Synthesis of Glucosamine Derivative with Double Caffeic Acid Moieties at *N*- and 6-*O*-Positions for Developments of Natural Based Materials

Kenta Yamatani,[1] Ryo Kawatani,[1] and Hiroharu Ajiro[1,2]*

[1]*Division of Materials Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, Takayama-cho 8916-5, Ikoma, Nara 630-0192, Japan*

[2]*Institute for Research Initiatives, Nara Institute of Science and Technology, Takayama-cho 8916-5, Ikoma, Nara 630-0192, Japan*

*Corresponding author Email address: ajiro@ms.naist.jp (H. Ajiro)
TEL +81-(0)743- 72-5507 FAX +81-(0)743- 72-5509

ABSTRACT

Glucosamine derivatives with double caffeic acid moieties were synthesized for developments of natural based materials. Caffeic acids were introduced to glucosamine derivatives through the synthesis roots as protection and de-protection reactions. Firstly, the silyl groups and *tert*-butoxycarbonyl groups were selected for the protection of each hydroxyl group and amino group. The designed glucosamine derivative showed reactivity of the double bonds, which was confirmed by UV spectra. Photoresponsivity was observed both in solution and heterogeneous suspension. The oligomerization was also confirmed in water suspension. The solubility and thermal stability were changed after the UV irradiation. This results show the potential of great progress a natural based material development.

Keywords: Glucosamine; Photosensitive; Caffeic acid

Introduction

Developments of materials using natural based polymers were imperative chemistry in order to contribute the solution of the depletion of natural energy source. Among them, chitosan derivatives were any one of keenly anticipated polymers for

natural based materials which were produced from crab and shrimp shells and many kind of biomaterials using them were reported, such as, nanofibers,[1,2] gels,[3] particles,[4-6] coagulated drug[7,8] and anti-oxidant agent.[9] However, almost chitosan derivatives show poor solubility in every solvents which behavior prevents the further developments of various applications.

On the other hand, the glucosamine is monomer of chitosan which is also produced at enough to industrial scale from natural sources. Solubilities of glucosamine derivatives were improved compared with chitosan derivatives because of low molecular compounds. Therefore, developments of natural based materials using glucosamine derivatives could be expanded for various applications, such as coagulated drug,[10] antitumor drug,[11] adjuvant drug[12] and gelator.[13] The main contents of these research were usually syntheses of glucosamine derivatives specifically focusing on selective introductions of functional compounds, probably syntheses of glucosamine derivatives are too difficult to achieve the evaluation of various applications easily. Therefore, the researches of glucosamine derivatives are not known so much due to longways synthesis roots derived from protection groups.[12-15] However, it is important to solve the problem of natural energy source, by the usages of bio-based materials which are developed by photo-polymerization

Similarly, caffeic acid is natural compound, which is one of cinnamic acid derivatives and also a photo-polymerizable monomer which is conjugated into the synthetic polymers in our previous work, thermal stabilities of poly(lactic acid) are improved about 100ºC by introduction of cinnamic acid derivatives.[16,17] Cinnamic acid derivatives were utilized for the creation of a bio-plastic[18,19] prepared by photo-polymerization.[20,21] In addition, caffeic acid include catechol unit in the chemical structure which are expected for adhesive materials[22-25] and anti-oxidant agent, although there are few data available concerning the modification of glucosamine with caffeic acid derivatives.[26]

In this study, we designed and synthesized the glucosamine derivatives bearing double caffeic acid moieties, which are selectively modified at the *N*- and 6-*O*- positions for developments of natural based materials, in order to produce a series of multiple introductions of polyphenol moieties into the monosaccharide. Then, the synthesized glucosamine derivatives were oligomerized by UV irradiation. It is noteworthy that the the compound includes the multiple interactive moieties and the regulated conformation. The multiple interaction moieties, such as hydroxyl, amide, polyphenol, double bond, and aromatic groups, would allow the combination of various molecules, as well as selective interaction due to the regulated conformation. In addition, the obtained

oligomers were analyzed by FT-IR, UV spectrometer and MALDI-TOF MS to confirm the dimerization reaction of double bond derived from caffeic acids.

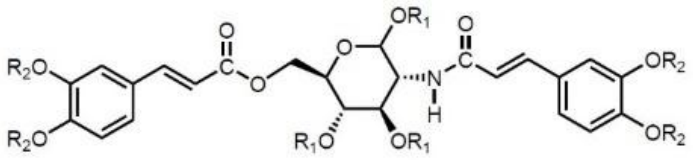


Figure 1. Chemical structure of the glucosamine derivative with symmetric caffeic acid moieties at the *N*- and 6-*O*-positions in this study.

Experimental sections

Materials and analytical apparatus

Sodium methoxide (CH_3ONa), di-*tert*-butyl dicarbonate, Caffeic acid, *N*,*N*-Diisopropylethylamine (DIPEA), Benzyl bromide (BnBr), Sodium hydride (NaH), triethylamine, Glucosamine, Tetrabutylammonium iodine (TBAI), Butyl(chloro)dimethylsilane), Dimethyltin dichloride (Me_2SnCl_2), trifluoroacetic acid were purchased from Tokyo Chemical Industry Co. Ltd (Japan). *tert*-Butyl(chloro)diphenylsilane (TBDPSCl) and imidazole were purchased from Aldrich Co. Ltd (USA).

^{1}H NMR was measured with JEOL JNM-ECM 400 (JEOL Ltd. Japan). FT-IR spectra were measured by the Spectrum 100 FT-IR and IRAffinity-1S ATR (Shimadzu Corporation, Japan). ESI-MASS was measured by JEOL AccuTOF, JMS-T100LC (JEOL Ltd., Japan). DART-MASS was measured by JEOL JMS-Q1000TD (JEOL Ltd., Japan). MALDI-TOF-MS was measured by Bruker Autoflex II (Bruker Daltonics K.K., Japan). UV spectra were monitored by UV-2600 (Shimadzu Corporation, Japan). TGA was analyzed by TGA-50 (Shimadzu Corporation Japan). DSC was analyzed by DSC-60 Plus and TAC/L system (Shimadzu Corporation, Japan). UV light was irradiated by SUPERCURE-352S (SAN-EI Electric Co. Ltd., Japan).

Syntheses

Synthesis of 2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**2**)

To a solution of D-(+)-Glucosamine hydrochloride (30.8 g, 143 mmol) and CH_3ONa (8.53 g, 158 mmol) in CH_3OH (400 mL) was stirred at room temperature for 1h in 1L egg plant shaped flask. After dissolving, di-*tert*-butyl dicarbonate (34 mL, 171 mmol) and triethylamine (20 mL, 143 mmol) were added to the solution. The mixture was

stirred at room temperature for 6 hours. The solvent was concentrated and the residue was recrystallized to give compound **2** as white solid (35.5 g, 127 mmol, 89%).
^{1}H NMR 400 MHz, D_2O): δ (ppm) 5.17 (d, 0.75H), 4.66 (d, 0.25H), 3.98-3.25 (m, 6H), 1.42 (s, 9H); R_f : 0.60 (CH_2Cl_2 : CH_3OH = 7 : 3 (v:v)).

Synthesis of 6-*O*-*tert*-Butyldiphenylsilyl-2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**3**)
To a solution of 2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**2**) (21.5 g, 77.0 mmol) and imidazole (4.18 g, 61.4 mmol) in dry DMF (250 mL) was stirred at room temperature for 1h under the nitrogen atmosphere in 500 mL 2-neck eggplant shaped flask. After dissolving, TBDPSCl (14 mL, 54.0 mmol) in dry THF (50 mL) were added to the solution in the ice bath. The mixture was stirred for 18 hours. After completion of the reaction, the residual TBDPSCl was quenched with H_2O. The solution was extracted with hexane, ethyl acetate and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate (1:1) to give compound **3** as colorless crystal (25.9 g, 50.0 mmol, 81%).
^{1}H NMR 400 MHz ($CDCl_3$): δ (ppm) 7.70-7.64 (m, 4H), 7.47-7.36 (m, 6H), 5.19 (s, 1H), 3.91-3.84 (m, 2H), 3.76-3.62 (m, 2H), 3.08 (s, 1H), 2.96 (s, 1H), 1.45 (s, 9H), 1.05 (s, 9H) ; R_f : 0.25 (Hexane : EtOAc = 1 : 1 (v:v)).

