



Design, synthesis and biological evaluation of photoaffinity probes of antiangiogenic homoisoflavonoids

Bit Lee^a, Wei Sun^a, Hyungjun Lee^a, Halesha Basavarajappa^{b,c}, Rania S. Sulaiman^{b,d,e}, Kamakshi Sishtla^b, Xiang Fei^a, Timothy W. Corson^{b,c,d}, and Seung-Yong Seo^{a,*}

^a College of Pharmacy and Gachon Institute of Pharmaceutical Sciences, Gachon University, Incheon 21936, South Korea

^b Eugene and Marilyn Glick Eye Institute, Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

^c Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

^d Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

^e Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

homoisoflavonoids

photoaffinity probes

antiangiogenic agents

human retinal microvascular endothelial cells

wet age-related macular degeneration

ABSTRACT

Cremastranone (**1**), a naturally occurring homoisoflavonoid, inhibited angiogenesis in vitro and in vivo. We developed an analogue SH-11037 (**2**) which is more potent than cremastranone in human retinal microvascular endothelial cells (HRECs) and blocks neovascularization in animal models. Despite its efficacy, the mechanism of this compound is not yet fully known. In the course of building on a strong foundation of SAR and creating a novel chemical tool for target identification of homoisoflavonoid-binding proteins, various types of photoaffinity probes were designed and synthesized in which benzophenone and biotin were attached to homoisoflavonoids using PEG linkers on either the C-3' or C-7 position. Notably, the photoaffinity probes linking on the phenol group of the C-3' position retain excellent activity of inhibiting retinal endothelial cell proliferation with up to 72 nM of GI₅₀.

2016 Elsevier Ltd. All rights reserved.

The identification of target proteins binding bioactive small molecules is an essential task in drug development that can extend the understanding of mechanisms and provide information for designing new drug candidates.¹⁻⁹ Among many approaches to identify cellular targets of bioactive molecules, biochemical affinity purification using synthetic affinity probes that mimic the structure of the bioactive compounds is a straightforward and powerful method for identifying target molecules. Recently, photoaffinity labeling has been considered a more efficient variant of this method, as it can enrich for low-abundance or transiently-interacting targets.¹⁰⁻¹⁴ So far, there have been a number of successful examples that have determined the target molecules and identified the binding site through the formation of a covalent bond between the ligand and the specific protein.¹⁵⁻²³ In general, photoaffinity probes contain three functional groups: a bioactive scaffold, a photoreactive group and a reporter tag.²⁴ A biotin tag is widely employed as a reporter tag because it binds streptavidin or avidin with extremely high affinity.

Cremastranone (**1**), a naturally occurring homoisoflavonone is reported to have antiangiogenic activity with some selectivity for blocking proliferation of human retinal endothelial cells over

other ocular cell types.²⁵⁻²⁸ It inhibits ocular angiogenesis, without substantial toxicity, in mouse models of laser-induced choroidal neovascularization (L-CNV) and oxygen-induced retinopathy (OIR). These animal models recapitulate features of the blinding eye diseases wet age-related macular degeneration and retinopathy of prematurity, respectively. Cremastranone impinges on multiple signaling pathways. It induces expression of p21WAF1 (CDKN1A), which inhibits the cyclin-dependent kinase Cdc2 (CDK1).²⁹ It also blocks prostaglandin synthesis and decreases activation of the mitogen activated protein kinases.^{30,31} Finally, it blocks nuclear translocation of NF-κB and production of inflammatory cytokines.^{25,31,32} Seeking more potent and selective compounds than cremastranone, we identified SH-11037 which is observed to have novel antiangiogenic activity against human retinal microvascular endothelial cells (HRECs) and blocks neovascularization in the OIR and L-CNV models.^{27,33} Although the studies of mechanisms and medicinal chemistry could contribute to many therapeutic facets of the antiangiogenic homoisoflavonoids, their exact mechanisms of action and cellular targets remain unknown, so the search for direct interacting proteins is valuable.^{34,35} Herein we report design and synthesis of homoisoflavonoid-biotin conjugates for inhibiting proliferation

* Corresponding author. Tel.: +82-32-820-4949; fax: +82-32-820-4829; e-mail: syseo@gachon.ac.kr

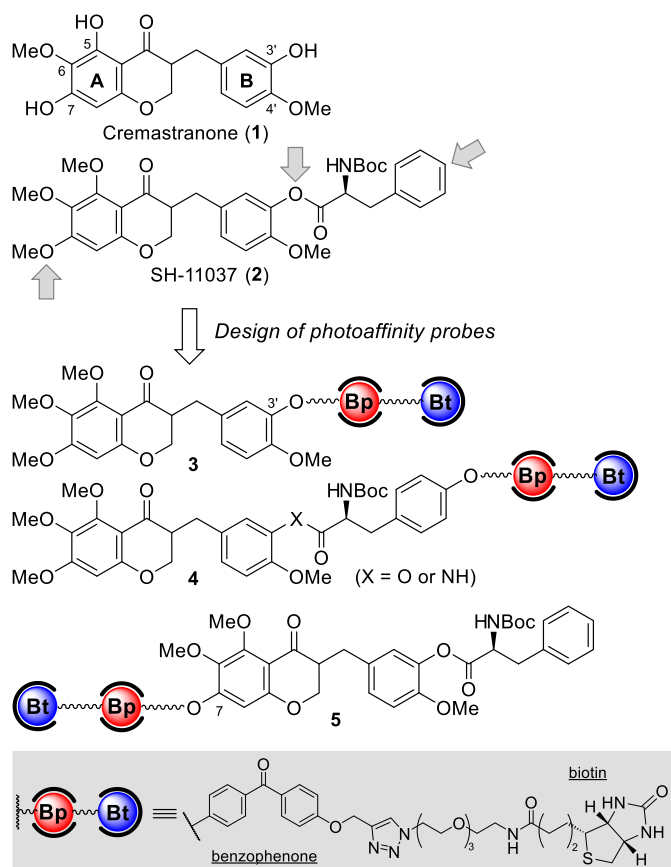


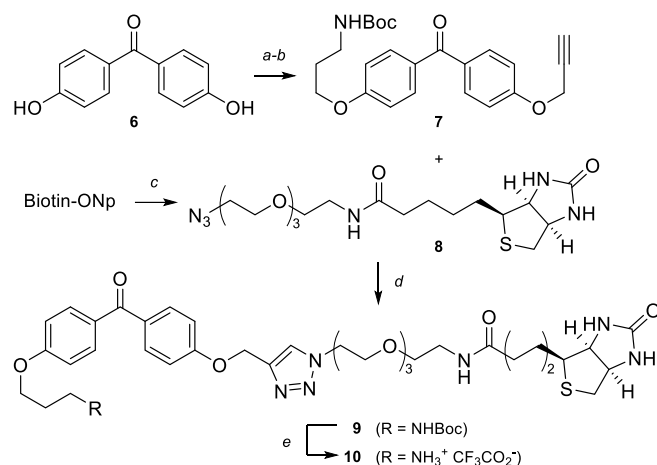
Figure 1. Structures of cremastranone (**1**), homoisoflavanone analogue SH-11037 (**2**) and photoaffinity probes (**3-5**). Bp: benzophenone, Bt: biotin.

of endothelial cells and identifying targets, adapting a synthetic strategy we previously employed to generate homoisoflavanones.

On the basis of our SAR investigations in which the A and B-ring modifications of homoisoflavanoids with trimethoxy and Boc-phenylalanyl groups, respectively, had the most beneficial effects on its activities and selectivities, trifunctional photoaffinity probes were designed. We synthesized three kinds of photoaffinity probes (**3-5**) by linking a trimethoxy derivative of cremastranone and peptidylated active analog SH-11037 (**2**) to benzophenone and biotin (Figure 1) plus a control compound which lacks the homoisoflavanone moiety, for the future goal of detecting proteins that interact with the homoisoflavanoid-labeled photoaffinity probes in cell lysates. In particular, we modified the homoisoflavanone scaffold at both C-7 and C-3' position with a benzophenone photoreactive group for crosslinking and a biotin reporter group through polyethylene glycol (PEG) as a linker. Thus, it was initially considered to prepare a photoaffinity reagent linking on the phenolic OH group of the C-3' position. It has been noted that the selected

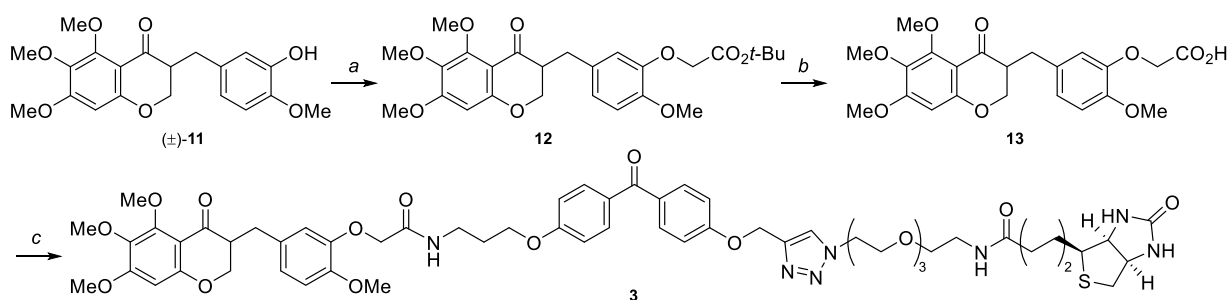
substituents such as *N*-Boc phenylalanyl moiety, which is added to the C-3' position of homoisoflavanone, are essential for the improved antiangiogenic effect. However, because it is uncertain where a linker might impinge on function, we anticipated that SH-11037 (**2**) could also be modified at the para-position of the phenylalanyl group as well as C-7 without a significant loss of activity by elongating a tethered biotin moiety with hydrophilic groups. Unfortunately, the introduction of a tether to the site of the HNBOc group in SH-11037 (**2**) was proven to be problematic due to the low reactivity of the amino group and instability of the ester group in the course of Boc-deprotection or linking with a carbamate.

The synthesis of intermediate **10** is outlined in Scheme 1.^{24,36} Similar to the procedures reported previously, the synthesis of biotinylated benzophenone was commenced with two different alkylations of 4,4'-dihydroxybenzophenone, which was treated with propargylic bromide and K_2CO_3 , followed by DIAD-mediated Mitsunobu reaction using 3-(Boc-amino)-1-propanol to afford the resulting bis-ether **7**. The azide **8** which was obtained from treatment of commercially available *p*-nitrophenyl ester of biotin (Biotin-ONp) with N_3 -PEG₃-CH₂CH₂NH₂ was coupled with bis-ether **7** by Cu-catalyzed Huisgen 1,3-dipolar cycloaddition,³⁷ followed by the treatment of the resulting 1,2,3-triazole **9** with TFA to afford benzophenone-linked biotin intermediate **10** in good yield. In future target identification studies, **10** can also be utilized as a negative control compound which lacks the homoisoflavanone moiety.

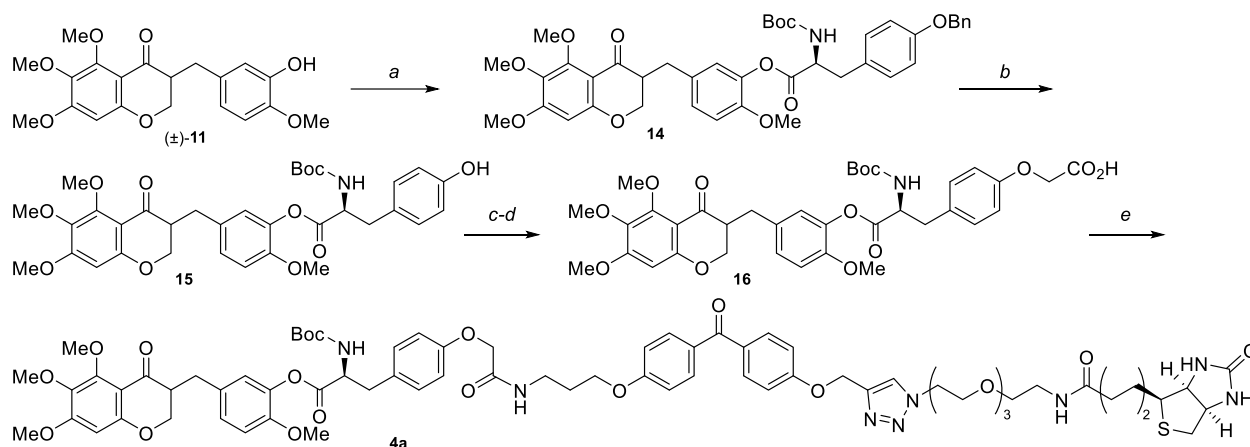


Scheme 1. Synthesis of benzophenone-linked biotin compound (**10**). Reagents and conditions: a) K_2CO_3 , propargylic bromide, acetone, 82%; b) DIAD, Boc-amino-propanol, PPh_3 , THF, 70%; c) 11-azido-3,6,9-trioxadecan-1-amine, iPr_2NEt , CH_2Cl_2 , 49%; d) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH/ H_2O , 40%; e) TFA, CH_2Cl_2 , 72%.

To prepare the first photoaffinity probe **3**, the bioactive and racemic 3-benzyl-4-chromanone (\pm)-**11** was chosen as a starting material (Scheme 2).²⁵⁻²⁷ The phenolic OH on the C-3' position



Scheme 2. Synthesis of photoaffinity probe (**3**). Reagents and conditions: a) *t*-butyl bromoacetate, K_2CO_3 , acetone, reflux, 95%; b) TFA, CH_2Cl_2 , 70%; c) **10**, HBTU, iPr_2NEt , DMF, 30%.



Scheme 3. Synthesis of photoaffinity probe (**4a**). Reagents and conditions: a) Boc-Tyr(Bn)-OH, EDCI, DMAP, rt, 94%; b) H₂, Pd/C, EtOAc, 88%; c) K₂CO₃, benzyl bromoacetate, acetone, reflux; d) H₂, Pd/C, EtOAc, 97% for 2 steps; e) **10**, HBTU, iPr₂NEt, DMF, 67%.

was alkylated with *tert*-butyl bromoacetate under basic conditions, followed by the acidic treatment of the ester **12** to afford the carboxylic acid **13**. Finally, the carboxylic acid **13** was coupled with the intermediate **10** by HBTU-mediated amidation to provide the desired photoaffinity probe **3** in moderate yield.

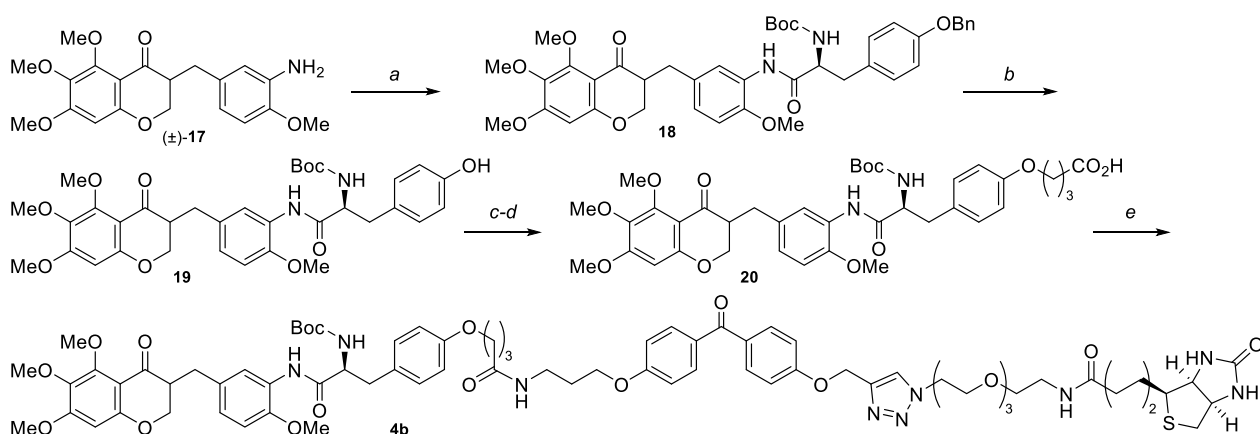
For the photoaffinity probes modified at the para-position of the phenylalaninyl group of **2**, Boc-Tyr(Bn)-OH was introduced into compound **11** at the C-3' position by using EDCI (Scheme 3). The debenzoylation of the ester **14** afforded the resulting compound **15**, which has a free OH group. The alkylation of **15** with benzyl bromoacetate and subsequent hydrogenolysis under H₂ and Pd/C generated the carboxylic acid **16**. In the end, the carboxylic acid **16** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the desired photoaffinity probe **4a** in good yield.

We next investigated the synthesis of an amide-containing photoaffinity probe, which might have increased stability compared to the ester probe **4a**. This amide compound **4b** was synthesized from homoisoflavanone **17**²⁷ having an amino group on the C3' position to which Boc-Tyr(Bn)-OH was added by EDCI-mediated amidation (Scheme 4). The resulting amide **18** was treated with H₂ and Pd/C to generate phenol **19**. With phenol **19** in hand, the alkylation with benzyl 4-bromobutyrate followed by debenzoylation provided the carboxylic acid **20**. Finally, the carboxylic acid **20** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the desired photoaffinity probe **4b** in good yield.

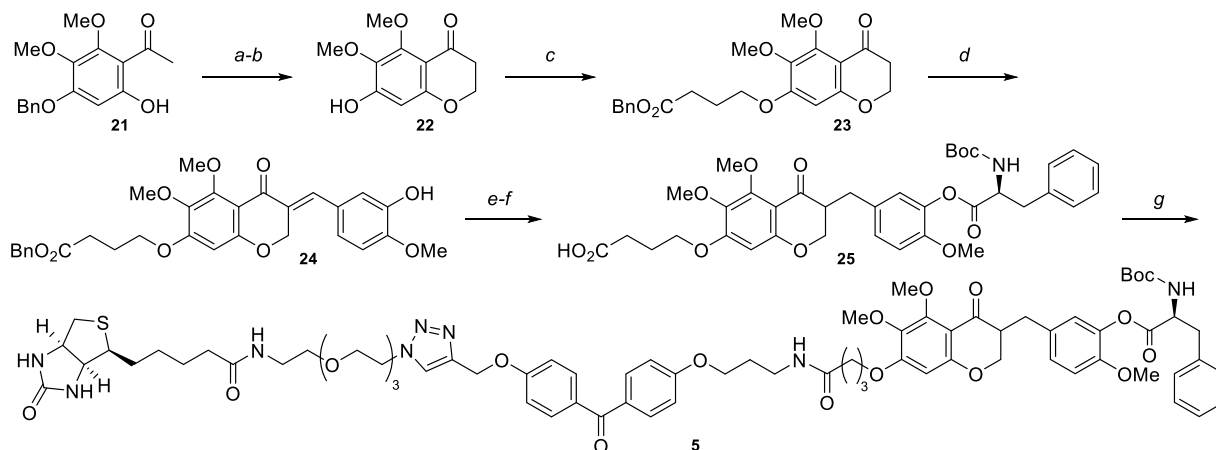
The linking site of the final photoaffinity probe was different

from that of the previous probes. Thus benzophenone-linked biotin could be attached at the C-7 position of **2** using our synthetic approach to cremastranone (Scheme 5). According to the procedures reported previously,²⁷ the known acetophenone **21** as a starting material was converted to 7-hydroxy-5,6-dimethoxy-4-chromanone **22** via 2 steps in good yield. The C-7 alkylation of the compound **22** with benzyl 4-bromobutyrate provided the compound **23**. Subsequently, the aldol condensation of **23** with isovanillin under acidic conditions gave the unstable 3-benzylidene-4-chromanone **24**. EDCI-mediated esterification of **24** with Boc-Phe-OH and the catalytic hydrogenation reaction using H₂, Pd/C resulted in the removal of the benzyl group and the saturation of the double bond to give the desired homoisoflavanone **25** having butyric acid at the C-7 position. The acid **25** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the final photoaffinity probe **5** in moderate yield.

It is obviously important that the synthesized probes retain potency in biological assays. Therefore, we tested the anti-proliferative activity of the probes as well as intermediates on human retinal microvascular endothelial cells (HRECs) and human umbilical vein endothelial cells (HUVECs). We also measured the endothelial cell specificity of these compounds by measuring their effects on the proliferation of non-endothelial ocular cell lines, Y79 (retinoblastoma cell line), 92-1 (uveal melanoma cell line) and ARPE19 (retinal pigment epithelium cell line). The results are summarized in Table 1. Among newly synthesized probes, **3** exhibited excellent inhibitory activity with GI₅₀ value of 72 nM against HRECs in spite of the incorporation



Scheme 4. Synthesis of photoaffinity probe (**4b**). Reagents and conditions: a) Boc-Tyr(Bn)-OH, EDCI, DMAP, rt, 72%; b) H₂, Pd/C, EtOAc, 99%; c) K₂CO₃, benzyl 4-bromobutyrate, acetone, reflux; d) H₂, Pd/C, EtOAc, 73% for 2 steps; e) **10**, HBTU, iPr₂NEt, DMF, 86%.



Scheme 5. Synthesis of photoaffinity probe (**5**). Reagents and conditions: a) $(\text{CH}_3\text{O})_2\text{CHN}(\text{CH}_3)_2$, toluene, 80 °C, then *c*-HCl, 50 °C, 97%; b) H_2 , Pd/C, MeOH, 99%; c) K_2CO_3 , benzyloxy 4-bromobutanoate, acetone, reflux, 80%; d) isovanillin, *p*-TsOH, benzene, reflux, 54%; e) Boc-Phe-OH, EDCl, DMAP, rt; f) H_2 , Pd/C, EtOAc, 70% for 2 steps; g) **10**, HBTU, *i*Pr₂NEt, DMF, 36%.

of the benzophenone photoreactive group and biotin reporter group. The probe **4a** ($\text{GI}_{50} = 210$ nM) also retained potency comparable to cremastranone (**1**) and SH-11037 (**2**), but lost some specificity. In contrast, amide probe **4b** and C-7 linking probe **5** had somewhat reduced activity on HREC cell proliferation. Therefore, the probes **3** and **4a** would be very promising tools for target identification of antiangiogenic homoisoflavonoid-binding proteins.

Table 1. Growth inhibitory activity (GI_{50} , μM) of photoaffinity probes and their intermediates on the proliferation of microvascular (HREC), macrovascular (HUVEC) and other ocular (92-1, Y79 and ARPE19) cells.

compd	HREC	HUVEC	92-1	Y79	ARPE19
1 ^a	0.22 (0.12 – 0.39) ^b	0.38 (0.24 – 0.59)	48 (17 – 132)	9.8 (2.1 – 45)	ND ^c
2 ^a	0.055 (0.032 – 0.094)	0.75 (0.37 – 1.5)	>100	12 (5.7 – 25)	ND
3	0.072 (0.00035 – 0.15)	>100	>100	>100	>100
4a	0.21 (0.041 – 1.1)	0.44 (0.15 – 1.3)	>100	>100	>100
4b	42 (4.1 – 444)	>100	>100	>100	>100
5	44 (11 – 171)	>100	>100	>100	>100
11 ^a	2 (0.81 – 5.1)	12 (2.7 – 55)	>100	>100	ND
14 ^a	0.51 (0.26 – 1.0)	>100	>100	>100	ND
16	0.047 (0.0062 – 0.36)	0.012 (0.0018 – 0.084)	>100	>100	>100
18 ^a	>100	>100	>100	0.39 (0.12 – 1.3)	ND
20	0.0079 (0.0018 – 0.035)	22 (3.0 – 168)	>100	>100	>100

^athe GI_{50} values reported in ref 27. ^b95% confidence interval shown in parentheses. ^cND, not determined.

In conclusion, we have designed and synthesized novel photoaffinity probes of antiangiogenic homoisoflavonoids with photoreactive benzophenone and biotin. In order to search for the photoaffinity probes which retain potency, the attachment of benzophenone and biotin was on C3' and C-7 position of homoisoflavonoids. Among them, the probes **3** and **4a** exhibited potent anti-proliferative and endothelial-cell specific activity when compared with the activity of the natural product cremastranone and improved analogue SH-11037.

Acknowledgments

This work was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2007151) and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare (HI14C1135) to S-YS; grants to TWC from the International Retinal Research Foundation, Carl Marshall and Mildred Almen Reeves Foundation, Ralph W. and Grace M. Showalter Research Trust, Retina Research Foundation, NIH NEI (R01EY025641) and NIH NCATS (KL2TR001106); and by an unrestricted grant from Research to Prevent Blindness, Inc..

References and notes

- Cong, F.; Cheung, A. K.; Huang, S. M. *Annu. Rev. Pharmacol. Toxicol.* **2012**, *52*, 57.
- Futamura, Y.; Muroi, M.; Osada, H. *Mol. Biosyst.* **2013**, *9*, 897.
- Heal, W. P.; Dang, T. H.; Tate, *Chem. Soc. Rev.* **2011**, *40*, 246.
- Lomenick, B.; Olsen, R. W.; Huang, J. *ACS Chem. Biol.* **2011**, *6*, 34.
- Park, J.; Koh, M.; Park, S. B. *Mol. Biosyst.* **2013**, *9*, 544.
- Rix, U.; Superti-Furga, G. *Nat. Chem. Biol.*, **2009**, *5*, 616.
- Schenone, M.; Dancik, V.; Wagner, B. K.; Clemons, P. A. *Nat. Chem. Biol.* **2013**, *9*, 232.
- Titov, D. V.; Liu, J. O. *Bioorg. Med. Chem.* **2012**, *20*, 1902.
- Ziegler, S.; Pries, V.; Hedberg, C.; Waldmann, *Angew Chem Int Ed Engl.* **2013**, *52*, 2744.
- Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64.
- Hatanaka, Y.; Sadakane, Y. *Curr. Top Med. Chem.* **2002**, *2*, 271.
- Lapinsky, D. J. *Bioorg. Med. Chem.* **2012**, *20*, 6237.
- Li, G.; Liu, Y.; Yu, X.; Li, X. *Bioconjug. Chem.* **2014**, *25*, 1172.
- Smith, E.; Collins, I. *Future Med. Chem.* **2015**, *7*, 159.
- Corson, T. W.; Cava, H.; Aberle, N.; Crews, C. M. *ChemBiochem* **2011**, *12*, 1767.

16. Kotoku, N.; Nakata, C.; Kawachi, T.; Sato, T.; Guo, X. H.; Ito, A.; Sumii, Y.; Arai, M.; Kobayashi, M. *Bioorg. Med. Chem.* **2014**, *22*, 2102.
17. Lee, K.; Ban, H. S.; Naik, R.; Hong, Y. S.; Son, S.; Kim, B. K.; Xia, Y.; Song, K. B.; Lee, H. S.; Won, M. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 10286.
18. Mizuhara, T.; Oishi, S.; Ohno, H.; Shimura, K.; Matsuoka, M.; Fujii, N. *Bioorg. Med. Chem.* **2013**, *21*, 2079.
19. Naik, R.; Won, M.; Ban, H. S.; Bhattarai, D.; Xu, X.; Eo, Y.; Hong, Y. S.; Singh, S.; Choi, Y.; Ahn, H. C.; Lee, K. *J. Med. Chem.* **2014**, *57*, 9522.
20. Park, J.; Oh, S.; Park, S. B. *Angew. Chem. Int. Ed. Engl.* **2012**, *51*, 5447.
21. Shi, H.; Zhang, C. J.; Chen, G. Y.; Yao, S. Q. *J. Am. Chem. Soc.* **2012**, *134*, 3001.
22. Suzuki, T.; Okamura, T.; Tomohiro, T.; Iwabuchi, Y.; Kanoh, N. *Bioconjug. Chem.* **2015**, *26*, 389.
23. Zhang, L.; Zhang, Y.; Dong, J.; Liu, J.; Zhang, L.; Sun, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1036.
24. Trippier, P. C. *ChemMedChem* **2013**, *8*, 190.
25. Basavarajappa, H. D.; Lee, B.; Fei, X.; Lim, D.; Callaghan, B.; Mund, J. A.; Case, J.; Rajashekhar, G.; Seo, S.-Y.; Corson, T. W. *PLoS One* **2014**, *9*, e95694.
26. Lee, B.; Basavarajappa, H. D.; Sulaiman, R. S.; Fei, X.; Seo, S.-Y.; Corson, T. W. *Org. Biomol. Chem.* **2014**, *12*, 7673.
27. Basavarajappa, H. D.; Lee, B.; Lee, H.; Sulaiman, R. S.; An, H.; Magana, C.; Shadmand, M.; Vayl, A.; Rajashekhar, G.; Kim, E. Y.; Suh, Y. G.; Lee, K.; Seo, S.-Y.; Corson, T. W. *J. Med. Chem.* **2015**, *58*, 5015.
28. Shim, J. S.; Kim, J. H.; Lee, J.; Kim, S. N.; Kwon, H. J. *Planta Med.* **2004**, *70*, 171.
29. Kim, J. H.; Kim, K. H.; Yu, Y. S.; Kim, Y. M.; Kim, K. W.; Kwon, H. J. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 848.
30. du Toit, K.; Drewes, S. E.; Bodenstein, J. *Nat. Prod. Res.* **2010**, *24*, 457.
31. Hur, S.; Lee, Y. S.; Yoo, H.; Yang, J. H.; Kim, T. Y. *J. Dermatol. Sci.* **2010**, *59*, 163.
32. Lee, Y. S.; Hur, S.; Kim, T. Y. *Allergy* **2014**, *69*, 453.
33. Sulaiman, R. S.; Merrigan, S.; Quigley, J.; Qi, X.; Lee, B.; Boulton, M. E.; Kennedy, B.; Seo, S.-Y.; Corson, T. W. *Sci. Rep.* **2016**, *6*, 25509.
34. La Clair, J. J. *Nat. Prod. Rep.* **2010**, *27*, 969.
35. Sulaiman, R. S.; Basavarajappa, H. D.; Corson, T. W. *Exp. Eye Res.* **2014**, *129*, 161.
36. Tae, H. S.; Hines, J.; Schneekloth, A. R.; Crews, C. M. *Org. Lett.* **2010**, *12*, 4308.
37. McKay, C. S.; Finn, M. G. *Chem. Biol.* **2014**, *21*, 1075.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi



Design, synthesis and biological evaluation of photoaffinity probes of antiangiogenic homoisoflavonoids

Bit Lee,^a Wei Sun,^a Hyungjun Lee,^a Halesha Basavarajappa,^{b,c} Rania Sulaiman,^{b,d,e} Kamakshi Sishtla,^b Xiang Fei^a, Timothy W. Corson,^{b,c,d} and Seung-Yong Seo^{a,*}

^aCollege of Pharmacy and Gachon Institute of Pharmaceutical Sciences, Gachon University, Incheon 21936, South Korea

^bEugene and Marilyn Glick Eye Institute, Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

^cDepartment of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

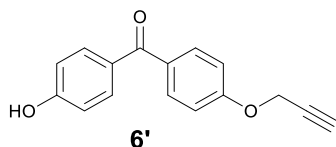
^dDepartment of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States of America

^eDepartment of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Synthetic Methodology

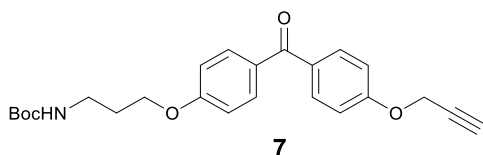
All starting materials and reagents were obtained from commercial suppliers and were used without further purification. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). ¹H and ¹³C NMR spectra were recorded on a Bruker 600 MHz spectrometer as solutions in deuteriochloroform (CDCl₃) or methanol-d₄. ¹H NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (*J*) in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-700 (FAB and EI) and an Agilent 6530 Q-TOF LC/MS/MS system (ESI).

(4-hydroxyphenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (6')



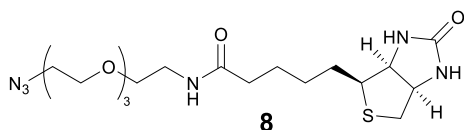
To a solution of 4,4'-dihydroxybenzophenone (500 mg, 2.33 mmol) in acetone (5.0 ml) at 0 °C, K₂CO₃ (161 mg, 1.17 mmol) and propargyl bromide (0.11 ml, 1.16 mmol) were added. The reaction mixture was stirred at 50 °C for 3 h, cooled to room temperature, and quenched with H₂O (10 mL). The mixture was extracted three times with ethyl acetate. The combined extracts were washed with brine, dried over MgSO₄, filtered, and concentrated. The residue was purified via Biotage (ethyl acetate / *n*-hexane = 1 : 2 Snap Ultra 25g column). The desired fractions were collected and concentrated to give propargylated benzophenone (**6'**) (240 mg, 82% BRSM). ¹H-NMR (600 MHz, CD₃OD) δ 7.73 (d, 2H, *J* = 8.4 Hz), 7.67 (d, 2H, *J* = 8.4 Hz), 7.09 (d, 2H, *J* = 9.0 Hz), 6.87 (d, 2H, *J* = 9.0 Hz), 4.82 (s, 2H), 3.00 (t, 1H, *J* = 2.4 Hz); ¹³C-NMR (150 MHz, CD₃OD) δ 195.4, 162.0, 161.0, 132.3, 131.6, 131.1, 128.9, 114.7, 114.7, 77.8, 75.9, 55.3; HRMS (ESI): mass calcd for C₁₆H₁₅O₃ [M + H]⁺, 253.0786; found, 253.0872.

