

Supporting Information

Genome Mining of Actinomycin Shunt products from *Kitasatospora* sp.

YINM00002

Zhou-Tian-Le Zhang^{a, 1}, Hui-Bing Sun^{a, 1}, Zhen Ren^{b, 1}, Tian-Peng Xie^a, Ying-Fang Wang^a, Yin Guo^a, Xiaoyu Su^a, Min Yin^{a, *}, Hao Zhou^{a, *} and Zhong-Tao Ding^{a, c}

Correspondence to: yinmin@ynu.edu.cn; haozhou@ynu.edu.cn

This PDF file includes:

Supplementary text

Figures S1 to S32

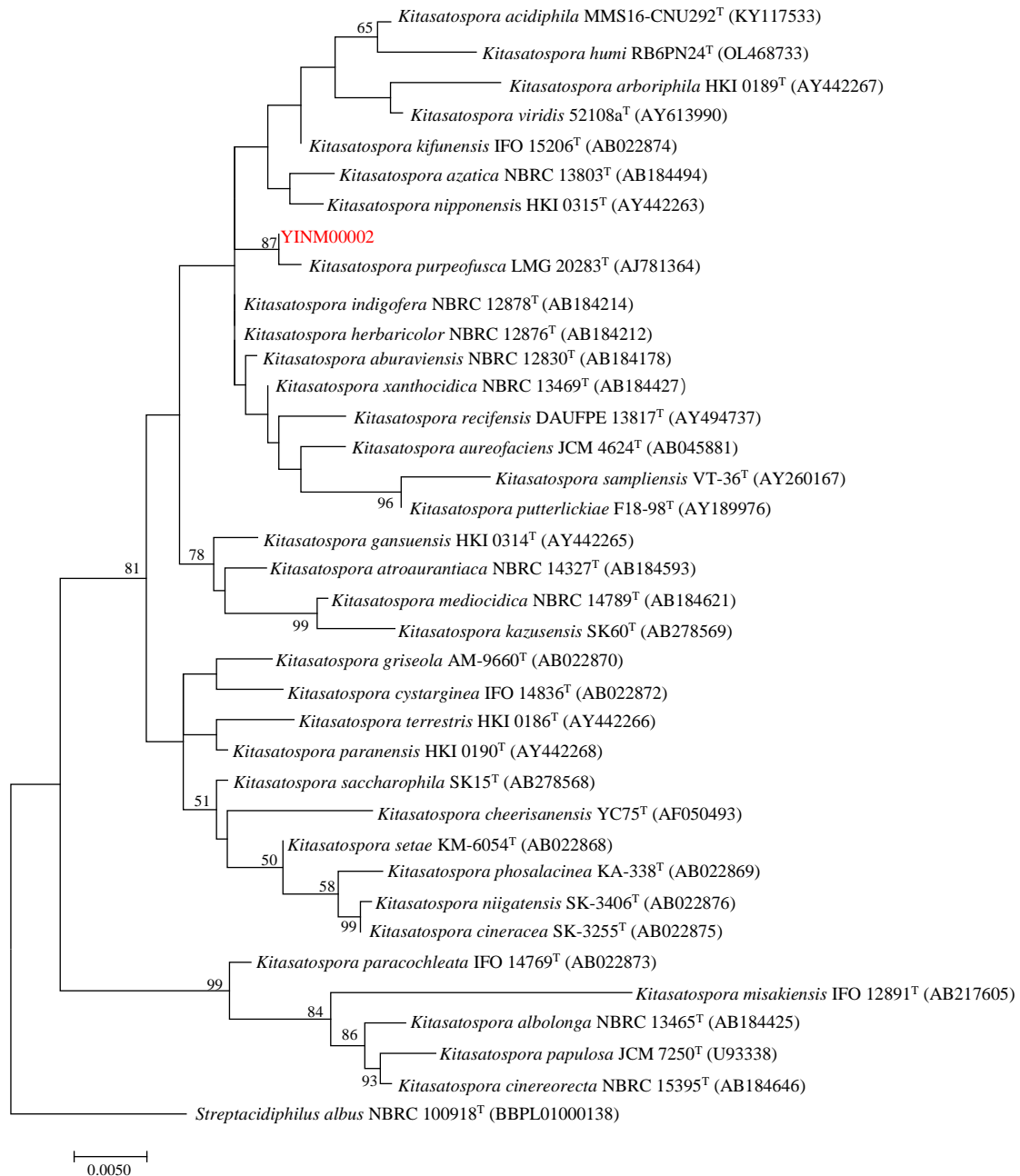


Fig. S1. Maximum-likelihood tree (MEGA 7.0) showing the relationships between strain YINM00002 and related members of the genus *Kitasatospora*. Bootstrap values (>50%) based on 1000 replicates are shown at the branch nodes. *Streptacidiphilus albus* NBRC 100918^T was used as the outgroup. Bar, 0.5% sequence divergence.

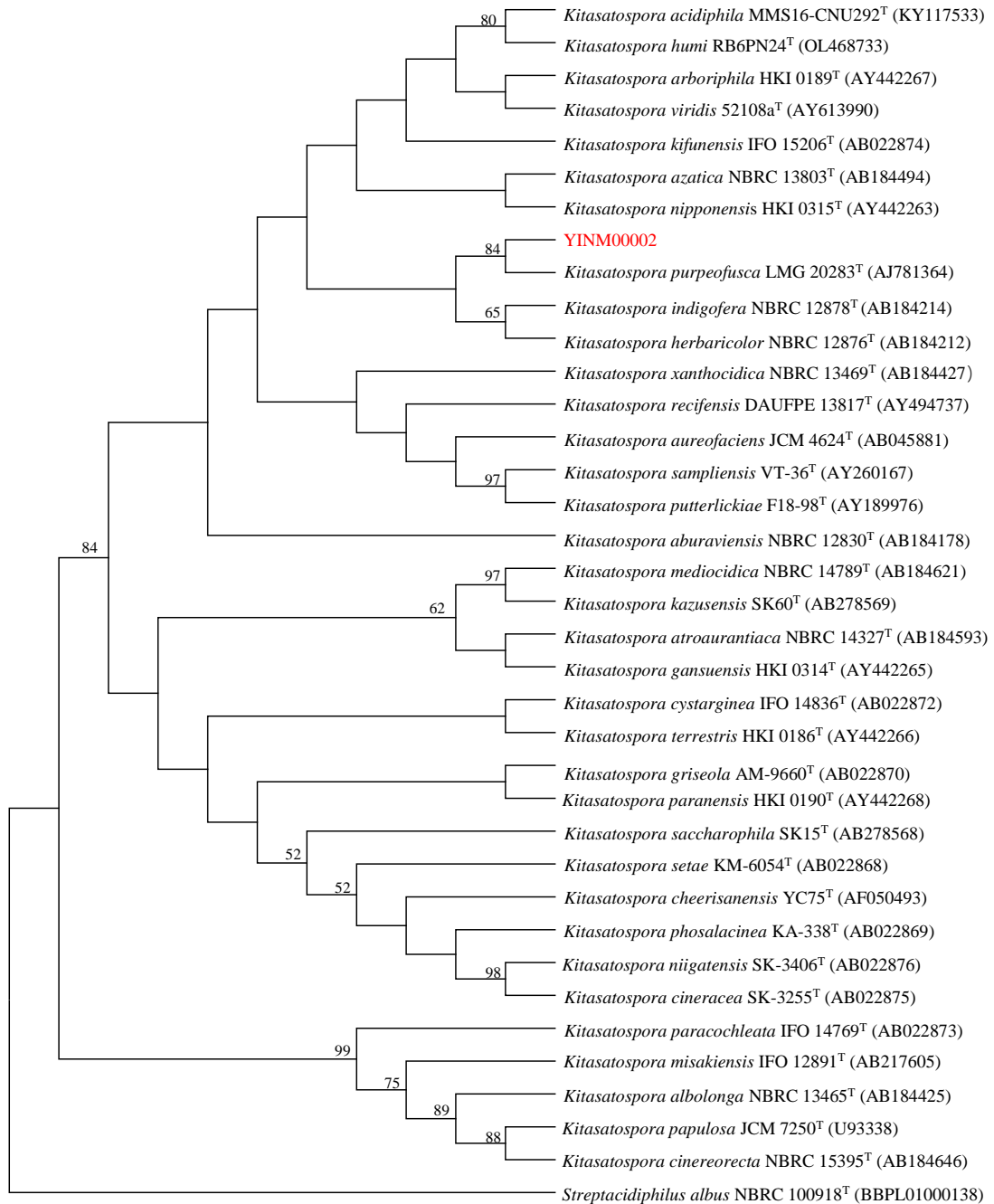


Fig. S2. Maximum-parsimony tree (MEGA 7.0) showing the relationships between strain YINM00002 and related members of the genus *Kitasatospora*. Bootstrap values (>50%) based on 1000 replicates are shown at the branch nodes. *Streptacidiphilus albus* NBRC 100918^T was used as the outgroup.

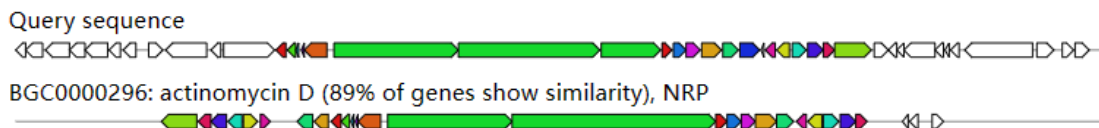


Fig. S3. The similarity of actinomycin D biosynthetic gene cluster in YINM00002 with the known one.

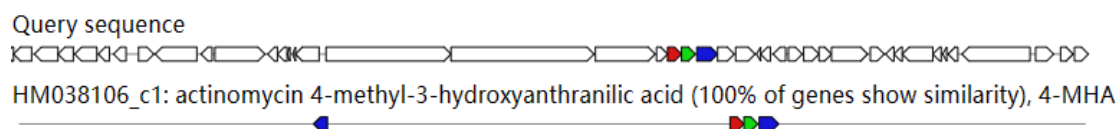


Fig. S4. The genes from actinomycin D biosynthetic gene cluster were high similar to 4-MHA biosynthetic genes.

