Structure–Activity Relationships of Morpholine-Modified Silicon(IV) Phthalocyanines as Potential Antidiabetic Agents

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Experimental

Chemicals and Reagents

Silicon(IV) phthalocyanine dichloride, iodomethane, 3-(4-morpholinyl)phenol, 1-bromo-3-chloropropane, 4-hydroxybenzyl alcohol, 3,5-dihydroxybenzyl alcohol, sodium hydride, toluene, 18-crown-6, ethanol, potassium carbonate, chloroform, aluminum oxide, silica gel, and activated basic aluminum oxide were obtained from commercial suppliers. Silicon phthalocyanine dichloride, 1-bromo-3-chloropropane, sodium hydride, toluene, 18-crown-6, potassium carbonate, silica gel, and aluminum oxide were purchased from Sigma-Aldrich. Iodomethane and ethanol were obtained from Merck, chloroform from Carlo Erba, and 3-(4-morpholinyl)phenol from Thermo Scientific Chemicals. Thin-layer chromatography (TLC) analyses were performed using Merck TLC plates. All chemicals were used as received without further purification.

Equipments

Fourier-transform infrared (FT-IR) spectra were recorded using a PerkinElmer 1600 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained on an Agilent 400 MHz NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvents, and the chemical shifts (δ) were referenced to tetramethylsilane (TMS) as an internal standard. Mass spectrometric analyses were carried out using a Bruker Microflex LT MALDI-TOF mass spectrometer and a Thermo Surveyor TSQ Quantum Access system. UV–Vis absorption measurements were performed at room temperature on a PerkinElmer Lambda 25 UV–Vis spectrophotometer.

In vitro α–glycosidase inhibition assay

All compounds' α–glycosidase inhibition properties were measured according to our previously published procedures [43]. The stock solutions of compounds were prepared by dissolving the compounds in dimethyl sulfoxide at 2 mM and subsequently diluted to 500 µM with distilled water. These solutions were then utilized in the study. Acarbose (Sigma–Aldrich, A8980) was the standard inhibitor. In a 96-well plate, 100 µL α–glycosidase (Sigma–Aldrich, G5003) (0.5 U/mL) was added to the compounds, and acarbose at different concentrations (12.5–100 µM) in 50 µL 0.1 M phosphate buffer (pH = 6.9). Then, the

mixtures were incubated at room temperature for 10 min. Afterward, 50 µL of *p*–nitrophenyl–*α*–D–glucopyranoside (4–pNPG) (Sigma–Aldrich, N1377) (5 mM) solution was added as substrate and incubated for 10 min at room temperature. Absorbance was detected at 405 nm using a microplate reader (Thermo Scientific Multiskan^{TM} Go Microplate Spectrophotometer), and the inhibition rate was calculated as follows:

$$Inhibition\% = \left[{}^{A_A}\!/_{A_B}\right] \times 100$$

Where A_A and A_B represent the absorbance of the without compound and with compound, respectively. IC_{50} value was calculated.

***In vitro* α–amylase inhibition assay**

All compound's α–amylase inhibition properties were conducted per the established procedure, with minor modifications [44]. Acarbose was used as the standard inhibitor. In a 96-well plate, 10 µL α–amylase (Sigma–Aldrich, A3176) (50 U/mL) was added to the compounds, and acarbose at different concentrations (12.5–100 µM) in 50 µL 100 mM phosphate buffer (pH = 6.8). Then, the mixtures were incubated at room temperature for 20 min. Afterward, 150 µL of 1% starch solution was added as substrate and incubated for 20 min at room temperature. Then, 20 µL of 3,5-dinitrosalicylic acid (DNSA) reagent (containing DNSA (Sigma-Aldrich, D0550), potassium sodium tartrate (Sigma–Aldrich, S6170) and sodium hydroxide (Tekkim, TK.170510) (NaOH)) and finally 20 µL of 2 N NaOH are added and incubated for 20 min at 100°C in a thermo shaker. Absorbance was detected at 540 nm using a microplate reader, and the inhibition rate was calculated as follows:

$$Inhibition\% = \left[{}^{A_A}\!/_{A_B}\right] \times 100$$

Where A_A and A_B represent the absorbance of the without compound and with compound, respectively. IC_{50} value was calculated.

Kinetic analysis

Kinetic analyses of the compounds (**MT-C3-H-Si** and **MT-C3-D-Si**) showed the highest effect on α–glycosidase was performed. The type of inhibition type and the K_i value were determined using the Lineweaver-Burk plot. In the graph, the value of K_m is determined by the corresponding value on the X axis (1/[S]), and the value of V_{max} is determined by the corresponding value on the Y axis (1/V). The K_i value, which represents the inhibition constant, is then calculated using the formula below [45]:

$$\frac{1}{V} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right) \times \frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

Where V is the reaction rate, K_m is the Michaelis-Menten constant, and V_{max} is the maximum rate the reaction reaches. [I] and [S] are the concentrations of inhibitor and substrate, respectively [46]. In both studies, the enzyme concentration was kept constant at 0.5 U/mL, and 2.5, 5, 10, and 20 mM substrates were used. As calculated IC_{50} values, 16.02 µM were studied for **MT-C3-H-Si** and 44.14 µM for **MT-C3-D-Si**. Then, α–glycosidase inhibitory properties of the compounds were performed according to the above procedure. The results were graphed using Microsoft Excel 13.

Statistical analysis

GraphPad Prism Software version 5.0 (San Diego, California, USA) was used for all the statistical analyses. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's tests for enzyme inhibition.

