Supplementary Material (ESI) for Chemical Communication

Electronic Supplementary Information

Highly Selective Recognition of Carbenicillin *via* Concerted Interactions in 100% Aqueous Solution

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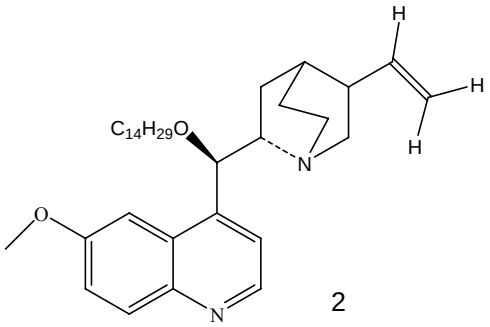
General Information

Fluorescence spectra were recorded on Hitachi (F-4500) spectrophotometers at 25 ^{o}C. The water was purified by Millipore filtration system. All analytical chemicals were purchased from Sigma Company for direct use, including carbenicillin, amoxicillin, ampicillin sodium, streptomycin sulfate, gentamycin sufate, penicillin, chloromycetin. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were determined on Bruker Avance П-400. Chemical shifts were reported relative to tetramethylsilane.

Synthesis of intermediate (2)



2

1.8g quinine was dissolved in 20 mL THF and 4.0g 50% KOH was added. The mixture was refluxed for 1h, then 1.0 mL $C_{14}H_{29}Br$ was added and refluxed for another 8h. The solution was concentrated *in vacuo* and 30 mL distilled water was added to the resulting residue. The solution was extracted with 3 × 10 mL CH_2Cl_2. The collected organic layer was dried with $MgSO_4$, and the CH_2Cl_2 was removed in vacuum. The crude product was purified by column chromatography on silica gel (ethyl acetate: petroleum ether = 10:1) to give **2** (1.18 g, 41%) as colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (t, 3H), 1.20–1.60 (m, 26H), 1.71–1.81 (s, 3H), 2.27 (s, 1H), 2.62-2.73 (m, 2H), 3.08-3.14 (m, 2H), 3.33-3.37 (t, 2H), 3.49-3.52 (s, 1H), 3.92 (s, 3H), 4.84-4.92 (m, 2H), 5.18 (s, 1H), 5.61-5.70 (m, 1H), 7.26-7.40 (m, 3H), 7.97-8.00 (d, 1H), 8.70-8.73 ppm (d, 1H). ^{13}C NMR(100 MHz, $CDCl_3$): δ 14,1, 22.7, 26.3, 27.9, 28.0, 29.4, 29.6, 30.1, 31.9, 38.5, 40.1, 43.3, 55.7, 57.3, 60.2, 69.6, 76.6, 77.2, 77.5, 101.2, 114.2, 118.7, 121.6, 127.4, 131.8, 142.0, 144.7, 145.2, 147.6, 157.7. HR-MS: m/z = 520.4020 M^{+}, calcd for $C_{34}H_{52}N_2O_2$ 520.4026.

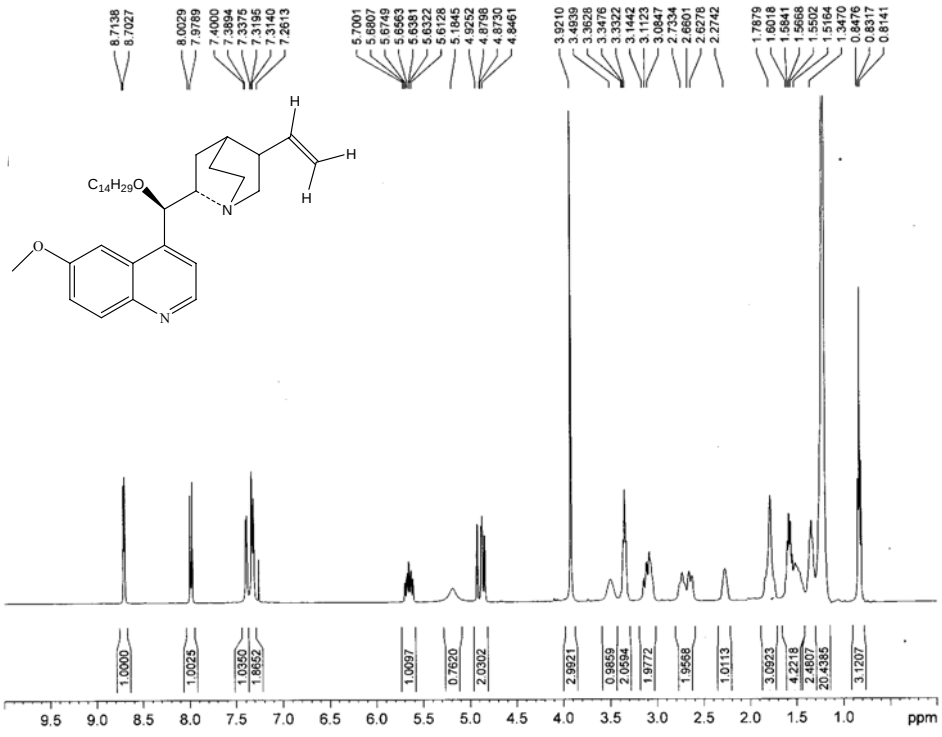


Figure S1. ^{1}H NMR of **2**

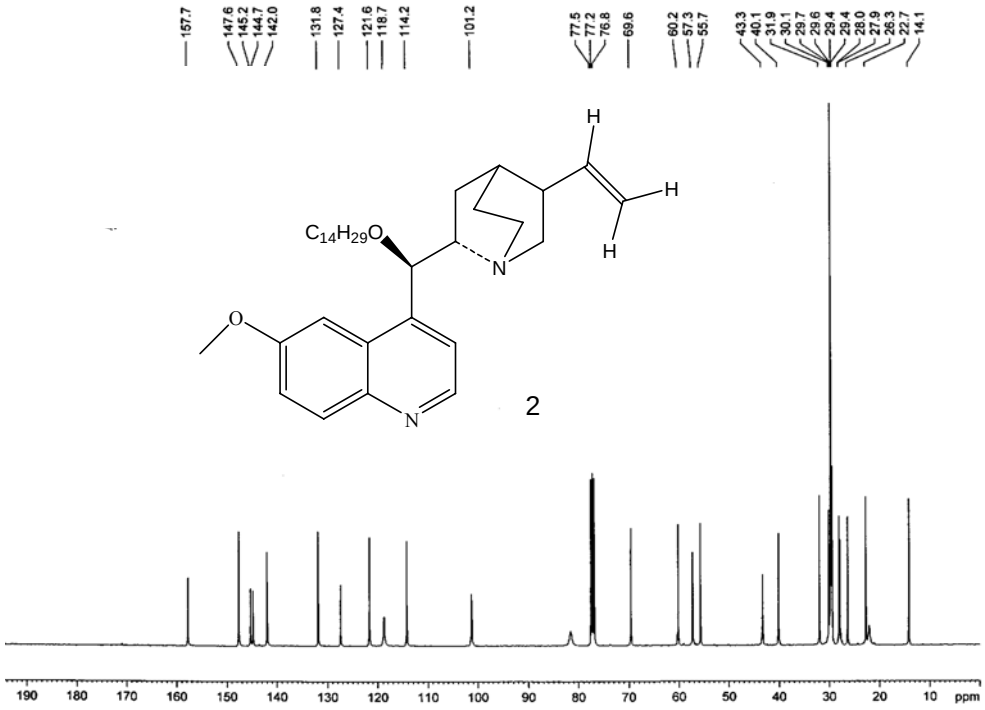


Figure S2. ^{13}C NMR of **2**

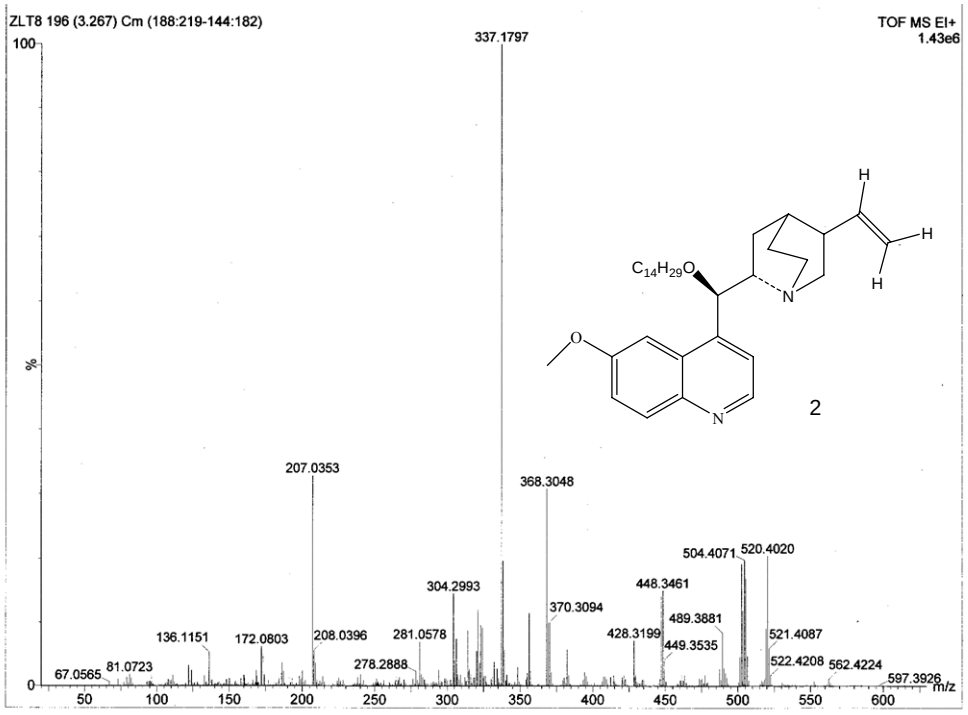
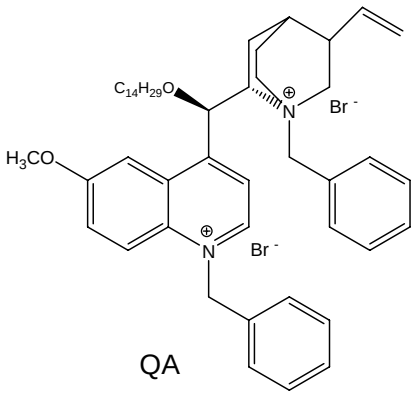


Figure S3. HR-MS of 2

Dibenzylquinicidal bromide (**QA**):



O-$C_{14}H_{29}$ quinine (1.06 g, 2 mmol) was dissolved in 10 mL CH_2Cl_2/CH_3OH (v/v = 3:2), then benzyl bromide (1.5 mL, 15 mmol) was added. The mixture was stirred at room temperature for 48h. After the reaction was completed, the solution was concentrated to a third of the volume, and the reaction residue was poured into 200 mL diethyl ether under stirring and was filtered. The solids were collected to afford 2.7g crude product. The crude product was further purified by column chromatography on silica gel

(CH_2Cl_2/CH_3OH = 100:8) to give **QA** (910 mg, 52%) as a light yellow solid.