Synthesis of 1,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*N*-*tert*-butoxy carbonyl-*D*-glucosamine (**4**)
To a solution of 6-*O*-*tert*-Butyldiphenylsilyl-2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**3**) (6.60 g, 12.8 mmol) and NaH 60% in oil (1.69 g, 42.1 mmol) in dry DMF (25 mL) was stirred at room temperature for 1h under the nitrogen atmosphere in 300 mL 2-neck eggplant shaped flask. TBAI (0.480 g, 1.28 mmol) and BnBr (5.0 mL, 42.1 mmol) were added to the solution in the ice bath. The mixture was stirred for 18 hours. After completion of the reaction, the residual BnBr was quenched with H_2O. The solution was extracted with hexane, ethyl acetate and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate (9:1) to give compound **4** as yellow oil (6.90 g, 8.76 mmol, 69%).
^{1}H NMR 400 MHz (DMSO-d_6): δ (ppm) 7.73-7.62 (m, 3H), 7.47-7.10 (m, 22H), 4.84 (m, 2H), 4.74-4.54 (m, 3H), 4.45 (d, *J*=8.0 Hz, 1H), 3.96-3.87 (m, 3H), 3.68-3.61 (m, 3H), 3.53 (m, 1H), 1.41 (s, 9H), 1.00 (s, 9H); R_f : 0.38 (Hexane : EtOAc = 4 : 1 (v:v)).

Synthesis of 1,3,4-Tri-*O*-benzyl-2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**5**) To a solution of

1,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**4**) (6.90 g, 8.76 mmol) and 1M TBAF / THF (17.5 mL, 17.5 mmol) in dry THF (8.7 mL) was stirred at room temperature for 24 hours under the nitrogen atmosphere in 300 mL 2-neck eggplant shaped flask. After completion of the reaction, the solution was extracted with ethyl acetate and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate (2:1) to give compound **5** as colorless crystal (1.88 g, 3.42 mmol, 39%).
^{1}H NMR 400 MHz ($CDCl_3$): δ (ppm) 7.38-7.27 (m, 15H), 4.89-4.47 (m, 7H), 3.96-3.32 (m, 6H), 1.44 (s, 9H); R_f : 0.38 (Hexane : EtOAc = 1 : 1 (v:v)).

Synthesis of 1,3,4-Tri-*O*-benzyl-*D*-glucosamine (**6**)
To a solution of 1,3,4-Tri-*O*-benzyl-2-*N*-*tert*-butoxycarbonyl-*D*-glucosamine (**5**) (1.88 g, 3.42 mmol) and trifluoroacetic acid (2.60 mL, 34.0 mmol) in CH_2Cl_2 (7.0 mL) was stirred at room temperature for 1 hours in 200 mL eggplant shaped flask. After completion of the reaction, the residual trifluoroacetic acid was quenched with $NaHCO_3$ aq. The solution was extracted with CH_2Cl_2 and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate / TEA (1:1:0.2) to give compound **6** as colorless crystal (1.45 g, 3.23 mmol, 94%).
^{1}H NMR 500 MHz ($CDCl_3$): δ (ppm) 7.37-7.27 (m, 15H), 4.97 (d, *J*=10.5 Hz, 1H), 4.86 (t, *J*=11.5 Hz, 2H), 4.73-4.61 (m, 3H), 4.37 (d, *J*=8.0 Hz, 1H), 3.89 (d, *J*=11.5 Hz, 1H), 3.75 (d, *J*=12.0 Hz, 1H), 3.63 (t, *J*=9.5 Hz, 1H), 3.47 (t, *J*=9.25 Hz, 1H), 3.42-3.39 (m, 1H), 2.90 (dd, *J*=8.0, 10.0 Hz, 1H); R_f : 0.13 (Hexane : EtOAc : NEt_3 = 1 : 2 : 0.1 (v:v:v)).

Synthesis of (*E*)-3-(3,4-bis((*tert*-Butyldimethylsilyl)oxy)caffeic acid (**8**)
To a solution of caffeic acid (1.51 g, 8.38 mmol) and imidazole (2.55 mL, 37.4 mmol) and TBSCl (*tert*-butyl(chloro)dimethylsilane) (5.66 g, 37.4 mmol) in dry DMF (16.0 mL) was stirred at room temperature for 20 hours under the nitrogen atmosphere in 300 mL 2-neck eggplant shaped flask. After completion of the reaction, the reaction was quenched
with H_2O. The solution was extracted with ethyl acetate and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate (1:1) and vacuum at 100 ^oC to give compound **8** as pale yellow crystal (3.05 g, 7.47 mmol, 90%).
^{1}H NMR 400 MHz ($CDCl_3$): δ (ppm) 7.67 (d, *J*=16.0 Hz, 1H), 7.05 (m, 1H), 6.83 (d,

J=8.8 Hz, 2H), 6.25 (d, *J*=16.0 Hz, 1H), 1.00 (s, 18H), 0.22 (s, 12H); R_f : 0.45 (Hexane : EtOAc = 2 : 1 (v:v)).

Synthesis of 2,6-Di-*O*,*N*-(*E*)-3,4-bis(*tert*-butyldimethylsilyloxy)caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**10**)

To a solution of (*E*)-3-(3,4-bis((*tert*-utyldimethylsilyl)oxy)caffeic acid (**8**) (0.767 g, 1.88 mmol) and 1M $SOCl_2$ / CH_2Cl_2 (7.5 mL, 7.50 mmol) in dry CH_2Cl_2 (1.8 mL) was stirred and reflux for 15 hours under the nitrogen atmosphere in 100 mL 2-neck eggplant shaped flask. After completion of the reaction, the solution was concentrated to obtain acid chloride (**9**).To a solution of 1,3,4-Tri-*O*-benzyl-*D*-glucosamine (**6**) (0.352 g, 0.783 mmol) and Me_2SnCl_2 (0.0188g, 0.0783 mmol) and DIPEA (*N*,*N*-Diisopropylethylamine) (0.55 mL, 3.13 mmol) in dry THF (1.0 mL) was stirred under the nitrogen atmosphere in 50 mL 2-neck eggplant shaped flask. Acid chloride (**9**) in dry THF (1.5 mL) was added to the solution at room temperature and the solution was stirred for 24 hours. After completion of the reaction, the solution was extracted with ethyl acetate and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with hexane / ethyl acetate (1:1) to give compound **10** as pale yellow crystal (0.535 g, 4.35 mmol, 55%).

^{1}H NMR 400 MHz ($CDCl_3$): δ (ppm) 7.59 (d, *J*=16.0 Hz, 1H), 7.47 (d, *J*=15.6 Hz, 1H), 7.37-7.27 (m, 15H), 7.02-6.96 (m, 4H), 6.82 (d, *J*=8.0 Hz, 2H), 6.26 (d, *J*=16.0 Hz, 1H), 5.93 (d, *J*=15.6 Hz, 1H), 5.38 (d, *J*=10.0 Hz, 1H), 4.97-4.59 (m, 6H), 4.51-4.45 (m, 3H), 4.36 (m, 1H), 3.97 (m 1H), 3.85-3.74 (m, 2H), 1.00 (s, 36H), 0.22 (s, 24H).

ESI-MS = $[M+Na]^+$=1253.69; FT-IR: 2929, 2858, 1712, 1508, 1286, 1251, 1165, 1124, 904, 837, 779, 694 cm^{-1}; R_f : 0.23 (Hexane : EtOAc = 4 : 1 (v:v)).

Synthesis of 2,6-Di-*O*,*N*-(*E*)-caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**11**)

To a solution of 2,6-Di-*O*,*N*-(*E*)-3,4-bis(*tert*-butyldimethylsilyloxy)caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**10**) (0.807 g, 0.656 mmol) and TBAF (3.2 mL, 3.15 mmol) in dry THF (0.60 mL) was stirred at room temperature for 3 hours under the nitrogen atmosphere in 50 mL 2-neck eggplant shaped flask. The solution was extracted with CH_2Cl_2 and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography with CH_2Cl_2 / CH_3OH (9:1) to give compound **11** as brown crystal (0.406 g, 0.525 mmol, 80%).

^{1}H NMR 400 MHz (DMSO-d_6): δ (ppm) 9.64 (s, 1H), 9.41 (s, 1H), 9.18 (s, 2H), 8.31 (d, *J*=9.2 Hz, 1H), 7.51 (s, 1H), 7.40-7.19 (m, 15H), 7.07-6.85 (m, 4H), 6.74 (d, *J*=8.0 Hz,

2H), 6.53 (d, *J*=15.6 Hz, 1H), 6.35 (d, *J*=15.6 Hz, 1H), 5.28 (s, 1H), 4.83-4.57 (m, 6H), 4.51 (m, 1H), 4.38 (m, 1H), 4.30-4.15 (m, 2H), 3.92-3.81 (m, 2H), 3.63 (t, 1H).
ESI-MS = $[M+Na]^+$=796.27; FT-IR: 3294, 2954, 2927, 1693, 1597, 1514, 1273, 1157, 1112, 1016, 977, 696 cm^{-1}; R_f : 0.53 (CH_2Cl_2 : CH_3OH = 9 : 1 (v:v)).

