공학석사 학위논문

Synthetic Progress on β-Cyclodextrin-based Multifunctional MRA Contrast Agent

베타-사이클로덱스트린 기반

다기능성 MRA 조영제 합성 연구

2023년 2월

서울대학교 대학원

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이 논문을 공학석사 학위논문으로 제출함

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김주희의 공학석사 학위논문을 인준함

2023년 2월

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Abstract

Synthetic Progress on β-Cyclodextrin-based Multifunctional MRA Contrast Agent

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MRI (Magnetic resonance imaging) is a high-resolution imaging technology that can create detailed anatomical images of the internal body. MRI is used especially for the examination of soft tissue lesions. For a better examination, it is often required to use an MRI contrast agent on patients before MRI scanning.

MR angiography (MRA) is a type of MR technology that mainly visualizes the vascular system to diagnose cardiovascular diseases. When it comes to MRA, it is necessary to prolong the systemic circulation time of contrast agents to visualize blood vessels down to the microvascular system.

So far, many researchers have developed MRI contrast agents composed of biocompatible β-cyclodextrin (β-CD) and Gd-DOTA by forming a covalent or non-covalent bond between them. Some have also conducted PEGylation on MRI contrast agents to increase their circulation time so that they can be used as MRA blood pool contrast agents (BPCA).

Herein, we devised a new BPCA by introducing six PEGs and one Gd-DOTA to one β-CD molecule. The material based on our design not only may prolong the

circulation time but can form a host-guest assembly with a hydrophobic molecule like an antibody or a targeting material for its further multiple utilization.

To conjugate two distinct functional groups of PEG and DOTA on β-CD, we adopted thiol-maleimide addition and alkyne-azide click reaction, respectively. Notably, a dimer intermediate of two β-CD was synthesized to prevent undesired reactions.

In this study, we attempted to produce a β-cyclodextrin-based multifunctional contrast agent and this design shows the potential for further utilization such as a theranostic application or targeted MRI.

Keywords: Magnetic resonance angiography, Blood pool contrast agent, β-Cyclodextrin, Total synthesis, Selective substitution, Disulfide bond

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List of Abbreviations

β	beta
Boc	*tert*-butyloxycarbonyl
br	broad
δ	chemical shift, ppm
d	doublet
DCM	methylene chloride
DMF	*N*,*N*-dimethylformamide
eq	equivalent
EtOAc	ethylacetate
FT-IR	Fourier transform infrared
g	gram(s)
H_2O	water
K_2CO_3	potassium carbonate
M	molarity
m	multiplet
MeOH	methanol
mmol	millimole(s)
mL	milliliter(s)
NMR	nuclear magnetic resonance
$NaHCO_3$	sodium hydrogen carbonate

NaOH	sodium hydroxide
NaOMe	sodium methoxide
PEG	polyethylene glycol
ppm	parts per million
quant.	quantitative
s	singlet
t	triplet
TEA	triethylamine
TFA	trifluoroacetic acid

Chapter 1. Introduction

1.1. MRI contrast agent

Magnetic resonance imaging (MRI) is a high-resolution three-dimensional diagnostic technology that enables non-invasive diagnosis of abnormal tissues by showing their contrast difference.[1] With the use of contrast agents (CA), MRI can provide more precise information about the lesion by increasing the signal intensity as shown in **Figure 1** below.[2]

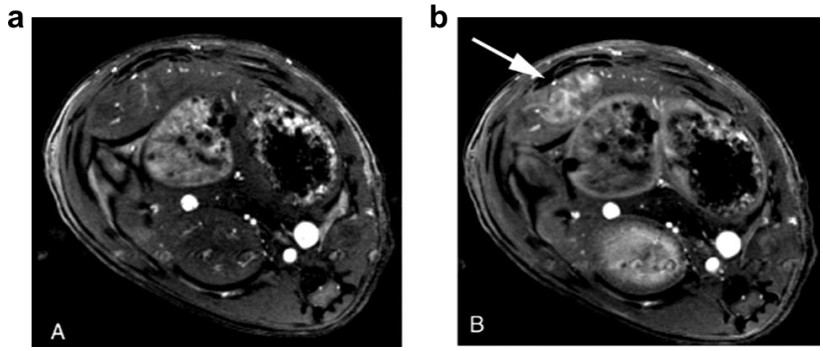


Figure 1. MRI image of the hepatic metastasis (a) before and (b) after the application of the MRI CA.[2]

Clinical MRI CAs such as Dotarem and Magnevist (**Figure 2**) contain gadolinium (Gd) in an organic compound to form a Gd-chelated structure. As these agents are low molecular weight molecules, they can be characterized by rapid excretion into extracellular regions after intravenous injections.

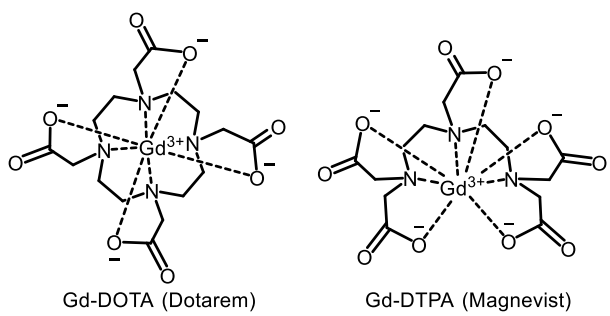


Figure 2. Structure of gadolinium chelate.

1.2. MR angiography

Magnetic resonance angiography (MRA) is a technology specialized for imaging blood vessels in the body. Unlike catheter-based angiography, MRA is not invasive while showing a high-contrast image of blood vessels. Therefore, MRA is frequently used in the research of vascular anatomy.[3] The MRA image in **Figure 3** shows mild stenosis (white arrow) and an irregular plaque in the internal carotid artery (black arrow) which can cause a stroke.[4]

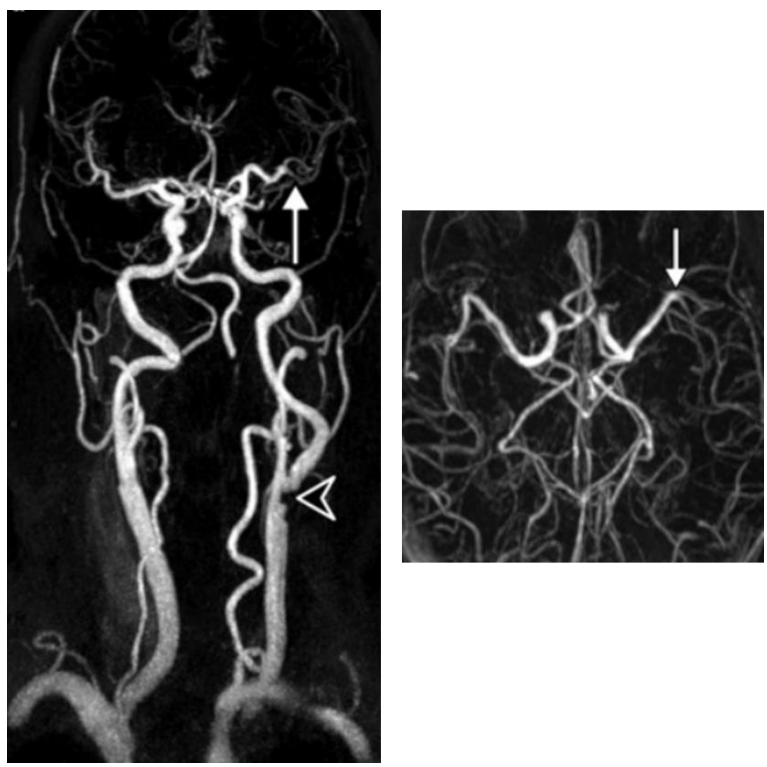


Figure 3. Contrast enhanced MRA image of the legion.[4]

1.3. Blood pool contrast agent

The initial MRA diagnosis was based on time-of-flight (TOF) techniques, a non-contrast-enhanced MRA which generates an MR signal by the natural blood flow.[5] With a contrast-enhanced MRA, however, lesions' location and morphological characteristics can be more efficiently identified by making the

blood flow easier to see.[6,7]

Thus, blood pool contrast agents (BPCA) have been developed to enhance the contrast of vascular spaces. Unlike the MRI CA for extracellular spaces, BPCAs should circulate exclusively within the blood vessel without escaping to the extracellular part.[8]

As mentioned above, clinically used MRI CAs extravasate rapidly out of the vessel into the extracellular space. For that reason, most BPCAs developed so far have sizes in the macromolecule scale. In a previous study, Gd chelate-grafted polymer nanoparticles have been reported as a macromolecular Gd contrast agent with a long circulation time in the vascular system (**Figure 4 (a)**).[9] Another study has reported Gd chelate-conjugated dendrimers that can be used to monitor the effect of anti-angiogenesis therapy and identify its location (**Figure 4 (b)**).[10] The macromolecular properties of these BPCAs certainly prevented their immediate excretion and consequently enhanced the contrast of blood vessels.

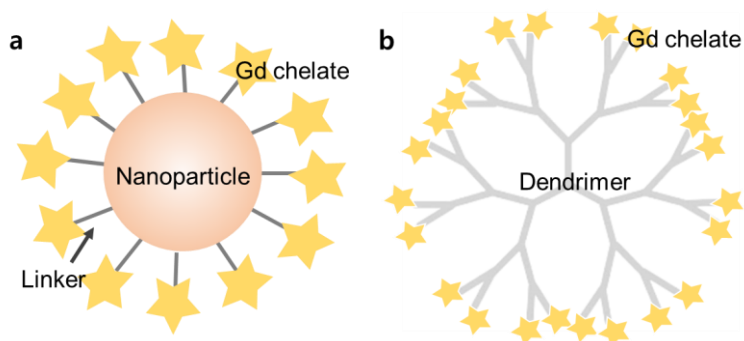


Figure 4. Examples of Gd contrast agents with macromolecule carriers.

β-cyclodextrin (β-CD) is also a common material used for contrast agents because β-CD can easily bind with Gd chelate by a covalent bond or by a host-guest inclusion at the cavity in a non-covalent method. The chemical structure of β-CD is shown below.

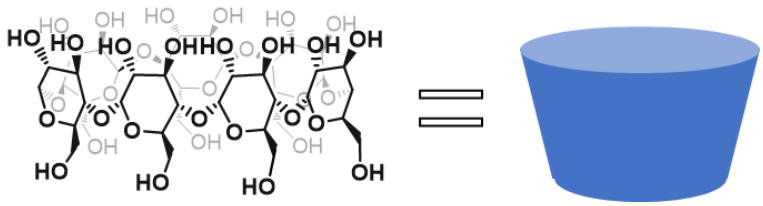


Figure 5. Structure of β-CD.

A contrast agent of rod-like polyrotaxanated cyclodextrins have been reported with a high relaxivity (r_1=23.83 $mM^{-1}s^{-1}$ per Gd chelate at 1.5 T) (**Figure 6 (a)**).[11] Another study developed a "CA tree" composed of numerous host-guest assemblies of the peptide residue and contrast agents and it was noted that the "CA tree" has a relaxivity of 32 $mM^{1}s^{-1}$ (**Figure 6 (b)**).[12]

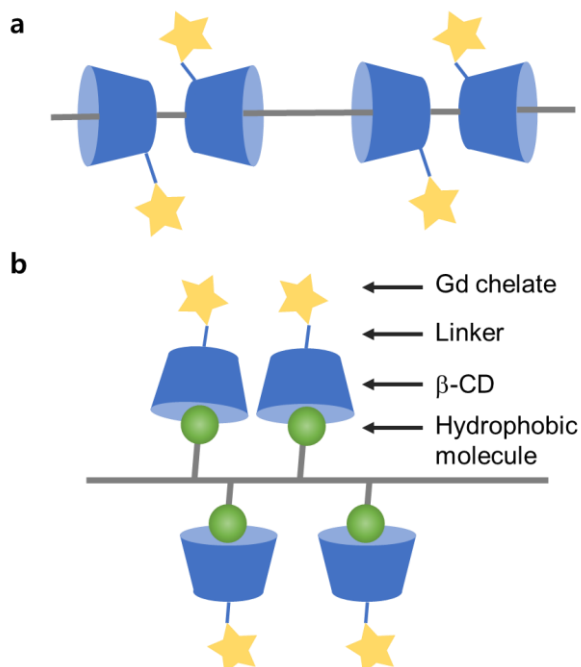


Figure 6. MRI CA with β-CD carrier.

However, these macromolecular contrast agents may increase the risk of Gd accumulation in the liver, which can cause nephrogenic systemic fibrosis (NSF).[13] Contrast agents should be decomposed or eliminated from the body after

completing their duty of contrast enhancement. It is necessary to develop a renal clearable Gd carrier to prevent the potential toxicity of Gd.

One previous research developed a small single β-CD-based MRI contrast agent (**Figure 7**).[14] With mono-substitution of Gd-DOTA on β-CD, the substance exhibited a high relaxivity of 8.50 $mM^{-1}s^{-1}$.

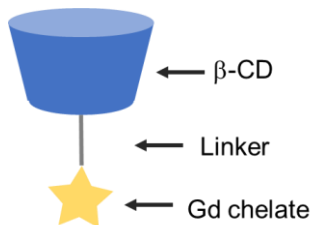


Figure 7. Mono-substituted DOTA on β-CD.

Nanomaterials of renal clearable size around 5.5 nm can obtain the properties of extended systemic circulation with PEG (polyethylene glycol). PEG is well-known for its anti-opsonizing function against phagocytic uptake.[15,16] A previous study noted that PEGylation of β-CD extended the circulation time of the contrast agent in the blood vessel.[17]

In our previous study, we synthesized a renal clearable material named VasCA, a non-covalent complex of PEGylated β-CD and a gadolinium chelate (Gd-DOTA) as described in **Figure 8**.[18] A β-CD molecule has a cavity that can load a hydrophobic molecule by host-guest inclusion. β-CD was covalently linked to a Gd chelate and the guest molecule with hydrophobic adamantane was loaded on the cavity.

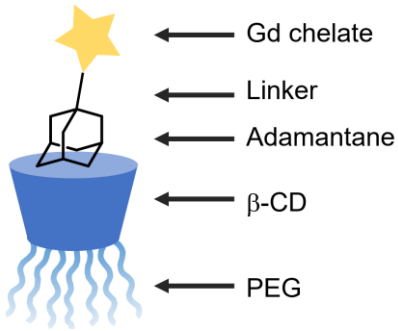


Figure 8. Renal clearable β-CD contrast agent.

It was confirmed that VasCA circulates the blood vessels longer than Dotarem while imaging the microvasculature and intratumoral vascular structure with a higher relaxivity (9.27 $mM^{-1}s^{-1}$) than Dotarem (3.87 $mM^{-1}s^{-1}$). In addition, it was mostly excreted to the kidneys through the glomerular filtration membrane. According to the experimental results, per-PEGylated cyclodextrin was proved to be a useful and biocompatible BPCA carrier.

1.4. Purpose of Research

Our new material was designed to have six PEG chains and one Gd-DOTA chelate on β-CD as shown in **Figure 9**.

The covalent bond between DOTA and β-CD can ensure their high binding stability. This will enable a precise biodistribution study of the intact material because β-CD and DOTA derivatives will not dissociate from each other when intravenously injected. In addition, PEGylation can extend the systemic circulation of this material. Therefore, this organic compound can serve as a long-circulating BPCA itself.

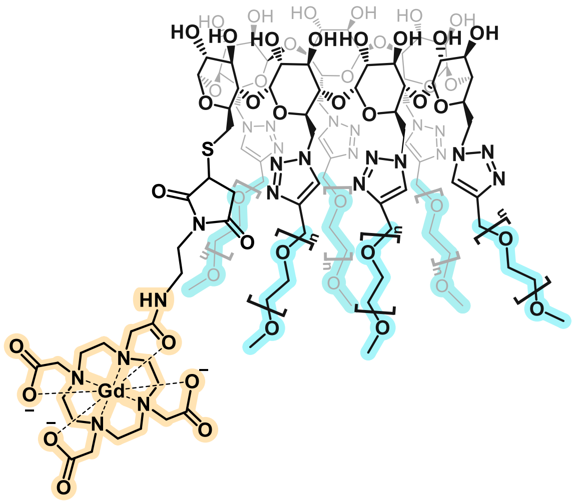


Figure 9. Structure of MRA contrast agent.

Notably, it also can be used as a theranostic agent if a drug is non-covalently loaded on the inner cavity of β-CD.[19-22] This structure can make it easier to track and monitor the drug after administered to patients. Targeted MRI can be also achieved with an antibody or a peptide that targets a specific region like a tumor.[23,24] Thus, our new material can act as a multifunctional MRA contrast agent.

2. Results and discussion

One β-CD molecule consists of seven glucose units. In each unit, every carbon and proton is designated by numbering them (**Figure 10**). Every C-6 has one primary hydroxyl group and each C-2 and C-3 has one secondary hydroxyl group.