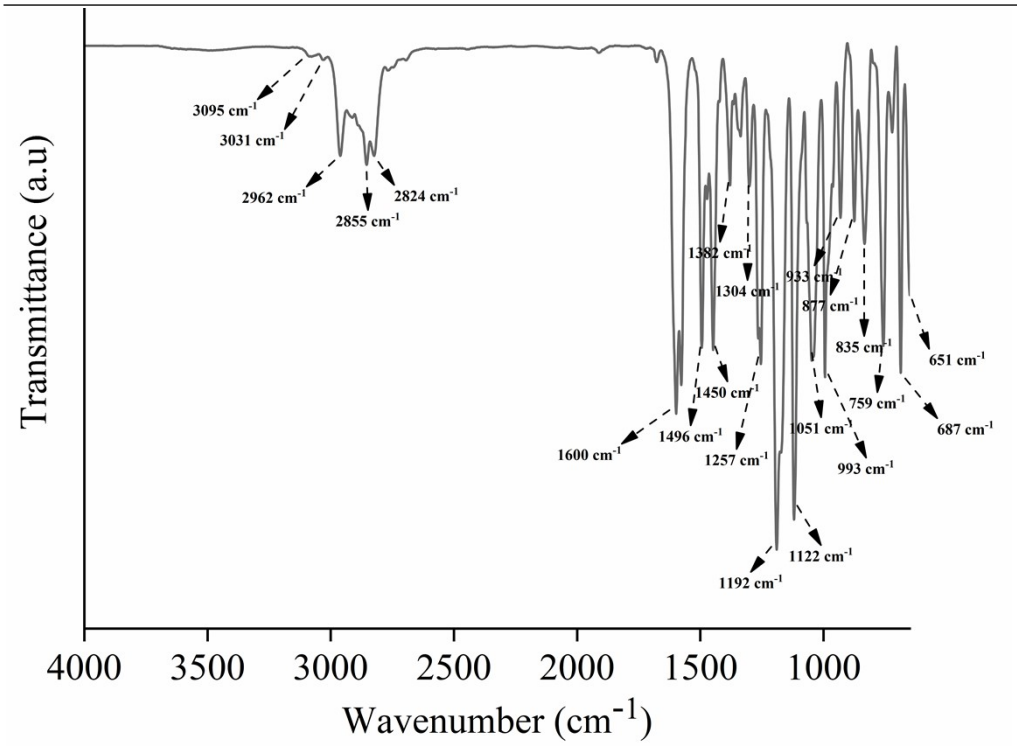

Figure S1. FT-IR spectrum of the MT-C3-Cl.

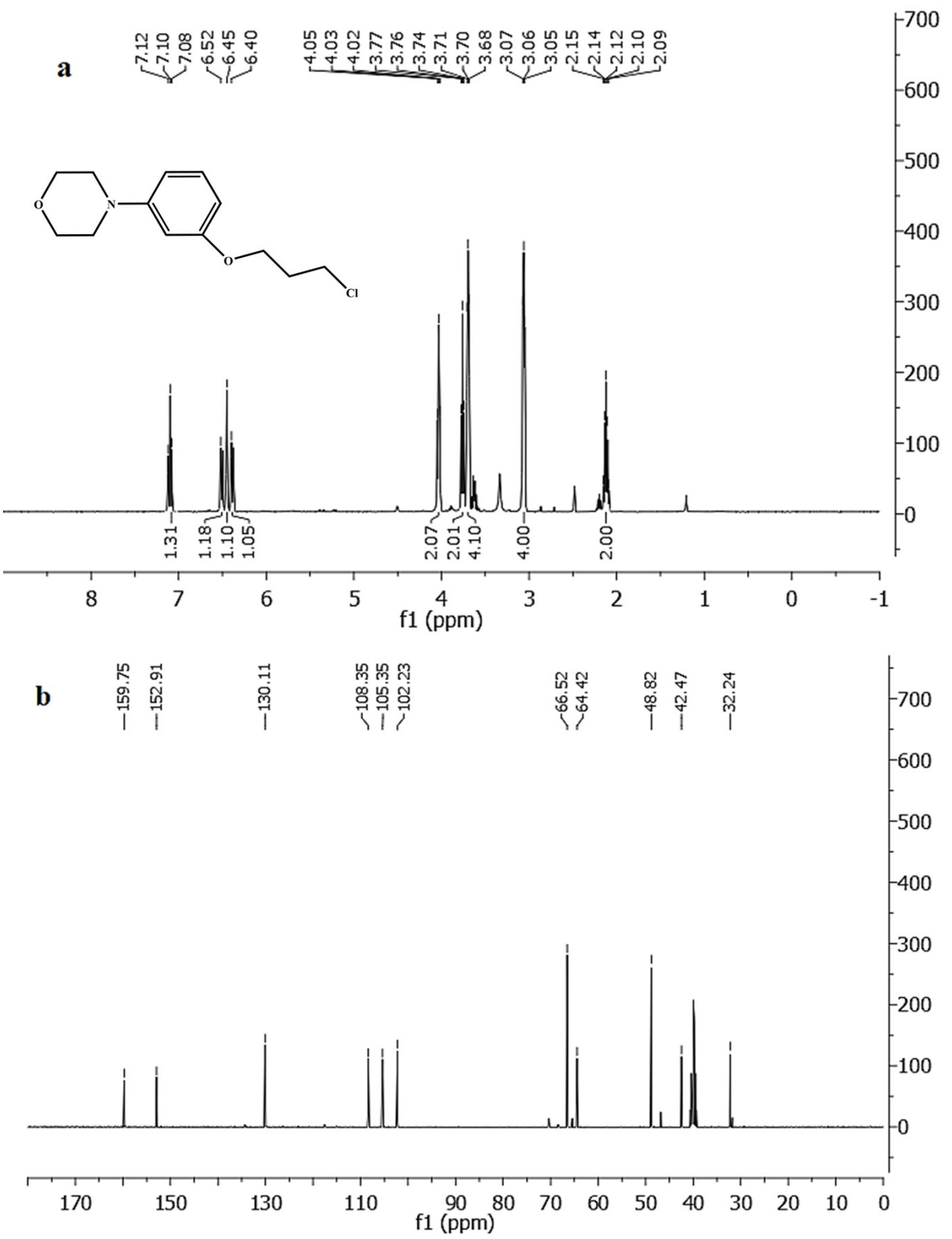

Figure S2. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of MT-C3-Cl recorded in DMSO-d_6.