^{1}H NMR (400 MHz, CD_3OD): δ 0.80-0.92 (t, 3H), 1.20–1.58 (m, 22H), 1.82-1.96 (m, 4H), 2.12 (m, 1H), 2.47-2.34 (m, 2H), 2.72 (m, 1H), 3.58-3.45(m, 2H), 3.63(t, 1H), 3.68-3.66(m, 2H), 3.97(m, 1H), 4.08-3.99 (m, 2H), 4.16(s, 3H),4.83-4.80(s, 1H), 5.17-5.05(m, 2H), 5.49 (d, 1H), 5.79(m, 1H), 6.34 (s, 1H), 6.72 (s, 1H), 7.90-7.43 (m, 12H), 8.39-8.37(d, 1H), 8.56-8.54 (d, 1H), 9.38-9.37 (d, 1H). ^{13}C NMR(100 MHz, $CDCl_3$): δ 14,2, 20.2, 22.7, 24.8, 26.8, 27.5, 29.4, 30.1, 32.8, 38.5, 50.8, 57.8, 58.6, 61.0, 64.2, 68.4, 70.3, 72.2, 76.8, 105.6, 118.5, 121.3, 127.6, 128.1, 129.2, 129.4, 130.5, 132.5, 133.5, 134.3, 136.1, 144.8, 154.3, 160.7. ESI-MS m/z =351.7 M^{2+}, calcd for $C_{48}H_{66}N_2O_2^{2+}$ 351.76.

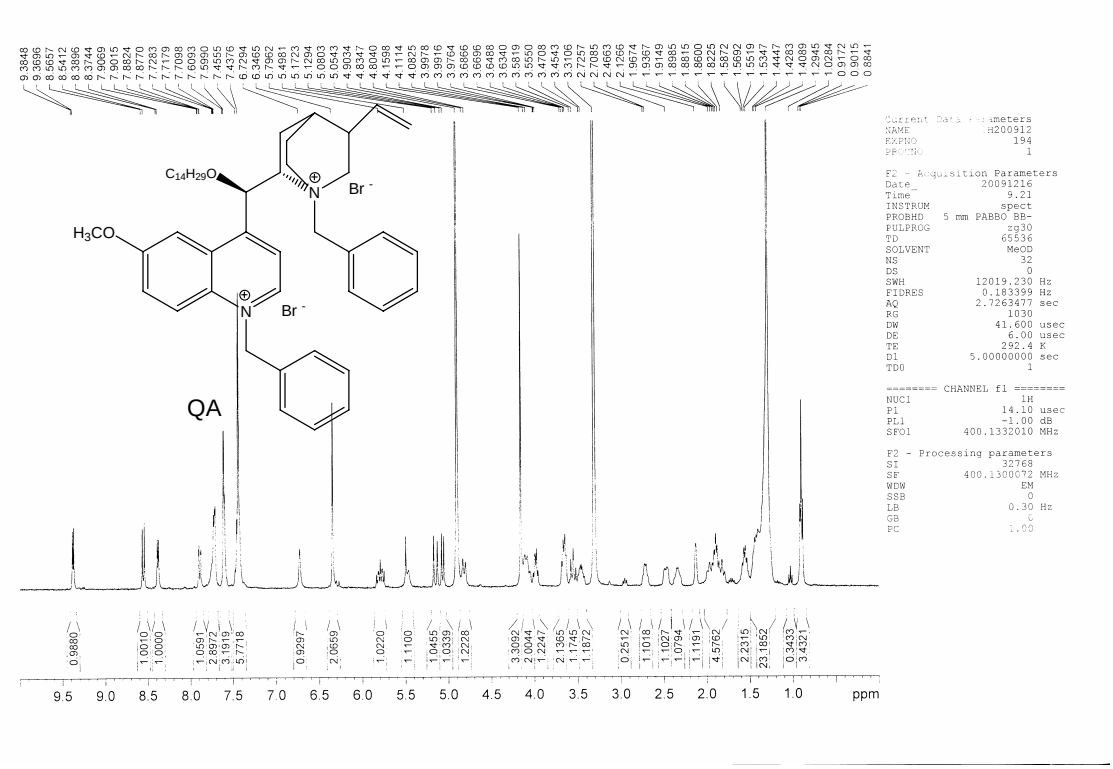


Figure S4. ^{1}H NMR of **QA**

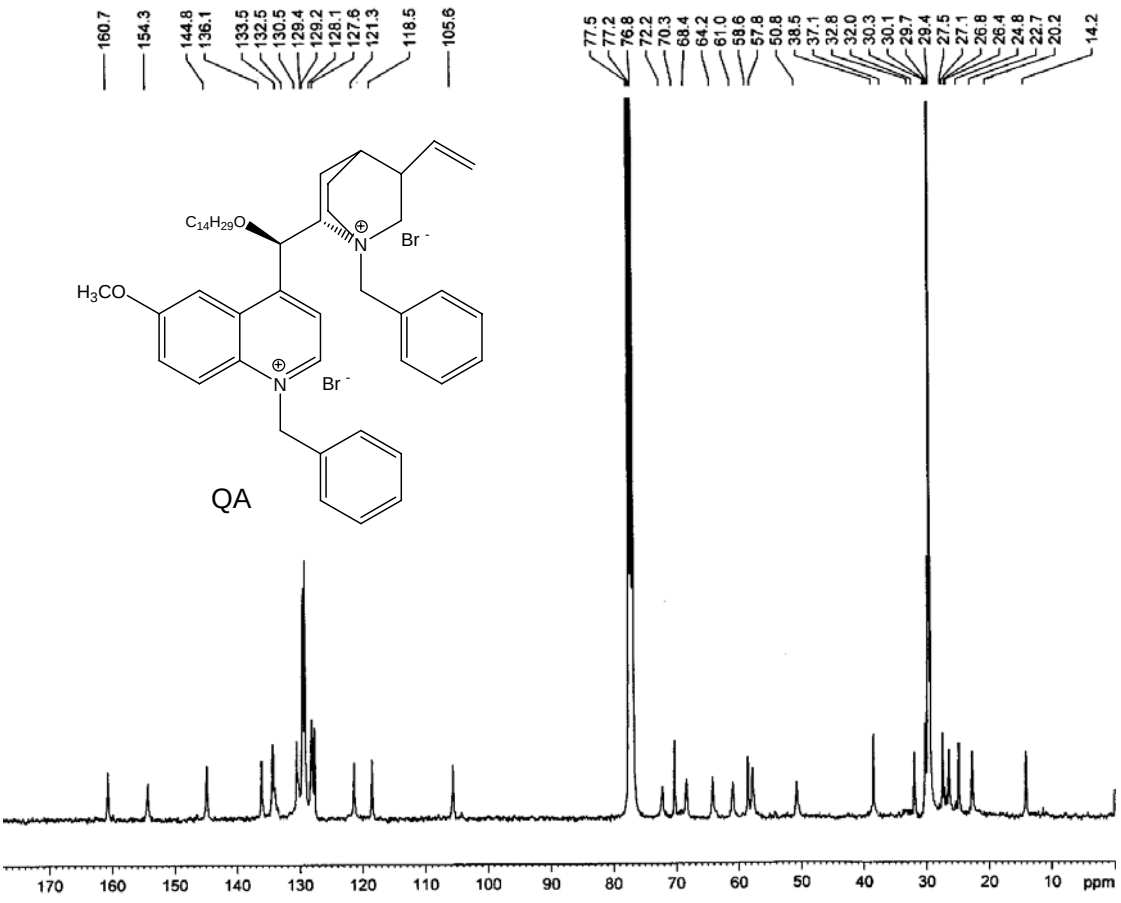


Figure S5. ^{13}C NMR of **QA**

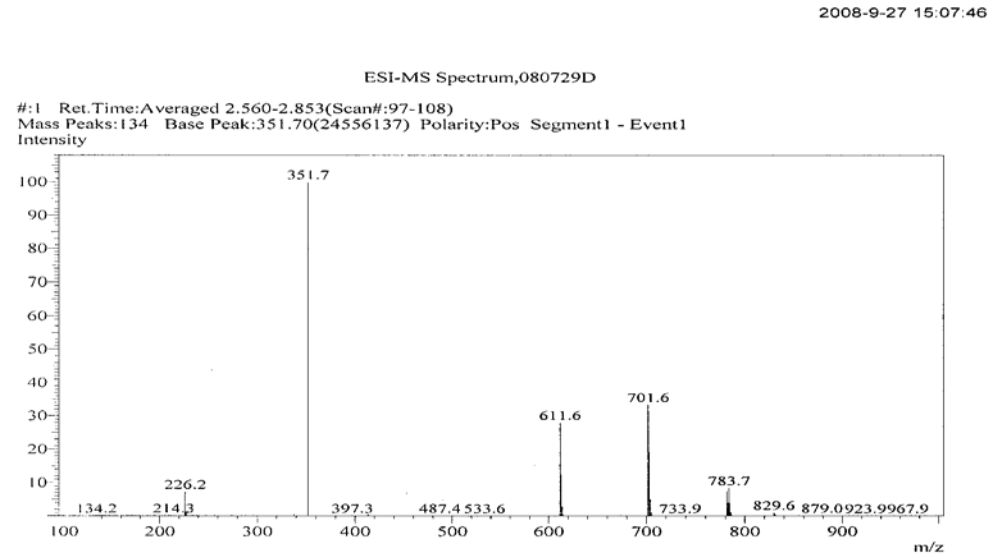
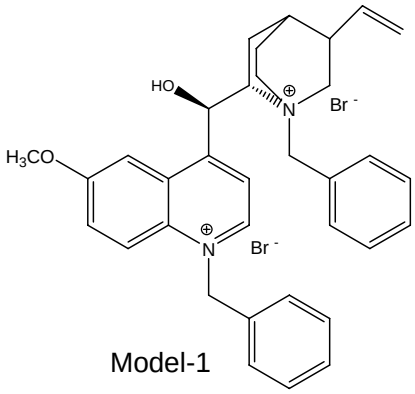


