



Spectral Data Analysis and Identification of Vancomycin Hydrochloride

Ye Tian[†], Xiaomeng Chong[†], Shangchen Yao* and Mingzhe Xu*

National Institutes for Food and Drug Control, Beijing, China

Objective: To establish a method for the determination of the chemical structure of vancomycin hydrochloride.

Methods: Nuclear magnetic resonance spectroscopy and mass spectrometry were conducted to analyze the chemical structure of vancomycin hydrochloride.

Results: In this study, the target compound (1) was identified as (S_α)-(3S, 6R, 7R, 22R, 23S, 26S, 36R, 38_αR)-44-[[[2-O-(3-amino-2, 3, 6-trideoxy-3-C-methyl- α -L-lysohexopyranosyl)- β -D-glucopyranosyl] oxy]-3-(carbamoylmethyl)-10, 19-dichloro-7, 22, 28, 30, 32-pentahydroxy-6-[[[(2R)-4-methyl-2-(methylamino) pentanoyl] amino]-2, 5, 24, 38, 39-pentaoxo-2, 3, 4, 5, 6, 7, 23, 24, 25, 26, 36, 37, 38, 38 α -tetradecahydro-22H-8, 11: 18, 21-dietheno-23, 36-(iminomethano)-13, 16: 31, 35-dimetheno-1H, 13H-[1, 6, 9] oxadiazacyclohexadecino [4, 5-m] [10, 2, 16]-benzoxadiazacyclotetracosine-26-carboxylic acid hydrochloride.

Conclusion: The method used in this study is accurate and can be used for the production and structural elucidation of vancomycin hydrochloride.

Keywords: NMR, MS, chemical shift, vancomycin, structure elucidation

OPEN ACCESS

Edited by:

Anna Napoli,
University of Calabria, Italy

Reviewed by:

Giuseppina De Luca,
University of Calabria, Italy
Wei-Lung Tseng,
National Sun Yat-sen University,
Taiwan

*Correspondence:

Shangchen Yao
yw_sc@sina.com
Mingzhe Xu
tianye@nifdc.org.cn

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Analytical Chemistry,
a section of the journal
Frontiers in Chemistry

Received: 04 August 2021

Accepted: 08 September 2021

Published: 20 September 2021

Citation:

Tian Y, Chong X, Yao S and Xu M
(2021) Spectral Data Analysis and
Identification of
Vancomycin Hydrochloride.
Front. Chem. 9:753060.
doi: 10.3389/fchem.2021.753060

INTRODUCTION

Vancomycin hydrochloride (**Figure 1**) is a glycopeptide antibiotic that exhibits significant antibacterial activity against various Gram-positive bacteria, including cocci and bacilli (McCormick et al., 1956). The mechanism of action of vancomycin hydrochloride is as follows: vancomycin binds to the alanine moiety of the precursor peptide present on the sensitive bacterial cell wall with high affinity, thereby preventing the synthesis of the polymer peptidoglycan that constitutes the bacterial cell wall, resulting in cell wall defects and bacterial death. Existing literature shows that vancomycin can selectively inhibit the synthesis of ribonucleic acid by altering the permeability of bacterial cell membranes (Wang et al., 2018). This property is listed in the Chinese Pharmacopoeia (2020 edition, Volume II), Japanese Pharmacopoeia (17th edition), United States Pharmacopoeia (43rd edition), and European Pharmacopoeia (10.0 edition).

China is an important supplier of the raw materials used in vancomycin synthesis. Presently, to the best of our knowledge, the structural analysis of vancomycin has not been reported. Moreover, due to its complex structure, the signals obtained in the nuclear magnetic resonance (NMR) spectrum of vancomycin tend to overlap, making it difficult to analyze and elucidate its structure. In this study, mass spectrometry (MS), one- (¹H, ¹³C) and two-dimensional (¹H-¹H) correlation spectroscopy (COSY), and heteronuclear multiple quantum coherence (HMQC) and heteronuclear