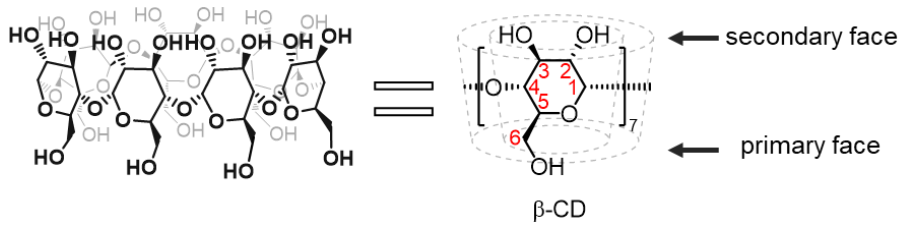
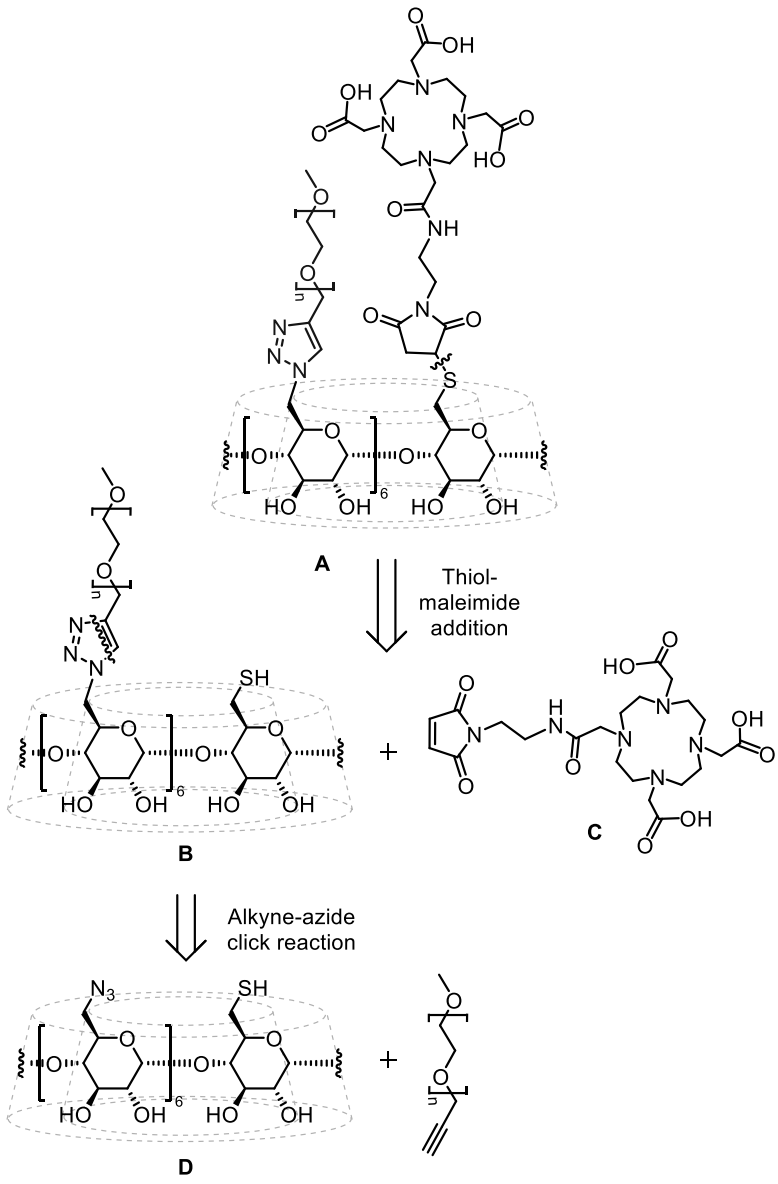


Figure 10. Structure of β-CD with the carbons numbered.

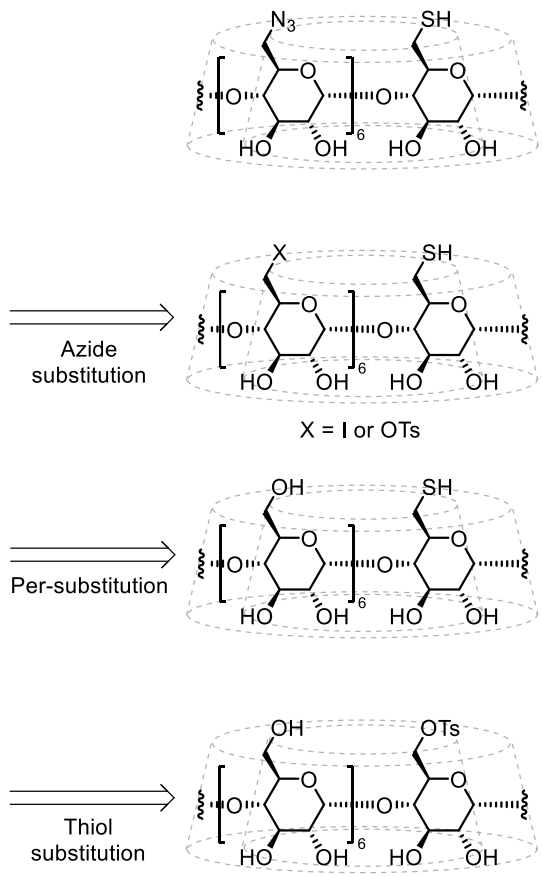
Our multifunctional MRA contrast agent can be produced by converting primary alcohols to PEG and DOTA derivatives. The retrosynthetic strategy for β-CD-derived multifunctional agent **A** is shown in **Scheme 1**. PEG and DOTA can be attached to β-CD respectively by applying different chemistries. Copper-catalyzed azide–alkyne cycloadditions (CuAAC) reaction was adopted as per-PEGylation method like it was conducted in the previous research of VasCA. To conjugate β-CD and DOTA by mono-substitution, we used thiol-maleimide Michael addition reaction which is known to form one thiosuccinimide product by simple conditions. Thus, maleimide conjugated-DOTA (**C**) and hexa-6-azido-mono-6-thio-CD (**D**) were determined as the key intermediates, as shown in **Scheme 1**.



Scheme 1. Retrosynthetic strategy for the multifunctional reagent.

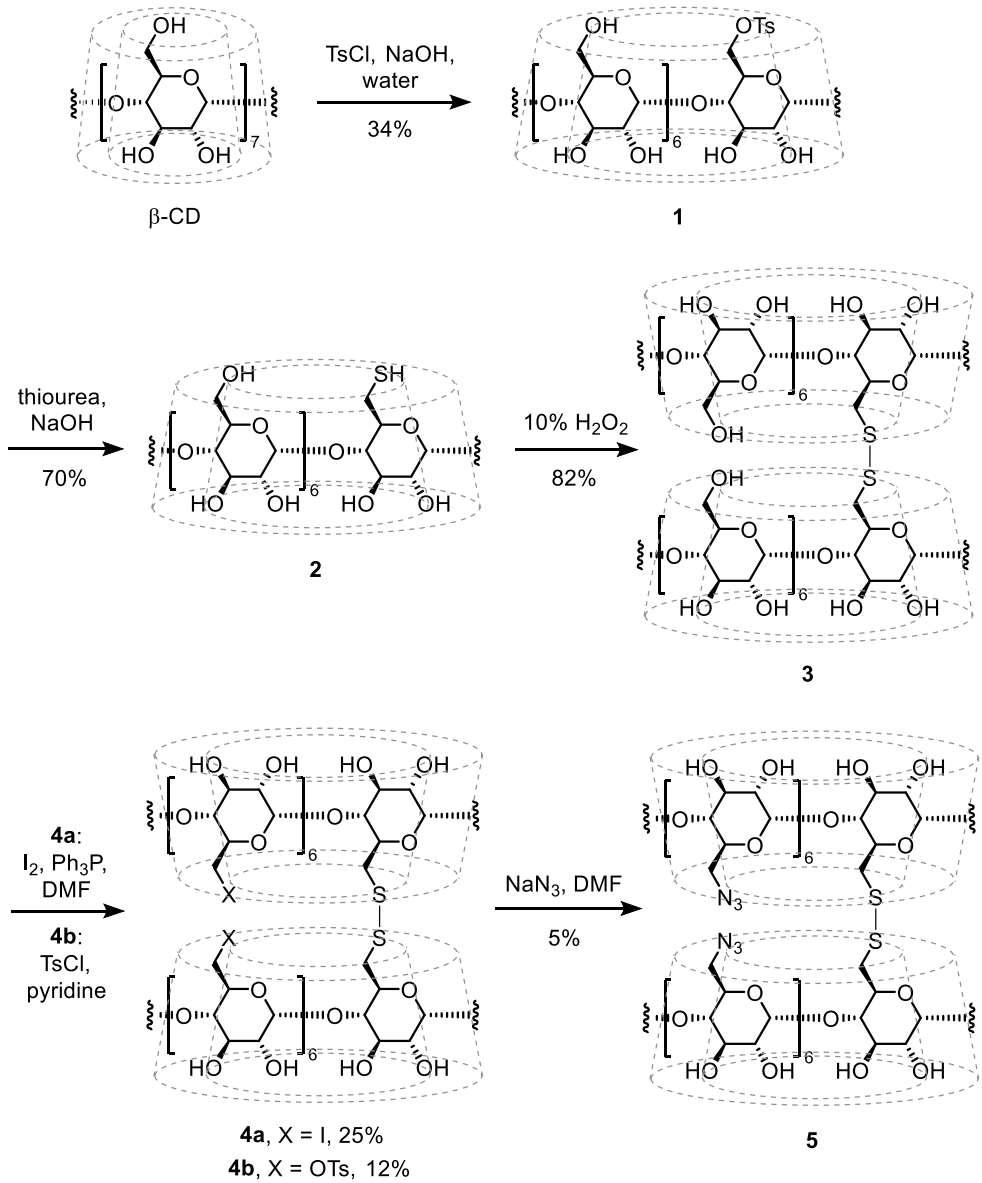
2.1. Synthesis of β-CD derivatives

To synthesize the β-CD derivative (**D**) in **Scheme 1**, mono-6-thio-CD has to be substituted with the leaving groups, which can be displaced with azido group in the next step. The azido group reacts with the propargyl PEG to generate a triazole ring via CuAAC click reaction. This retrosynthetic strategy of **D** is described in **Scheme 2**.



Scheme 2. Retrosynthetic strategy to the mono-6-tosyl-CD.

Based on the retrosynthetic strategy, the synthetic scheme for β-CD derivative was designed as shown below (**Scheme 3**).



Scheme 3. Synthetic scheme for **5**.

As reported in previous studies, β-CD can be readily mono-substituted using tosyl-chloride (TsCl), and tosyl group is a good leaving group that can be exchanged with another substituent including thiol.[25]

According to the scheme, mono-6-tosyl-CD (**1**) was prepared first. 2.5 M NaOH solution was slowly added to β-CD suspension in water. Then, TsCl was added in several portions and stirred for 2 h at room temperature. Unreacted TsCl

solids were removed by filtration and the filtrate was neutralized to form a white precipitate. After removing the remaining TsCl by washing with acetone, pure **1** was obtained with a 34% yield. [26]

For thiol substitution, **1** and thiourea were dissolved in DMF and refluxed for 2 days to obtain cyclodextrin sulfonium. The mixture was added to diethyl ether for precipitation and washed with acetone to remove unreacted thiourea. After filtration, the residue was dissolved in 10% NaOH to form mono-6-thio-CD (**2**) as a product and urea as a byproduct.[27] The impurities could be removed by washing with acetone and water. In this reaction, DMF, or 80% MeOH/H_2O was used as the solvent. 70% and 20% yield of the product were obtained as pure **2** by using DMF and 80% MeOH/H_2O, respectively. The ^{1}H, ^{13}C, and 2D-HSQC NMR spectra of **2** are shown in **Figure 11**. Methylene protons were detected at 2.98 and 2.78 ppm (green arrow) and correlated with the secondary carbon at 22.5 ppm (green arrow), which refers to C-6, in 2D-HSQC NMR of **2**. In addition, thiol proton was detected at 2.01 ppm in the triplet state (orange arrow). As a result, we can tell the thiol group has successfully replaced the tosyl group.

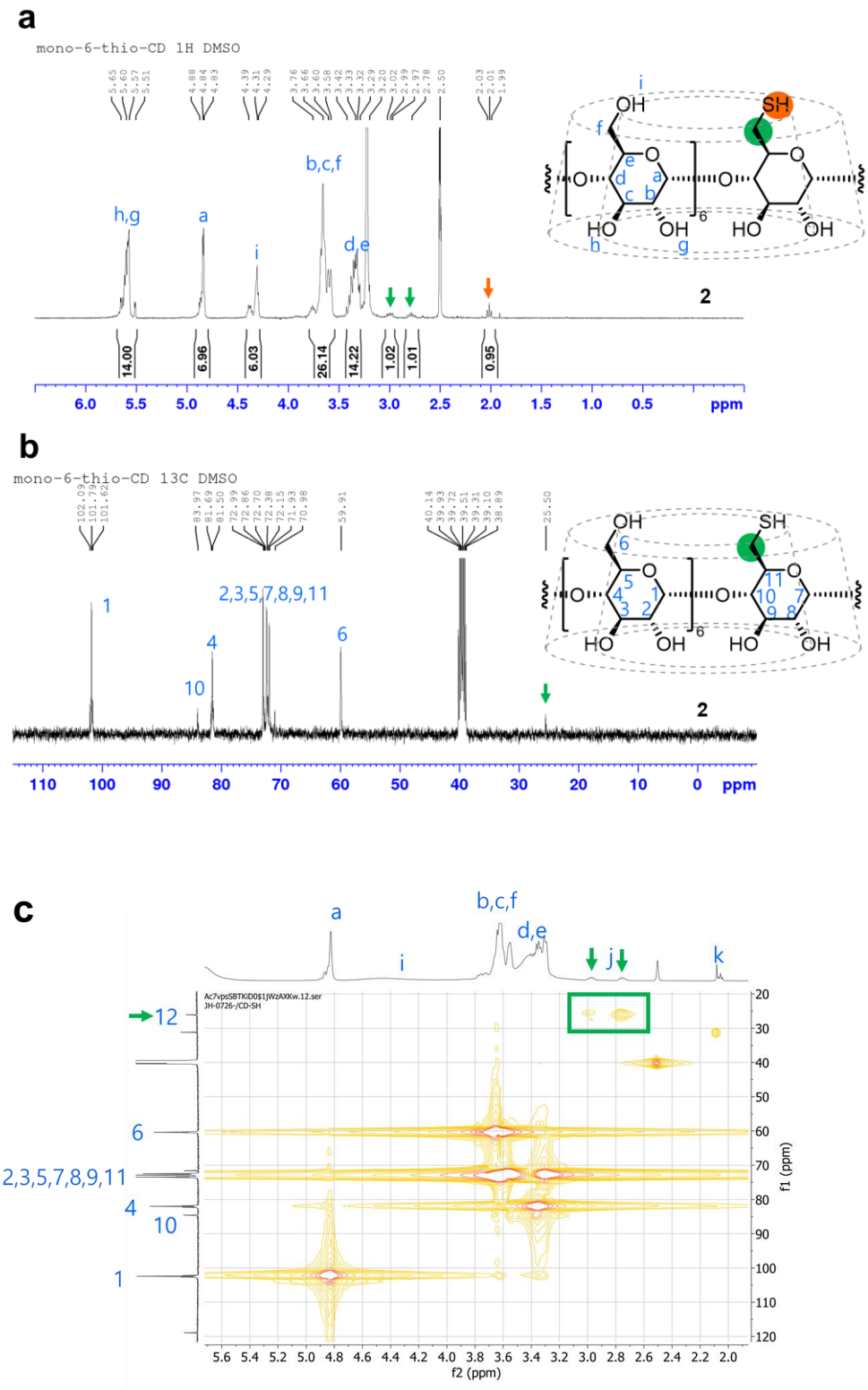


Figure 11. (a) ^{1}H, (b) ^{13}C, (c) 2D-HSQC NMR spectrum of **2**.

Then we tried per-iodination of mono-6-thio-CD with triphenylphosphine (PPh_3) to introduce another functional group to β-CD. But from the ^1H NMR spectrum, we found that the thiol proton of the crude material was missing. Since thiol is a good nucleophile, it seemed to make the undesired product during the reaction. So, we protected the thiol group before functionalizing the 6 free primary alcohols to avoid any potential undesired reaction. We used benzyl chloroformate (CbzCl) or hydrogen peroxide (H_2O_2) to protect the thiol group (**Figure 12**).

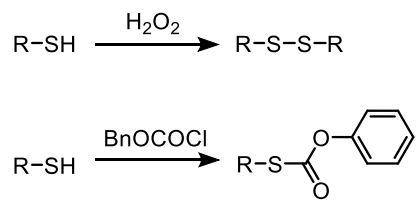


Figure 12. Protection of thiol group.

First, we mixed **2** with CbzCl and TEA to form mono-6-S-Cbz-CD but ended up finding that unreacted **2** remained. On the other hand, 82% of pure disulfide-linked cyclodextrin dimer **3** was obtained by stirring **2** in H_2O_2 solution at room temperature.[28] The ^1H NMR spectrum (**Figure 13)** shows that thiol was oxidized to disulfide in this way.

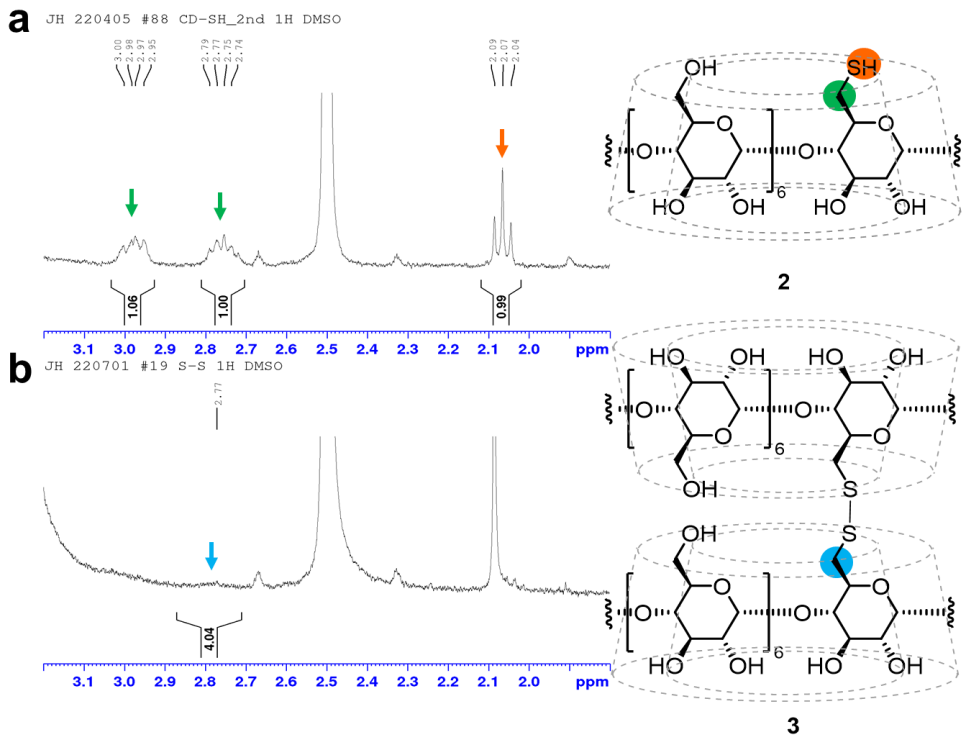


Figure 13. ^{1}H NMR spectrum of (a) **2** and (b) **3**.