***tert*-Butyl (3-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)propyl)carbamate (7)**



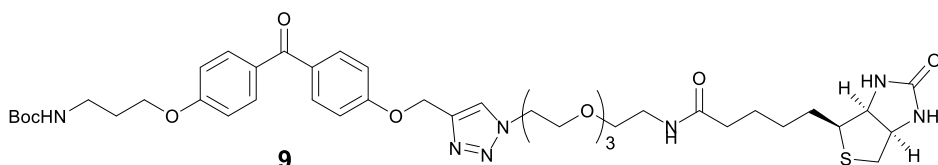
To a THF solution (2 mL) of *N*-Boc-3-aminopropanol (117 mg, 0.76 mmol) were added the propargylated benzophenone (**6'**) (140 mg, 0.56 mmol), PPh₃ (150 mg, 0.76 mmol), and DIAD (130 μL, 0.56 mmol) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was diluted with ethyl acetate and the combined organic phase was washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 3) to afford the dialkylated dihydroxybenzophenone (**7**) (160 mg, 70%). ¹H-NMR (600 MHz, CDCl₃) δ 7.77 (dd, 4H, *J* = 9.0 and 5.0 Hz), 7.03 (d, 2H, *J* = 9.0 Hz), 6.93 (d, 2H, *J* = 9.0 Hz), 4.82 (bs, 1H), 4.75 (d, 2H, *J* = 1.2 Hz), 4.09 (t, 2H, *J* = 6.0 Hz), 3.33 (m, 2H), 2.56 (t, 1H, *J* = 2.4 Hz), 2.01 (m, 1H), 1.42 (s, 9H), 1.25 (m, 1H); ¹³C-NMR (150 MHz, CDCl₃) δ 194.3, 162.1, 160.6, 156.0, 132.2, 132.1, 131.5, 130.6, 114.3, 113.9, 77.8, 76.1, 65.9, 55.8, 37.8, 29.5, 28.4, 14.2; HRMS (ESI): mass calcd for C₂₄H₂₇NO₅ [M + H]⁺, 410.1889; found, 410.1956.

***N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-5-((3*a*S,4*S*,6*a*R)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (8)**



To a THF solution (1.0 mL) of biotin-ONp (50 mg, 0.14 mmol) were added 11-azido-3,6,9-trioxaundecan-1-amine (27 μ L, 0.14 mmol) and Et₃N (57 μ L, 0.41 mmol) at room temperature. After stirring for 12 h at room temperature, the reaction mixture was quenched with H₂O (1 mL). The reaction mixture was extracted three times with ethyl acetate and the combined organic phase was washed with brine, dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH₂Cl₂ = 1 : 10) to afford the azide (**8**) (30 mg, 49%) ¹H-NMR (600 MHz, CDCl₃) δ 6.83 (m, 1H), 6.73 (m, 1H), 5.78 (m, 1H), 4.50 (m, 1H), 4.30 (m, 1H), 3.65 (m, 8H), 3.62 (m, 2H), 3.56 (m, 2H), 3.42-3.37 (m, 4H), 3.13 (m, 1H), 2.90 (m, 1H), 2.74 (d, 1H, *J* = 13 Hz), 2.22 (t, 2H, *J* = 7.8 Hz), 1.75-1.62 (m, 4H), 1.44-1.41 (m, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 173.4, 164.3, 70.6, 70.4, 70.0, 70.0, 69.9, 60.2, 55.7, 50.6, 40.5, 39.1, 36.0, 28.3, 28.1, 25.56; HRMS (ESI): mass calcd for C₁₈H₃₂N₆O₅S [M + H]⁺, 445.2155; found, 445.3135.

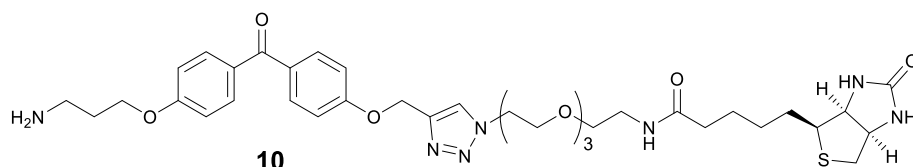
Boc-containing benzophenone-biotin (**9**).



To a *t*-BuOH/H₂O solution (2 mL, 1 : 1) of the benzophenone (**7**) (35 mg, 0.086 mmol) and the PEG-Biotin (**8**) (38 mg, 0.086 mmol), CuSO₄·5H₂O (2 mg, 0.086 mmol) and sodium ascorbate (1.0 M in H₂O, 2 drops) were added at room temperature. After stirring at room temperature for 24 h, the reaction mixture was diluted with H₂O (1 mL) and extracted with ethyl acetate and the combined organic phase was washed with brine, dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH₂Cl₂ = 1 : 10) to afford the 1,2,3-triazole (30 mg, 40%). ¹H-NMR (600 MHz, CDCl₃) δ 7.86 (s, 1H), 7.77 (dd, 4H, *J* = 8.4 and 2.4 Hz), 7.06 (d, 2H, *J* = 9.0 Hz), 6.94 (d, 2H, *J* = 8.4 Hz), 6.67 (bs, 1H), 6.45 (bs, 1H), 5.49 (bs, 1H), 5.28 (s, 2H), 4.82 (bs, 1H), 4.57 (t, 2H, *J* = 4.8 Hz), 4.47 (m, 1H), 4.28 (m, 1H), 4.10 (t, 2H, *J* = 6.0 Hz), 3.89 (t, 2H, *J* = 4.8 Hz), 3.65 (m, 2H), 3.60 (m, 2H), 3.56 (m, 6H), 3.52 (t, 2H, *J* = 4.8 Hz), 3.41-3.33 (m, 4H), 3.12 (m, 1H), 2.88 (m, 1H), 2.73 (m, 1H), 2.19 (t, 2H, *J* = 12 Hz),

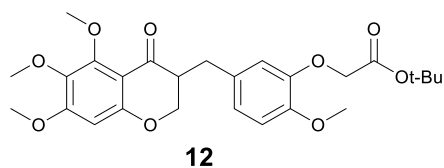
2.03 (m, 2H), 1.75-1.58 (m, 6H), 1.43 (s, 9H), 1.41 (m, 2H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ 194.4, 173.2, 163.9, 162.1, 161.4, 156.0, 143.2, 132.2, 132.2, 131.2, 130.5, 124.3, 114.3, 113.9, 79.3, 70.6, 70.5, 70.4, 70.3, 70.1, 69.9, 69.3, 65.9, 62.0, 61.7, 60.1, 55.5, 50.6, 50.3, 40.5, 39.1, 37.8, 35.9, 29.5, 28.4, 28.1, 25.5; HRMS (ESI): mass calcd for $\text{C}_{42}\text{H}_{59}\text{N}_7\text{O}_{10}\text{S}$ $[\text{M} + \text{H}]^+$, 854.4044; found, 854.4143.

Ammonium salt of benzophenone-biotin (**10**).



To a CH_2Cl_2 solution (2 mL) of the Boc-protected benzophenone-biotin **9** (20 mg, 0.028 mmol) was added TFA (0.4 mL) at 0 °C. After stirring for 2.5 h at room temperature, the reaction mixture was concentrated under reduced pressure to afford the crude amine **10** (16 mg, 72%). The crude ammonium salt was used for the next reaction without further purification. $^1\text{H-NMR}$ (600 MHz, CD_3OD) δ 8.17 (s, 1H), 7.77 (d, 4H, $J = 8.4$ Hz), 7.16 (d, 2H, $J = 8.4$ Hz), 7.08 (d, 2H, $J = 8.4$ Hz), 5.28 (s, 2H), 4.62 (m, 4H), 4.23 (t, 2H, $J = 6.0$ Hz), 3.91 (t, 2H, $J = 5.0$ Hz), 3.65 (m, 2H), 3.59-3.55 (m, 8H), 3.48 (m, 2H), 3.18 (t, 2H, $J = 12$ Hz), 3.12-3.01 (m, 2H), 2.20 (m, 4H), 1.89 (m, 2H), 1.66 (m, 4H); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ 165.1, 174.8, 164.7, 162.1, 161.8, 159.2, 158.9, 132.0, 132.0, 130.7, 130.4, 125.1, 114.2, 113.9, 70.0, 70.0, 69.9, 69.8, 69.1, 68.8, 65.1, 62.0, 61.1, 60.3, 55.5, 53.3, 50.2, 39.6, 38.9, 37.1, 35.3, 28.3, 28.0, 26.8, 25.4; HRMS (ESI): mass calcd for $\text{C}_{37}\text{H}_{51}\text{N}_7\text{O}_8\text{S}$ $[\text{M} + \text{H}]^+$, 754.3520; found, 754.3623.

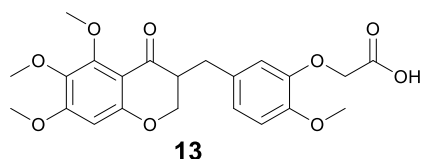
tert-butyl 2-(2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenoxy)acetate (**12**)



To an acetone solution (5 mL) of 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**11**) (102 mg, 0.272 mmol) were added *tert*-butyl bromoacetate (48 μL , 0.326 mmol) and K_2CO_3 (104 mg, 0.816 mmol). After refluxing for 3 h, the reaction mixture was diluted with ethyl acetate and the combined organic phase was washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl

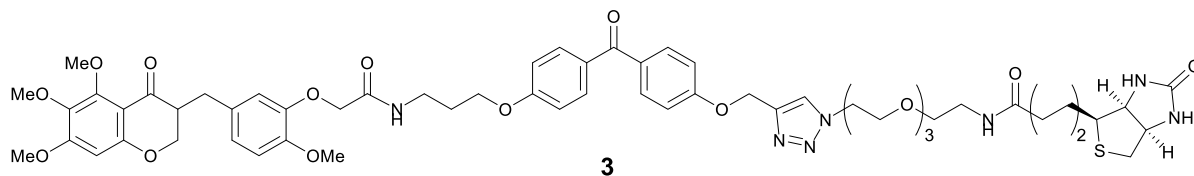
acetate / *n*-hexane = 1 : 2) to afford the *tert*-butyl aryloxyacetate (**12**) (105 mg, 79%). ¹H-NMR (600 MHz, CDCl₃) δ 6.78 (t, 2H, *J* = 8.4 Hz), 6.61 (d, 1H, *J* = 1.8 Hz), 6.16 (s, 1H), 4.51 (s, 2H), 4.20 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.02 (dd, 1H, *J* = 11.4 and 7.8 Hz), 3.88 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.11 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.66 (m, 1H), 2.55 (dd, 1H, *J* = 13.8 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 193.2, 169.6, 161.7, 161.3, 156.4, 150.2, 149.3, 139.4, 132.7, 124.4, 116.6, 114.1, 110.6, 97.9, 84.1, 70.9, 68.4, 63.5, 63.2, 58.0, 58.0, 50.4, 34.3, 30.0, 30.0.

2-(2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenoxy)acetic acid (13)



To a CH₂Cl₂ solution (2.0 mL) of the *tert*-butyl acetate (**12**) (66 mg, 0.135 mmol) was added TFA (0.7 mL). After stirring for 2.5 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH₂Cl₂ = 1 : 10) to afford the aryloxyacetic acid (**13**) (62 mg, 84%). ¹H-NMR (600 MHz, CDCl₃) δ 6.83 (s, 2H), 6.77 (s, 1H), 6.24 (s, 1H), 4.70 (s, 2H), 4.25 (dd, 1H, *J* = 11.4 and 3.6 Hz), 4.09 (dd, 1H, *J* = 11.4 and 6 Hz), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.05 (dd, 1H, *J* = 13.2 and 4.2 Hz), 2.75 (m, 1H), 2.69 (dd, 1H, *J* = 13.2 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 194.1, 173.5, 160.5, 160.4, 154.2, 148.3, 146.8, 137.1, 130.7, 123.8, 116.4, 112.3, 107.9, 96.2, 68.6, 66.8, 61.8, 61.4, 56.2, 55.9, 48.0, 32.7; HRMS (ESI): mass calcd for C₂₂H₂₄O₉ [M + Na]⁺, 455.1420; found, 455.1419.

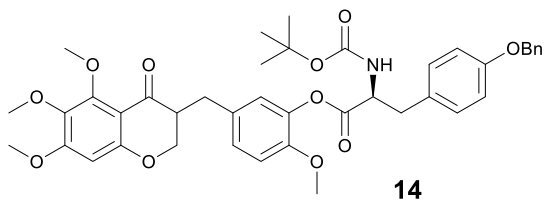
***N*-(2-(2-(2-(2-(4-((4-(4-(3-(2-(2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenoxy)acetamido)propoxy)benzoyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)-5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (3)**



To a DMF solution (1 mL) of the carboxylic acid (**13**) (24 mg, 0.044 mmol) were added HBTU (20 mg, 0.051 mmol) and DIPEA (22 μL, 0.129 mmol). After stirring for 30 min, a DMF solution (0.5

mL) of the ammonium salt (**9**) (38 mg, 0.043 mmol) was added to the reaction mixture. After stirring for 24 h, the reaction mixture was diluted with ethyl acetate, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH₂Cl₂ = 1 : 10) to afford the cremastranone-benzophenone-biotin (**3**) (16 mg, 32%). ¹H-NMR (400 MHz, DMSO-d₆) δ 8.21 (s, 1H), 8.01 (t, 1H, *J* = 5.6 Hz), 7.78 (t, 1H, *J* = 5.6 Hz), 7.66 (dd, 4H, *J* = 8.8 and 6.8 Hz), 7.16 (d, 2H, *J* = 8.8 Hz), 6.99 (d, 2H, *J* = 8.8 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 6.80 (s, 1H), 6.77 (d, 1H, *J* = 8.4 Hz), 6.38 (s, 2H), 6.32 (s, 1H), 5.22 (s, 2H), 4.53 (t, 2H, *J* = 5.2 Hz), 4.43 (s, 2H), 4.26-4.23 (m, 1H), 4.21 (dd, 1H, *J* = 11.2 and 4.4 Hz), 4.08-3.99 (m, 4H), 3.80 (d, 2H, *J* = 5.2 Hz), 3.77 (s, 3H), 3.71 (s, 3H), 3.69 (s, 3H), 3.60 (s, 3H), 3.50-3.47 (m, 2H), 3.44-3.42 (m, 6H), 3.34-3.31 (m, 4H), 3.14 (q, 2H, *J* = 5.6 Hz), 3.05-3.01 (m, 1H), 2.99 (dd, 1H, *J* = 14.0 and 4.8 Hz), 2.78-2.74 (m, 2H), 2.54 (d, 1H, *J* = 12.4 Hz), 2.00 (t, 2H, *J* = 7.2 Hz), 1.90 (t, 2H, *J* = 6.4 Hz), 1.56(m, 1H), 1.48 (m, 3H), 1.24 (m, 2H); ¹³C-NMR (100 MHz, DMSO-d₆) δ 193.5, 190.5, 172.5, 168.3, 163.1, 162.2, 161.7, 159.5, 159.2, 153.9, 148.2, 147.5, 142.4, 137.2, 132.2, 132.1, 131.3, 130.6, 130.2, 125.5, 122.8, 115.9, 114.8, 114.5, 112.6, 108.6, 96.8, 70.1, 70.0, 69.9, 69.5, 69.3, 69.0, 68.9, 66.1, 61.7, 61.6, 61.4, 61.1, 59.5, 56.5, 55.9, 55.8, 49.8, 47.6, 40.2, 38.8, 35.8, 35.5, 31.8, 29.1, 28.6, 28.4, 25.6; HRMS (ESI): mass calcd for C₅₉H₇₃N₇O₁₆S [M + H]⁺, 1168.4835; found, 1168.5145.

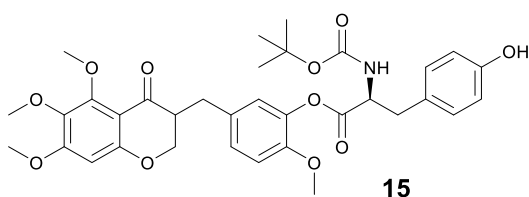
2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (2S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (14**).**



To a CH₂Cl₂ solution (2 mL) of **11** (63 mg, 0.16 mmol) were added Boc-Tyr(Bzl)-OH (75 mg, 0.19 mmol), EDCI (40 mg, 0.24 mmol) and DMAP (4 mg, 0.03 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14**) (115 mg, 94%). ¹H-NMR (600 MHz, CDCl₃) δ 7.39 (d, 2H, *J* = 7.2 Hz), 7.33 (t, 3H, *J* = 7.2 Hz), 7.28 (t, 1H, *J* = 7.2 Hz), 7.19 (d, 2H, *J* = 8.4 Hz), 7.05 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.92 (m, 3H), 6.22 (d, 1H, *J* = 2.4 Hz), 5.01 (s, 2H), 4.80 (m, 1H), 4.26 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.06 (dd, 1H, *J* = 7.8 and 3.6 Hz), 3.89 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.26 (dd, 1H, *J* = 14.4 and 6.0 Hz), 3.16

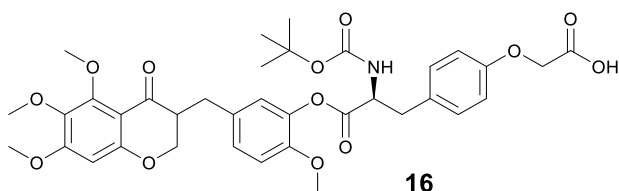
(dd, 1H, $J = 13.8$ and 4.2 Hz), 2.72 (m, 1H), 2.62 (m, 1H), 1.40 (s, 9H); ^{13}C -NMR (150 MHz, CDCl_3) δ 191.0, 170.1, 159.6, 159.3, 157.9, 155.0, 154.4, 149.6, 139.2, 137.5, 136.9, 131.0, 130.6, 128.5, 128.2, 127.9, 127.6, 127.4, 123.4, 114.8, 112.6, 108.6, 96.0, 79.9, 70.0, 68.9, 61.6, 61.3, 56.0, 55.8, 54.4, 48.2, 37.3, 31.8, 28.3; HRMS (ESI): mass calcd for $\text{C}_{41}\text{H}_{45}\text{NO}_{11}$ $[\text{M} + \text{H}]^+$, 727.2993; found, 727.302.

2-Methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl (*tert*-butoxycarbonyl)-L-tyrosinate (15**)**



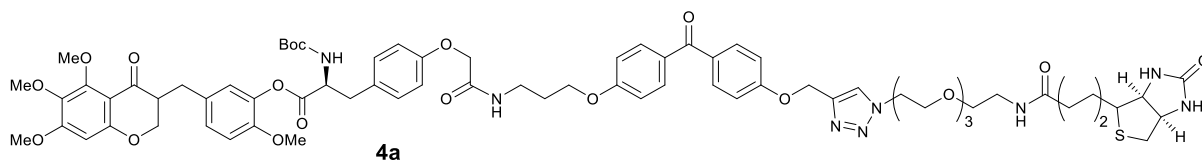
A solution of **14** (161 mg, 0.22 mmol) and 10% Pd/C (23 mg) in ethyl acetate (3 mL) was placed under an atmosphere of hydrogen. After stirring for 48 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford (**15**) (112 mg, 88%). ^1H -NMR (600 MHz, CDCl_3) δ 7.10 (bs, 2H), 7.01(dd, 1H, $J = 8.4$ and 1.8 Hz), 6.85 (m, 3H), 6.44 (bs, 1H), 6.25 (s, 1H), 5.10 (m, 1H), 4.80 (m, 1H), 4.25 (d, 1H, $J = 9.0$ Hz), 4.08 (m, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.17 (m, 1H), 3.09 (m, 1H), 3.03 (t, 1H, $J = 10.2$ Hz), 2.66 (m, 2H), 1.42 (s, 9H); ^{13}C -NMR (150 MHz, CDCl_3) δ 192.0, 170.3, 159.8, 159.7, 155.5, 155.0, 154.4, 149.8, 139.2, 137.5, 130.9, 130.8, 127.6, 123.4, 115.8, 112.4, 108.4, 96.1, 80.1, 68.6, 61.6, 61.3, 56.1, 55.9, 54.7, 53.4, 48.4, 38.0, 31.9, 28.3; HRMS (ESI): mass calcd for $\text{C}_{34}\text{H}_{39}\text{NO}_{11}$ $[\text{M} + \text{H}]^+$, 638.2601; found, 638.2601.

2-(4-((2*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenoxy)-3-oxopropyl)phenoxy)acetic acid (16**)**



To an acetone (3 mL) solution of **15** (26 mg, 0.04 mmol), benzyl bromoacetate (10 μ L, 0.08 mmol) and K_2CO_3 (16 mg, 0.12 mmol) were added. After stirring for 8 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate / *n*-hexanes = 1 : 1) to afford the benzyl ester (30 mg, 96%). And then a solution of benzyl ester (30 mg, 0.038 mmol) and 10% Pd/C (5 mg) in ethyl acetate (3 mL) was placed under an atmosphere of hydrogen. After stirring for 4 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH_2Cl_2 = 1 : 10) to afford **16** (26 mg, 99%). 1H -NMR (400 MHz, DMSO- d_6) δ 12.93 (bs, 1H), 7.42 (d, 1H, J = 8 Hz), 7.21 (d, 2H, J = 8.4 Hz), 7.08 (q, 2H, J = 8.4 Hz), 6.85 (s, 1H), 6.82 (d, 2H, J = 8.4 Hz), 6.41 (s, 1H), 4.59 (s, 2H), 4.33-4.28 (m, 1H), 4.27 (dd, 1H, J = 11.2 and 4.8 Hz), 4.07 (t, 1H, J = 10 Hz), 3.79 (s, 3H), 3.70 (s, 6H), 3.62 (s, 3H), 3.12 (dd, 1H, J = 13.6 and 4.4 Hz), 3.01 (dd, 1H, J = 14 and 5.2 Hz), 2.92 (dd, 1H, J = 14.4 and 10 Hz), 2.85-2.78 (m, 1H), 2.60 (dd, 1H, J = 13.6 and 9.2 Hz), 1.31 (s, 9H); ^{13}C -NMR (100 MHz, DMSO- d_6) δ 190.5, 170.8, 170.7, 159.6, 159.3, 156.9, 155.9, 154.0, 149.7, 139.4, 137.3, 136.8, 131.5, 130.6, 130.3, 123.5, 114.6, 113.4, 108.6, 96.9, 78.8, 69.4, 64.9, 61.7, 61.7, 61.2, 56.6, 56.3, 47.5, 35.9, 31.3, 28.6; HRMS (ESI): mass calcd for $C_{36}H_{41}NO_{13}$ $[M + H]^+$, 696.2656; found, 696.2656.

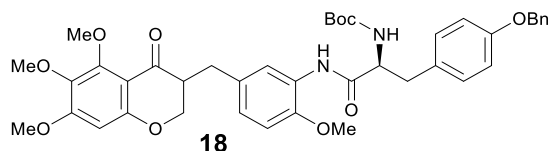
2-Methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(2-oxo-2-((3-(4-(4-((1-(13-oxo-17-((3aS,4S,6aR)-2-oxohexahydro-1H-Thieno[3,4-d]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1H-1,2,3-triazol-4-yl)methoxy)benzoyl)phenoxy)propyl)amino)ethoxy)phenyl)propanoate (4a)



To a solution of **16** (16 mg, 0.023 mmol) in DMF (1 mL) at room temperature were added HBTU (10 mg, 0.027 mmol) and DIPEA (12 μ L, 0.069 mmol). The reaction mixture was stirred at room temperature for 0.5 h and a solution of benzophenone-biotin amine (**10**) (20 mg, 0.023 mmol) in DMF (0.5 mL) was added to the mixture. The resulting mixture was stirred at room temperature for 18 h and quenched at 0 $^{\circ}C$ with H_2O . The mixture was extracted three times with ethyl acetate and the

combined extracts were washed with brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($\text{MeOH} / \text{CH}_2\text{Cl}_2 = 1 : 30$) to afford the amide (**4a**) (22 mg, 67%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.17 (t, 1H, $J = 6\text{Hz}$), 7.78 (t, 1H, $J = 6\text{Hz}$), 7.67 (d, 4H, $J = 8\text{ Hz}$), 7.42 (d, 1H, $J = 8\text{ Hz}$), 7.23 (d, 2H, $J = 8.4\text{ Hz}$), 7.16 (d, 2H, $J = 8.8\text{ Hz}$), 7.07-7.00 (m, 4H), 6.88-6.85 (m, 3H), 6.41 (s, 1H), 6.37 (s, 1H), 6.31 (s, 1H), 5.21 (s, 2H), 4.52 (t, 2H, $J = 5.6\text{ Hz}$), 4.41 (s, 2H), 4.29 (m, 1H), 4.26-4.22 (m, 2H), 4.08-4.01 (m, 4H), 3.79 (m, 5H), 3.69 (s, 6H), 3.61 (s, 3H), 3.49-3.47 (m, 2H), 3.44-3.42 (m, 5H), 3.34 (t, 2H, $J = 6\text{ Hz}$), 3.27 (d, 2H, $J = 2\text{ Hz}$), 3.13 (d, 2H, $J = 6\text{ Hz}$), 3.08 (m, 1H), 3.05 (m, 1H), 3.00-2.99 (m, 1H), 2.97-2.95 (m, 1H), 2.92-2.86 (m, 1H), 2.80 (m, 1H), 2.78 (dd, 1H, $J = 16.8$ and 4.8 Hz), 2.59-2.56 (m, 1H), 2.54 (d, 1H, $J = 12.4\text{ Hz}$), 2.02 (t, 2H, $J = 6.8\text{ Hz}$), 1.90 (t, 2H, $J = 6.4\text{ Hz}$), 1.56-1.52 (m, 1H), 1.44 (m, 3H), 1.30 (s, 9H), 1.24 (m, 2H); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6) δ 193.5, 190.4, 172.5, 170.8, 168.1, 163.1, 162.3, 161.7, 159.5, 159.2, 156.8, 155.8, 154.0, 149.6, 142.4, 139.3, 137.2, 132.2, 132.1, 131.4, 130.7, 130.6, 130.5, 130.2, 125.5, 123.4, 114.9, 114.8, 114.6, 113.3, 108.6, 96.8, 78.8, 70.1, 70.0, 69.9, 69.5, 69.3, 69.0, 67.5, 66.1, 61.7, 61.7, 61.4, 61.2, 59.6, 56.6, 56.2, 55.9, 55.8, 49.8, 47.5, 40.2, 38.8, 35.9, 35.8, 35.5, 31.3, 29.1, 28.6, 28.5, 28.4, 28.2, 25.6; HRMS (ESI): mass calcd for $\text{C}_{73}\text{H}_{90}\text{N}_8\text{O}_{20}\text{S} [\text{M} + \text{H}]^+$, 1431.6070; found, 1431.6067.

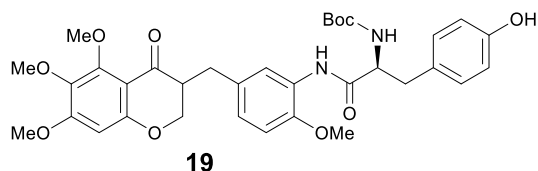
***tert*-Butyl ((2*S*)-3-(4-(benzyloxy)phenyl)-1-((2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl)amino)-1-oxopropan-2-yl)carbamate (**18**)**



To a CH_2Cl_2 solution (6 mL) of **17** (35 mg, 0.09 mmol) were added Boc-Tyr(Bn)-OH (35 mg, 0.09 mmol), EDCI (18 mg, 0.1 mmol) and DMAP (2 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford **18** (50 mg, 72%). $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ 8.22 (s, 1H), 8.10 (bs, 1H), 7.41 (d, 2H, $J = 7.8\text{ Hz}$), 7.37 (t, 2H, $J = 7.2\text{ Hz}$), 7.31 (t, 1H, $J = 7.2\text{ Hz}$), 7.16 (d, 2H, $J = 8.4\text{ Hz}$), 6.90 (d, 3H, $J = 8.4\text{ Hz}$), 6.75 (d, 1H, $J = 7.8\text{ Hz}$), 6.24 (s, 1H), 5.01 (s, 2H), 4.46 (bs, 1H), 4.29 (dd, 1H, $J = 10.8$ and 3.6 Hz), 4.12 (m, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.73 (s, 3H), 3.23 (dd, 1H, $J = 14.4$ and 3.6 Hz), 3.10 (m, 2H), 2.81 (m, 1H), 2.63 (m, 1H), 1.42 (s, 9H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ 191.3, 169.4, 159.7, 159.2, 157.8, 154.4, 146.7, 137.4, 136.9,

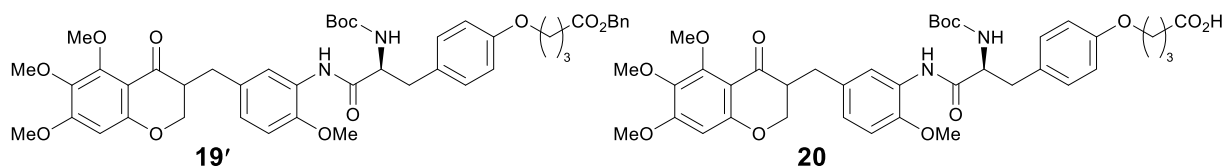
131.0, 130.3, 128.5, 127.9, 127.4, 127.0, 124.4, 120.3, 115.0, 110.0, 108.7, 96.0, 70.0, 69.2, 69.2, 61.6, 61.3, 56.0, 55.6, 48.3, 48.3, 37.7, 32.2, 30.9, 29.7, 28.2; HRMS (ESI): mass calcd for C₄₁H₄₆N₂O₁₀ [M + H]⁺, 727.3230; found, 727.3228.

***tert*-Butyl (3-(4-hydroxyphenyl)-1-((2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl)amino)-1-oxopropan-2-yl)carbamate (19)**



A solution of **18** (50 mg, 0.068 mmol) and 10% Pd/C (8 mg) in ethyl acetate (3 mL) was placed under an atmosphere of hydrogen. After stirring for 17 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford **19** (43 mg, 99%). ¹H-NMR (600 MHz, CDCl₃) δ 8.13 (s, 1H), 8.03 (bs 1H), 7.06 (d, 2H, *J* = 8.4 Hz), 6.80 (d, 1H, *J* = 7.8 Hz), 6.72 (m, 3H), 5.14 (bs, 1H), 4.42 (bs, 1H), 4.14 (dd, 1H, *J* = 10.4 and 1.8 Hz), 3.95 (d, 1H, *J* = 12.4 Hz), 3.88 (d, 3H, *J* = 6.6 Hz), 3.80 (t, 6H, *J* = 1.2 Hz), 3.73 (d, 3H, *J* = 1.8 Hz), 3.15 (m, 2H), 2.50 (dd, 1H, *J* = 13.8 Hz), 2.40 (m, 1H), 2.25 (t, 1H, *J* = 7.8 Hz), 1.41 (s, 9H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.6, 169.6, 159.8, 159.3, 155.5, 155.3, 154.3, 146.9, 137.3, 130.8, 130.3, 127.7, 126.9, 124.6, 120.5, 115.7, 110.0, 108.6, 96.0, 80.3, 69.2, 61.6, 61.3, 56.9, 56.1, 55.7, 48.3, 37.8, 32.3, 28.2; HRMS (ESI): mass calcd for C₃₄H₄₀N₂O₁₀ [M + H]⁺, 637.2761; found, 637.2758.

4-(4-((2*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl)amino)-3-oxopropyl)phenoxy)butanoic acid (20)

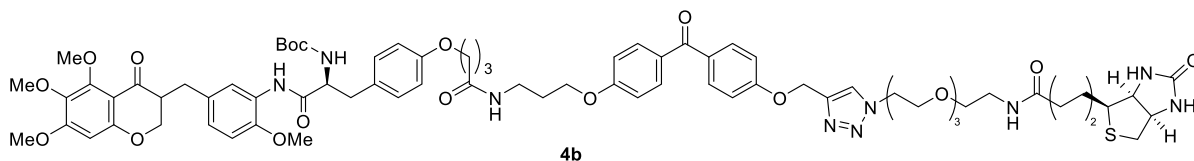


To an acetone (3 mL) solution of **19** (77 mg, 0.067 mmol), benzyl 4-bromobutanoate (28 mg, 0.1 mmol) and K₂CO₃ (30 mg, 0.2 mmol) were added. After refluxing for 24 h, the reaction mixture was

diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate / *n*-hexanes = 1 : 1) to afford the benzyl butanoate **19'** (35 mg, 64%). $^1\text{H-NMR}$ (600 MHz, CD_3OD) δ 8.21 (s, 1H), 8.04 (bs, 1H), 7.33-7.23 (m, 5H), 7.11 (d, 2H, J = 8.4 Hz), 6.88 (d, 1H, J = 7.8 Hz), 6.77 (d, 2H, J = 8.4 Hz), 6.73 (d, 1H, J = 7.8 Hz), 6.23 (d, 1H, J = 1.8 Hz), 5.10 (s, 2H), 4.42 (bs, 1H), 4.28 (dd, 1H, J = 10.8 and 3.6 Hz), 3.94 (t, 2H, J = 6 Hz), 3.90 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.71 (s, 3H), 3.22 (dd, 1H, J = 13.8 and 3.6 Hz), 3.09 (bs, 1H), 3.03 (bs, 1H), 2.78-2.74 (m, 1H), 2.61(t, 1H, J = 13.8 Hz), 2.55 (t, 2H, J = 7.2 Hz), 2.10 (m, 2H), 1.41 (s, 9H); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ 191.3, 173.0, 171.1, 169.3, 159.7, 159.2, 154.4, 146.7, 137.4, 135.9, 131.0, 130.3, 128.5, 128.2, 128.2, 124.4, 120.3, 114.7, 110.0, 108.7, 96.0, 69.2, 66.6, 66.3, 61.6, 61.3, 60.4, 56.1, 55.6, 53.4, 48.3, 48.3, 37.7, 32.2, 31.5, 30.8, 28.3, 24.6, 22.6.