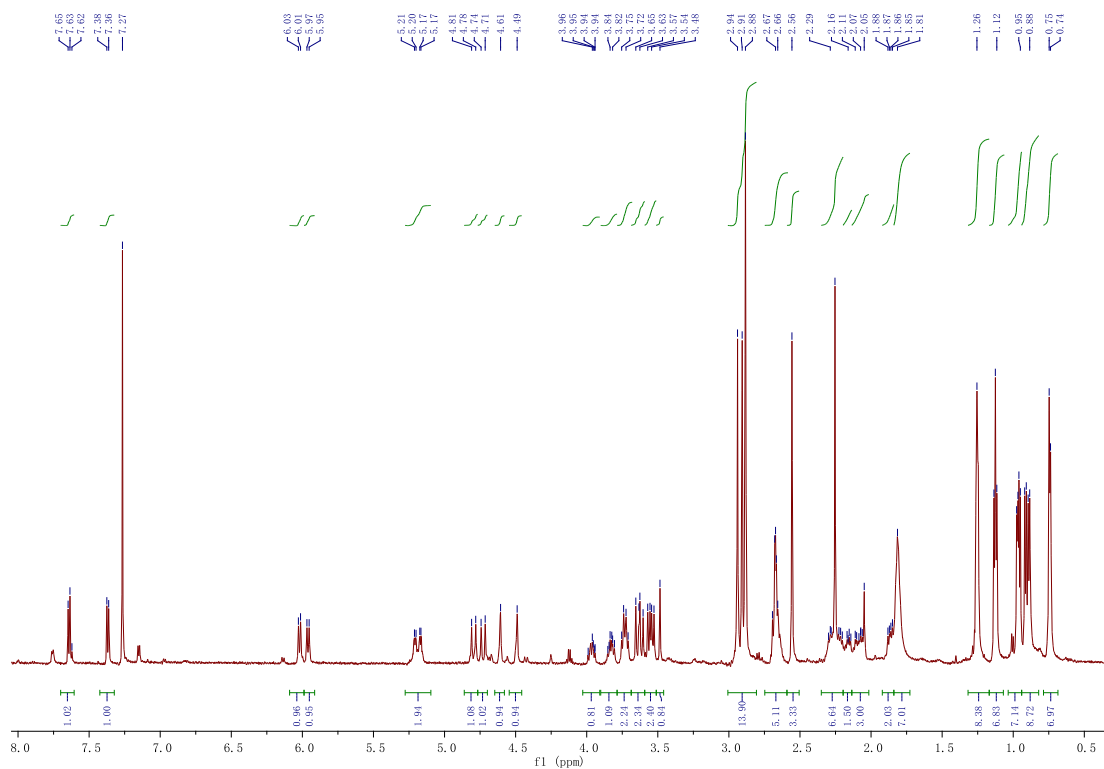


Fig. S5. ^1H NMR (600 MHz) spectrum of actinomycin D (**1**, in CDCl_3-d)

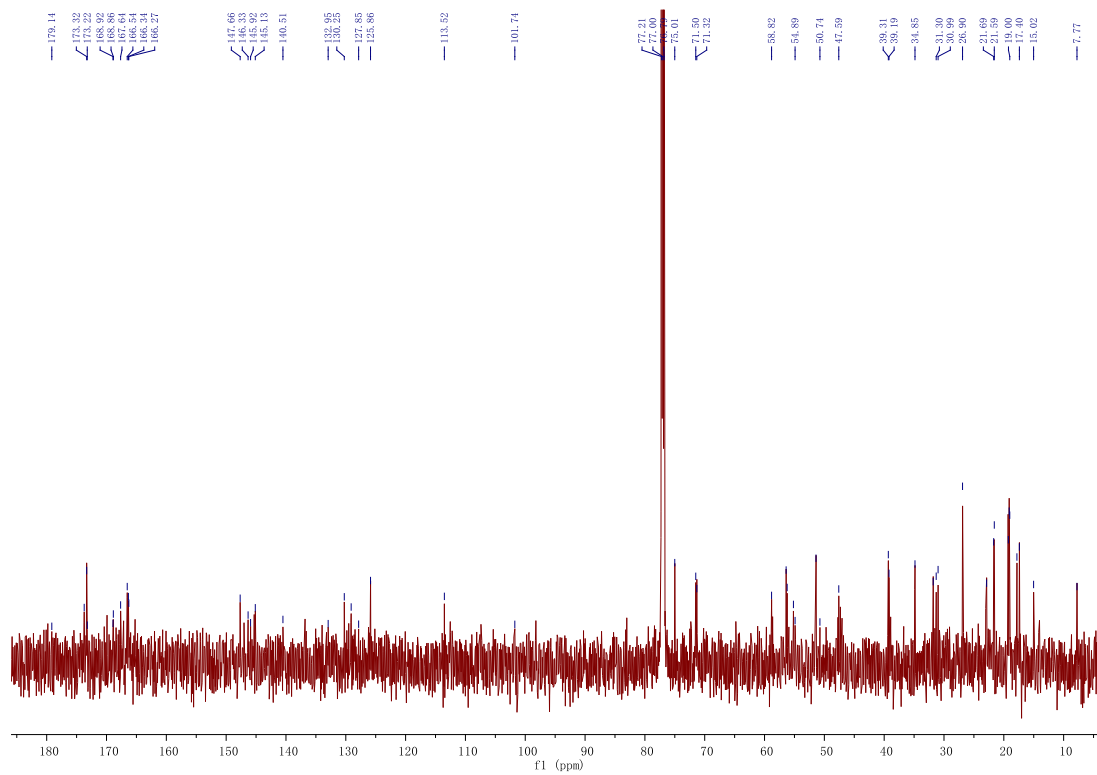


Fig. S6. ^{13}C NMR (150 MHz) spectrum of actinomycin D (**1**, in $\text{CDCl}_3\text{-}d$)

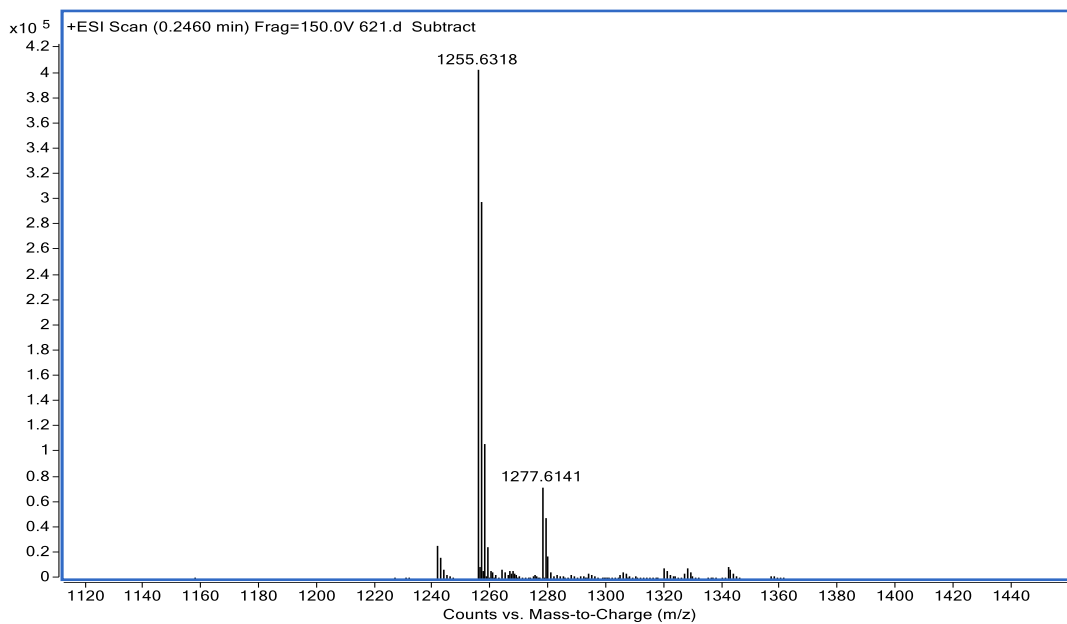


Fig. S7. (+) HRESIMS data of actinomycin D (**1**)

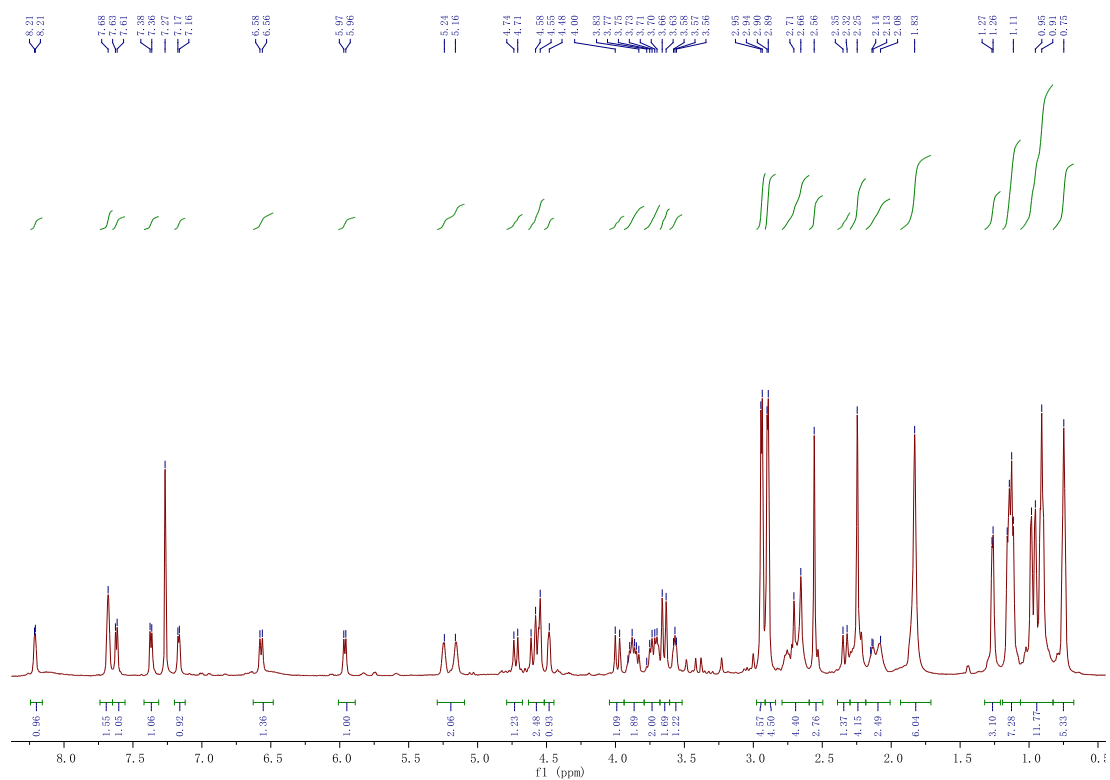


Fig. S8. ^1H NMR (600 MHz) spectrum of actinomycin V (**2**, in CDCl_3-d)