Then we tried two methods to per-substitute **3**. First, per-iodination using PPh_3 and I_2 was adopted.[29,30] Under argon condition, PPh_3 was dissolved in dry DMF and then a solution of I_2 in DMF was added dropwise. After 10 min, a solution of **3** in DMF was also added slowly. The reaction proceeded overnight at reflux. The reaction was conducted under the argon atmosphere. The mixture was concentrated, and then 3 M NaOMe/MeOH was added. The mixture was stirred for 30 min and poured into excess MeOH to form a precipitate. Finally, 25% of pure **4a** was obtained by washing with diethyl ether and acetone. In **Figure 14**, the twelve free primary alcohol protons (blue arrow) that existed in compound **3** were found to be absent in **4a**, which means the hydroxyl group was successfully substituted with iodine.

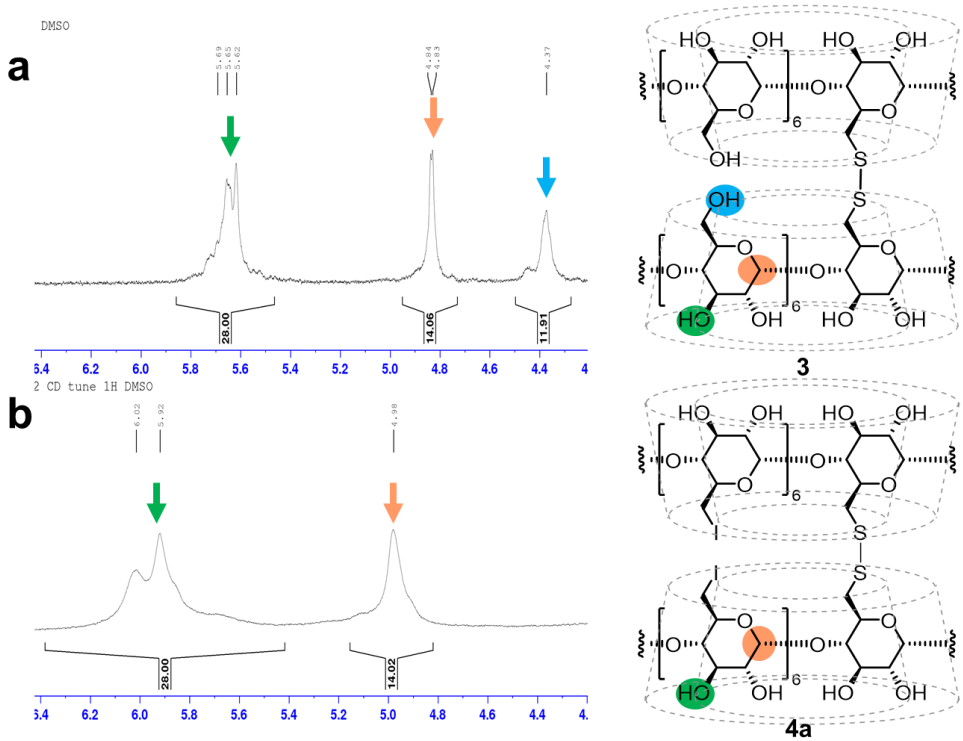


Figure 14. ^{1}H NMR spectrum of (a) **3** and (b) **4a**.

For the second approach to per-substitute **3**, **3** was per-tosylated with TsCl and pyridine to form **4b.** Under anhydrous conditions, a solution of TsCl in anhydrous pyridine was slowly added to a solution of **3** in anhydrous pyridine. After most pyridine was removed under vacuum, the precipitate can be obtained by adding excess water. 12% of pure **4b** was gained after washing the precipitate with hexane and acetone.[31] In **Figure 15,** we can check protons at 7.71, 7.39, and 2.38 ppm, which indicate tosyl group (orange arrow) was newly detected.

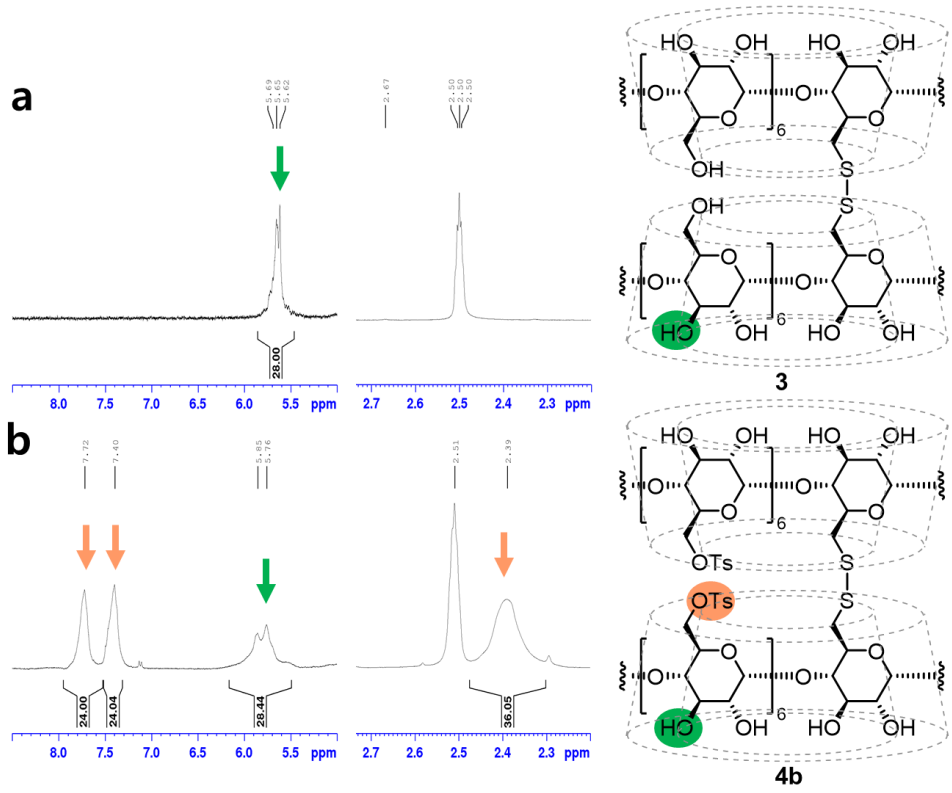


Figure 15. ^{1}H NMR spectrum of (a) **3** and (b) **4b**.

In the FT-IR spectrum, the existence of tosyl group in **4b** was confirmed from characteristic wavenumber at 1358.9, and 1450.5 cm^{-1}, which indicate S=O bond and aromatic ring C-C bond, respectively (**Figure 16**).

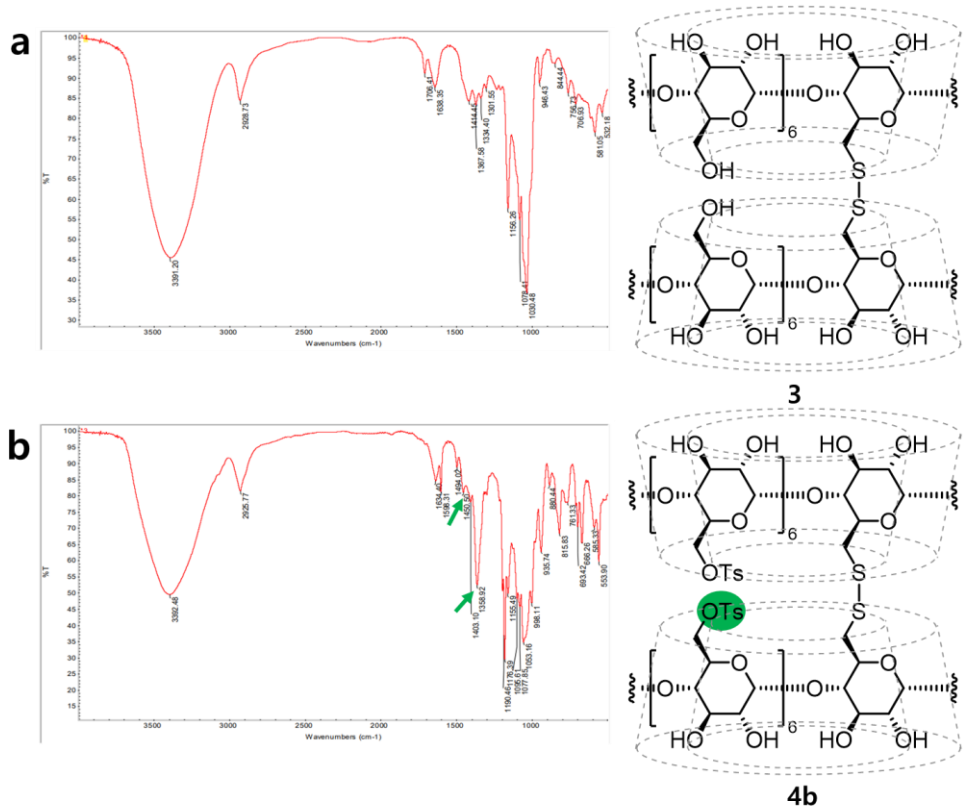


Figure 16. FT-IR spectrum of (a) **3** and (b) **4b**.

We decided to choose **4a** for the next azide substitution step due to its higher yield. **4a** was dissolved in dry DMF and sodium azide (NaN_3) was added to the solution. It was stirred for 20 h at reflux condition and then concentrated. With excess water added, an insoluble precipitate was obtained with 5% yield.

To confirm the structure, we could refer to the ^1H NMR spectrum from our previous research.[26] In **Figure 17,** as per-6-iodo-CD is changed into per-6-azido-CD, 14 protons of the secondary alcohol (green box) at 6.05 and 5.95 ppm of per-6-iodo-CD moved upfield to 5.93 and 5.77 ppm of per-6-azido-CD. Protons at C-1 (orange box) are also more shielded for per-6-azido-CD than per-6-iodo-CD. They moved upfield from 4.99 ppm to 4.90 ppm.

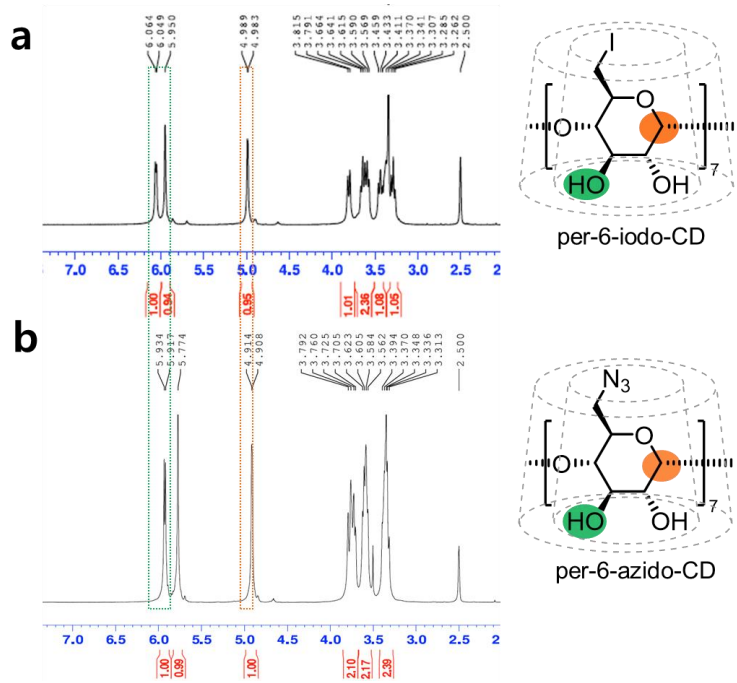


Figure 17. ^{1}H NMR spectrum of (a) per-6-iodo-CD and (b) per-6-azido-CD.[26]

In **Figure 18**, similar to the previous result, 28 secondary alcohol protons (green arrow) at 6.02 and 5.92 ppm moved upfield to 5.90 and 5.77 ppm as **4a** was converted into **5**, respectively. We could observe that the C-1 proton (orange arrow) of **4a** at 4.98 ppm became shielded as it shifted to 4.91 ppm, as well.

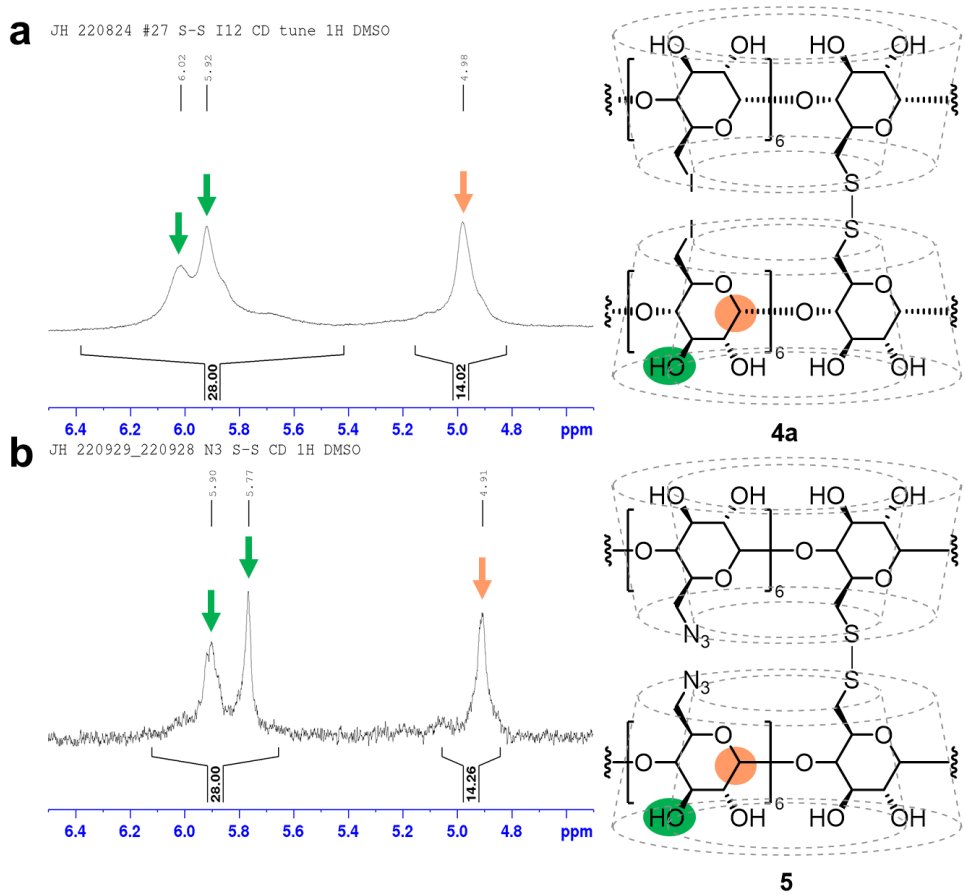


Figure 18. ^{1}H NMR spectrum of (a) **4a** and (b) **5**.

So far, we introduced two different functional groups of azide and disulfide to β-CD. According to **Scheme 1** and **Scheme 2,** before we conduct the conjugation between the azide group of **5** and propargyl PEG, we had to reduce the disulfide bond to free thiol. This is because the bulky dimer structure might hinder the per-substitution of long PEG chains. However, considering that thiol is easily oxidized during storage, we tried to synthesize maleimide conjugated-DOTA in advance.

Figure 18 below shows the whole ^{1}H NMR spectrum of **β-CD, 1, 2, 3, 4a,** and **5.** Each product was confirmed to be successfully synthesized by examining the chemical shift of each proton.

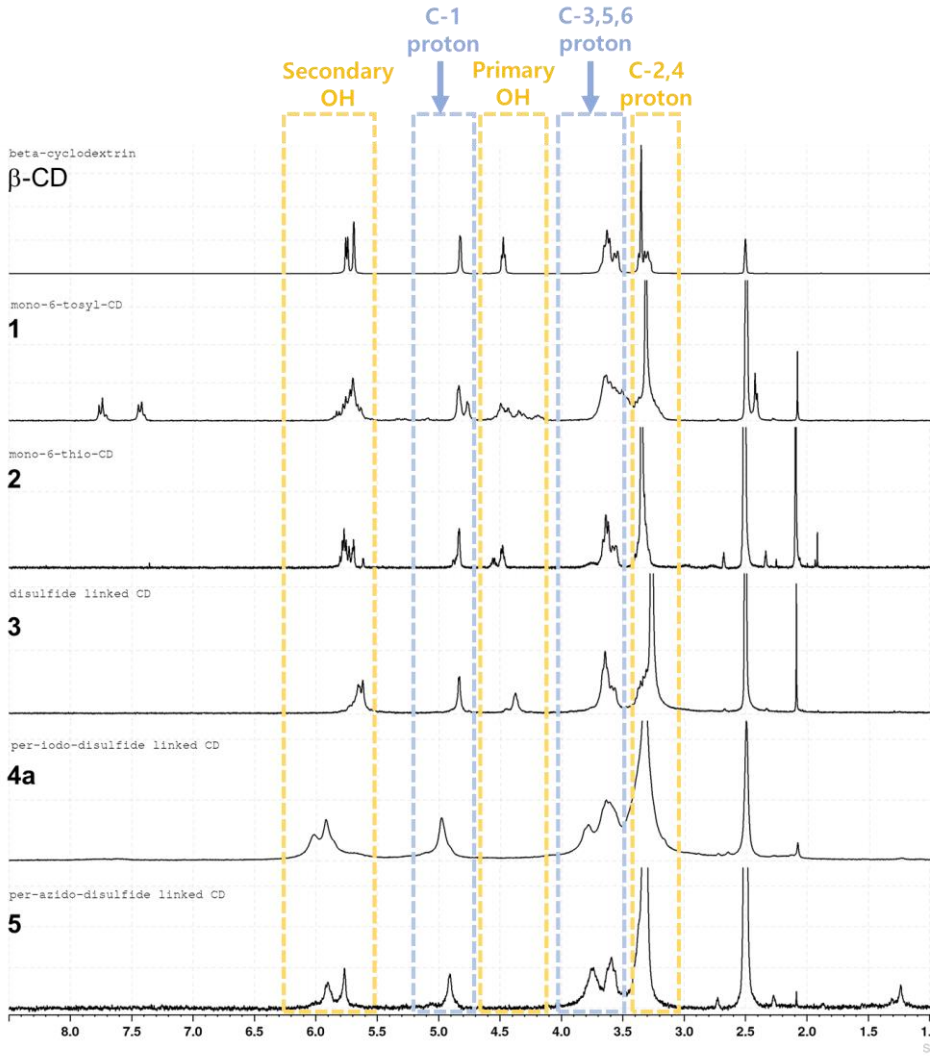
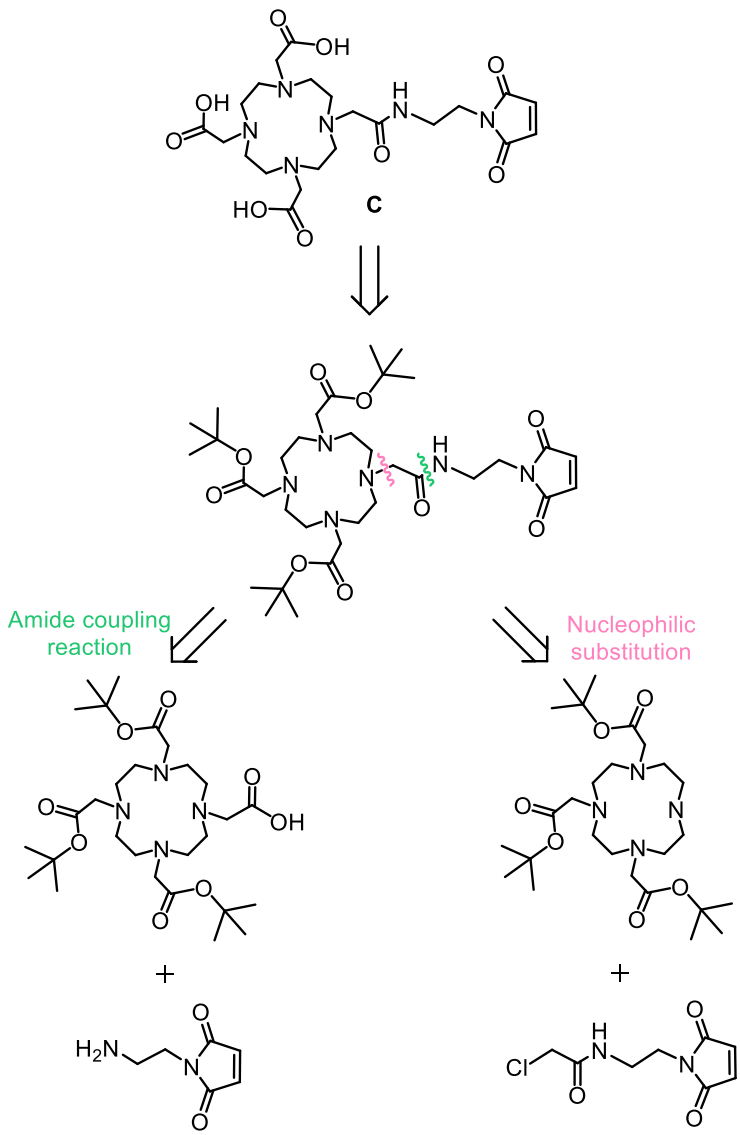


