

Supporting Information

Practical and scalable one-pot synthesis of Arbekacin

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1. Density functional theory (DFT) calculation of Dibekacin

1.1 Calculation of N-H bond length of Dibekacin molecule

All calculations were performed in the gas phase using the density functional theory (DFT) calculation method. The Gaussian 09 software was utilized to optimize the structure of Dibekacin and minimize its energy value, employing B3LYP/6-31G (d, p) as the basis set. Subsequently, the N-H bond length of the amino group of Dibekacin was determined.

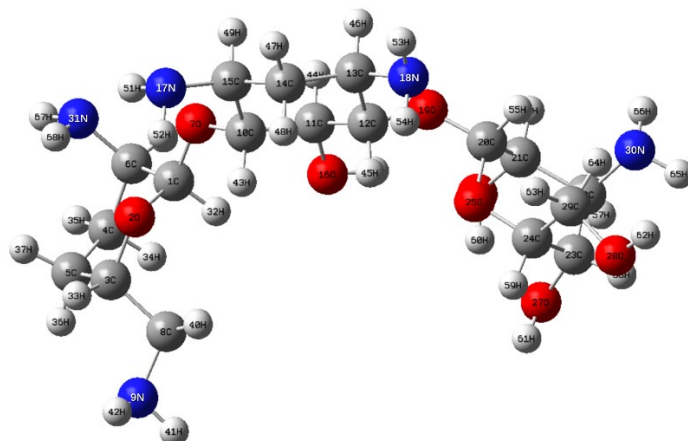


Fig. S1. Ball-and-stick model and atomic number of Dibekacin molecule

Table S1. N-H bond length of Dibekacin molecule

	Bond name	Bond length (pm)
9N	9N-41H	1.03480
	9N-42H	1.03489
17N	17N-51H	1.03535
	17N-52H	1.03492
18N	18N-53H	1.03470
	18N-54H	1.03503
30N	30N-65H	1.03482
	30N-66H	1.03470
31N	31N-67H	1.03535
	31N-68H	1.03500

Cartesian coordinates for the stationary points

C	-3.3375	2.0155	-1.2198
O	-3.3294	3.3521	-0.7638
C	-4.2773	3.6121	0.235
C	-5.7978	1.9042	-0.8592
C	-5.6926	3.3136	-0.265
C	-4.669	1.6906	-1.8762
O	-2.3082	1.9772	-2.1867
C	-3.9163	2.9354	1.5615
N	-4.7038	3.4978	2.6715
C	-1.014	1.909	-1.6447
C	-0.489	0.4818	-1.6715

C	0.9547	0.3574	-1.183
C	1.8826	1.2857	-1.9752
C	1.3363	2.7251	-1.9921
C	-0.1046	2.7851	-2.508
O	-1.2776	-0.3425	-0.8444
N	-0.6047	4.1718	-2.4698
N	3.2272	1.3652	-1.3732
O	1.235	-1.0119	-1.3214
C	2.4612	-1.4977	-0.824
C	2.4626	-3.0075	-0.9517
C	3.7189	-3.6011	-0.324
C	3.8884	-3.0487	1.0893
C	3.7181	-1.5355	1.1698
O	2.5416	-1.1207	0.5298
O	1.3334	-3.5425	-0.2969
O	2.9111	-3.6424	1.9152
O	6.0393	-1.0415	1.5348
C	4.9211	-0.7068	0.7377
N	4.8976	-3.2953	-1.1507
N	-4.8727	2.5571	-3.052
H	-3.1171	1.3054	-0.3966
H	-4.2103	4.715	0.4208
H	-5.7181	1.1438	-0.0472
H	-6.7927	1.7568	-1.3417
H	-6.4366	3.4435	0.555
H	-5.9626	4.056	-1.0544
H	-4.6782	0.6293	-2.2255
H	-4.0936	1.8373	1.5332
H	-2.8365	3.0973	1.79
H	-4.4106	3.0747	3.5692
H	-4.5136	4.5098	2.7748
H	-0.9943	2.303	-0.6023
H	-0.5723	0.0734	-2.7064
H	0.9731	0.6411	-0.1049
H	1.9808	0.9149	-3.0235
H	1.9868	3.3695	-2.6306
H	1.3713	3.1478	-0.9597
H	-0.1361	2.4379	-3.5692
H	-0.8733	-1.2152	-0.8238
H	-1.587	4.1927	-2.7963
H	-0.6473	4.5148	-1.4943
H	3.7741	2.1229	-1.8175
H	3.1579	1.6303	-0.3751
H	3.2918	-1.0742	-1.4216
H	2.3837	-3.3066	-2.0236
H	3.6404	-4.715	-0.2935
H	4.8688	-3.368	1.5109
H	3.5654	-1.2818	2.2511
H	1.4327	-3.4123	0.6496
H	3.1925	-3.6075	2.8314
H	6.7927	-0.5108	1.2667
H	4.7326	0.3803	0.8872
H	5.2213	-0.8614	-0.3182
H	5.7483	3.7164	-0.7386
H	4.8053	-3.7311	-2.0846
H	-5.874	2.5907	-3.3106
H	-4.6132	3.5332	-2.8259

1.2 Fukui function calculation

All calculations were performed in the gas phase using Gaussian 09 software at the B3LYP/6-31G (d, p) theoretical level. The charge distribution of natural atoms was calculated at 298K. To assess the degree of unpaired spin and charge at each site of the Dibekacin molecule, the electron spin density based on natural bond orbital (NBO) analysis was utilized. The regioselectivity of Dibekacin molecules was explained using the Fukui index.

Fukui function is defined as:

$$f(r) = \left[\frac{\partial \rho(r)}{\partial N} \right]_v$$

In the formula, $\rho(r)$ represents the electron density at the point r in space. N denotes the number of electrons in the system, and the constant term v in the partial derivative represents the external potential. The condensed version of the Fukui function utilizes the atomic number to quantify the distribution of electron density around the atom. The concentrated Fukui function can be calculated as follows:

$$\text{Nucleophilic attack: } f_k^+ = q_N^k - q_{N+1}^k$$

$$\text{Electrophilic attack: } f_k^- = q_{N-1}^k - q_N^k$$

$$\text{Radical attack : } f_k^0 = (q_{N-1}^k - q_{N+1}^k) \div 2$$

Where q_N^k is the atomic charge of k atom in the corresponding state. One of the most suitable methods for calculating the Fukui index is the natural population analysis (NPA) charge, which is used to study the reaction sites.³ Microsoft Excel 2019 is used to sort the calculated values, and the results are shown in Table S2:

Table S2 : Natural Population Analysis (NPA) charge distribution on Dibekacin at different electron state and calculated Fukui index (f^-) levels.

Atom	Charge (+1) ($e/\text{\AA}^3$)	Charge (0) ($e/\text{\AA}^3$)	Charge (-1) ($e/\text{\AA}^3$)	f^-
N31	-0.90365	-0.89896	-0.73689	0.16207
N17	-0.91523	-0.90851	-0.76145	0.14706
N18	-0.91296	-0.91908	-0.82617	0.09291
N9	-0.91654	-0.90834	-0.8495	0.05884
N30	-0.93352	-0.92372	-0.86609	0.05763
H57	0.22674	0.25398	0.28187	0.02789
H67	0.35069	0.37311	0.40078	0.02767
H38	0.26053	0.26931	0.29549	0.02618
O26	-0.77832	-0.76112	-0.73663	0.02449
H34	0.2329	0.24103	0.2625	0.02147
H49	0.25132	0.25929	0.27893	0.01964
H52	0.36451	0.36958	0.38893	0.01935
H53	0.36588	0.38356	0.40186	0.0183
H35	0.22228	0.24991	0.2681	0.01819

H47	0.24669	0.26209	0.27941	0.01732
H4	0.33224	0.3864	0.4034	0.017
H36	0.26228	0.27668	0.29351	0.01683
H65	0.334	0.38577	0.40233	0.01656
H46	0.24874	0.25656	0.27279	0.01623
H68	0.3683	0.38175	0.39754	0.01579
O19	-0.59683	-0.58959	-0.57395	0.01564
H40	0.18472	0.21722	0.23249	0.01527
H51	0.39543	0.40123	0.41549	0.01426
H54	0.36154	0.37778	0.39172	0.01394
H33	0.21304	0.24615	0.25995	0.0138
H42	0.31463	0.37363	0.38703	0.0134
H58	0.24148	0.25226	0.26556	0.0133
H59	0.24842	0.2562	0.26913	0.01293
H66	0.3026	0.38983	0.40247	0.01264
H32	0.23973	0.23846	0.25077	0.01231
H48	0.21853	0.2276	0.23981	0.01221
H44	0.22911	0.23818	0.24971	0.01153
H56	0.24064	0.25613	0.26752	0.01139
O25	-0.60081	-0.59593	-0.58458	0.01135
H50	0.49649	0.49913	0.50924	0.01011
H43	0.225	0.22731	0.23681	0.0095
H61	0.37218	0.4877	0.49714	0.00944
O28	-0.77509	-0.76761	-0.75862	0.00899
H62	0.41356	0.47682	0.48517	0.00835
H45	0.24349	0.24369	0.25147	0.00778
H39	0.22486	0.23874	0.24503	0.00629
O16	-0.77896	-0.77562	-0.76969	0.00593
H37	0.20582	0.23805	0.24337	0.00532
H60	0.48876	0.49758	0.50214	0.00456
H55	0.24448	0.24734	0.25083	0.00349
C10	0.03632	0.03774	0.04035	0.00261
O7	-0.56981	-0.56343	-0.56129	0.00214
O27	-0.7937	-0.76832	-0.76746	0.00086
O2	-0.60702	-0.60539	-0.6046	0.00079
C20	0.34376	0.3436	0.34439	0.00079
H63	0.16665	0.20756	0.20812	0.00056
C4	-0.49631	-0.49965	-0.49943	0.00022
C11	0.05356	0.05218	0.05019	-0.00199
C29	-0.15814	-0.14838	-0.15106	-0.00268
C21	0.02864	0.03114	0.02815	-0.00299
C5	-0.51043	-0.515	-0.51831	-0.00331
C1	0.35504	0.35495	0.35095	-0.004
C12	0.03388	0.036	0.03199	-0.00401

C23	0.00057	0.03469	0.03032	-0.00437
C24	0.02624	0.0204	0.01544	-0.00496
H64	0.22567	0.243	0.23801	-0.00499
C3	0.0555	0.05225	0.04707	-0.00518
C14	-0.49717	-0.49897	-0.50519	-0.00622
C22	-0.13189	-0.12284	-0.12925	-0.00641
C13	-0.10276	-0.10367	-0.11106	-0.00739
C8	-0.31503	-0.31196	-0.32071	-0.00875
C15	-0.08715	-0.08938	-0.10011	-0.01073
C6	-0.11612	-0.1181	-0.13323	-0.01513

1.3 Bond dissociation energy calculation

All calculations are carried out in the gas phase. The bond dissociation energy was calculated by Gauss09 software at the B3LYP/6-31G (d, p) theoretical level. After measuring the bond understanding energy of these molecules, the single point energy and Enthalpy at 298 K was calculated according to the following equation:

$$\Delta H_{f, 298K}(X - H) = \Delta H_{f, 298K}(X) + \Delta H_{f, 298K}(H) - D_0(X - H)$$

The calculated enthalpy of molecular formation is shown in Table S3:

Table S3. Single point energy and enthalpy of formation of different amino groups after removal of hydrogen protons

Name	Single point energy (Hartree)	Enthalpy (Hartree)	Result 1 ^a (Kcal mol ⁻¹)	Result 2 ^b (Kcal mol ⁻¹)
Dibekacin	-1583.634615	-1583.004776	/	/
H	-0.500273	-0.497912	/	/
9N(-41H)	-1583.054116	-1582.440418	50.39059967	41.73540024
9N(-42H)	-1583.055608	-1582.442205	49.45361651	40.61296758
17N(-51H)	-1583.055188	-1582.441426	49.7177305	41.10226531
17N(-52H)	-1583.059408	-1582.445567	47.06714379	38.5012616
18N(-53H)	-1583.054667	-1582.440617	50.04461781	41.61040634
18N(-54H)	-1583.060068	-1582.445697	46.65209495	38.4196073
30N(-65H)	-1583.05387	-1582.440327	50.54524665	41.92194892
30N(-66H)	-1583.055369	-1582.441433	49.60409911	41.09786854
31N(-67H)	-1583.056484	-1582.442982	48.90355541	40.12492608
31N(-68H)	-1583.054063	-1582.440121	50.42407166	41.79255825

^aThe result is calculated by single point energy. ^bThe result is calculated from the enthalpy of formation.

1.4 Hydrogen bond interaction and conformational inversion speculation of Dibekacin

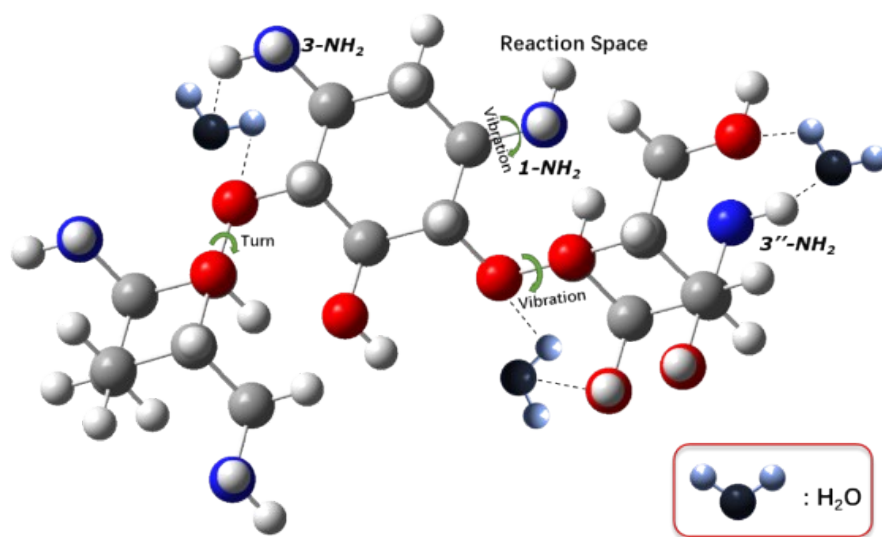


Fig. S2. Speculative twisted view of the structure of Dibekacin

2. Scale-up experiment data

Table S4 The feeding batch of scale-up synthesis and the yield of each component

entry ^a	Dibekacin/g	composition of the crude product by HPLC (%) ^b				
		Arbekacin	1a	1b	Dibekacin	1c
1	100.0	38.8	21.8	5.4	21.5	12.5
2	120.0	40.4	20.1	5.7	22.2	11.6
3	150.0	37.9	21.5	5.7	23.9	11.0

^aUnless indicated otherwise, the specific reaction conditions are the same as those of the pilot-scale optimization. ^bAfter all the reactions were completed, HPLC detection was performed, the results are show in Fig. S27-S29.