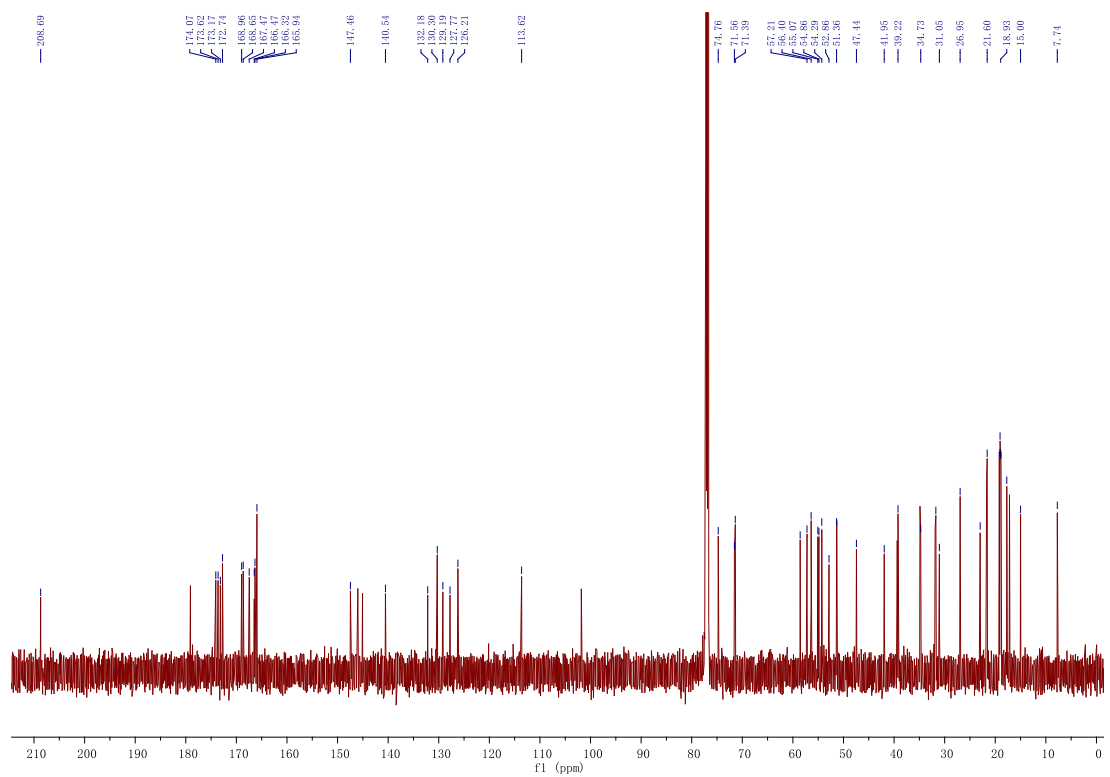


Fig. S9. ^{13}C NMR (150 MHz) spectrum of actinomycin V (**2**, in CDCl_3-d)

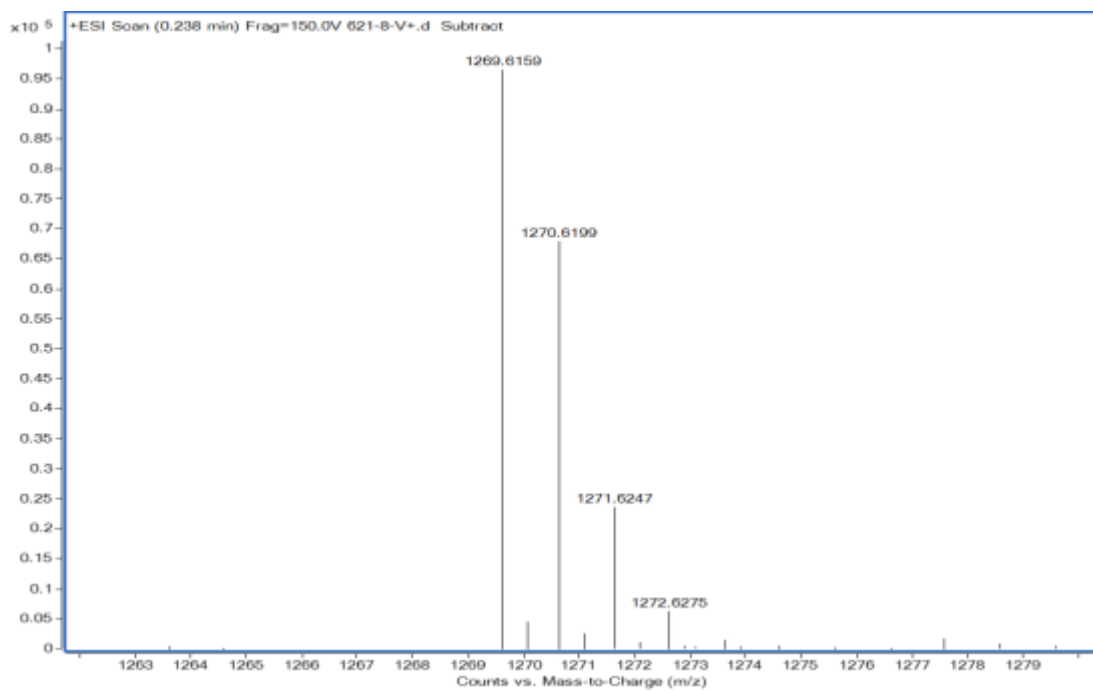


Fig. S10. (+) HRESIMS data of actinomycin V (2)

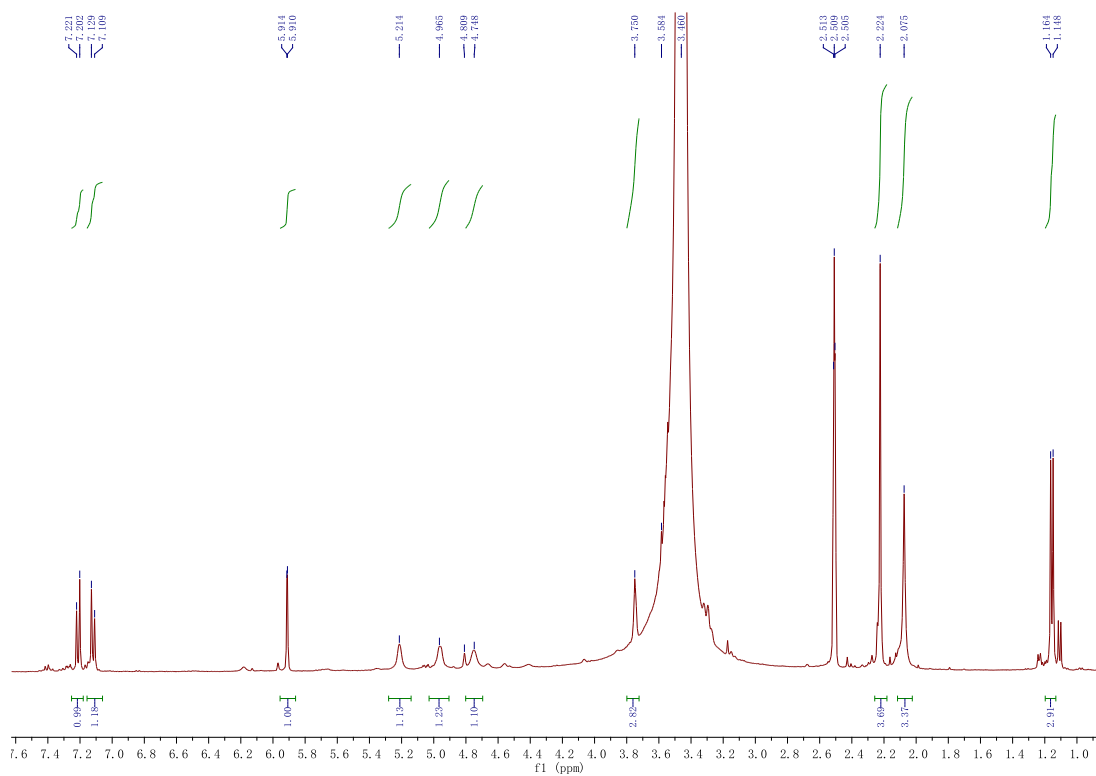


Fig. S11. ¹H NMR (400 MHz) spectrum of actinrhater A (3, in DMSO-d₆)