Figure 19. Whole ^{1}H NMR spectrum (DMSO-d_6) of β-CD, **1**, **2**, **3**, **4a** and **5**.

2.2. Synthesis of DOTA derivatives

The retrosynthetic strategy for maleimide conjugated-DOTA is shown in **Scheme 4**. It can be generated by deprotecting the maleimide conjugated-DO3A, which can be fragmented into maleimide moiety and DO3A-*tert*-butyl ester (DO3A(tBu)$_3$).



Scheme 4. Retrosynthetic strategy for the maleimide conjugated DOTA.

We tried to conjugate the maleimide moiety and DO3A(tBu)$_3$ with amide coupling reaction or nucleophilic substitution.

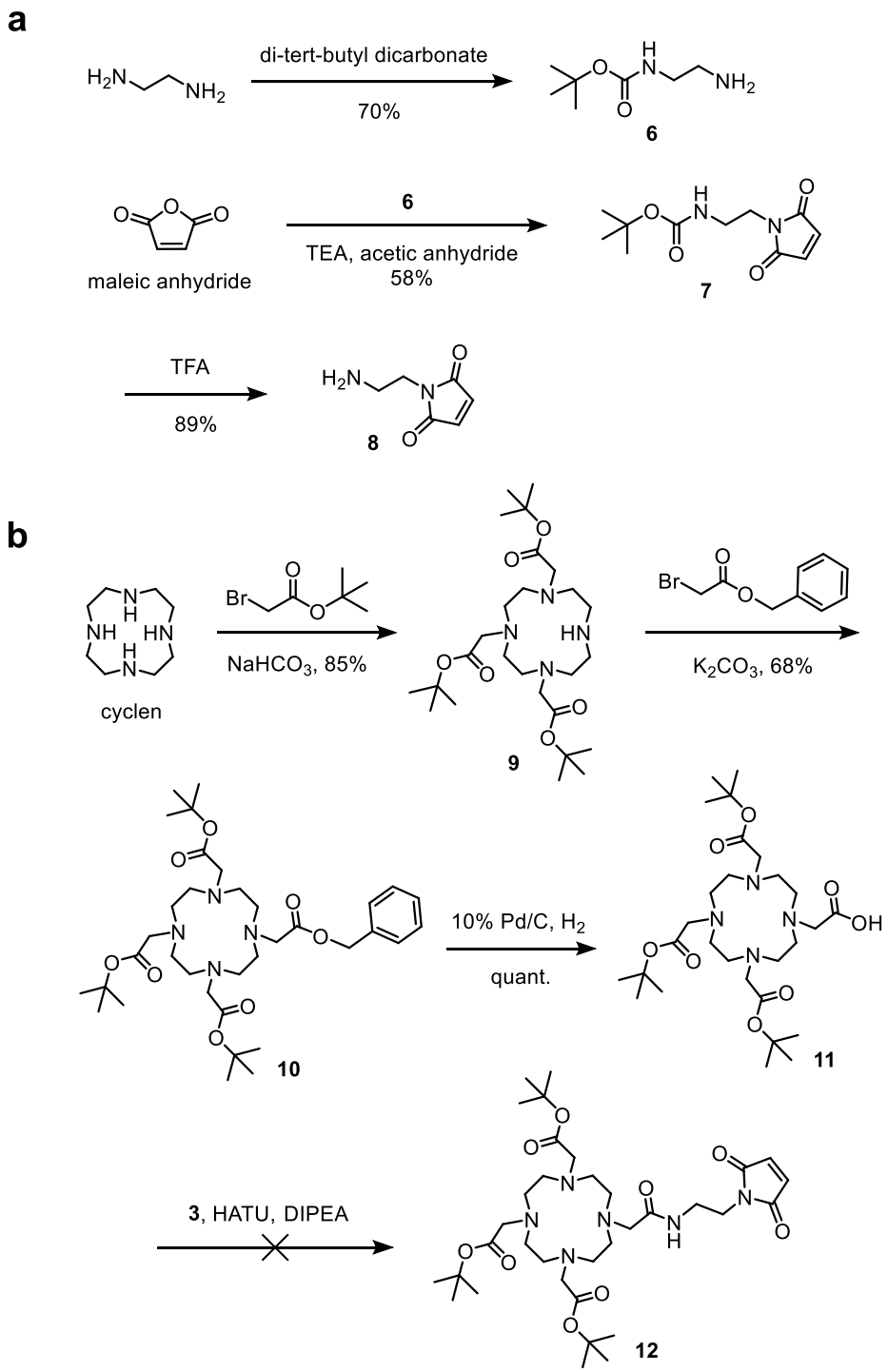
2.2.1. 1st Approach for DOTA derivative C

Our first approach to synthesize **C** was the amide coupling reaction as described in **Scheme 5**.

We started with synthesizing *N*-Boc-ethylenediamine (**6**) by reacting ethylenediamine with di-*tert*-butyl bicarbonate.[32] **6** was then dissolved in diethyl ether at 0 °C with TEA, followed by dropwise addition of maleic anhydride. The reaction was carried out for 4 h and additional 2 equivalents of TEA were added and stirred at reflux. We gained pure **7** with a yield of 58% by purifying the crude product through column chromatography. Then **7** was mixed with TFA in DCM overnight to obtain pure **8**.[33]

To synthesize DO3A(tBu)$_3$ (**9**), cyclen and $NaHCO_3$ were dissolved in acetonitrile and *tert*-butyl bromoacetate in acetonitrile was added dropwise. The reaction progressed overnight. Pure **9** was obtained by precipitation with toluene and excess diethyl ether. Two steps were further taken to conjugate carboxylic acid on **9**. **9** was reacted with benzyl bromoacetate and K_2CO_3 in acetonitrile at reflux condition overnight. Column chromatography was conducted to obtain pure **10**. Through reduction with 10% Pd/C and hydrogen gas, **11** was obtained as a product.

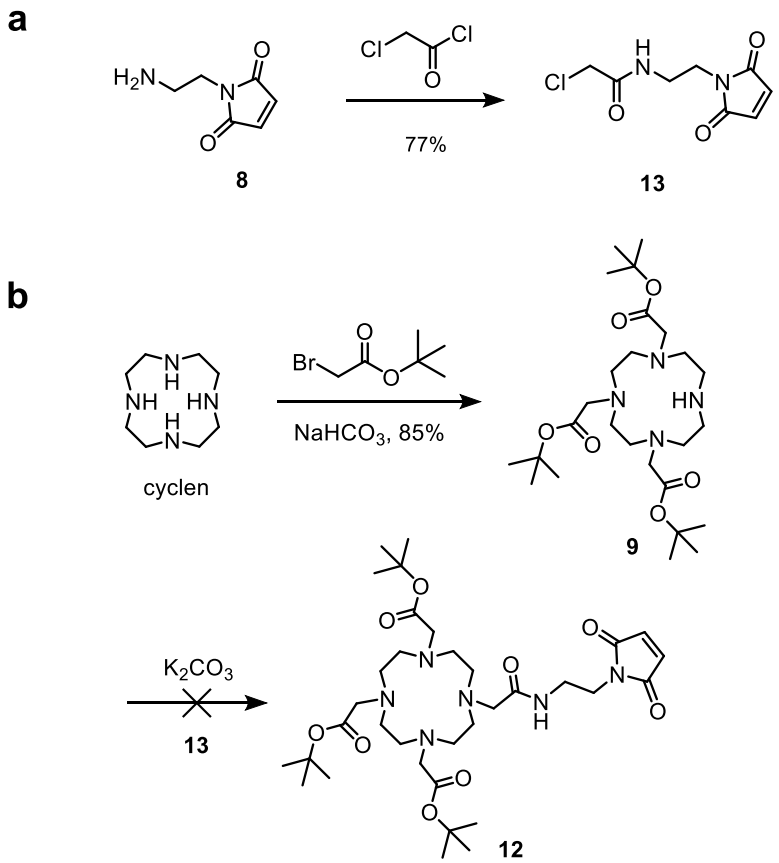
To conjugate the maleimide moiety (**8**) and DO3A-carboxylic acid (**11**), HATU and DIPEA were used as coupling reagents. The mixture was stirred in DMF under argon conditions at room temperature and the crude was concentrated. However, due to remaining moisture, it seemed that **8** did not undergo amide coupling with **11**. As the amide coupling reaction failed, we tried another synthetic scheme that does not require the anhydrous condition.



Scheme 5. Synthetic scheme for (a) **8** and (b) **12**.

2.2.2. 2nd Approach for DOTA derivative C

We tried the nucleophilic substitution between maleimide and DO3A moiety for the second approach, as demonstrated in **Scheme 6**.



Scheme 6. Synthetic scheme for (a) **13** and (b) **12**.

To incorporate a leaving group in **8**, a solution of **8** in DCM was added with chloroacetyl chloride dropwise. Through column chromatography, pure **13** was obtained. Then we tried to conjugate **9** with **13**. **9** was dissolved in dry acetonitrile with K_2CO_3, and subsequently, **13** was added dropwise. The reaction was conducted under argon condition at reflux and the mixture was filtered. Checking the crude NMR spectrum, we found the product was not formed and the starting material remained.

To sum up, we tried two respective schemes for synthesizing maleimide conjugated-DOTA and made it to **8** and **11** in **Scheme 3**, and **13** and **9** in **Scheme 4**.

3. Conclusion

The MRI contrast agents currently used in clinical practice are unsuitable for imaging the blood vessels since they extravasate into extracellular parts rapidly due to their low molecular weight. This study devised a new MRA contrast agent with the prolonged systemic circulation.

We designed a β-CD derivative substituted with one DOTA and six PEGs. To conjugate those two different functional groups, we applied thiol-maleimide click reaction and alkyne-azide click reaction, respectively.

At first, we substituted one hydroxyl group of β-CD with thiol group and subsequently conducted protection of thiol to form a disulfide dimer to prevent potential undesired reactions. Then we substituted the remaining free primary alcohols of a β-CD dimer with iodine, which was followed by azido substitution for the further click reaction with propargyl PEG. For DOTA derivatives, we tried two approaches to make maleimide conjugated-DOTA. We tried an amide coupling reaction as the first approach and nucleophilic substitution as the second approach.

Our final product of β-CD derivatives contains two different functional groups which can be substituted with DOTA and PEG. This design showcases the potential for multifunctional BPCAs that can be utilized for theranostic application.

4. Experimental

4.1 General information

Materials were purchased from commercial suppliers and used without further purification. Reactions that are sensitive to moisture or air proceeded with flame-dried glassware and magnetic stirring bars under argon conditions.

^{1}H, ^{13}C, and 2D-HSQC spectra were recorded on a Bruker Advance III 400 MHz spectrometer. DMSO-d_6, MeOD, and $CDCl_3$ were used as solvents. FT-IR spectra were measured using Vertex-80V/Hyperion2000.

4.2 Synthetic procedure

Synthesis of compound 1

β-CD (26.4 mmol, 1 eq) was suspended in 270 mL H_2O and 105.6 mL of 2.5 M NaOH solution was added to the suspension slowly. After 5 min, TsCl (39.7 mmol, 15 eq) was added gradually to the reaction mixture over 10 min. The reaction mixture was stirred for 2 h at room temperature. The unreacted TsCl was filtered off. The filtrate was neutralized with concentrated HCl. The resulting white precipitate was filtered and washed with water and acetone.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.73(d, 2H), 7.42(d, 2H), 5.87-5.66 (m, 14H), 4.85-4.83 (m, 5H), 4.77 (d, 2H), 4.59-4.43(m, 5H), 4.40-4.35 (m, 1H), 4.39-4.31 (m, 2H), 4.21-4.16 (m, 1H), 3.65-3.43 (m, 28H), 3.40-3.20 (m, overlapping with water), 2.43 (s, 3H).

Synthesis of compound 2

1 (4.5 mmol, 1 eq) and thiourea (45 mmol, 10 eq) were dissolved in 80 mL DMF. The solution was stirred for 2 days at 75 °C. After it cooled to room temperature, the mixture was added to 500 mL diethyl ether and stirred for 10 min. The resulting

precipitate was filtered, washed with ether, and suspended in acetone (230 mL). The solution was stirred for 2 h at 60 °C, then cooled to room temperature. After filtering the precipitate, the residue was dissolved with 10 wt% NaOH solution (100 mL) and stirred at 50 °C. After 5 h, the mixture was adjusted to pH 2 with concentrated HCl. The resulting solution was added to 25 mL trichloroethylene and stirred overnight. The white precipitate was filtered, washed with water, and dried.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.89-5.52 (m, 14H), 4.95-4.74 (m, 7H), 4.57-4.38 (m, 6H), 3.88-3.47(m, 26H), 3.42-3.24 (m, overlapping with water), 3.00-2.95 (m, 1H), 2.77-2.74 (m, 1H), 2.07 (t, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 101.786, 83.97, 81.59, 72.99, 72.86, 72.70, 72.38, 72.15, 71.94, 70.98, 59.92, 25.50.

Synthesis of compound 3

2 (0.1 mmol) was dissolved in 3 mL of 10% H_2O_2 solution by heating and stirred overnight at room temperature. The mixture was added to acetone. The resulting white precipitate was filtered and dried.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.86-5.47 (m, 28H), 4.93-4.75 (m, 14H), 4.49-4.28 (m, 12H), 3.80-3.50 (m, 52H), 3.46-3.15 (m, overlapping with water), 2.88-2.72 (m, 4H); FT-IR (KBr): ν = 3391.2, 2928.7, 1706.4, 1638.4, 1414.5, 1367.6, 1301.6 cm^{-1}.

Synthesis of compound 4a

To a solution of Ph_3P (7 mmol, 35 eq) in 5 mL dry DMF, the solution of I_2 (7.4 mmol, 37 eq) in 5 mL dry DMF was added dropwise. After 15 min, the solution of **3** (0.2 mmol, 1 eq) in 5 mL dry DMF was added dropwise to the mixture and stirred for 24 h at 80 °C under argon atmosphere. The solution was concentrated to 1/3 of the original volume. 7 mL of 3 M NaOMe in MeOH was added slowly to the residue at 0 °C, stirred for 30 min, and poured into excess MeOH. The resulting precipitate was filtered and washed with MeOH.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.37-5.42 (m, 28 H), 5.18-4.82 (m, 14H), 4.95-

3.73 (m, 12H), 3.96-3.13 (m, overlapping with water). FT-IR (KBr): ν = 3386.0, 2916.6, 1703.3, 1637.2, 1414.3, 1367.9, 1223.5, 1152.1, 1062.5, 1041.0, 943.7, 750.6, 683.3, 587.2, 524.3 cm^{-1}.

Synthesis of compound 4b

12 (0.11 mol, 1 eq) was dissolved in dry pyridine 3 mL at 0 °C. To the solution, TsCl (2.32 mmol, 21 eq) in dry pyridine 8 mL was added dropwise and stirred for 24 h at room temperature. Pyridine was mostly removed by vacuum and the residue was poured into water. The resulting precipitate was filtered and washed with hexane and acetone.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.72 (br s, 24H), 7.40 (br s, 24H), 6.17-5.22 (m, 28H), 5.13-2.98 (m, overlapping with water), 2.39 (br s, 36H). FT-IR (KBr): ν = 3392.5, 2925.8, 1634.4, 1598.3, 1494.0, 1450.5, 1403.1, 1358.9, 1190.5, 1176.4, 1155.5, 1096.6, 1077.9, 1053.2, 998.1, 935.7, 880.4, 815.8, 761.3, 693.4, 666.3, 583.3, 535.9 cm^{-1}.