Figure S6. ESI-MS of **QA**

Synthesis of Model-1



Model-1

1.30 g quinine was dissolved in 10.0 mL CH_2Cl_2/CH_3OH (v/v = 3:2), and 1.5 mL benzyl bromide was added into the mixed solution. The mixture was stirred at room temperature overnight. After the reaction was complete, the reaction residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 100:8) to give **Model-1** (1.58g, yield 85%) as a light yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ1.78–2.53 (m, 6H), 3.04-3.07 (t, 1H), 3.34-3.38 (t, 1H), 4.22 (s, 3H), 4.58-4.67(t, 1H), 4.87-4.97(t,1H), 5.05-5.36(m, 4H),5.87-5.91(m, 2H), 5.94-6.35 (m, 2H), 6.93 (s, 1H), 7.26 (s, 1H), 7.30-7.50 (m, 9H), 7.58 (s, 1H), 7.31-7.63(m, 9H), 8.03-8.04(d,1H), 8.30-8.32(d, 1H), 8.42-8.44(d, 1H), 9.38-9.40 (d, 1H) ppm. ^{13}C NMR(100 MHz, $CDCl_3$): δ 21.8, 25.0, 27.6, 38.7, 51.2, 57.8, 59.4, 61.5, 62.8, 64.9, 68.3, 105.7,118.6, 120.9, 122.2, 127.5, 127.8, 129.2, 129.3, 129.9, 130.3, 130.6, 131.9, 133.8, 134.3, 136.4, 144.2, 156.7, 160.6 ppm. ESI-MS m/z =253.3 M^{2+}, calcd for $C_{34}H_{38}N_2O_2^{2+}$ 253.2.

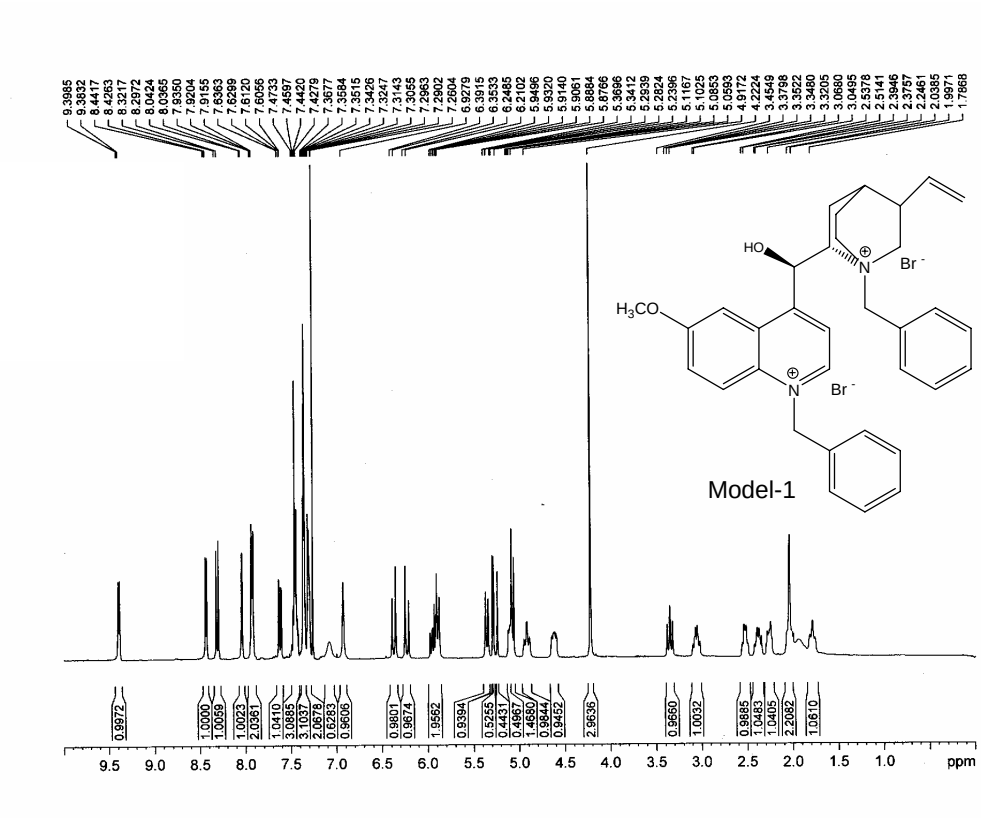


Figure S7. ^{1}H NMR of **Model-1**

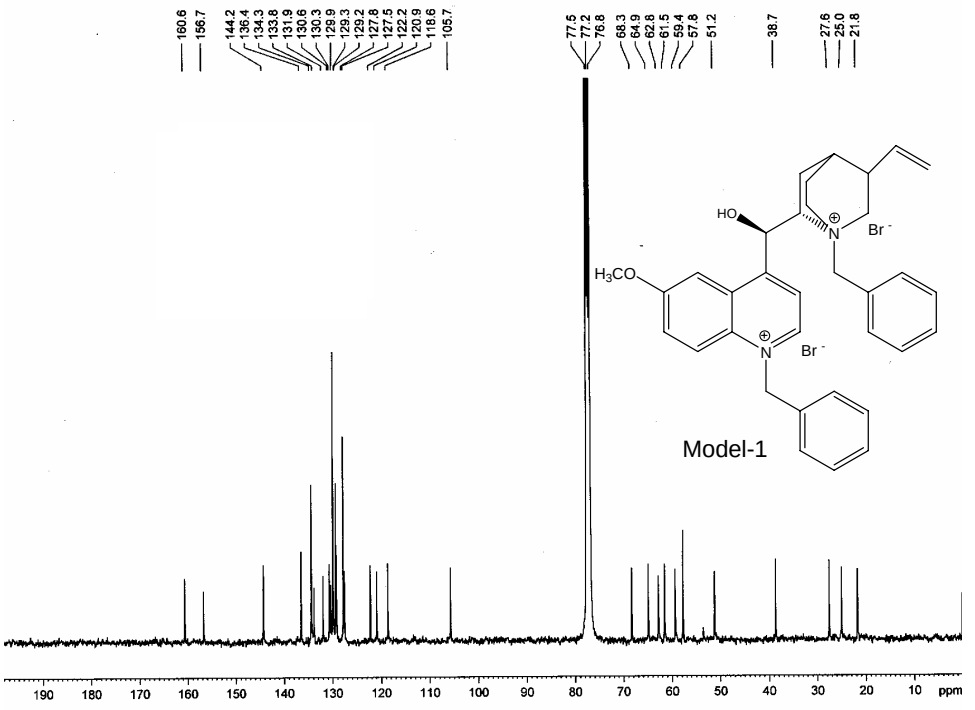


Figure S8. ^{13}C NMR of **Model-1**

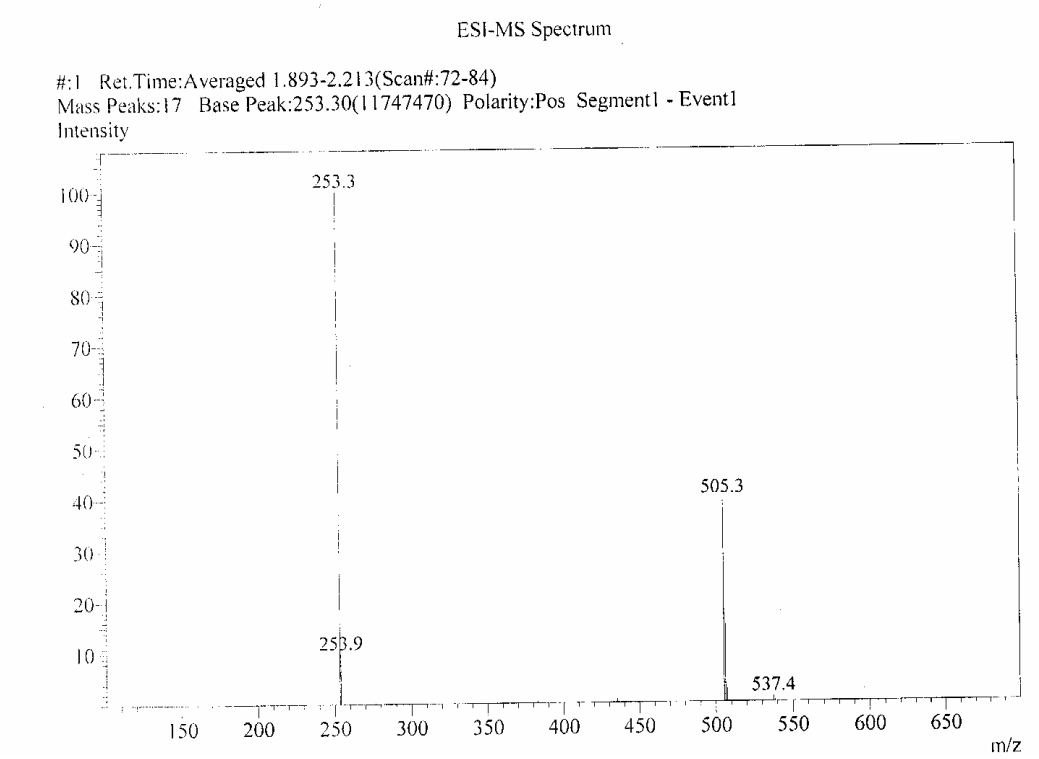
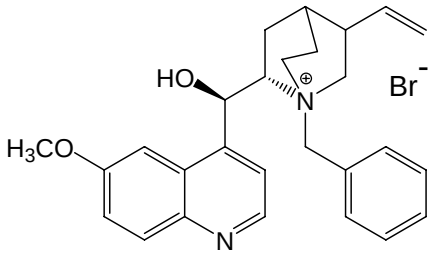


Figure S9. ESI-MS of **Model-1**

Synthesis of Model-2



Model-2

1.0g quinine was dissolved in 10.0mL CH_3OH, and 0.1mL benzyl bromide was added into the mixed solution. The mixture was stirred at room temperature overnight. After the reaction was complete, the reaction residue was poured into 400mL absolute ether under stirring, the precipitate was filtered and dried. The crude product was further recrystallized by CH_3OH to give **Model-2** (1.29g, yield 85%) as a colorless crystal.