Photo reactivity

Photo reactivity of 2,6-Di-*O*,*N*-(*E*)-3,4-bis(*tert*-butyldimethylsilyloxy)caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**10**) in CH_3OH

2,6-Di-*O*,*N*-(*E*)-3,4-bis(*tert*-butyldimethylsilyloxy)caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**10**) was prepared so as to be 45 μM CH_3OH in quartz cell. The solution was treated with nitrogen bubbling. While stirring, the solution was irradiated by UV light using UV irradiation machine (Hg lump, λ > 280 nm, 56 mW / cm^2) in the iced bath under the nitrogen atmosphere to occur the [2+2] photocyclization. The irradiation condition
(irradiated area: 3 cm^2, distance to the source of UV light: 5 cm) was fixed. The UV-Vis spectra of solution was measured at 0, 10, 20, 30 s, 1, 2, 3, 5, 10, 20, 30, 60, 120, 180 and 240 min.

Photo reactivity of 2,6-Di-*O*,*N*-(*E*)-caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**11**) in CH_3OH

2,6-Di-*O*,*N*-(*E*)-caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**11**) was prepared so as to be 65 μM CH_3OH in quartz cell. The solution was treated with nitrogen bubbling. While stirring, the solution was irradiated with UV light using UV irradiation machine (Hg lump, λ > 280 nm, 56 mW / cm^2) in the iced bath under the nitrogen atmosphere to occur the [2+2] photocyclization. The irradiation condition (irradiated area: 3 cm^2, distance to the source
of UV light: 5 cm) was fixed. The solution of UV-Vis spectra were measured at 0, 10, 20, 30 s, 1, 2, 3, 5, 10, 20, 30, 60, 120, 180 and 240 min.

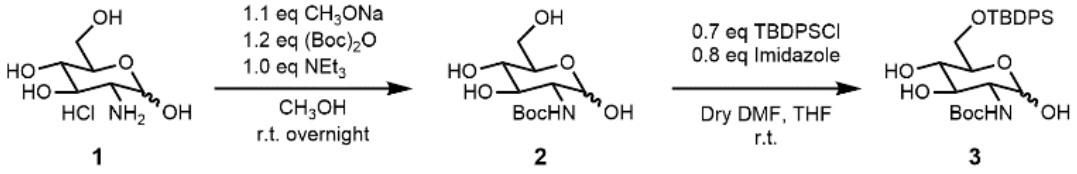
Photo reactivity of 2,6-Di-*O*,*N*-(*E*)-caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**11**) in H_2O

2,6-Di-*O*,*N*-(*E*)-caffeoyl-1,3,4-tri-*O*-benzyl-*D*-glucosamine (**11**) was prepared so as to be 1.0 mM aqueous solution in screw bial. The solution was treated with nitrogen bubbling. While stirring, the solution was irradiated with UV light using UV irradiation machine (Hg lump, λ > 280 nm, 56 mW / cm^2) in the iced bath under the nitrogen

atmosphere to occur the [2+2] photocyclization. The irradiation condition (distance to the source of UV light: 5 cm) was fixed. The solution was irradiated by UV light for 4 hours. After irradiation, the mixture was washed with CH_3OH and acetone. The soluble part (0.009 g, 12 %) and insoluble part (0.068 g, 88 %) were collected respectively.

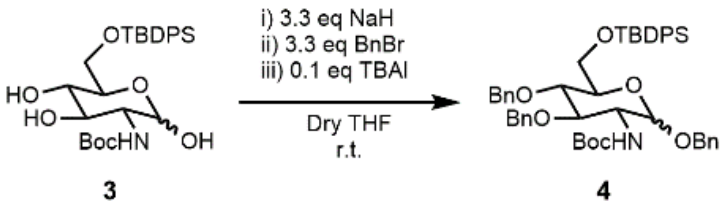
Results and Discussion

Initially, we had planned to synthesize the target compound directly without protecting groups, however, it was very difficult to confirm whether the product was target compound or not by thin layer chromatography (TLC). So, the protective groups were utilized for the *N*-position and 6-*O*-position as shown in Scheme 1.



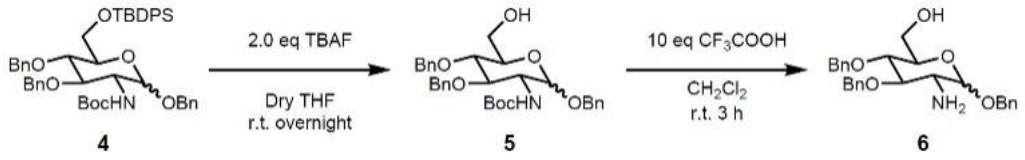
Scheme 1. Synthesis of compound **3**.

Considering the introduction of functional groups at *N*- and 6-*O*- positions with different reactivities from the other positions, it was necessary to protect the rest of 2-*O*-, 3-*O*-, and 4-*O*- hydroxyl positions at first. So, the careful selection of protecting groups for 2-*O*-, 3-*O*-, and 4-*O*- hydroxyl positions was required to keep the other protecting groups stable at *N*- and 6-hydroxyl positions under their deprotection reaction at 2-*O*-, 3-*O*-, and 4-*O*- hydroxyl positions. We selected the Boc group which could be removed under acidic conditions for the protection of the amino group at the *N*-position, and we selected the silyl group for the selective protection of the primary alcohol at the 6-*O*-position. Next, the protection of the three hydroxyl groups was attempted as shown in Scheme 2.



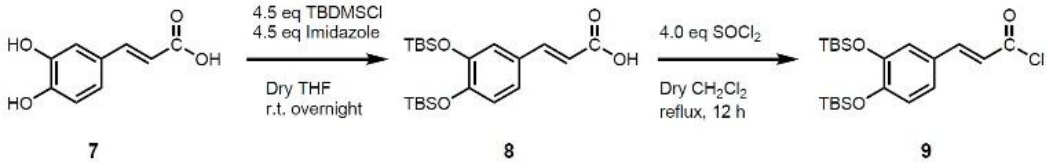
Scheme 2. Synthesis of compound **4**.

Actually, the compound **4** could not be obtained under some conditions, although the reason was unclear. For example, the proton at the *N*-position also reacted under an excessive amount of NaH (6 eq). No reaction was confirmed under the milder alkaline condition, such as the triethylamine and the potassium carbonate at room temperature. Furthermore, some reactions could not proceed because of the poor reactivity after protections and the failure of the selective deprotections. Thus, we pursue the different pathway to synthesize the target compound. That was the reason why we synthesized **6** through the synthesis roots in Scheme 2 and the following Scheme 3.



Scheme 3. Synthesis of compound **6**.

Using compound **4**, selective deprotection of TBDPS was achieved in THF at room temperature. Successively, the deprotection of the Boc group proceeded under an acidic condition with 10eq. of the trifluoro acetic acid at room temperature. Although compound **6** is a reported compound elsewhere,[12] it is noteworthy that the total steps of the synthesis decreased with the higher yield. In order to conjugate with compound **6**, the caffeic acid derivative **9** was synthesized by the following reaction as shown in Scheme 4, using the *N*-position and 6-*O*-position of compound **6**.

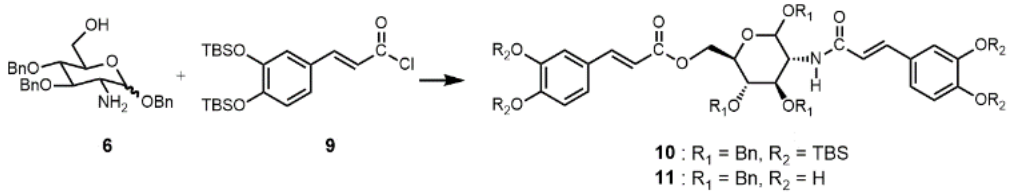


Scheme 4. Synthesis of compound **9**.

Two aromatic hydroxyl groups were protected by the *t*-butyldimethylsilyl group, then the tionyl chloride was treated with compound **8**, in order to react with the amide or the primary alcohol of compound **6**. The obtained compound **9** was directly combined with the compound **6** as shown in Scheme 5.

The condensation reaction of glucosamine and caffeic acid was depicted in Scheme 5.[27]

DIPEA was employed for trapping the acid which was generated in the reaction, and Me_2SnCl_2 was utilized for the acceleration of the reaction as an acid. Then, compound **10** was successfully obtained. Generally, the protection of the aromatic hydroxyl groups was easier than aliphatic hydroxyl groups when TBDPS groups was used. Then the deprotection reaction of aromatic hydroxyl groups were also proceeded effectively, resulting in compound **11**. The catechol groups were confirmed by ^{1}H NMR in DMSO-d_6, as well as the confirmation of hydroxyl groups by FT-IR spectrometer at around 3000 cm^{-1}.



Scheme 5. Synthesis of **10** and **11**.