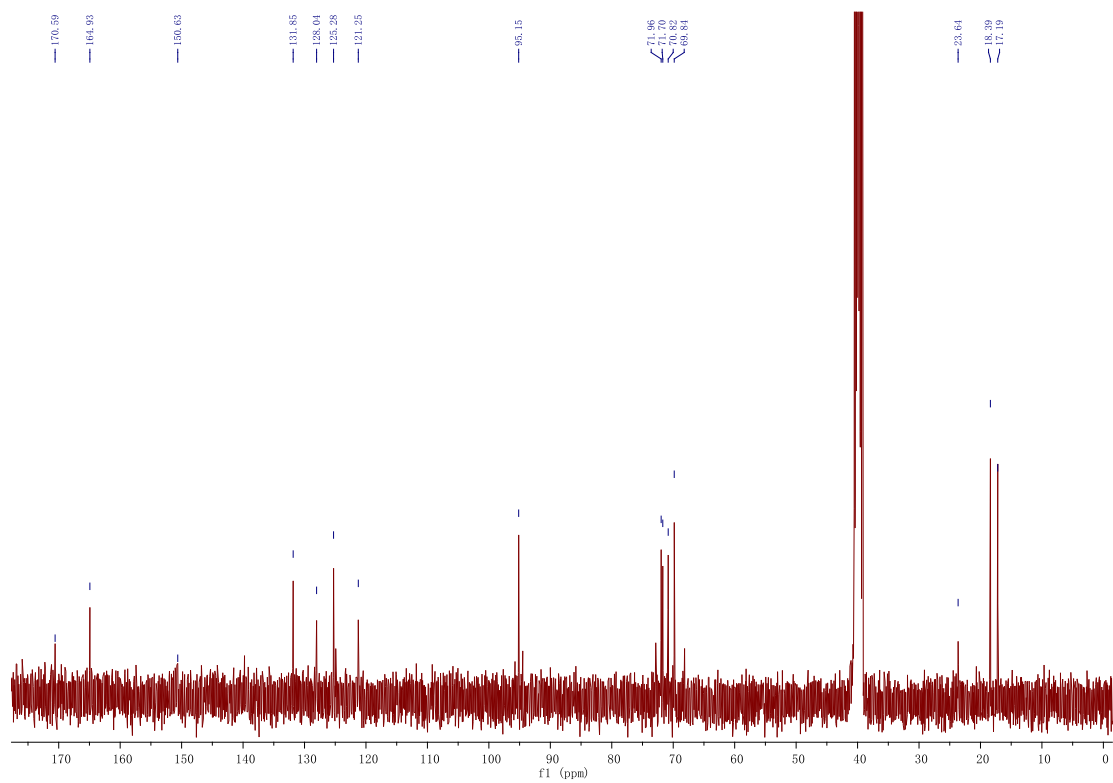


Fig. S12. ^{13}C NMR (100 MHz) spectrum of actinrhater A (**3**, in $\text{DMSO-}d_6$)

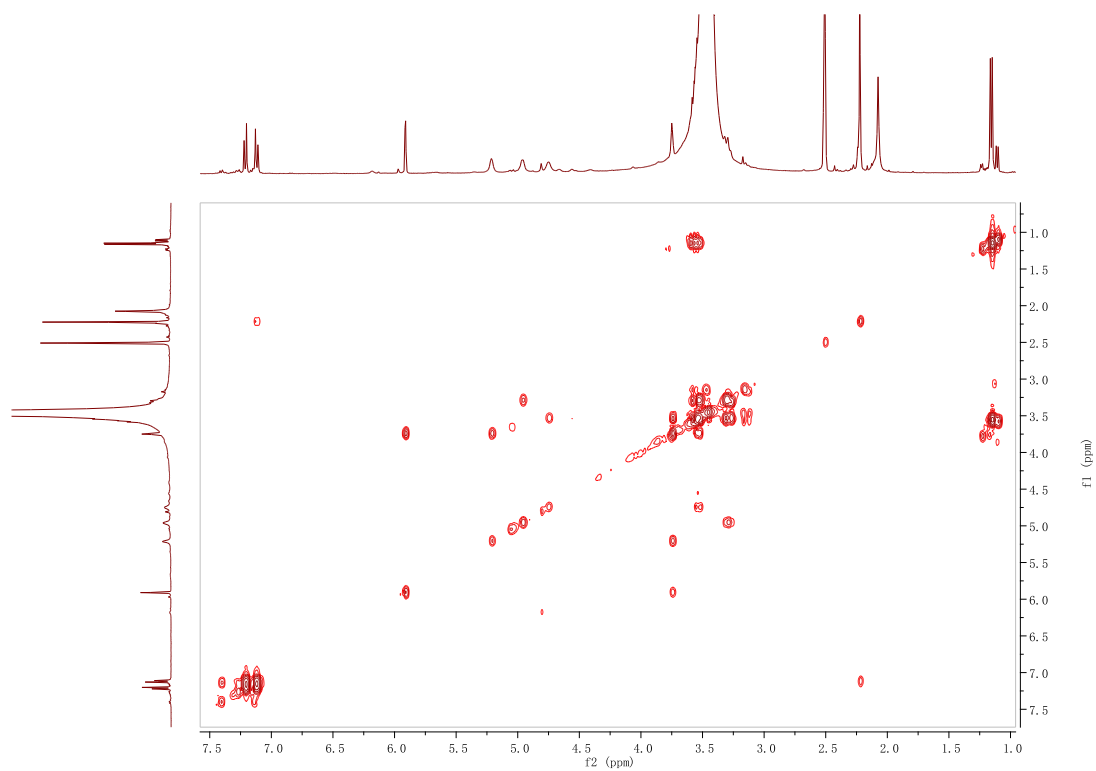


Fig. S13. COSY spectrum (400 MHz) of actinrhater A (**3**, in $\text{DMSO-}d_6$)

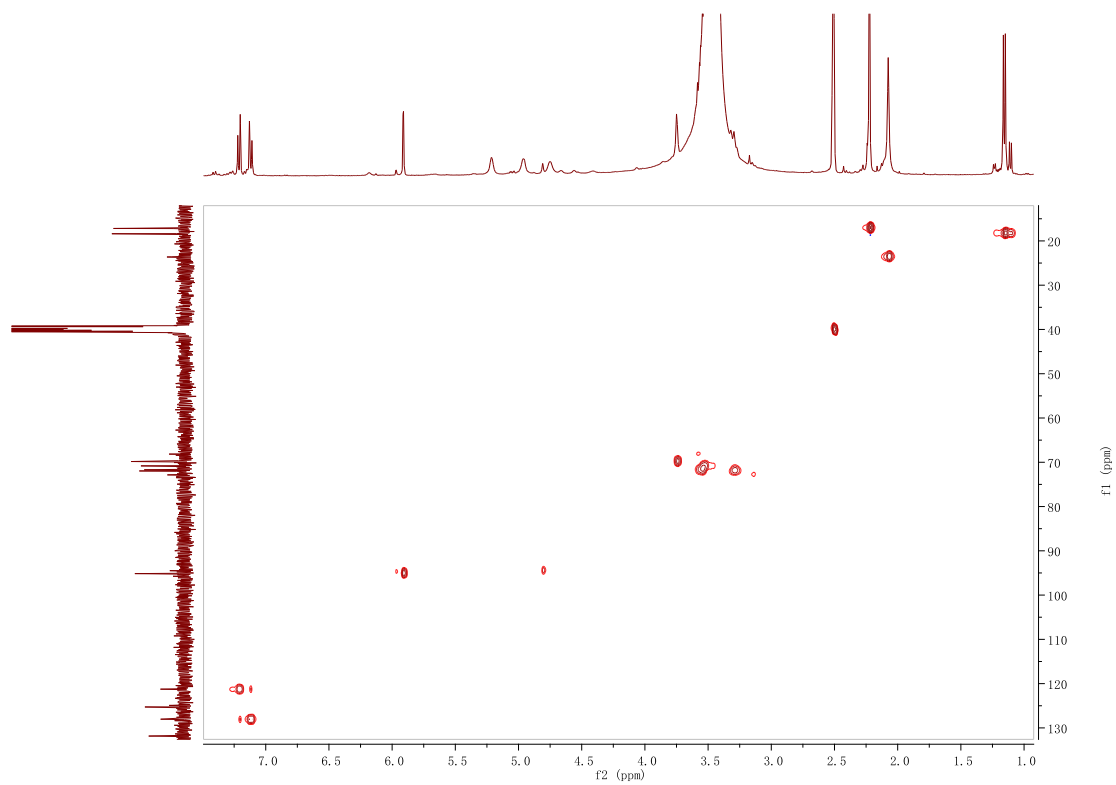


Fig. S14. HSQC spectrum (400 MHz) of actinrhater A (**3**, in DMSO- d_6)

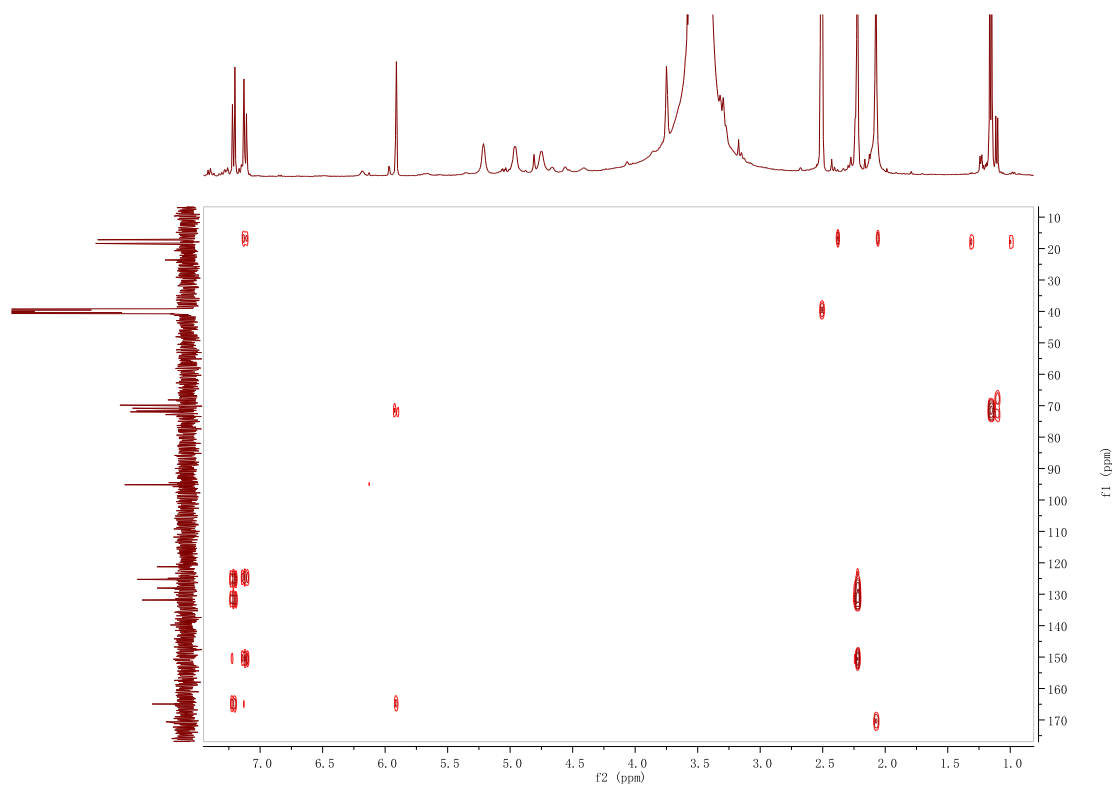


Fig. S15. HMBC spectrum (400 MHz) of actinrhater A (**3**, in DMSO- d_6)