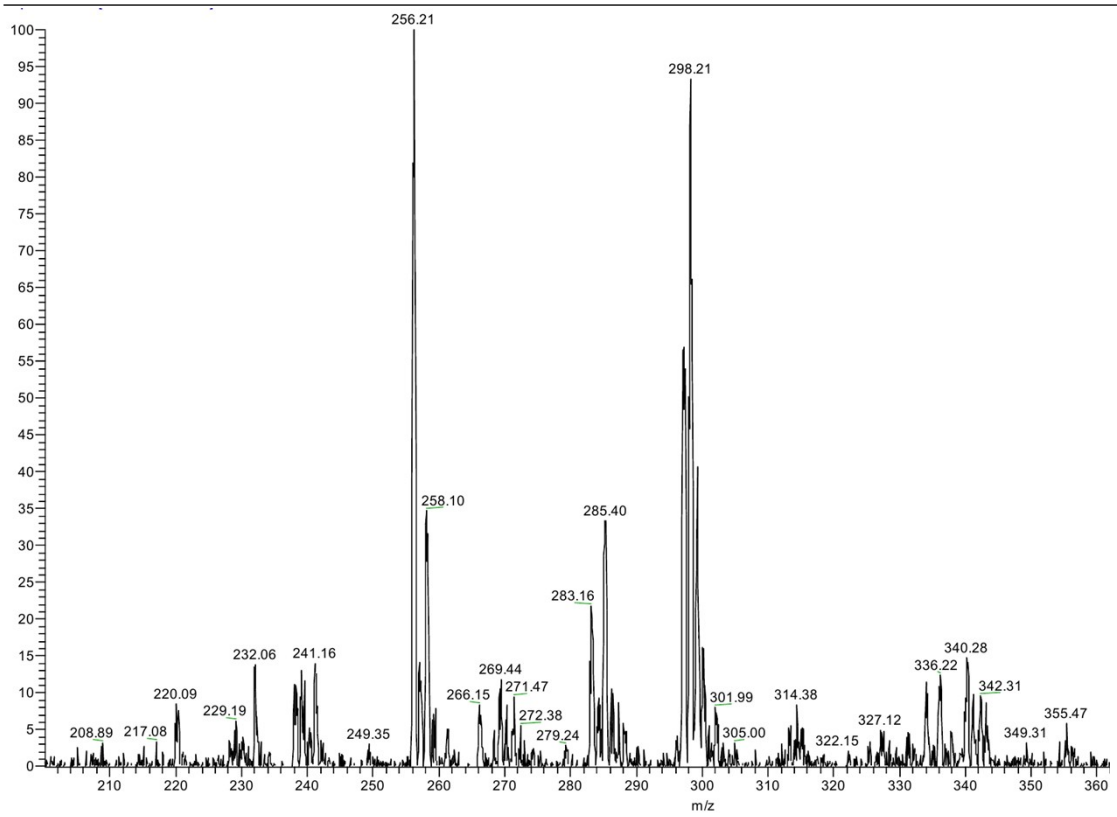

Figure S3. ESI–MS spectrum of MT-C3-Cl.

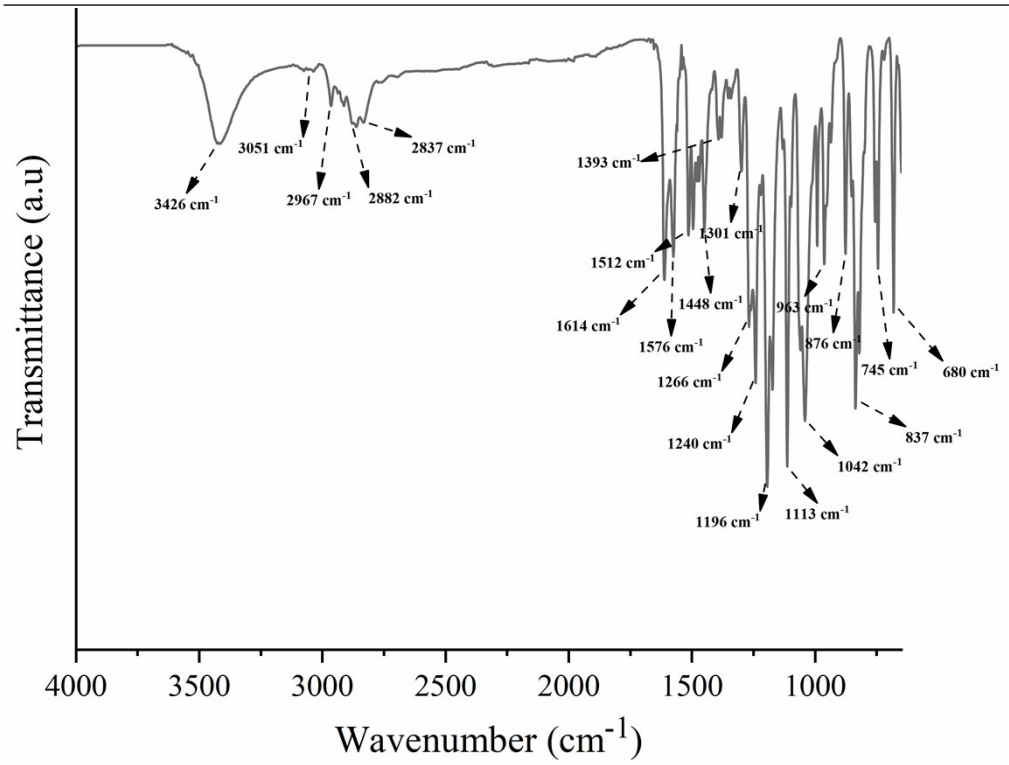

Figure S4. FT-IR spectrum of MT-C3-H-OH.

