Synthesis of Novel 3,4-Chloroisothiazole-Based Imidazoles as Fungicides and Evaluation of Their Mode of Action

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Supporting Information

Table 4. Scores of molecular designation by docking between the designed target compounds and BcCYP51 protein

	Binding Affinity				
Compd.	(kcal/mol)	Compd.	(kcal/mol)	Compd.	(kcal/mol)
***R*-1**	6.6	***R*-7**	8.7	***S*-1**	6.6
***R*-2**	6.5	***R*-8**	8.2	***S*-11**	7.1
***R*-3**	7.1	***R*-9**	7.7	***S*-12**	9.1
***R*-4**	7.8	***R*-10**	8.4	tioconazole	8.4
***R*-5**	7.8	***R*-11**	7.3	imazalil	7.3
***R*-6**	7.5	***R*-12**	9.4		

Amino acid sequence homology of CYP51 between fungi and plants

The amino acid sequences of the CYP51s of plant pathogens and of plants were obtained from GenBank. The fourteen sequences are as follows: *B. maydis* (ENI03630.1); *B. oryzae* (EUC44554.1); *B. graminis* (CCU76296.1); *B. cinerea* (XP_001549961.1); *F. graminearum* (XP_381176.1); *F. oxysporum* (EMT62075.1); *F. verticillioides* (EWG37542.1); *M. oryzae* (EHA53720.1); *R. solani* (EUC64781.1); *S. sclerotiorum* (EDO02329.1); *S. turcica* (EOA80713.1); *U. maydis* (XP_759809.1); *O. sativa* (EKV48970.1) and *A. thaliana* (NC_003070.9). The sequence alignment was performed by the program Clustal X 2.

Cytochrome P450 14α-demethylase of plants and fungi have six substrate binding region (SRS) domains and one heme binding domain. As shown in Figure 8, the heme binding domain is highly conserved and is usually used to identify CYP51 members, while SRS4, SRS5 and SRS6 are substrate identification domains. Among these domains, SRS4 can form a binding pocket with the substrate. In addition, the conserved residues in SRS2 and SRS3 have great influence on their catalytic activity. The sequences in fungi and plants were identified by their SRS domains and heme binding domain and aligned by the program Clustal X 2, and the results are listed in Table 5. The CYP51 of fungi exhibited 27-35% amino acid sequence identities with plants. The CYP51 sequence identities in the plant pathogen were 47 to 93%. These results indicated that the target compounds in this study could be safe for crops when used as fungicides.