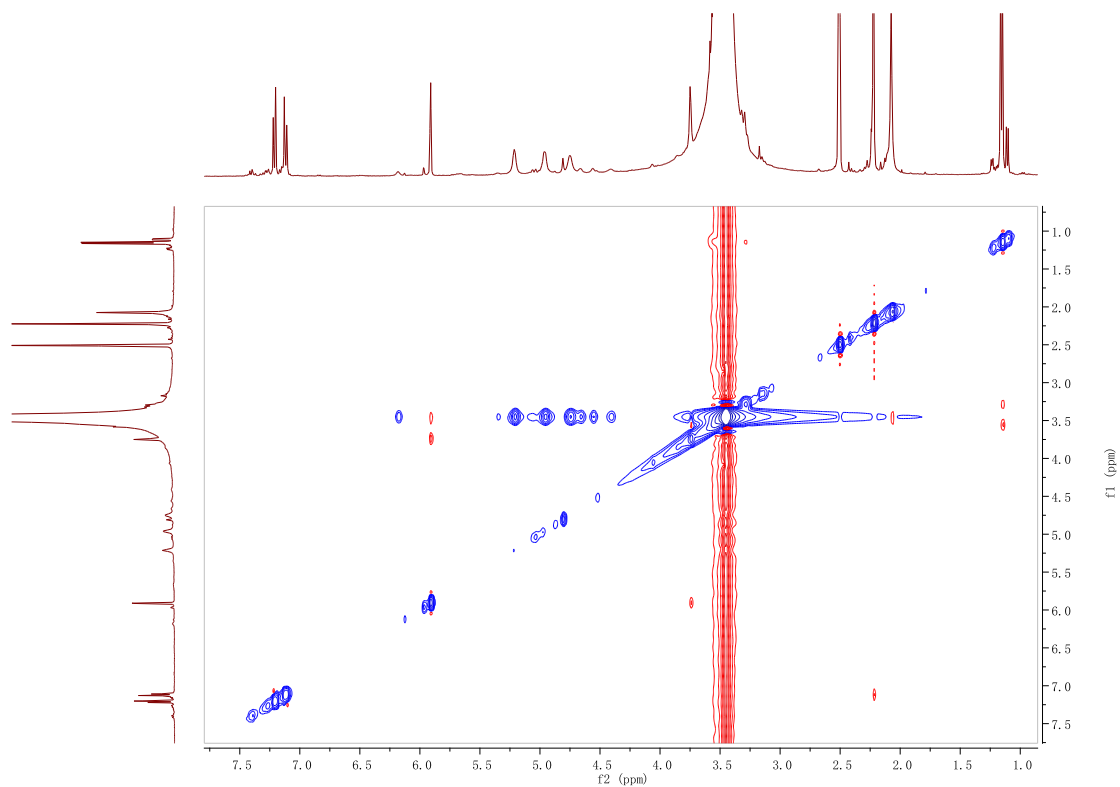


Fig. S16. NOESY spectrum (400 MHz) of actinrhater A (**3**, in DMSO- d_6)

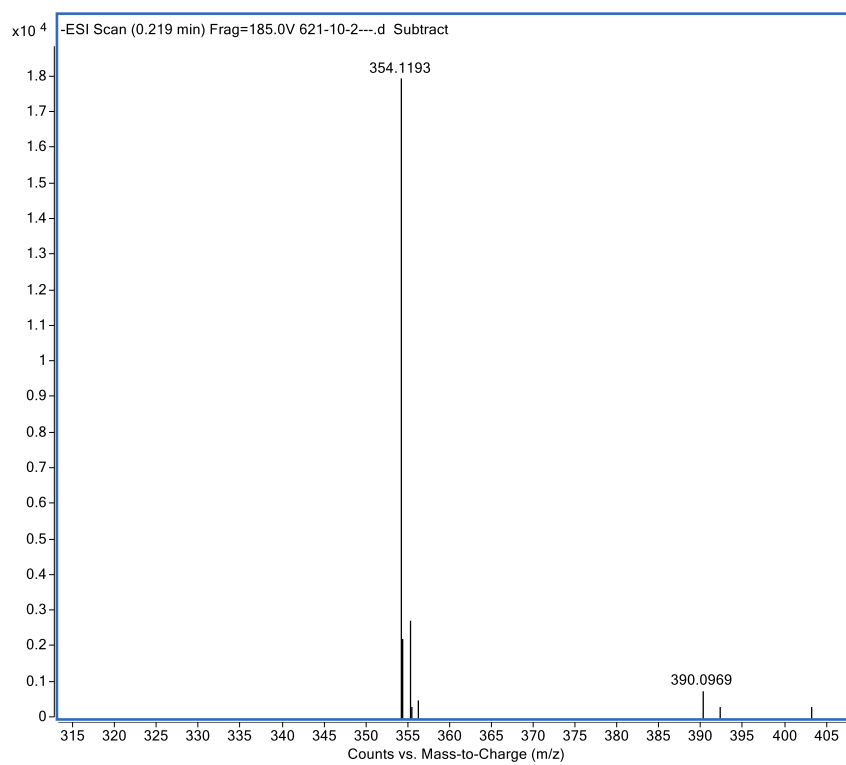


Fig. S17. (-) HRESIMS data of actinrhater A (**3**)

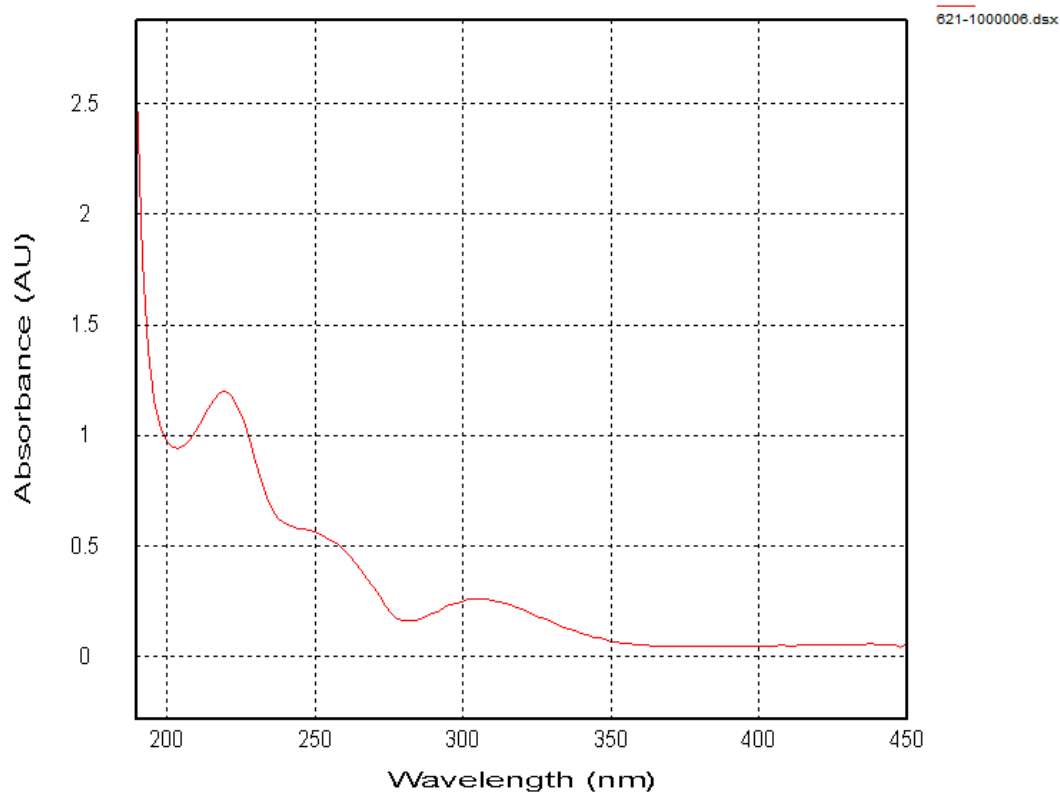


Fig. S18. The UV spectrum of actinrhater A (3)

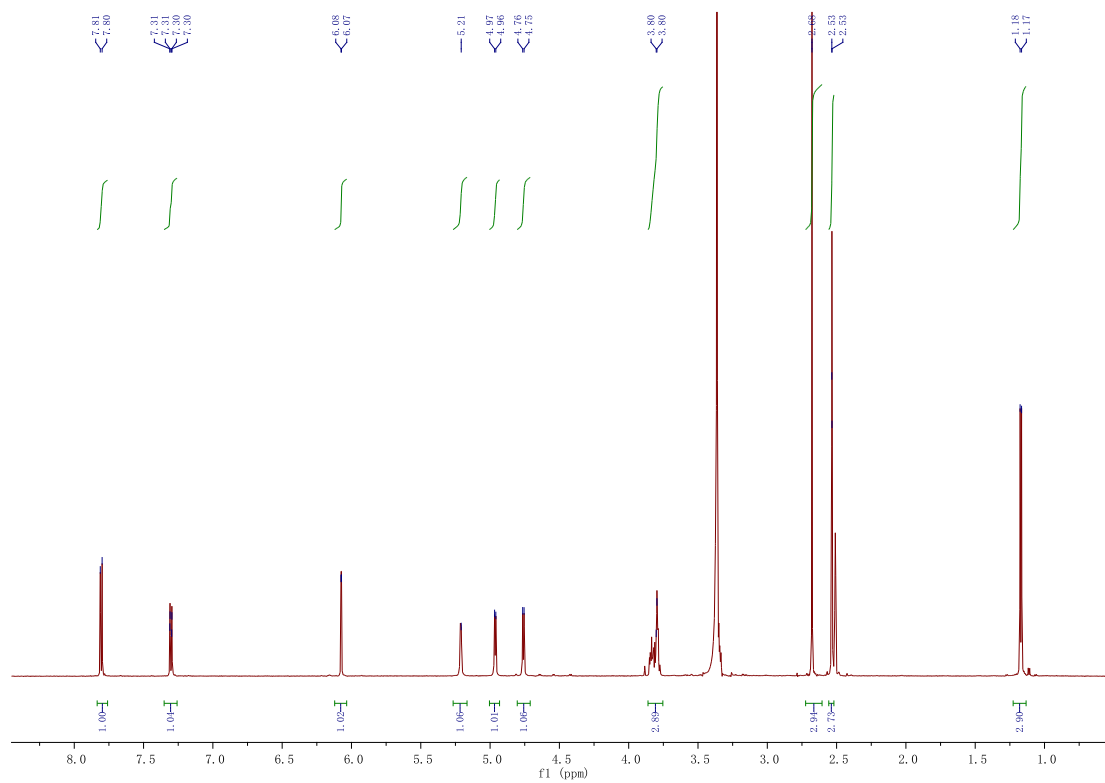


Fig. S19. ¹H NMR (600 MHz) spectrum of actinrhater B (4, in DMSO-*d*₆)