3. NMR Spectra, HRMS and HPLC

3.1 NMR Spectra

¹H NMR (600 MHz, D₂O)

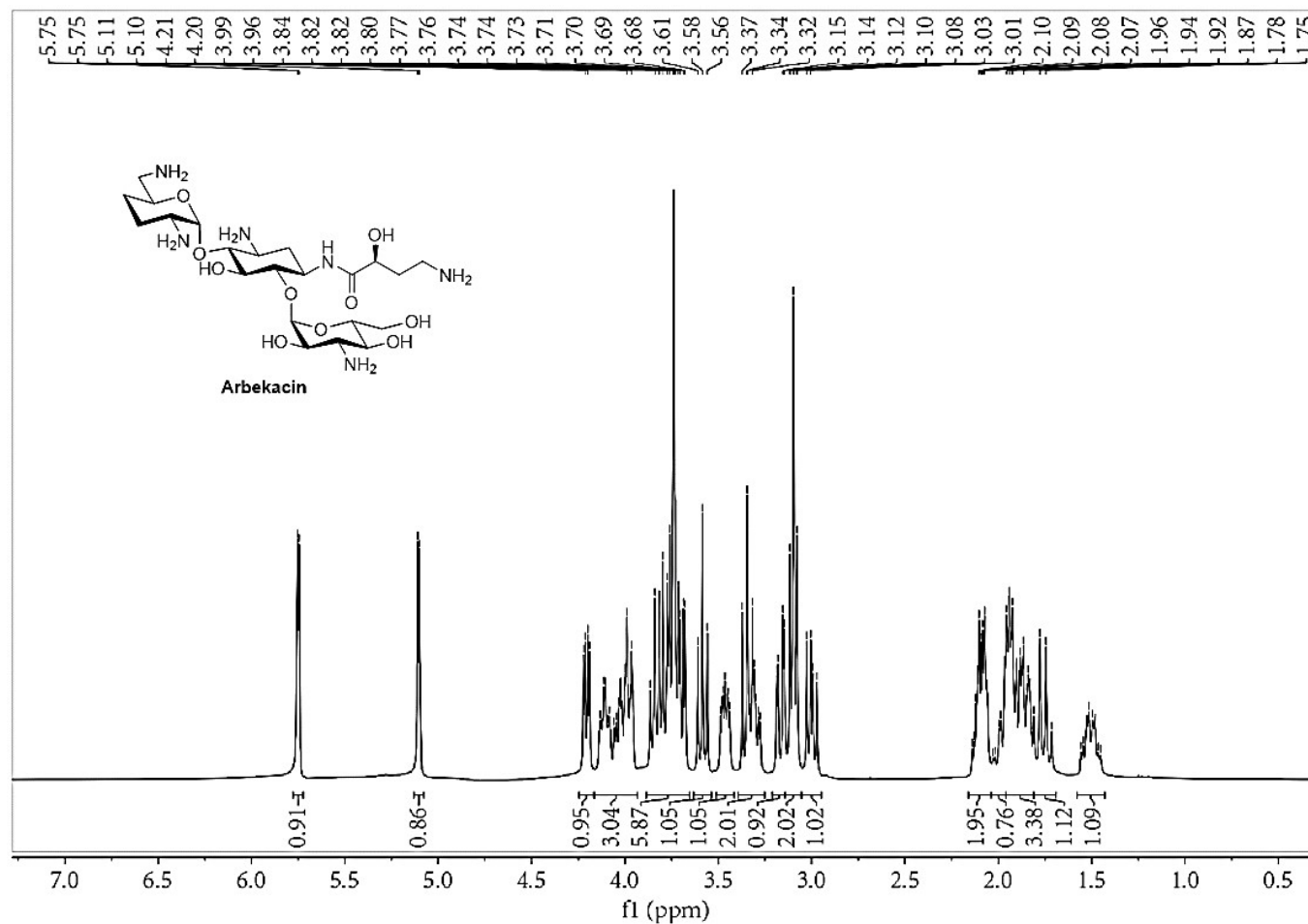


Fig. S3. ¹H NMR of Arbekacin

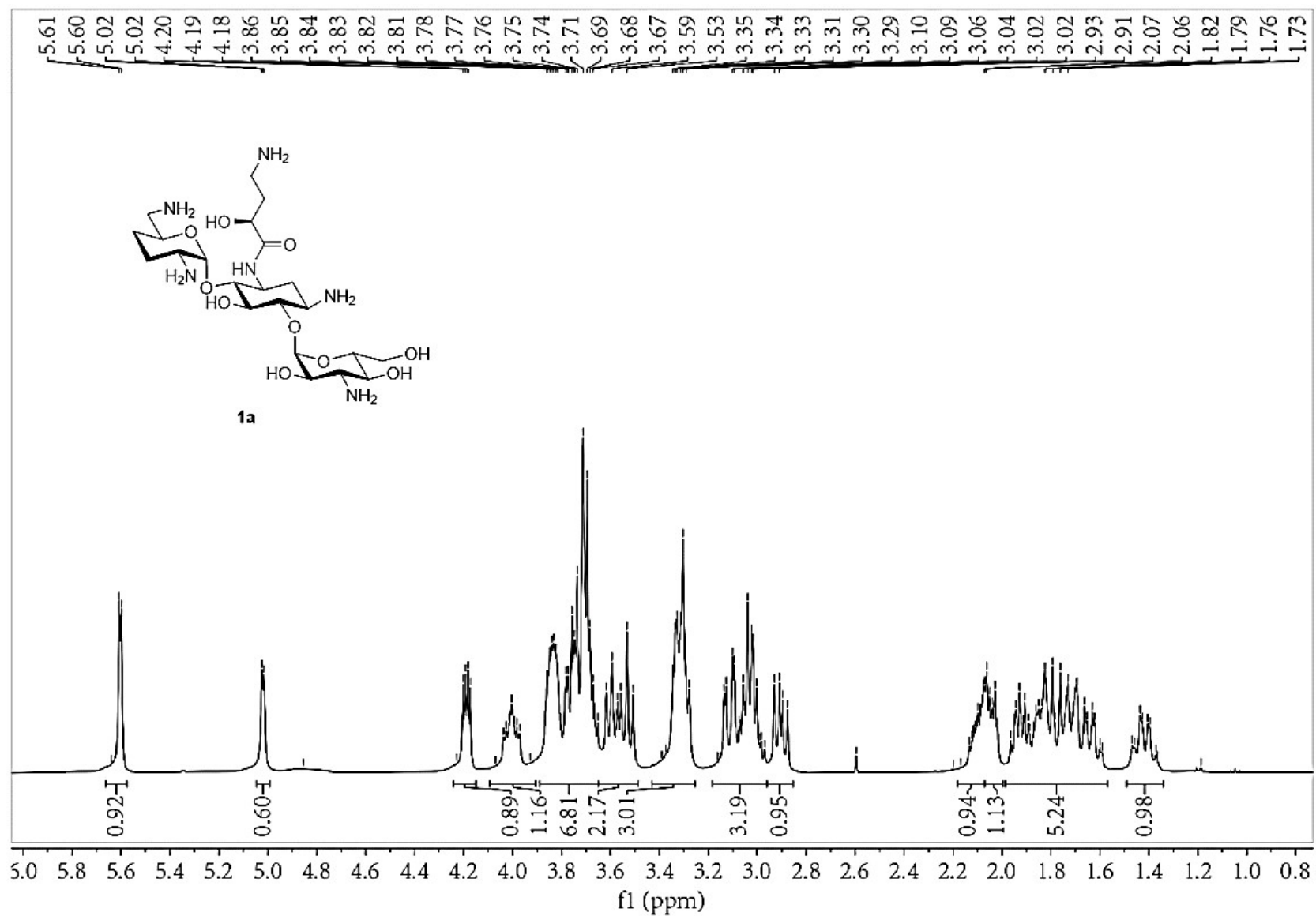


Fig. S4. ^1H NMR of **1a**

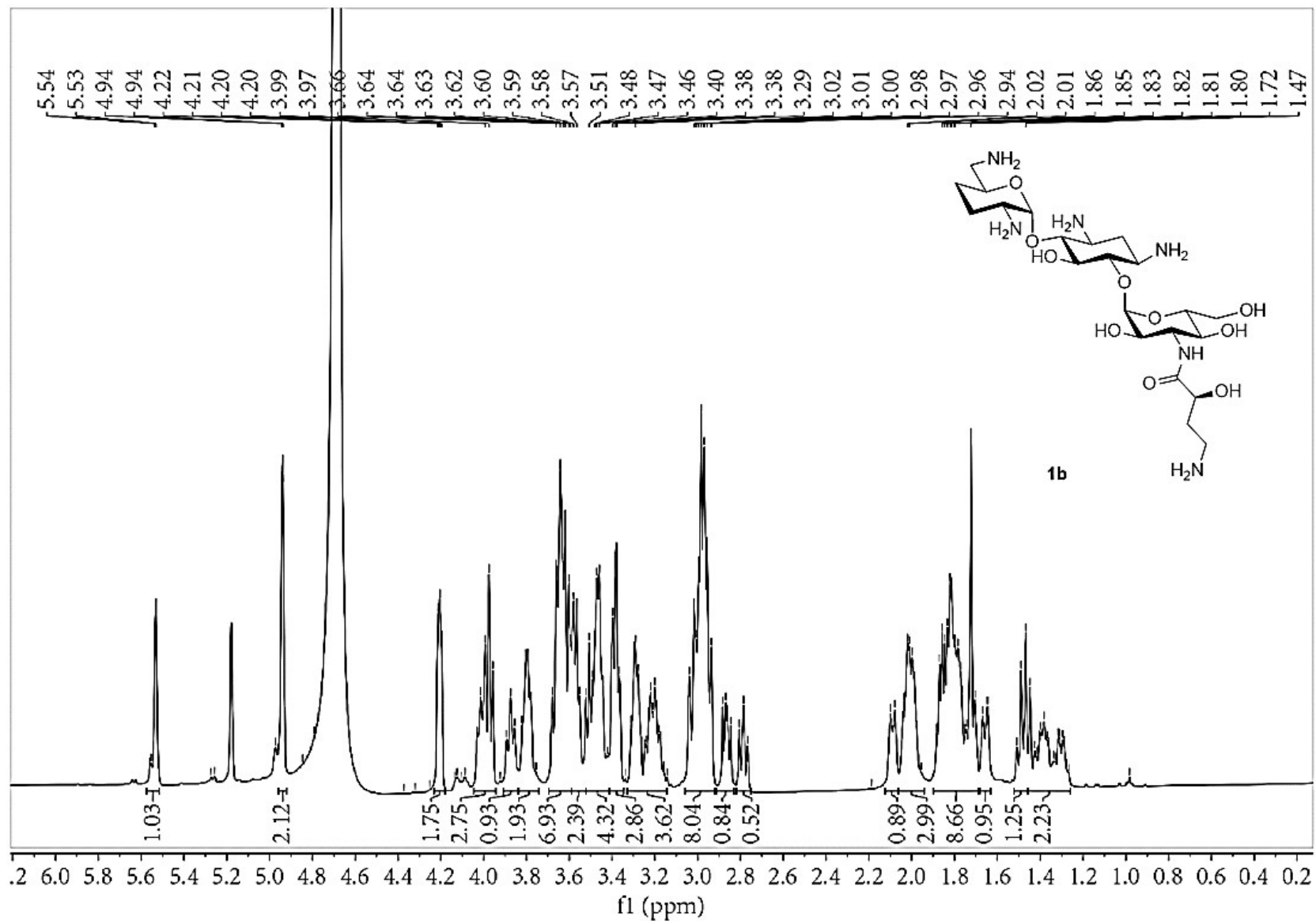


Fig. S5. ^1H NMR of **1b**

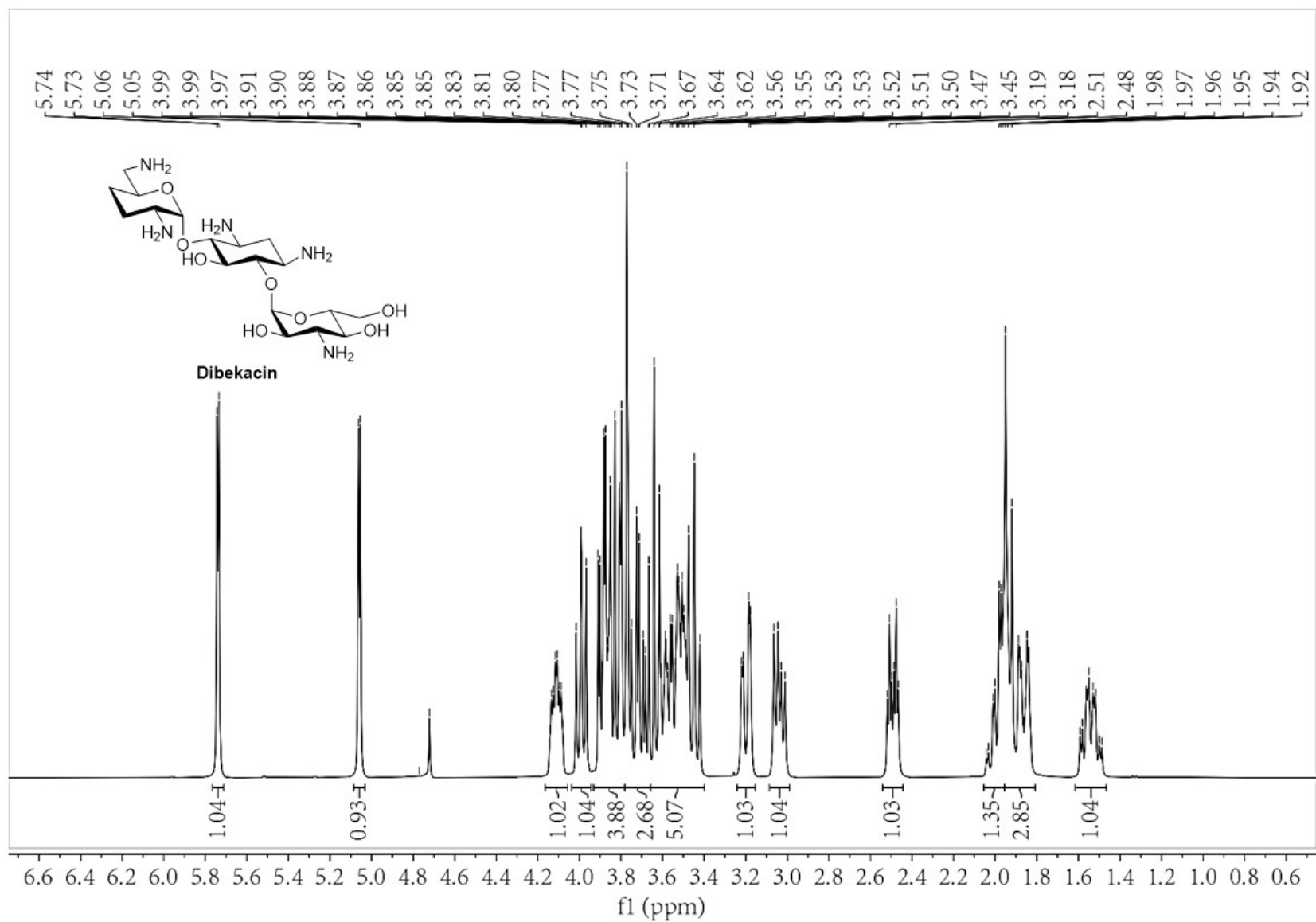


Fig. S6. ¹H NMR of Dibekacin

^{13}C NMR (150 MHz, D_2O)