As a result, the monomer of the photo-polymerization was successfully synthesized, using the glucosamine as a core moiety, accompanied by double caffeic acid moieties. During

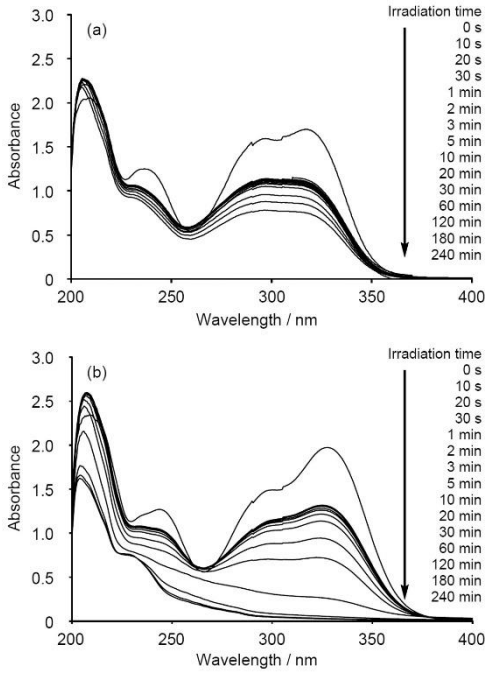


Figure 2. UV-vis spectra of compound **10** (45 μM) (a) and compound **11** (65 μM) (b) in methanol UV irradiation (λ>280 nm) with each elapsed time.

the reaction, the protecting groups played important roles in the aspects of both solubility and selectivity. After the successful synthesis of compounds **10** and **11**, their characteristics were examined. At first, the photo-reactivity of the double bonds was investigated in methanol (Figure 2). Compound **10** was irradiated by UV light at 45 μM after nitrogen bubbling, and the UV-vis adsorption spectra were monitored. The peak top at 317 nm decreased after the irradiation as shown in Figure 2a, indicating that the photo-generated radical was consumed. The same tendency as compound **10** was confirmed for compound **11** at 65μM as shown in Figure 2b. The peak top at 328 nm similarly decreased. However, compound **11** showed a complete decrease of the peak intensity of the double bonds. This results probably based on the different bulkiness between **10** and **11**, supported by the molecular simulation (Supporting Information, Figure S13 and S14). The change of UV spectral pattern at the double bond was also confirmed in THF as a solvent. As a model of glucosamine materials for film formation, the aggregated behavior of the compound was also investigated. 77.3 mg (0.1 mmol) of compound **11** was suspended in 100 mL of ion exchanged water, and then irradiated with UV light. After the UV irradiation, 9 mg (12%) of the methanol soluble part and 68 mg (88%) of the methanol insoluble part were recovered. The insoluble part was analyzed by FT-IR and MALDI-TOF/MS. The FT-IR spectra of the samples before and after UV irradiation are compared in Figure 3.

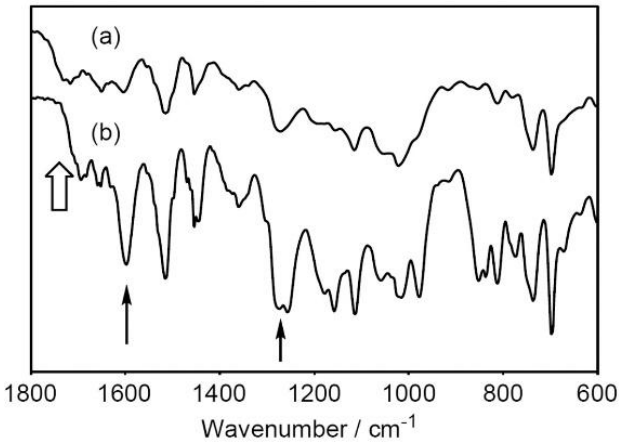


Figure 3. FT-IR spectra zoom of compound **11** before (a) and after (b) UV irradiation.

The peak intensity of the stretching and twisting of the double bond decreased on the

FT-IR spectra after the UV irradiation (Figure 3). This change has been reported for the dimerization of caffeic acid.[28] This result indicated the decrease of the double bond. At the same time, a new peak appeared at 1750 cm^{-1} unexpectedly, which was assigned as a benzoquinone group, suggesting that the crosslinking had occurred.

Figure 4 shows the MALDI-TOF/MS of compound **11** after UV irradiation. Since the molecular weight of compound **11** was 773.26 g/mol, the oligomer peaks were confirmed at dimer, trimer, tetramer, and pentamer. This result demonstrates that the obtained compound **11** is photosensitive to form the oligomers.

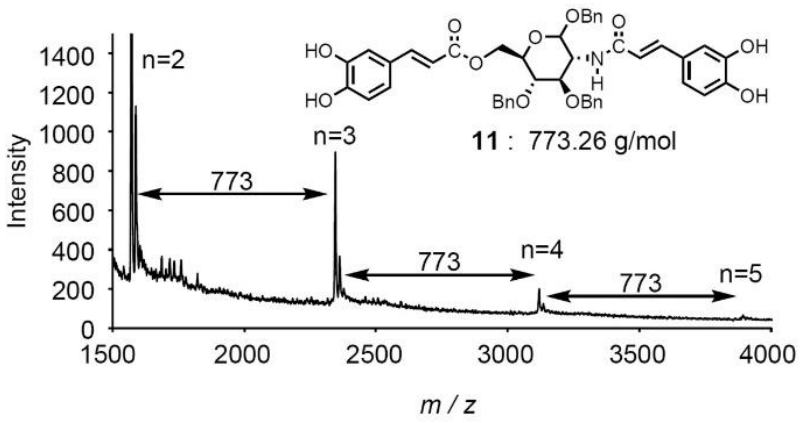


Figure 4. MALDI-TOF-MS of insoluble part of the compound **11**.

Finally, the physical properties of compound **11** were analyzed. It was soluble in most of the common organic solvents, such as methanol, ethanol, isopropanol, acetone, diethyl ether, and ethyl acetate, whereas it was not soluble in water, dichloromethane, and hexane. However, it could not be dissolved in any of the abovementioned solvents after UV irradiation. The DSC analysis of compound **11** after UV irradiation showed no glass transition temperature and melting points, while the degradation temperature was 291 ˚C at the 10% weight loss temperature. These results suggest that the solubility and thermal stability were improved after UV irradiation. Since the oligomer was composed of the six membered ring of glucosamine and aromatic groups of cinnamic acid derivatives, the high rigid and strong heat resistant polymer materials are expected. Currently, the further medication were underway, which might lead to the high performance polymer materials that can be controlled by photo-responsive functionality.

Conclusion

We designed and synthesized a glucosamine derivative bearing double caffeic acid moieties for developments of natural based materials. The properly selected protection and deprotection reactions of each the hydroxyl group and the amino group led to the successful synthesis of compound **10** and **11**. Their synthesis roots contribute to the trim of syntheses steps and the improvement of a final yield. In addition, synthesized glucosamine derivatives were polymerized by UV irradiation and obtained oligomers were investigated by FT-IR, UV spectrometer and MALDI-TOF-MS. These results indicated that dimerization reactions of double bond at caffeic acid were occurred and their reactivity were inferenced by the structural bulkiness. Furthermore, the solubility and thermal stability were improved after the UV irradiation. The insights from the present study should contribute to developments of natural based materials using glucosamine derivatives.

Acknowledgments

The authors are grateful to Ms. Y. Nishikawa for the measurements of mass spectra. This work is partly supported by Bilateral program: Joint research Thailand-Japan (JSPS-NRCT).

References and notes

1. Ohkawa K, Minato K, Kumagai G, Hayashi S, Yamamoto H. Biomacromolecules 2006:7:3291-3294.
2. Kadokawa J, Egashira N, Yamamoto K. Biomacromolecules 2018:19:3013-3019.
3. Khan M, Koivisto FT, Hukka TI, Hokka M, Kellomaki M. ACS Appl. Mater. 2018:10:11950-11960
4. Chochottiros C, Yoksan R, Chirachanchai S. Polymer 2009:50:1877-1886.
5. Jirawutthiwongchai J, Klaharn I, Hobang N, Mai-ngam K, Klaewsongkram J, Sereemaspun A, Chirachanchai S. Carbohydrate Polym. 2016:141:41-53.
6. Engkagul V, Klaharn I, Sereemaspun A, Chirachanchai S. Nanomed. Nanotech. Bio. Med. 2017:13:2523-2531.
7. Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Carbohydrate Polym. 2003:15:337-342.
8. Dowling MB, MacIntire IC, White JC, Narayan M, Duggan MJ, King DR, Raghavan SR. ACS Biomater. Sci. Eng. 2015:1:440-447.
9. Anraku M, Fujii T, Kondo Y, Kojima E, Hata T, Tabuchi N, Tsutsumi H, Kadowaki D, Maruyama T, Otagiri M, Tomida H. Carbohydrate Polym.