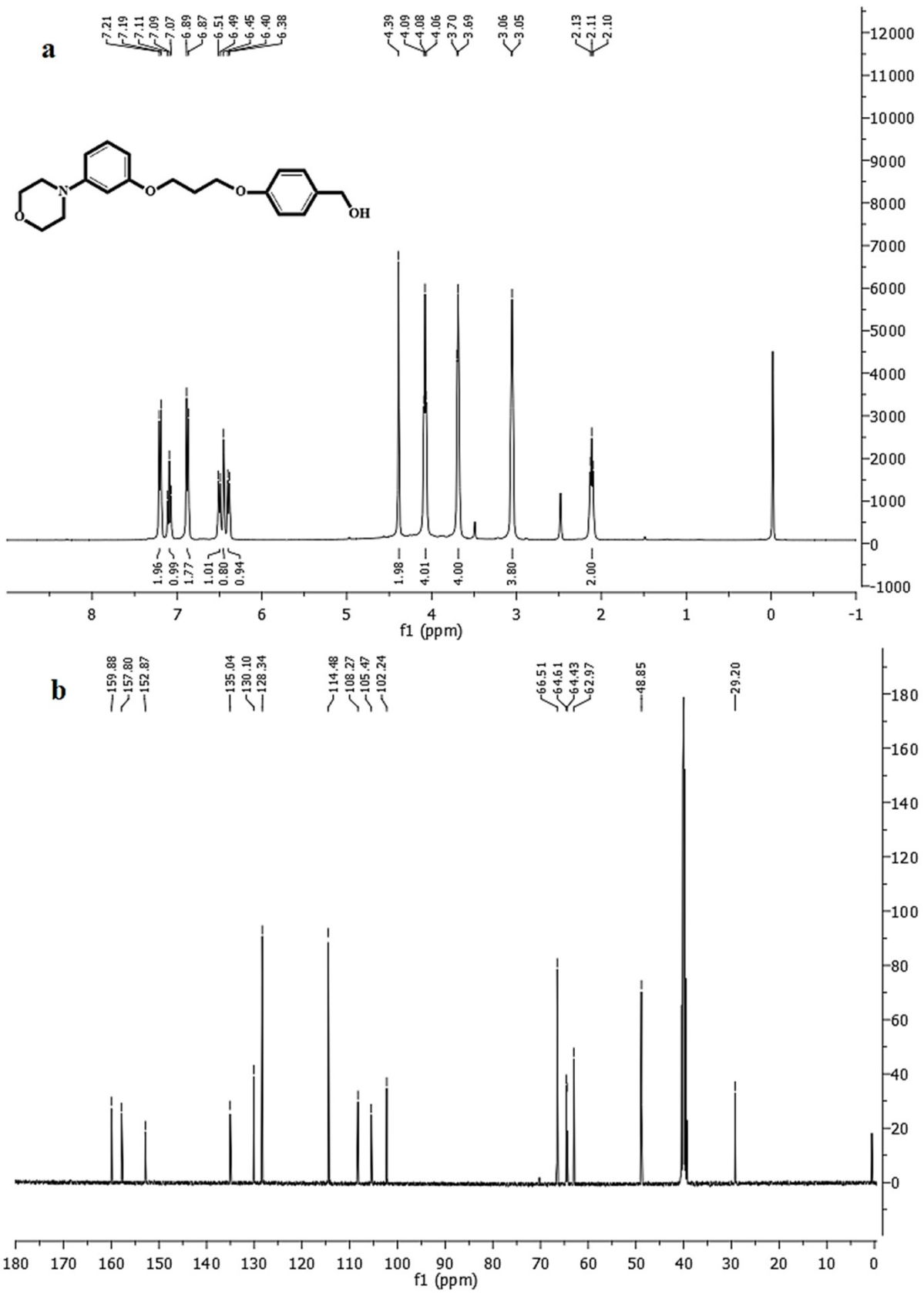

Figure S5. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of MT-C3-H-OH recorded in DMSO-d_6.