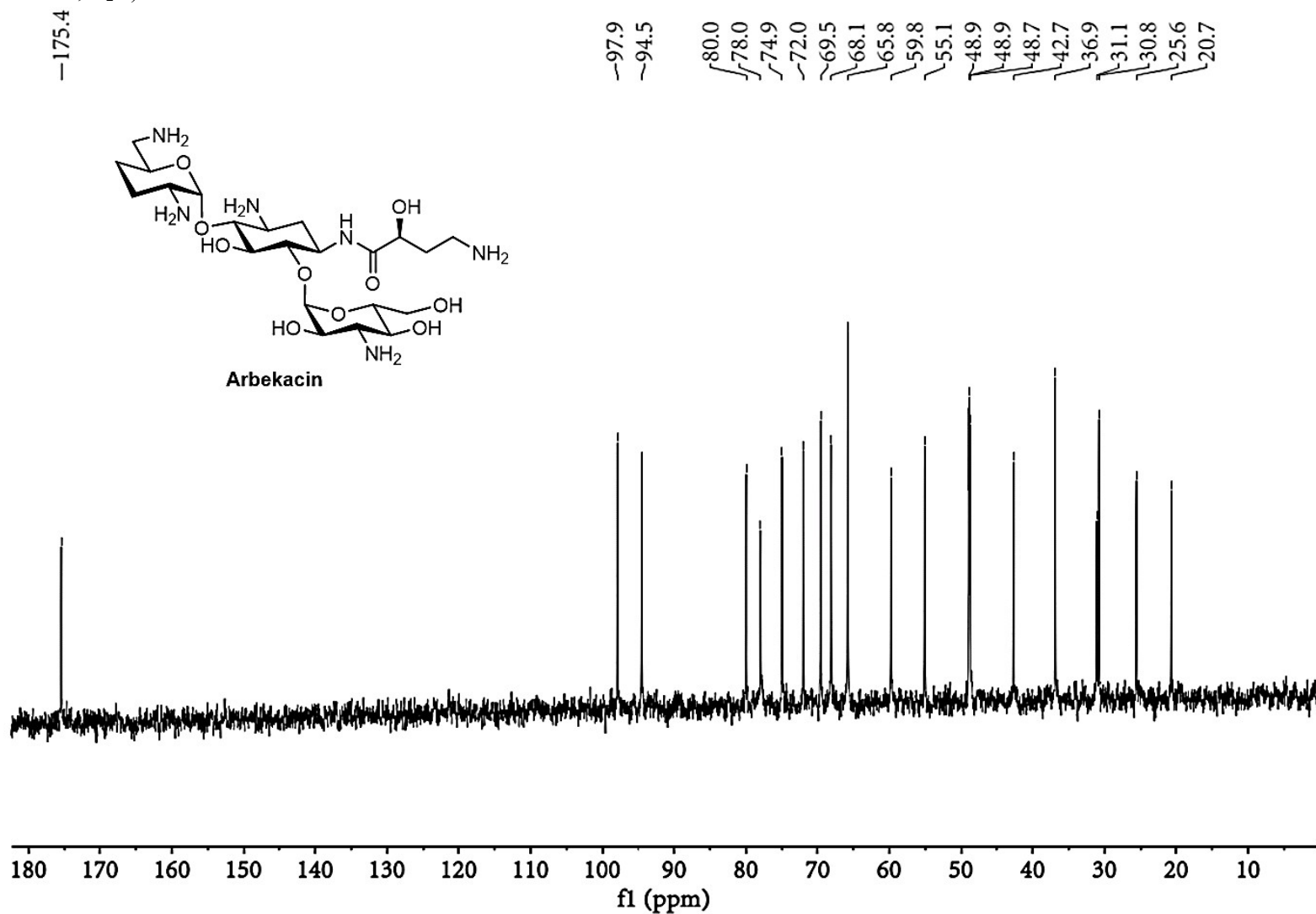


Fig. S7. ^{13}C NMR of Arbekacin

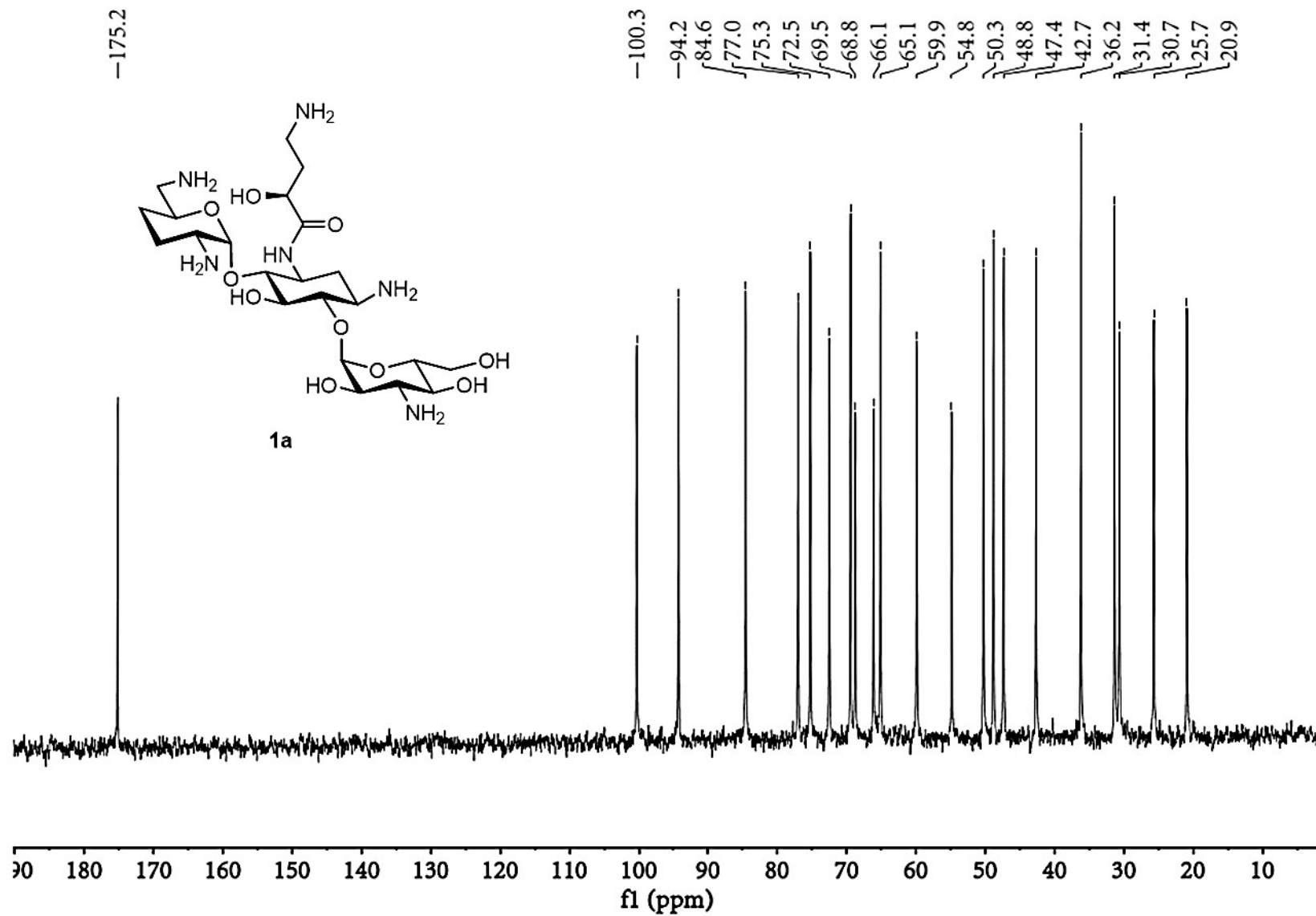


Fig. S8. ^{13}C NMR of 1a

-177.4

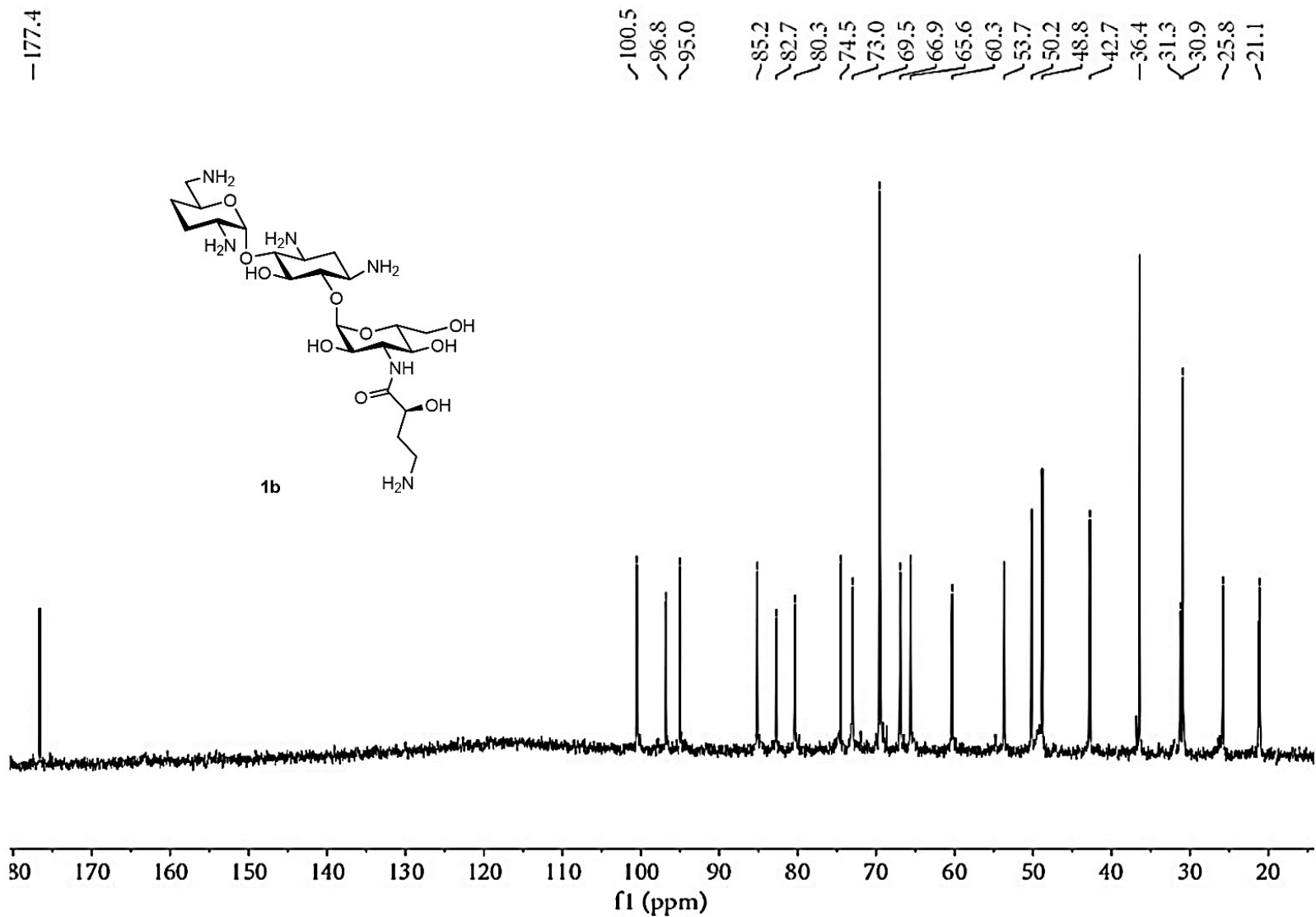


Fig. S9. ¹³C NMR of 1b

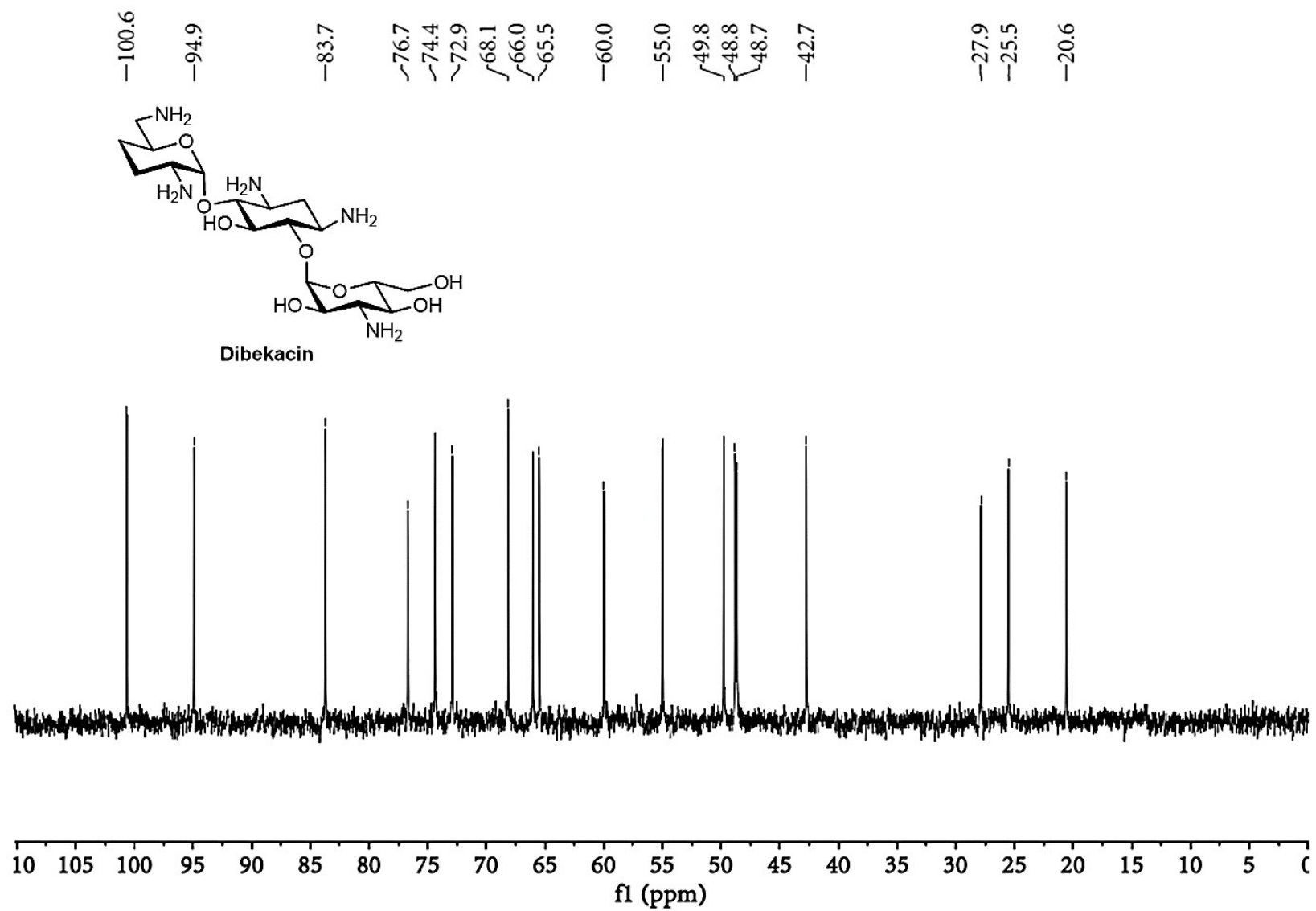


