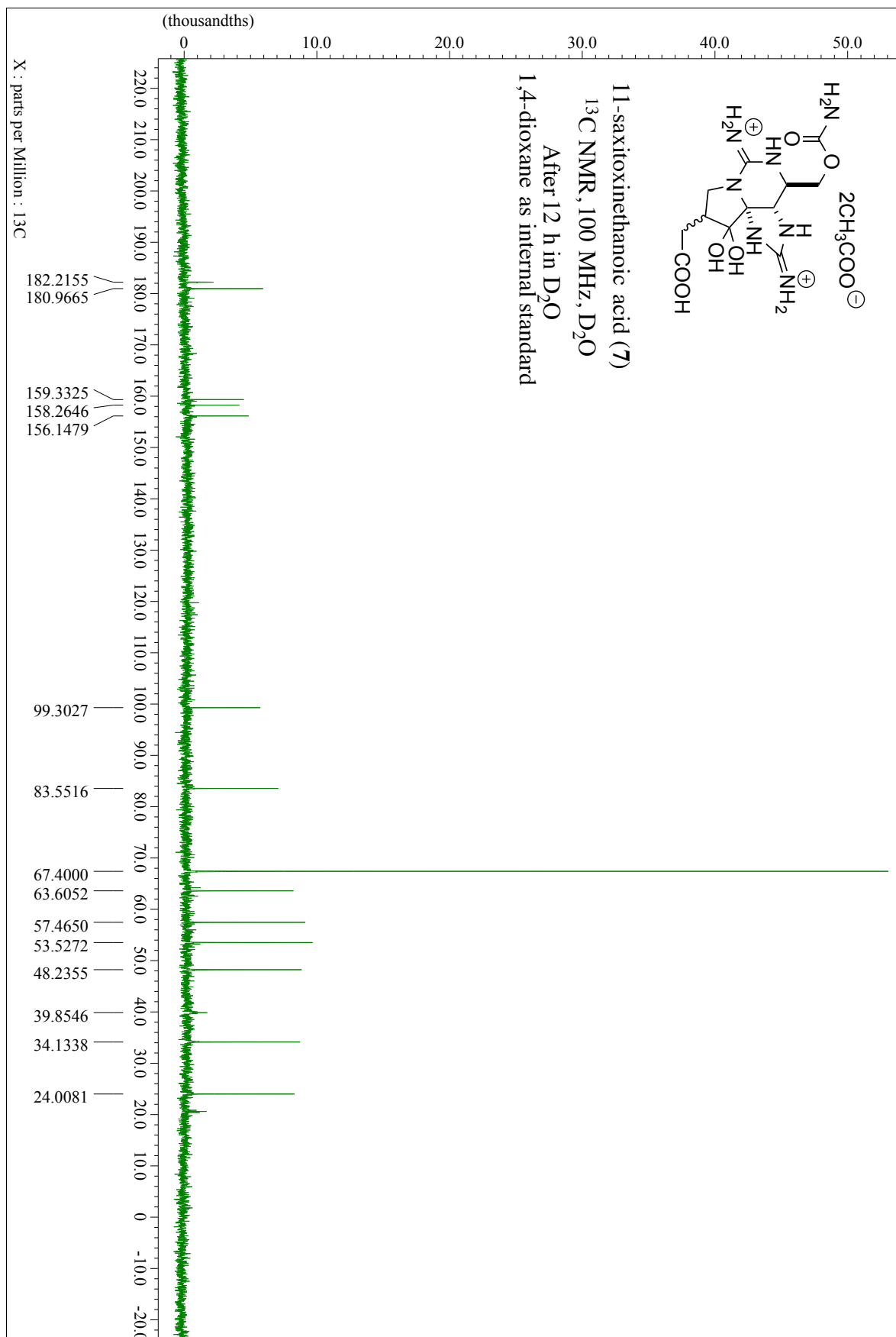


Precise assignment of 7

 (β) -SEA

SEA (keto form)

 (α) -SEA



Spectral data for synthetic and natural SEA (7)**¹H NMR data for synthetic and natural SEA (7).**

After H-D exchange, signal of H-11 was disappeared.