2011:83:501-505.
10. Orgueira HA, Bartolozzi A, Schell P, Litfens REJN, Palamacci ER, Seeberger PH. C. Chem. Eur. J. 2003:9:140-169.
11. Zhang L, Liu W, Han B, Wang D. Carbohydrate Polym. 2007:69:644–650.
12. Jiang Z, Budzynski WA, Qiu D, Yalamati D, Koganty RR. Carbohydrate Res. 2007:784-796.
13. Goyal N, Cheuk S, Wang G. Tetrahedron. 2010:66:5962-5971.
14. Stubbs KA, Macauley MS, Vocadlo DJ, Innov. Carbohydrate Res. 2006:341, 1764-1769.
15. Zhang J, Zhao M, Peng S. Carbohydrate Res. 2011:346:1997-2003.
16. Thi TH, Matsusaki M, Akashi M. Biomacromolecules 2008:3918-3920.
17. Ajiro H, Hsiao Y, Thi TH, Fujiwara T, Akashi M. Chem. Commun. 2012:48:8478-8480.
18. Kaneko T, Thi TH, Shi DJ, Akashi M. Nat. Mater. 2006:5:966-970.
19. Suvannasara P, Tateyama S, Miyasato A, Matsumura K, Shimoda T, Ito T, Yamagata Y, Fujita T, Takaya N, Kaneko T. Macromolecules 2014:47:1586-1593.
20. Koch H, Laschewsky A, Ringsdorf H, Teng K. Makromol. Chem. 1986:187:1843-1853.
21. Coates GW, Dunn AR, Henling LM, Ziller JW, Lobkovsky EB, Grubbs RH. J. Am. Chem. Soc. 1998:120:3641-3649.
22. Lee H, Scherer NF, Messersmith PB. PNAS 2006:103:12999-13003.
23. Xu J, Soliman M, Barralet F, Cerruti M. Langmuir 2012:28:14010-14017.
24. Yabu H, Saito Y, Shimomura M, Matsuo Y. J. Mater. Chem. C. 2013:1:1558-1561.
25. Zhan K, Kim C, Sung K, Ejima H, Yoshie N. Biomacromolecules 2017:18:2959-2966.
26. Brzonova I, Steiner W, Zankel A, Nyanhongo GS, Guebitz GM. Eur. J. Pharm. Biopharm. 2011:79:294-303.
27. Khong DT, Judeh MA. Tetrahedron Lett. 2017:58:109.
28. Wang S, Kaneko D, Okajima M, Yasaki K, Tateyama S, Kaneko T, Angew. Chem. Int. Ed. 2013:52:1143.

Supplementary Material

Supplementary material is available in Internet. Analyses data of the comopounds, such as ^{1}H NMR, FT-IR, and MALDI-TOF/MS. Molecular simulation.

Supporting Information

Synthesis of Glucosamine Derivative with Double Caffeic Acid Moieties at *N*- and 6-*O*-Positions for Developments of Natural Based Materials

Kenta Yamatani[a] Ryo Kawatani[a] and Hiroharu Ajiro[a,b]

[a]Graduate School of Science and Technology, Division of Materials Science, Nara Institute of Science and Technology, Takayama-cho 8916-5, Ikoma, Nara 630-0192, Japan
[b]Institute for Research Initiatives, Nara Institute of Science and Technology, Takayama-cho 8916-5, Ikoma, Nara 630-0192, Japan

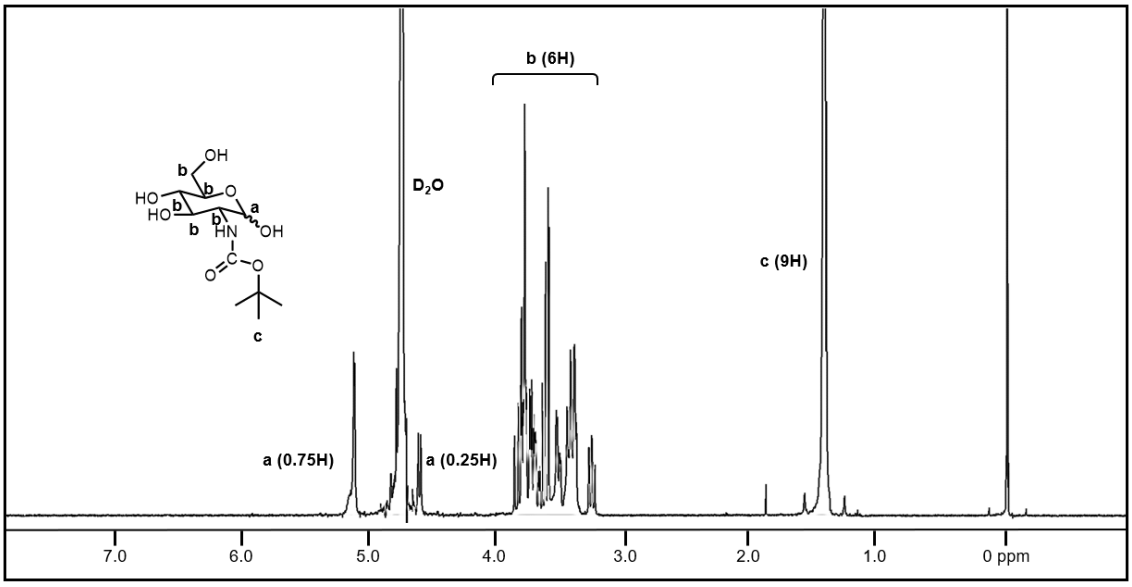


Figure S1. ^{1}H NMR spectrum of **2**.

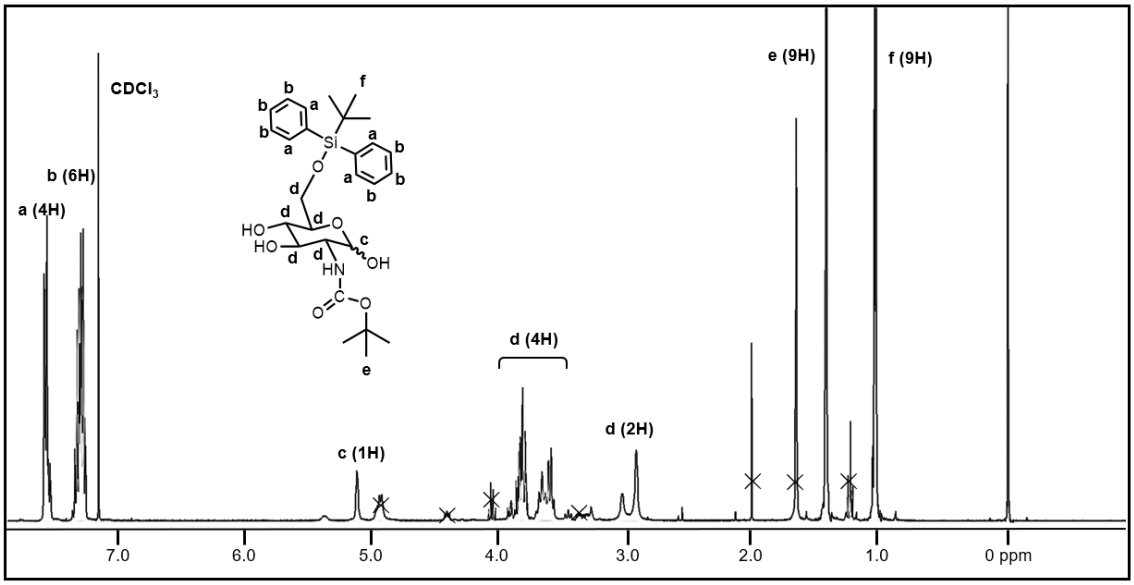


Figure S2. ^{1}H NMR spectrum of **3**.

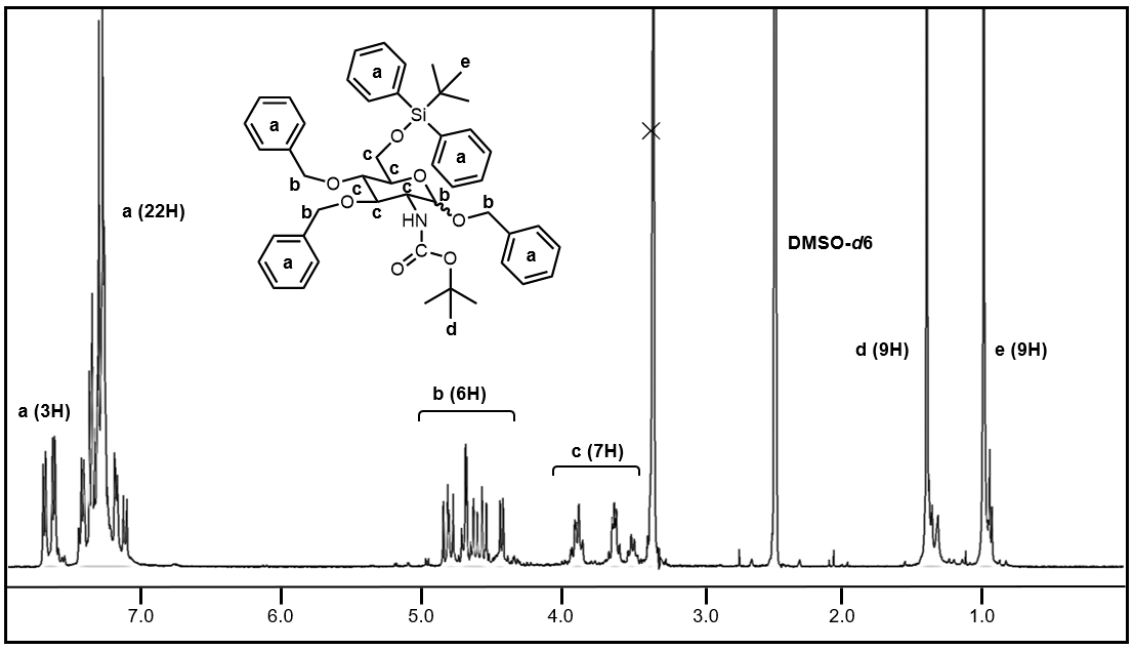


Figure S3. ^{1}H NMR spectrum of **4**.