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Synthesis of compound 5

4a (0.017 mmol, 1 eq) was dissolved in 1.5 mL dry DMF, and NaN_3 (0.36 mmol, 20 eq) was added to the solution. After the mixture was heated up to 60 °C, it was stirred for 20 h. DMF was removed under reduced pressure and the residue was precipitated with water. The insoluble product was filtered and washed with water and dried.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.09-5.66 (m, 28H), 5.03-7.82 (m, 14H), 4.00-3.20 (m, overlapping with water).

Synthesis of compound 6

To the solution of ethylenediamine (50 mmol, 10 eq) in 25 mL DCM, a solution of di-*tert*-butyl-bicarbonate (5 mmol, 1 eq) in 25 mL was added dropwise at room

temperature. The reaction mixture was stirred overnight. After filtering the insoluble water, the mixture was concentrated under reduced pressure to produce colorless transparent oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.88 (br s, 1H), 3.19-3.15 (m, 2H), 2.81-2.78 (m, 2H), 1.44 (s, 9H).

Synthesis of compound 7

6 (3.5 mmol, 1 eq) and TEA (5.25 mmol, 1.5 eq) were dissolved in 7 mL diethyl ether at 0 ºC. The solution of maleic anhydride in 7 mL diethyl ether was added dropwise and the reaction mixture was stirred for 4 h. After the solution was concentrated, it was dissolved in acetone with TEA (7 mmol, 2 eq). The mixture was heated to reflux and acetic anhydride (5.25 mmol, 1.5 eq) was added and stirred for 20 h. The solvent was removed and the crude was purified by column chromatography (30% EtOAc/hexane) to obtain brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.70 (s, 2H), 4.75 (br s, 1H), 3.65 (t, 2H), 3.38-3.22 (m, 2H), 1.39 (s, 9H).

Synthesis of compound 8

7 (0.6 mmol, 1 eq) was dissolved in 1 mL DCM at 0 ºC. To this solution, 1 mL TFA was added and stirred for overnight at room temperature. After concentrating the solvent, precipitate was obtained by adding diethyl ether to the residue. 3 was obtained by filtration and evaporation as a white solid.

^{1}H NMR (MeOD, 400 MHz) δ 6.91 (s, 2H), 3.81 (t, 2H), 3.17-3.14 (m, 2H).

Synthesis of compound 9

Cyclen (5.8 mmol, 1eq) $NaHCO_3$(19 mmol, 3.28 eq) was dissolved in 100 mL ACN and stirred for 15 min. The solution of *tert*-butyl bromoacetate (19 mmol, 3.28 eq) in 50 mL ACN was added dropwise to the mixture. After stirring overnight at 85 ºC, the reaction mixture was evaporated. The crude was dissolved in warm

toluene and cooled down to room temperature to obtain a precipitate. The precipitate was filtered and washed by diethyl ether.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.32 (4H, br s), 3.23 (2H, br s), 3.04 (4H, br s), 2.87-2.82 (12H, m), 1.41-1.39 (27H, m).

Synthesis of compound 10

9 (5.64 mmol, 1 eq) was dissolved in 50 mL dry acetonitrile and added K_2CO_3 (16.9 mmol, 3 eq). Benzyl bromoacetate (8.46 mmol, 1.5 eq) was added subsequently and the mixture was stirred at reflux under argon atmosphere for 16 h. the precipitated solid was removed by filtration, and the filtrate was concentrated. The crude was purified by column chromatography (10% MeOH/DCM) to give a brown solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.29 (m, 5H), 5.15-5.08 (m, 2H), 3.64-1.88 (m, 24H), 1.45-1.42 (m, 27H).

Synthesis of compound 11

The mixture of **10** (0.62 mmol) and 40 mg of 10% Pd/C in 18 mL MeOH was stirred under hydrogen atmosphere for 6 h. The catalyst was removed by celite filtration. The crude was concentrated and purified under column chromatography (10% MeOH/DCM) as a brown solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.42-3.12 (m, 24H), 1.62-1.26 (m, 27H).

Synthesis of compound 13

11 (1 eq, 1 mmol) and TEA (2.04 eq, 2.04 mmol) were dissolved in 10 mL DCM at room temperature. To the solution, chloroacetyl chloride (1.04 eq, 1.04 mmol) was added dropwise. The reaction was stirred overnight and evaporated. The crude was purified by column chromatography (5% MeOH/DCM) to a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (br s, 1H), 6.74 (s, 2H), 4.00 (s, 2H), 3.76-3.73 (m, 2H), 3.51 (q, 2H).

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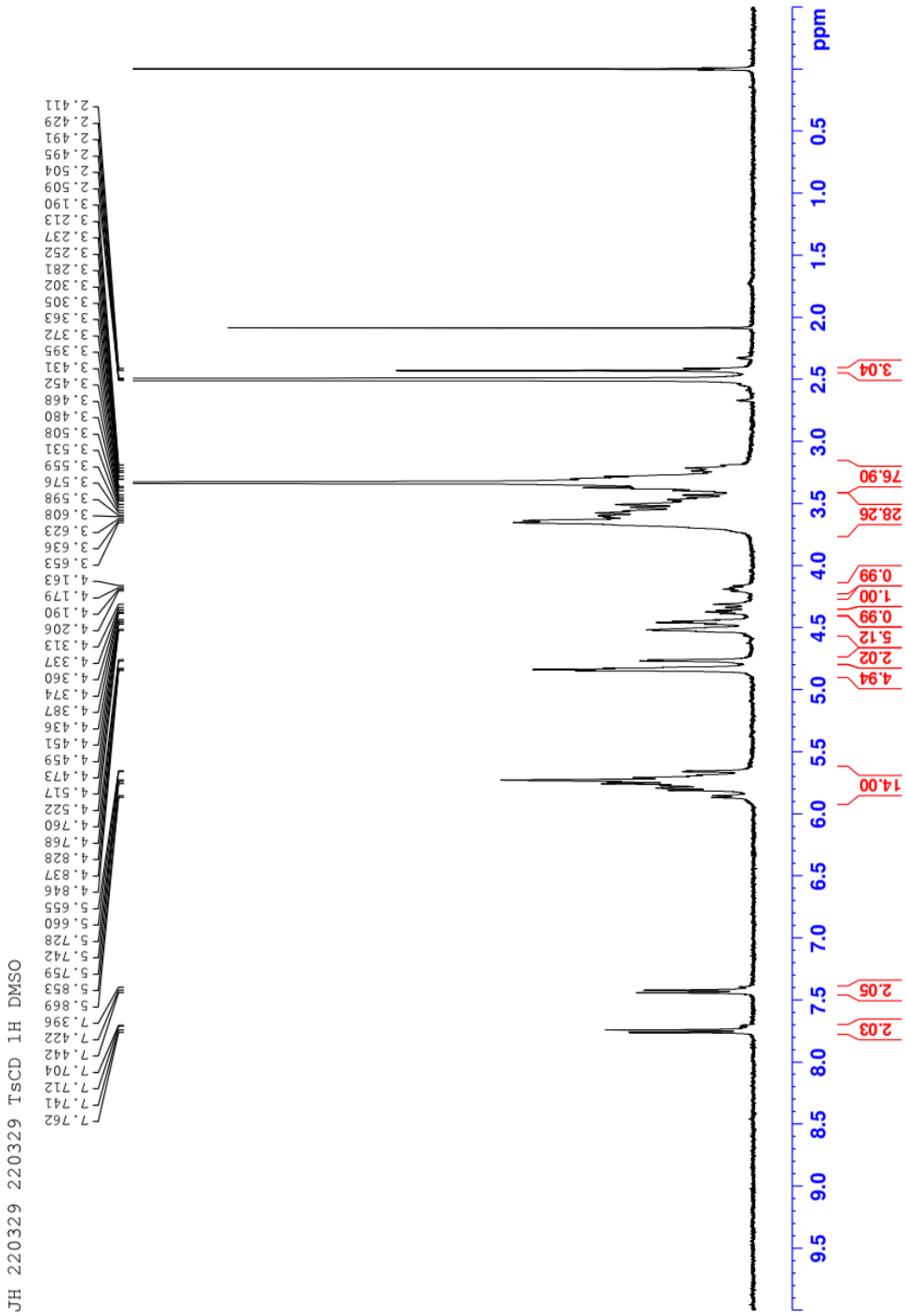
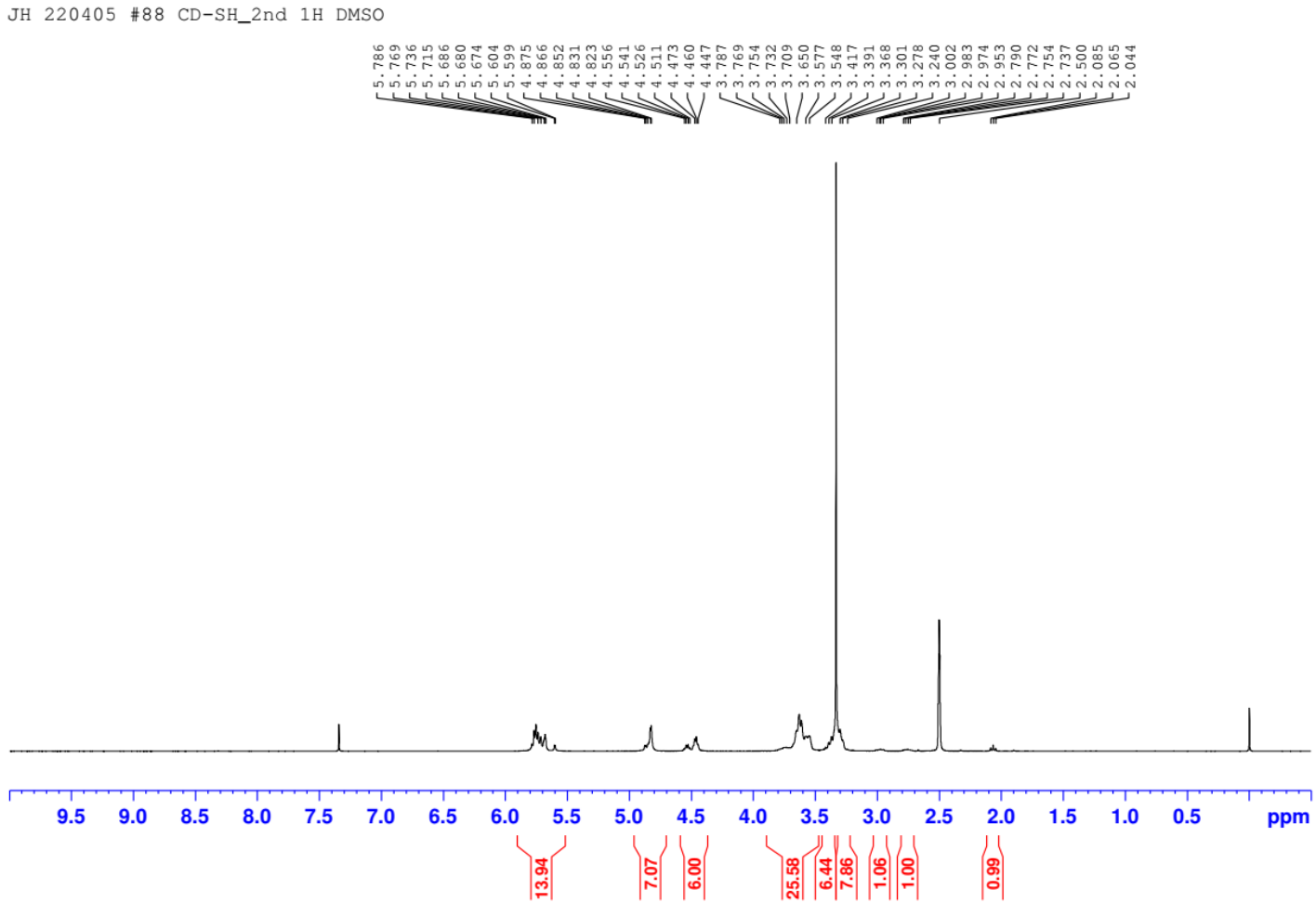
Appendices

List of ^{1}H NMR Spectra of Synthesized Compounds

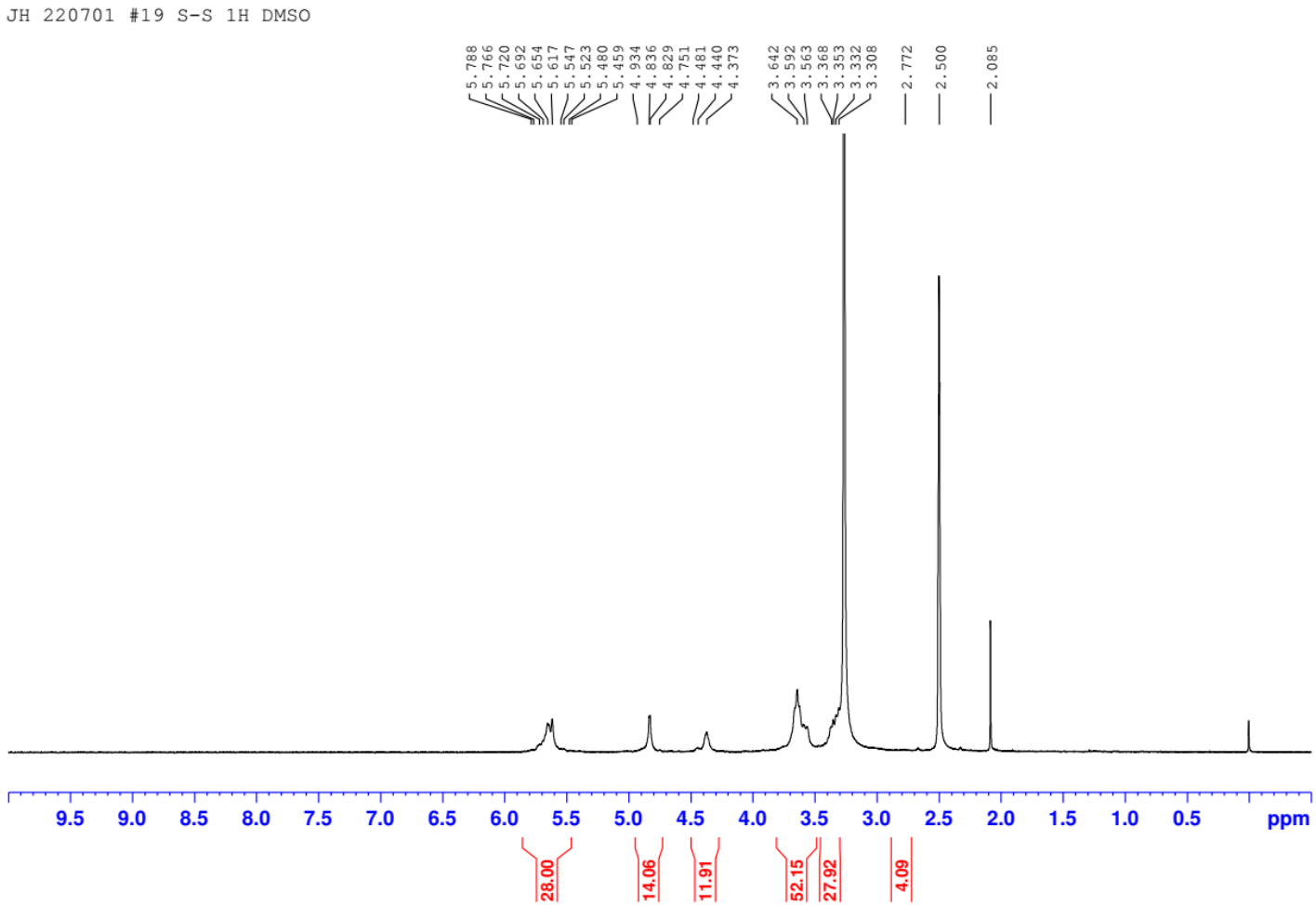
1. ^{1}H NMR spectrum (DMSO-d_6) of **1**
2. ^{1}H NMR spectrum (DMSO-d_6) of **2**
3. ^{1}H NMR spectrum (DMSO-d_6) of **3**
4. ^{1}H NMR spectrum (DMSO-d_6) of **4a**
5. ^{1}H NMR spectrum (DMSO-d_6) of **4b**
6. ^{1}H NMR spectrum (DMSO-d_6) of **5**
7. ^{1}H NMR spectrum ($CDCl_3$) of **6**
8. ^{1}H NMR spectrum ($CDCl_3$) of **7**
9. ^{1}H NMR spectrum (MeOD) of **8**
10. ^{1}H NMR spectrum ($CDCl_3$) of **9**
11. ^{1}H NMR spectrum ($CDCl_3$) of **10**
12. ^{1}H NMR spectrum ($CDCl_3$) of **11**
13. ^{1}H NMR spectrum ($CDCl_3$) of **13**