^{1}H NMR (400 MHz, $CDCl_3$): δ1.32-1.50(t,1H), 1.72-1.80(t, 1H), 2.01(s, 1H), 2.12-2.22(t, 2H), 2.61(s, 1H), 3.20-3.64 (m, 3H), 3.72 (t, 1H), 3.94(s, 3H), 4.24(t, 1H), 4.64(d, 1H),

4.79-5.08(m, 2H), 5.12-5.16(d, 1H), 5.48-5.57(m, 2H), 6.53 (s, 1H), 7.25 (s, 1H), 7.45-7.58 (m, 6H), 7.74(d, 1H), 7.93-7.96(d, 1H), 8.68(d, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 15.8, 21.6, 24.9, 26.9, 38.2, 51.2, 51.2, 56.5, 58.4, 60.9, 63.8, 64.0, 69.8, 102.2, 118.1, 120.6, 121.4, 126.2, 127.0, 129.3, 130.7, 131.8, 134.0, 136.4, 143.6, 144.1, 147.3, 158.3 ppm, Chemical fomula: $C_{27}H_{31}N_2O_2$.

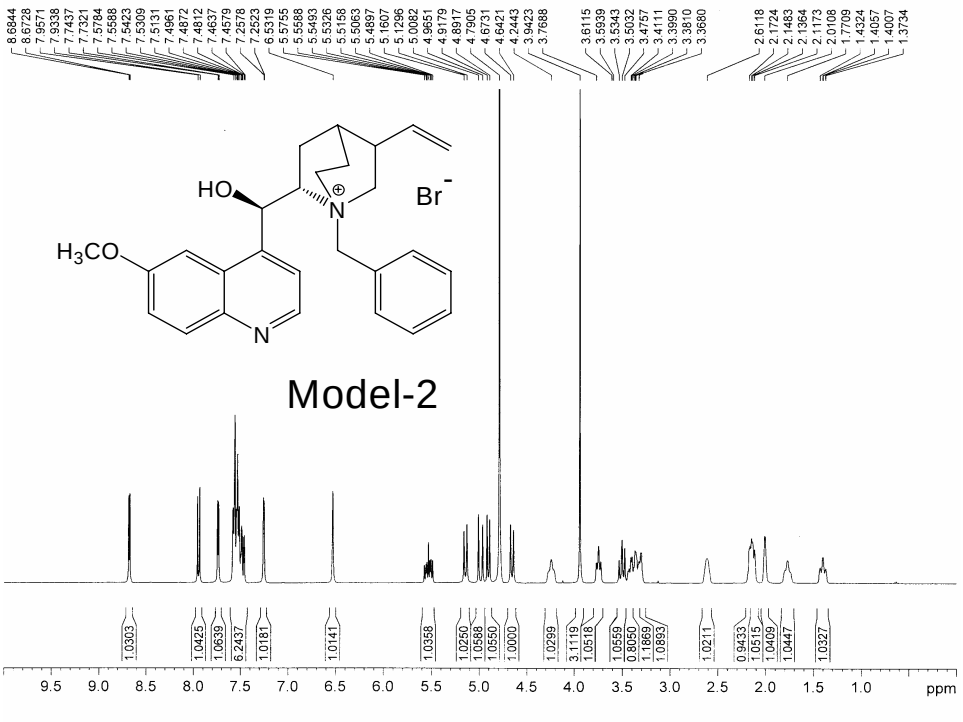


Figure S10. ^{1}H NMR of **Model-2**

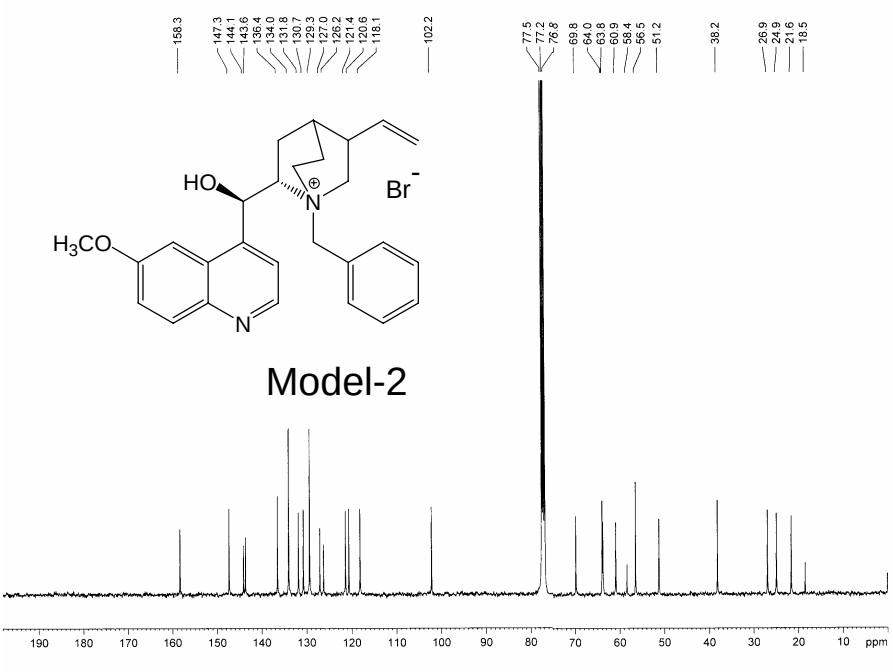
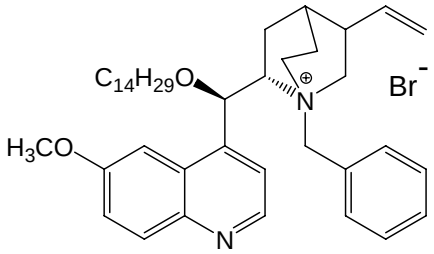


Figure S11. ^{13}C NMR of **Model-2**

Synthesis of Model-3



Model-3

O-$C_{14}H_{29}$ quinine (1.06 g, 2 mmol) was dissolved in 10.0 mL CH_3OH, and 0.2 mL benzyl bromide was added into the solution. The mixture was stirred at room temperature overnight. After the reaction was complete, the reaction residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 100:8) to give **Model-3** (0.45 g, yield 32%) as a colorless solid.

^{1}H NMR (400 MHz, CD_3OD): δ 0.88 (t, 3H),1.32-1.50 (m, 22H), 1.97-1.84 (m, 2H), 2.12(s, 1H), 2.35 (t, 2H), 2.54 (s, 1H), 2.71-2.69 (m, 1H), 3.48-3.46 (m, 1H), 3.52-3.50 (m, 2H), 3.64-3.60 (m,1H), 3.92-3.80 (m, 2H), 4.04(s, 3H), 4.22-4.18(t, 1H), 4.72-4.69 (d, 2H), 5.16-5.02 (m, 2H), 5.40-5.34(d, 1H), 5.80-5.65(m, 1H), 6.40 (s, 1H), 7.40 (s, 1H),

7.68-7.52 (m, 6H), 7.77(d, 1H), 8.05-8.07(d, 1H), 8.81(d, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 14.3, 22.8, 25.5, 26.7, 27.3, 29.5, 29.6, 29.8, 30.2, 32.0, 38.1, 51.4, 59.6, 62.7, 70.0, 118.6, 127.4, 129.4, 130.7, 131.5, 134.2, 136.4 ppm, Chemical fomula: $C_{41}H_{59}N_2O_2^+$. ESI-MS m/z = 611.5 M^+, calcd for $C_{41}H_{59}N_2O_2^+$ 611.46.