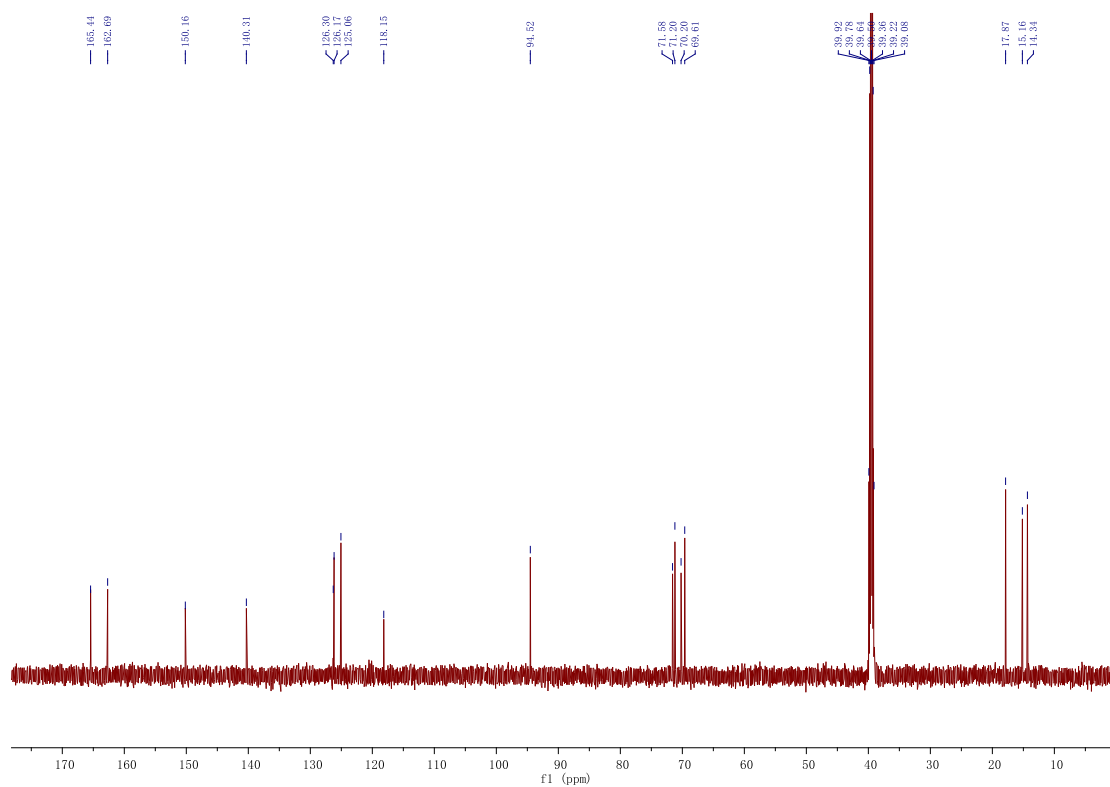


Fig. S20. ^{13}C NMR (150 MHz) spectrum of actinrhater B (**4**, in $\text{DMSO-}d_6$)

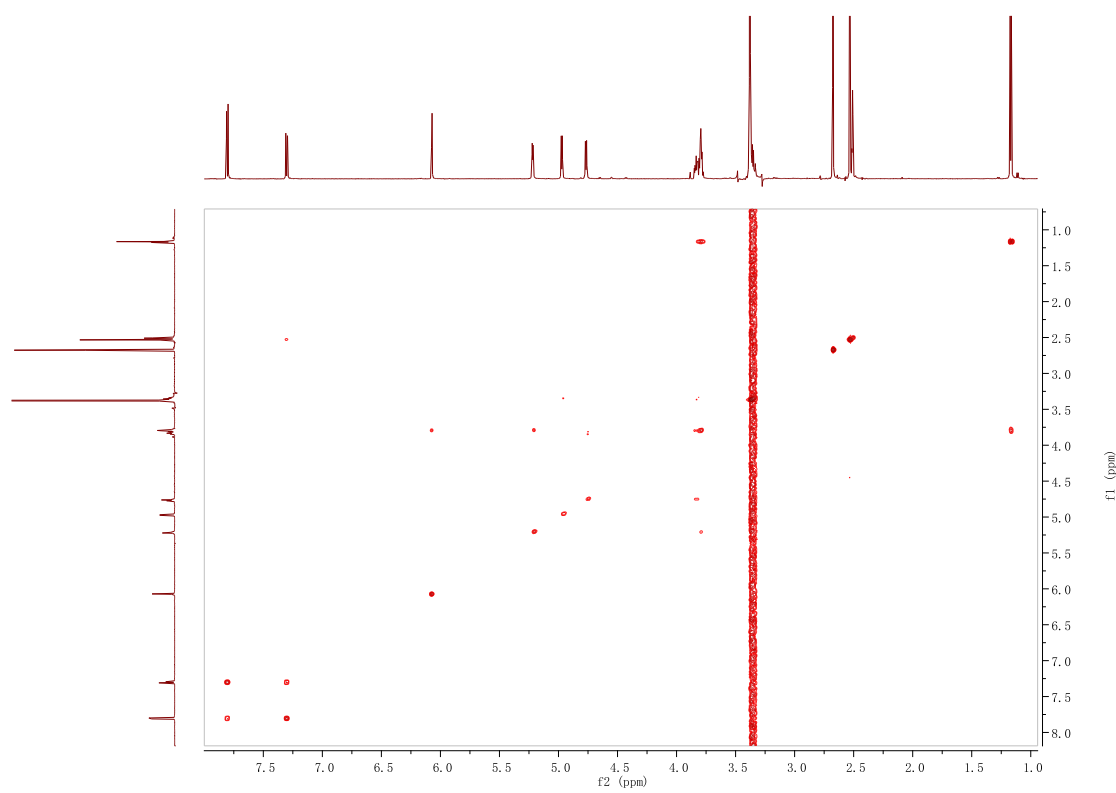


Fig. S21. COSY (600 MHz) spectrum of actinrhater B (**4**, in $\text{DMSO-}d_6$)

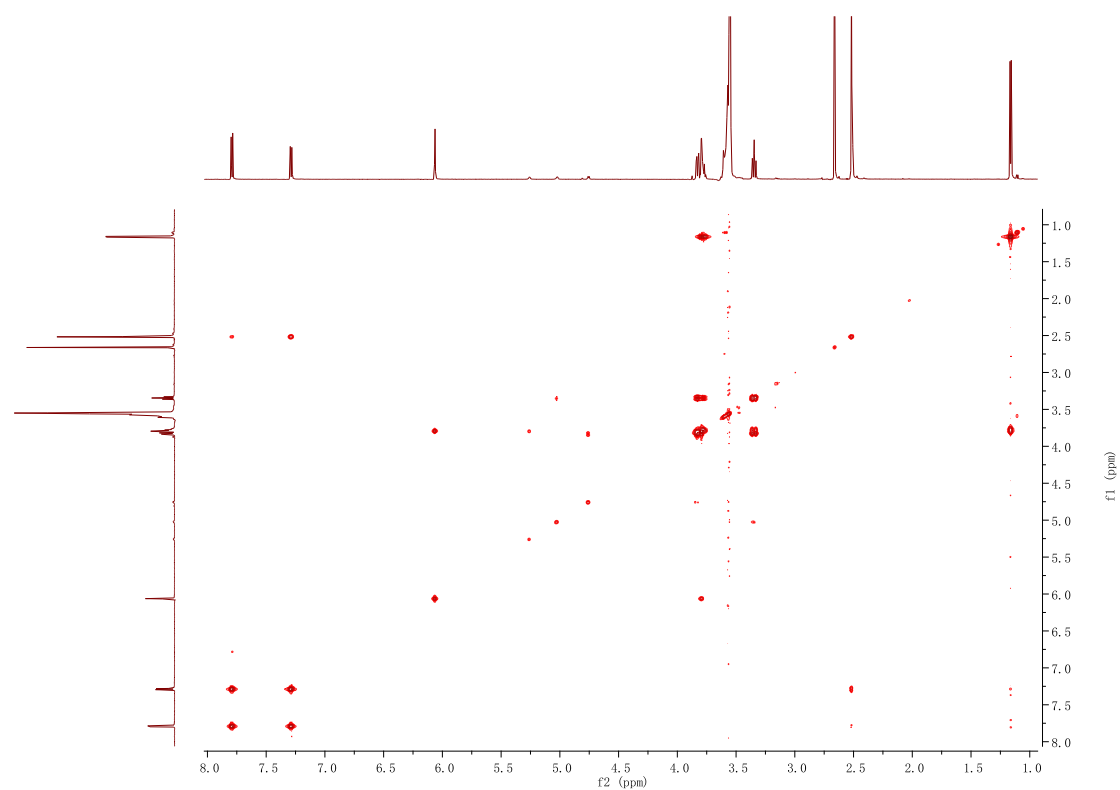


Fig. S22. COSY (600 MHz) spectrum of actinrhater B (**4**, in DMSO- d_6 + D $_2$ O)