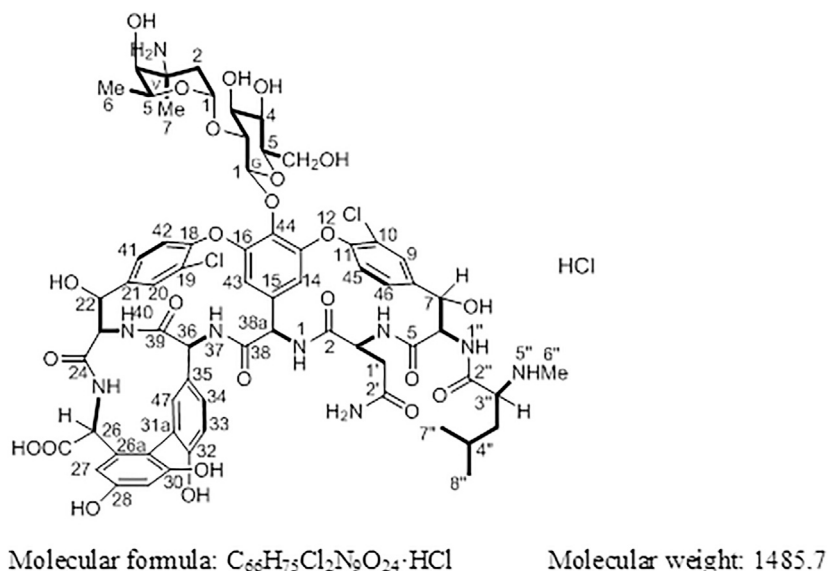


FIGURE 1 | Structure of vancomycin hydrochloride (1).

multiple bond coherence (HMBC)) NMR spectroscopy were comprehensively performed to analyze the structure on the basis of the spectral data of vancomycin hydrochloride. The obtained results will provide a scientific basis for the qualitative analysis of this compound.

MATERIALS AND METHODS

Reagents

Formic acid (Fluka, GR), acetonitrile (Fisher, HPLC), Watsons purified deionized water (Xellia pharmaceuticals, batch number: A3250080), and vancomycin hydrochloride reference standard (National Institutes for Food and Drug Control, batch number: 130360-201302) were used in this study. Deuterated dimethyl sulfoxide (Merck) was used for NMR analysis of 1.

Instruments

Mass spectra were recorded using an AB Sciex 3200 Qtrap mass spectrometer (AB SCIEX, United States). NMR spectra were obtained using a Varian INOVA 600 MHz NMR spectrometer.

RESULTS

Compound 1 is a white solid. The positive ion electrospray ionization mass spectra [(+)-ESIMS] of 1 revealed a quasi-molecular ion peak at (m/z) 1448 [$M+H$]⁺. High-resolution (+)-ESIMS afforded the accurate mass-to-charge ratio of the quasi-molecular ion at m/z 1448.4380 [$M+H$]⁺, suggesting that the molecular composition of the test product was $C_{66}H_{75}Cl_2N_9O_{24}$ (the calculated value was 1448.4375, and the