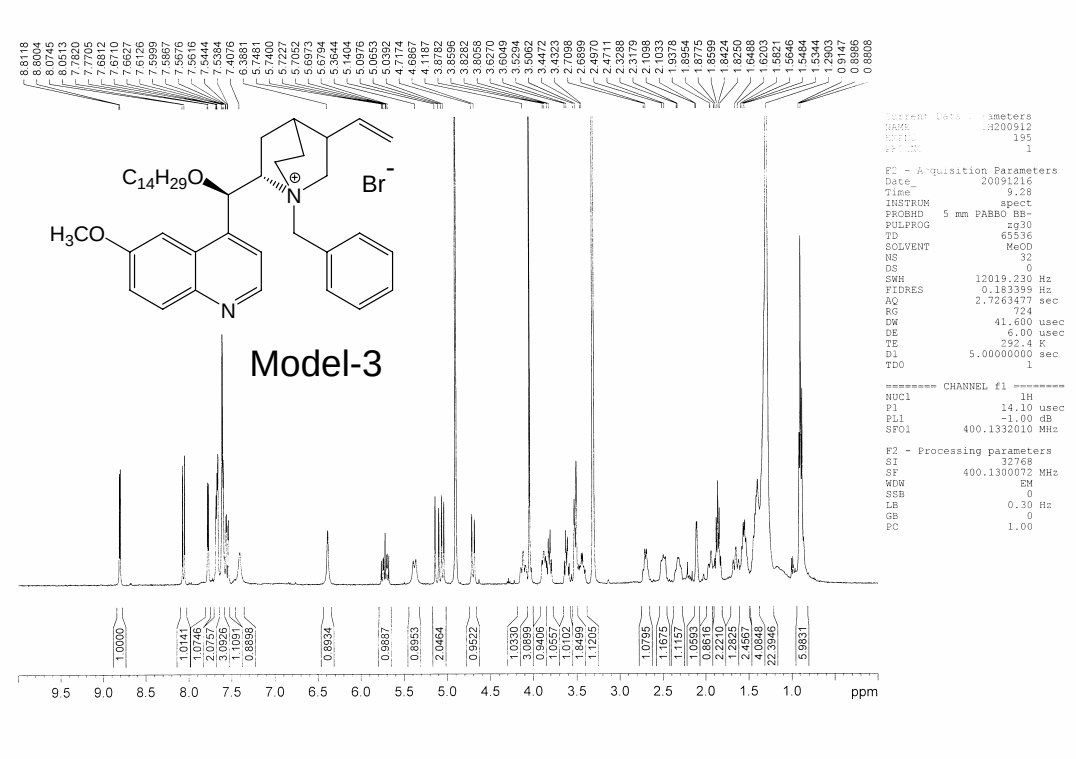


Figure S12. ^{1}H NMR of **Model-3**

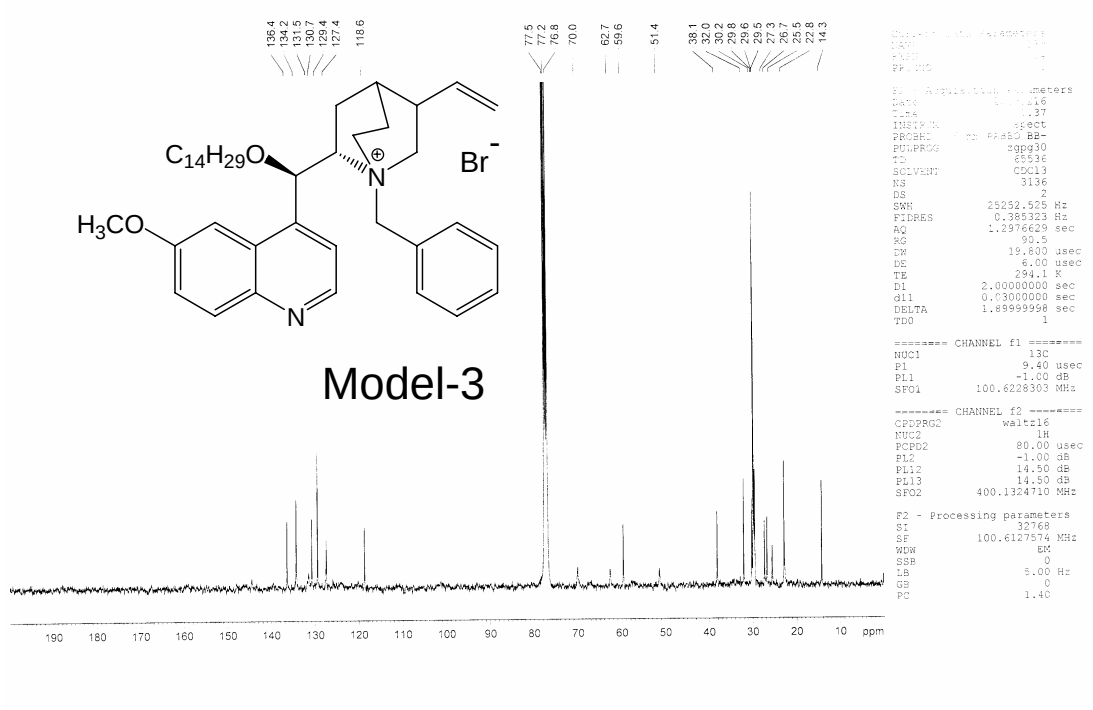


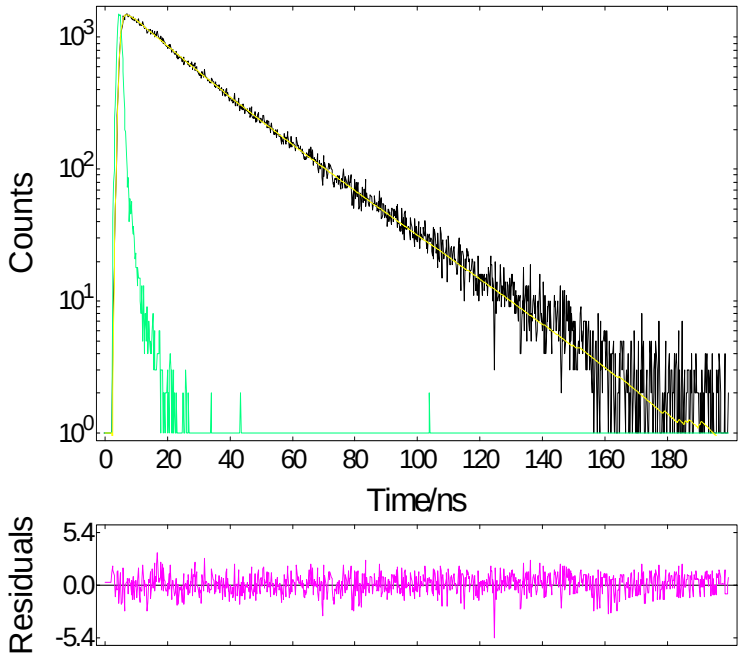
Figure S13. ^{13}C NMR of **Model-3**

Fluorescence spectra detection of carbenicillin

For the fluorescence detection of carbenicillin, a buffer solution of QA [5.0×10^{-6} M in HEPES buffer solution (10.0mM, pH = 7.4)] and aliquots of carbenicillin stock solution were carefully mixed for fluorescence spectral measurement.

Determination of fluorescence quenching rate

A buffer solution of **QA** [5.0×10^{-6} M in HEPES buffer solution (10.0 mM, pH = 7.4)] and aliquots of carbenicillin stock solution were carefully mixed for fluorescence spectral measurements. The quenching rate k_q was calculated following the Stern-Volmer equation: $I_0/I = 1 + k_q\tau_0$ [QA]



Fit Parameters

Fit = A + B*exp (-t/τ_0) τ_0 = 26.2 ns

Figure S14. Time-resolved emission spectra of **QA**

Job's Plot

In order to determine the complex ratio of **QA** and carbenicillin, 50 mL **QA** at he concentration of 5.0 $\times 10^{-5}$ M and 50 mL carbenicillin solution (5.0 $\times 10^{-5}$ M) were prepared in HEPES buffer solution (10.0 mM, pH = 7.4). According to the volume ratio of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 between **QA** and carbenicillin, the two solutions were mixed and the fluorescent spectra were measured. The fluorescence intensity at 458.6nm was recorded and was plotted as the fraction of carbenicillin.

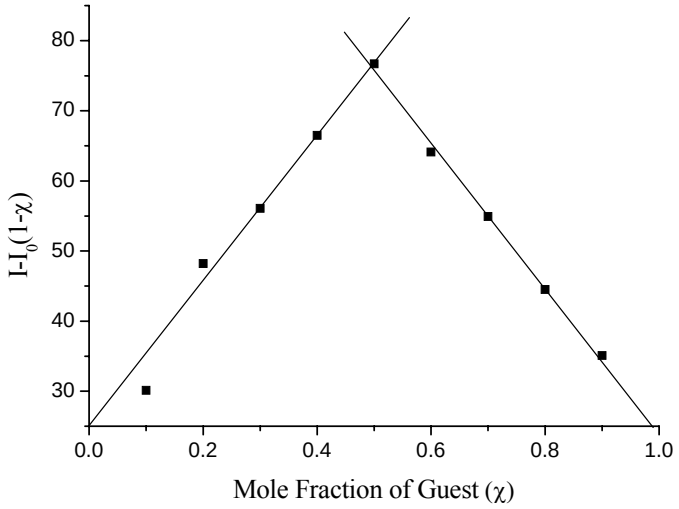


Figure S15. The Job's plot of sensor and guest (c = 5.0 x 10^{-5} M)

Determination of the association constant (*K*s)

1:1 Association constants of **QA** and **Model-1** with anions are calculated by nonlinear least-squares curve fitting using the following equation in Origin 6.0:

$$I / I_0 = 1 + \frac{I_{\lim} / I_0 - 1}{2}\left[1 + \frac{C_A}{C_H} + \frac{1}{K_S C_H} - \sqrt{\left(1 + \frac{C_A}{C_H} + \frac{1}{K_S C_H}\right)^2 - 4\frac{C_A}{C_H}}\right]$$

Where I_0 is fluorescent intensity of host without anions, I_{lim} is fluorescent intensity reaching a limitation by adding excessive anions, C_A is the concentration of anions added, and C_H is the concentration of host molecule.

By allowing $1/K_aC_H$ and I_{lim}/I_0 to be floating parameters, we can obtain the K_a and I_{lim}/I_0 values by a nonlinear least-squares analysis of I/I_0 versus C_A/C_H.

CD measurement

CD spectra were recorded on a JASCO J-815 spectrodichrometer using 1.0 cm quartz cell for 5.0x10^{-4} M **QA** in methanol solution. The changes of CD signal in the presence of carbenicillin were recorded. The concentrations of carbenicillin in the tested **QA** solution (5.0x10^{-4} M) were 0, 2.5x10^{-3} M and 5.0x10^{-3} M, respectively, which were corresponding to 0 equiv., 5 equiv. and 10 equiv. of **QA**.