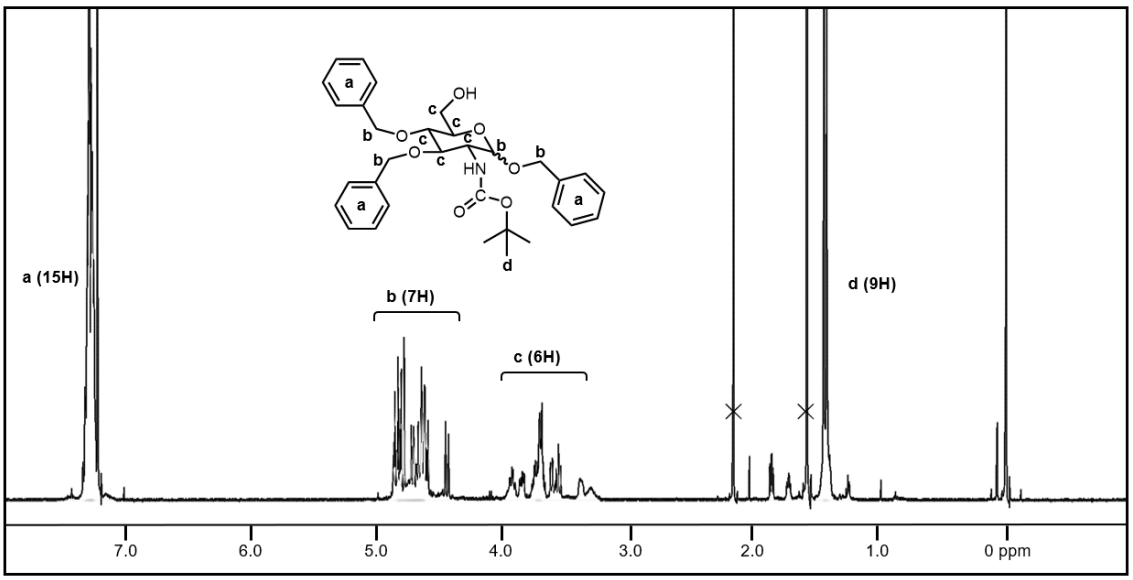


Figure S4. ^{1}H NMR spectrum of **5**.

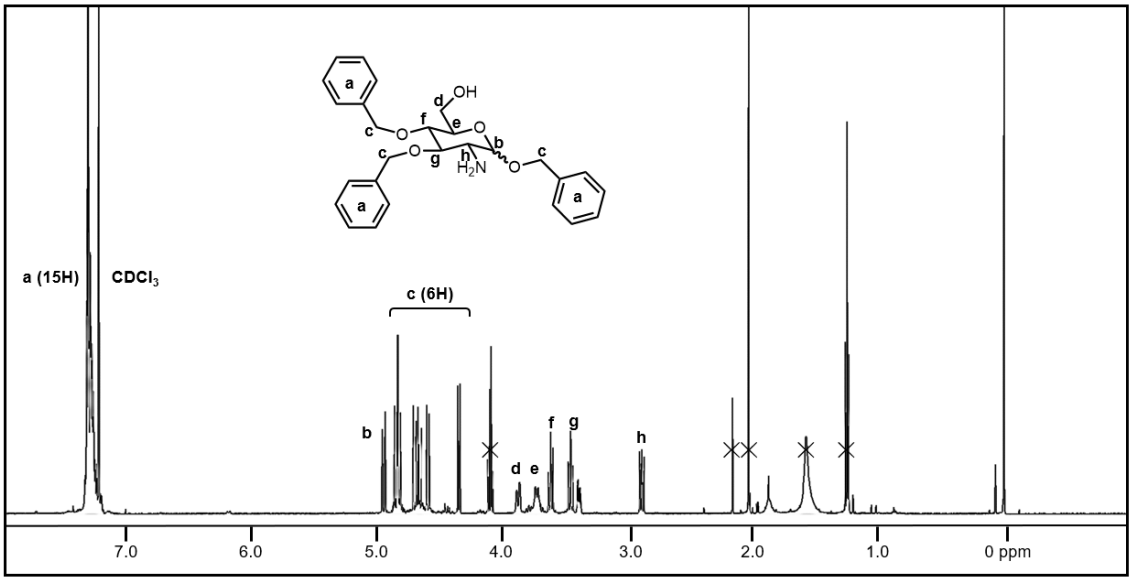


Figure S5. ^{1}H NMR spectrum of **6**.

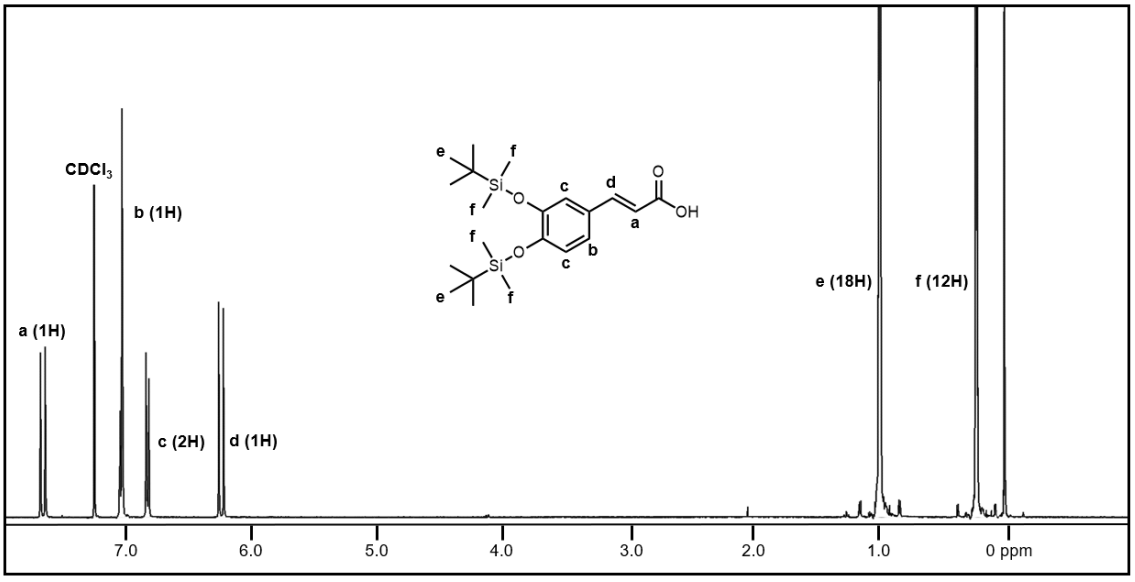


Figure S6. ^{1}H NMR spectrum of **8**.