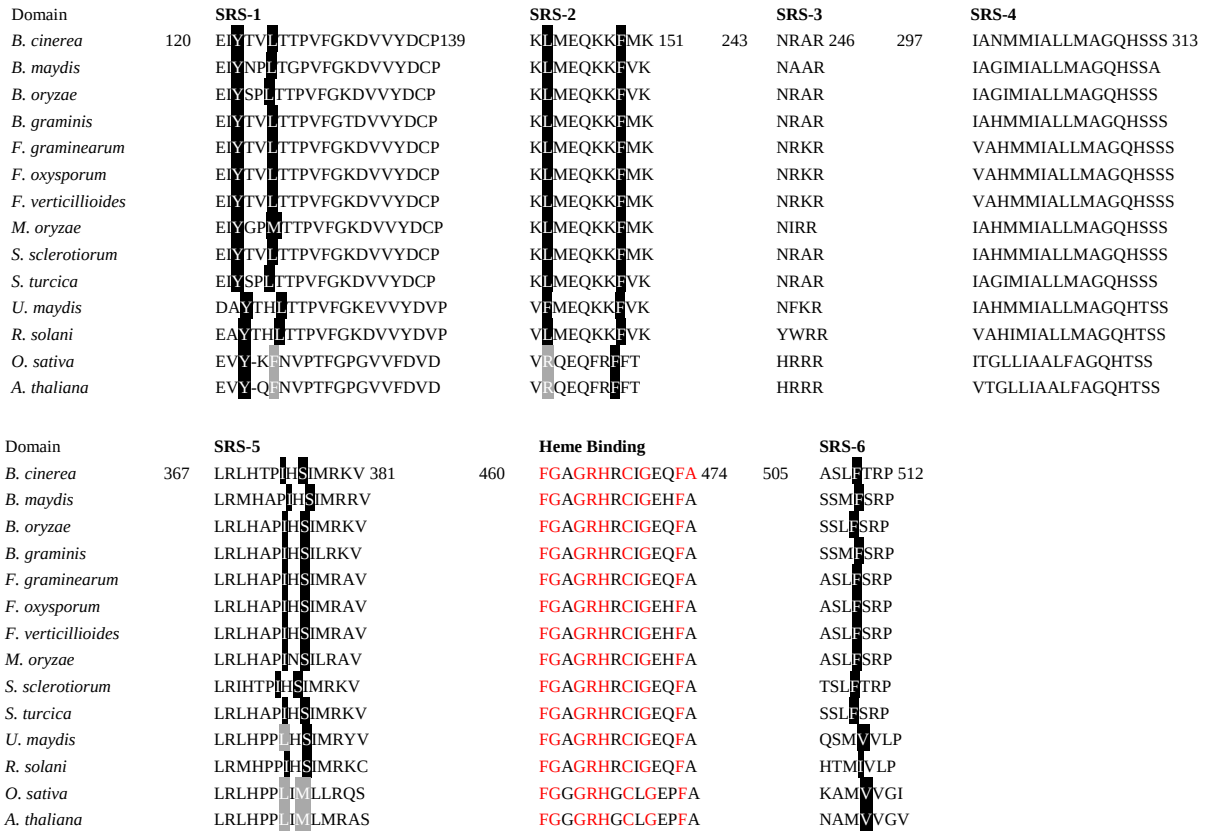

Figure 7. Sequences Alignment of the SRS domain and Heme binding domain

Table 5. Sequence homology of CYP51 between fungi and plant.

	B. c	*B. m*	*B. o*	*B. g*	*F. g*	*F. o*	*F. v*	*M. o*	*S. s*	*S. t*	*U. m*	*R. s*	*O. s*	*A. t*
B. c	100	62.1	64.5	72.2	69.7	68.5	68.5	65.1	92.5	64.6	50	46.5	26.7	27.6
B. m	—	100	78.5	57.6	60.3	60	60	58	61.7	77.7	48.5	47.1	28.8	29.8
B. o	—	—	100	58.3	61.6	60.7	60.7	58.6	64.7	87.5	47.6	46	29	29.3
B. g	—	—	—	100	64.8	65	65	62.6	71.6	58.9	47.8	47	28	28.7
F. g	—	—	—	—	100	93	93.5	72.5	69.7	61.9	49.1	47.7	28.5	30.3
F. o	—	—	—	—	—	100	98.7	72.1	68.9	61.3	48.8	46.8	29.1	31.1
F. v	—	—	—	—	—	—	100	72.3	69.5	61.7	48.8	47.2	29.3	31.3
M. o	—	—	—	—	—	—	—	100	64.3	59.3	46.9	44.3	28.8	29.1
S. s	—	—	—	—	—	—	—	—	100	64.4	49.8	46.9	27.5	27.8
S. t	—	—	—	—	—	—	—	—	—	100	47.6	46.3	28.8	29.9
U. m	—	—	—	—	—	—	—	—	—	—	100	56.4	33.6	35.2
R. s	—	—	—	—	—	—	—	—	—	—	—	100	31.4	32.9
O. s	—	—	—	—	—	—	—	—	—	—	—	—	100	76.7
A. t	—	—	—	—	—	—	—	—	—	—	—	—	—	100

Note, *B. m (B. maydis); B. o (B. oryzae); B. g (B. graminis); B. c (B. cinerea); F. g (F. graminearum); F. o (F. oxysporum); F. v (F. verticillioides); M. o (M. oryzae); R. s (R. solani); S. s (S. sclerotiorum); S. t(S. turcica); U. m (U. maydis); O. s (O. sativa); A. t (A. thaliana).*