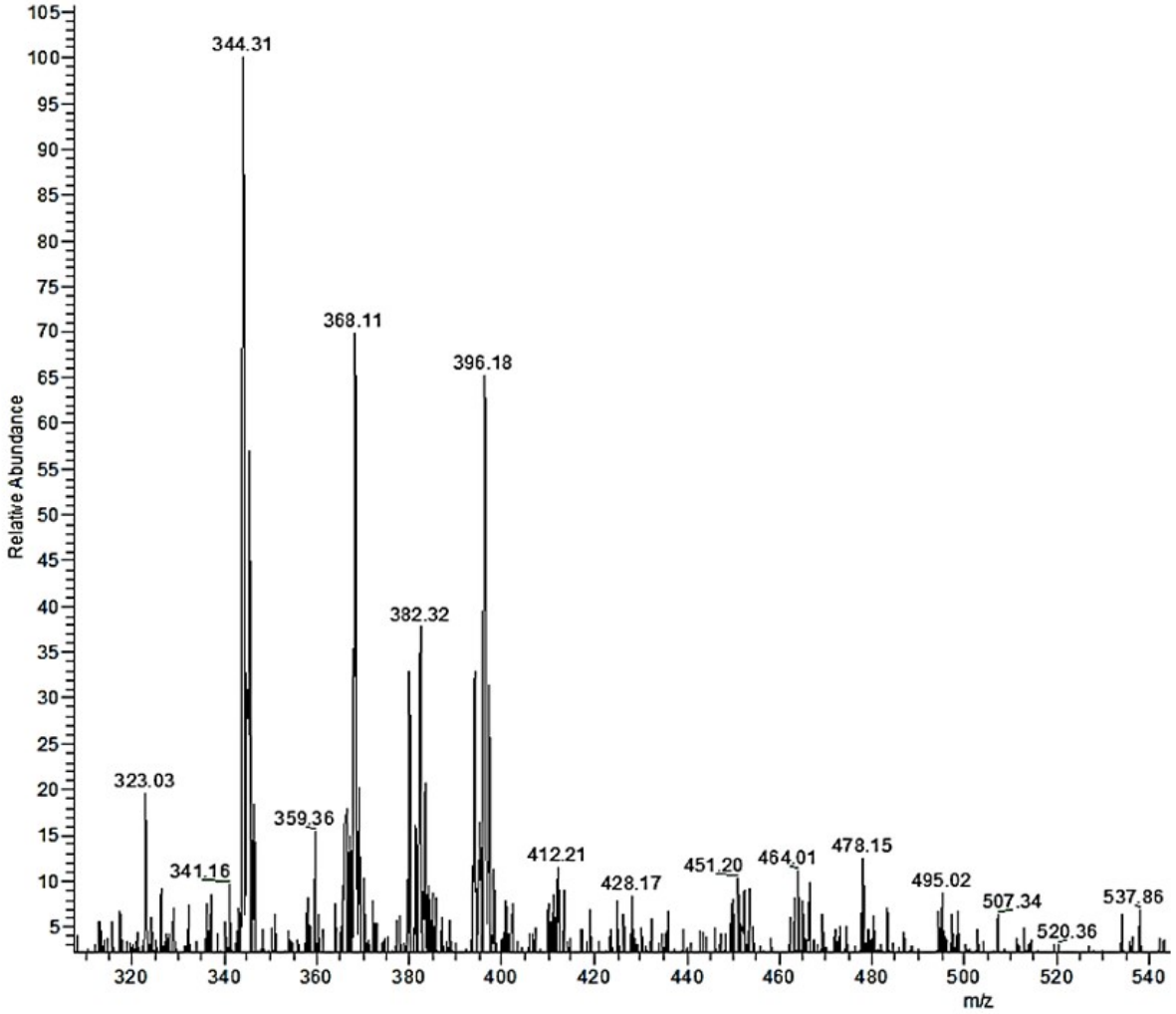

Figure S6. ESI–MS spectrum of MT-C3-H-OH.

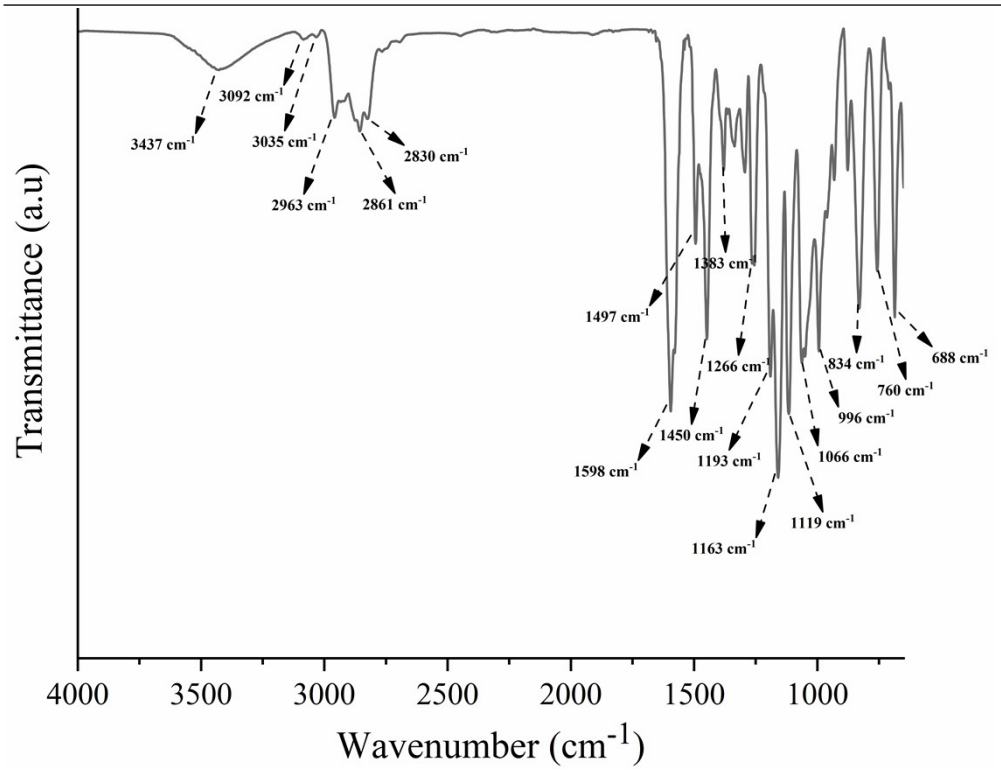

Figure S7. FT-IR spectrum of MT-C3-D-OH.