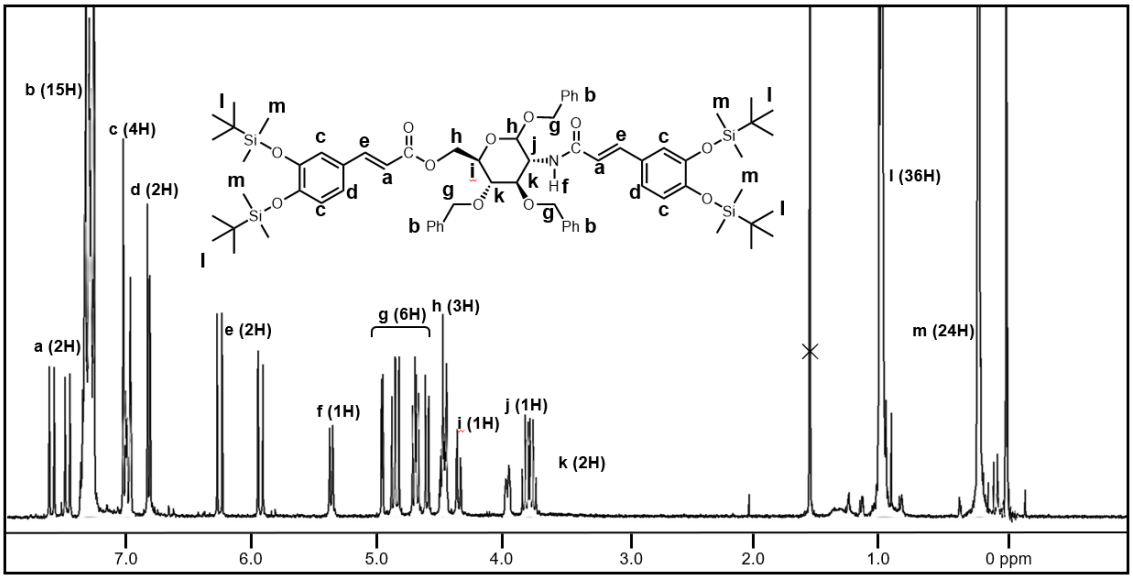


Figure S7. ^{1}H NMR spectrum of **10**.

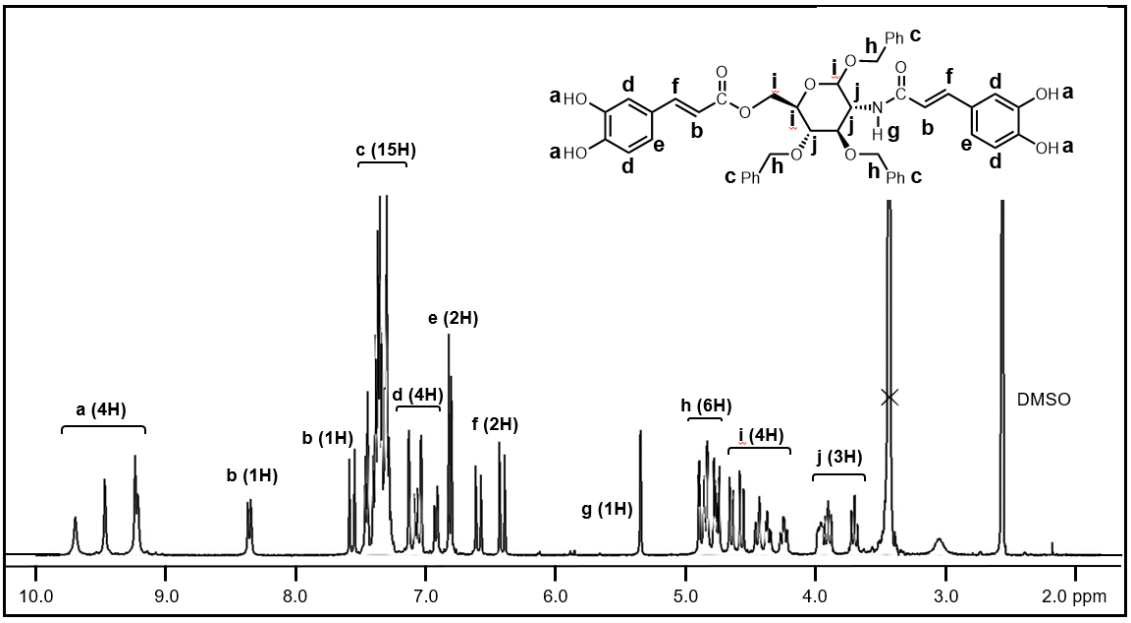


Figure S8. ^{1}H NMR spectrum of **11**.

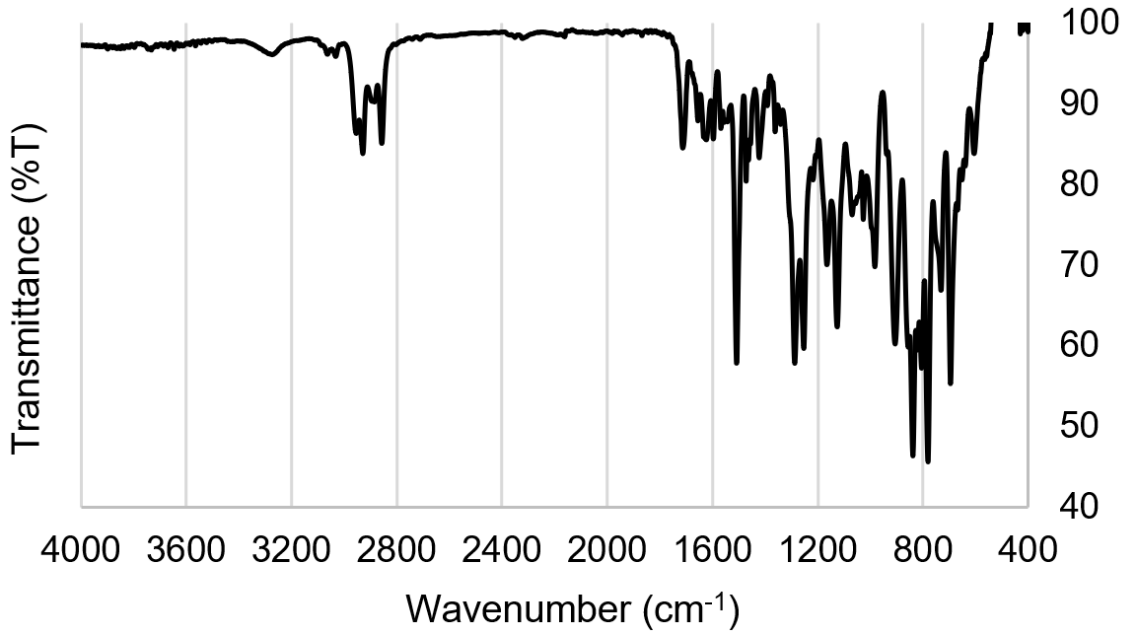


Figure S9. FT-IR spectrum of **10**.

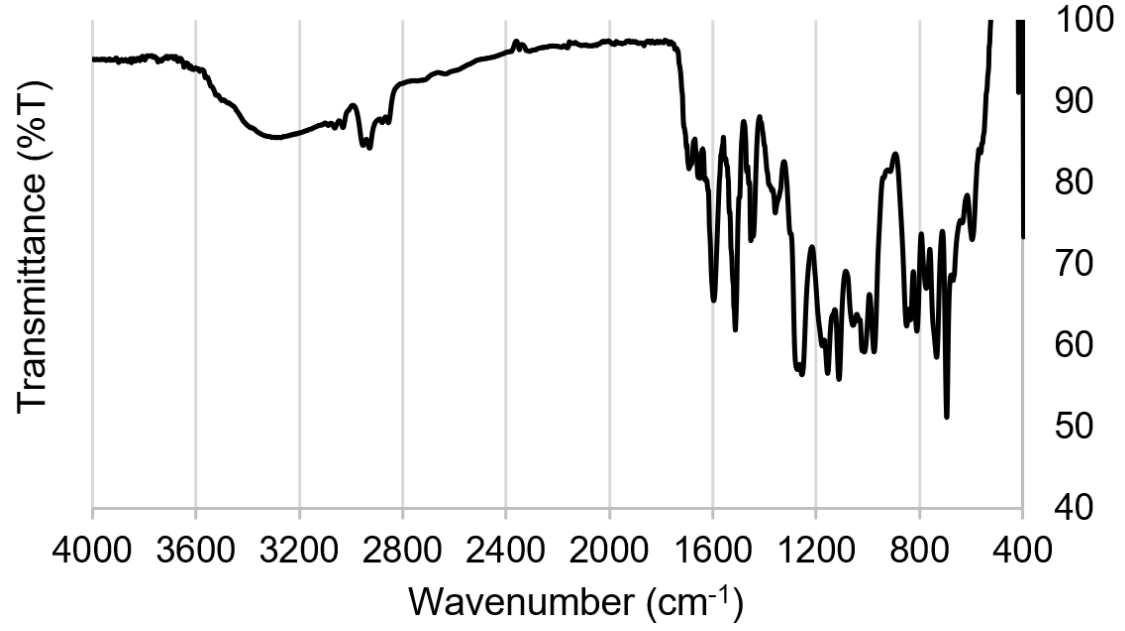


Figure S10. FT-IR spectrum of **11**.