	Synthetic	Natural
Position	¹ H	¹ H
5	4.74 (s, 1H)	4.74 (s, 1H)
6	3.80 (dd, <i>J</i> = 5.2, 8.9 Hz, 1H)	3.80 (dd, <i>J</i> = 5, 9 Hz, 1H)
10	3.90 (d, <i>J</i> = 10 Hz, 1H); 3.18 (d, <i>J</i> = 10 Hz, 1H)	3.91 (d, <i>J</i> = 10 Hz, 1H); 3.18 (d, <i>J</i> = 10 Hz, 1H)
11	----	----
13	4.25 (dd, <i>J</i> = 9.3, 11.7 Hz, 1H) 4.02 (dd, <i>J</i> = 5.5, 11.7 Hz, 1H)	4.24 (dd, <i>J</i> = 9, 12 Hz, 1H) 4.03 (dd, <i>J</i> = 5, 12 Hz, 1H)
15	2.68 (d, <i>J</i> = 16.5 Hz, 1H) 2.49 (d, <i>J</i> = 16.1 Hz, 1H)	2.68 (d, <i>J</i> = 16 Hz, 1H) 2.51 (d, <i>J</i> = 16 Hz, 1H)

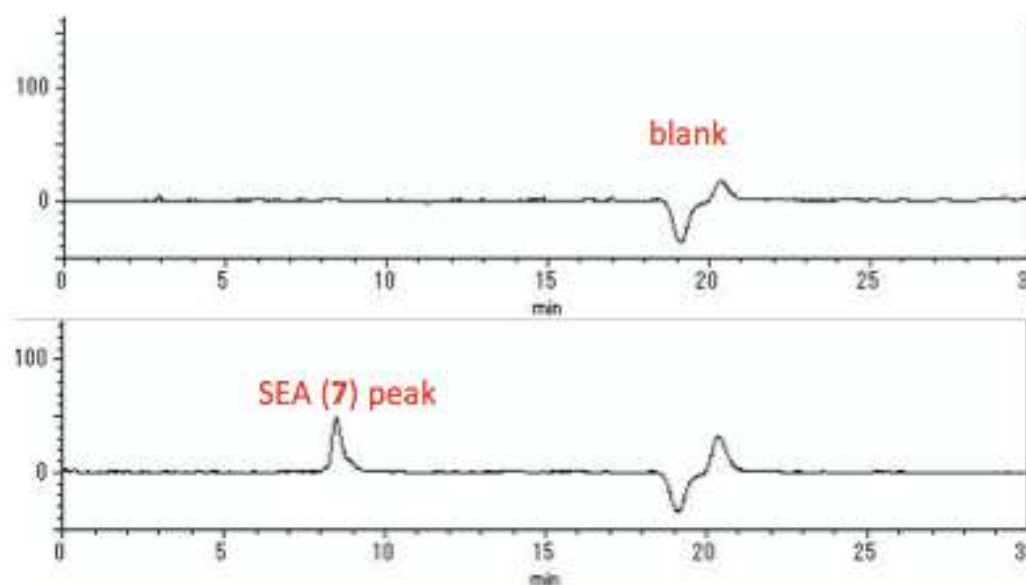
¹³C NMR data for synthetic and natural SEA (7)