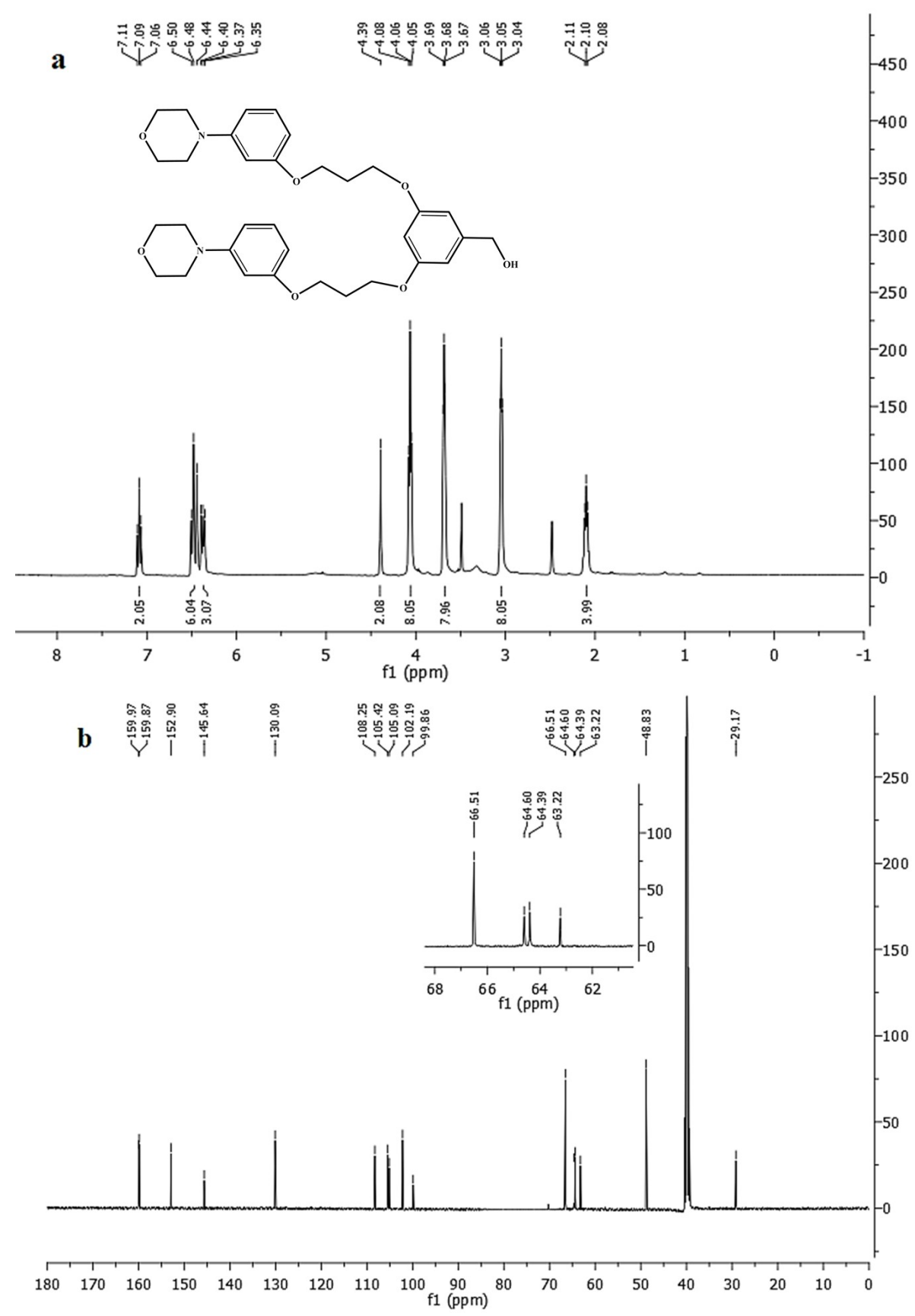

Figure S8. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of MT-C3-D-OH recorded in DMSO-d_6.