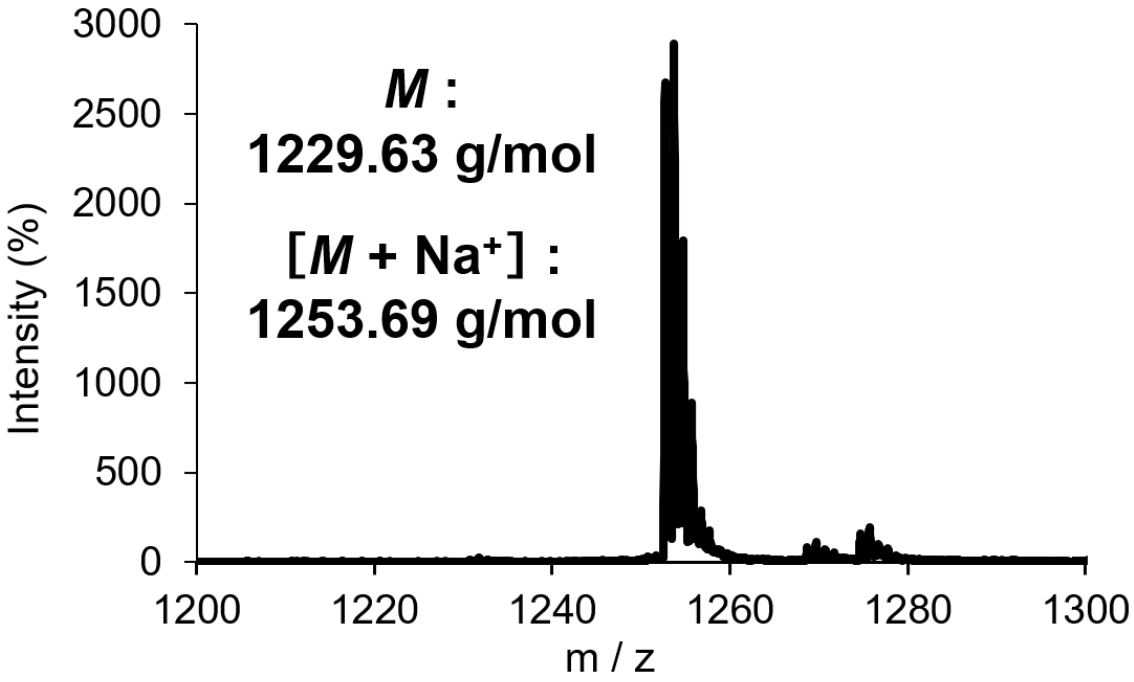


Figure S11. MALDI-TOF/MS spectrum of **10**.

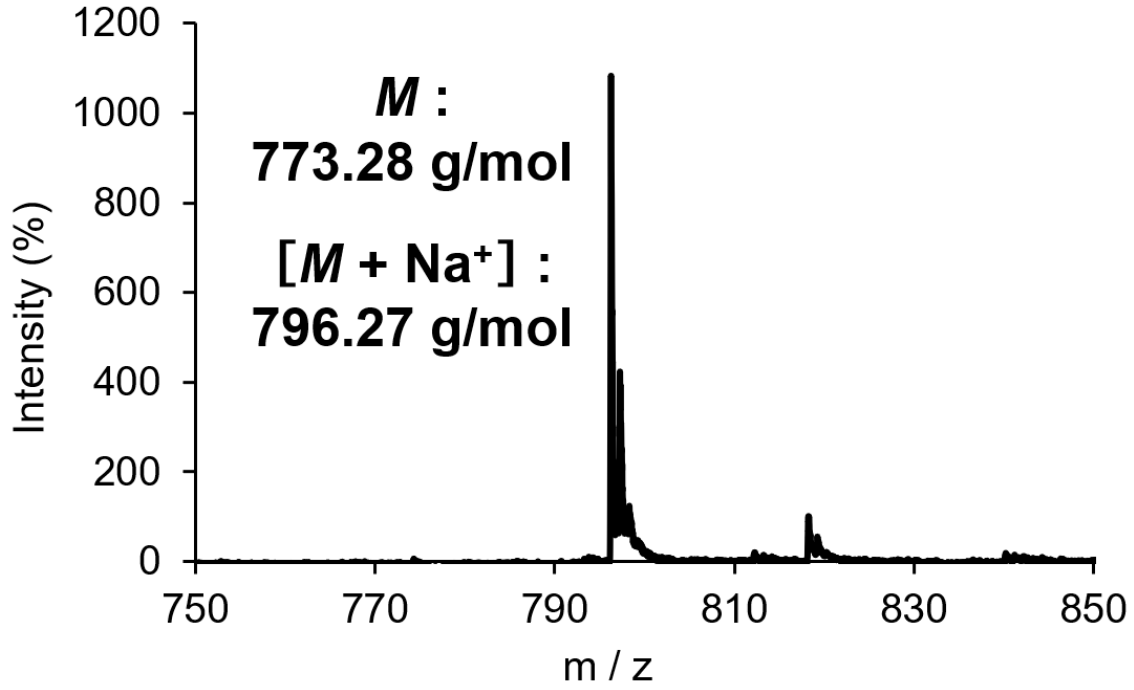


Figure S12. MALDI-TOF/MS spectrum of **11**.

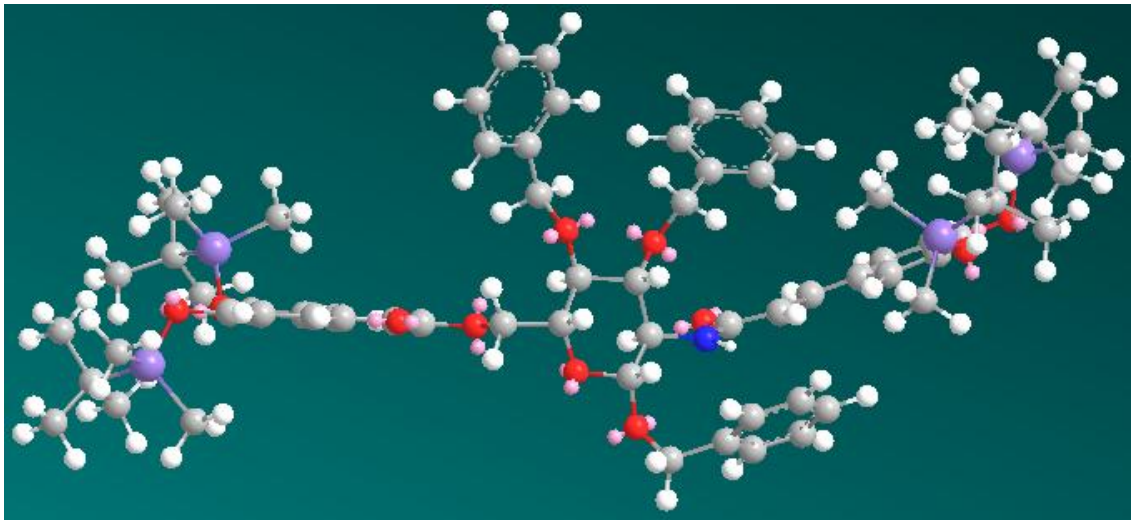


Figure S13. Molecular structure of **10** by Chem3D Pro MM2 calculations.

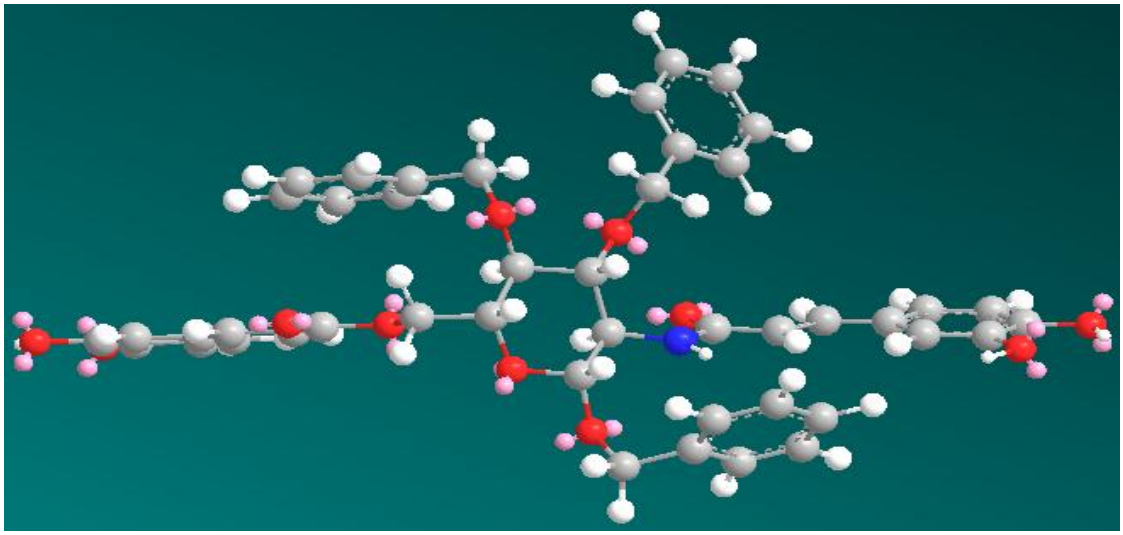


Figure S14. Molecular structure of **11** by Chem3D Pro MM2 calculations.

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