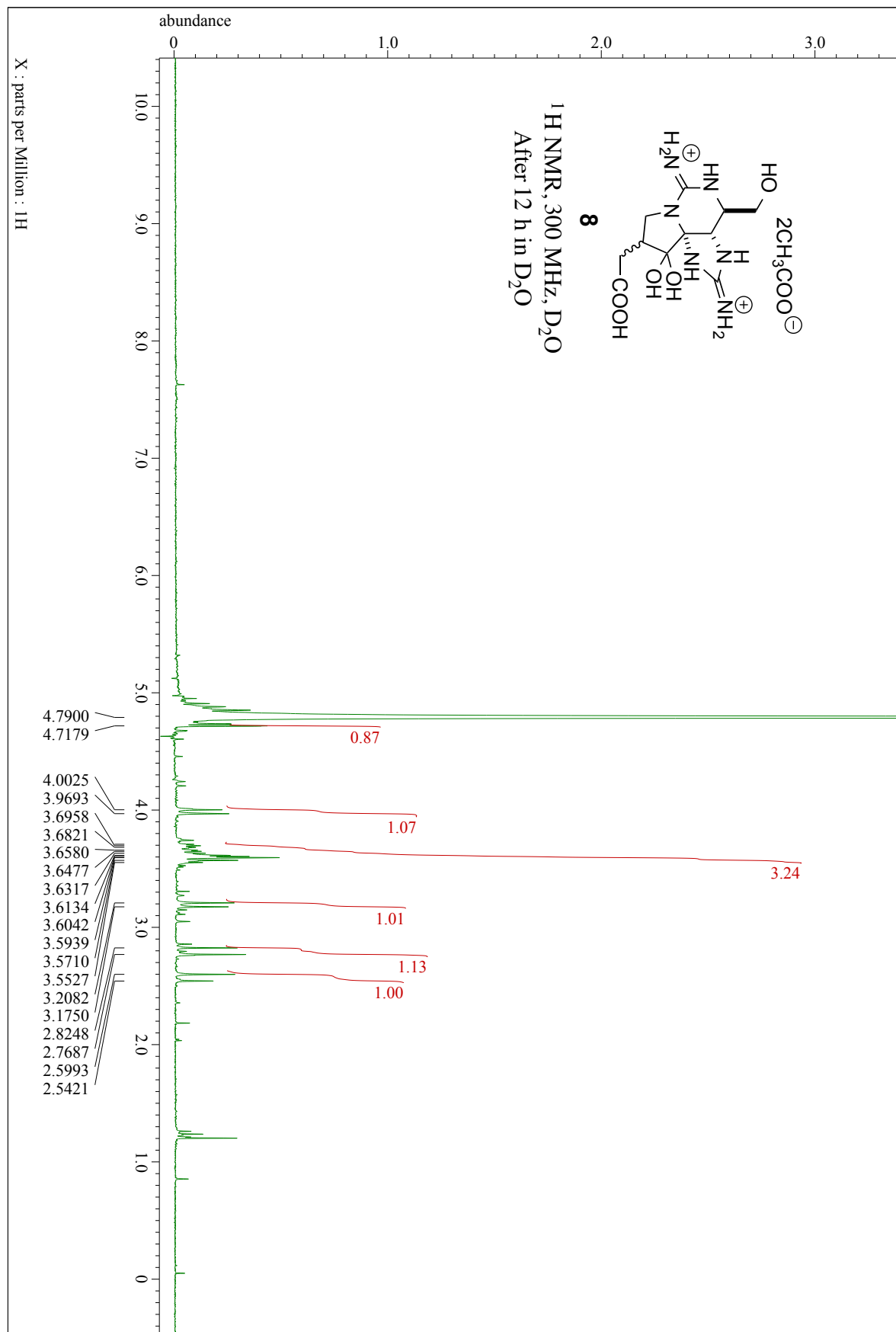
	Synthetic	Natural
Position	¹³ C NMR	¹³ C NMR
C-2	156.1	156.2
C-4	83.6	83.5
C-5	57.5	57.5
C-6	53.5	53.5
C-8	158.3	158.3
C-10	48.2	48.2
C-11	39.9	39.9
C-12	99.3	99.3
C-13	63.6	63.6
C-14	159.3	159.3
C-15	34.1	33.9
C-16*	182.2	180.6

* The ¹³C NMR chemical shift values of C16 were greatly influenced by pH.

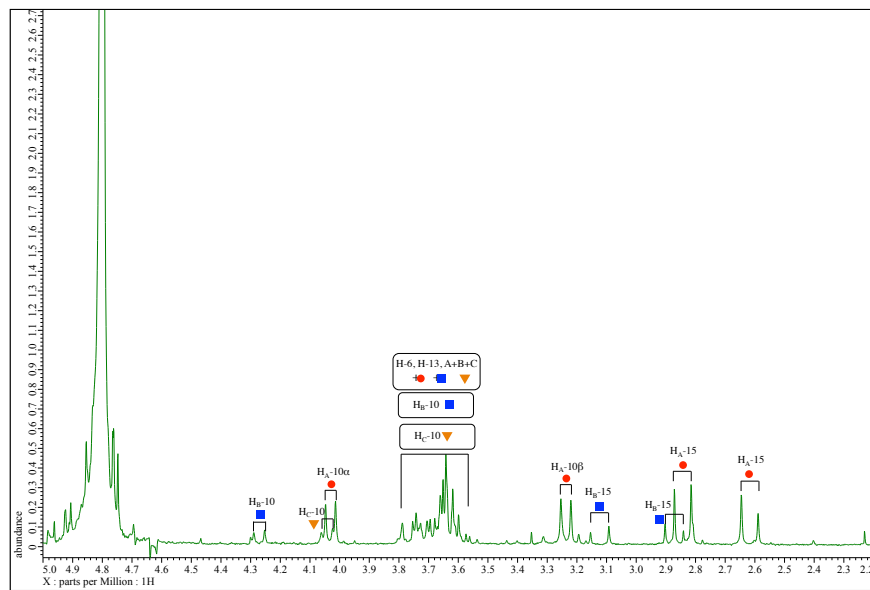
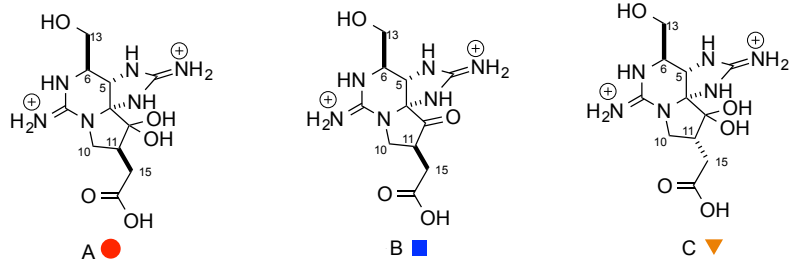
HPLC data of the SEA (7)