Figure 8. The ^{1}H NMR (400 MHz, $CDCl_3$) of **2.**

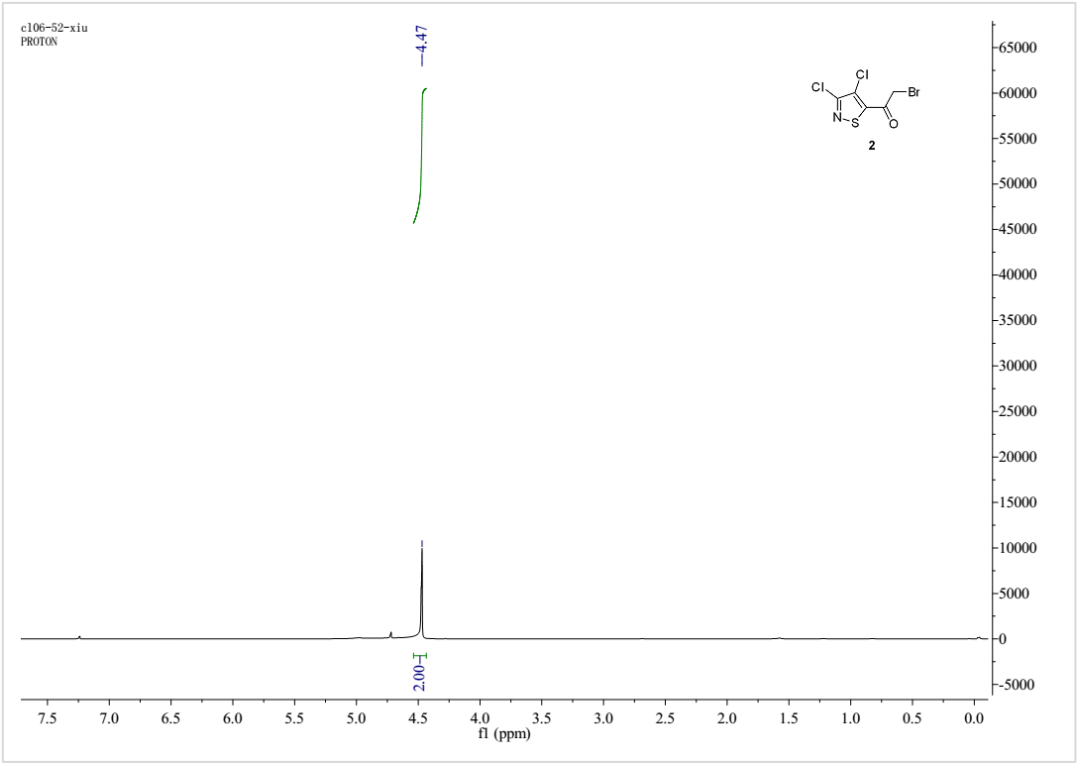

Figure 9. The ^{13}C NMR (101 MHz, $CDCl_3$) of **2**.