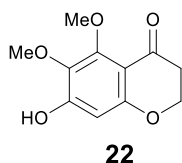
A solution of the benzyl butanoate **19'** (30 mg, 0.036 mmol) and 10% Pd/C (10 mg) in ethyl acetate (3 mL) was placed under an atmosphere of hydrogen. After stirring for 2 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 1) to afford **20** (23 mg, 86%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 7.89 (s, 1H), 7.23 (d, 1H, J = 7.6 Hz), 7.18 (d, 2H, J = 7.6 Hz), 6.96 (d, 1H, J = 8.4 Hz), 6.90 (d, 1H, J = 8.4 Hz), 6.80 (d, 2H, J = 7.6 Hz), 6.42 (s, 1H), 4.26 (m, 2H), 4.10-4.06 (m, 1H), 3.90 (t, 2H, J = 6 Hz), 3.80 (s, 3H), 3.78 (s, 3H), 3.70 (s, 3H), 3.62 (s, 3H), 2.98-2.95 (m, 2H), 2.71 (m, 2H), 2.57 (m, 1H), 2.23 (t, 2H, J = 6.8 Hz), 1.86 (t, 2H, J = 6.8 Hz), 1.30 (s, 9H). A carboxylic acid proton in the butyric acid linked to tyrosine moiety was not observed; $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6) δ 190.5, 171.0, 159.6, 159.3, 157.6, 155.9, 154.0, 137.2, 130.7, 130.6, 130.2, 130.2, 127.3, 121.7, 121.6, 121.6, 114.5, 111.5, 108.6, 96.9, 78.8, 69.3, 67.3, 61.7, 61.2, 57.2, 56.6, 56.3, 48.0, 36.4, 32.1, 31.7, 28.6, 25.3; HRMS (ESI): mass calcd for $\text{C}_{38}\text{H}_{46}\text{N}_2\text{O}_{12}$ $[\text{M} + \text{H}]^+$, 723.3129; found, 723.3129.

***tert*-Butyl ((2*S*)-1-((2-methoxy-5-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl)amino)-1-oxo-3-(4-(4-oxo-4-((3-(4-(4-((1-(13-oxo-17-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzoyl)phenoxy)propyl)amino)butoxy)phenyl)propan-2-yl)carbamate. (**4b**)**



To a solution of **20** (20 mg, 0.028 mmol) in DMF (1 mL) at room temperature were added HBTU (13 mg, 0.033 mmol) and DIPEA (15 μ L, 0.084 mmol). The reaction mixture was stirred at room temperature for 0.5 h and a solution of benzophenone-biotin amine (**9**) (24 mg, 0.028 mmol) in DMF (0.5 mL) was added to the mixture. The resulting mixture was stirred at room temperature for 18 h and quenched at 0 °C with H₂O. The mixture was extracted three times with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH / CH₂Cl₂ = 1 : 30) to afford the amide (**4b**) (36 mg, 87%). ¹H-NMR (400 MHz, DMSO-d₆) δ 9.04 (s, 1H), 8.20 (s, 1H), 7.94 (t, 1H, *J* = 5.2 Hz), 7.88 (s, 1H), 7.78 (t, 1H, *J* = 5.2 Hz), 7.67 (d, 4H, *J* = 7.6 Hz), 7.23 (d, 1H, *J* = 8 Hz), 7.18 (t, 4H, *J* = 8.8 Hz), 7.03 (d, 2H, *J* = 8.8 Hz), 6.95 (d, 1H, *J* = 8 Hz), 6.90 (d, 1H, *J* = 8.4 Hz), 6.79 (d, 2H, *J* = 8 Hz), 6.42 (s, 1H), 6.38 (s, 1H), 6.32 (s, 1H), 5.22 (s, 2H), 4.52 (t, 2H, *J* = 4.8 Hz), 4.26 (m, 3H), 4.10-4.02 (m, 4H), 3.88 (t, 2H, *J* = 6 Hz), 3.80 (s, 4H), 3.77 (s, 3H), 3.70 (s, 3H), 3.62 (s, 3H), 3.48 (d, 2H, *J* = 4.4 Hz), 3.42 (s, 5H), 3.30 (s, 8H), 3.19 (d, 2H, *J* = 5.6 Hz), 3.13 (d, 2H, *J* = 5.6 Hz), 3.03 (m, 1H), 2.98 (d, 2H, *J* = 13.2 Hz), 2.78-2.70 (m, 3H), 2.57 (m, 2H), 2.21 (t, 2H, *J* = 7.2 Hz), 2.02 (t, 2H, *J* = 7.2 Hz), 1.87 (t, 4H, *J* = 6.4 Hz), 1.52 (m, 1H), 1.46-1.44 (m, 3H), 1.29 (s, 9H), 1.22-1.20 (m, 2H); ¹³C-NMR (100 MHz, DMSO-d₆) δ 193.5, 190.5, 172.5, 172.0, 171.0, 163.1, 162.3, 161.7, 159.6, 159.3, 157.5, 155.9, 154.1, 142.4, 137.3, 132.3, 132.2, 130.8, 130.8, 130.7, 130.6, 130.6, 130.3, 127.3, 125.6, 124.9, 114.9, 114.6, 114.5, 111.4, 108.6, 96.9, 78.8, 70.1, 70.0, 69.9, 69.6, 69.3, 69.1, 67.2, 66.0, 61.8, 61.8, 61.7, 61.7, 61.4, 61.2, 59.6, 57.2, 56.6, 56.3, 56.3, 55.8, 49.9, 48.0, 38.8, 35.8, 35.5, 32.2, 32.1, 31.8, 29.2, 28.6, 28.5, 28.4, 25.7, 25.4; HRMS (ESI): mass calcd for C₇₅H₉₅N₉O₁₉S [M + H]⁺, 1458.6543; found, 1458.6518.

7-Hydroxy-5,6-dimethoxychroman-4-one (**22**).

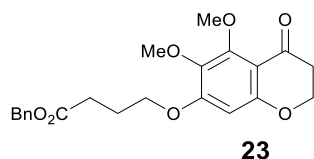


To a solution of 1-(4-(benzyloxy)-6-hydroxy-2,3-dimethoxyphenyl)ethanone **21** (100 mg, 0.33 mmol) in toluene (2.0 mL) was added *N,N*-dimethylformamide dimethyl acetal (0.052 mL, 0.39 mmol). After stirring for 18 h at 80 °C, the mixture was cooled to 0 °C and *c*-HCl (0.2 mL) was added. After stirring for 1 h at 50 °C, The reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure and purified by flash column chromatograph on silica gel (ethyl

acetate : *n*-hexane = 1 : 2) to afford 7-(benzyloxy)-5,6-dimethoxy-4*H*-chromen-4-one **21'** (101 mg, 97%). ¹H-NMR (CDCl₃, 600 MHz) δ 7.63 (d, 1H, *J* = 6.0 Hz), 7.46-7.40 (m, 4H), 7.37 (t, 1H, *J* = 6.6 Hz), 6.7 (s, 1H), 6.1 (d, 1H, *J* = 6.0 Hz), 5.20 (s, 2H), 3.97 (s, 3H), 3.92 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 176.2, 156.8, 154.6, 152.9, 140.7, 135.5, 128.8, 128.4, 127.2, 114.2, 113.8, 97.6, 70.9, 62.1, 61.5, 30.9.

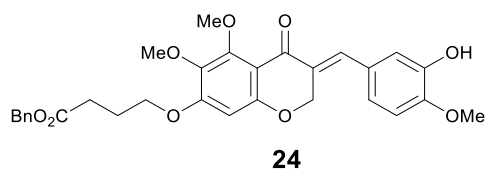
An anhydrous MeOH solution of chromen-4-one **21'** (46.8 mg, 0.15mmol) and 10% Pd/C (15.9mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with EtOAc, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexanes = 1 : 2) to afford the hydrogenated compound **22** (33 mg, 99%); ¹H-NMR (CDCl₃, 600 MHz) δ 6.31 (s, 2H), 4.43 (t, 2H, *J* = 6.6 Hz), 3.90 (s, 3H), 3.90 (s, 3H), 2.72 (d, 2H, *J* = 6.6 Hz). ¹³C-NMR (150 MHz, CDCl₃) δ 189.3, 160.1, 155.5, 153.3, 135.1, 109.6, 98.9, 66.6, 61.5, 61.4, 38.7; HRMS (ESI): mass calcd for C₁₁H₁₂O₅ [M + H]⁺, 224.0685; found, 224.0677.

Benzyl 4-((5,6-dimethoxy-4-oxochroman-7-yl)oxy)butanoate (**23**)



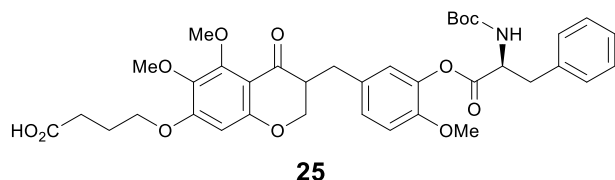
To an acetone (3 mL) solution of 7-hydroxy-5,6-dimethoxychroman-4-one (**22**) (77 mg, 0.34 mmol), benzyl 4-bromobutanoate (88 mg, 0.34 mmol) and K₂CO₃ (142 mg, 1.0 mmol) were added. After refluxing for 24 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate / *n*-hexanes = 1 : 2) to afford the butanoate (**23**) (110 mg, 80%). ¹H-NMR (600 MHz, CDCl₃) δ 7.30 (s, 5H), 6.18 (s, 1H), 5.09 (s, 2H), 4.39 (t, 2H, *J* = 6.0 Hz), 4.01 (t, 2H, *J* = 6.0 Hz), 3.87 (s, 3H), 3.73 (s, 3H), 2.67 (t, 2H, *J* = 6.0 Hz), 2.57 (t, 2H, *J* = 7.2 Hz), 2.17 (m, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 189.1, 172.6, 159.9, 158.5, 154.4, 137.4, 135.8, 128.5, 128.3, 128.2, 109.6, 96.8, 67.5, 66.8, 66.4, 61.6, 61.2, 38.7, 30.5, 24.2; HRMS (EI): mass calcd for C₂₂H₂₄O₇ [M + H]⁺, 400.1522; found, 400.1529.

Benzyl (E)-4-((3-(3-hydroxy-4-methoxybenzylidene)-5,6-dimethoxy-4-oxochroman-7-yl)oxy)butanoate (24)



To solution of butanoate (**23**) (56 mg, 0.139 mmol) in benzene (2 mL) was added isovanillin (22 mg, 0.139 mmol) and *p*-toluenesulfonic acid (3 mg, 0.01 mmol) at 0 °C. The reaction mixture was refluxed for 20 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford **24** (40 mg, 54%). ¹H-NMR (600 MHz, CDCl₃) δ 7.71 (s, 1H), 7.32 (m, 5H), 6.88 (d, 1H, *J* = 8.4 Hz), 6.85 (d, 1H, *J* = 1.8 Hz), 6.83 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.19 (s, 1H), 5.21 (s, 2H), 5.11 (s, 2H), 4.04 (t, 2H, *J* = 6.0 Hz), 3.95(s, 3H), 3.92 (s, 3H), 3.77 (s, 3H), 2.59 (t, 2H, *J* = 7.2 Hz), 2.19 (m, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.5, 172.7, 159.3, 158.4, 154.8, 147.5, 145.5, 137.9, 136.2, 135.8, 130.2, 128.6, 128.3, 128.2, 128.1, 123.2, 115.7, 110.7, 110.5, 96.9, 67.6, 67.5, 66.4, 61.6, 61.3, 56.0, 30.5, 24.3; HRMS (ESI): mass calcd for C₃₀H₃₀O₉ [M + H]⁺, 535.1968; found, 535.1987.

4-((3-(3-(((*tert*-Butoxycarbonyl)-L-phenylalanyl)oxy)-4-methoxybenzyl)-5,6-dimethoxy-4-oxochroman-7-yl)oxy)butanoic acid (25)

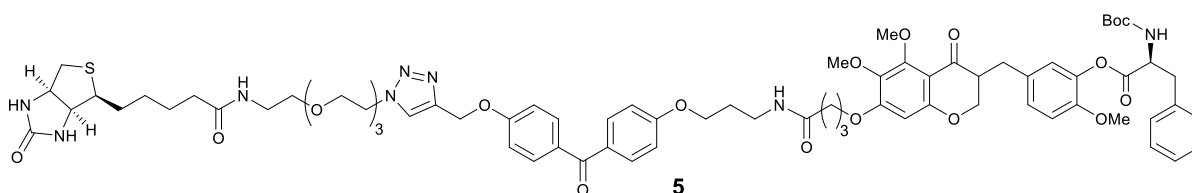


To a CH₂Cl₂ solution (2 mL) of benzylidene (**24**) (18.8 mg, 0.035 mmol) were added Boc-Phe-OH (10 mg, 0.035 mmol), EDCI (8 mg, 0.052 mmol) and DMAP (1 mg, 0.007 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford the acylated benzylidene **24'** (19 mg, 70%). ¹H-NMR (600 MHz, CDCl₃) δ 7.70 (s, 1H), 7.33(m, 10H), 7.16 (dd, 1H, *J* = 9.0 and 2.4 Hz), 7.00 (d, 1H, *J* = 9.0 Hz), 6.87 (s, 1H), 6.21 (s, 1H), 5.16 (d, 2H, *J* = 1.8 Hz), 5.12 (s, 2H), 4.88 (m, 1H), 4.05 (t, 2H, *J* = 6.6 Hz), 3.96 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.32 (dd, 1H, *J* = 14.4

and 6.0 Hz), 3.22 (dd, 1H, $J = 13.8$ and 6.0 Hz), 2.60 (t, 2H, $J = 7.2$ Hz), 2.20 (m, 2H), 1.41(s, 9H); ^{13}C -NMR (150 MHz, CDCl_3) δ 179.3, 172.7, 171.1, 170.0, 159.3, 158.5, 154.9, 151.8, 139.2, 137.9, 135.8, 134.9, 130.7, 129.5, 128.6, 128.3, 128.2, 127.5, 127.1, 124.1, 112.4, 110.6, 96.9, 67.6, 67.4, 66.4, 61.6, 61.3, 55.9, 54.3, 38.2, 31.5, 30.5, 29.9, 29.7, 28.3, 28.1, 24.3; HRMS (ESI): mass calcd for $\text{C}_{44}\text{H}_{47}\text{NO}_{12}$ $[\text{M} + \text{H}]^+$, 782.3175; found, 782.3164.

A solution of the acylated benzylidene **24'** (19 mg, 0.024 mmol) and 10% Pd/C (4 mg) in ethyl acetate (2 mL) was placed under an atmosphere of hydrogen. After stirring for 24 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford the butanoic acid (**25**) (16 mg, 99%). ^1H -NMR (600 MHz, CDCl_3) δ 7.32 (m, 5H), 7.05 (d, 1H, $J = 7.8$ Hz), 6.89 (d, 1H, $J = 8.4$ Hz), 6.80 (s, 1H), 6.24 (s, 1H), 4.85 (m, 1H), 4.24(dd, 1H, $J = 11.4$ and 3.6 Hz), 4.07 (m, 3H), 3.90 (s, 3H), 3.78 (s, 3H), 3.77 (s, 3H), 3.32 (dd, 1H, $J = 7.8$ Hz), 3.20 (m, 2H), 2.70 (m, 1H), 2.60 (t, 1H, $J = 12$ Hz), 2.43 (t, 2H, $J = 7.2$ Hz), 2.15 (m, 2H). A carboxylic acid proton in the C7-butyric acid moiety was not observed; ^{13}C -NMR (150 MHz, CDCl_3) δ 191.0, 177.4, 170.1, 159.6, 159.0, 155.0, 154.4, 149.6, 139.2, 137.6, 136.0, 131.1, 129.6, 128.5, 127.6, 127.0, 123.3, 112.6, 108.4, 96.7, 79.9, 68.9, 68.5, 65.0, 61.6, 61.2, 55.8, 54.3, 52.0, 48.2, 40.8, 38.2, 32.3, 31.9, 29.7, 29.6, 28.3, 25.1; HRMS (ESI): mass calcd for $\text{C}_{37}\text{H}_{43}\text{NO}_{12}$ $[\text{M} + \text{H}]^+$, 694.2864; found, 694.2852.

5-((5,6-Dimethoxy-4-oxo-7-(4-oxo-4-((3-(4-(4-((1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxo-12-azapentacycl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzoylphenoxy)propyl)amino)butoxy)chroman-3-yl)methyl)-2-methoxyphenyl (tert-butoxycarbonyl)-L-phenylalaninate (5)



To a solution of **25** (12 mg, 0.016 mmol) in DMF (1 mL) at room temperature were added HBTU (9 mg, 0.019 mmol) and DIPEA (2 μL , 0.048 mmol). The reaction mixture was stirred at room temperature for 0.5 h and a solution of benzophenone-biotin amine (**9**) (14 mg, 0.016 mmol) in DMF (0.5 mL) was added to the mixture. The resulting mixture was stirred at room temperature for 18 h and quenched at 0 $^\circ\text{C}$ with H_2O . The mixture was extracted three times with ethyl acetate and the

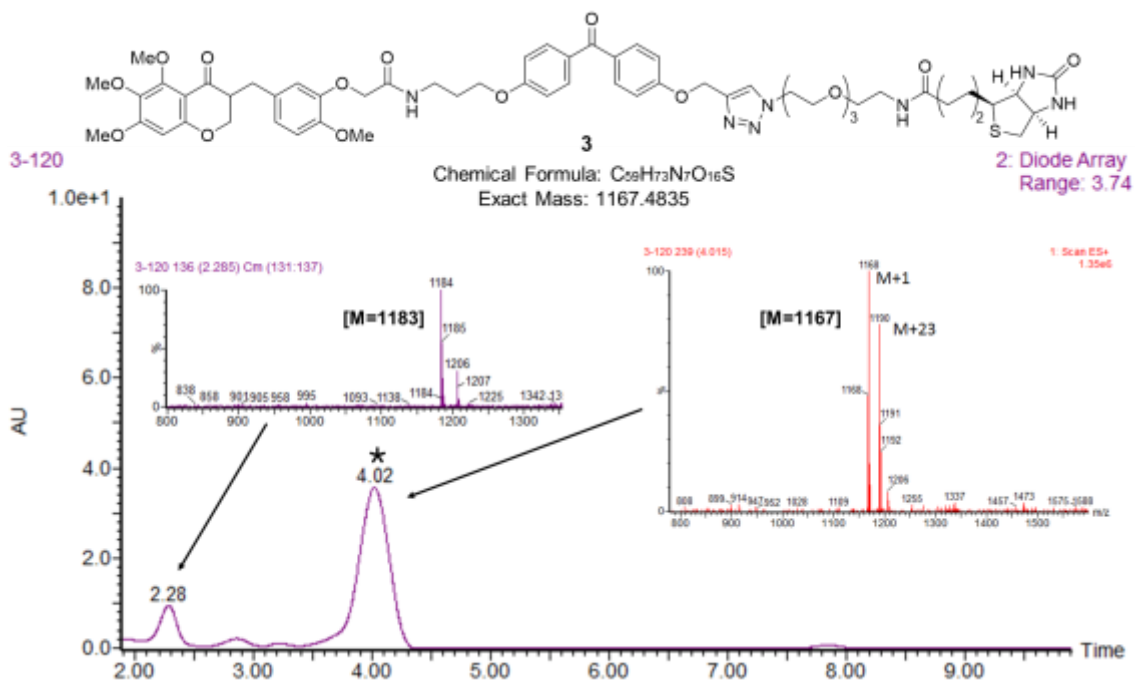
combined extracts were washed with brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($\text{MeOH} / \text{CH}_2\text{Cl}_2 = 1 : 30$) to afford the amide (**5**) (8.2 mg, 36%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.86 (s, 1H), 7.75 (dd, 4H, $J = 8.8$ and 2.8 Hz), 7.31-7.25 (m, 4H), 7.05 (d, 3H, $J = 8.8$ Hz), 6.91 (t, 3H, $J = 8.4$ Hz), 6.80 (s, 1H), 6.53 (s, 1H), 6.22 (s, 1H), 6.18 (s, 1H), 5.91 (s, 1H), 5.26 (s, 2H), 5.04 (d, 1H, $J = 8$ Hz), 4.84 (d, 1H, $J = 6.8$ Hz), 4.56 (t, 2H, $J = 4.8$ Hz), 4.45 (m, 1H), 4.25 (m, 2H), 4.08 (m, 4H), 3.88 (m, 5H), 3.77 (s, 3H), 3.77 (s, 3H), 3.64-3.53 (m, 10H), 3.48-3.44 (m, 4H), 3.39 (t, 2H, $J = 5.2$ Hz), 3.33 (dd, 1H, $J = 14.4$ and 6 Hz), 3.21-3.09 (m, 4H), 2.86 (m, 1H), 2.72 (m, 2H), 2.61 (m, 1H), 2.42 (t, 2H, $J = 7.6$ Hz), 2.18-2.12 (m, 4H), 2.03-1.98 (m, 2H), 1.63-1.56 (m, 4H), 1.46-1.39 (m, 11H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 194.3, 191.0, 173.5, 172.3, 171.1, 170.1, 164.0, 162.0, 161.4, 159.7, 158.5, 154.4, 149.6, 143.1, 139.2, 137.4, 136.0, 132.2, 132.2, 131.0, 131.0, 130.5, 129.6, 128.5, 127.6, 127.0, 124.4, 123.3, 114.3, 113.9, 112.6, 108.6, 96.9, 79.9, 70.4, 70.3, 70.2, 70.0, 69.9, 69.8, 69.3, 68.9, 68.0, 66.1, 61.9, 61.8, 61.7, 61.3, 60.4, 60.2, 55.8, 55.5, 54.3, 50.6, 50.3, 48.1, 42.5, 40.4, 39.1, 38.2, 36.9, 35.7, 32.6, 31.7, 29.0, 28.3, 28.1, 25.5, 24.8; HRMS (ESI): mass calcd for $\text{C}_{74}\text{H}_{92}\text{N}_8\text{O}_{19}\text{SNa} [\text{M} + \text{Na}]^+$, 1451.6097; found, 1451.6054.

LC-MS analysis of four photoaffinity probes

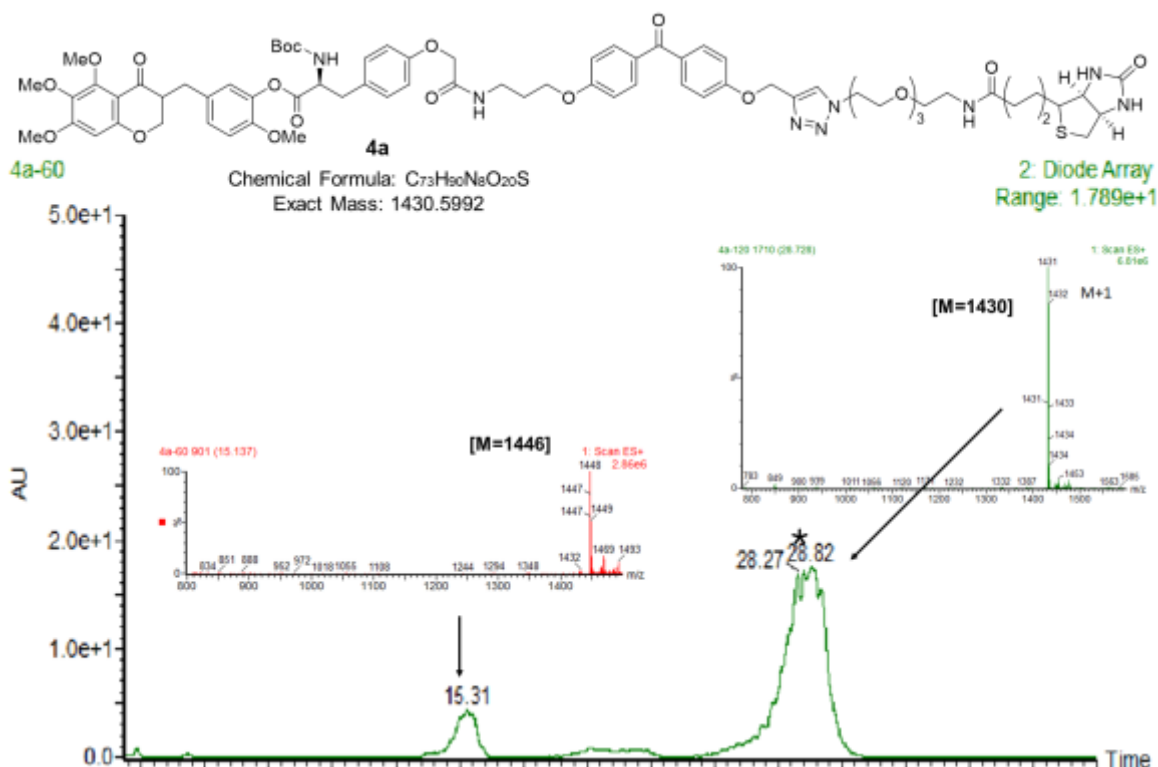
LC-MS data were obtained using a Waters system (Waters 2489 UV/Visible Detector, Waters 3100 Mass, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters Reagent Manager, and Waters 2767 Sample Manager) by using SunFire C18 column (4.6 × 50 mm, 5 μm particle size), isocratic elution at 40% acetonitrile in water for 60 min; flow rate: 1.4 mL/min

LC conditions	MS conditions
System : Waters Alliance HPLC system	System: Waters 3100 mass detector
Detection: PDA detector	Mode: Positive electrospray (ES+)
Flow rate: 1.4 mL/min	Capillary: 3.5 kV
Mobile phase A: H ₂ O	Cone: 50 V
Mobile phase B: Acetonitrile	Extractor: 1 V
Column: Sunfire C18 5.0 μm 4.6 x 50 mm Column	RF Lens: 0.1 V
Sample diluent: DMSO	Source temp.: 120 °C
Injection volume: 10 μL	Desolvation temp.: 350 °C
PDA scan range: 200 to 600 nm	Desolvation Gas: 650 L/h
Data management: MassLynx Software	
Isocratic: 40% Acetonitrile in H ₂ O	

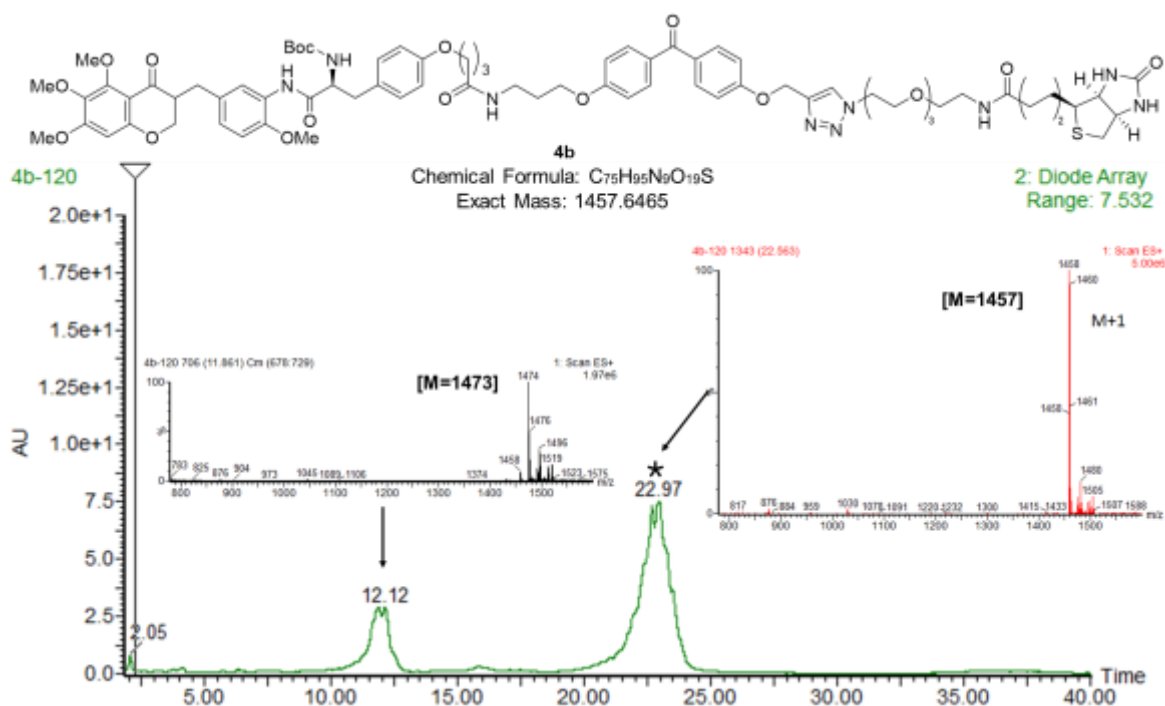
1) Compound 3



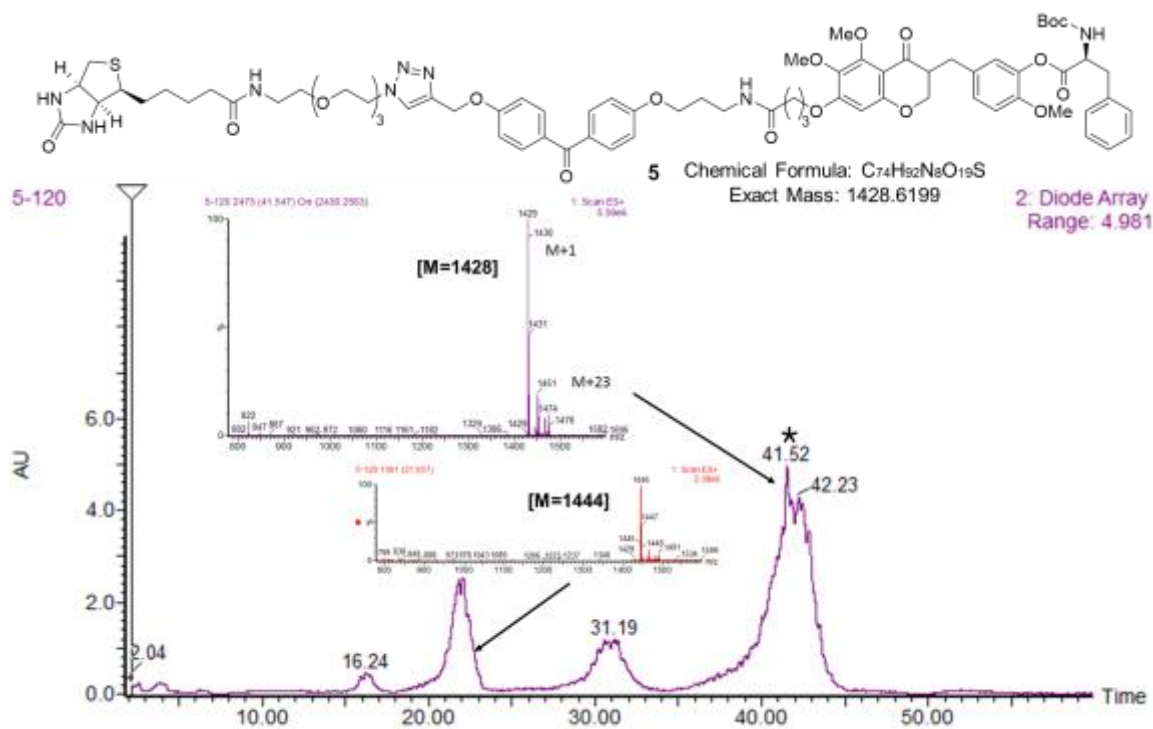
2) Compound 4a



3) Compound 4b



4) Compound 5



Biological Methods

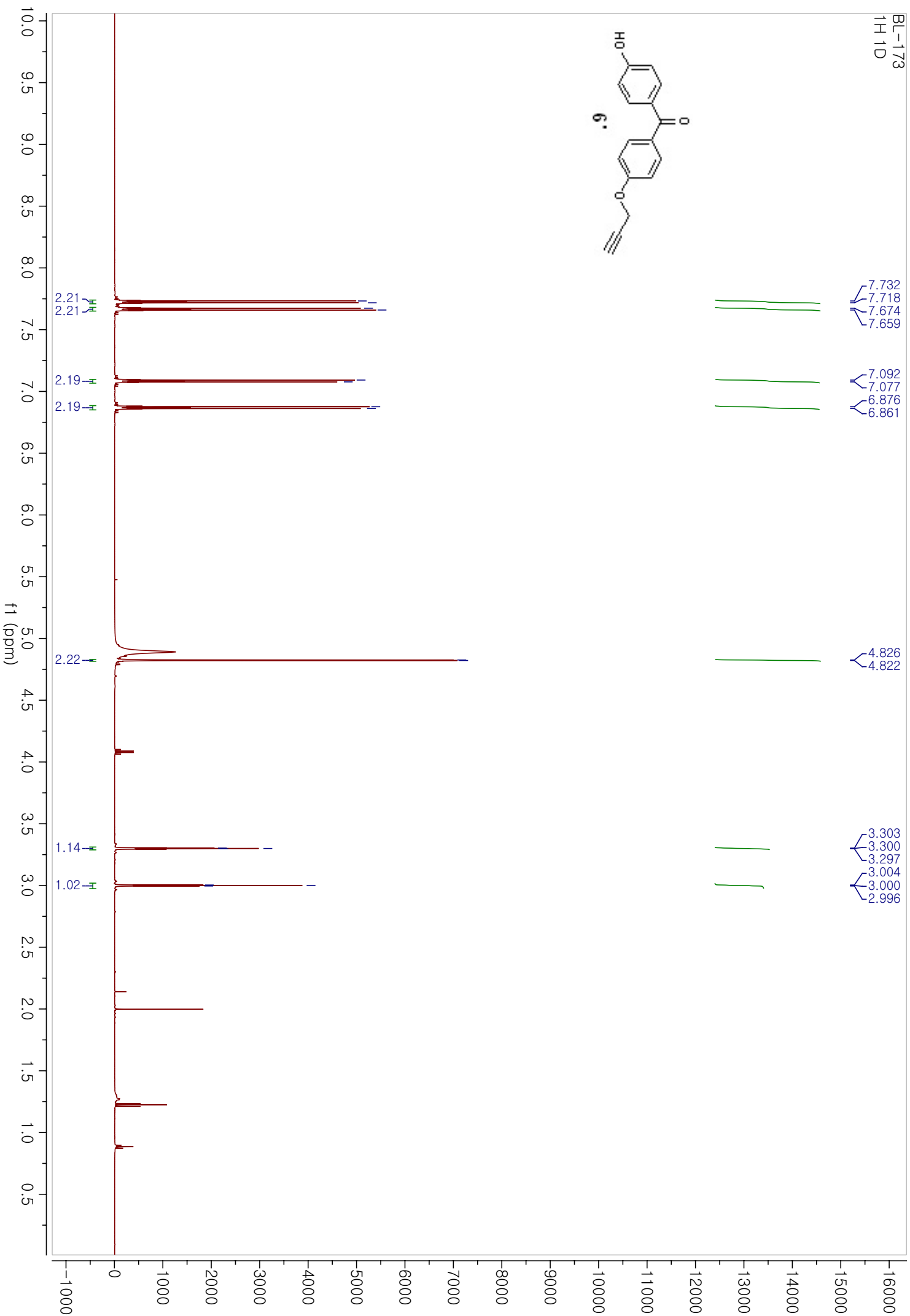
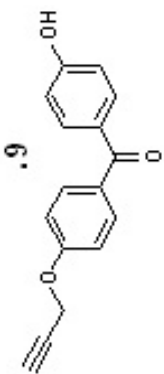
Materials

EBM-2 and IMDM growth media were purchased from Lonza (Walkersville, MD, USA). RPMI and Ham's/F-10 media were purchased from Thermo Scientific (Waltham, MA, USA). HRECs and Attachment Factor were purchased from Cell Systems (Kirkland, WA, USA). Clonetics® HUVECs were purchased from Lonza. All endothelial cells were used between passages 5 and 8. Endothelial Growth Medium (EGM-2) was prepared by mixing the contents of an EGM-2 "Bullet Kit" (Cat. no. CC-4176) with Endothelial Basal Medium (EBM) (Lonza). The EGM-2 "Bullet Kit" contains hydrocortisone, human fibroblast growth factor, vascular endothelial growth factor, R3-insulin like growth factor, ascorbic acid, human epidermal growth factor, gentamycin and heparin along with 2% fetal bovine serum (FBS). 92-1 cells¹ (a kind gift from Dr. Martine Jager) were grown in RPMI medium containing 10% FBS and 1% penicillin-streptomycin (pen-strep). Y79 cells² (a kind gift from Dr. Brenda Gallie) were grown in RB medium (IMDM + 10% FBS + 55 μ M β -mercaptoethanol + 10 μ g/mL insulin + 1% pen-strep). ARPE19 cells (ATCC, Manassas, VA, USA) were grown in Ham's/F-10 + 10% FBS + 1% pen-strep. Identity of 92-1 and Y79 cell lines was confirmed by short tandem repeat profiling.