corresponding molecular composition was $C_{66}H_{76}Cl_2N_9O_{24}$). The signals in the ¹H-NMR spectrum of 1 were assigned to five groups of aromatic proton as follows: 1) three groups of ABX-system aromatic proton: δ_H 7.38 (1H, br s, H-9), 7.52 (1H, brd, J = 8.4 Hz, H-46), and 7.26 (1H, brd, J = 8.4 Hz, H-45); δ_H 7.86 (1H, s, H-20), 7.47 (1H, brd, J = 8.4 Hz, H-41), and 7.34 (1H, brd, J = 8.4 Hz, H-42); δ_H 7.18 (1H, br s, H-47), 6.77 (1H, brd, J = 8.4 Hz, H-34), and 6.72 (1H, brd, J = 8.4 Hz, H-33); and 2) two groups of 3-substituted aromatic proton signals: δ_H 6.42 (1H, br s, H-29), 6.27 (1H, br s, H-27), and δ_H 5.54 (1H, br s, H-14), 5.21 (1H, br s, H-43)]. Moreover, three signals of methyl protons bonded to methyldiene [δ_H 0.90 (3H, d, J = 6.6 Hz, H3-8''), 0.86 (3H, d, J = 6.6 Hz, H3-9''), and 1.07 (3H, d, J = 6.0 Hz, H3-V6)], a signal of methyl protons bonded to a nitrogen atom [δ_H 2.37 (3H, s, H3-7'')], a signal of methyl protons bonded to a quaternary carbon [δ_H 1.32 (3H, s, H3-V7)], and 19 signals of protons bonded to various heteroatoms [δ_H 5.75 (1H, d, J = 7.8 Hz, H-38a), 5.27 (1H, d, J = 7.2 Hz, H-G1), 5.24 (1H, br s, H-V1), 5.16 (1H, br s, H-7), 5.13 (1H, br s, H-22), 4.88 (1H, br s, H-6), 4.68 (1H, q, J = 6.6 Hz, H-V5), 4.43 (1H, o, H-36), 4.42 (1H, d, J = 6.0 Hz, H-26), 4.35 (1H, o H-3), 4.19 (1H, d, J = 11.4 Hz, H-23), 3.68 (1H, d, J = 10.2 Hz, H-G6a), 3.57 (1H, t, J = 8.5 Hz, H-G2), 3.56 (1H, o, H-G6b), 3.46 (1H, o, H-G3), 3.35 (1H, o, H-G5), 3.35 (1H, o, H-G4), 3.35 (1H, o, H-3''), and 3.20 (1H, br s, H-V4)] were observed in the NMR spectra of 1. After deuterium exchange, the NMR spectra of 1 exhibited at least 16 exchangeable active proton signals [δ_H 9.44 (1H, br s, OH), 9.11 (1H, br s, OH), 8.63 (1H, br s, NH-37), 8.47 (1H, d, J = 6.0 Hz, NH-25), 8.25 (1H, br s, NH-1), 7.93 (1H, br s, NH-1''), 7.37 (2H, br s, CONH2), 6.91 (2H, br s, CONH2), 6.67 (1H, d, J = 11.4 Hz, NH-40), 6.62 (1H, br s, NH-4), 5.96 (1H, d, J = 4.2 Hz, OH-22), 5.83 (1H, br s, OH-7), 5.43 (1H, br s, OH-V4), 5.38 (1H, br s, OH-G3), 5.11 (1H, br s, OH-G4),

TABLE 1 | ^{13}C -NMR and ^1H -HMBC data of 1.

No.	Type	Chemical shift (^{13}C)	Chemical shift (^1H)	No.	Type	Chemical shift (^{13}C)	Chemical shift (^1H)
1	NH		8.25 (1H, br s)	36	CH	53.7	4.43 (1H, o)
2	C	170.7		37	NH		8.63 (1H, br s)
3	CH	50.9	4.35 (1H, o)	38	C	169.6	
4	NH		6.62 (1H, br s)	38a	CH	54.9	5.75 (1H, d, 7.8)
5	C	167.2		39	C	169.1	
6	CH	58.3	4.88 (1H, br s)	40	NH		6.67 (1H, d, 11.4)
7	CH	71.1	5.16 (1H, br s)	41	CH	127.3	7.47 (1H, br d, 8.4)
8	C	127.2		42	CH	123.4	7.34 (1H, br d, 8.4)
9	CH	128.7	7.38 (1H, br s)	43	CH	104.6	5.21 (1H, br s)
10	C	139.8		44	C	131.9	
11	C	149.8		45	CH	124.3	7.26 (1H, br d, 8.4)
12				46	CH	127.2	7.52 (1H, br d, 8.4)
13	C	152.2		47	CH	135.7	7.18 (1H, br s)
14	CH	107.0	5.54 (1H, br s)	1'	CH ₂	37.3	2.42 (1H, o); 2.14 (1H, dd, 15.6, 4.8)
15	C	134.5		2'	C	171.0	
16	C	151.3		1''	NH		7.93 (1H, br s)
17				2''	C	173.7	
18	C	148.3		3''	CH	61.8	3.35 (1H, o)
19	C	126.2		4''	CH ₂	40.7	1.53 (1H, m, 6.6); 1.53 (1H, m, 6.6)
20	CH	127.3	7.86 (1H, s)	5''	CH	24.1	1.17 (1H, non, 6.6)
21	C	142.5		6''			
22	CH	71.6	5.13 (1H, br s)	7''	CH ₃	33.2	2.37 (3H, s)
23	CH	61.9	4.19 (1H, d, 11.4)	8''	CH ₃	22.9	0.90 (3H, d, 6.6)
24	C	167.6		9''	CH ₃	22.6	0.86 (3H, d, 6.6)
25	NH		8.47 (1H, d, 6.0)	G1	CH	101.3	5.27 (1H, d, 7.2)
26	CH	56.9	4.42 (1H, d, 6.0)	G2	CH	78.1	3.57 (1H, t, 8.5)
26a	C	136.4		G3	CH	76.7	3.46 (1H, o)
26-COOH	C	172.8		G4	CH	70.1	3.35 (1H, o)
27	CH	105.9	6.27 (1H, br s)	G5	CH	76.7	3.35 (1H, o)
28	C	157.2		G6	CH ₂	61.2	3.68 (1H, d, 10.2); 3.56 (1H, o)
29	CH	102.3	6.42 (1H, br s)	V1	CH	96.8	5.24 (1H, br s)
30	C	156.5		V2	CH ₂	33.2	1.90 (1H, brd, 9.0); 1.75 (1H, brd, 9.0)
31	C	118.0		V3	C	53.9	
31a	C	121.7		V4	CH	70.7	3.20 (1H, br s)
32	C	155.1		V5	CH	63.1	4.68 (1H, q, 6.6)
33	CH	116.2	6.72 (1H, br d, 8.4)	V6	CH ₃	16.8	1.07 (3H, d, 6.0)
34	CH	125.4	6.77 (1H, br d, 8.4)	V7	CH ₃	22.3	1.32 (3H, s)
35	C	126.2					