Fig. S10. ^{13}C NMR of Dibekacin

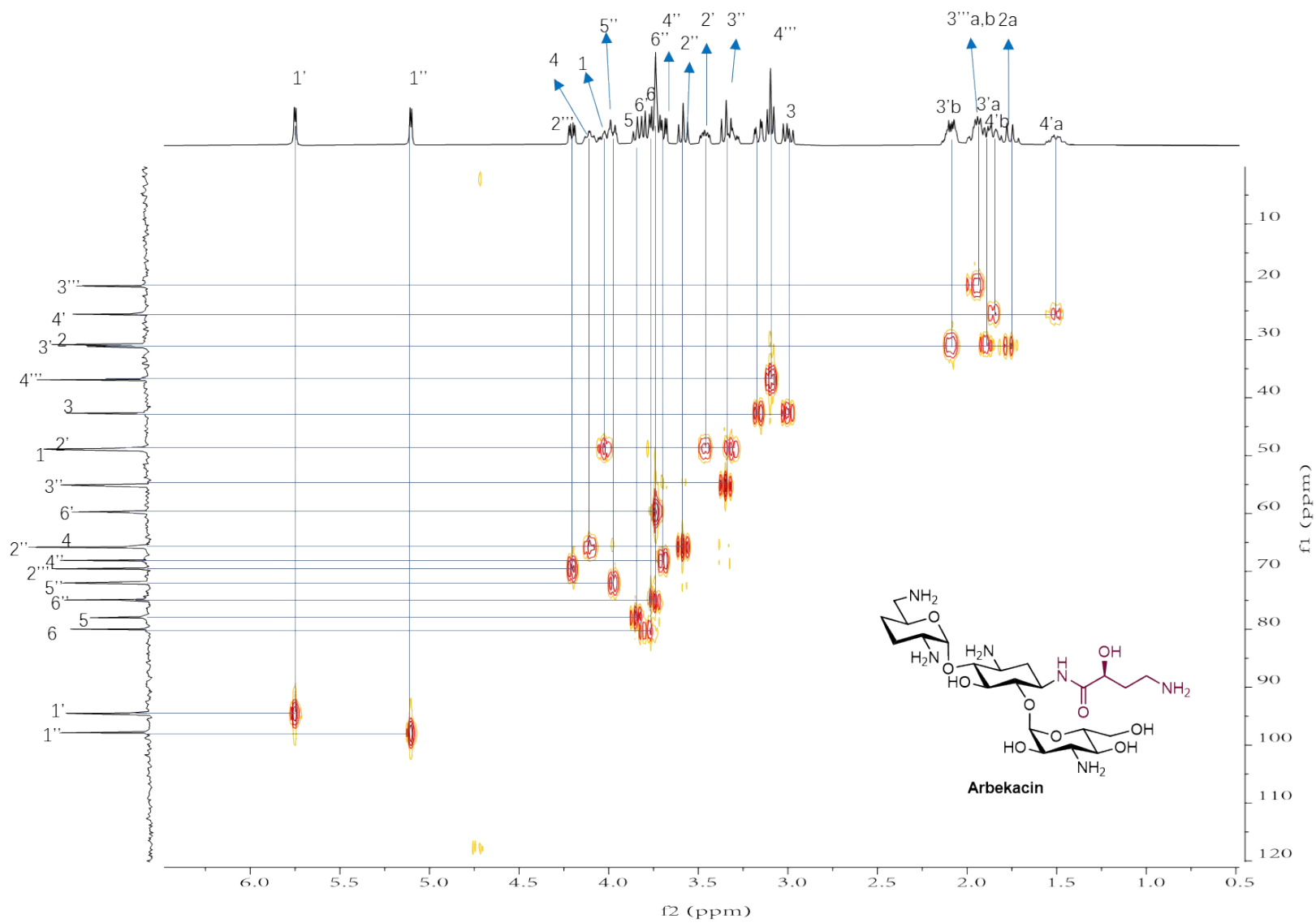


Fig. S12. HSQC of Arbekacin

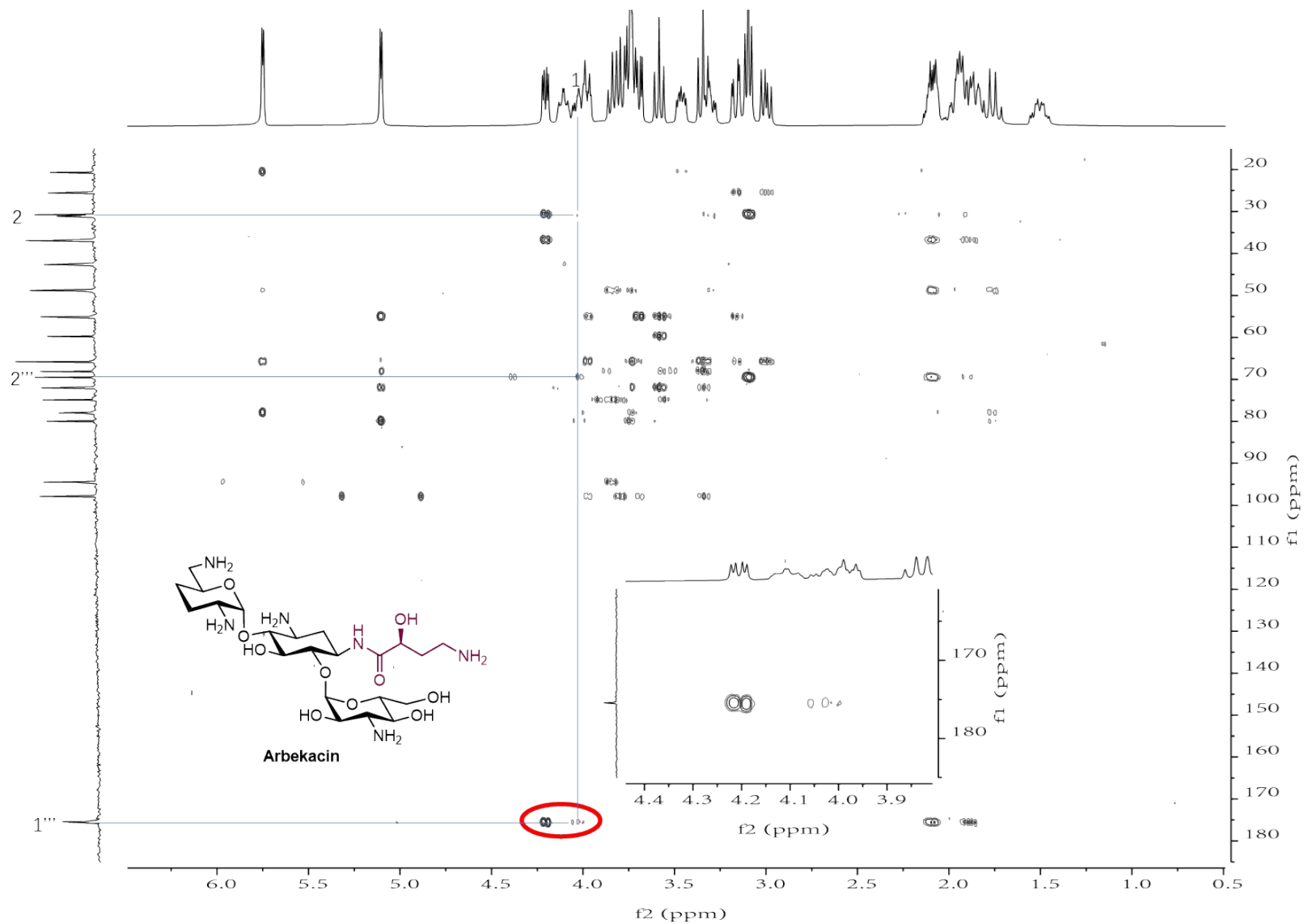


Fig. S13. HMBC of Arbekacin

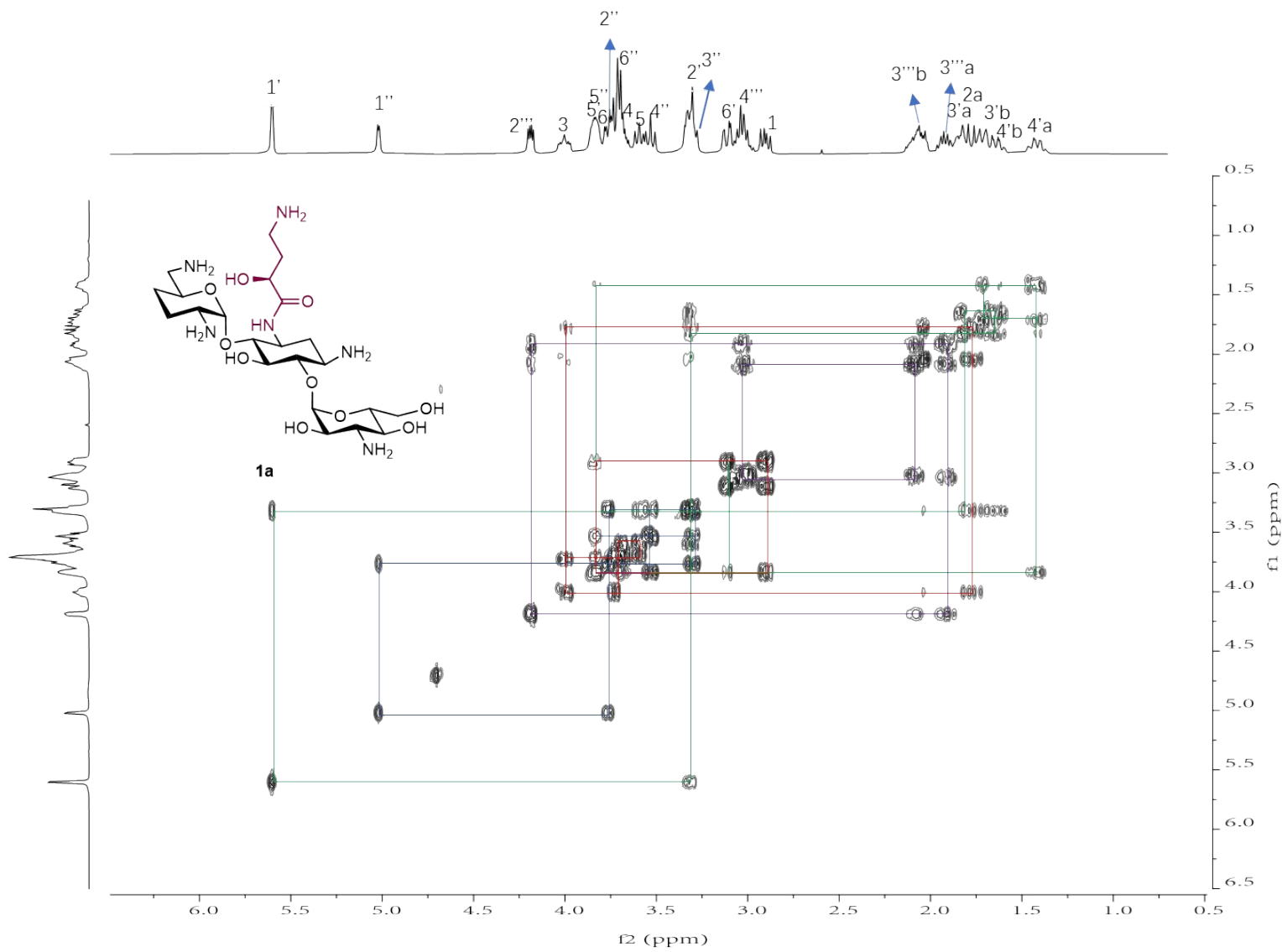