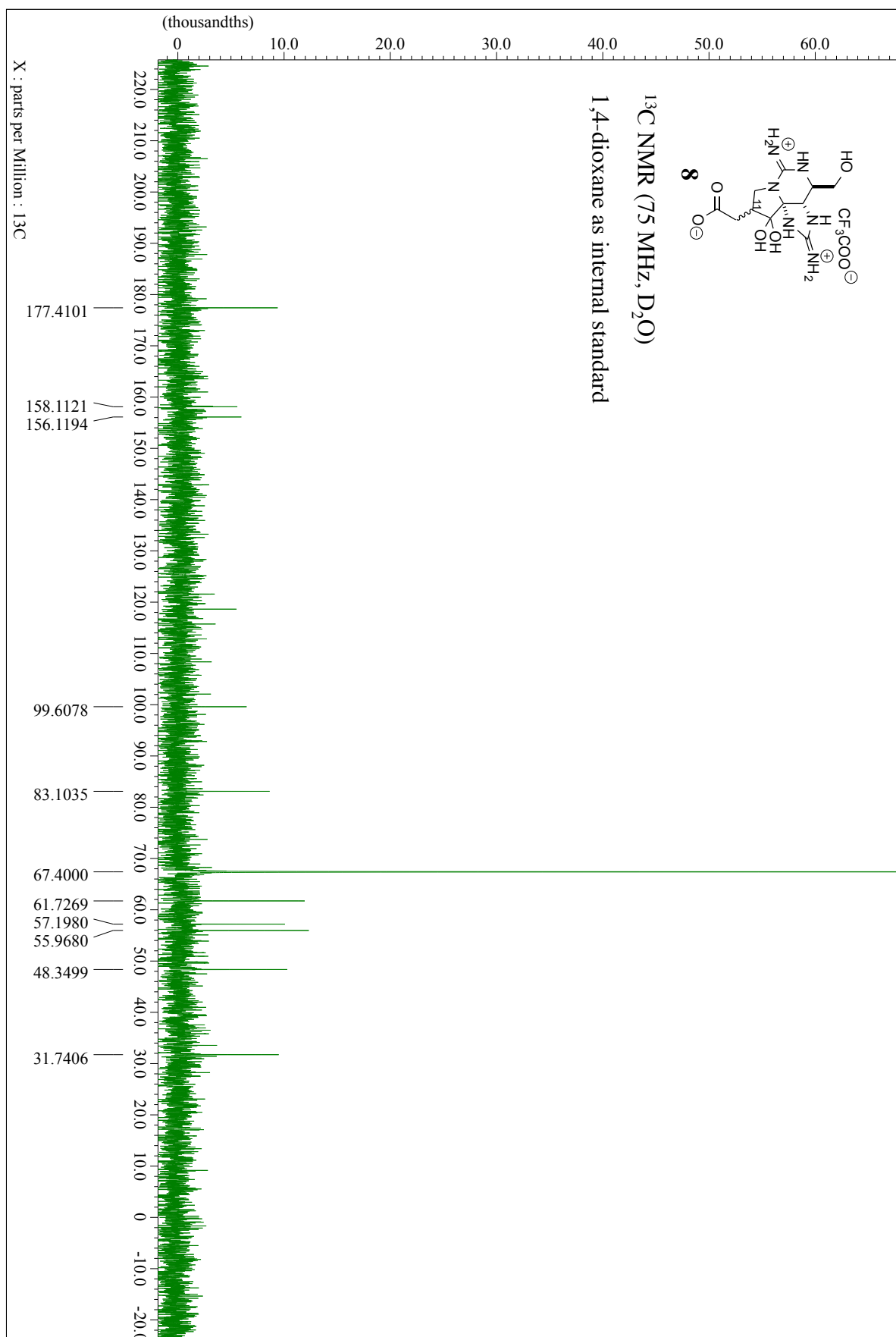
The purity of the purified SEA (7) was checked by using reverse phase HPLC (CH₃CN: 0.1% CH₃COOH aq. = 15: 85, Shiseido Cancel Pak AQ C18 column, Rt = 8.5 min, 214 nm UV detection).