Sensor selectivity test

To check the selectivity of sensor ($5.0x10^{-6}$ M) for carbenicillin, other typical antibiotics and some important biological molecules were tested. The concentration of antibiotics in the sensor solution was at $7.5x10^{-5}$ M, including amoxicillin, ampicillin sodium, streptomycin sulfate, penicillin, proctaphlin sodium (Proc) and chloromycetin. Some carboxyl acids were also examined, such as acetic sodium, oxalic sodium, malonic acid (malonic), (+)-tartatic acid, glutaric acid, terephthalic acid (teteph), aspartic acid, adipic acid (adipic), the concentration was $7.5x10^{-5}$ M in 10 mM HEPES buffer solution (pH = 7.4). Phosphate, pyrophosphate (PPi), sufide and adenine triphosphate (ATP) were at $7.5x10^{-5}$ M in 10 mM HEPES buffer solution (pH = 7.4). The solution of sensor with was carefully mixed with $7.5x10^{-5}$ M carbenicillin for fluorescence spectral measurements. For all measurements, excitation was at 375 nm. Both excitation and emission slit widths were 2 nm.

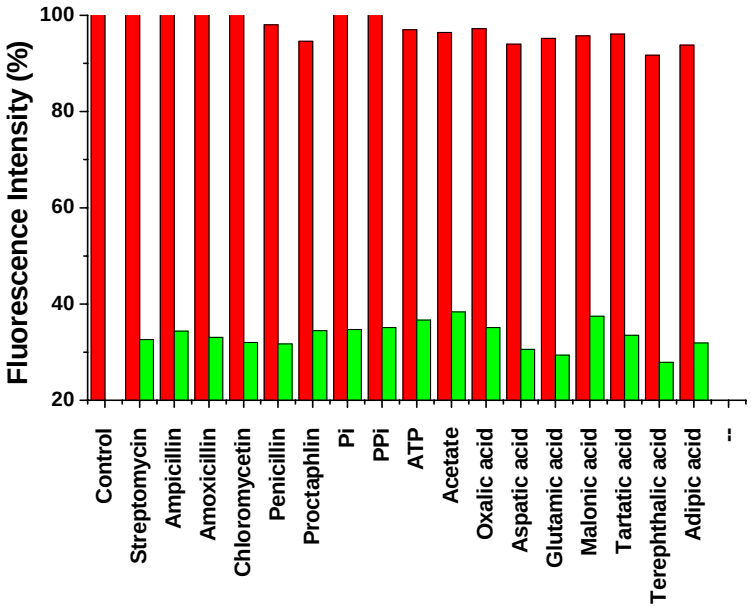
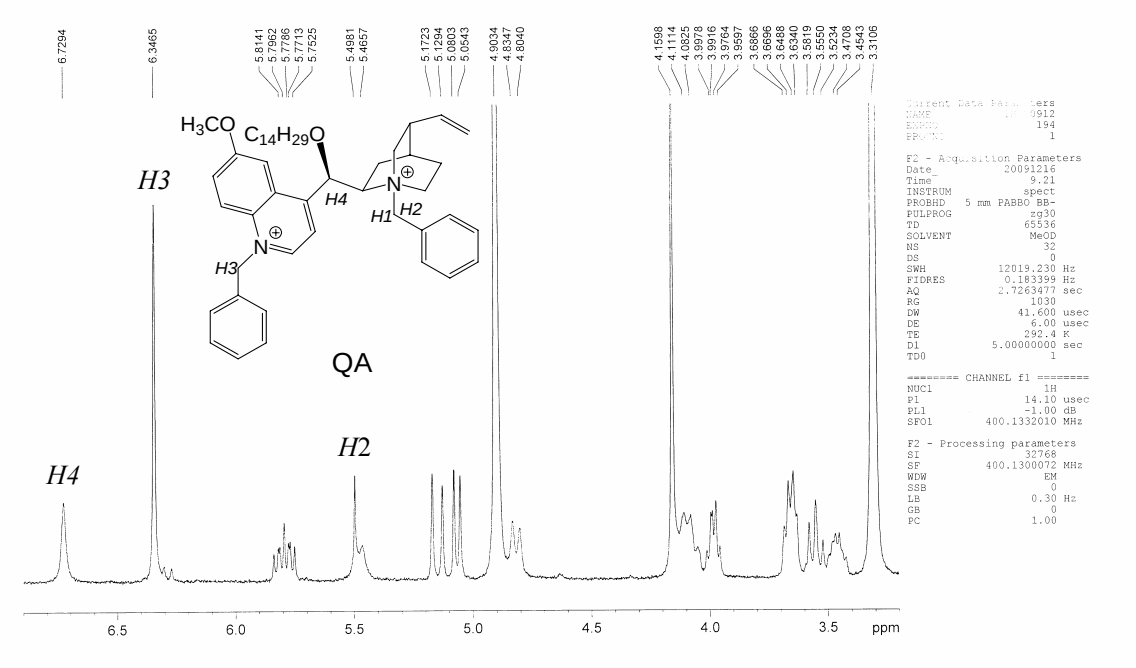


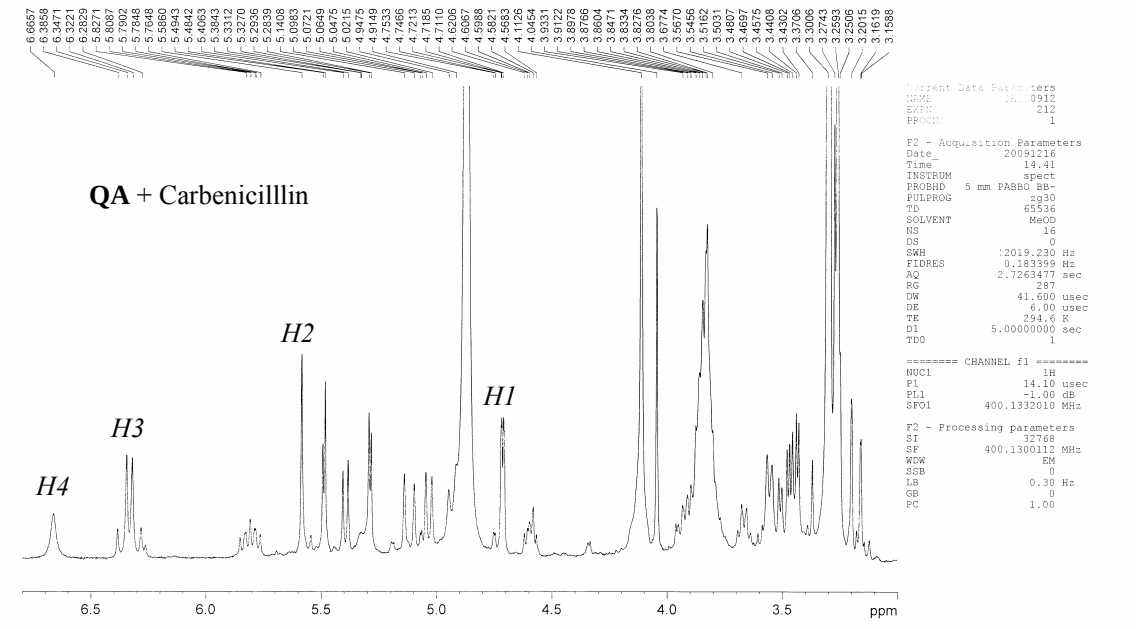
Figure S16. The fluorescence responses of **QA** (5 μM) to carbenicillin. Red bar represent the fluorescence intensity of **QA** in the presence of 15 equiv of streptomycin, ampicillin, amoxicillin, chloromycetin, penicillin, proctaphlin sodium, phosphate, pyrophosphate, ATP (adenosine triphosphate), acetate, oxalic acid, aspartic acid, glutamic acid, malonic acid, tartatic acid, terephthalic acid and adipic acid in 10.0 mM HEPES buffer pH 7.40.

Green bar represent the fluorescence intensity of **QA** in the presence of indicated species, followed by 15 equiv of carbenicill.