Fig. S14. ^1H - ^1H COSY of **1a**

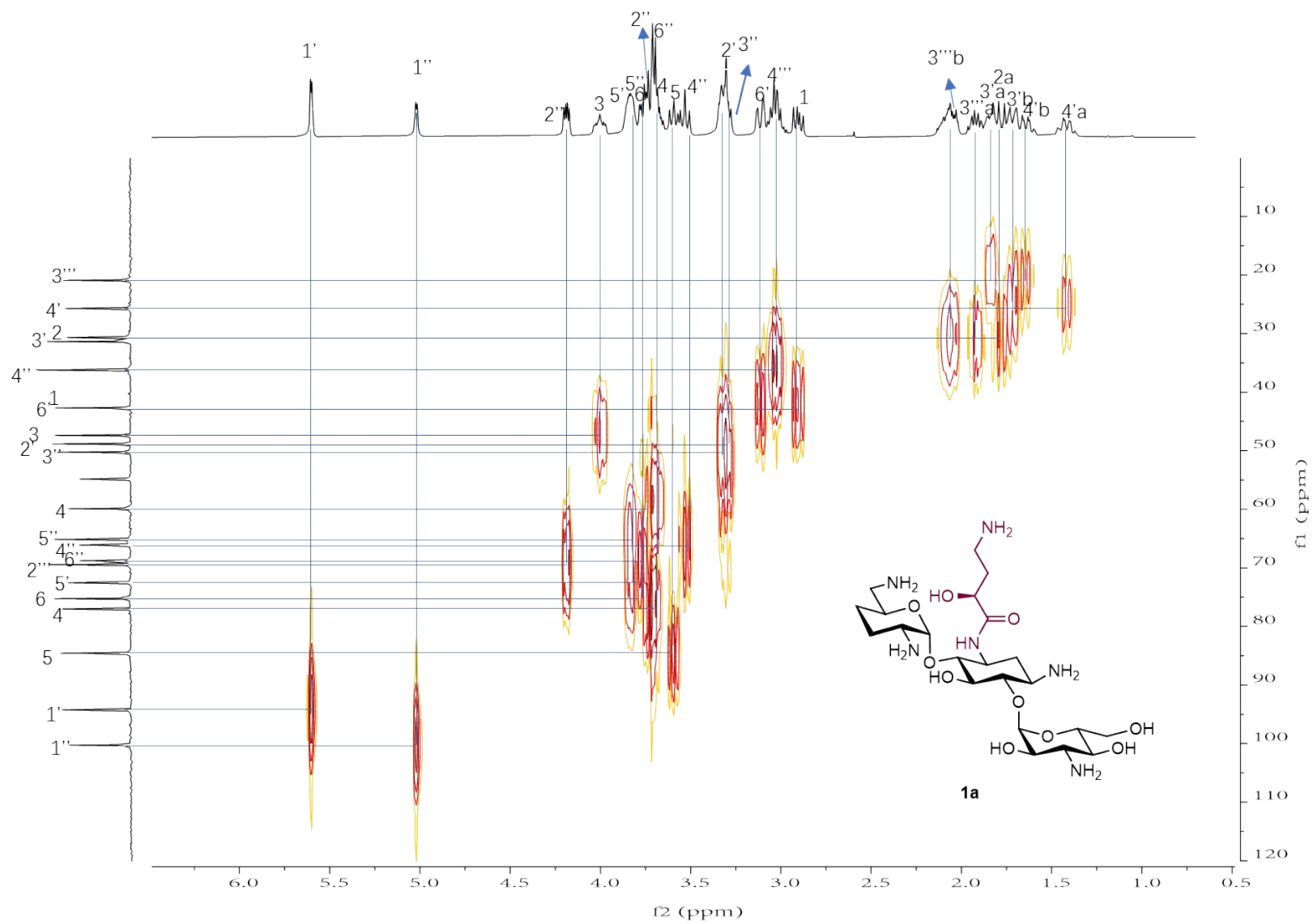


Fig. S15. HSQC of 1a

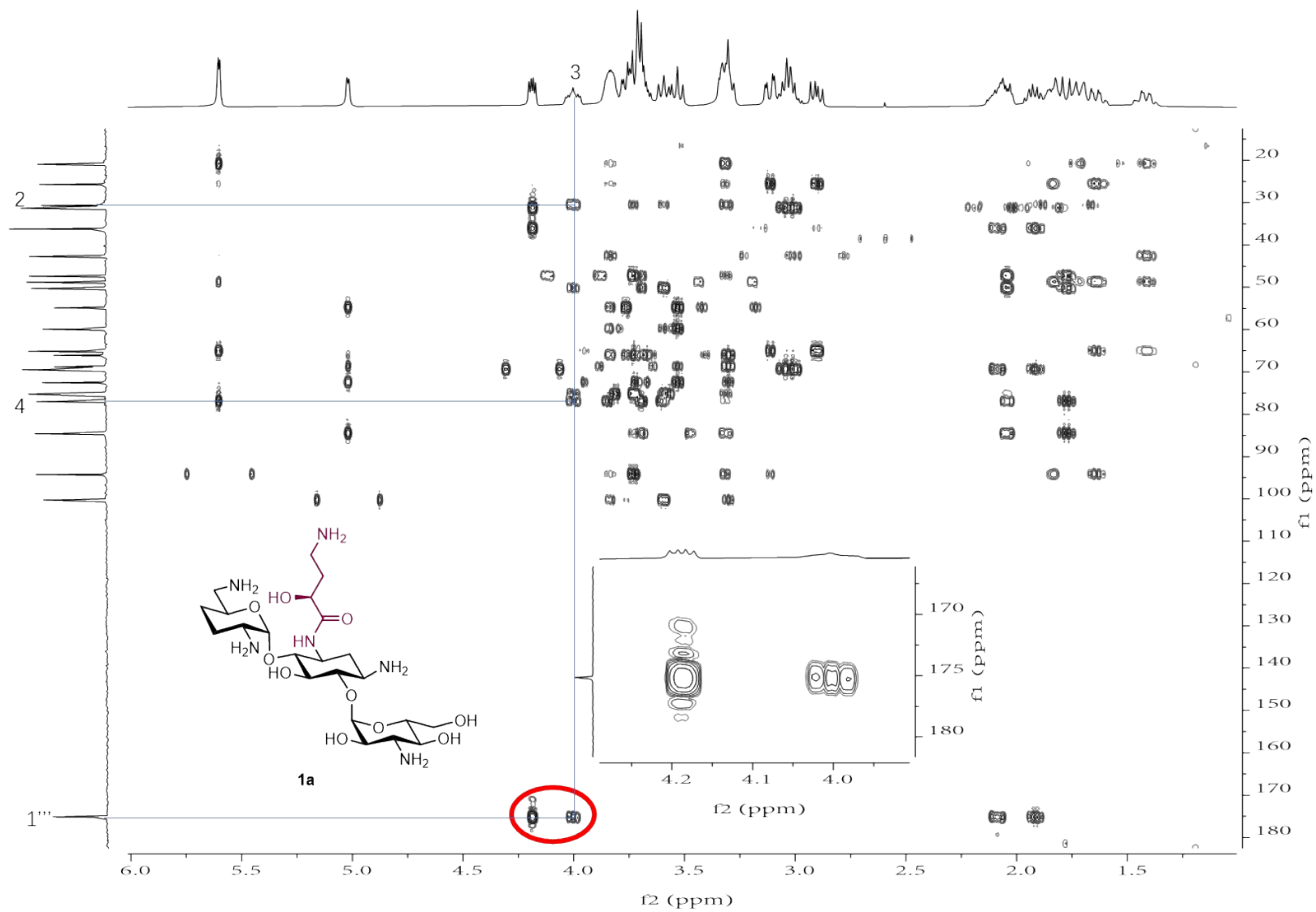


Fig. S16. HMBC of 1a

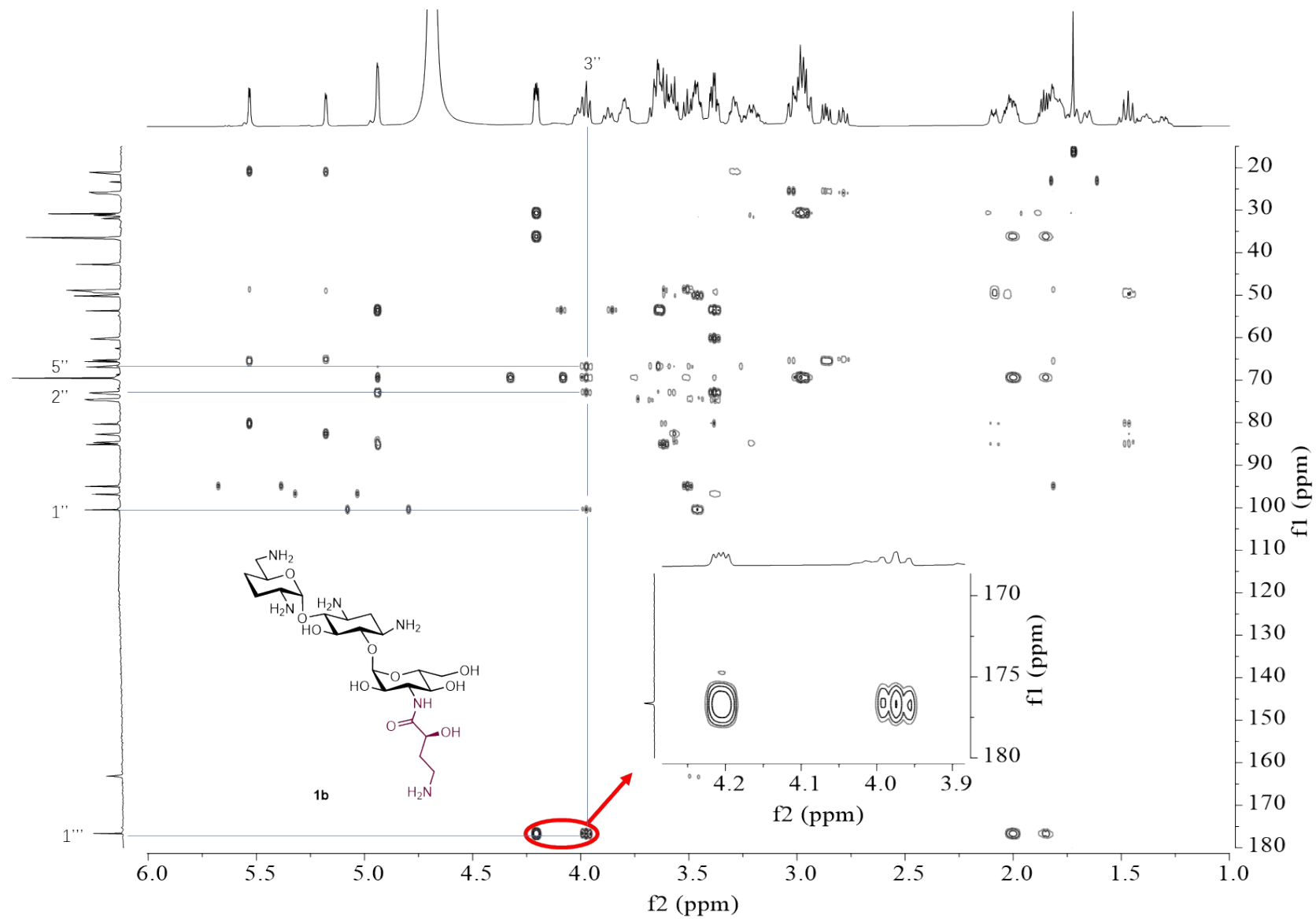


Fig. S17. HMBC of 1b