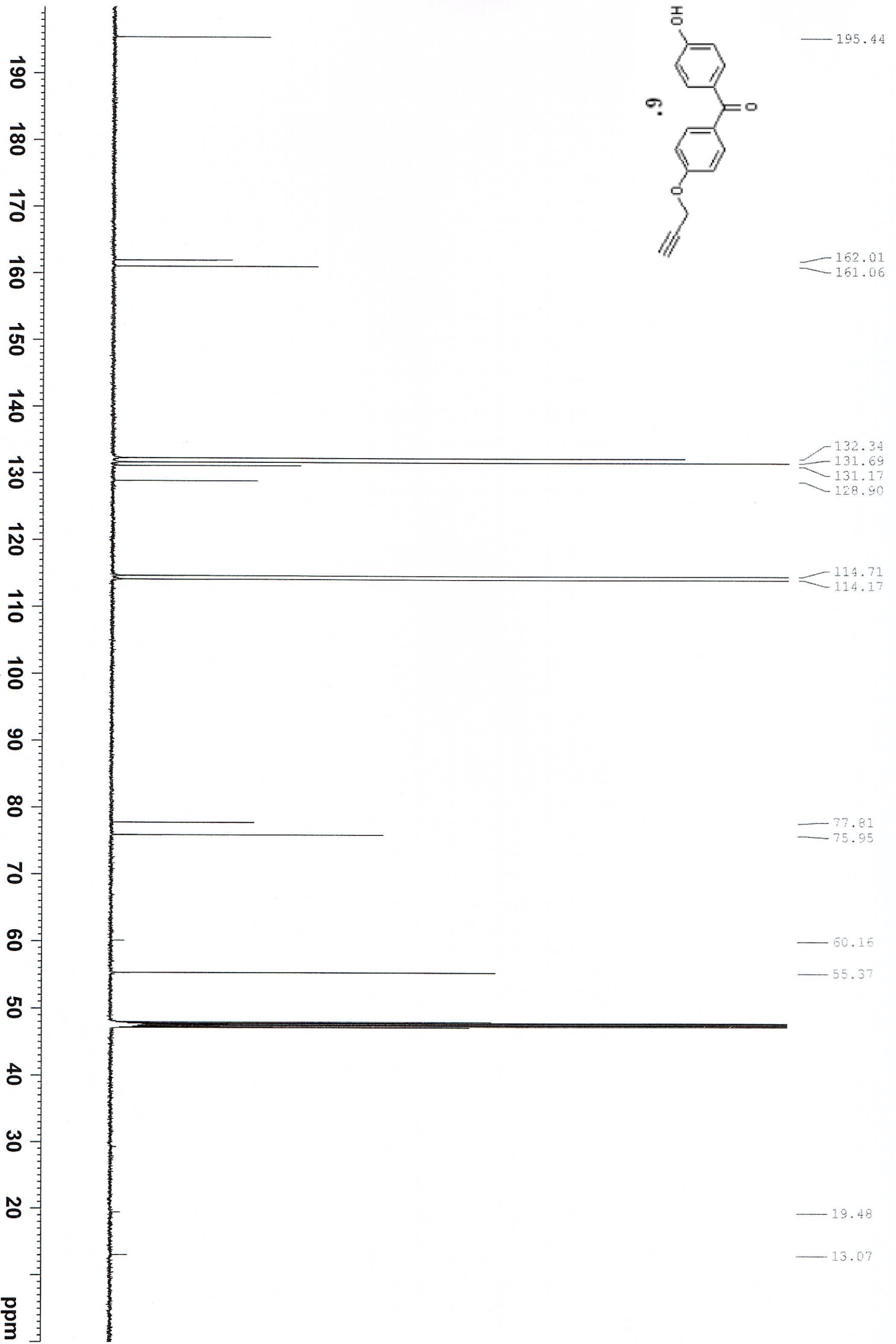
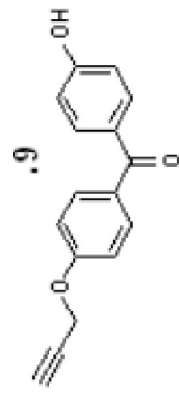
Cell Proliferation Assay

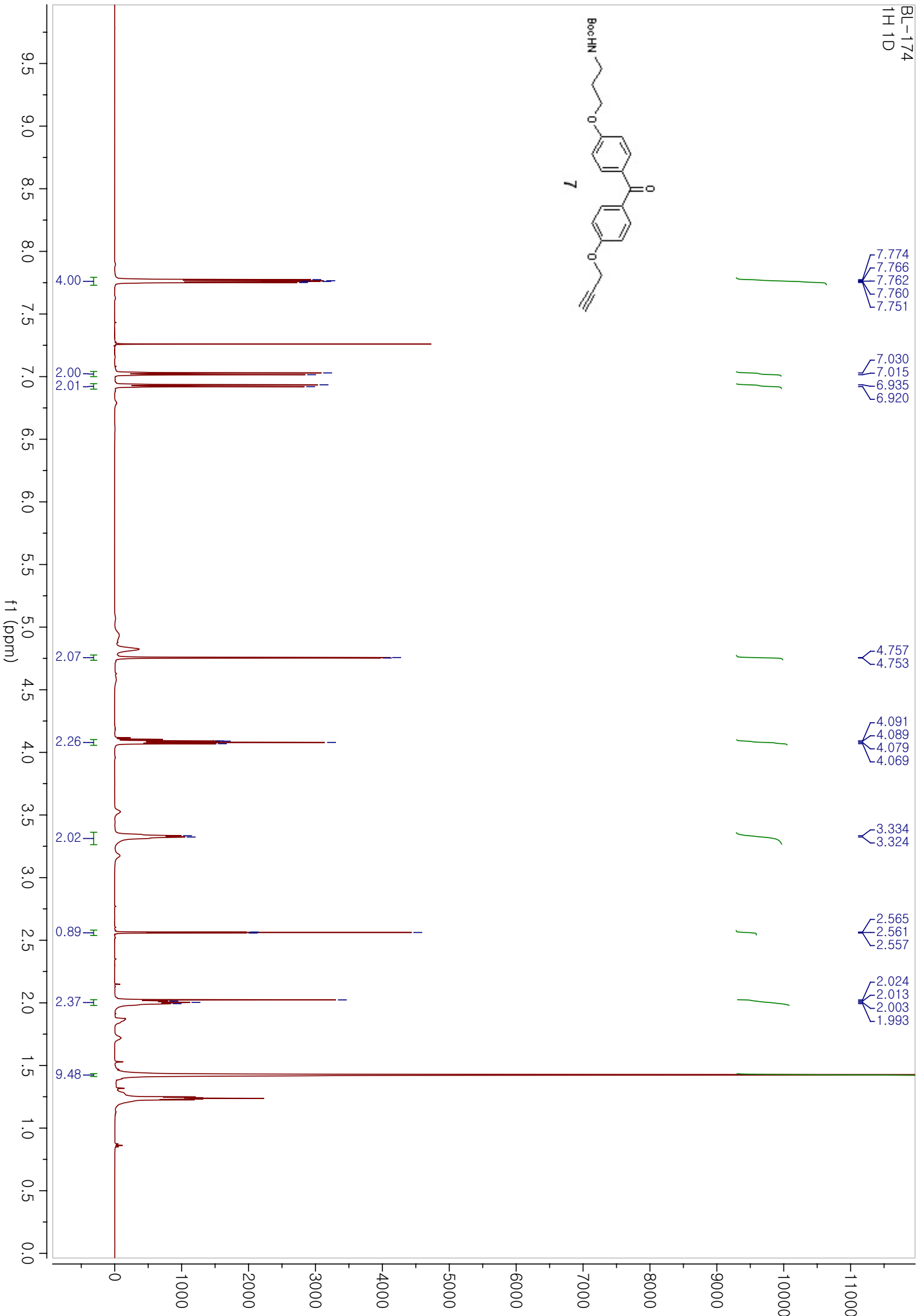
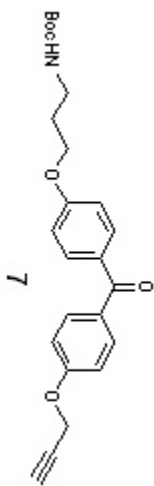
The proliferation of cells was monitored by an alamarBlue based fluorescence assay. Five cell types were used: HRECs, HUVECs, 92-1, Y79 and ARPE19. Briefly, 2,500 cells in 100 μ L growth medium were incubated in 96-well clear bottom black plates for 24 hours followed by 48 hours' incubation with different concentrations of each test compound (range: 0.5 nM to 500 μ M). At the end of the incubation, 11.1 μ L of alamarBlue reagent was added and 4 hours after, fluorescence readings were taken with excitation and emission wavelengths of 560 nm and 590 nm respectively. Data were analyzed and dose response curves generated using GraphPad Prism software (v. 6.0). Only those compounds that reduced cell number by 50% or more at the highest concentration tested (relative to DMSO control) were reported as having a GI₅₀ < 100 μ M.

1. I. De Waard-Siebinga, D. J. Blom, M. Griffioen, P. I. Schrier, E. Hoogendoorn, G. Beverstock, E. H. Danen and M. J. Jager, *Int. J. Cancer*, 1995, **62**, 155-161.
2. T. W. Reid, D. M. Albert, A. S. Rabson, P. Russell, J. Craft, E. W. Chu, T. S. Tralka and J. L. Wilcox, *J. Natl. Cancer Inst.*, 1974, **53**, 347-360.

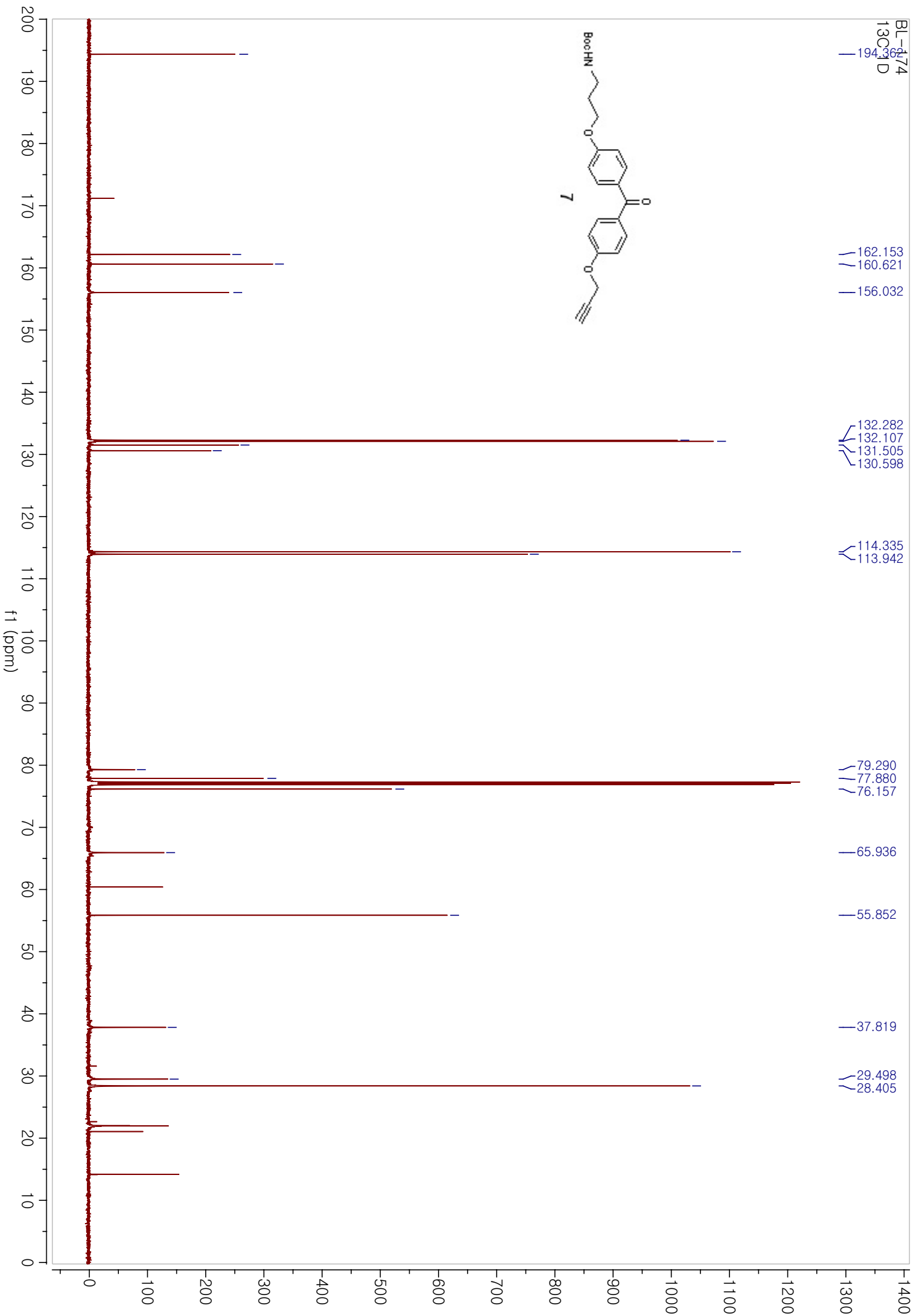
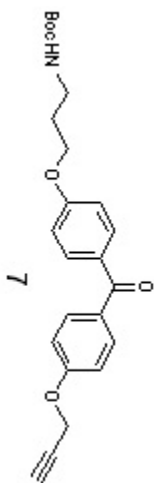


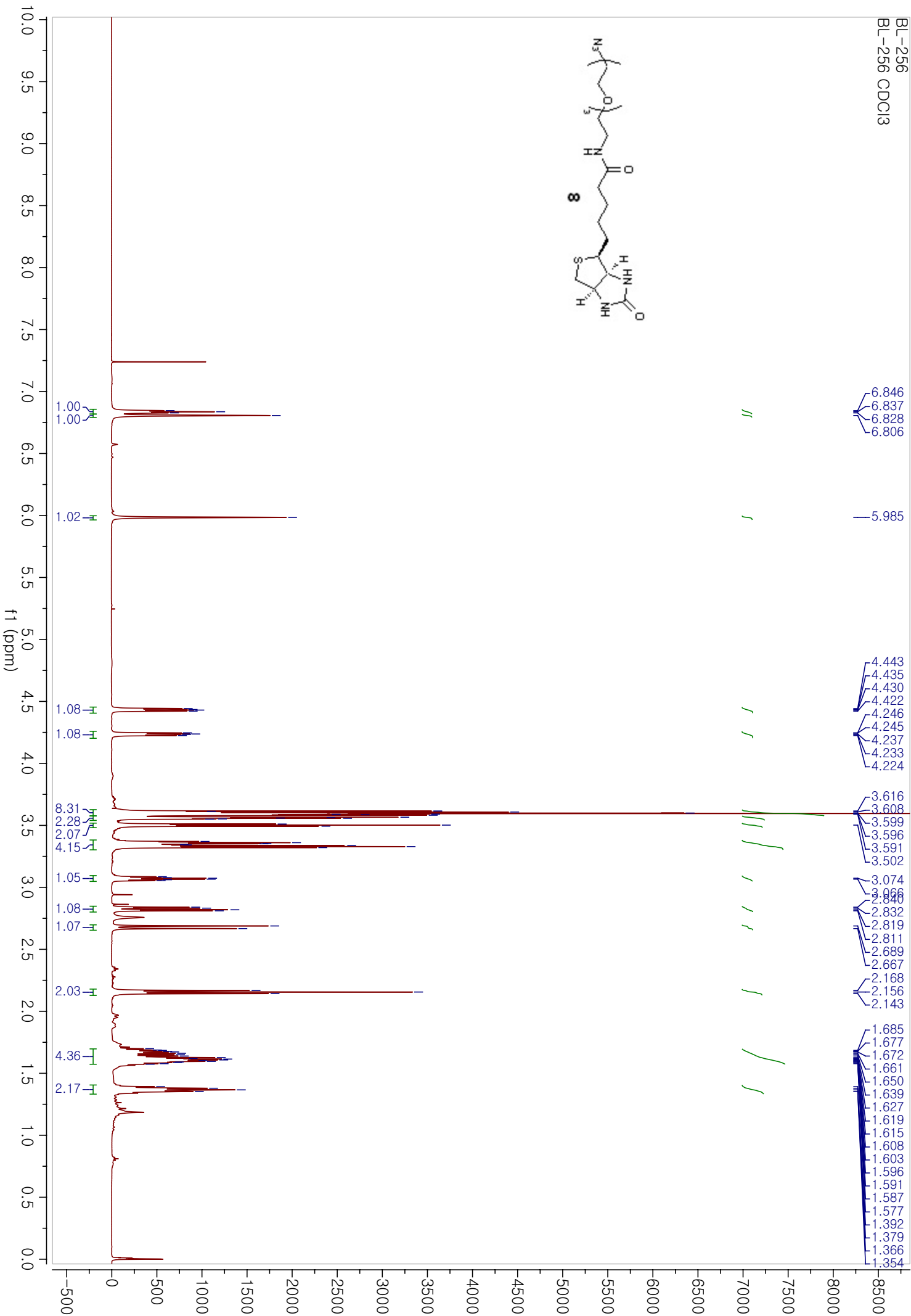
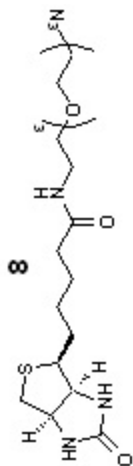
Carbon 1D



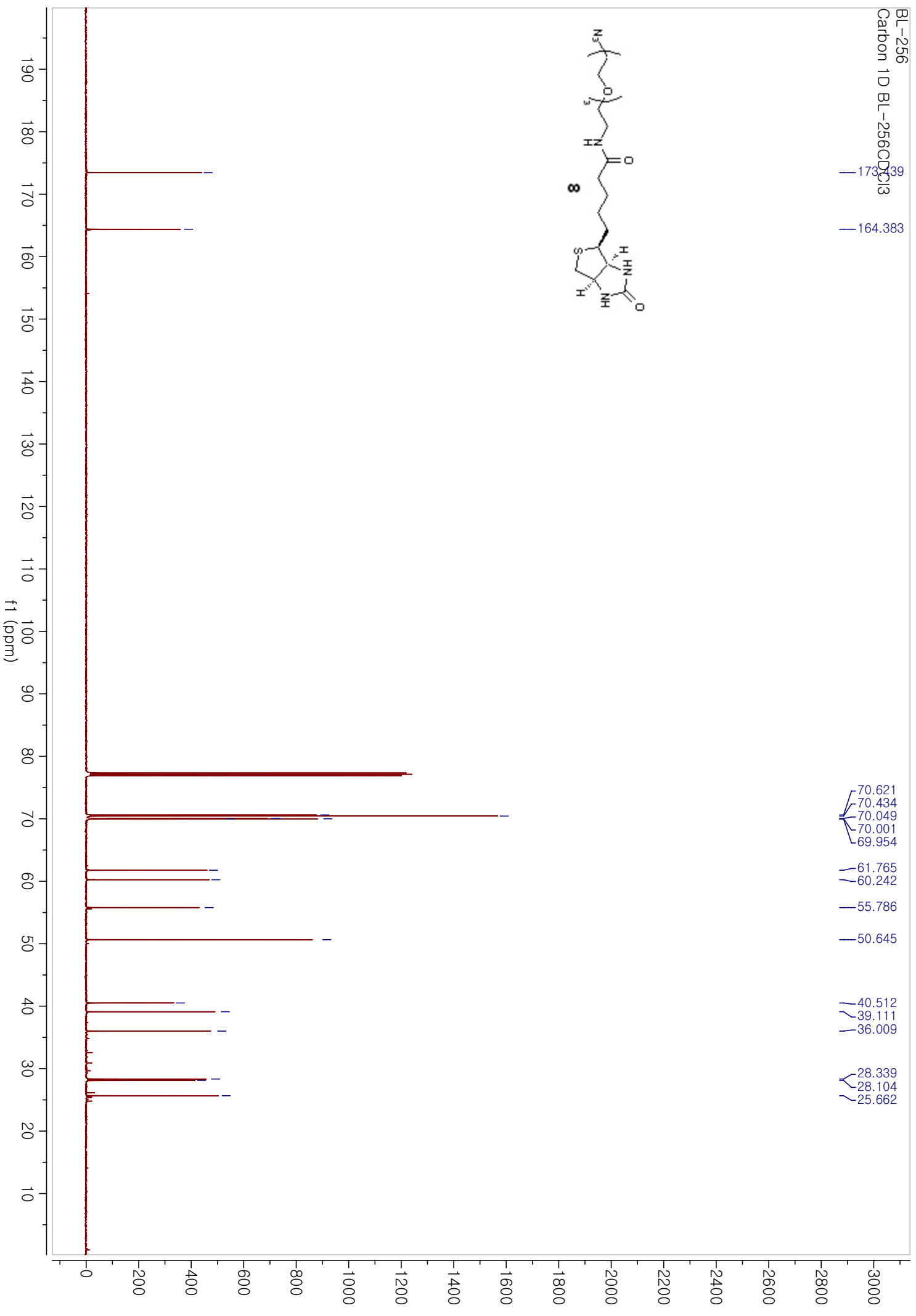
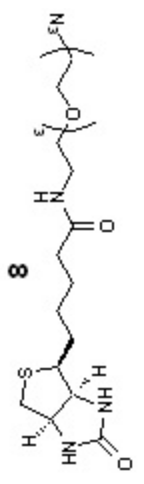


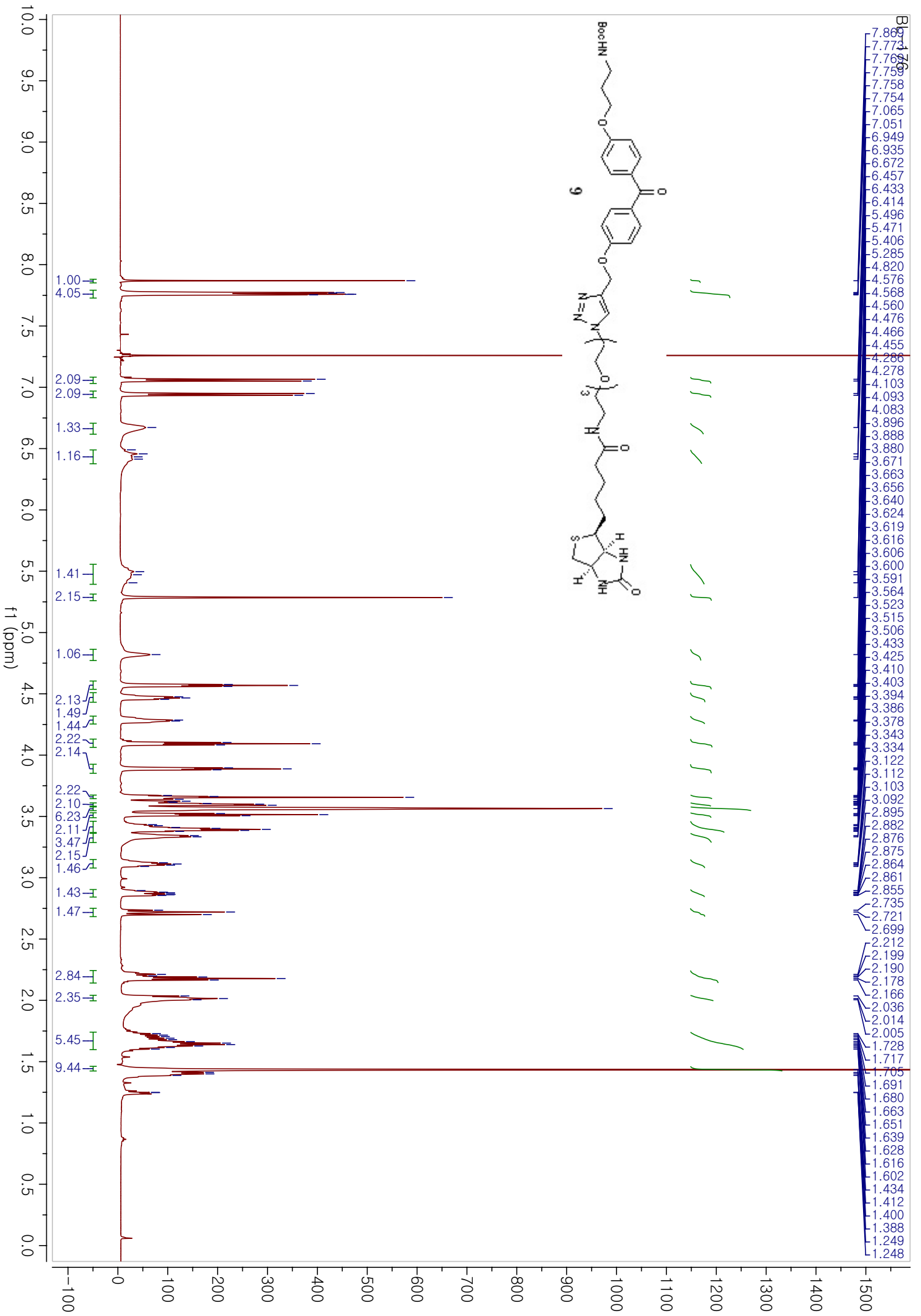
BL-474
13C NMR



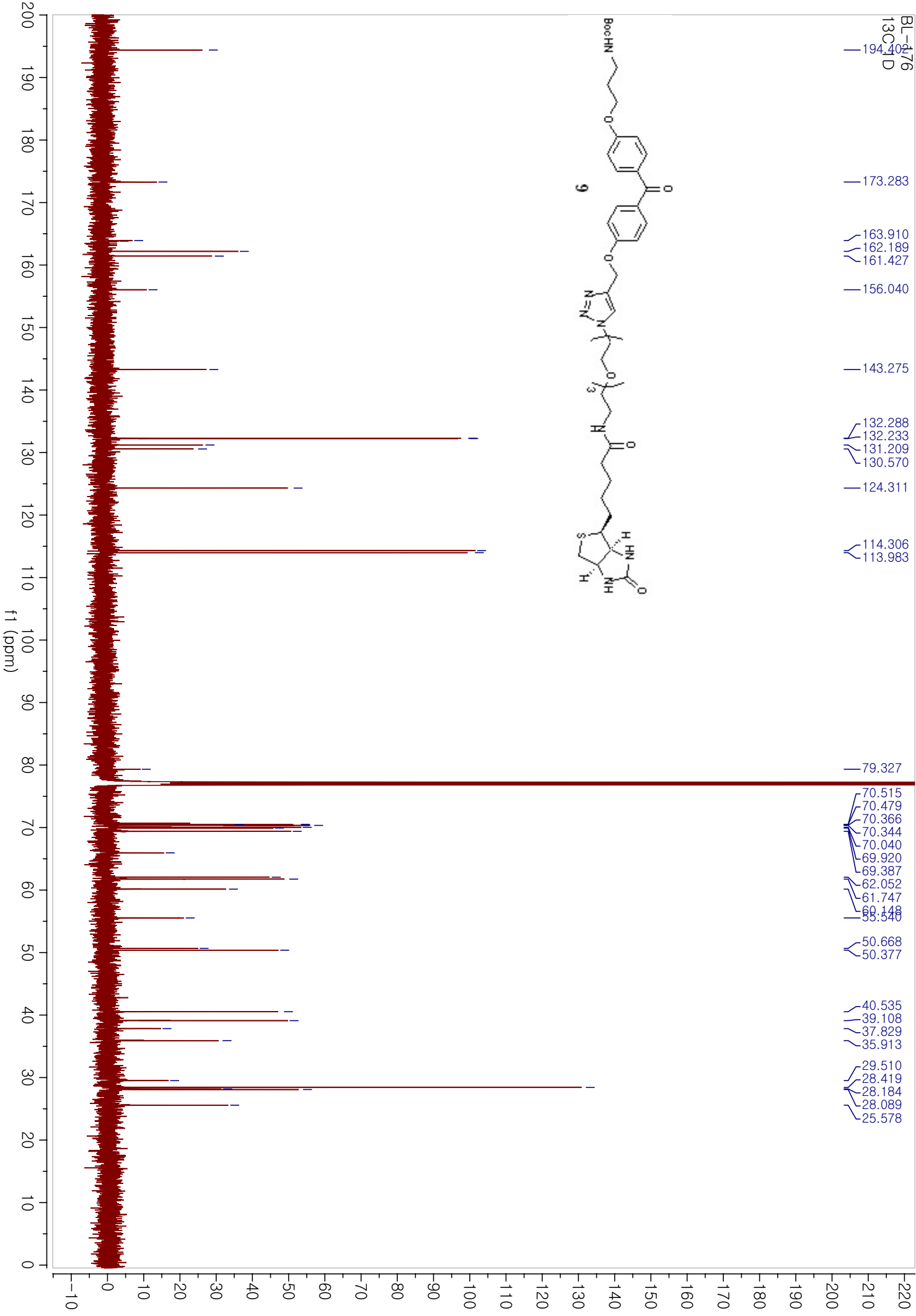
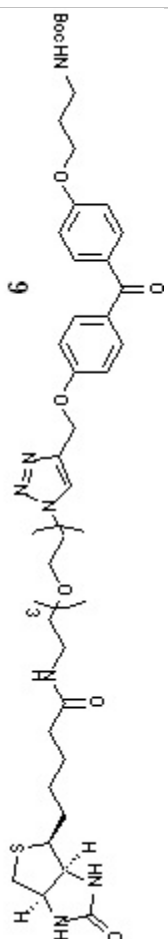