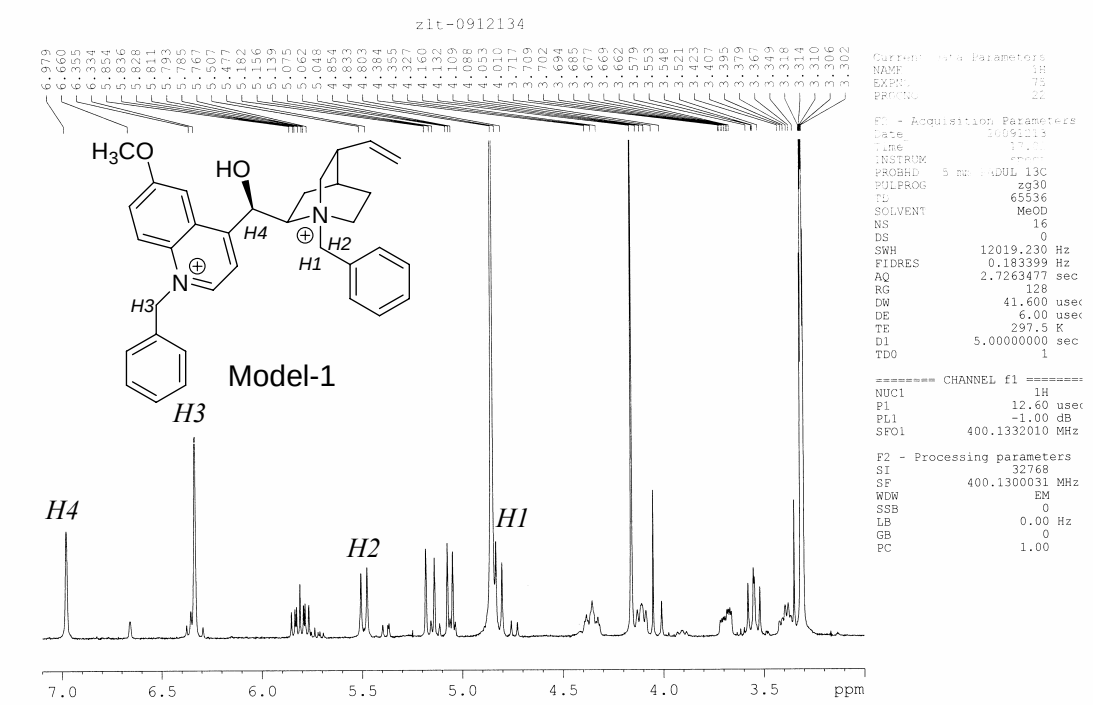
^{1}NMR titration



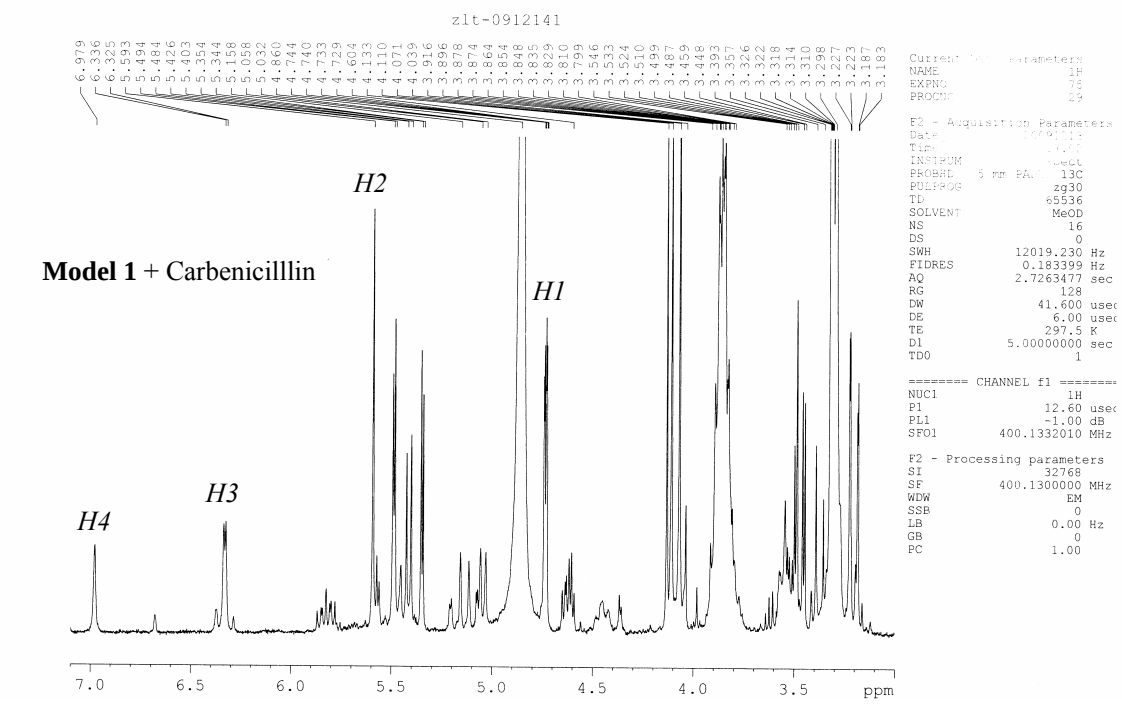
(a) ^{1}H NMR chemical shift of QA in the absence of carbenicillin (solvent: CD_3OD)



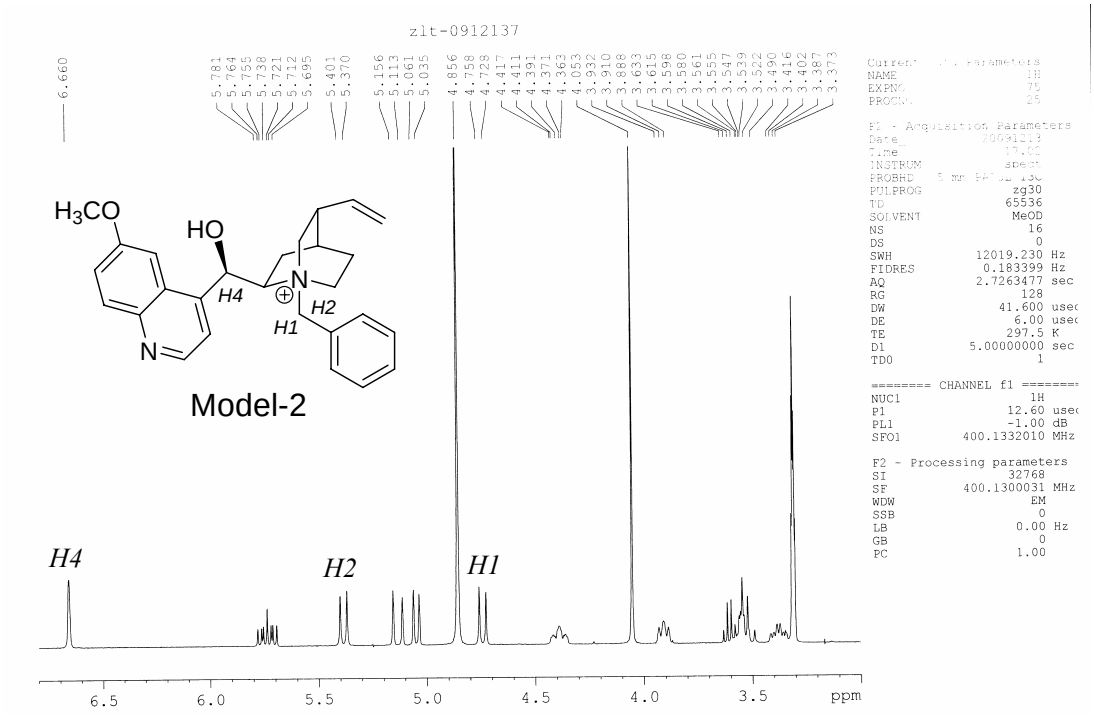
(b) ^{1}H NMR chemical shift of QA in the presence of carbenicillin (solvent: CD_3OD)



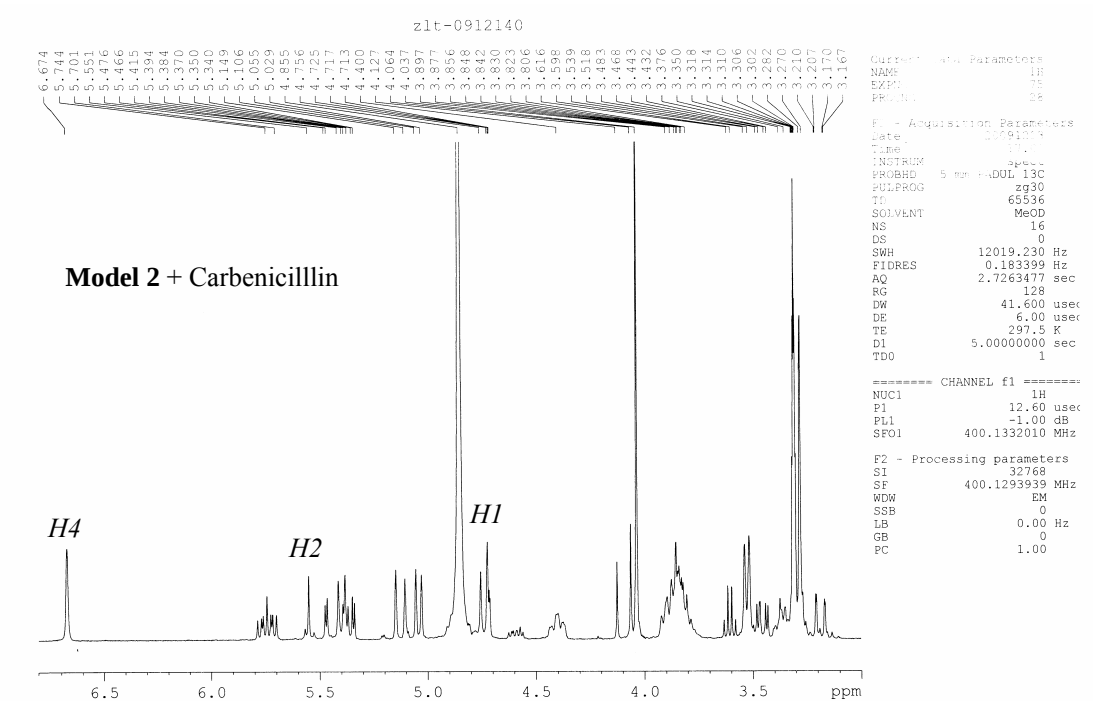
(c) ^{1}H NMR chemical shift of Model-1 in the absence of carbenicillin (solvent: CD_3OD)