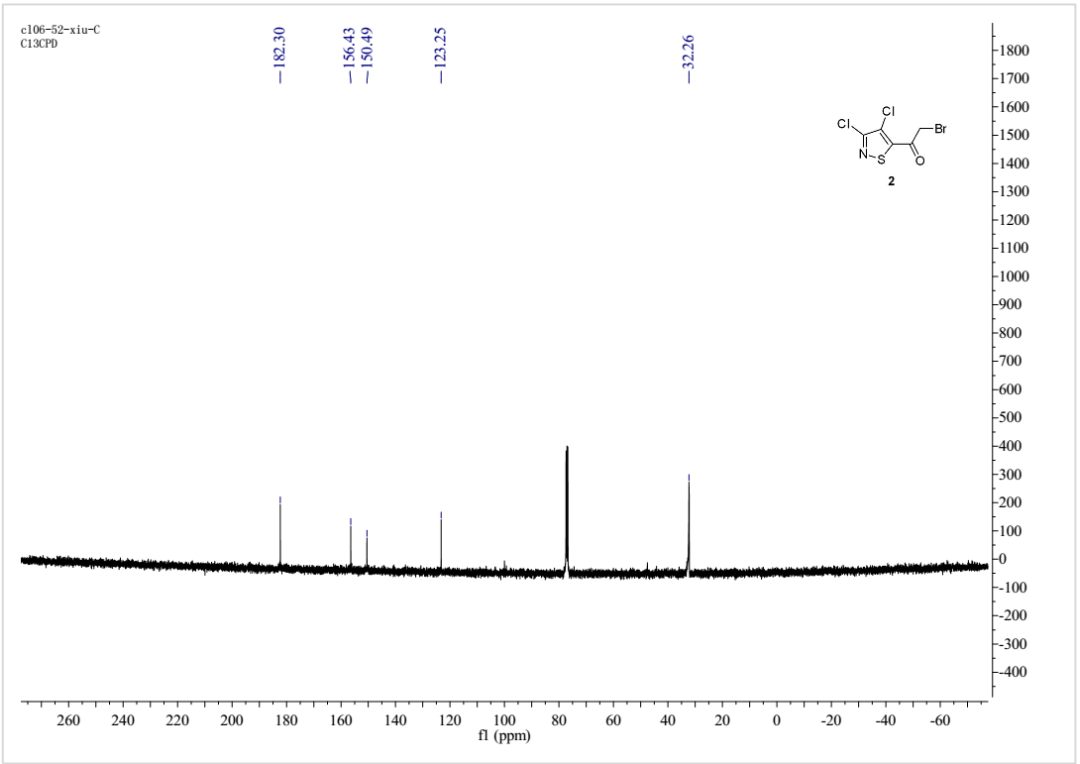

Figure 10. The ^{1}H NMR (400 MHz, $CDCl_3$) of **3a**.

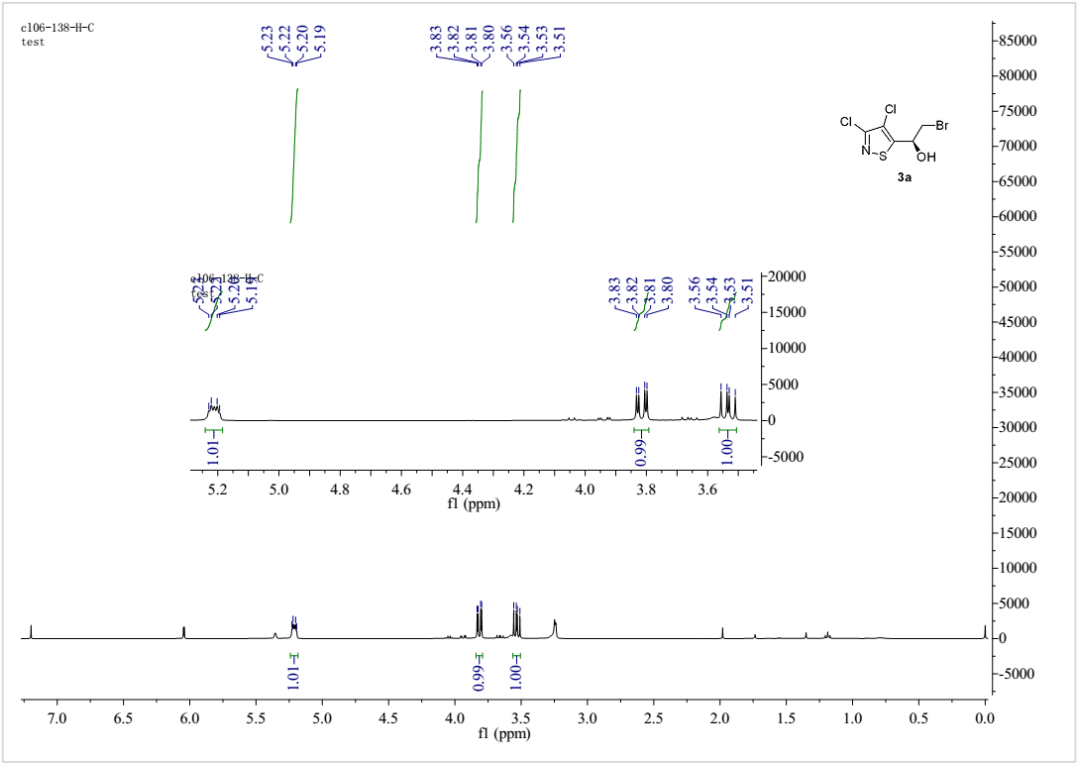

Figure 11. The ^{13}C NMR (101 MHz, $CDCl_3$) of **3a**.