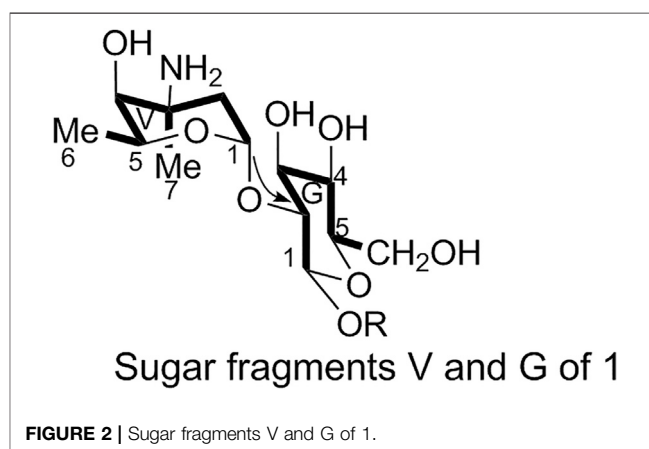
and 4.05 (1H, br s, OH-G5)]. In addition, multiple aliphatic hydrogen signals were observed in the upfield region. The above spectral data show that 1 is a glycopeptide containing multiple amino acid fragments (Harris et al., 1983; Zhou et al., 2004).

The ^1H and ^{13}C NMR signals of 1 were accurately assigned using HSQC and ^1H - ^1H COSY; the details are listed in **Table 1**. The ^1H - ^1H COSY spectrum of 1 showed the cross-peak correlation between the protons. The arrangement of the structural fragments of 1 was determined on the basis of the proton couplings in the structure (represented by thick lines in **Figure 5**).