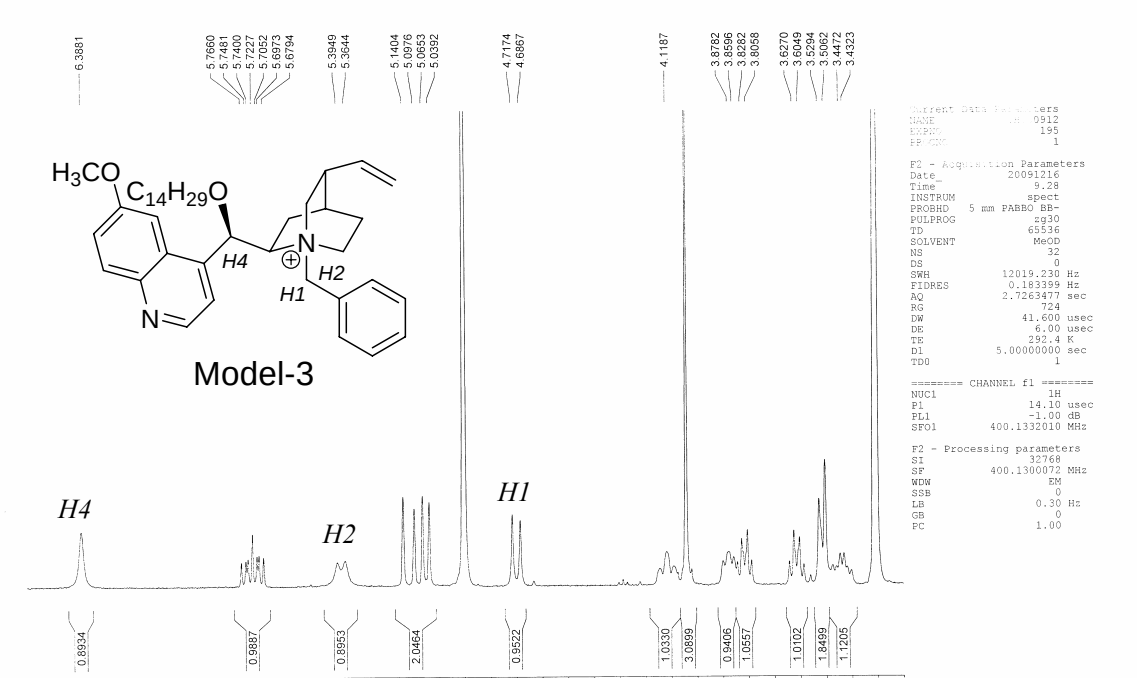
(d) ^{1}H NMR chemical shift of Model-1 in the presence of carbenicillin (solvent: CD_3OD)



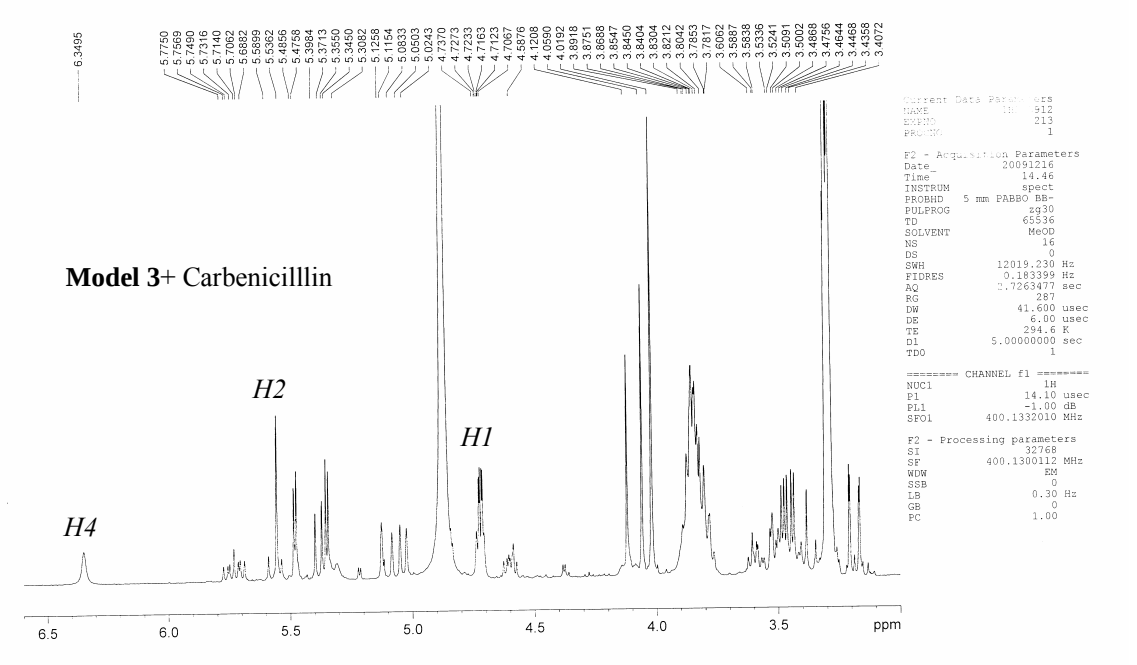
(e) ^{1}H NMR chemical shift of Model-2 in the absence of carbenicillin (solvent: CD_3OD)



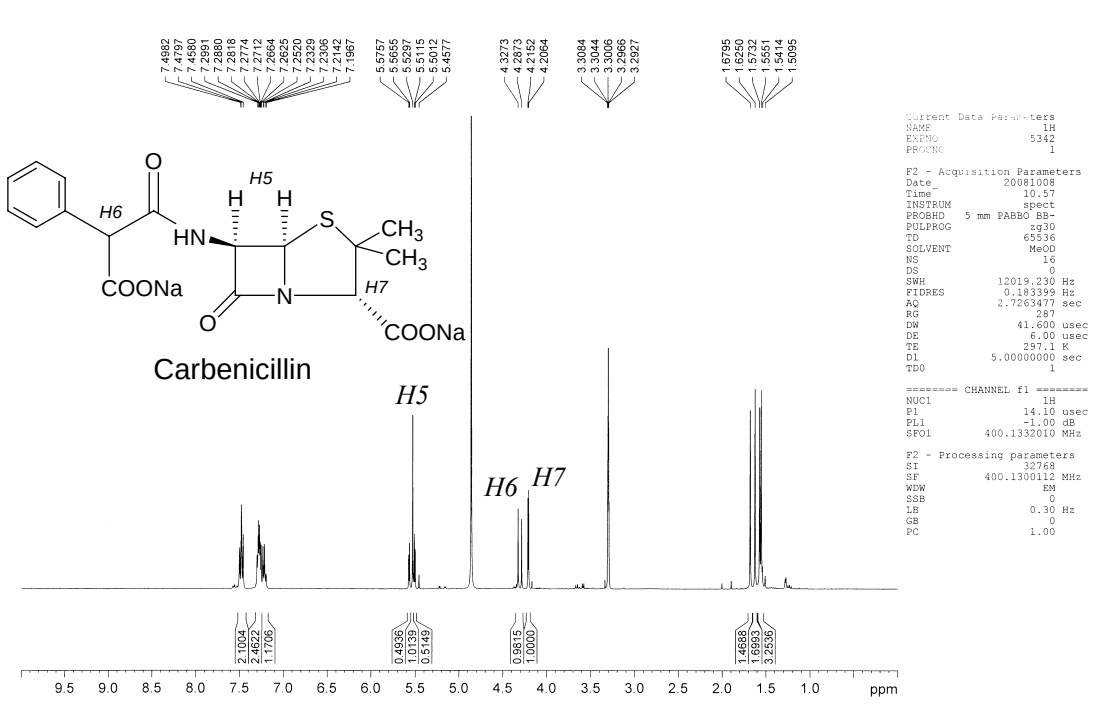
(f) ^{1}H NMR chemical shift of Model-2 in the presence of carbenicillin (solvent: CD_3OD)



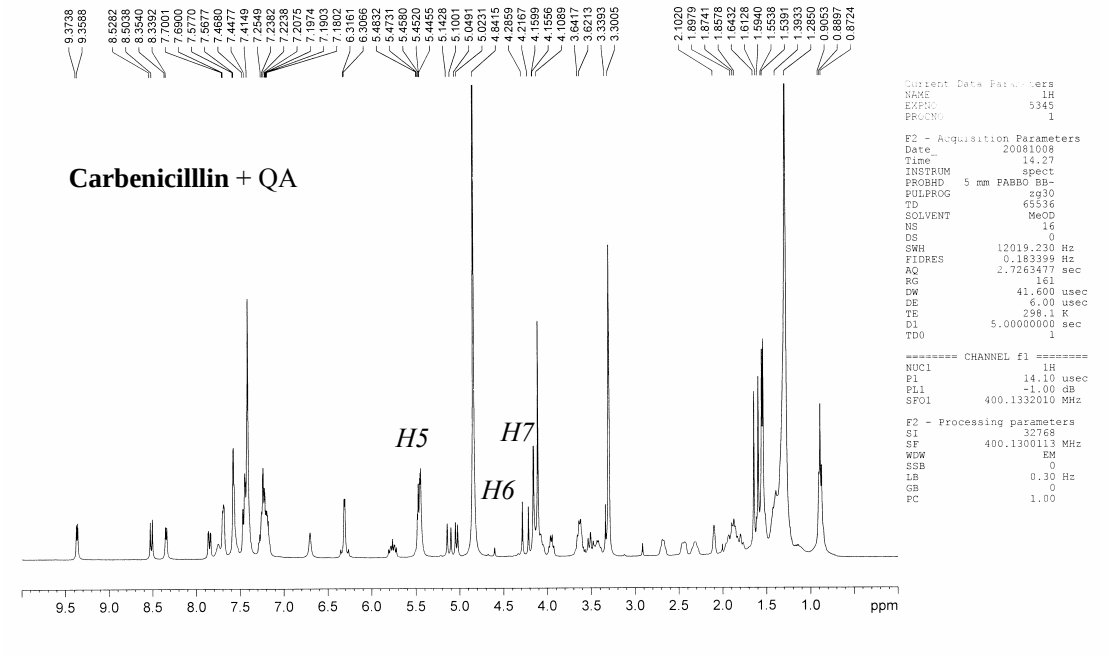
(g) ^{1}H NMR chemical shift of Model-3 in the absence of carbenicillin (solvent: CD_3OD)



(h) ^{1}H NMR chemical shift of Model-3 in the presence of carbenicillin (solvent: CD_3OD)



(j) ^{1}H NMR chemical shift of carbenicillin in the absence of QA (solvent: CD_3OD)



(j) ^{1}H NMR chemical shift of carbenicillin in the presence of QA (solvent: CD_3OD)

Figure S17. ^{1}H NMR chemical shifts of **QA** and model compounds in the presence of carbenicillin in CD_3OD.

Supporting Information References

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(S2) M. Greenwald, D. Wessely, E. Katz, I. Willner and Y. Cohen, *J. Org. Chem.*, 2000, **65**, 1050.

(S3) Z.Q. Guo, W.H. Zhu, L.J. Shen, H. Tian, *Angew. Chem., Int. Ed.,* 2007, **46**, 5549.