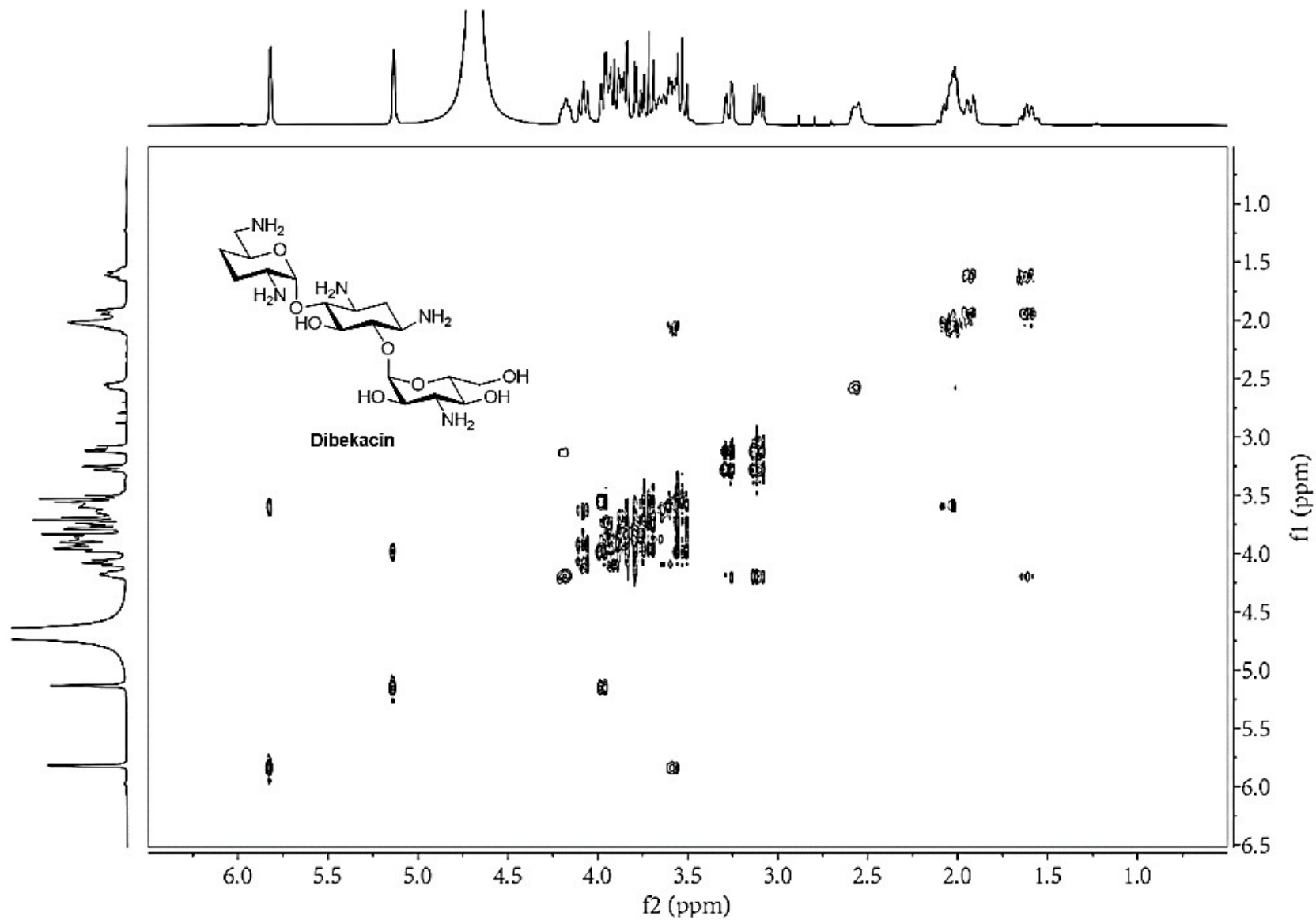


Fig. S18. ^1H - ^1H COSY of Dibekacin

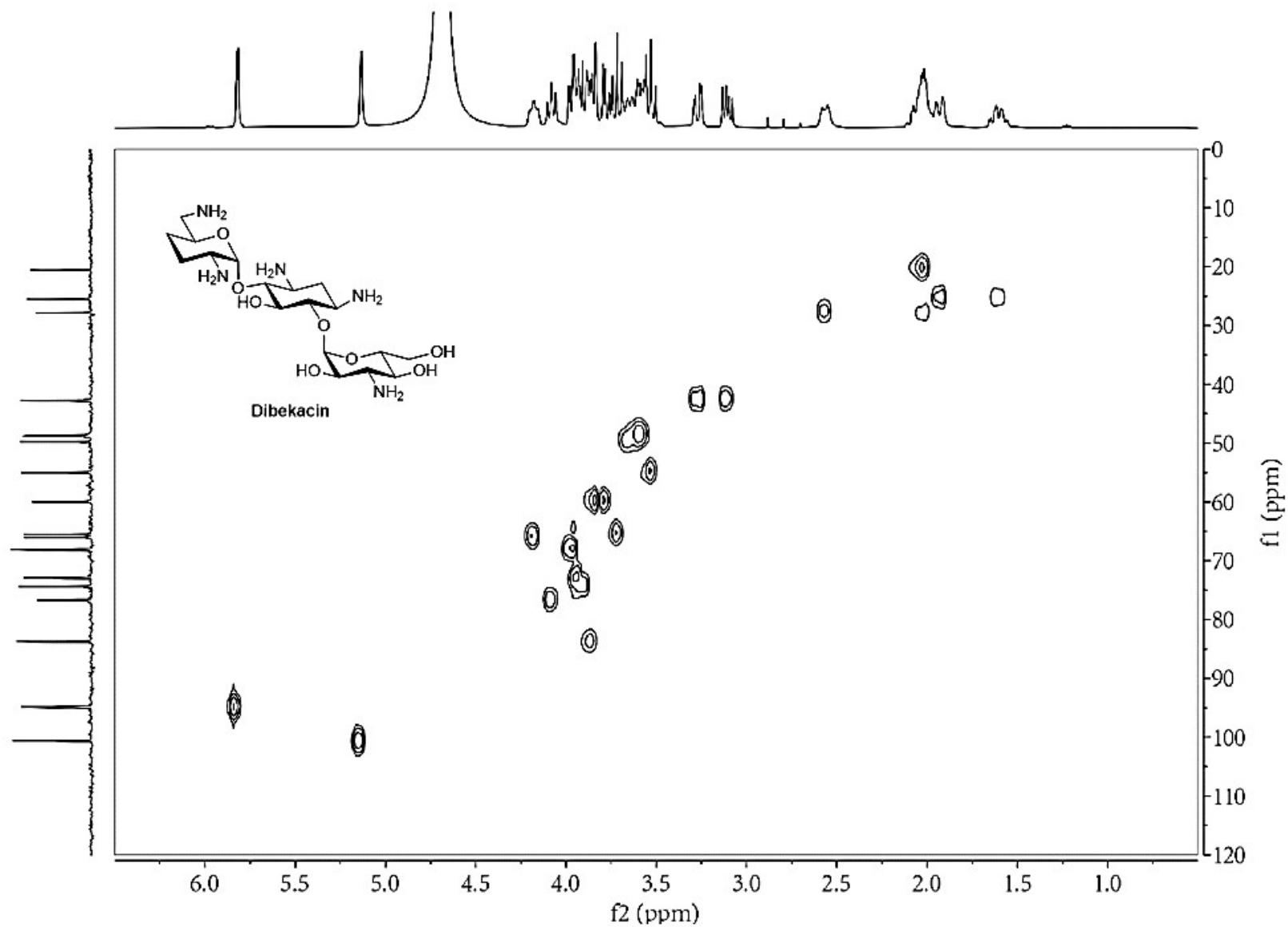


Fig. S19. HSQC of Dibekacin

3.2 HRMS and analysis

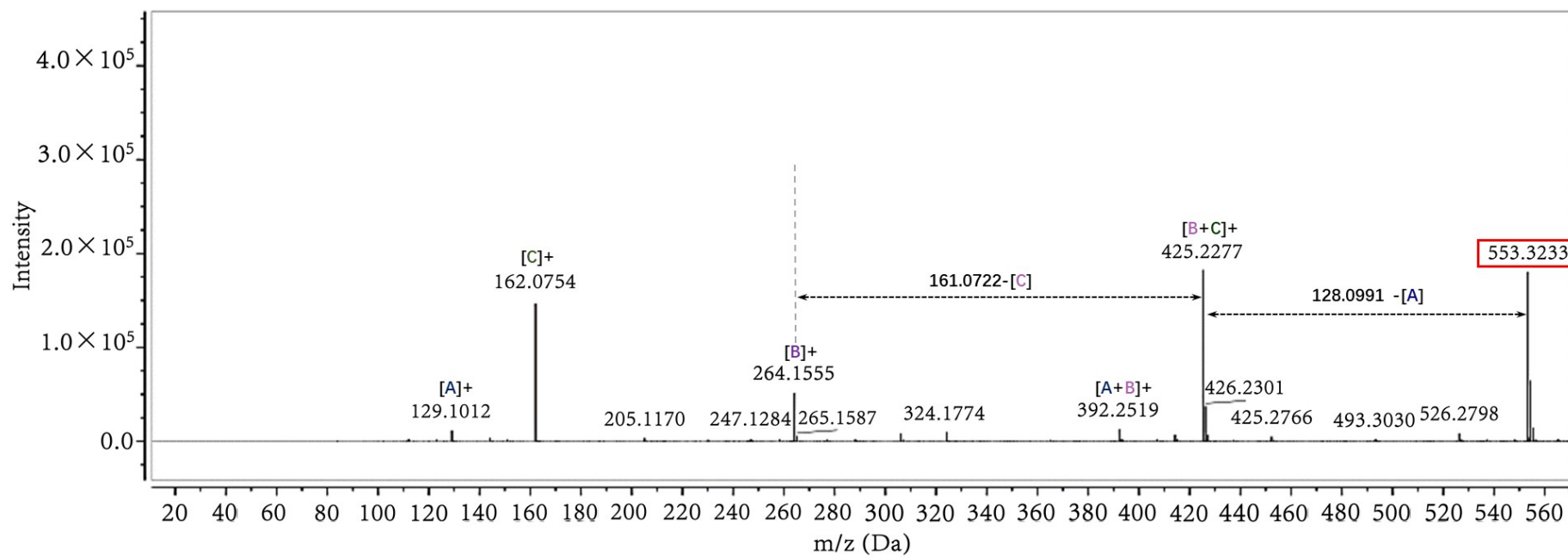
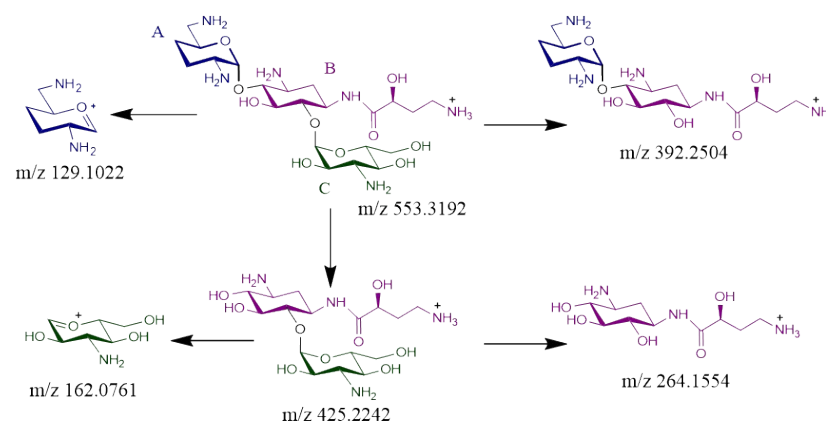