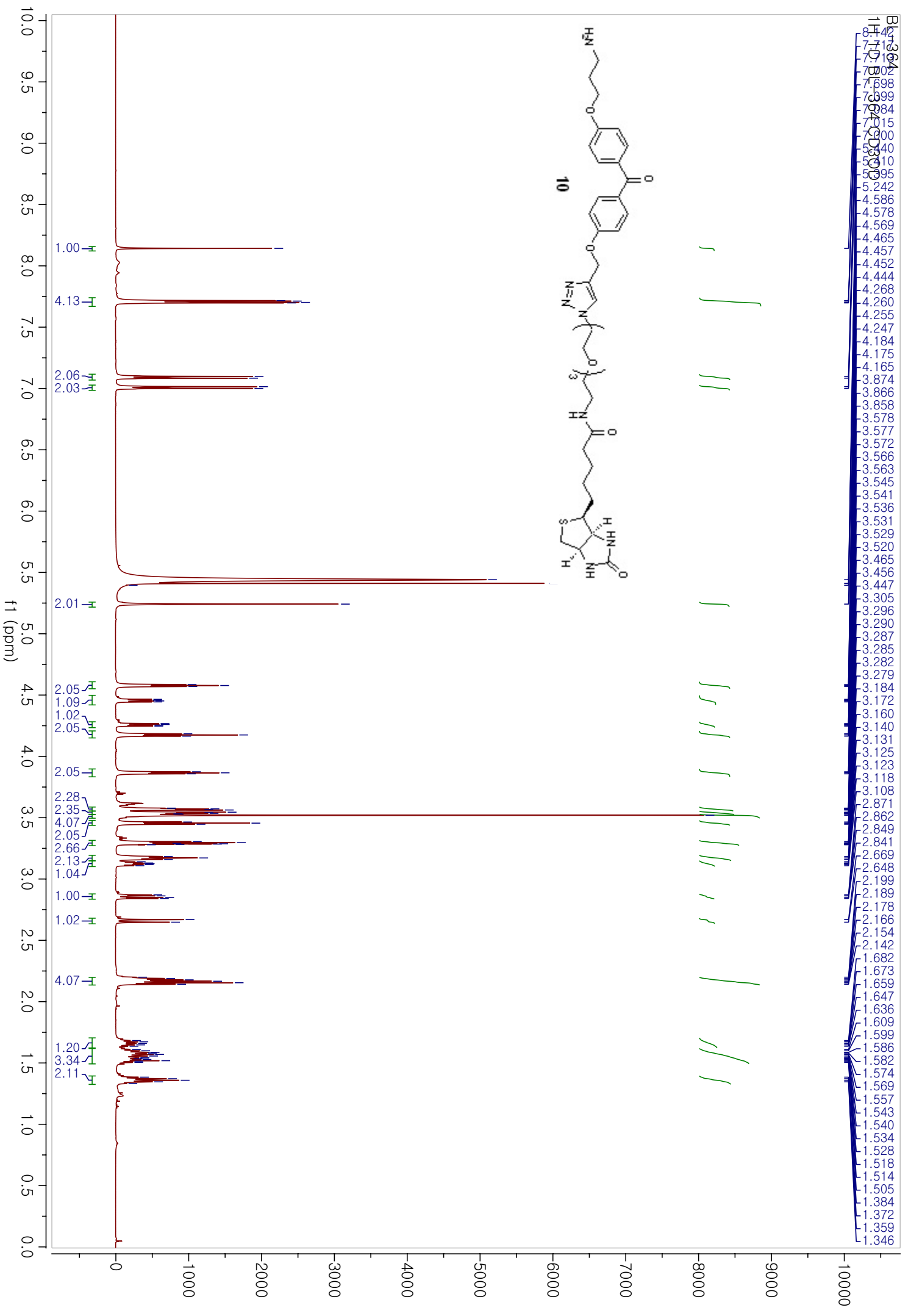
173.389
164.383



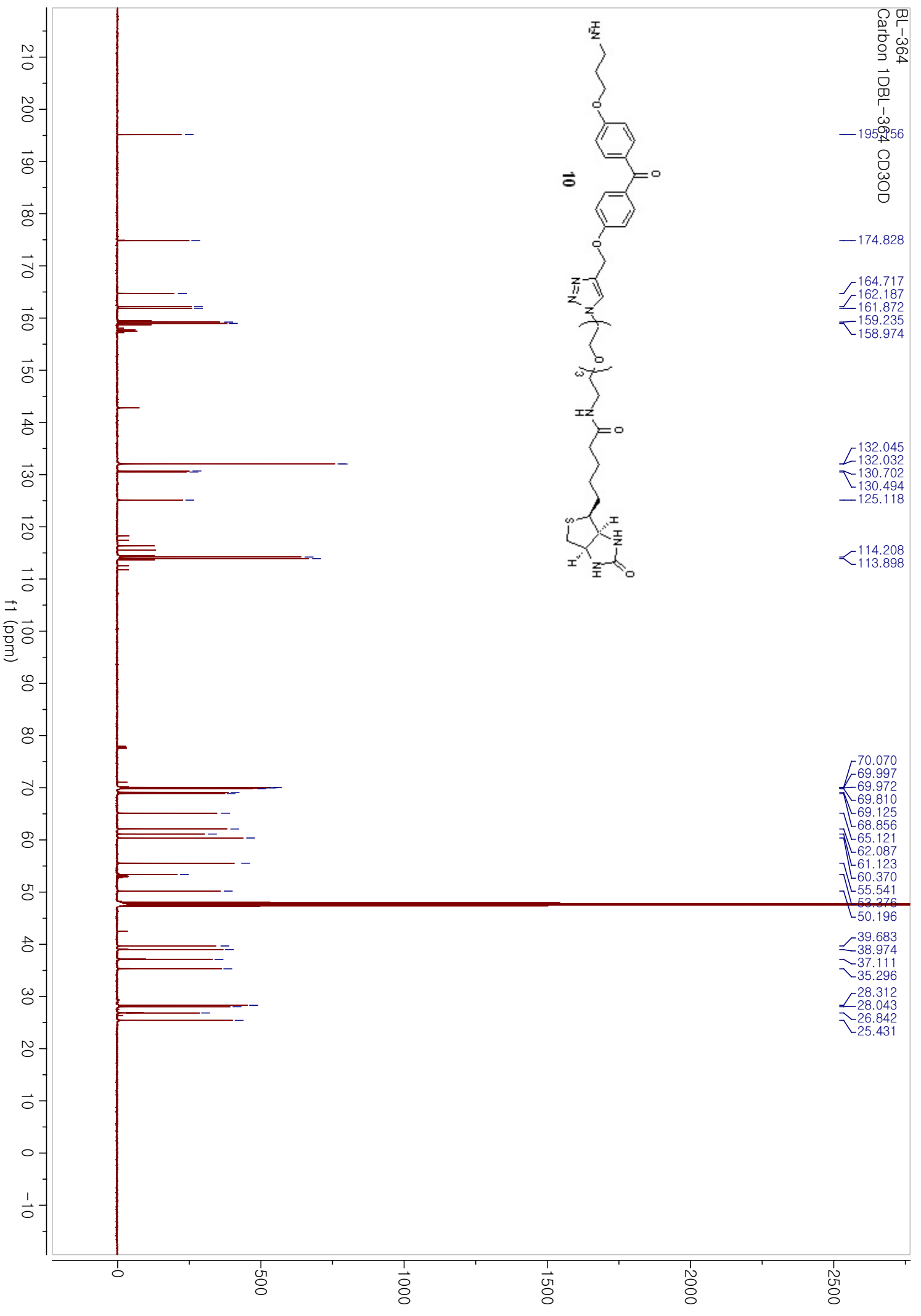
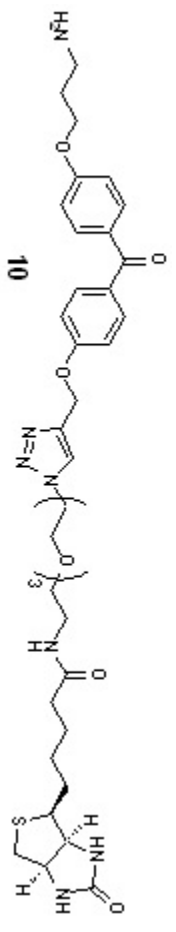


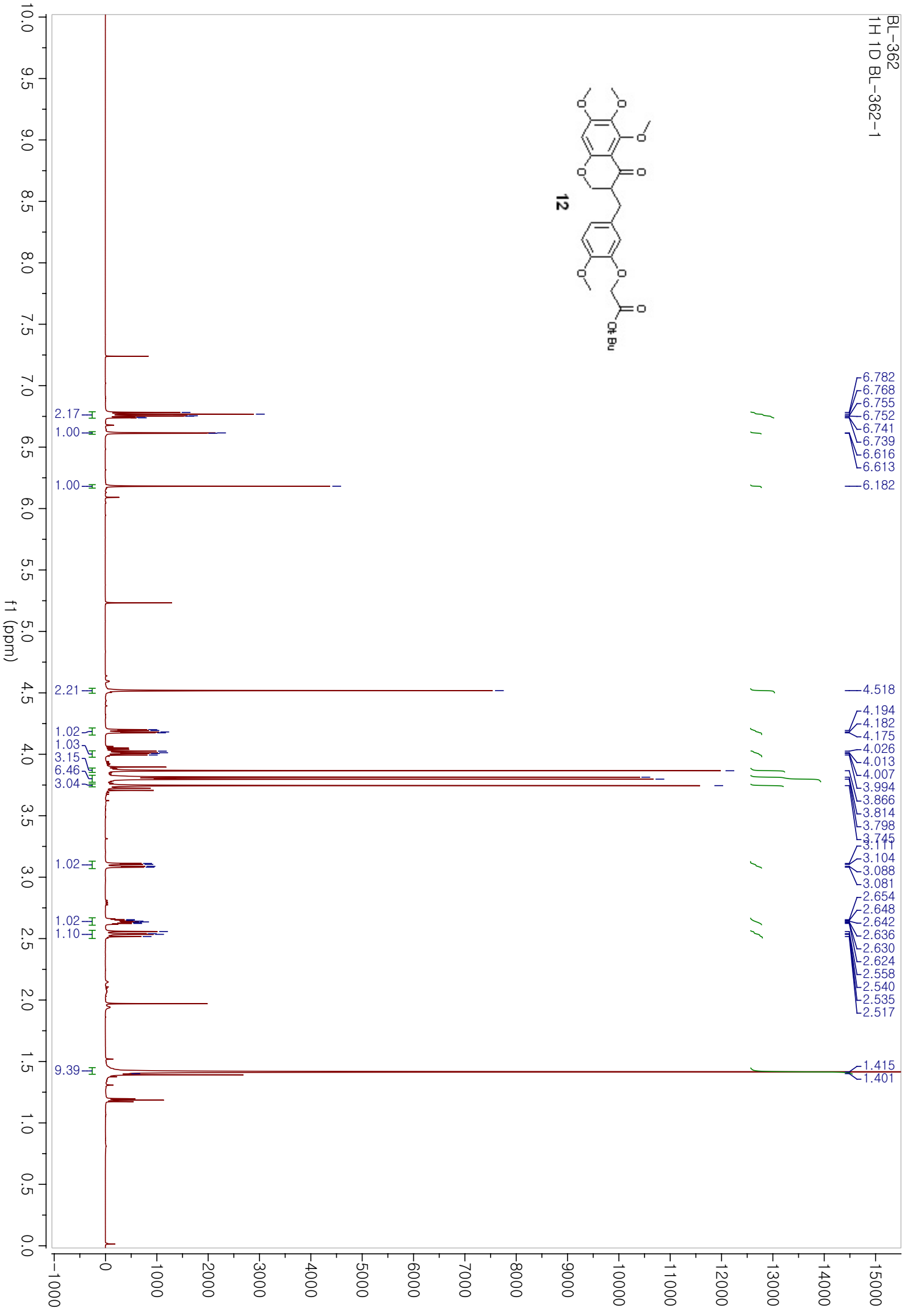
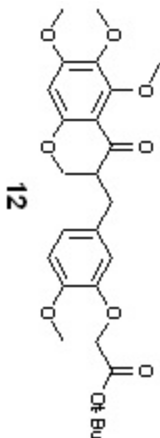
BL-476
13C NMR



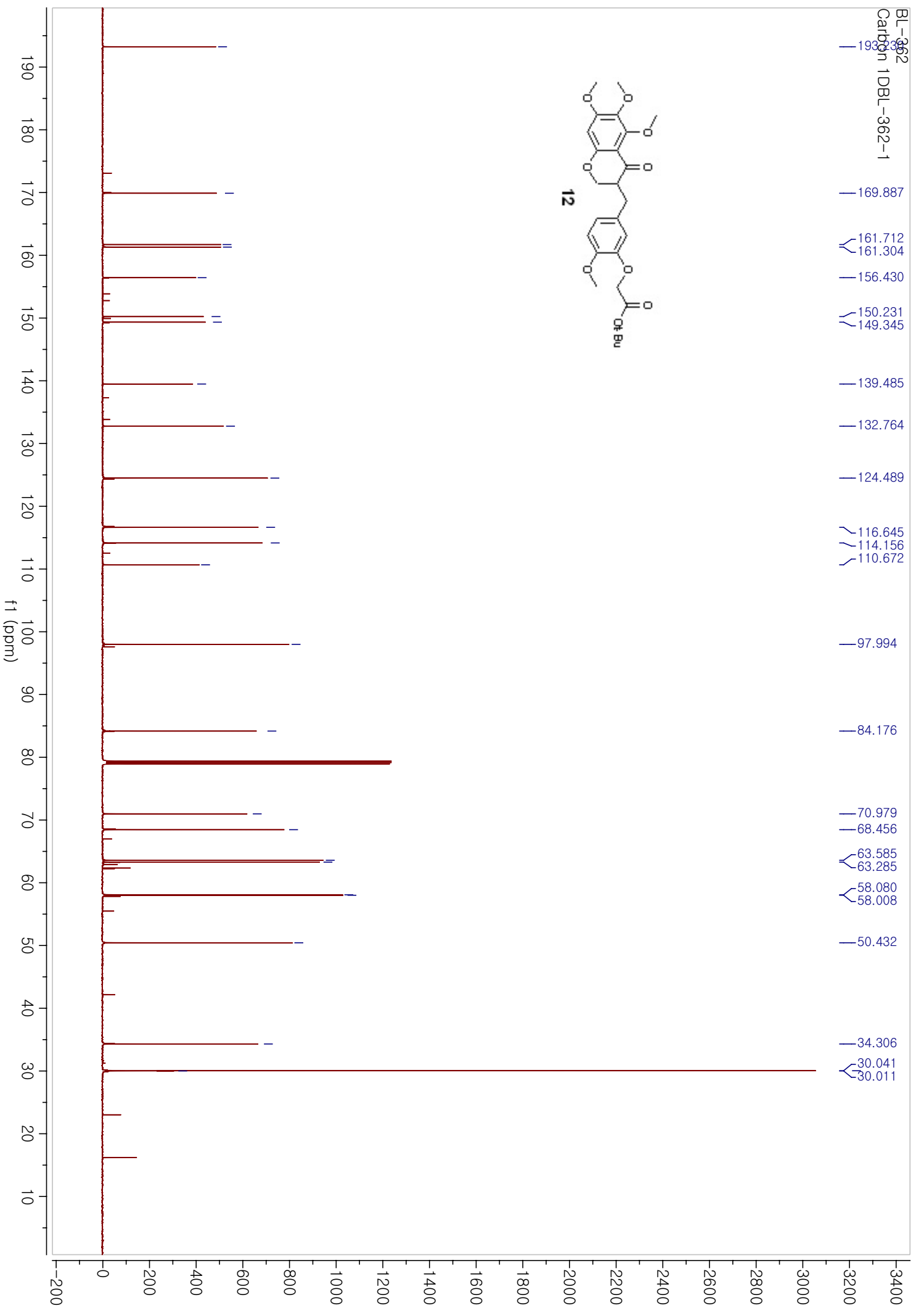
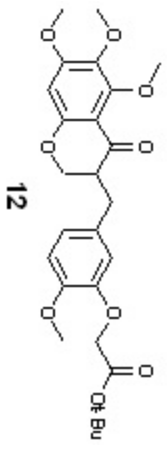


BL-364
Carbon 1 DBL-364 CD3OD

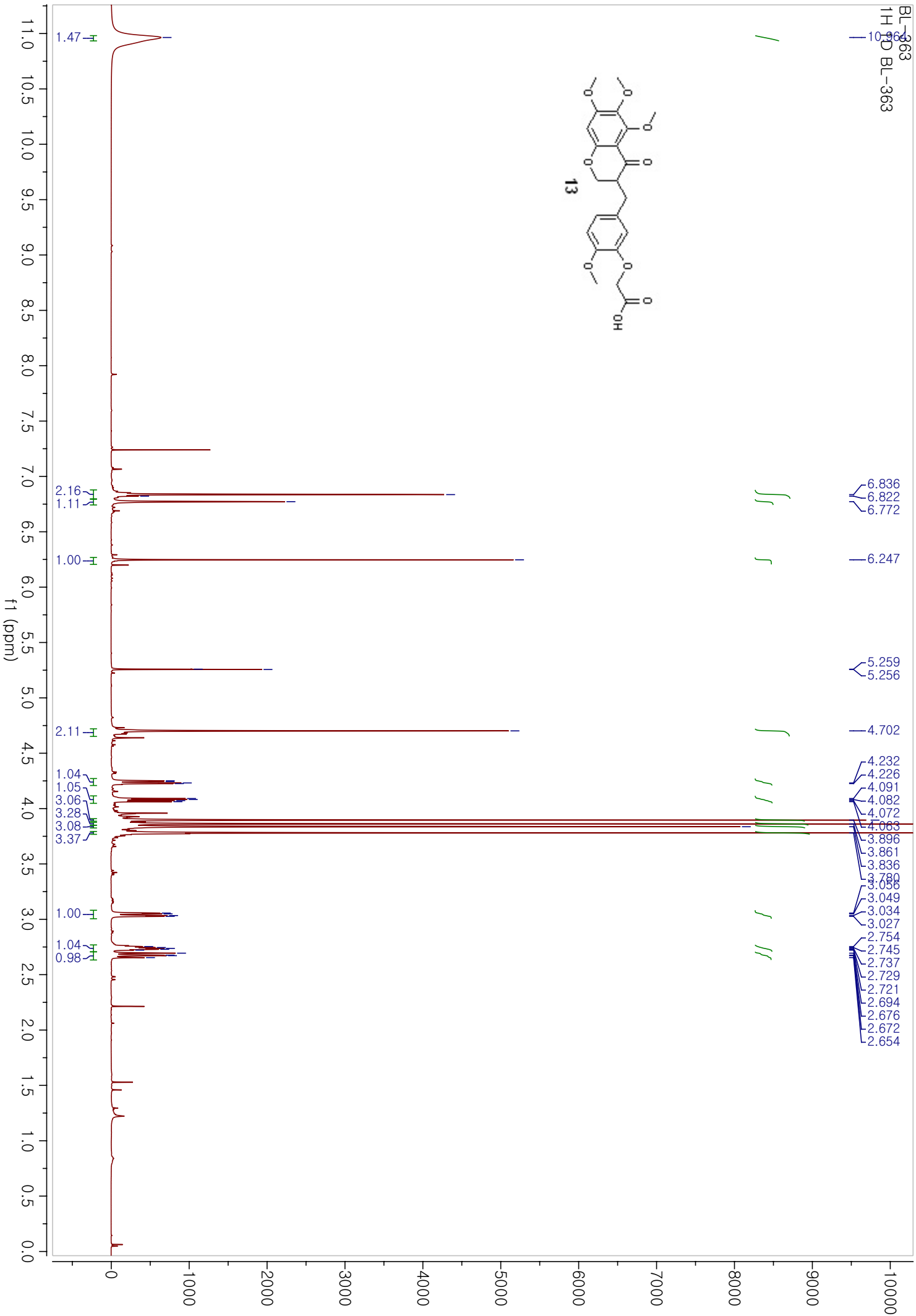
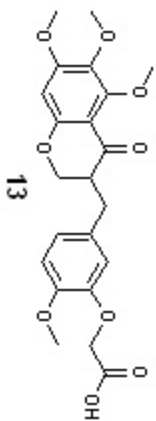




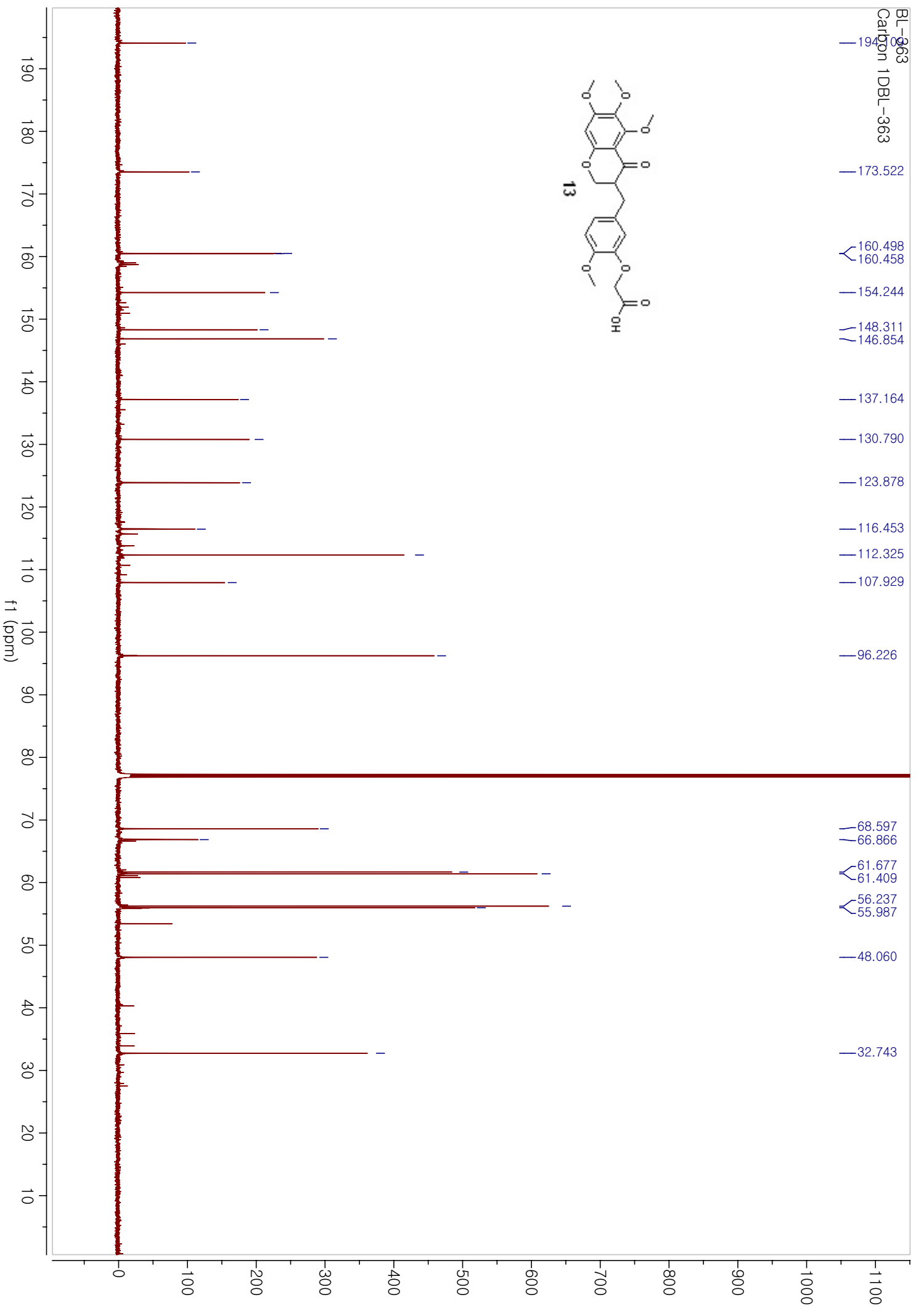
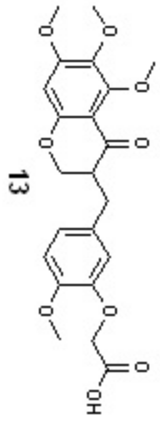
BL-362
Carbon 1 DBL-362-1

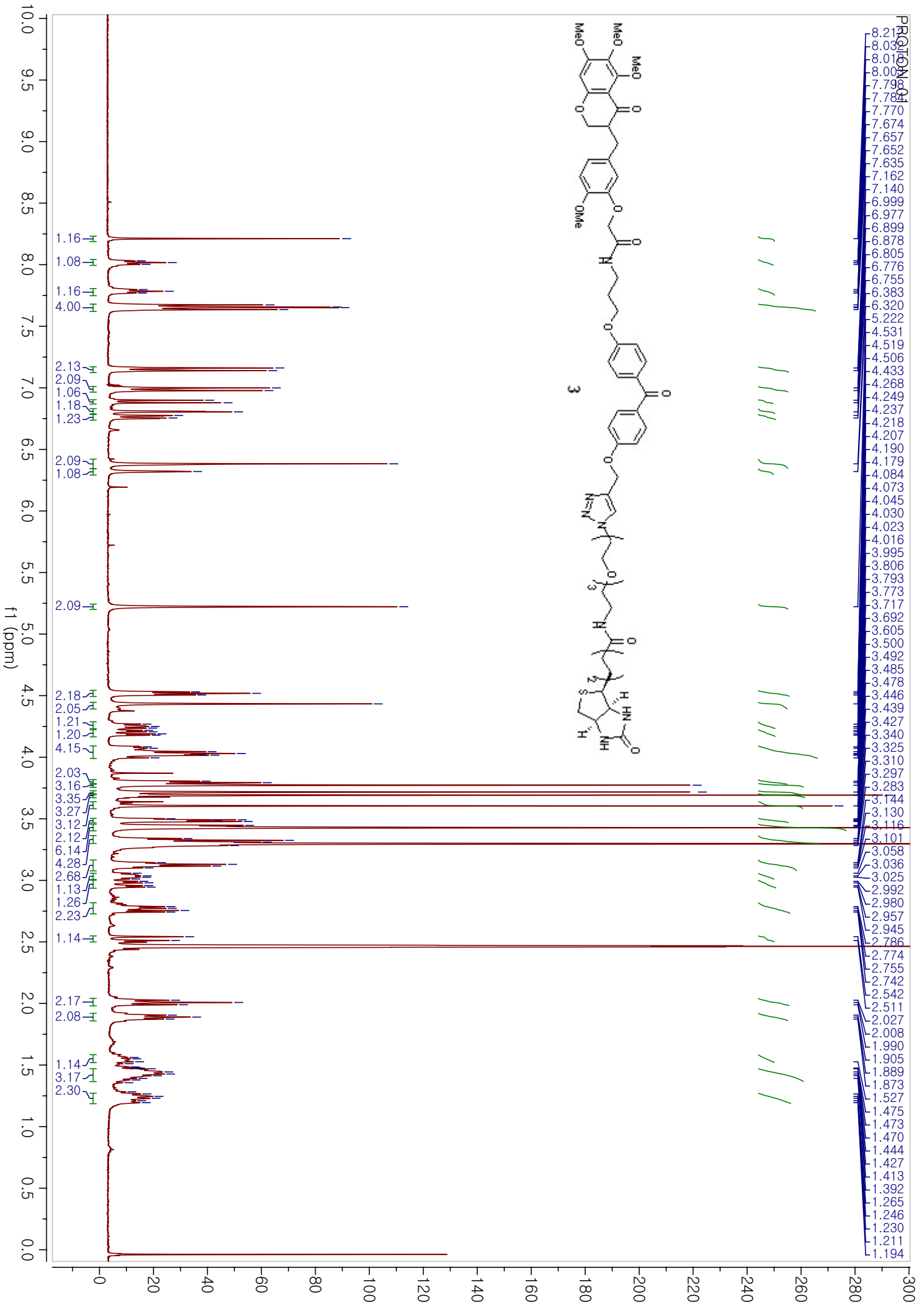


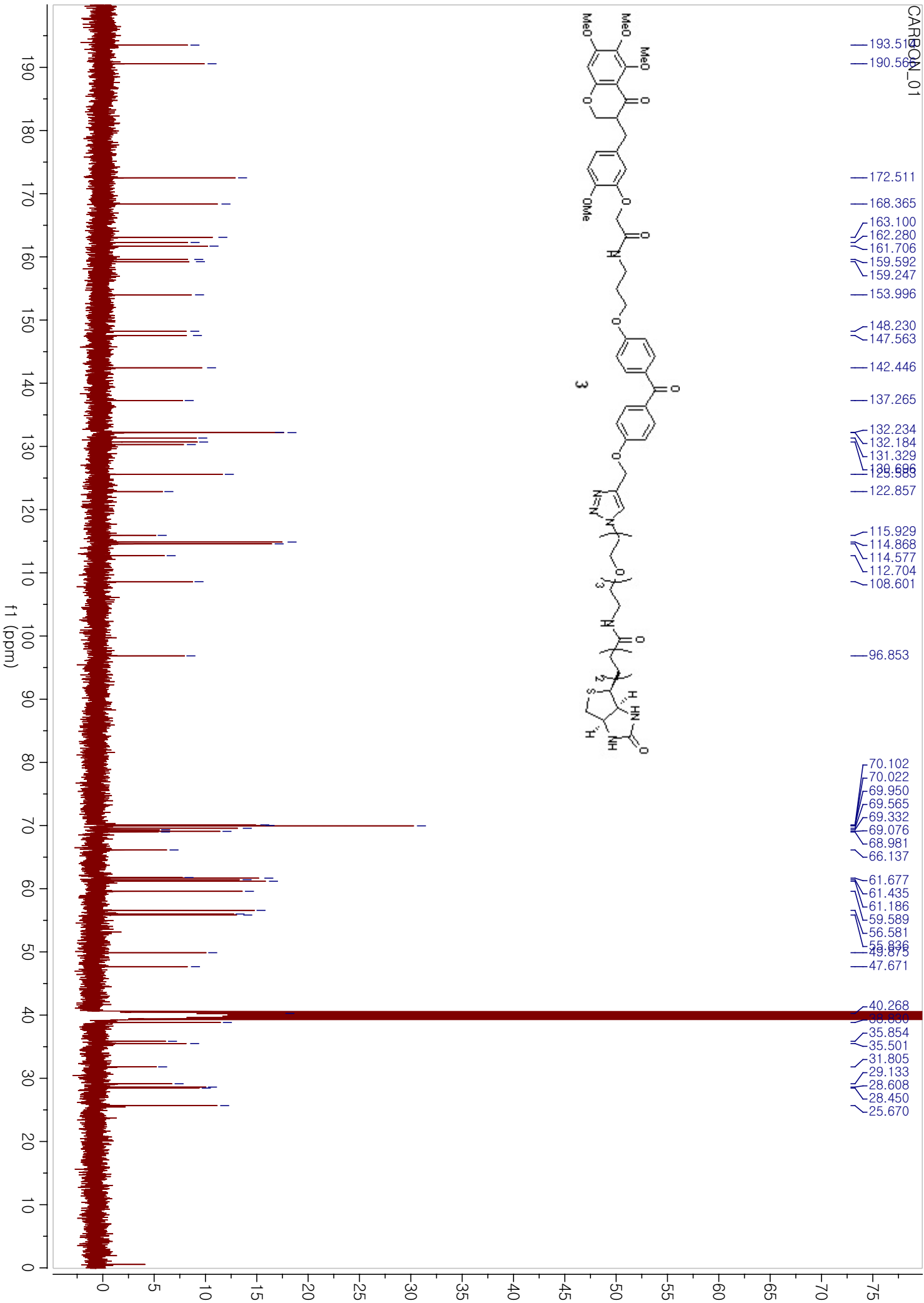
BL-363
1H NMR BL-363

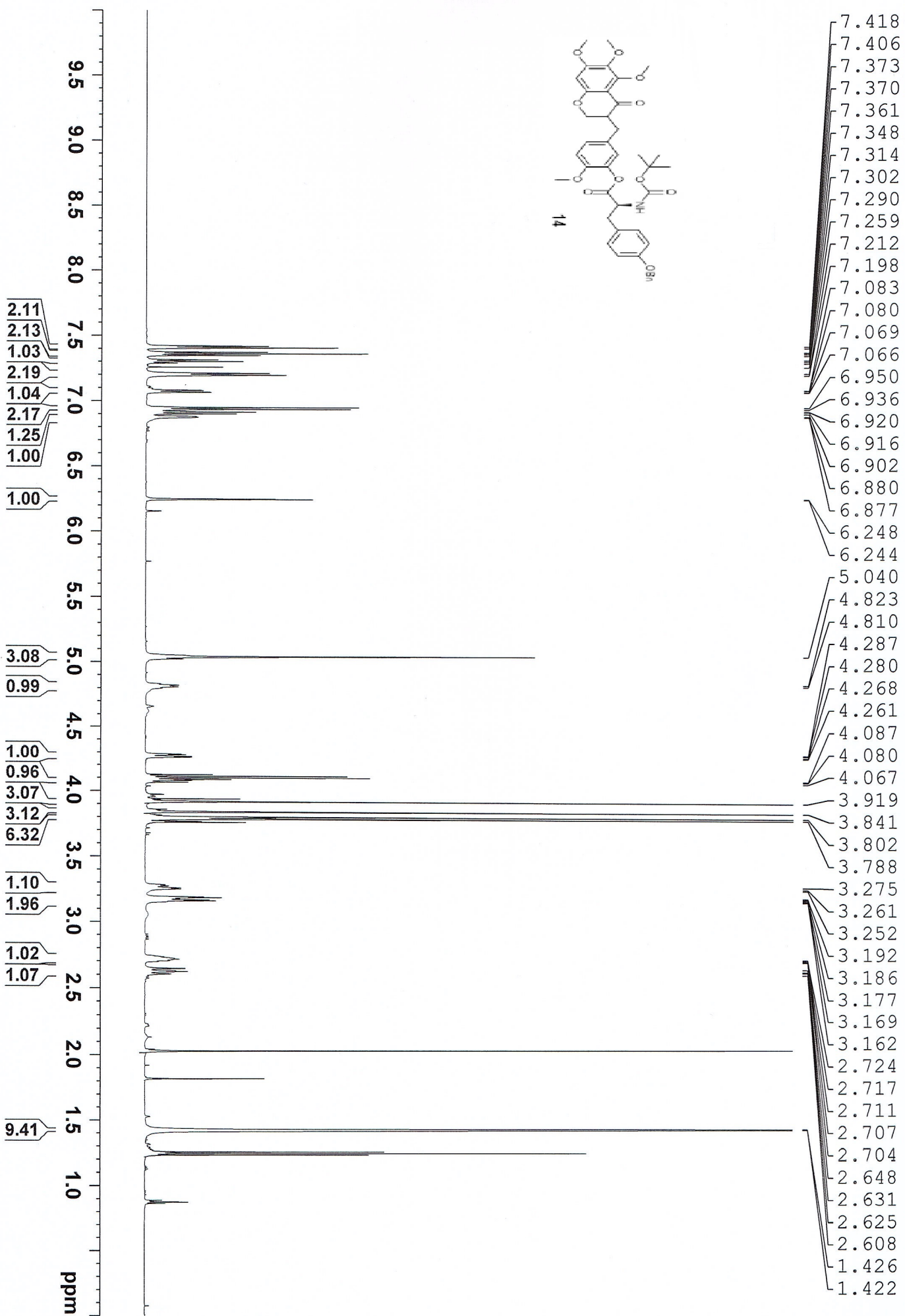
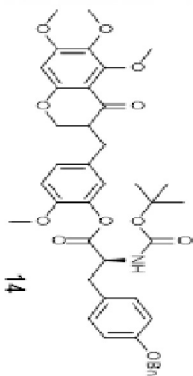