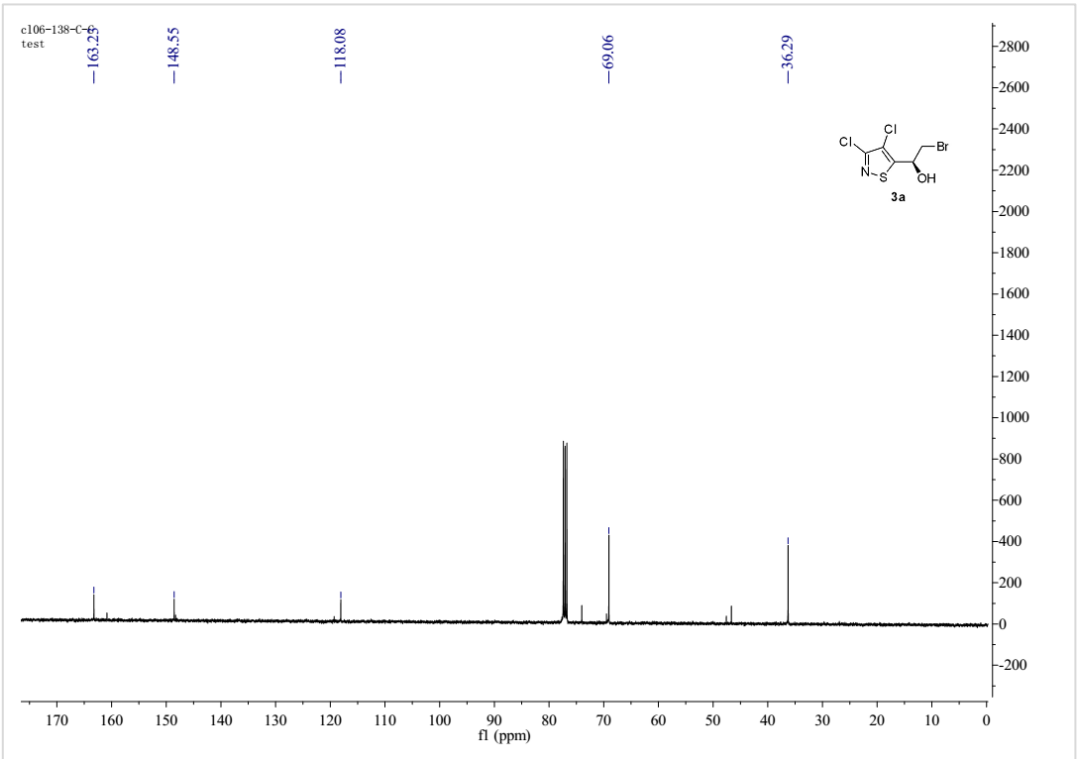

Figure 12. The ^{1}H NMR (400 MHz, $CDCl_3$) of **3b**.