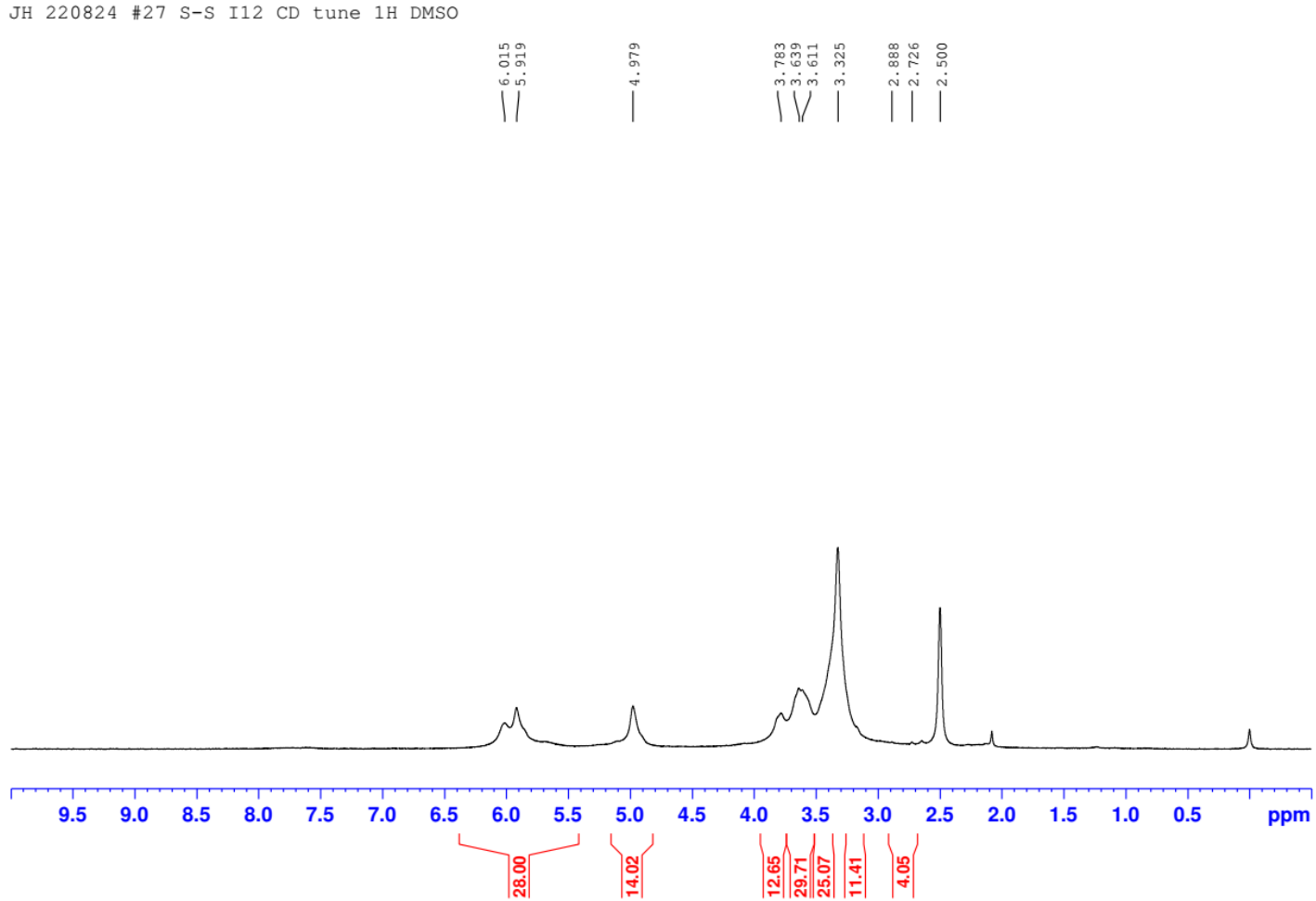
^{1}H NMR spectrum (DMSO-d_6) of **1**



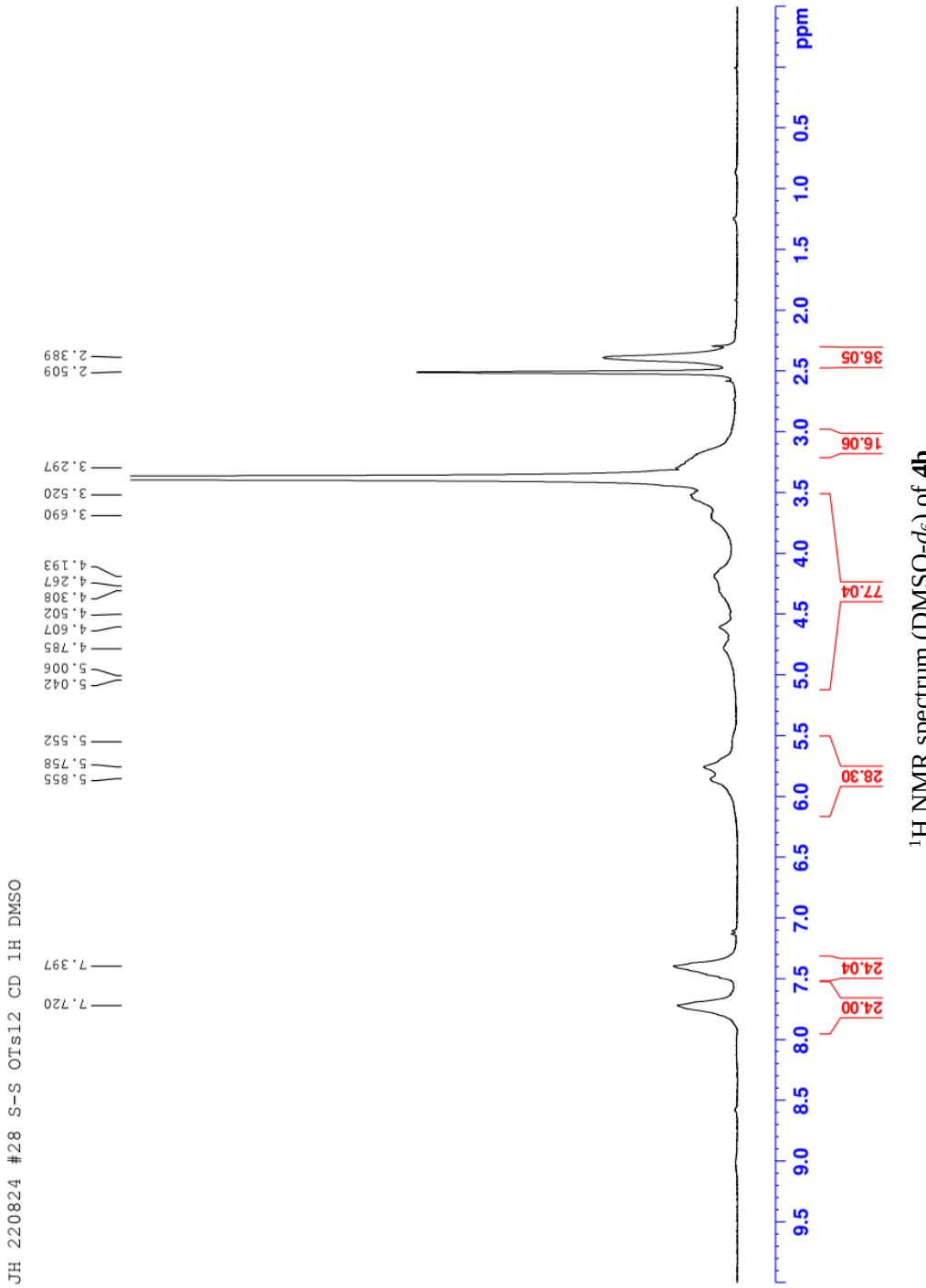
^{1}H NMR spectrum (DMSO-d_6) of **2**



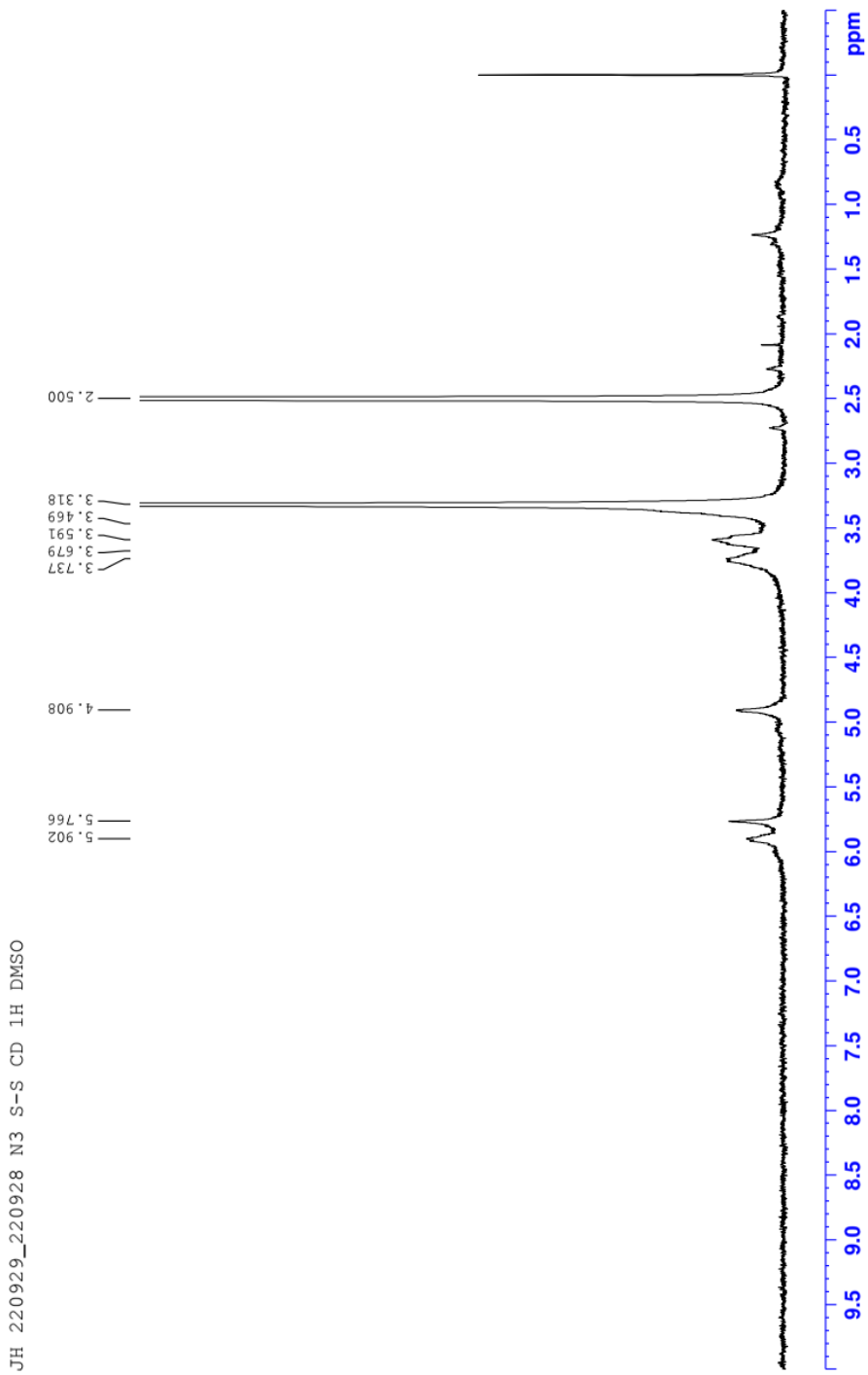
^{1}H NMR spectrum (DMSO-d_6) of **3**



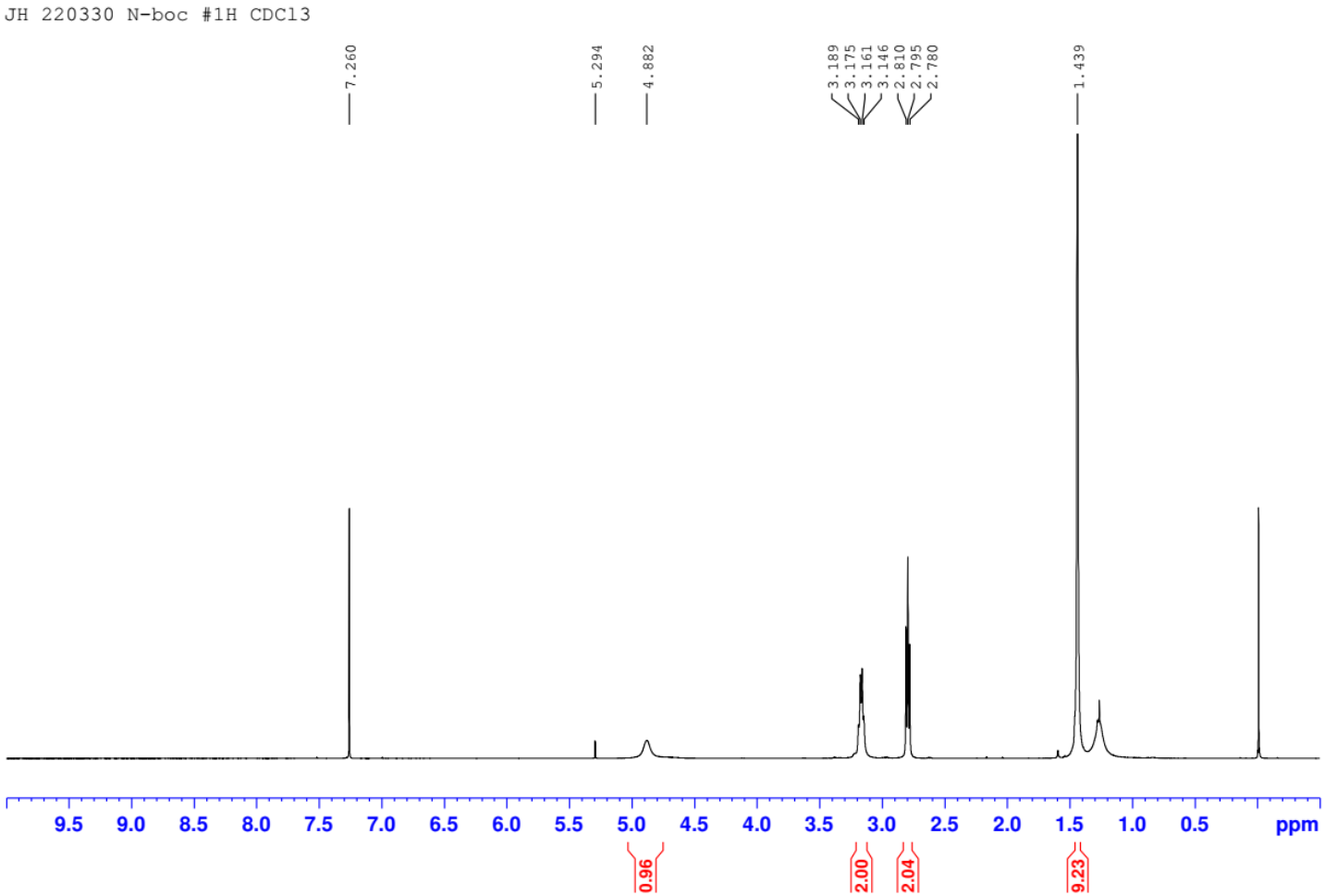
^{1}H NMR spectrum (DMSO-d_6) of **4a**



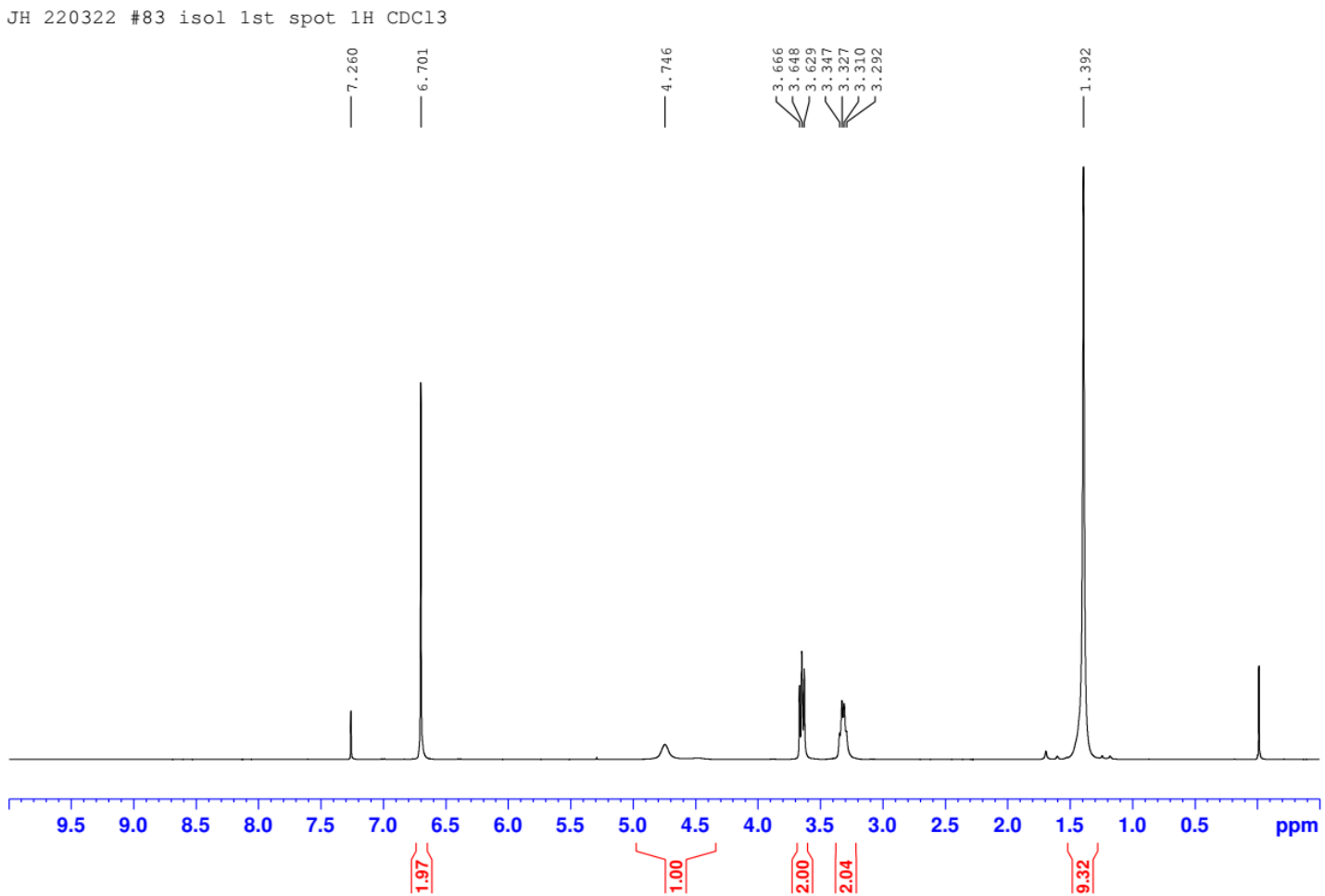
^{1}H NMR spectrum (DMSO-d_6) of **4b**



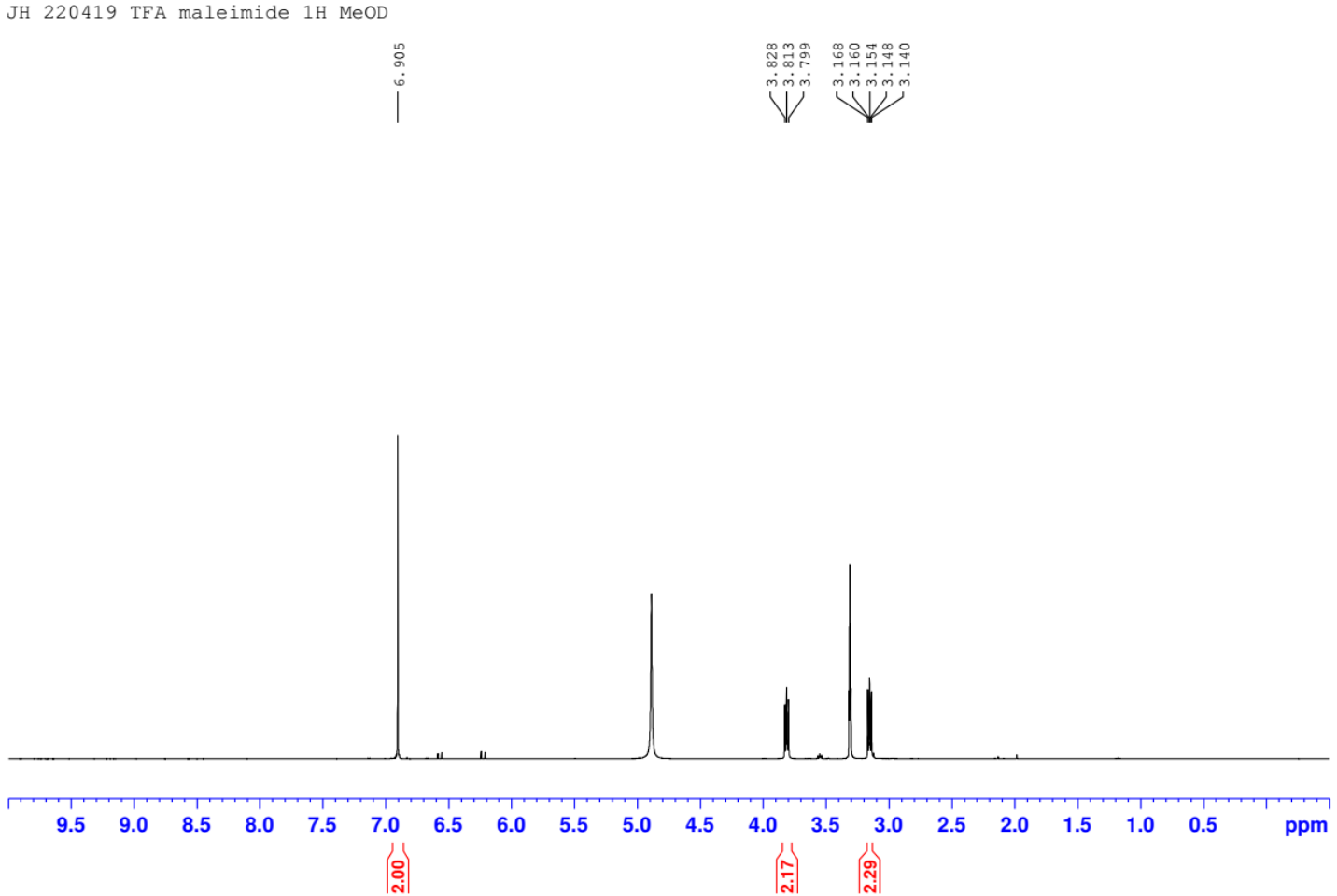
^{1}H NMR spectrum (DMSO-d_6) of **5**



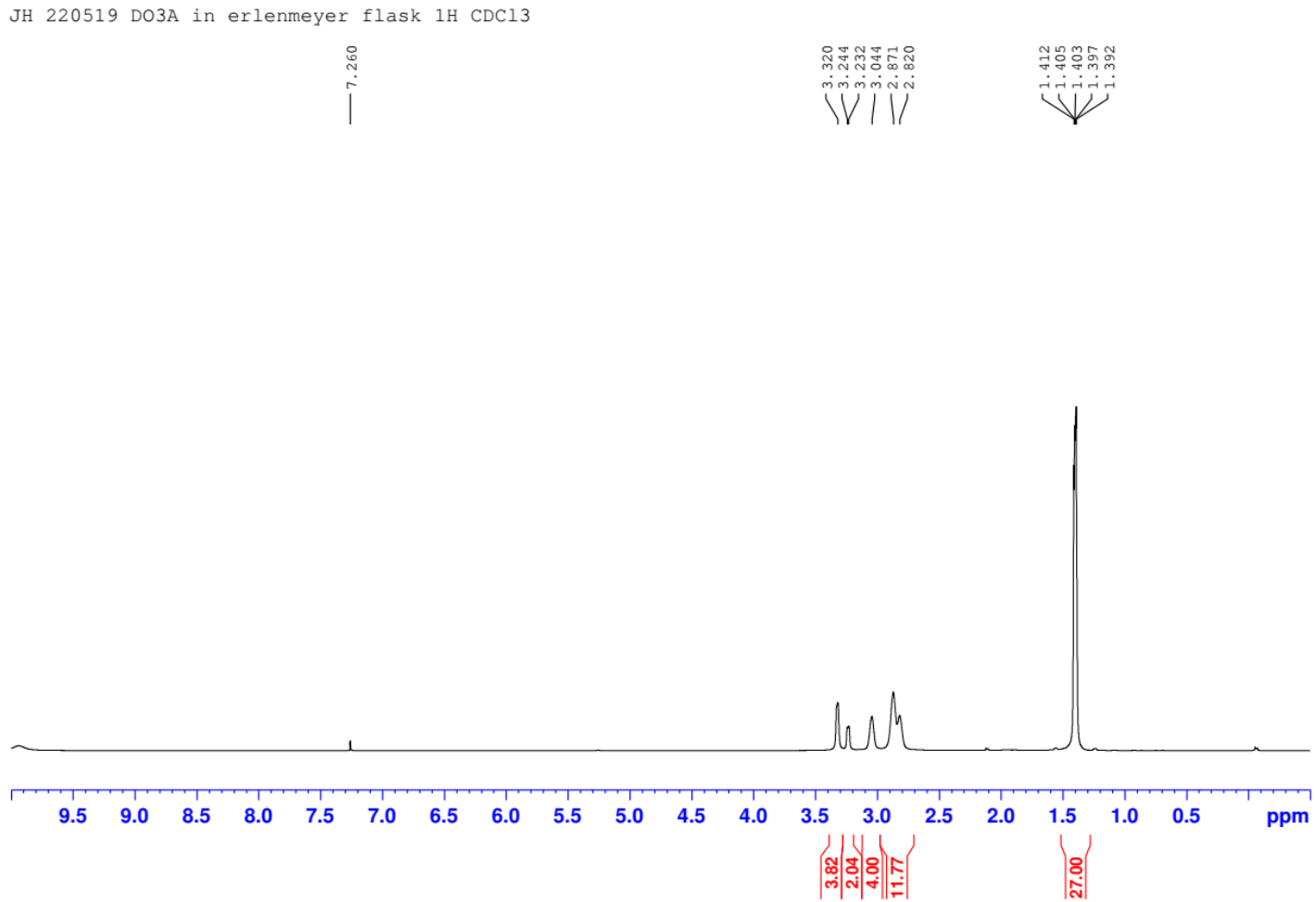
^{1}H NMR spectrum ($CDCl_6$) of **6**



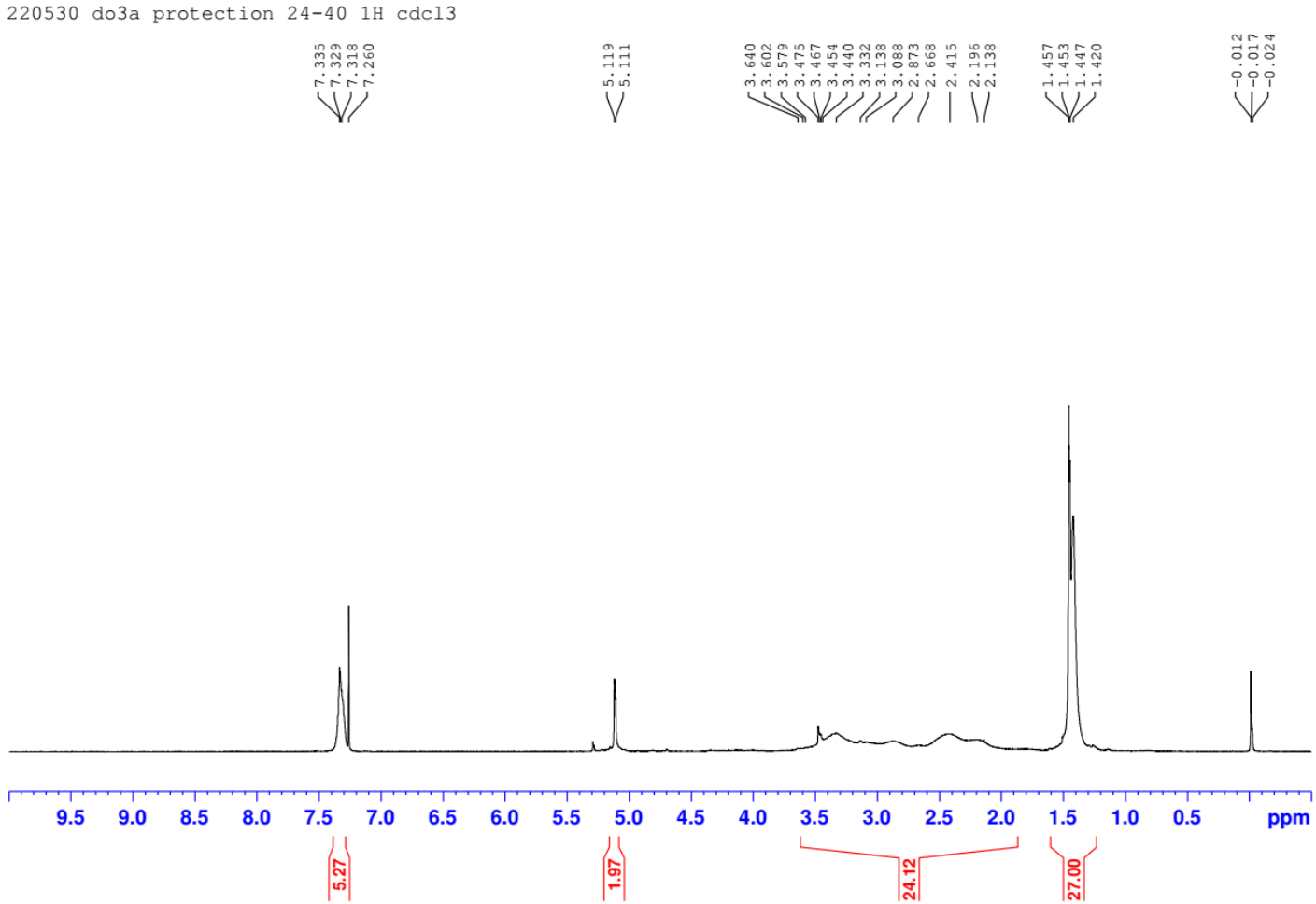
^{1}H NMR spectrum ($CDCl_6$) of **7**



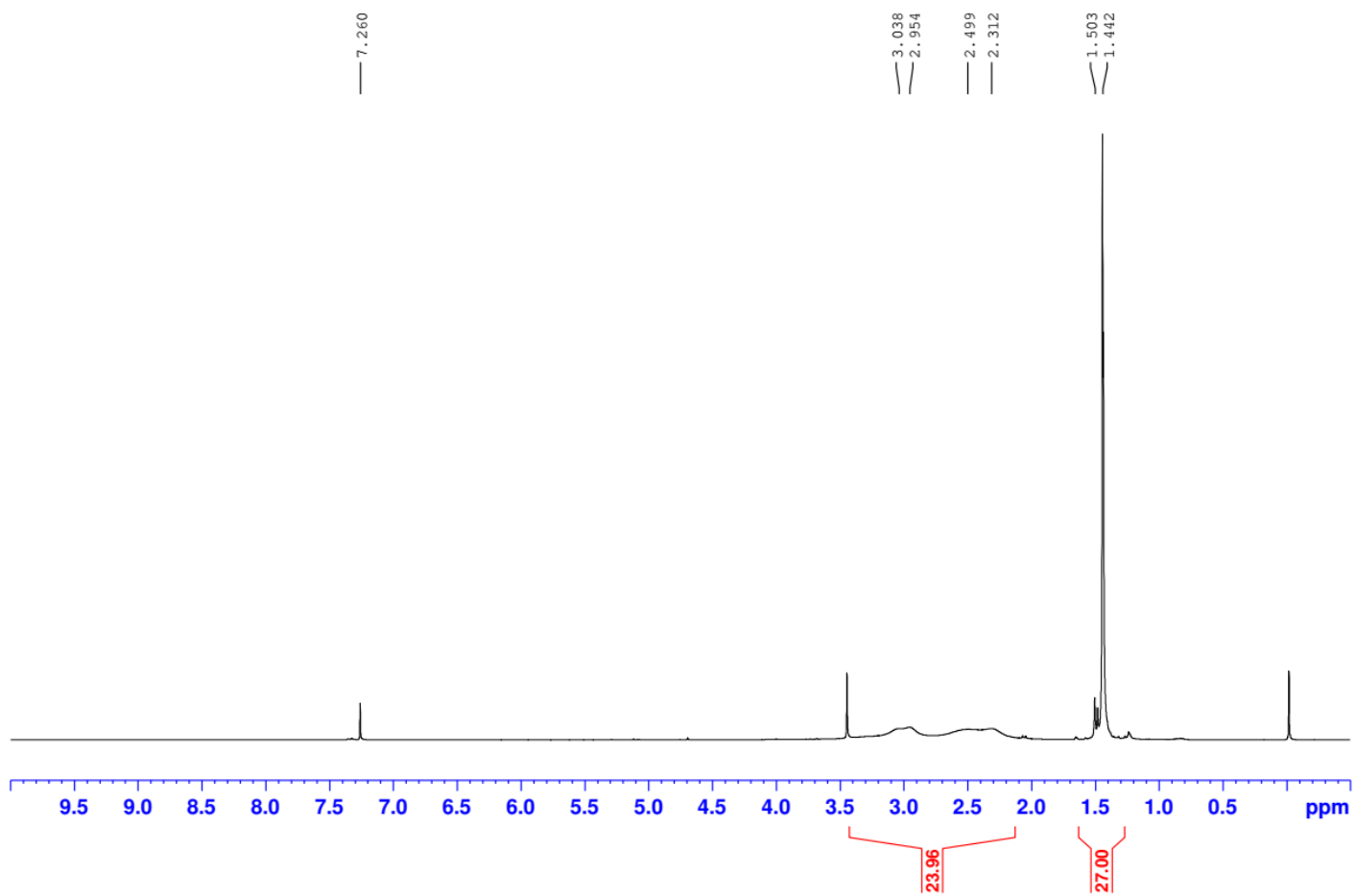
^{1}H NMR spectrum (MeOD) of **8**



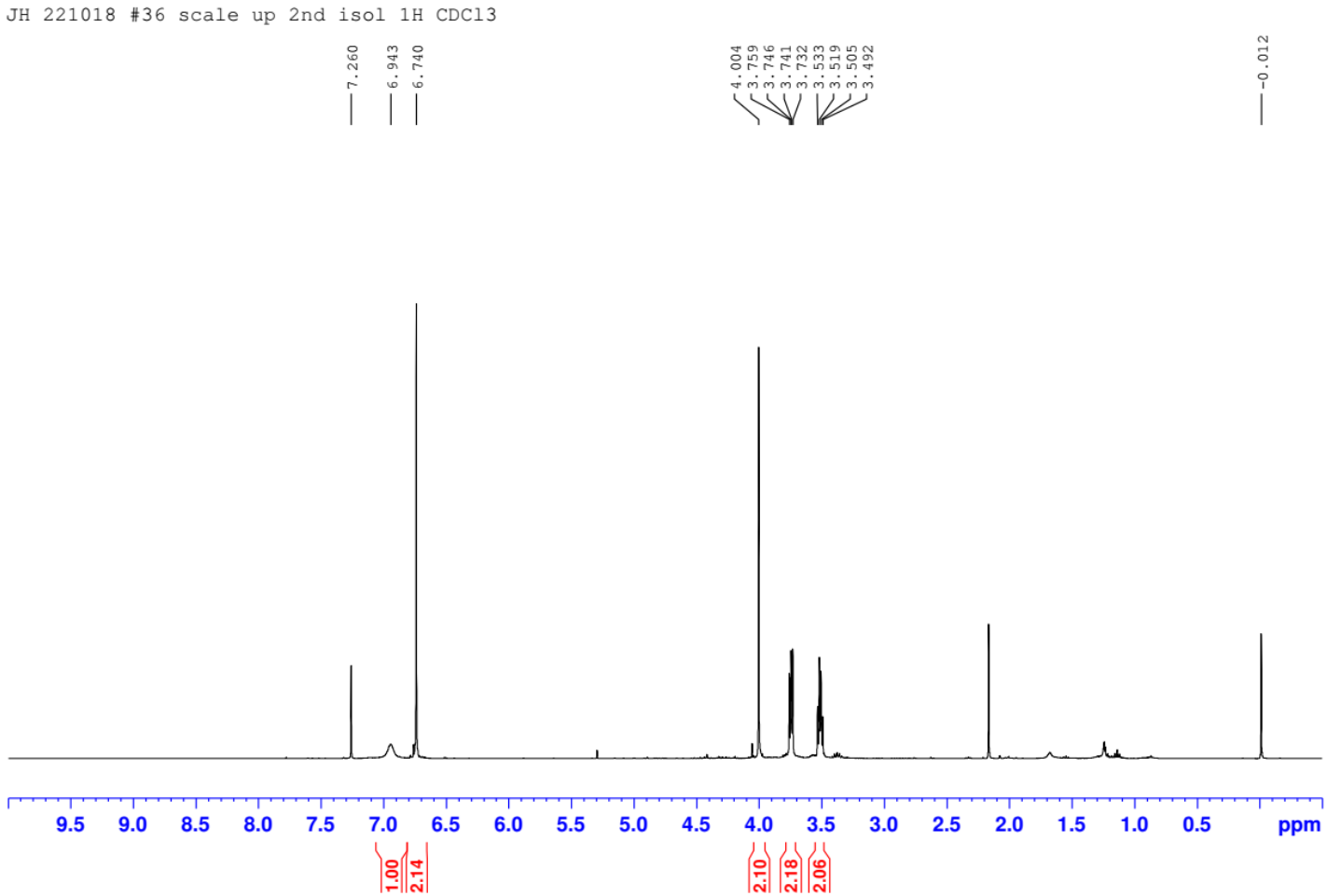