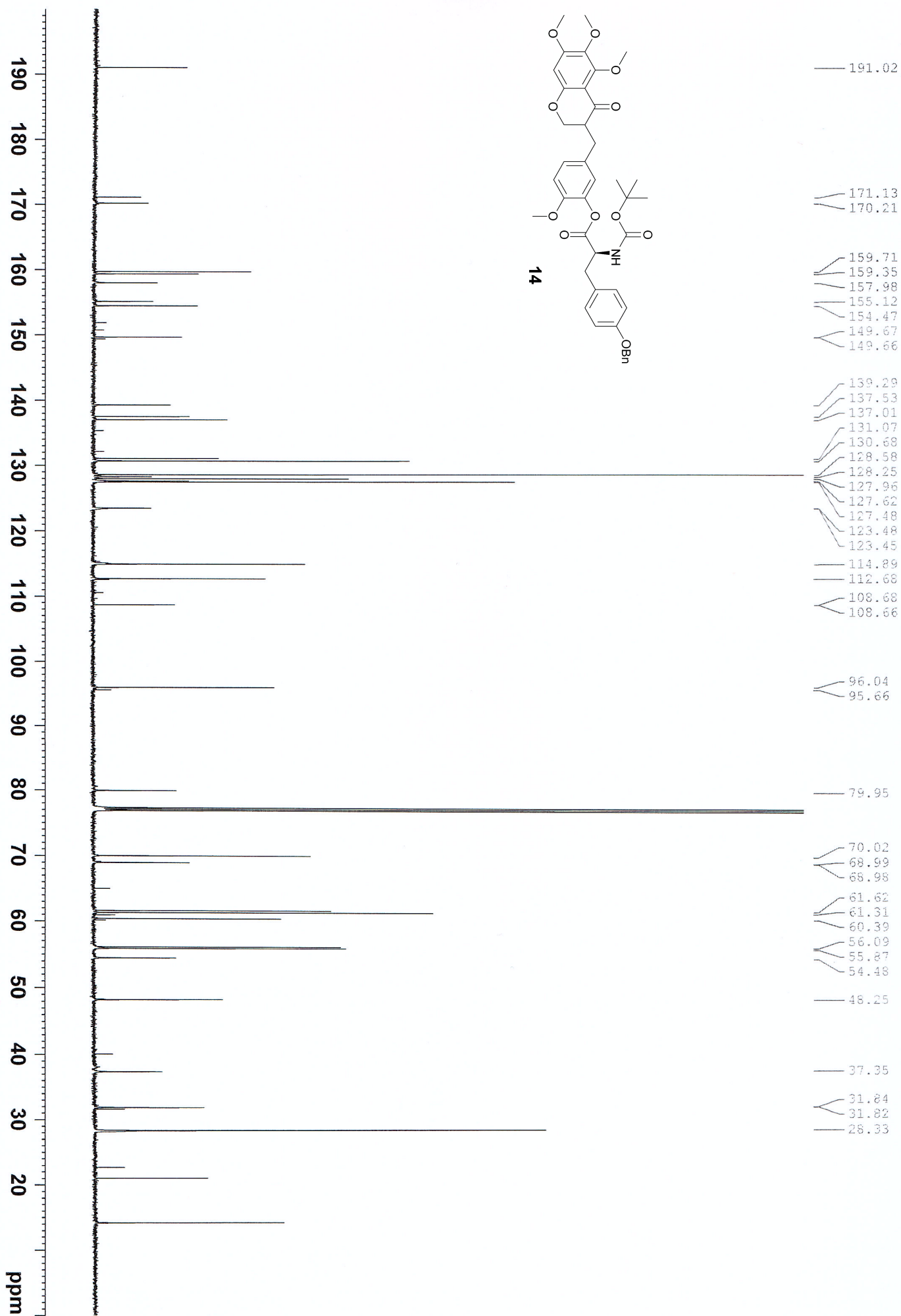
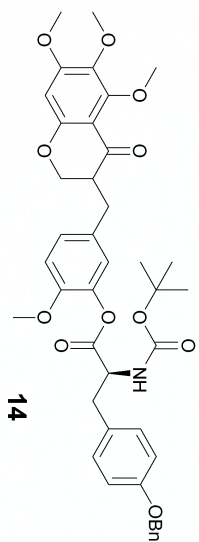
BL-363
Carbon 1 DBL-363

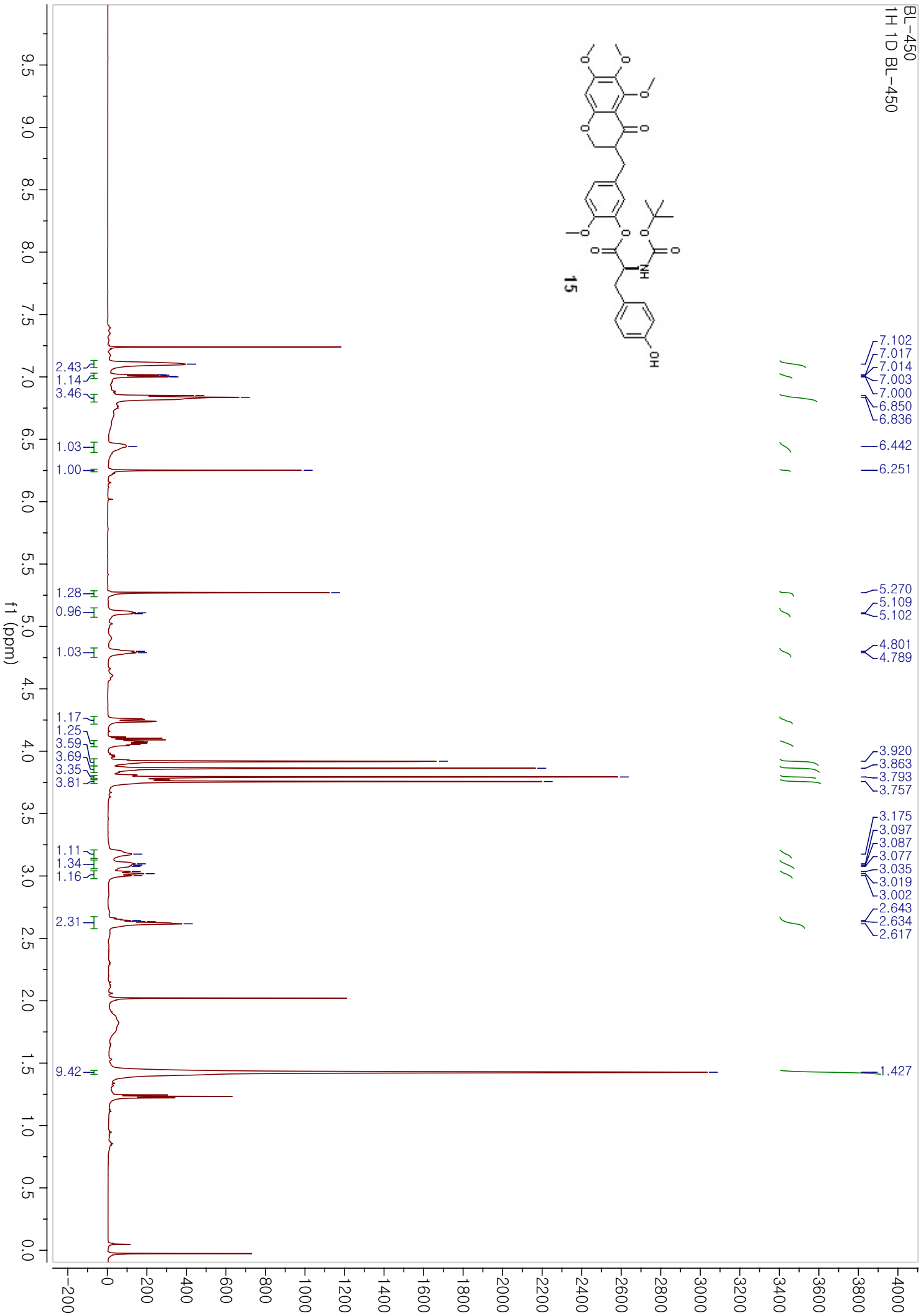
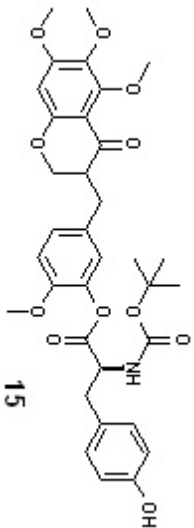




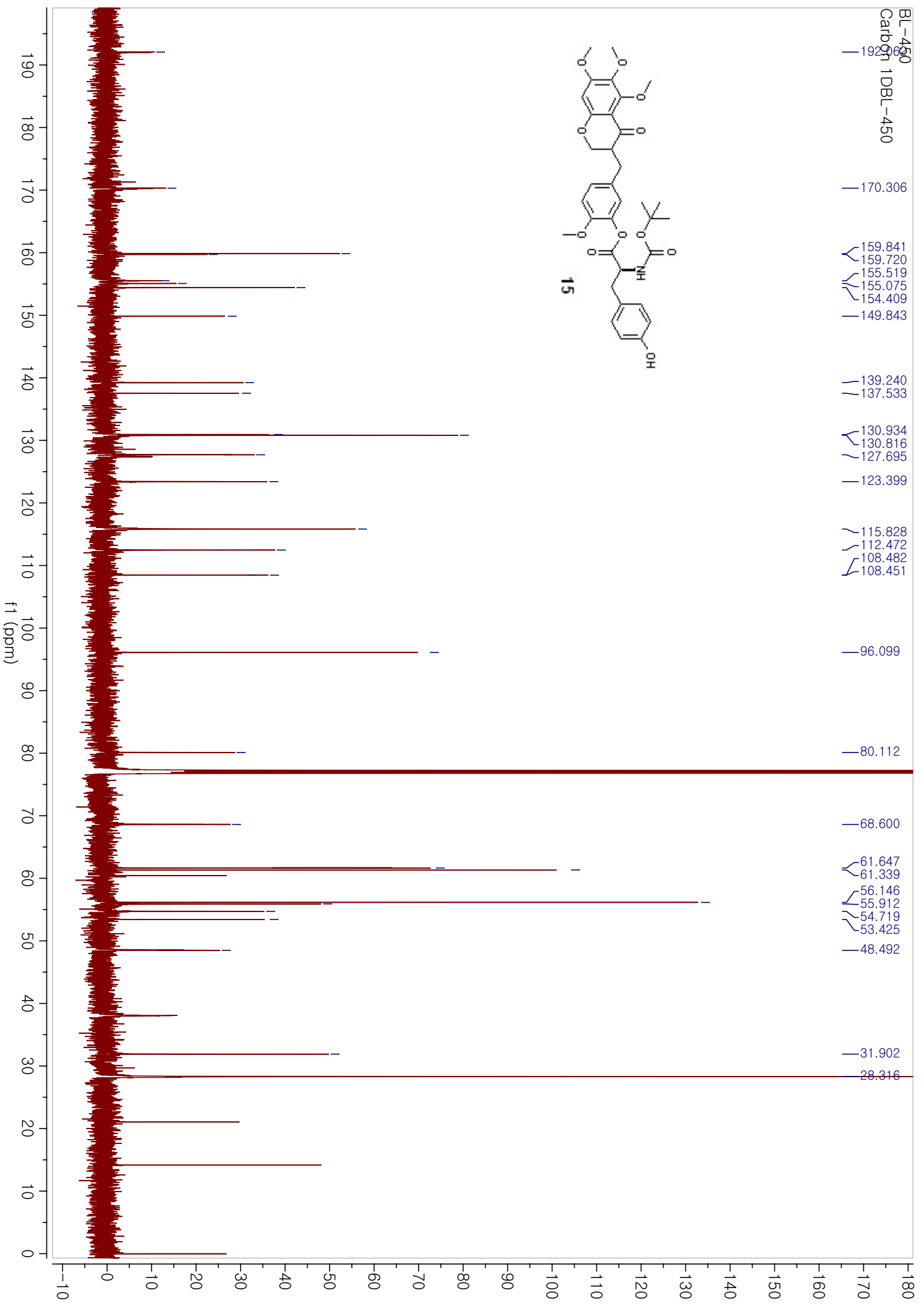
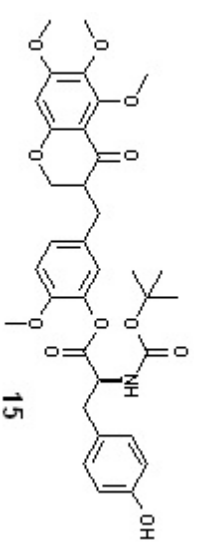


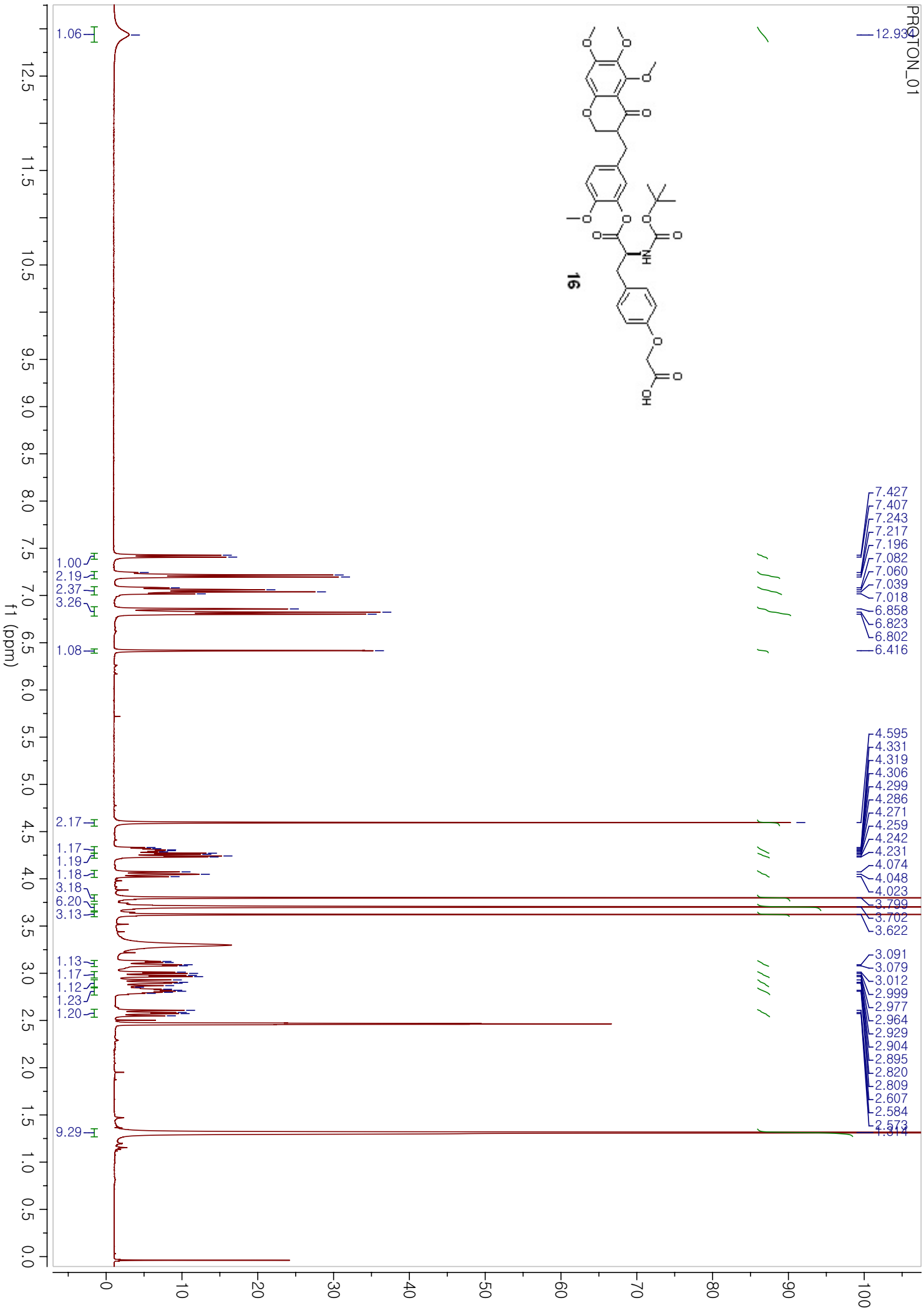
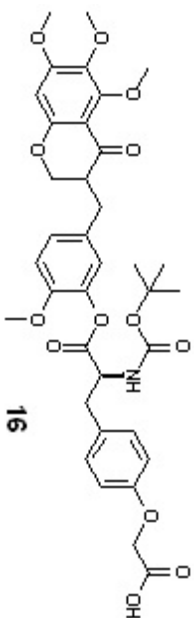




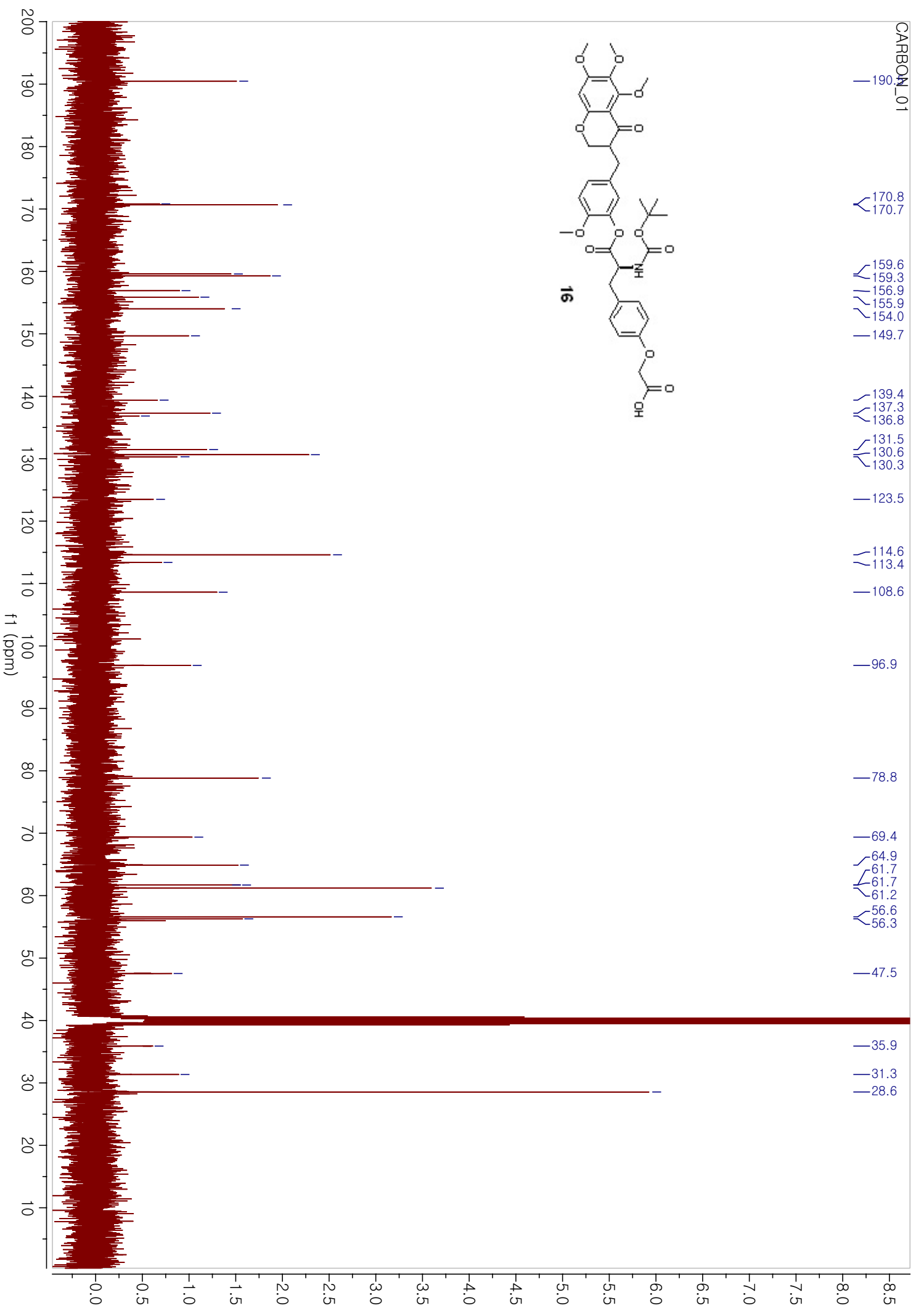
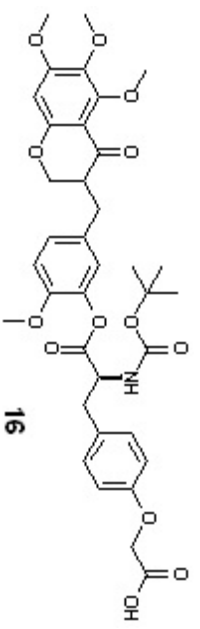


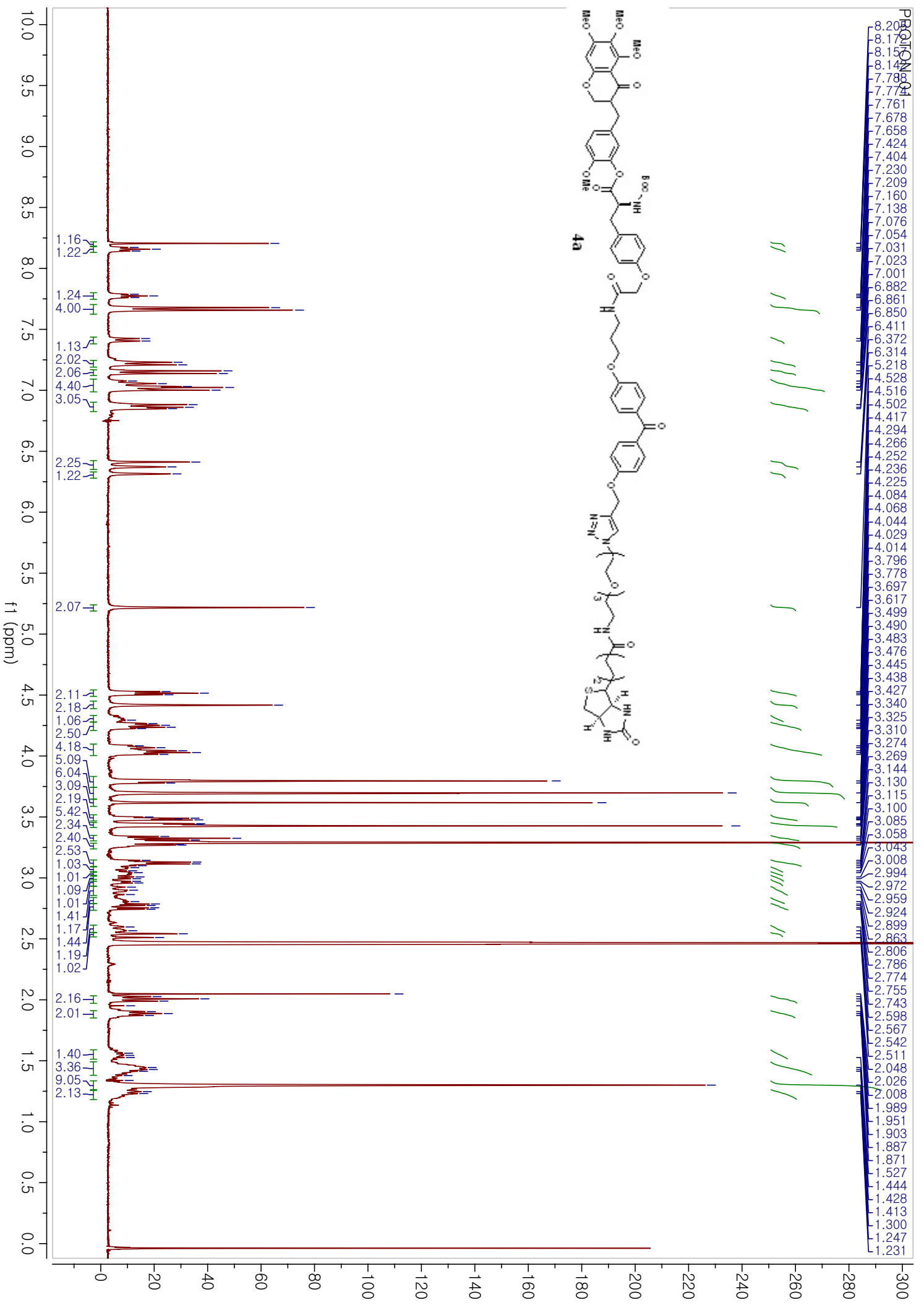
BL-450
Carb 0n 1 DBL-450

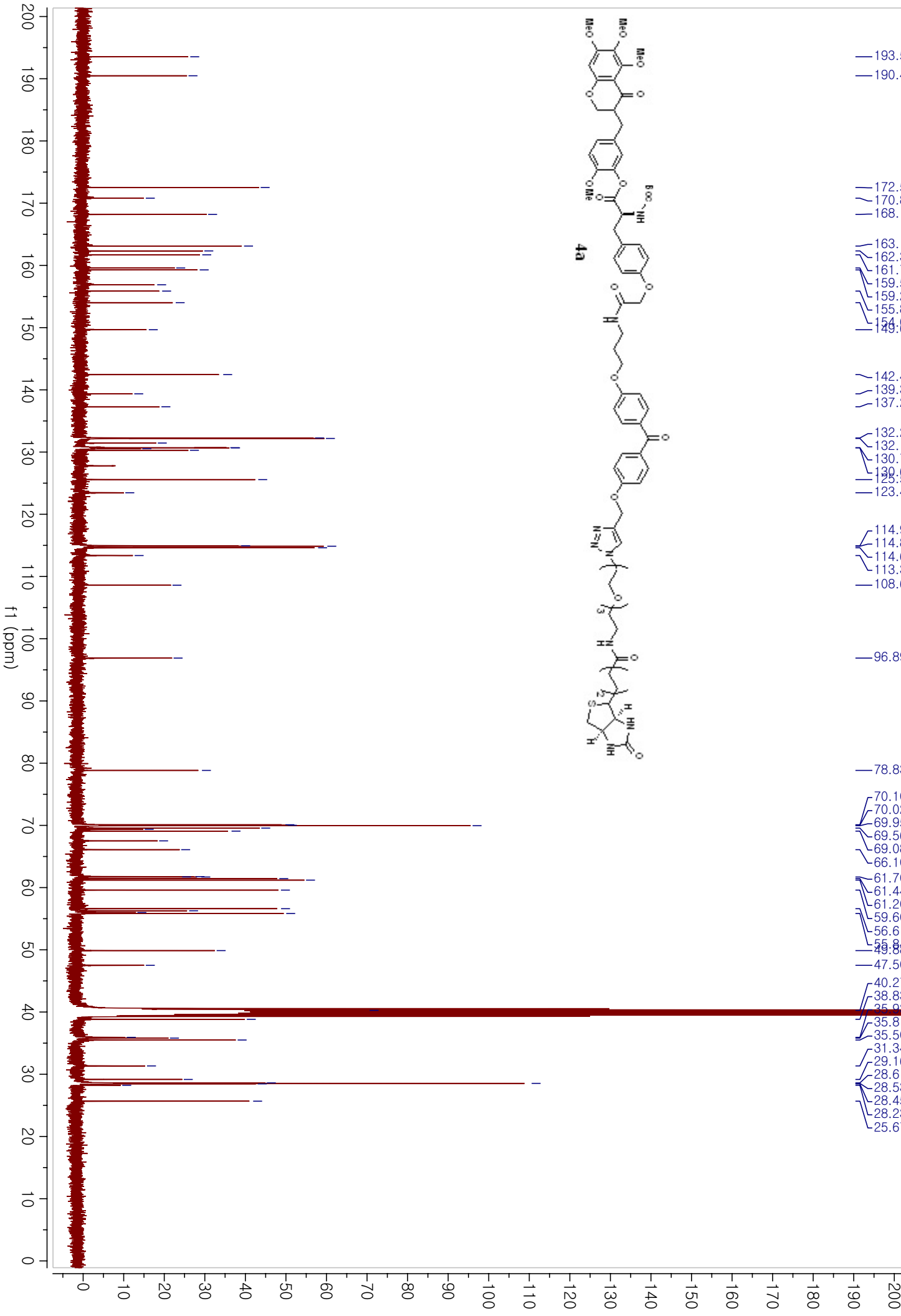
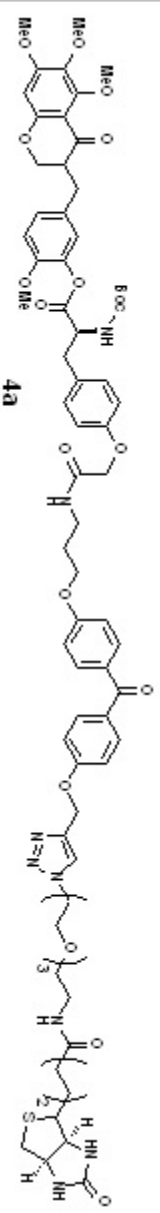


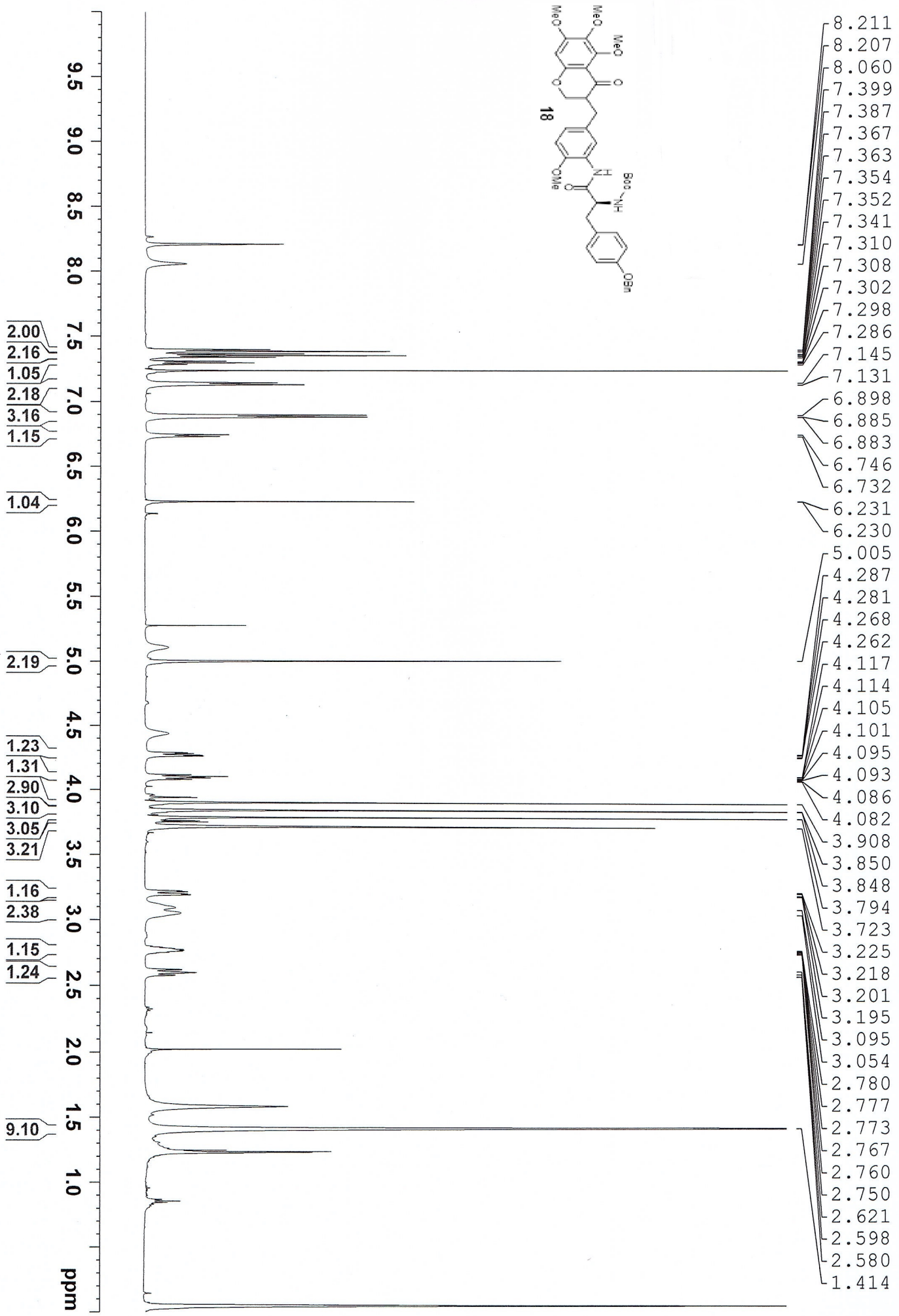
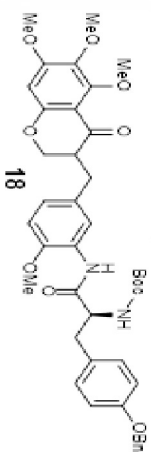