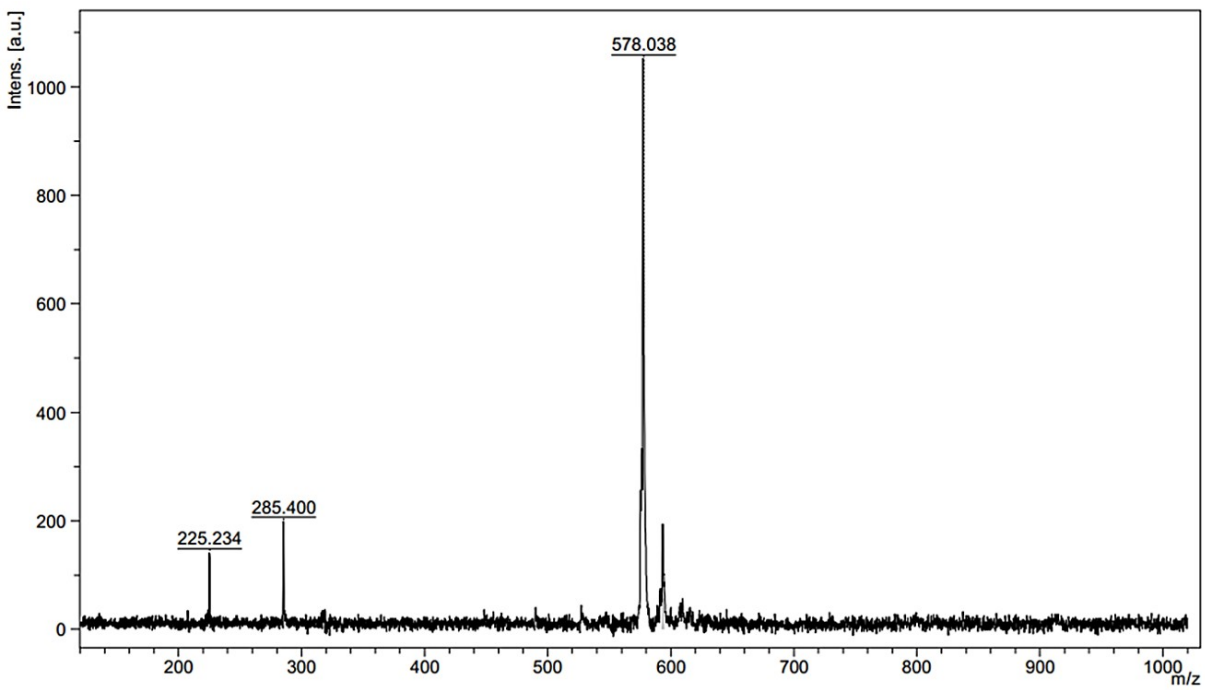

Figure S9. MALDI-TOF MS spectrum of MT-C3-D-OH.

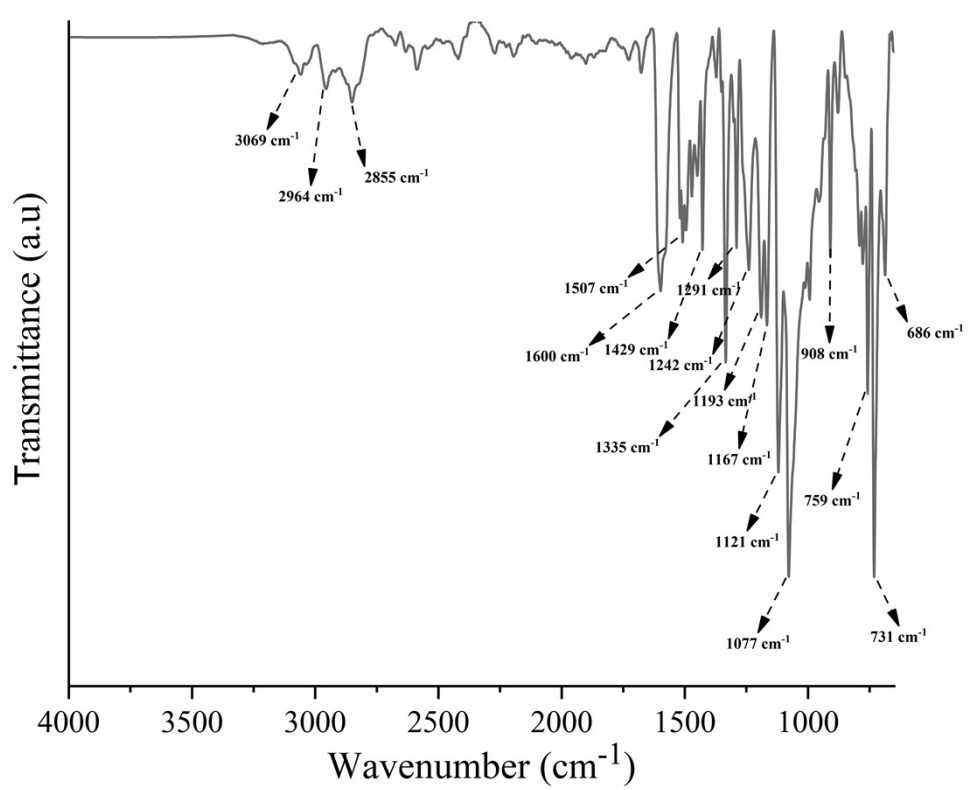

Figure S10. FT-IR spectrum of MT-C3-H-Si.

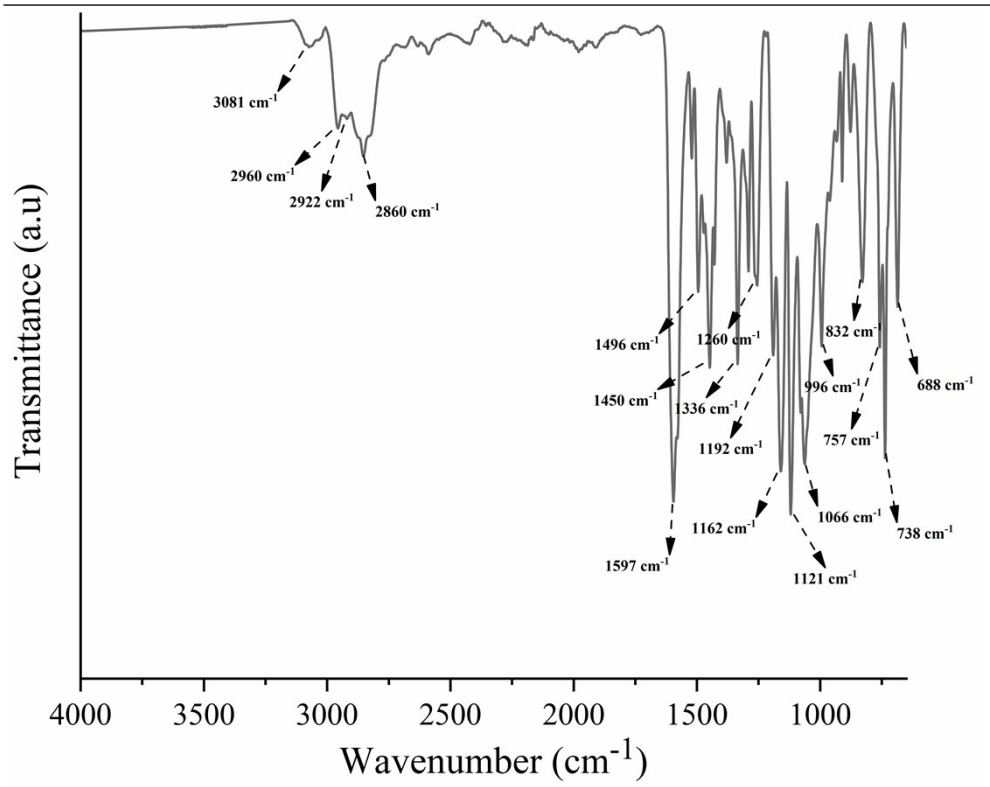

Figure S11. FT-IR spectrum of MT-C3-D-Si.