Fig. S20. HRMS and fragment ion analysis of Arbekacin

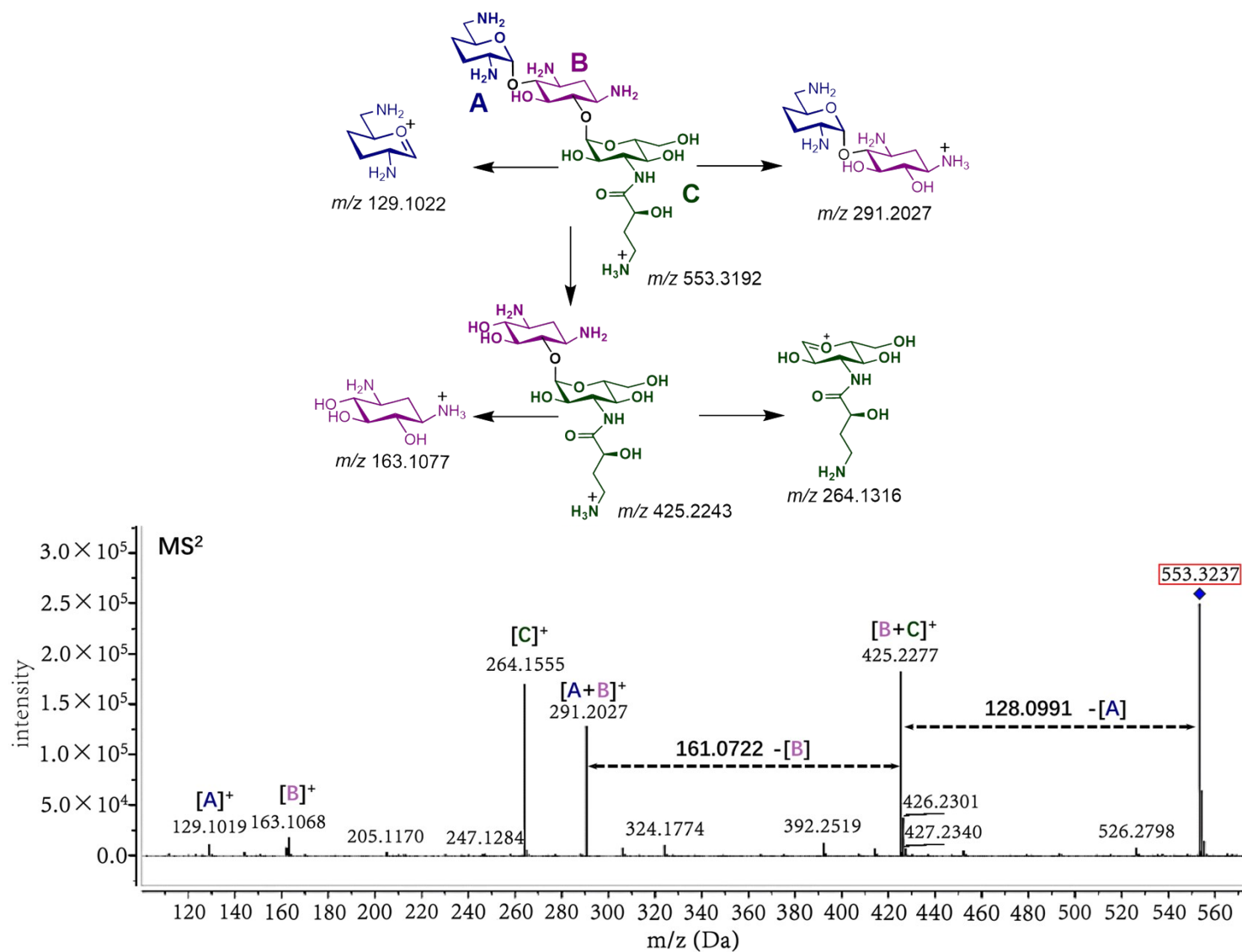


Fig. S22. HRMS and fragment ion analysis of **1b**

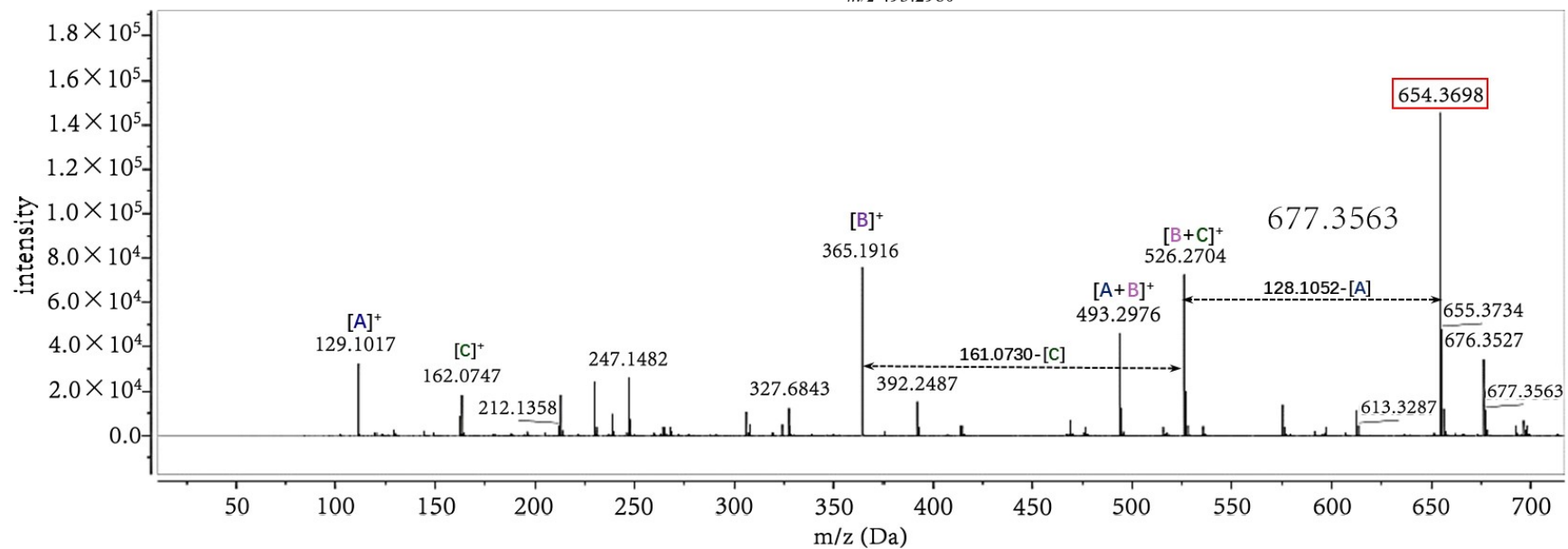
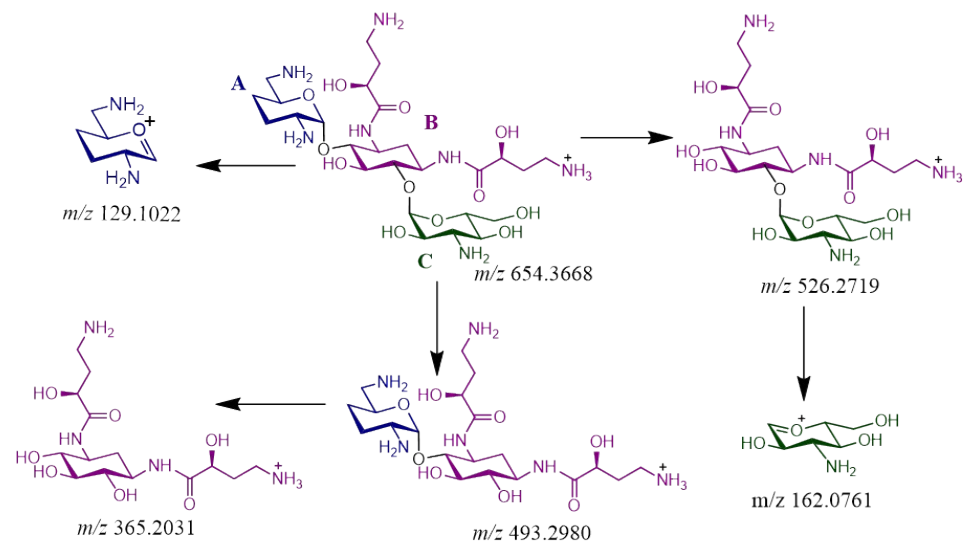


Fig. S23. HRMS and fragment ion analysis of **1c**

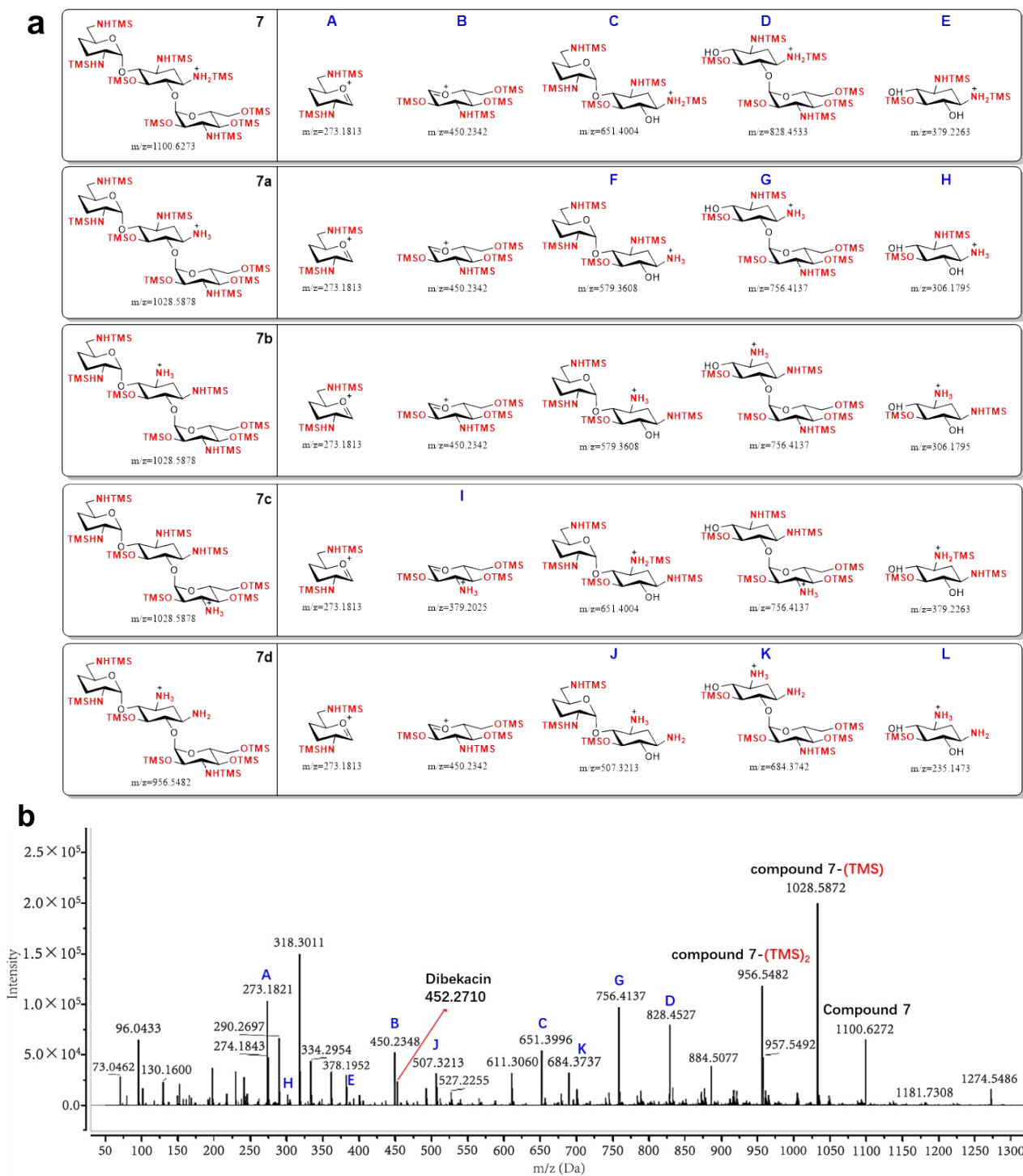
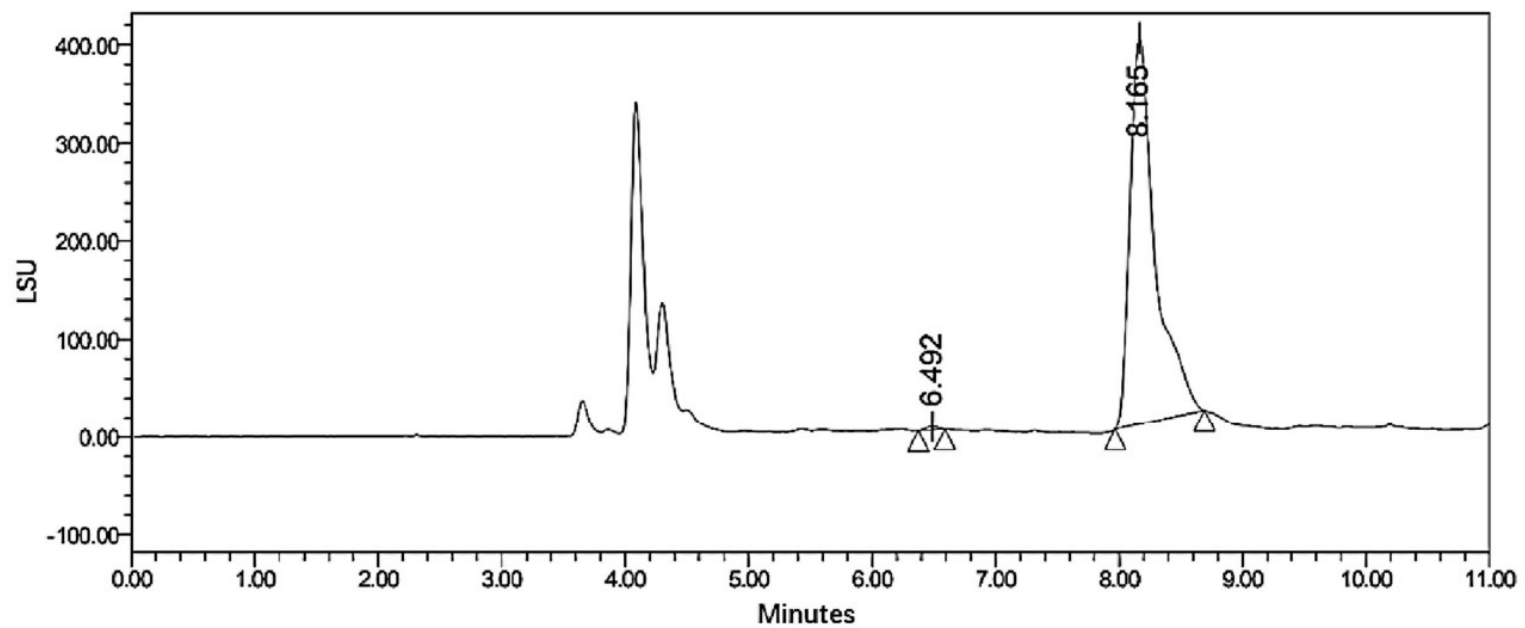


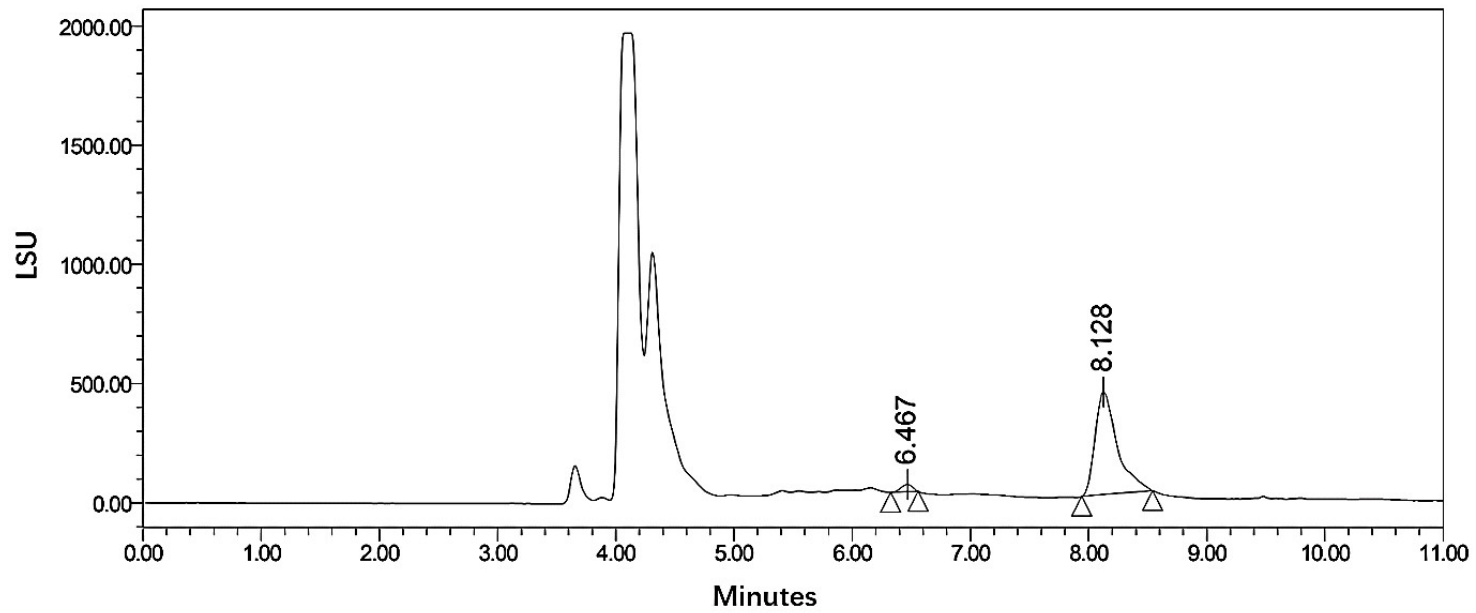
Fig. S24. a) The compounds and their mass spectrometry fragments that could potentially be present in the system after redissolving compound 7 in a mixed solvent of acetone and water. b) The mass spectrometry detection spectrum of the system after adding a mixed solvent of acetone and water.