^{1}H NMR spectrum ($CDCl_6$) of **9**



^{1}H NMR spectrum ($CDCl_6$) of **10**



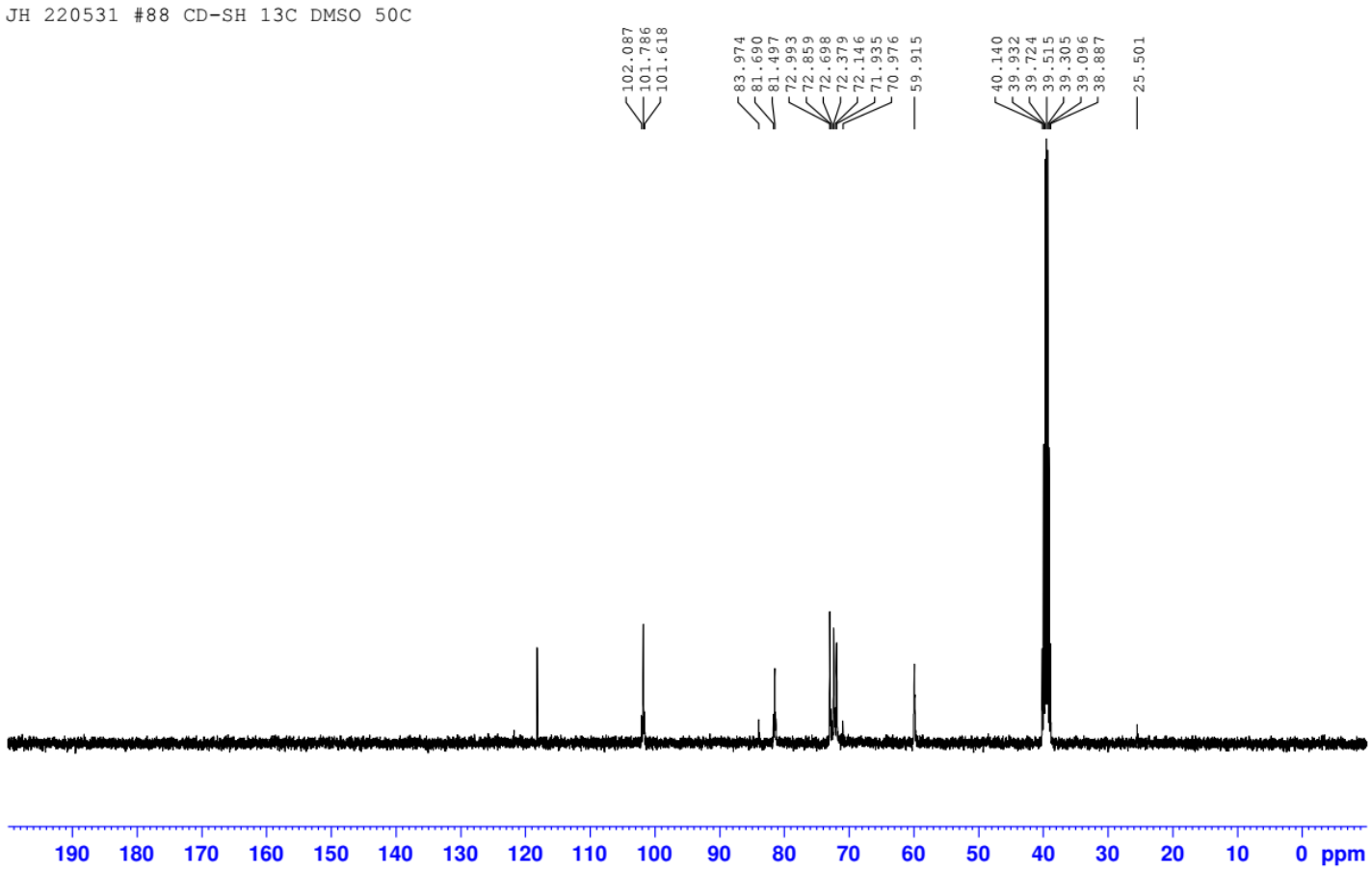
^{1}H NMR spectrum ($CDCl_6$) of **11**



^{1}H NMR spectrum ($CDCl_6$) of **13**

List of ^{13}C NMR Spectra of Synthesized Compounds

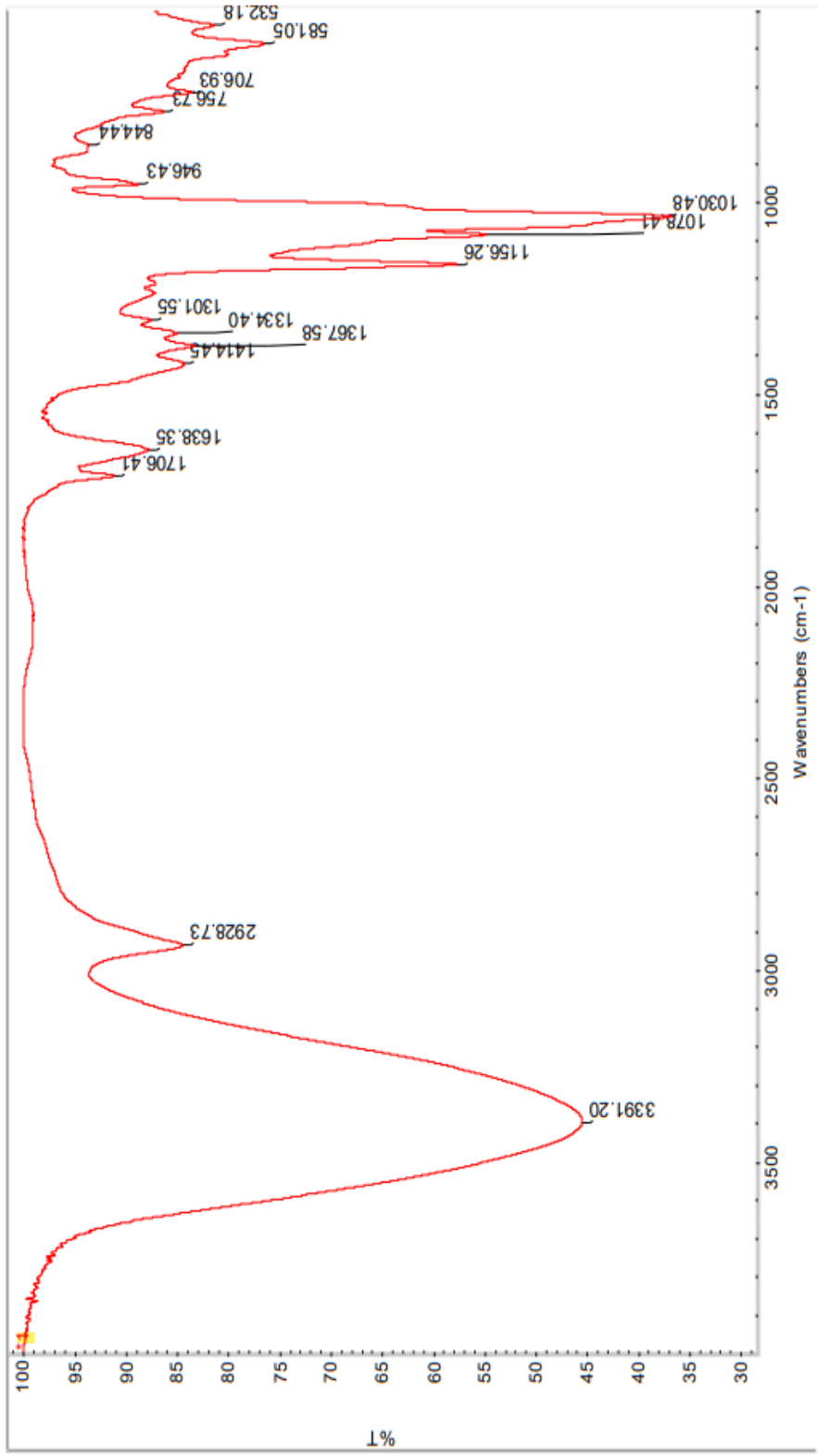
1. ^{13}C NMR spectrum (DMSO-d_6) of **2**



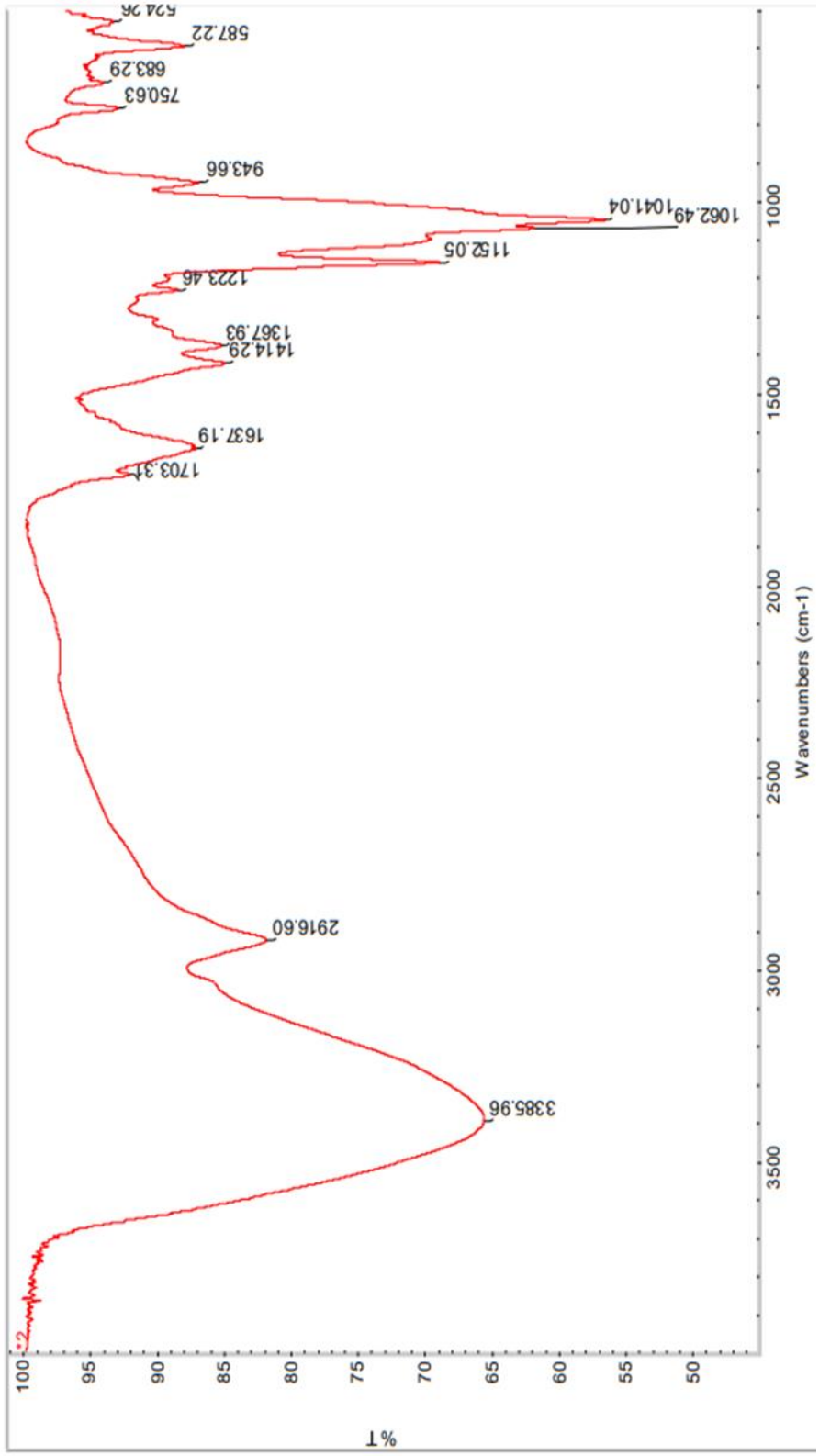
^{13}C NMR spectrum (DMSO-d_6) of **2**

List of FT-IR Spectra of Synthesized Compounds

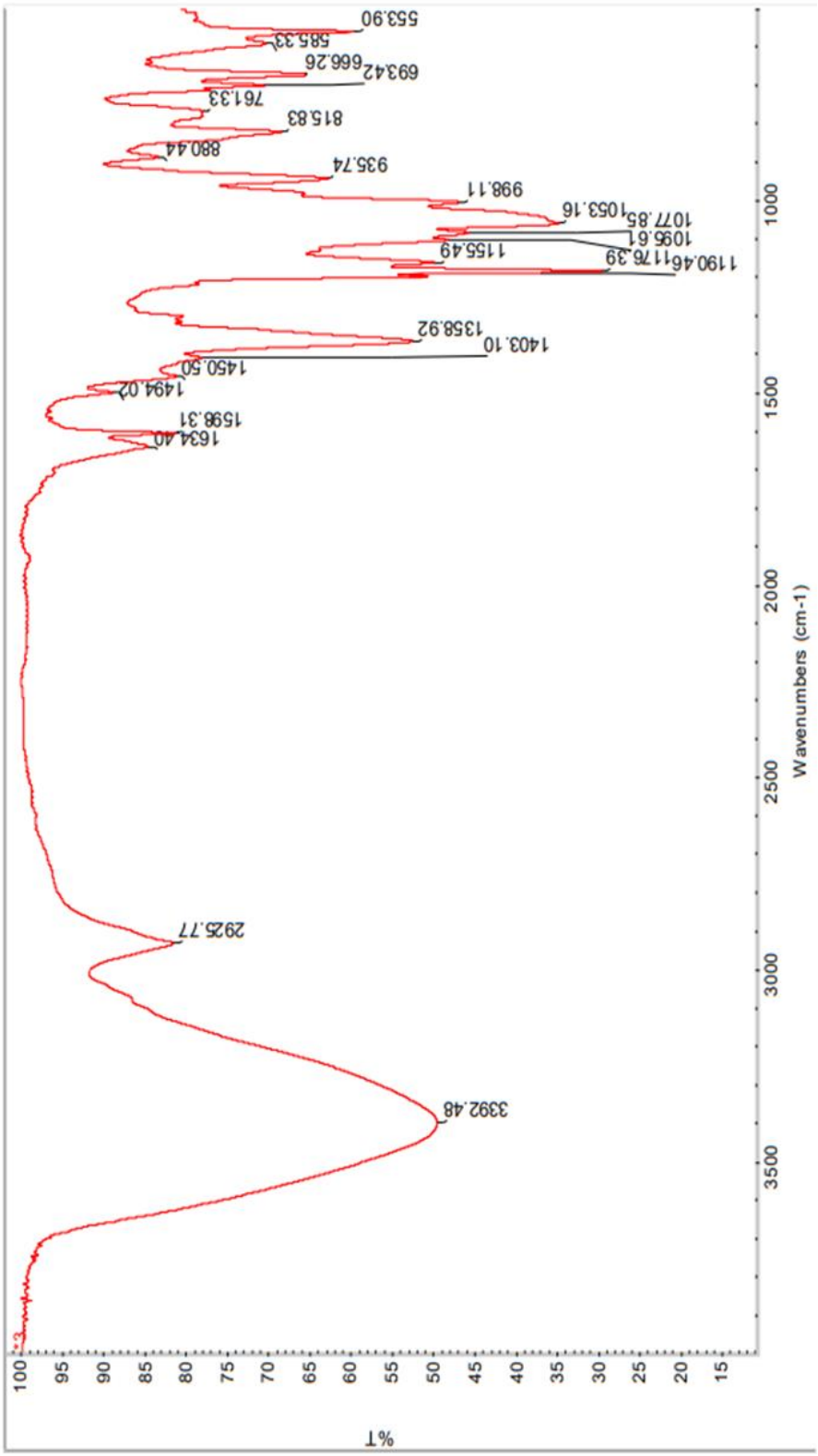
1. FT-IR spectrum of **3**
2. FT-IR spectrum of **4a**
3. FT-IR spectrum of **4b**



FT-IR spectrum of **3**



FT-IR spectrum of **4a**



FT-IR spectrum of **4b**

Abstract in Korean

자기공명영상(MRI)은 체내 조직의 암이나 염증 등의 진단에 사용되는 고해상도 3차원 이미징 기술이다. 병변의 정확한 진단을 위해 MRI 스캔 전에 조영제가 투여된다. 한편 자기공명혈관조영술(MRA)은 핵자기공명 원리로 조직 대신 혈관을 조영하는 기술로, 심혈관질환의 진단에 사용된다.