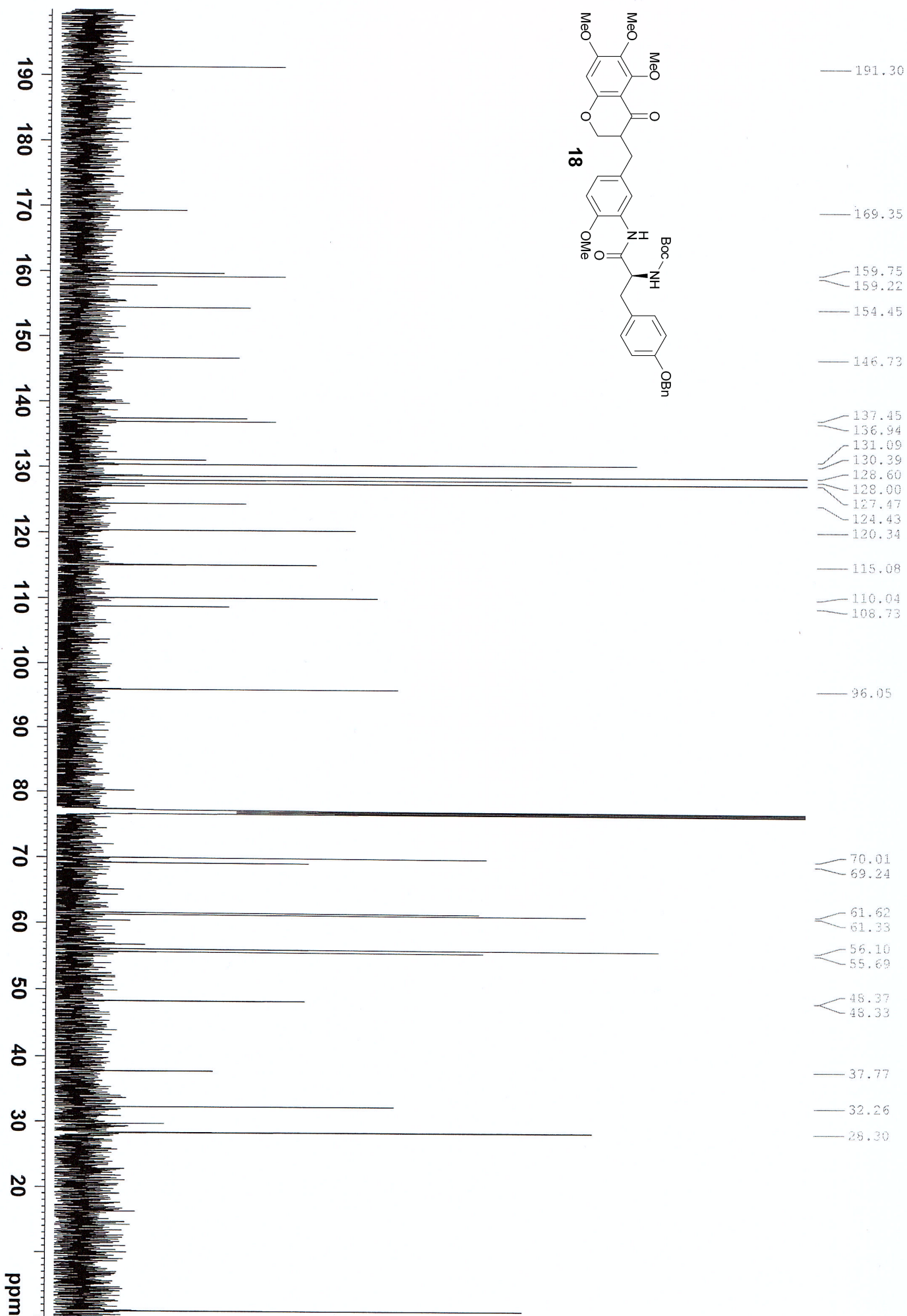
CARBON_01



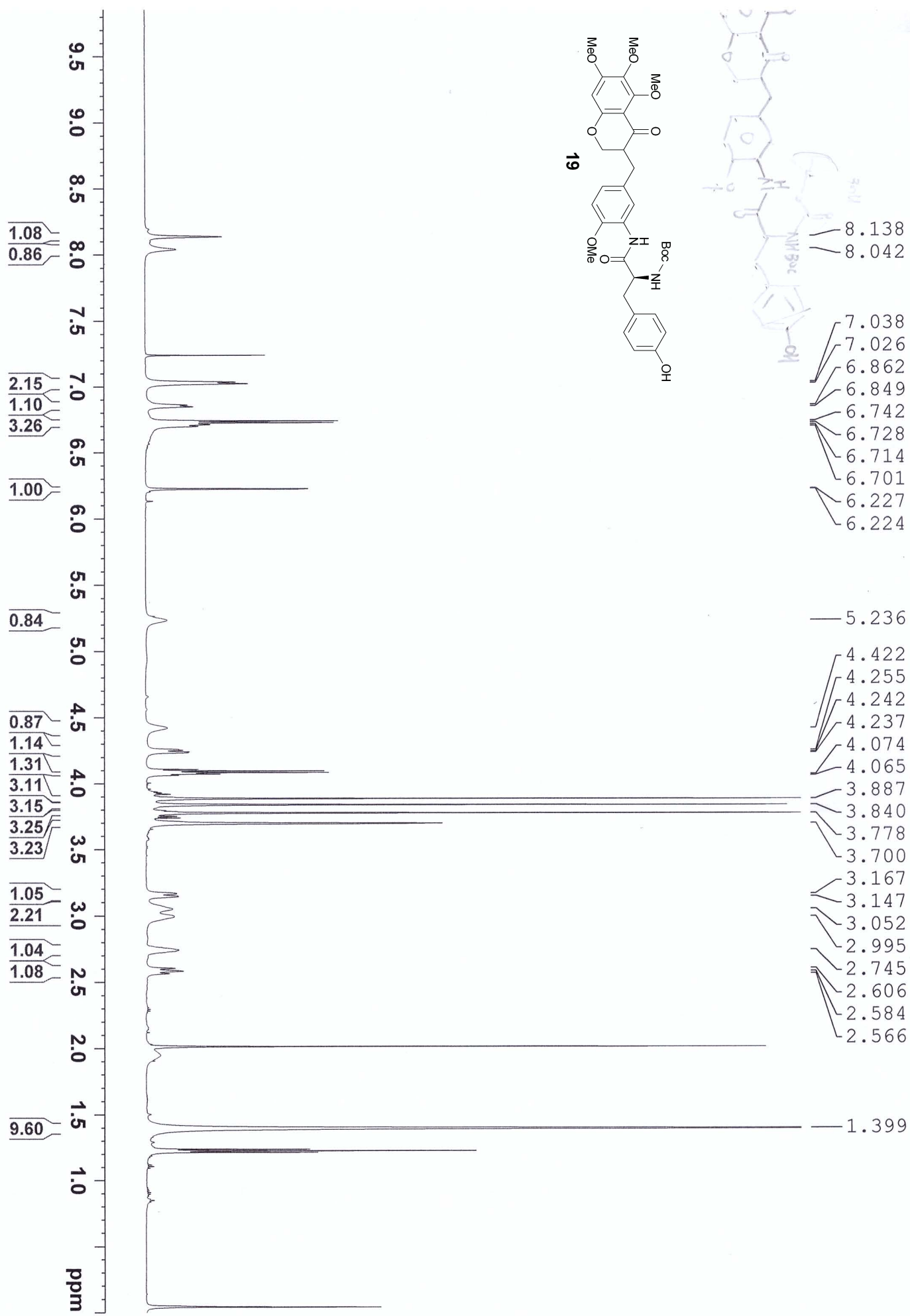
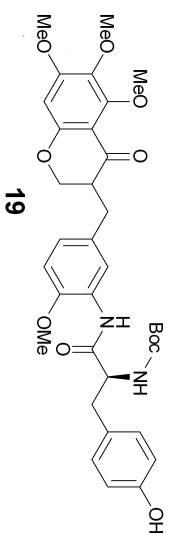
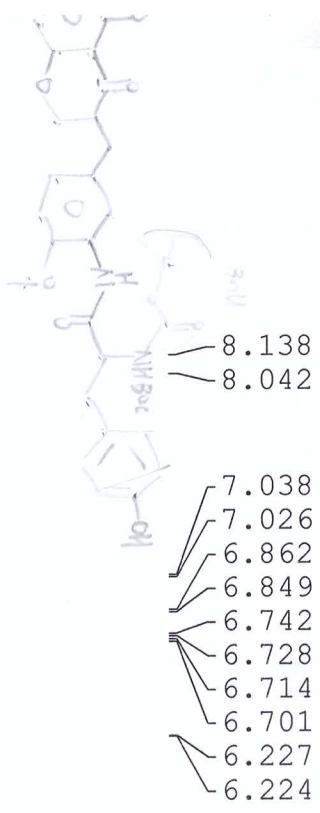


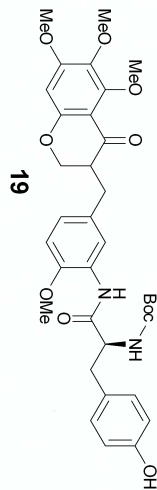






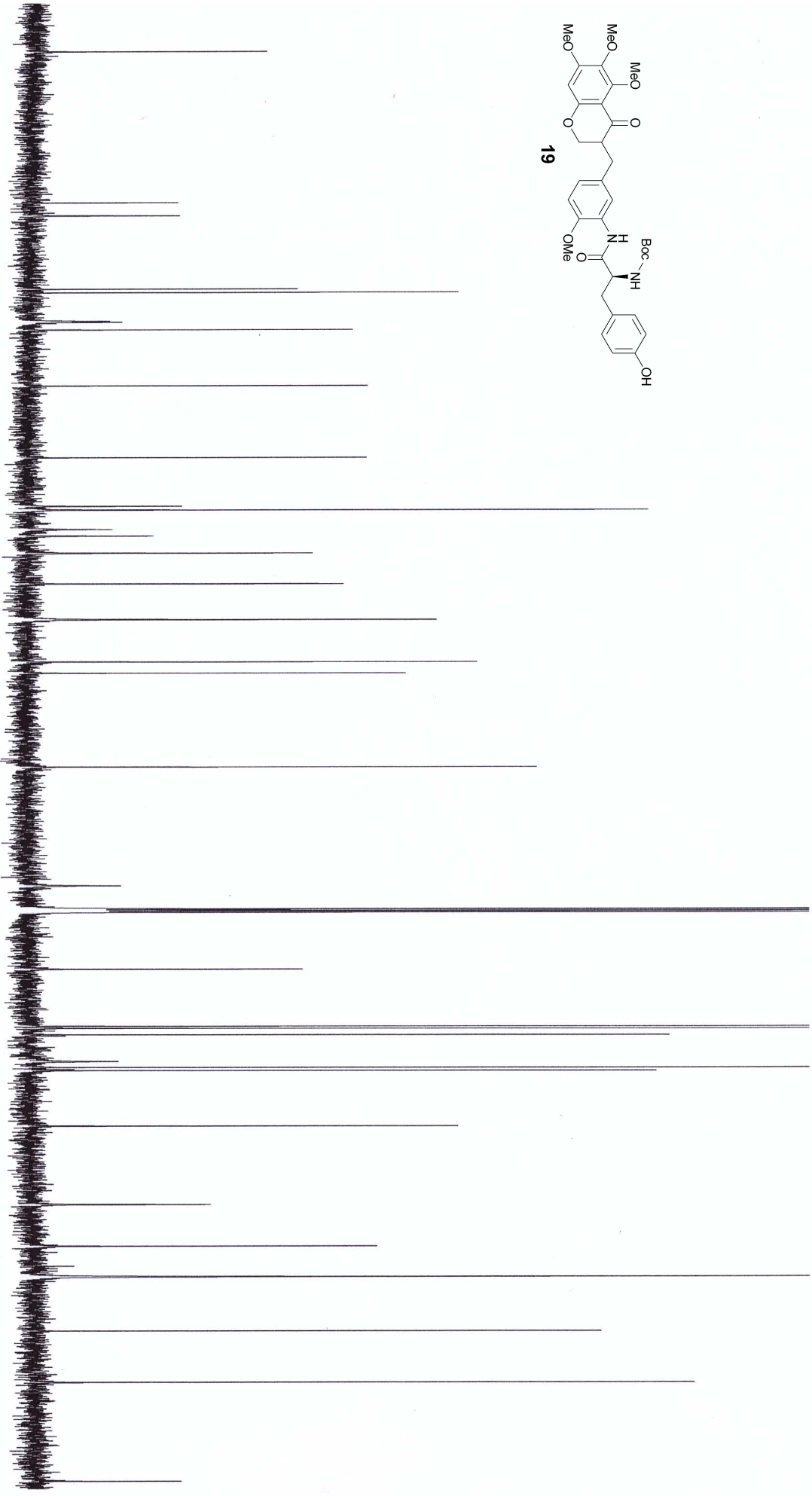
16



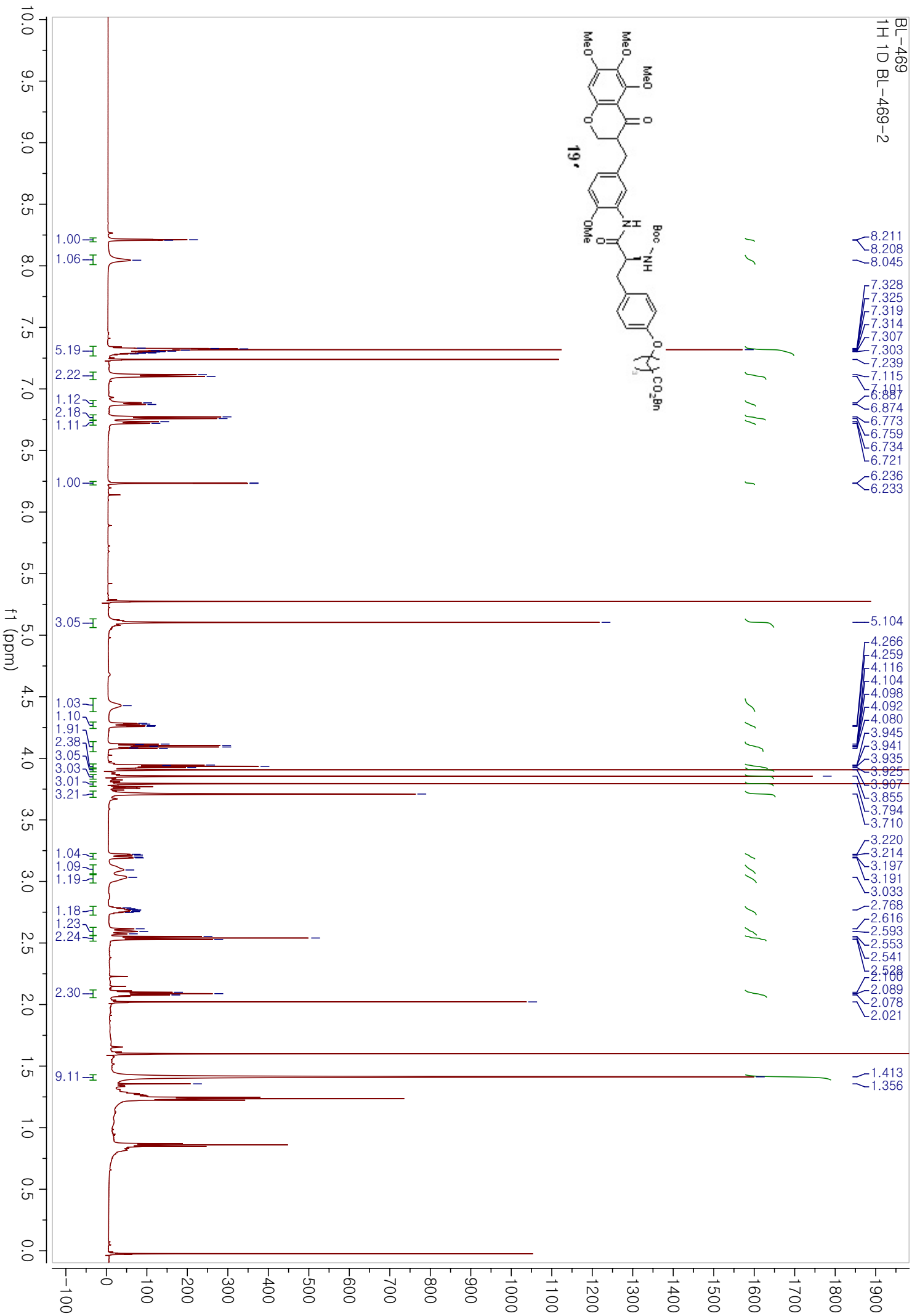
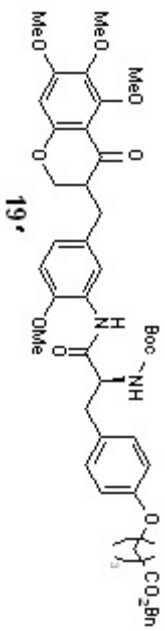


- 191.61
- 171.35
- 169.60
- 159.81
- 159.80
- 159.34
- 155.51
- 155.39
- 154.38
- 146.93
- 137.38
- 130.89
- 130.39
- 127.78
- 126.90
- 124.61
- 120.54
- 120.52
- 115.72
- 110.09
- 108.61
- 96.09
- 80.35
- 69.22
- 69.20
- 61.62
- 61.33
- 60.48
- 56.92
- 56.11
- 55.70
- 48.30
- 37.87
- 32.33
- 28.29

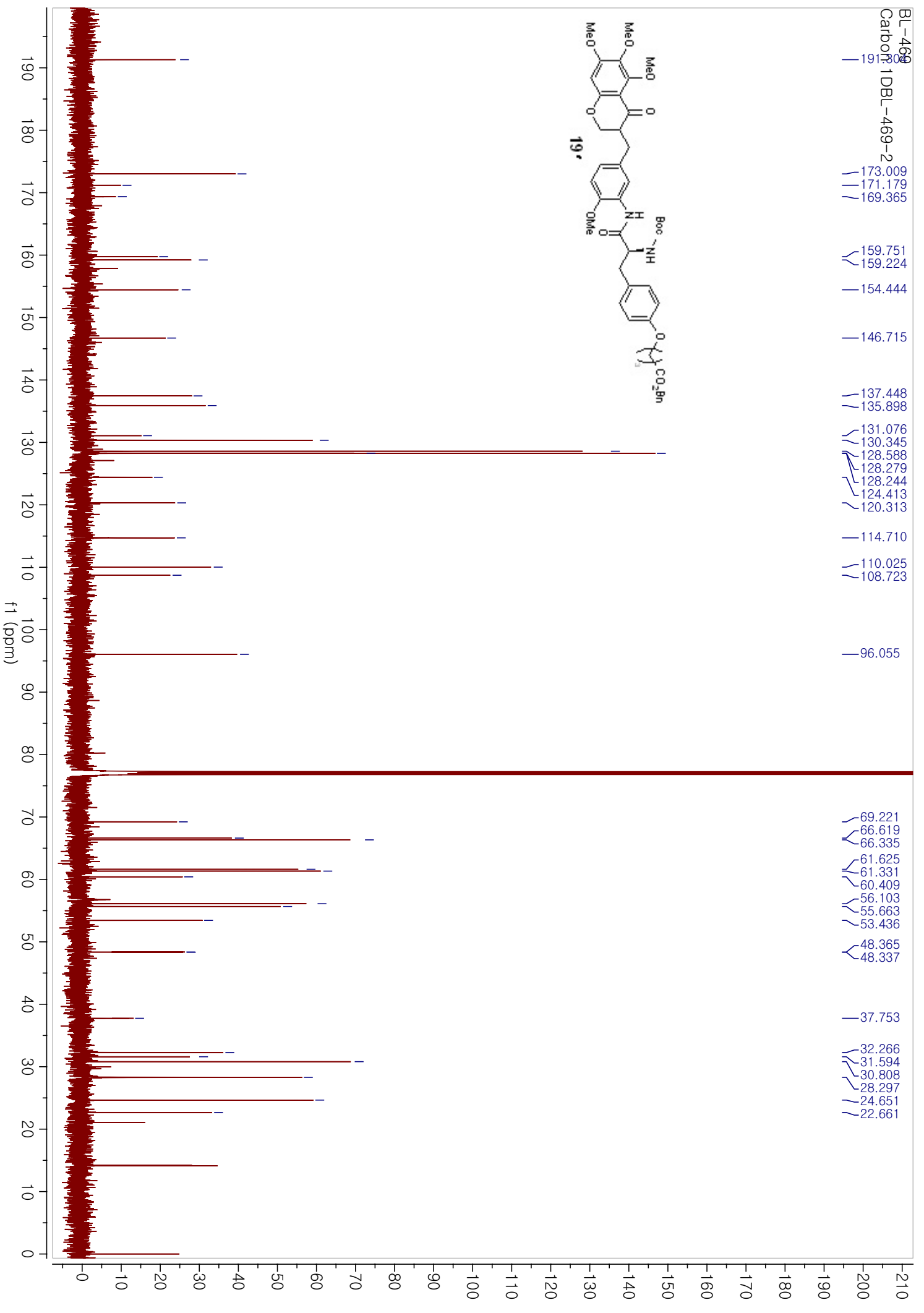
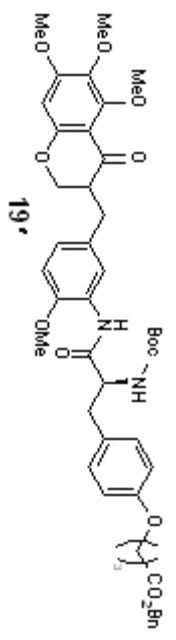
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

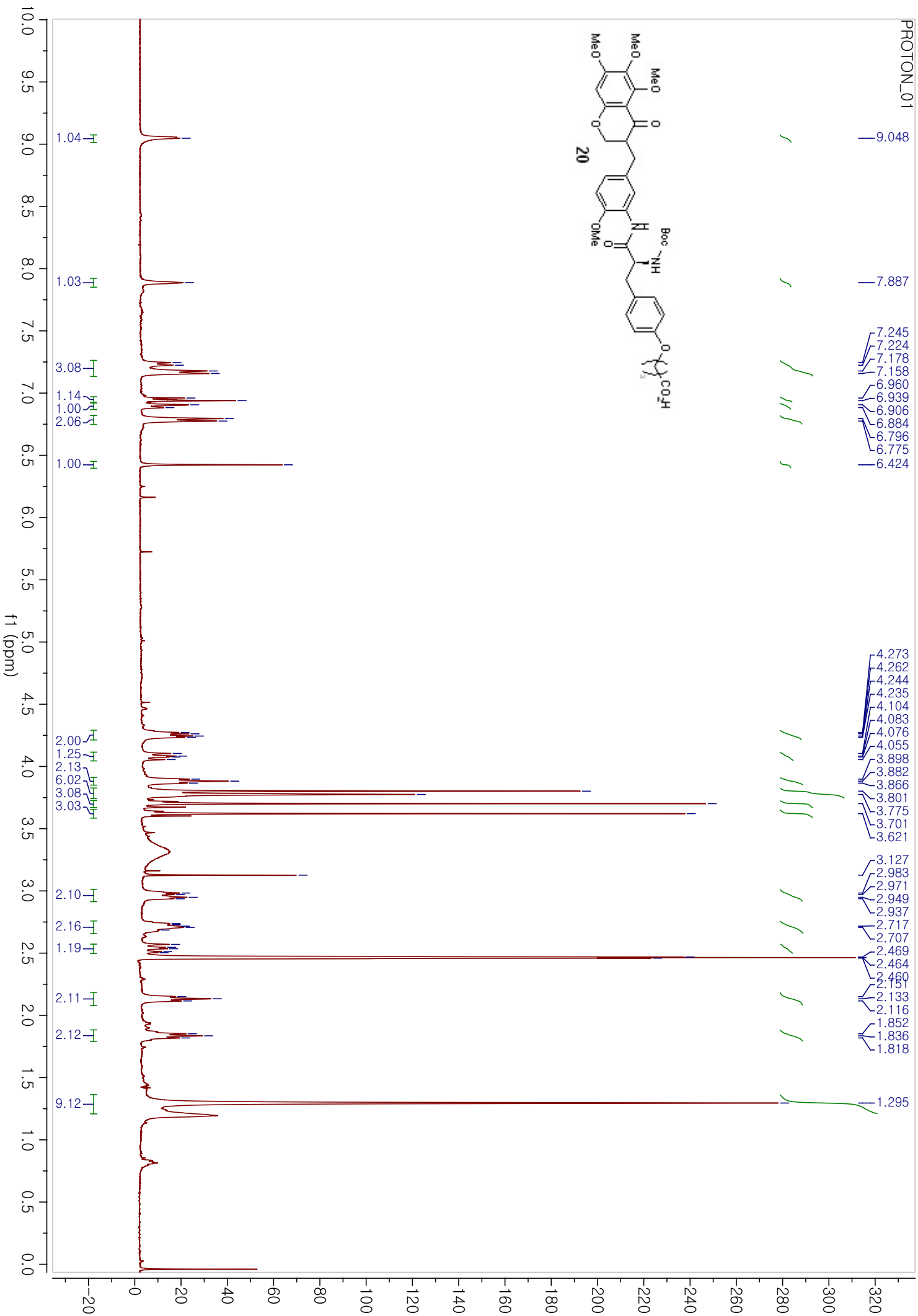
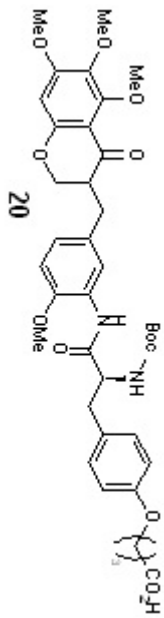


BL-469
1H 1D BL-469-2

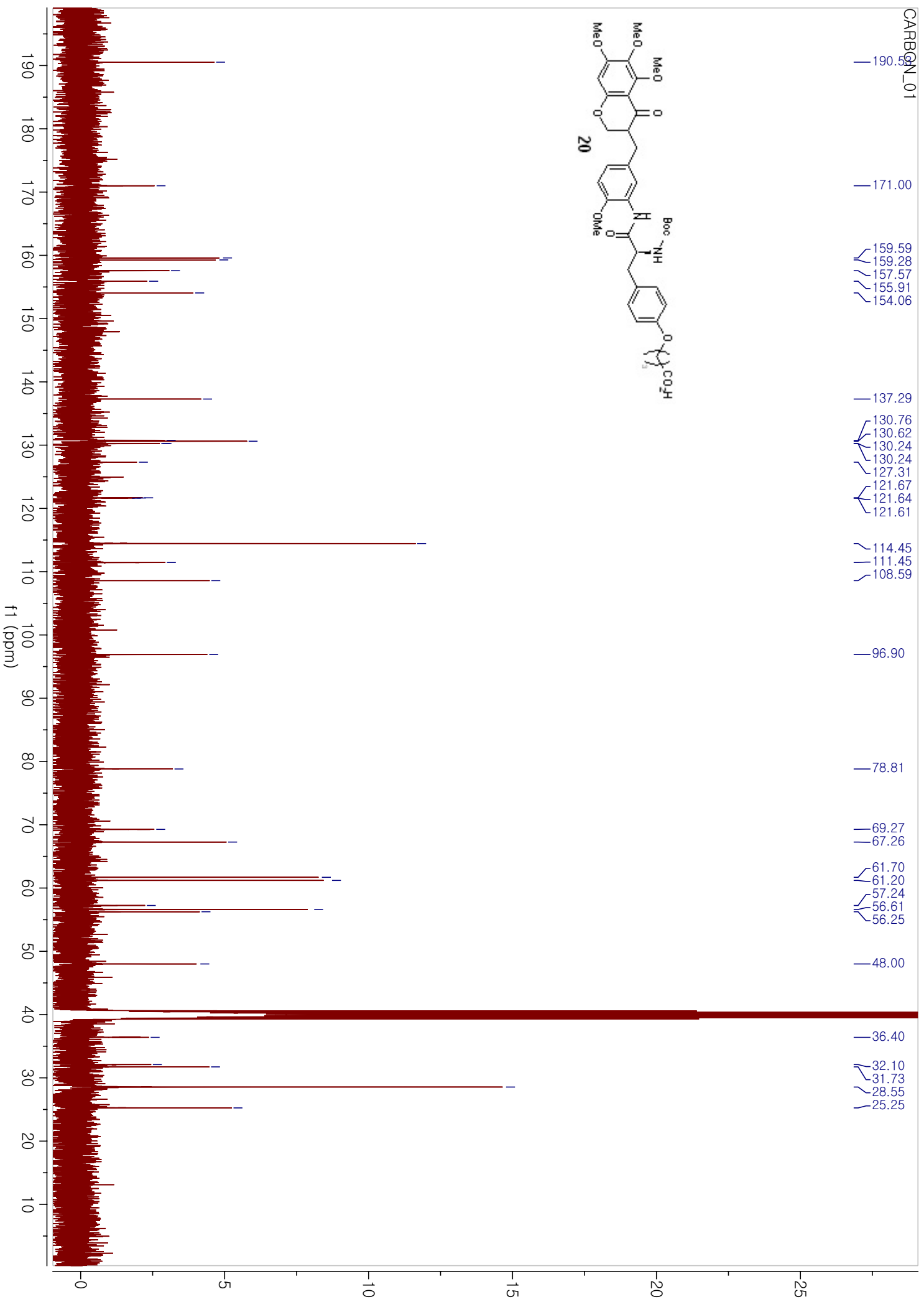
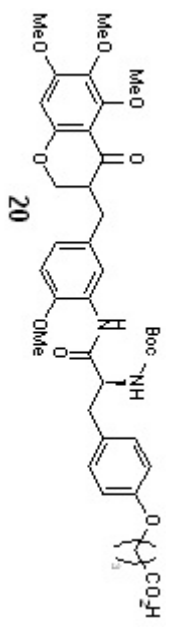


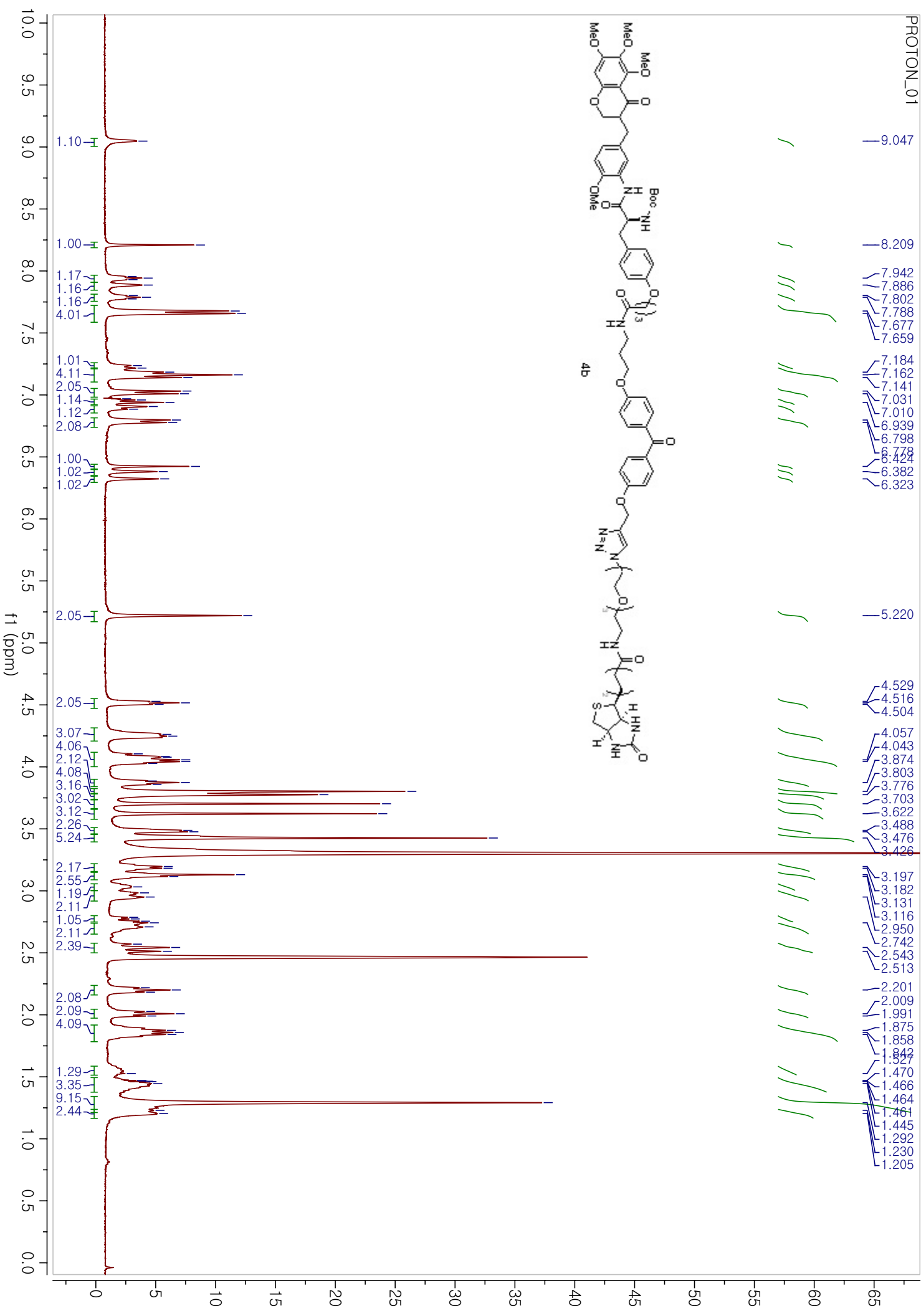
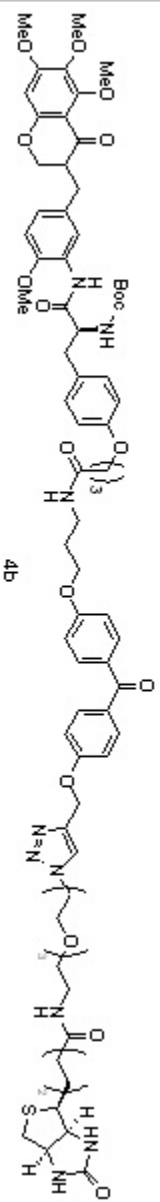
BL-469
Carbonyl DBL-469-2