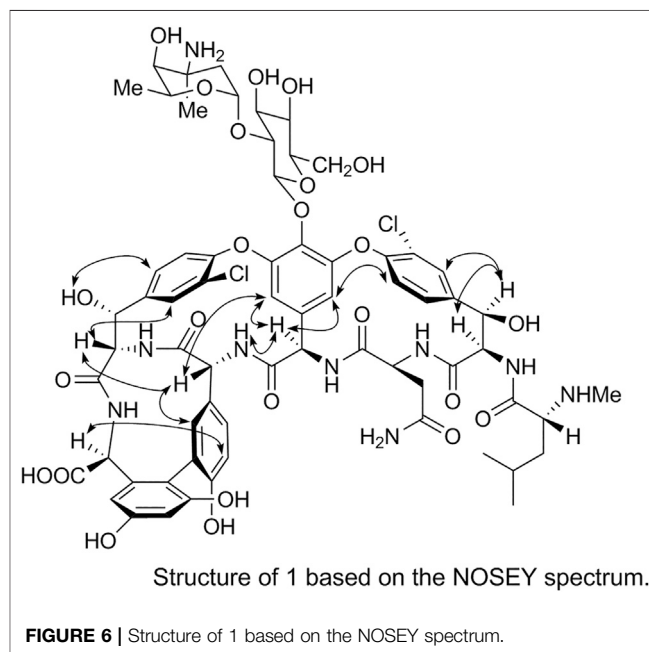
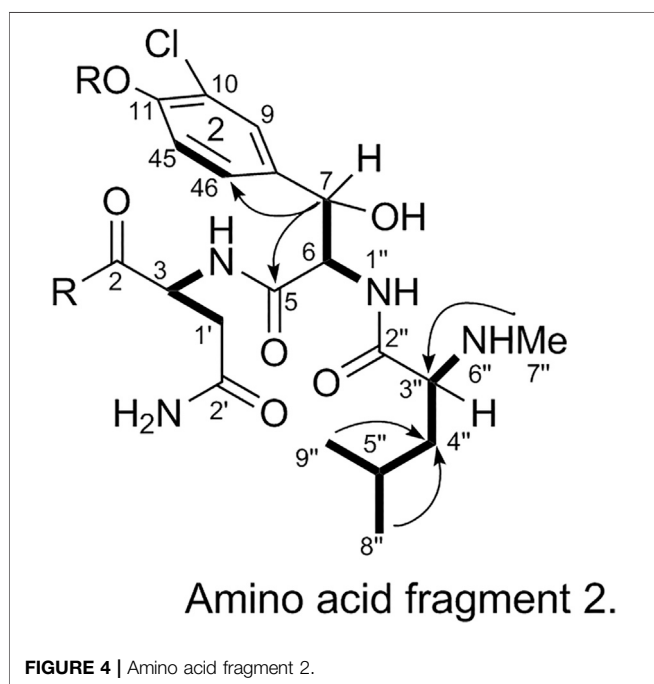
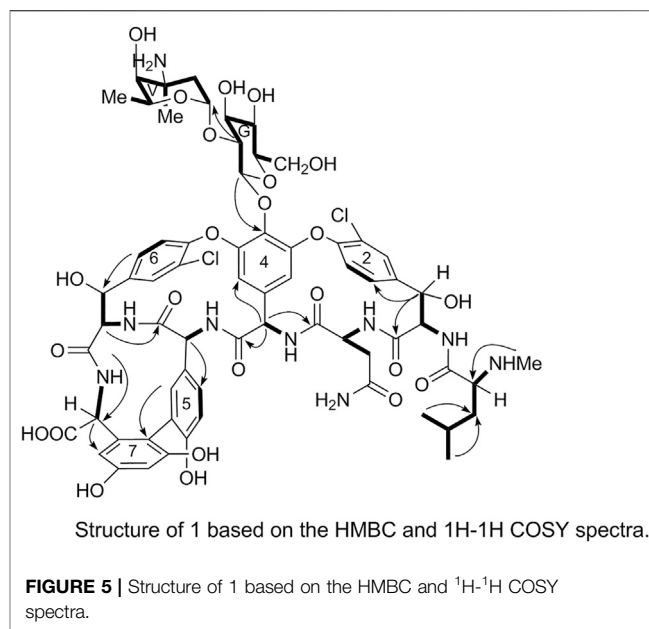
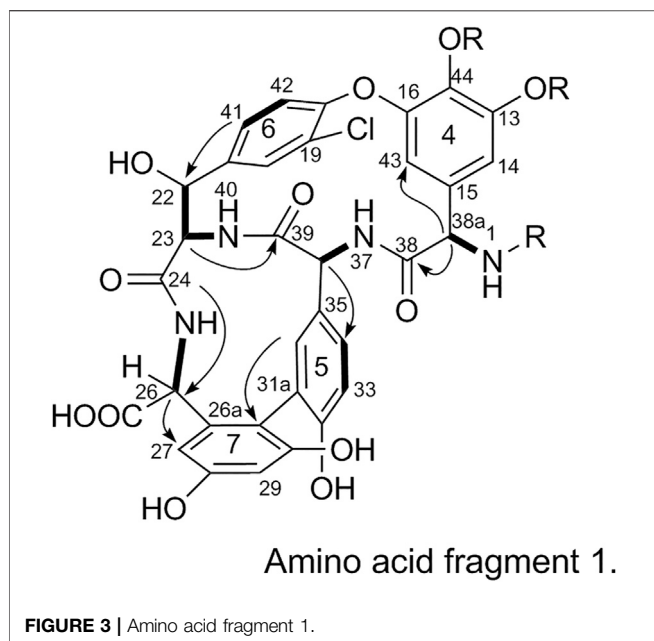
The fragments were then arranged based on the HMBC spectrum as follows:

1) Linkage of sugar fragments:

Two end group carbon signals were observed in the ^1H -NMR and ^{13}C -NMR spectra of 1. The structures and the linkage locations of the two sugar fragments (represented by V and G, respectively in **Figure 2**) were confirmed based on the



HSQC, ^1H - ^1H COSY, and HMBC spectra. Especially, in the HMBC spectrum, H-V1 can be attributed to C-G2, thereby confirming the α -L-vancosaminyl-(1 \rightarrow 2)-O- β -D-glucosyl linkage between the two sugars.



2) Linkage of amino acid fragments 4, 5, 6, and 7:

The substitution patterns of benzene rings 4, 5, 6, and 7 were determined using the δ_{H} , δ_{C} , and $J_{\text{H-H}}$ values observed in the ^1H -NMR and ^{13}C -NMR spectra of 1. In the HMBC spectrum of 1, H-38a corresponds to C-43 and C38, H-36 corresponds to C-34, H-41 corresponds to C-22, H-23 corresponds to C-39, and H-26 corresponds to C-24 and C-27. Thus, it can be inferred that 1 contains a 4-amino acid cyclic peptide structure (represented by thick lines in **Figure 3**).

3) Linkage of amino acid fragments 1, 2, and 3:

The substitution pattern of benzene ring 2 was confirmed using the δ_{H} , δ_{C} , and $J_{\text{H-H}}$ values observed in the ^1H -NMR and ^{13}C -NMR spectra of 1. In the HMBC spectrum, H₃-7'' corresponds to C-3'', H-8'' and H-9'' correspond to C-1a, H-7 corresponds to C-11 and C-2, and H-3 corresponds to C-2. Therefore, it could be inferred that there was another 3-amino acid fragment linkage in 1 (represented by thick lines in **Figure 4**).

4) Linkage of amino acid and sugar fragments.