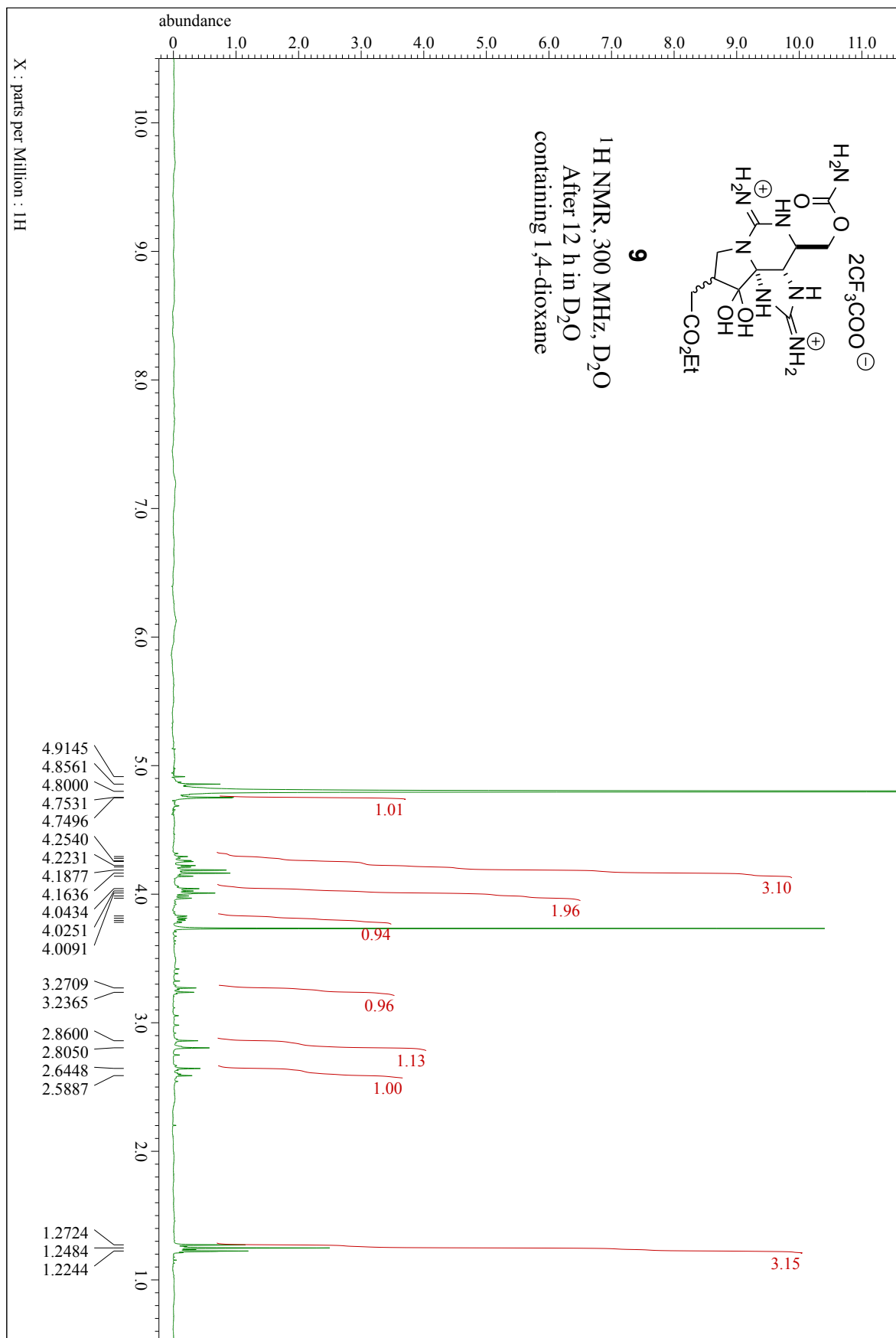




Precise assignment of 8







Precise assignment of **9**