혈관 폐색이나 협착증 등의 정밀진단을 위해서는 가돌리늄 기반 조영제를 사용하여 조영효과를 높여야 한다. 그러나 MRA 조영제로는 현재 임상 승인된 물질이 없으며, 저분자 가돌리늄 킬레이트 구조인 MRI 조영제는 혈관 외로 빠르게 배출되므로 혈관 조영제로 사용하기에 적합하지 않다.

따라서 혈관을 오래 순환하는 MRA 조영제를 개발하기 위한 연구가 진행되고 있다. 특히 베타-사이클로덱스트린은 생체적합성이 높아 조영제의 전달체로 자주 활용되는데, 여기에 펙을 붙이면 전달체의 혈관 순환 시간을 늘릴 수 있다는 연구 결과가 보고됐다.

이 연구에서는 베타-사이클로덱스트린에 하나의 가돌리늄 킬레이트와 여섯개의 펙이 공유결합으로 연결된 새로운 혈관 조영제의 합성법을 고안했다. 이 합성법을 바탕으로, 베타-사이클로덱스트린에 서로 다른 두 가지 작용기를 달아 다기능성 베타-사이클로덱스트린 조영제의 전구물질을 합성할 수 있었다.

본 연구에서 개발된 합성법에 따른 최종 물질에는 소수성 분자를 로딩할 수 있게 된다. 로딩되는 분자 종류에 따라 자기공명혈관영상 진단과 치료를 병행할 수 있는 테라노스틱스 물질 또는 동맥경화 등의 특정 병변 위주로 이미징하는 표적 MRI 물질 등으로 활용될 수 있을

것이다.

주요어: 자기공명혈관영상, 혈관 조영제, 베타-사이클로덱스트린, 전합성, 선택적 합성, 다이설파이드 본드

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