3.3 HPLC-ELSD



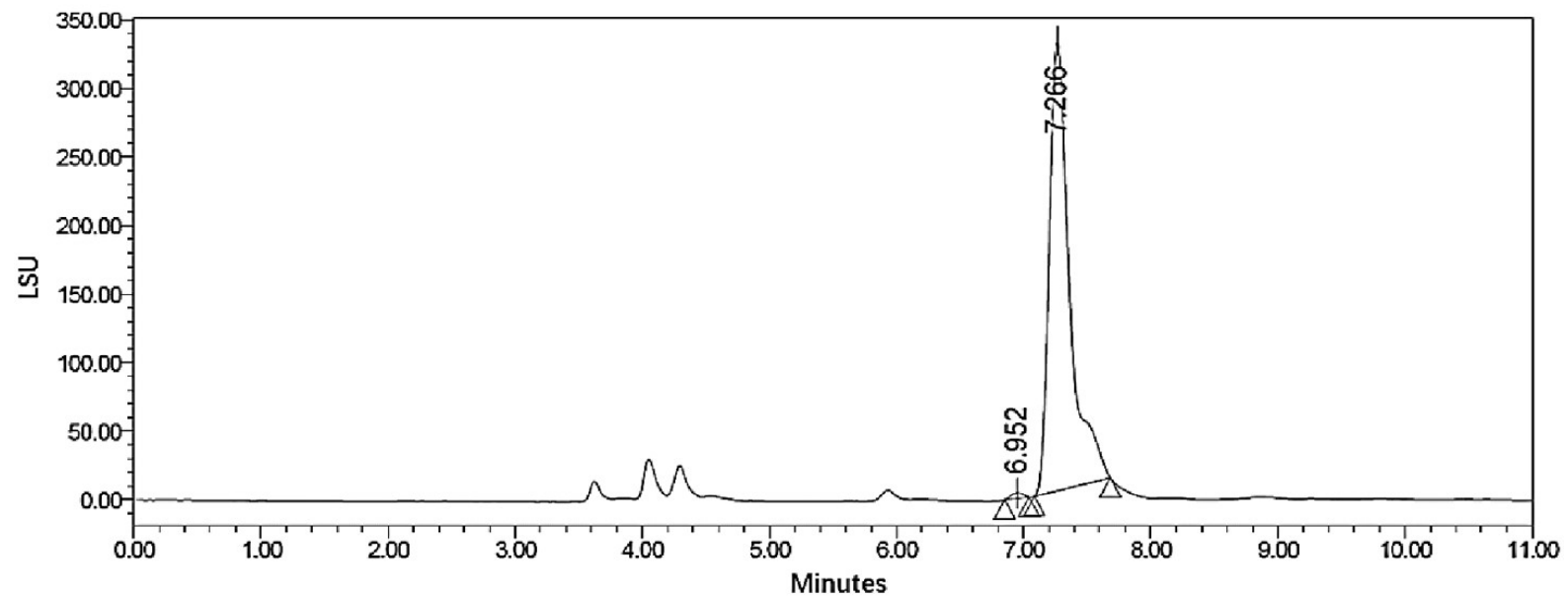
	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.492	24450	0.44	3600
2	ELSD signal	8.165	5593870	99.56	393791

Fig. S25. HPLC-ELSD data of Dibekacin



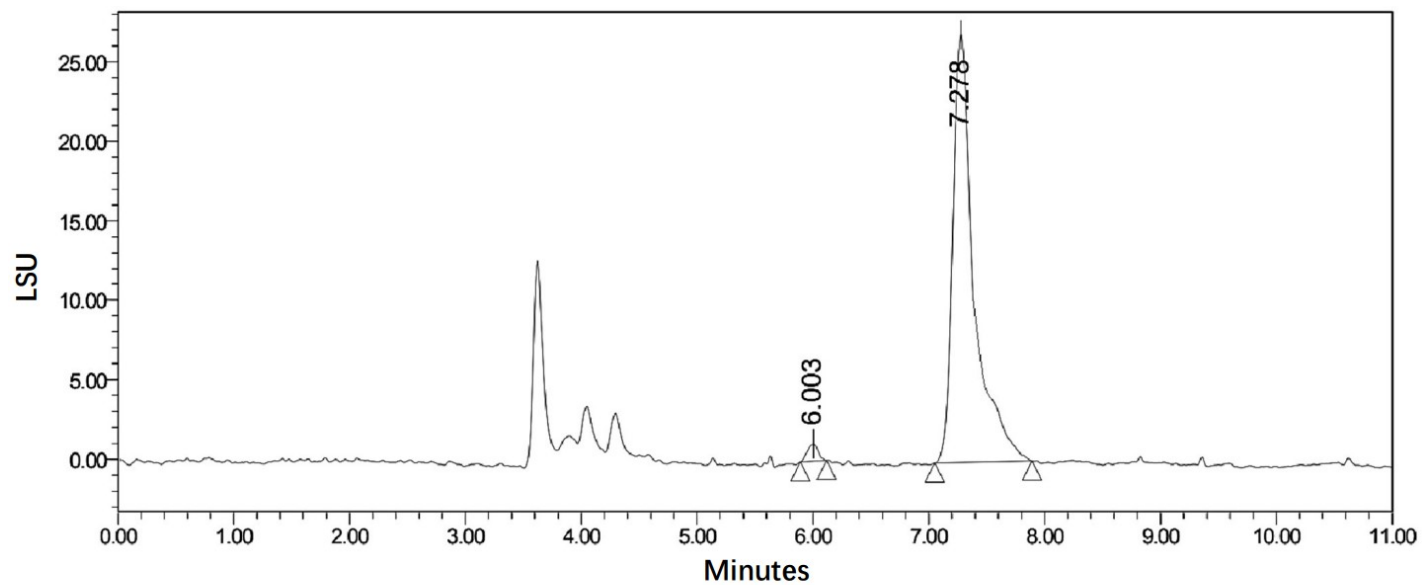
	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.467	49655	0.88	6254
2	ELSD signal	8.128	5593041	99.12	430943

Fig. S26. HPLC-ELSD data of recovered Dibekacin



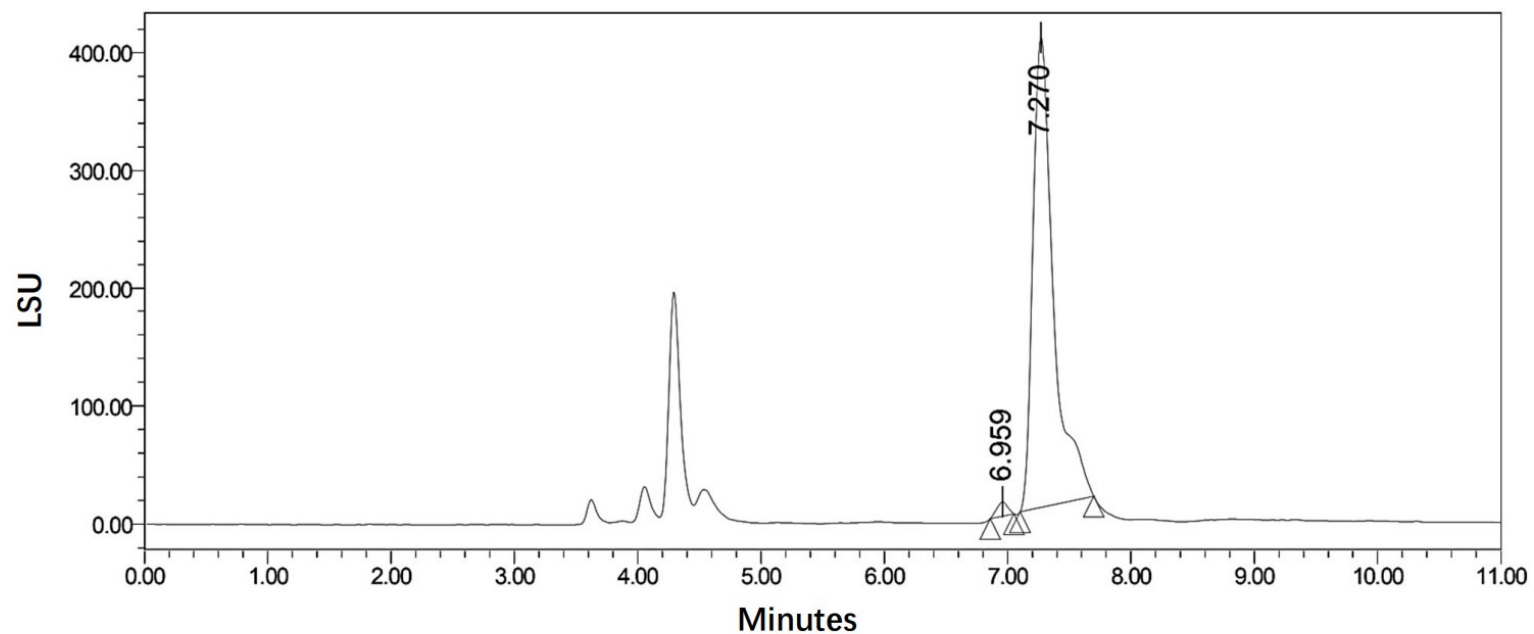
	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.952	24832	0.67	3651
2	ELSD signal	7.266	3667864	99.33	328180

Fig. S27. HPLC-ELSD data of Arbekacin



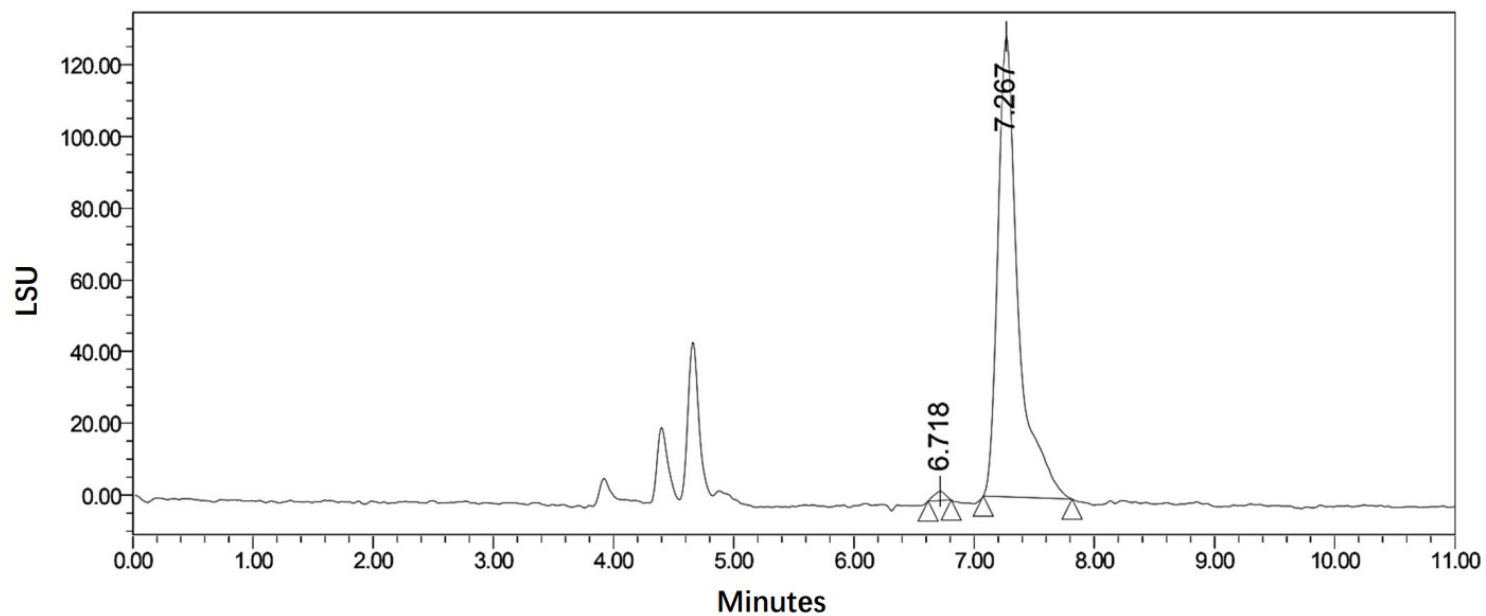
	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.003	1585	0.48	214
2	ELSD signal	7.278	328737	99.52	26913

Fig. S28. HPLC-ELSD data of Arbekacin (Table S4 entry 1)



	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.959	16282	0.35	2429
2	ELSD signal	7.270	4635963	99.65	399423

Fig. S29. HPLC-ELSD data of Arbekacin (Table S4 entry 2)



	Processing channel	Retention time (minutes)	Area	% area	Peak height
1	ELSD signal	6.718	6859	0.45	1229
2	ELSD signal	7.267	1517557	99.55	128354

Fig. S30. HPLC-ELSD data of Arbekacin (Table S4 entry 3)

4. Optical rotary power

Table S5 The Optical rotary power of the synthesized target compound

Experiment	Wavelength /(nm)	Measuring temperature /(°C)	Length of measuring tube /(dm)	Optical rotation measured value /(°)	Concentration ^a /(g/100 mL)	Specific rotation /(°)	Average specific rotation /(°)
1		20.06		0.738		+74	
2	589	20.02	1	0.736	1.0018	+74	+74
3		20.02		0.735		+74	

^aThe solvent is pure water.

The Optical rotary power is calculated by the following formula:

$$[\alpha]_D^t = \frac{100\alpha}{Lc}$$

In the formula, $[\alpha]$ represents the specific rotation, D represents the D line of the sodium spectrum, t represents the temperature at the time of determination, L represents the length of the measuring tube, α represents the measured optical rotation, and c represents the weight of the substance to be measured per 100 mL solution.

5. References

- (1) Oláh, J.; Van Alsenoy, C.; Sannigrahi, A. Condensed Fukui Functions Derived from Stockholder Charges: Assessment of Their Performance as Local Reactivity Descriptors. *The Journal of Physical Chemistry A* **2002**, *106* (15), 3885–3890.