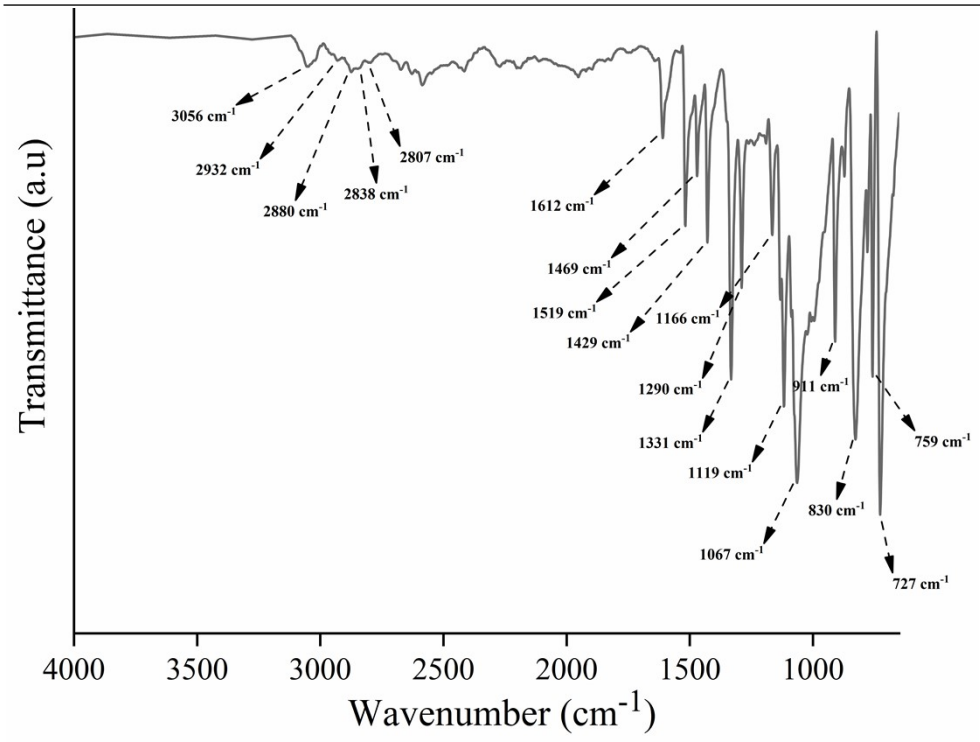

Figure S12. FT-IR spectrum of MT-C3-H-SiQ.

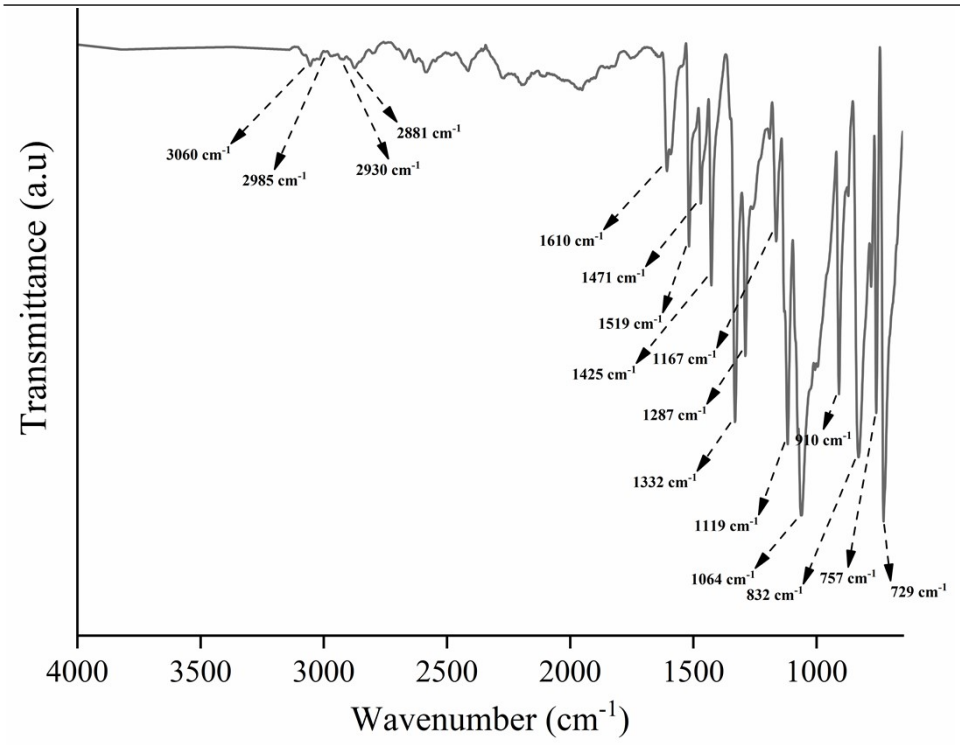

Figure S13. FT-IR spectrum of MT-C3-D-SiQ.