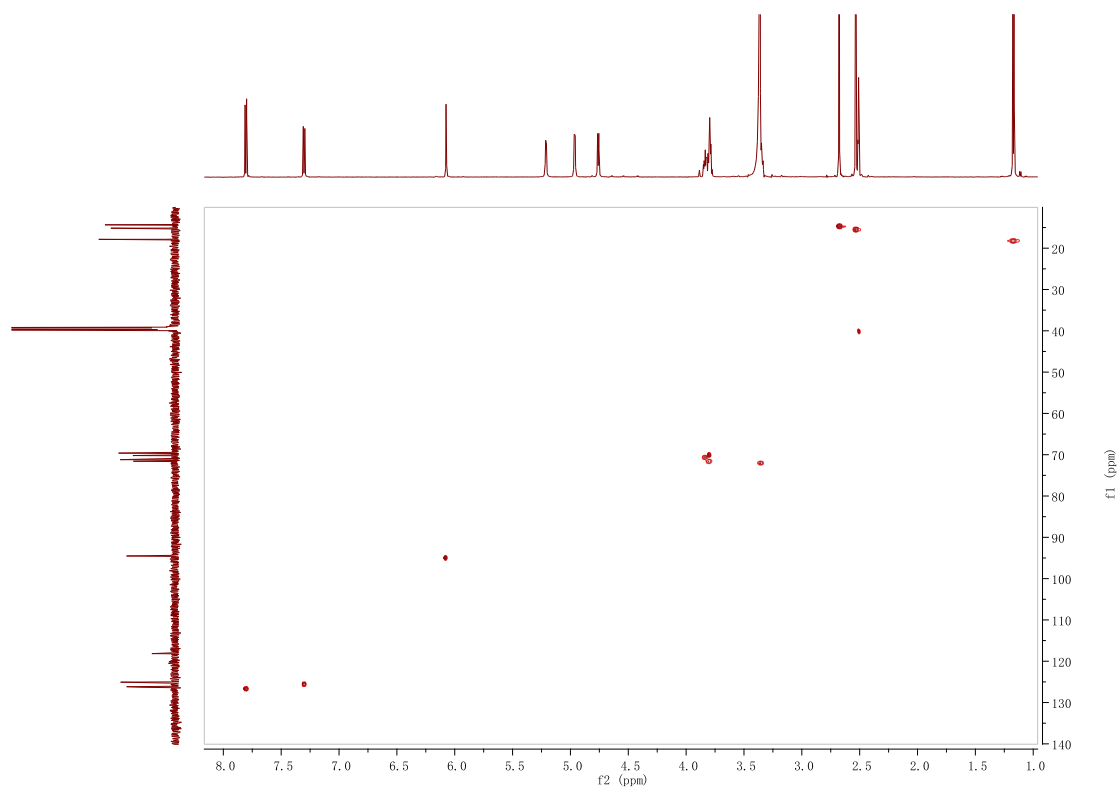


Fig. S23. HSQC (600 MHz) spectrum of actinrhater B (**4**, in DMSO- d_6)

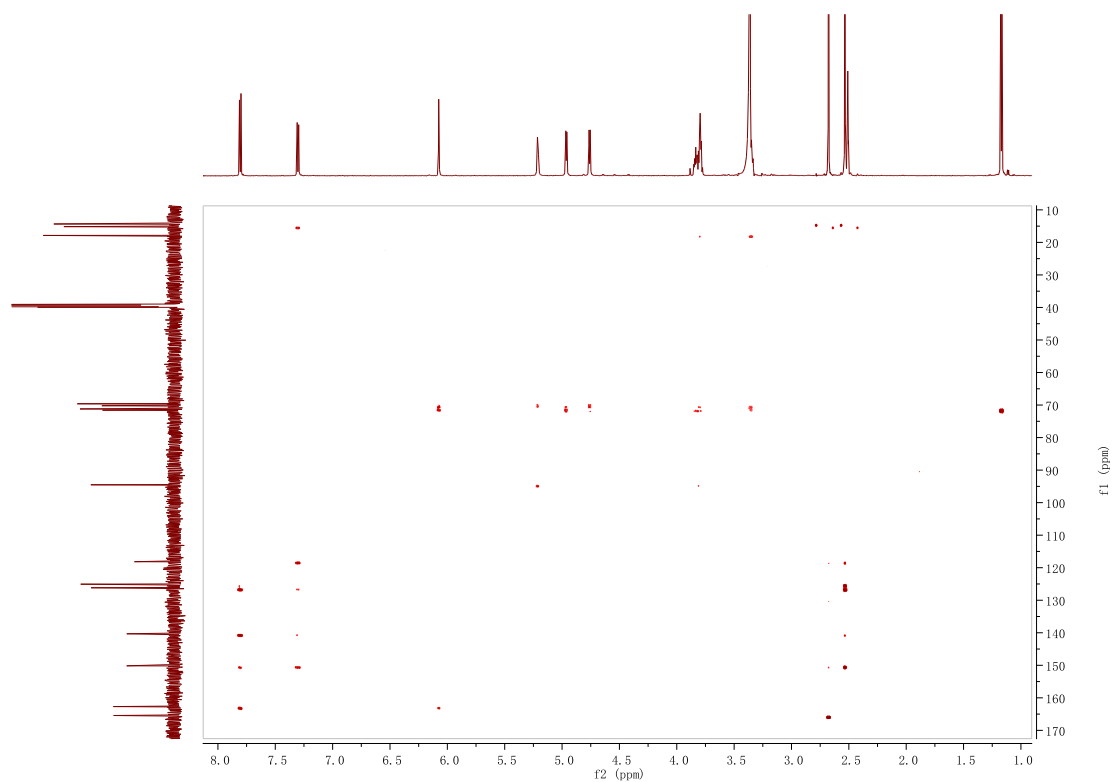


Fig. S24. HMBC (600 MHz) spectrum of actinrhater B (**4**, in DMSO- d_6)

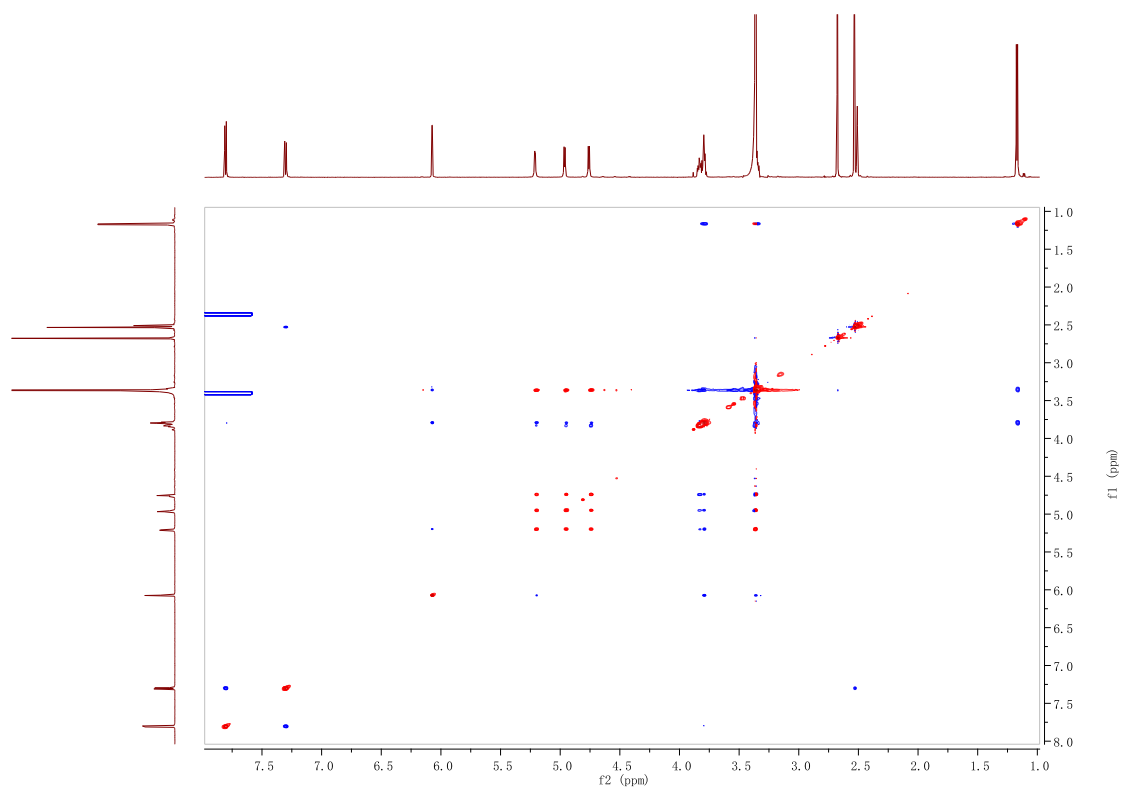


Fig. S25. ROESY (600 MHz) spectrum of actinrhater B (**4**, in DMSO- d_6)

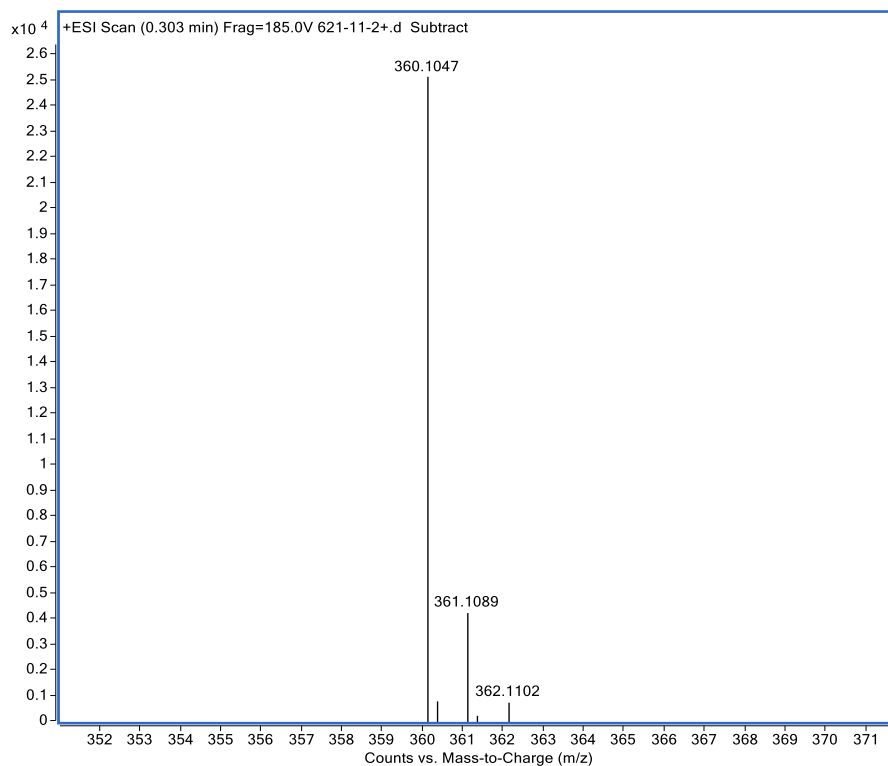


Fig. S26. (+) HRESIMS data of actinrhater B (4)