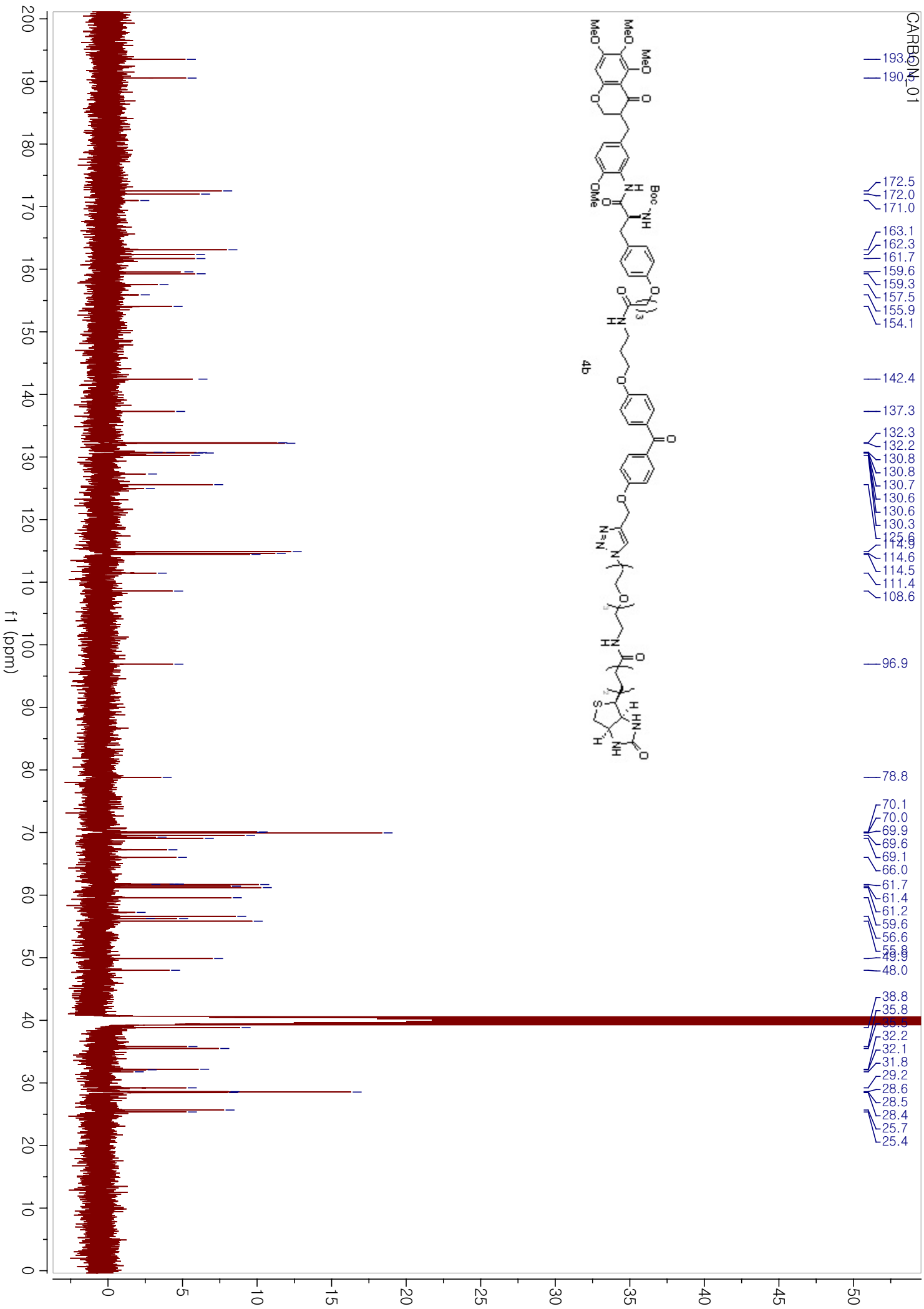




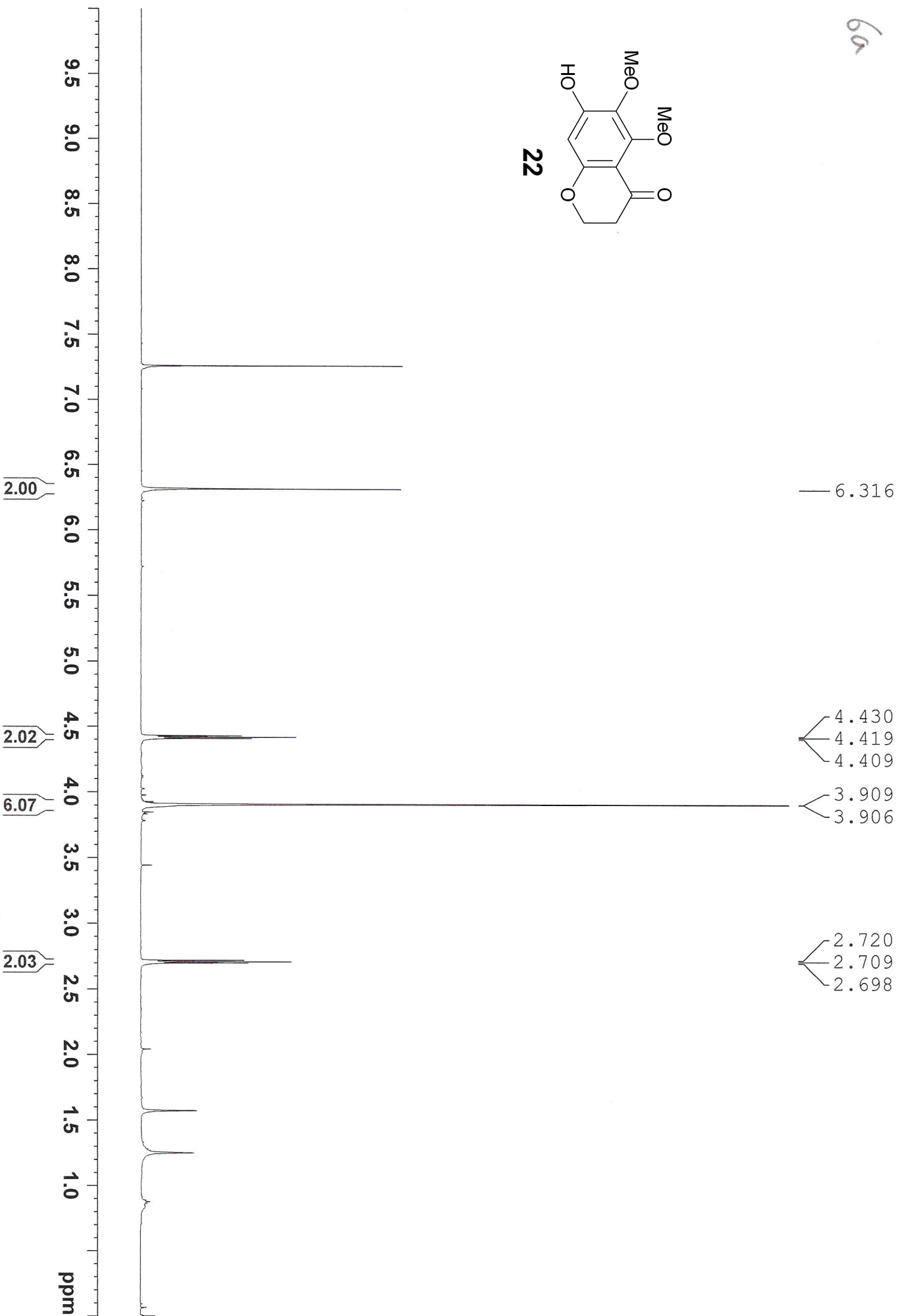
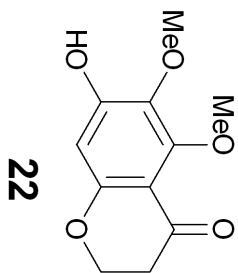
CARBON_01

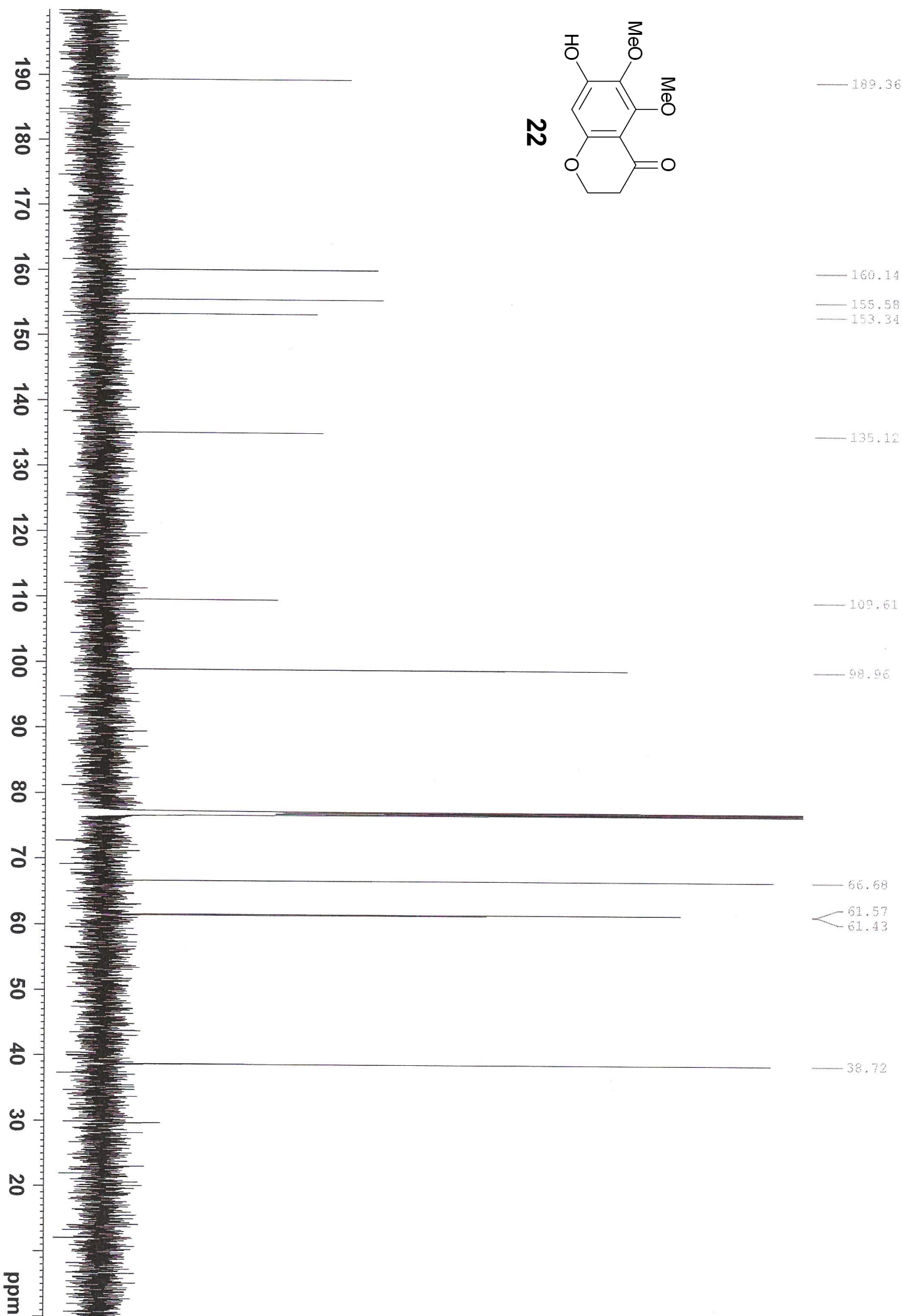
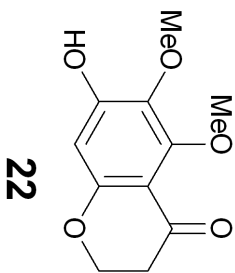


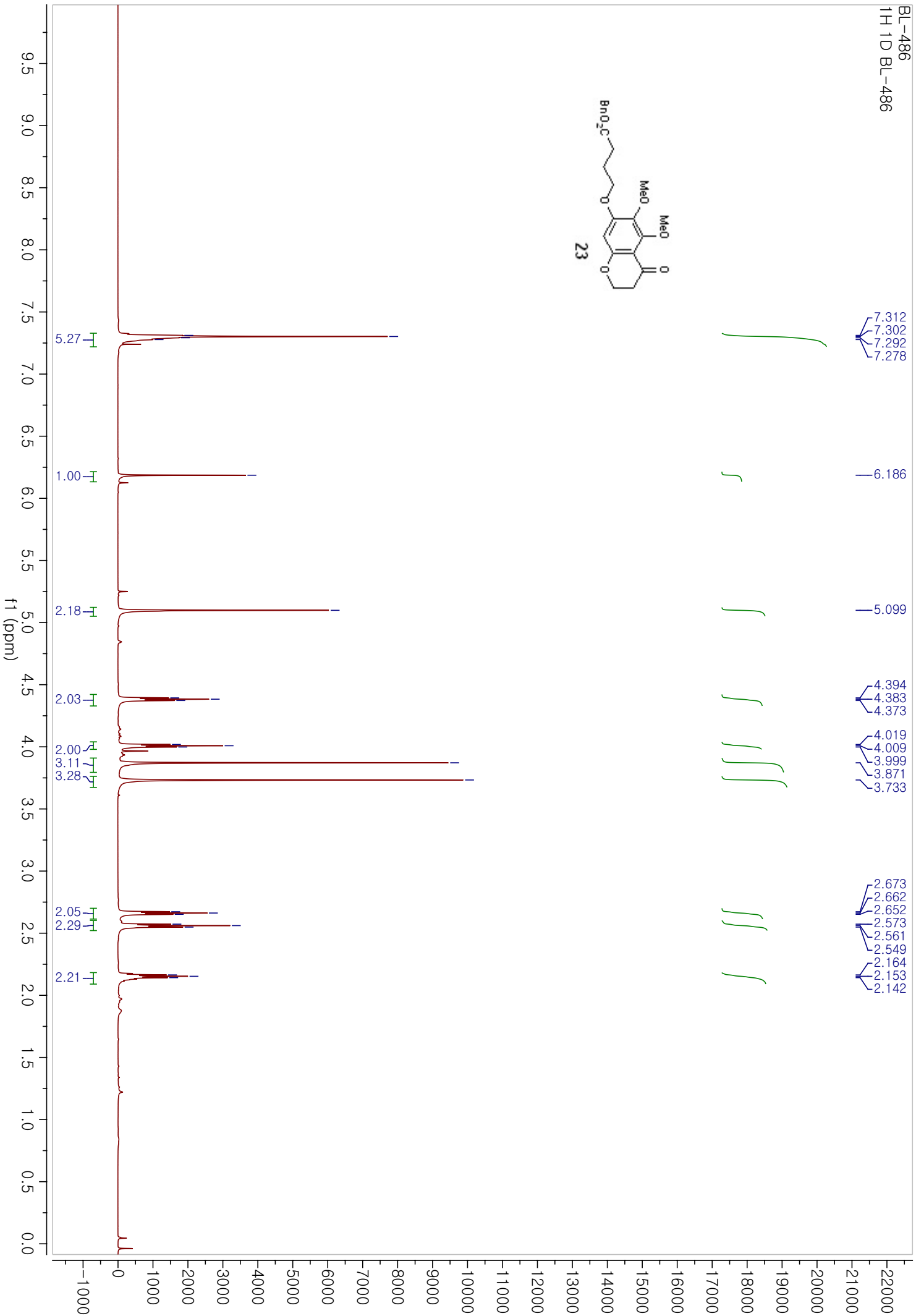
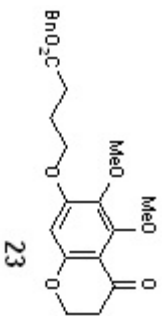




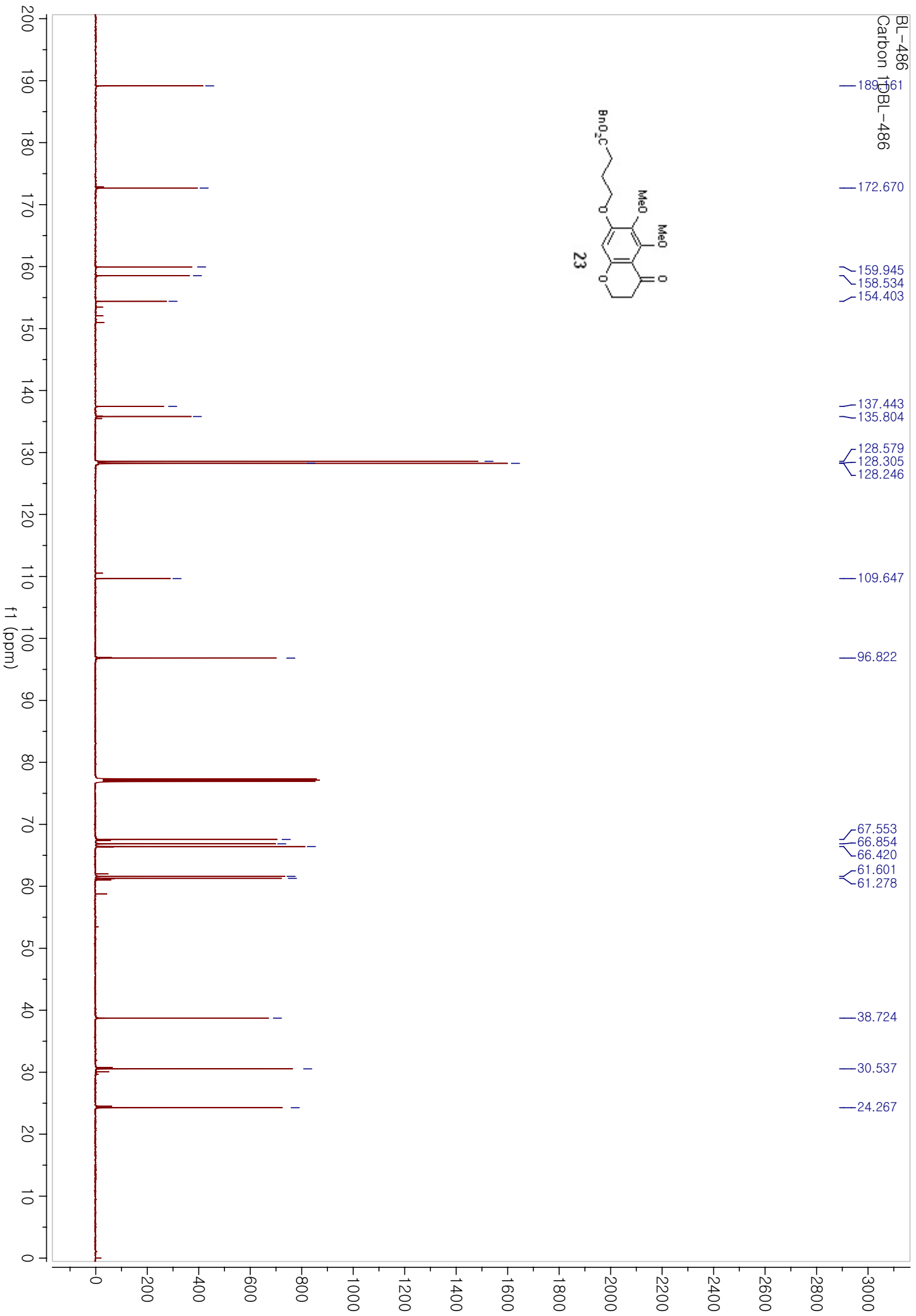
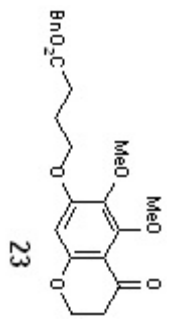
6a

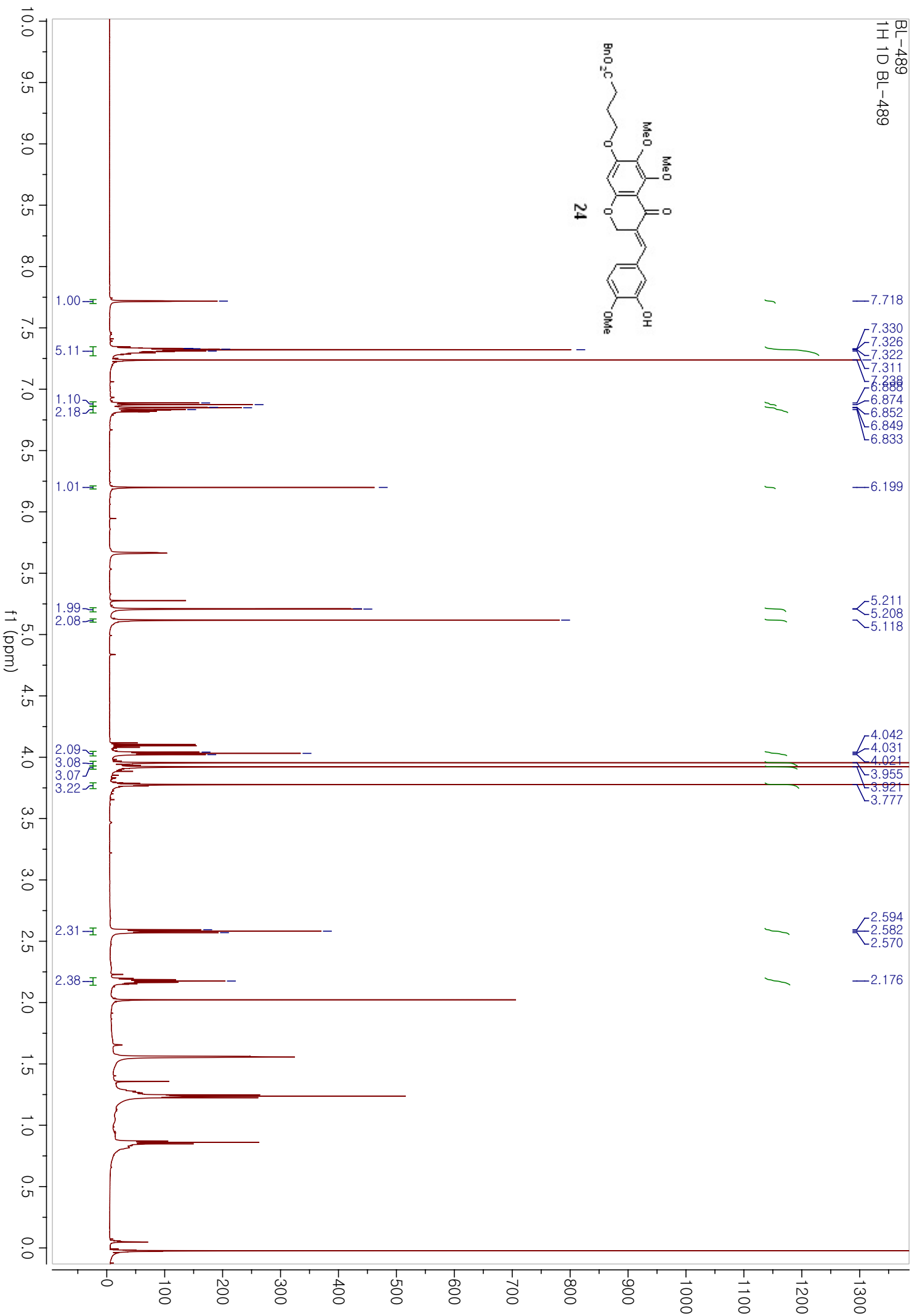
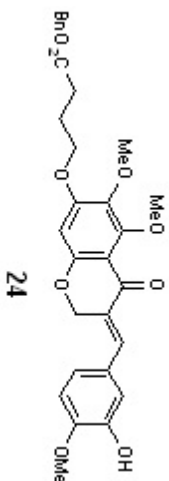




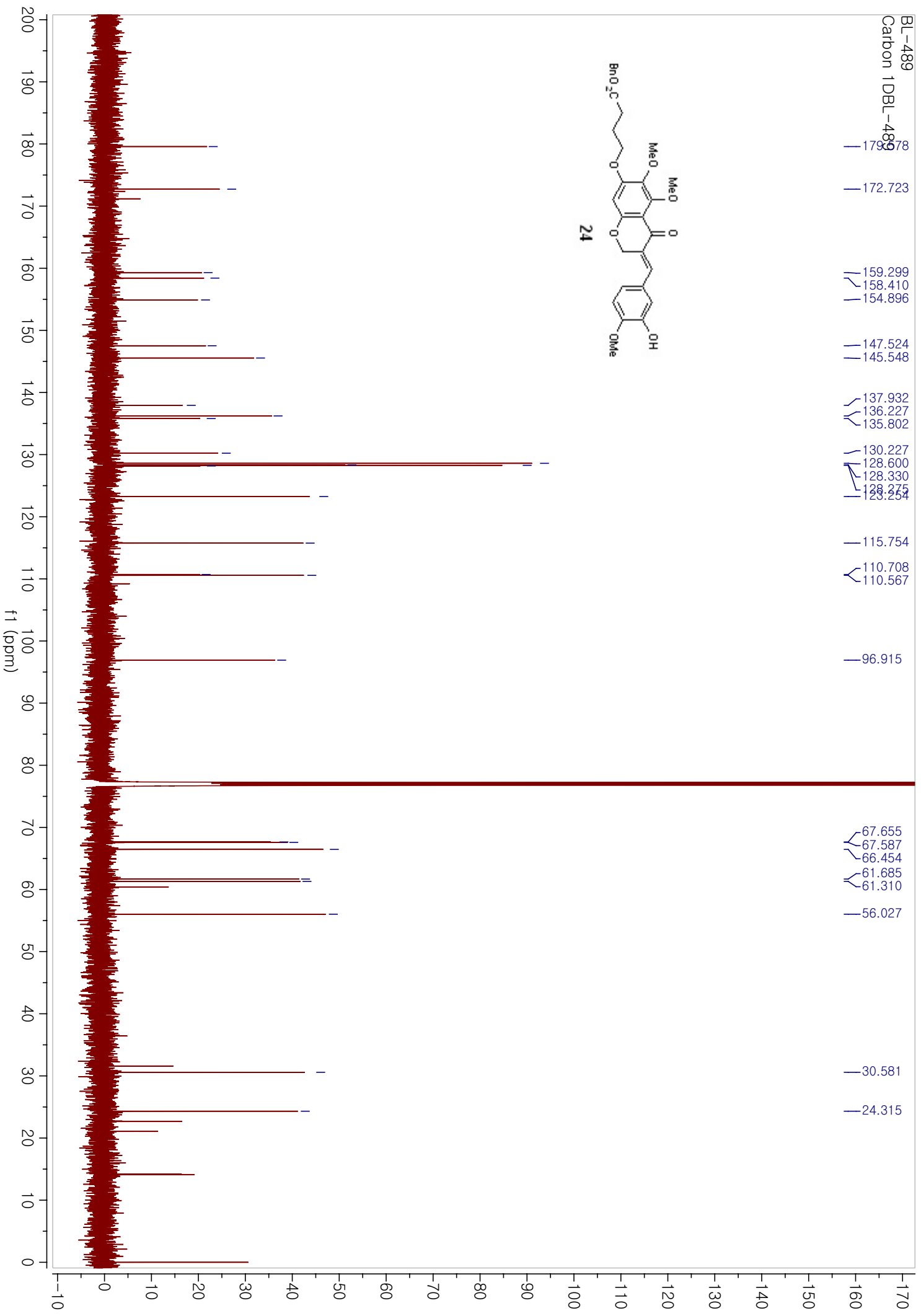
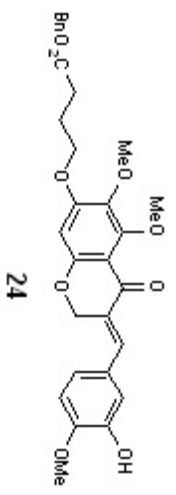


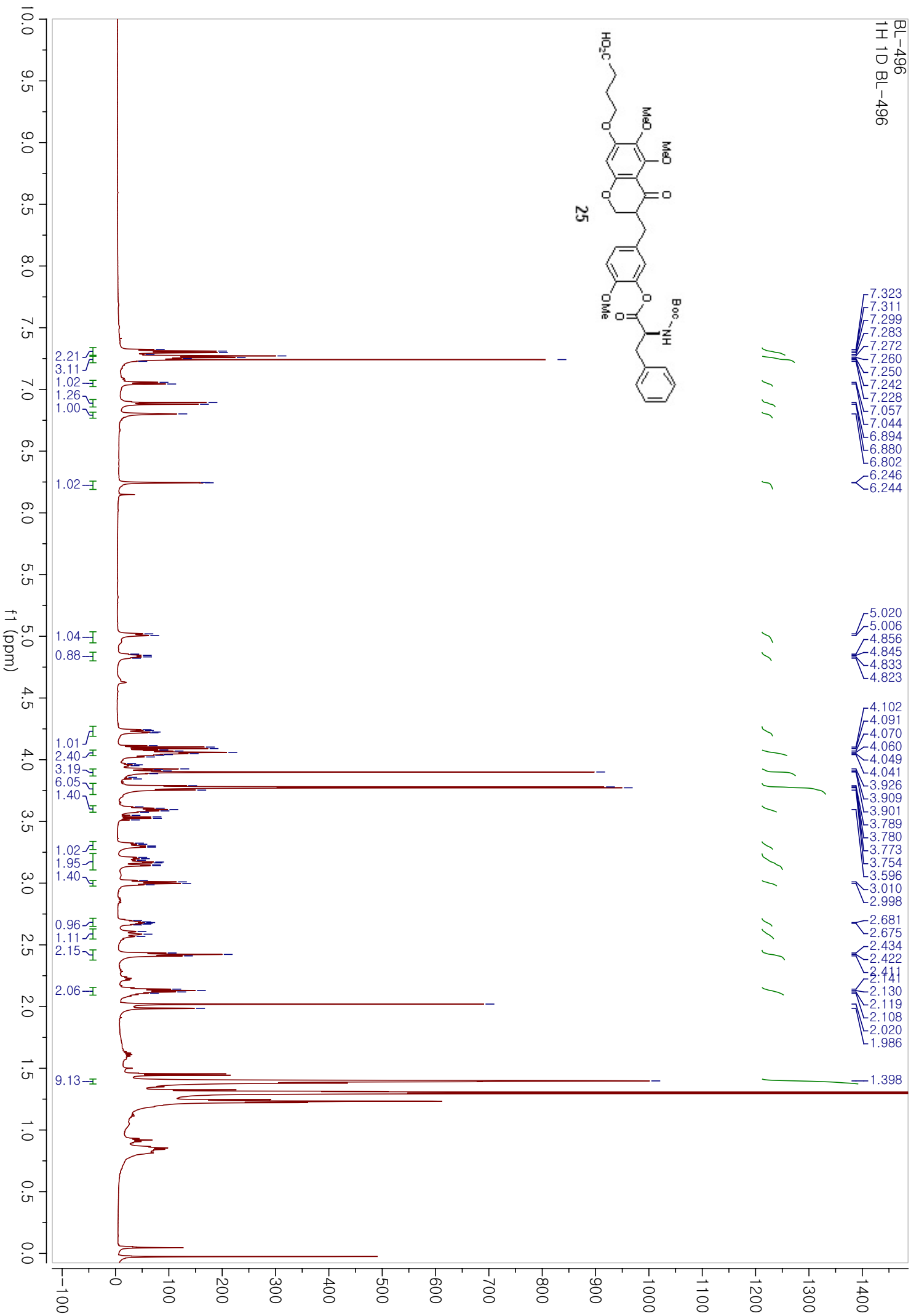
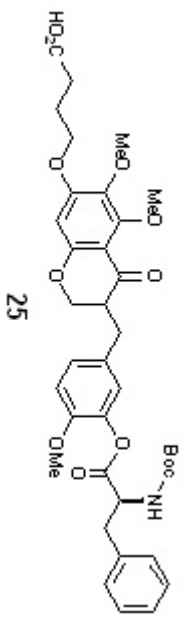
BL-486
Carbon 13





BL-489
Carbon 1 DBL-489





BL-496

Carbon-13 DBL-496