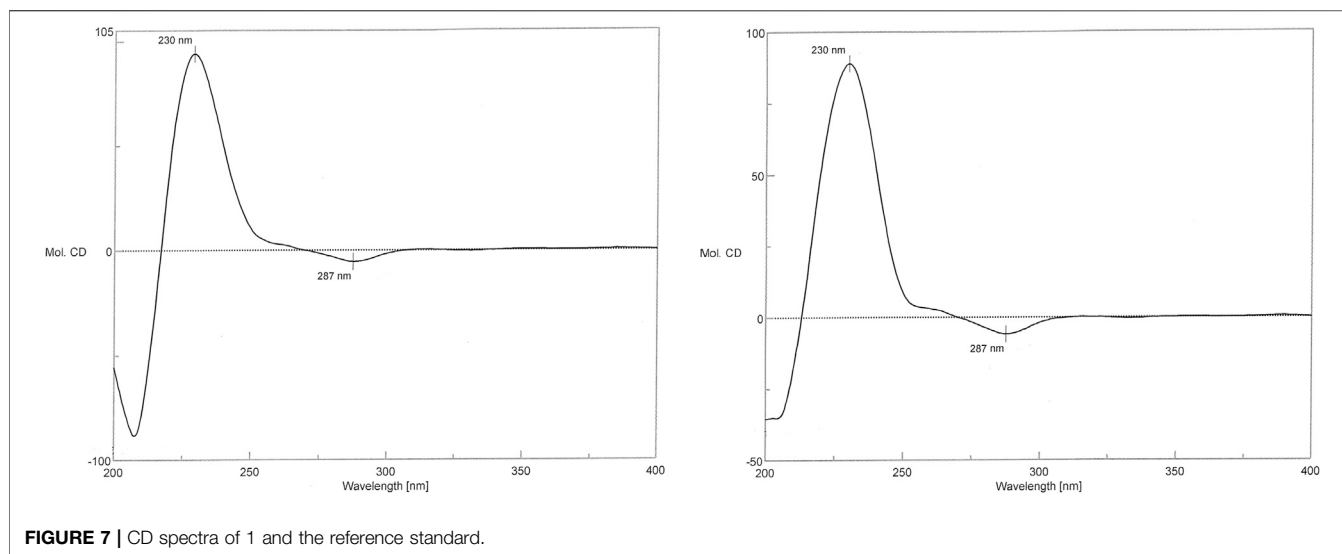


FIGURE 7 | CD spectra of 1 and the reference standard.

In the HMBC spectrum, H-G1 corresponds to C-44 and H-38a corresponds to C-2. These results confirmed the proposed arrangements of the above-mentioned three fragments (marked by arrows in **Figure 5**). The above analyses show that 1 is a glycopeptide antibiotic with a cyclic peptide core consisting of seven amino acids and two sugars linked by an oxygen glycosidic bond; its planar structure is identical to that of vancomycin (McCormick et al., 1956).

The spatial structure of 1 was determined by comparing its NOSEY and circular dichroism (CD) spectra with those of the reference standard. First, the relative configuration of 1 was determined using the NOSEY experimental data (indicated by arrows in **Figure 6**). Moreover, the δ_{H} , $J_{\text{H-H}}$, and δ_{C} values and the NOSEY spectra of 1 were consistent with those of the reference standard. These results confirm that 1 has a configuration identical to that of the vancomycin hydrochloride reference standard.

In the CD spectrum of 1, a negative Cotton effect was observed at 287 nm, while a positive Cotton effect was observed at 230 nm. The CD spectrum of 1 was identical to that of the vancomycin hydrochloride reference standard (2) (**Figure 7**). Therefore, 1 exhibited the three-dimensional structure identical to that of the vancomycin hydrochloride reference standard.

DISCUSSION

Through MS and NMR spectroscopy, 1 was identified as vancomycin hydrochloride. Moreover, the ^1H and ^{13}C NMR signals were accurately assigned on the basis of the one- and two-dimensional NMR spectra of 1. Thus, this method can be effectively utilized for the structural and qualitative analysis of complex compounds.

REFERENCES

Harris, C. M., Kopecka, H., and Harris, T. M. (1983). Vancomycin: Structure and Transformation to CDP-I. *J. Am. Chem. Soc.* 105, 6915–6922. doi:10.1021/ja00361a029

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found in the European Nucleotide Archive (ebi.ac.uk) under accession number PRJEB47449.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

Details of all funding sources should be provided, including grant numbers if applicable. Please ensure to add all necessary funding information, as after publication this is no longer possible.

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.cn) for English language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2021.753060/full#supplementary-material>

McCormick, M. H., Mcguire, J. M., Pittenger, G. E., Pittenger, R. C., and Stark, W. M. (1956). Vancomycin, a New Antibiotic. I. Chemical and Biologic Properties. *Antibiot. Annu.* 3, 606–611. doi:10.1021/ja00361a029

Wang, C., Wang, L. X., and Hu, C. Q. (2018). Quality Assessment of the Domestic Vancomycin Hydrochloride for Injection. *Chin. J. Antibiot.* 43, 262–268.

Zhou, C. C., Stoner, E. J., Kristensen, E. W., Stewart, K. D., Rasmussen, R. R., Hollis, L. S., et al. (2004). Formation, Isolation and Characterization of an AB-Biaryl Atropisomer of Oritavancin. *Tetrahedron* 60, 10611–10618. doi:10.1016/j.tet.2004.09.019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of

the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Tian, Chong, Yao and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.