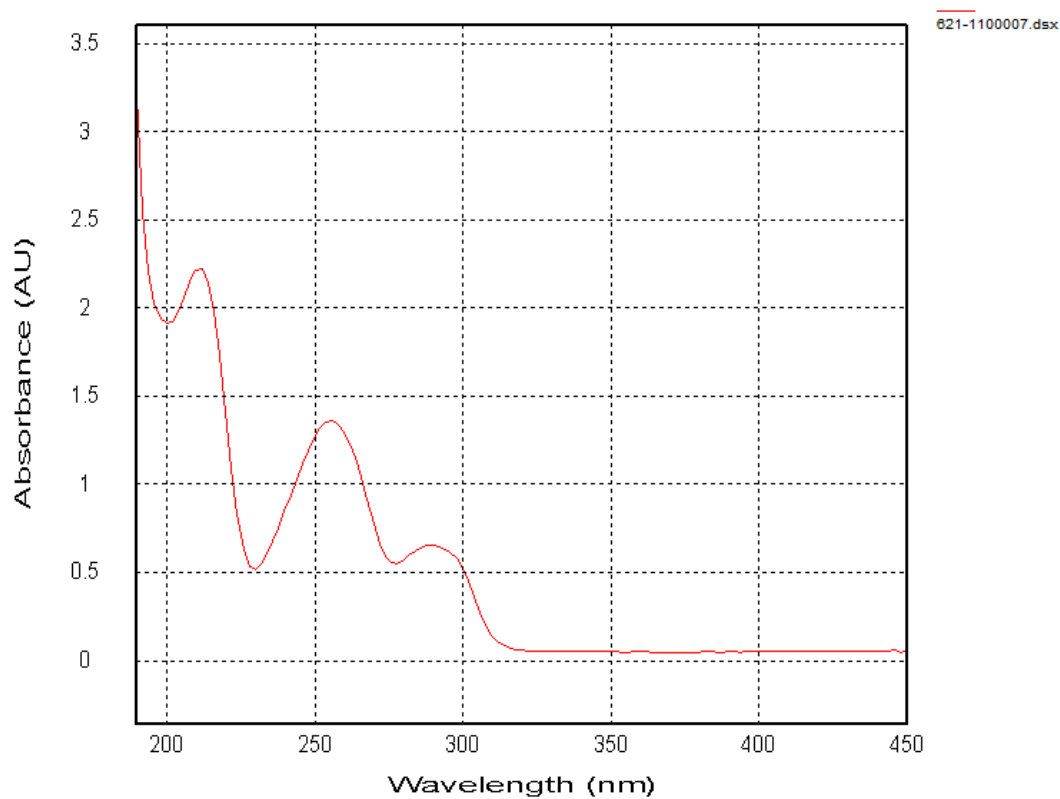


Fig. S27. The UV spectrum of actinrhater B (4)

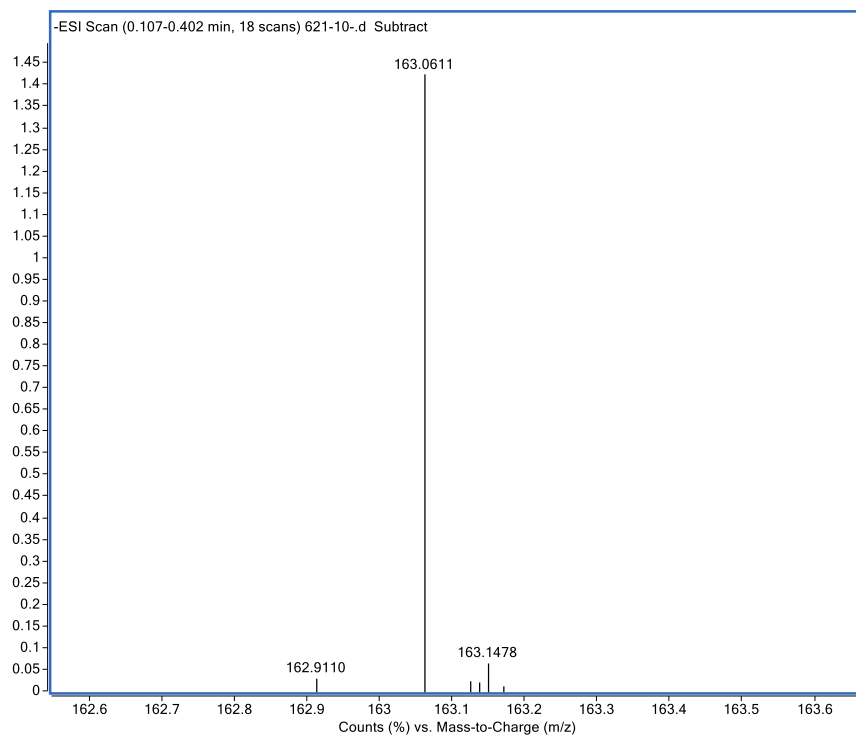


Fig. S28. (-) HRESIMS data of rhamnose from actinrhater A (3)

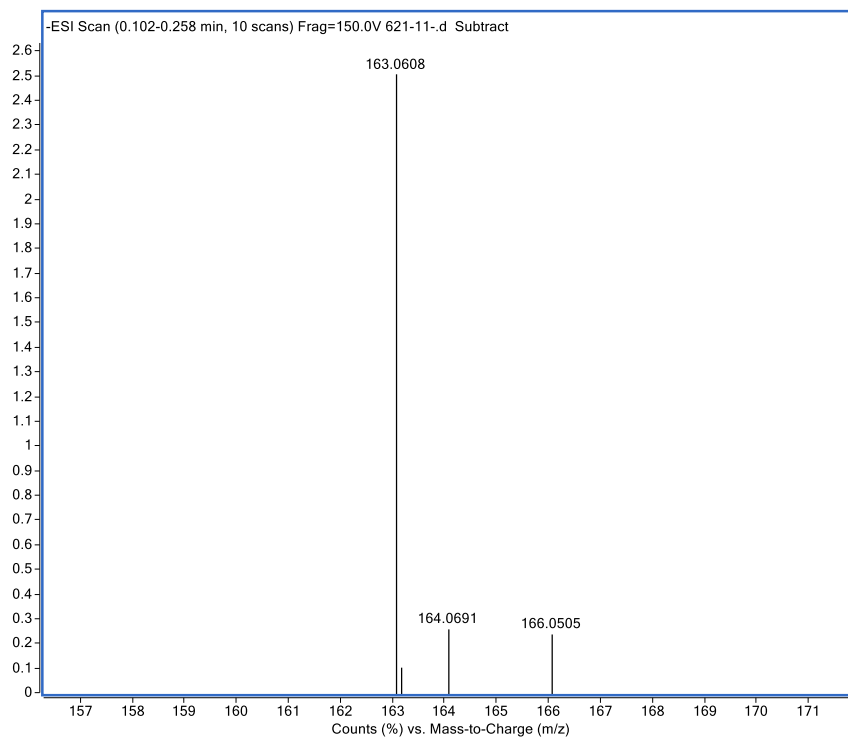


Fig. S29. (-) HRESIMS data of rhamnose from actinrhater B (4)

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2022/11/11
Method Name : Specific Rotation @25C
Lot Identifier : L-SLT
Set Temperature : 25.0°C
Time Delay : 5
Delay between measurement : 2 Sec

N	Avg.	Std.Dev.	%RSD	Min	Max
5	-4.920	0.438	-8.90	-5.400	-4.600

S.No	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Temp
1	11:24:16	-4.600	SR	-0.0046	589	100	25.0°C
2	11:24:24	-5.400	SR	-0.0054	589	100	25.0°C
3	11:24:32	-5.400	SR	-0.0054	589	100	25.0°C
4	11:24:39	-4.600	SR	-0.0046	589	100	25.0°C
5	11:24:47	-4.600	SR	-0.0046	589	100	25.0°C

Fig. S30. The rotation values of L-rhamnose-standard (0.1 mg/mL).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2022/11/11
Method Name : Specific Rotation @25C
Lot Identifier : 621-10-1
Set Temperature : 25.0°C
Time Delay : 5
Delay between measurement : 2 Sec

N	Avg.	Std.Dev.	%RSD	Min	Max
5	-8.560	0.428	-5.00	-9.000	-8.100

S.No	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Temp
1	11:35:01	-9.000	SR	-0.0090	589	100	25.0°C
2	11:35:09	-8.100	SR	-0.0081	589	100	25.0°C
3	11:35:17	-8.800	SR	-0.0088	589	100	25.0°C
4	11:35:25	-8.100	SR	-0.0081	589	100	25.0°C
5	11:35:33	-8.800	SR	-0.0088	589	100	25.0°C

Fig. S31. The rotation values of rhamnose actinrhater A (3) (0.1 mg/mL).

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2022/11/11
Method Name : Specific Rotation @25C
Lot Identifier : 621-11
Set Temperature : 25.0°C
Time Delay : 5
Delay between measurement : 2 Sec

N	Avg.	Std.Dev.	%RSD	Min	Max
5	-6.864	0.173	-2.51	-7.100	-6.640

S.No	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Temp
1	11:44:10	-7.100	SR	-0.0355	589	100	25.0°C
2	11:44:18	-6.940	SR	-0.0347	589	100	25.0°C
3	11:44:26	-6.940	SR	-0.0347	589	100	25.0°C
4	11:44:34	-6.800	SR	-0.0340	589	100	25.0°C
5	11:44:42	-6.640	SR	-0.0332	589	100	25.0°C

Fig. S32. The rotation values of rhamnose from actinrhater B (4) (0.1 mg/mL).