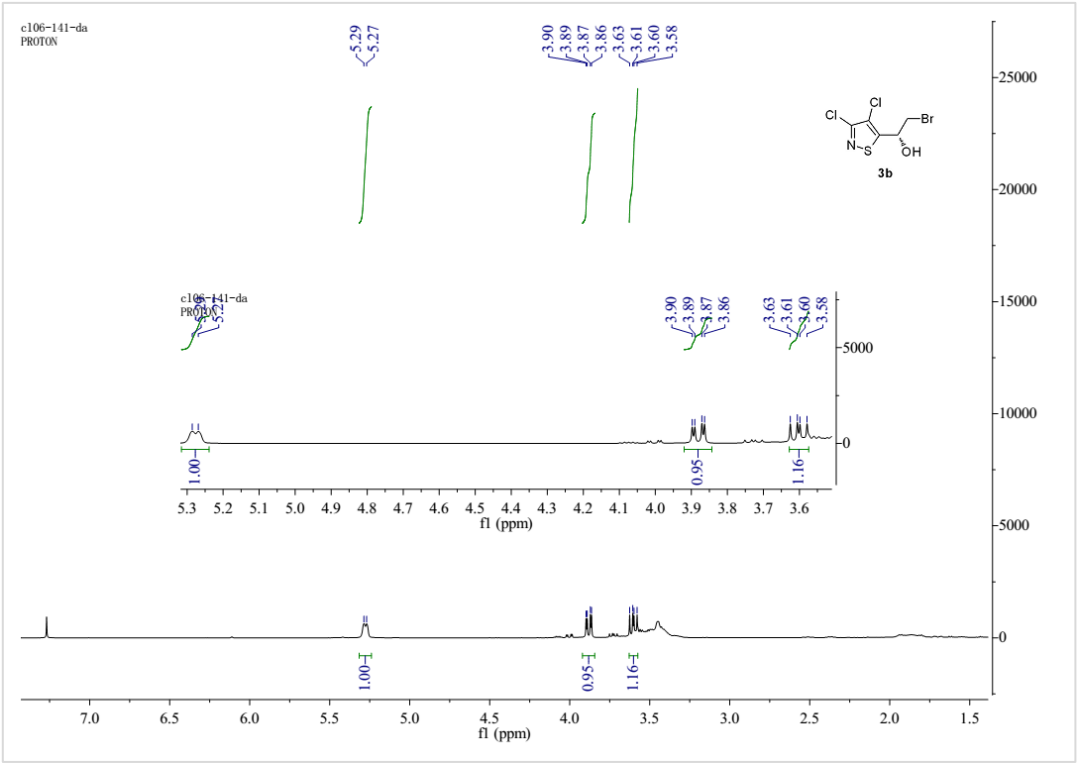

Figure 13. The ^{13}C NMR (101 MHz, $CDCl_3$) of **3b**.

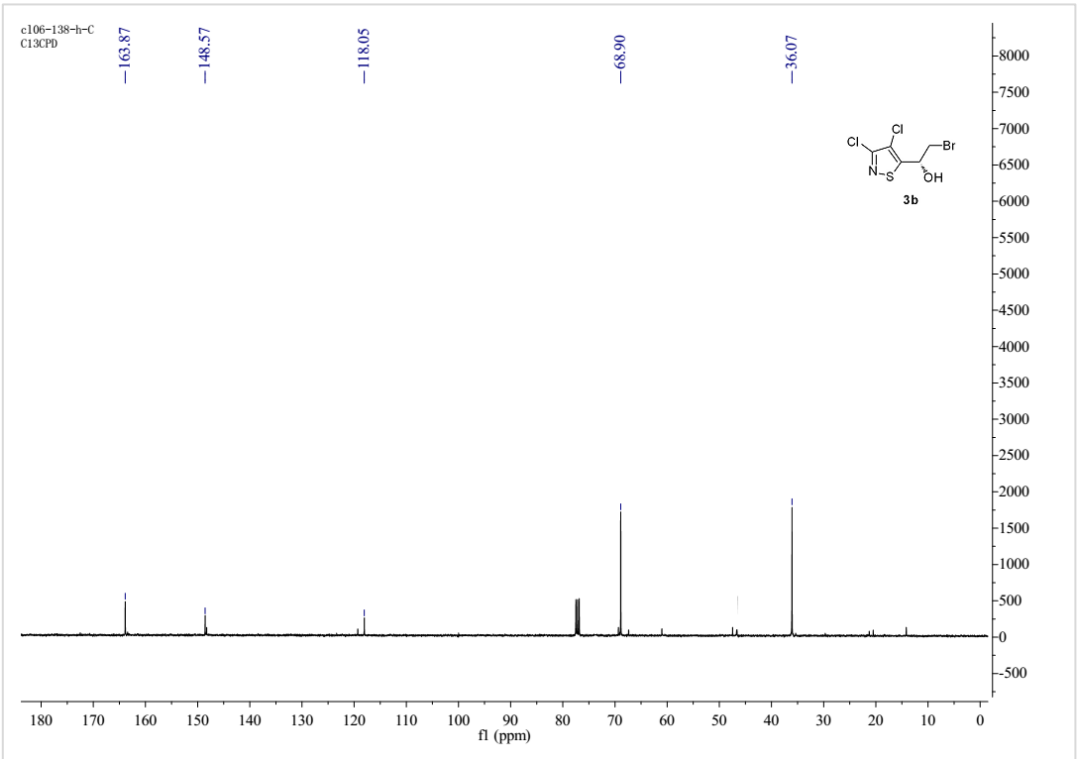

Figure 14. The ^{1}H NMR (400 MHz, $CDCl_3$) of **4a.**

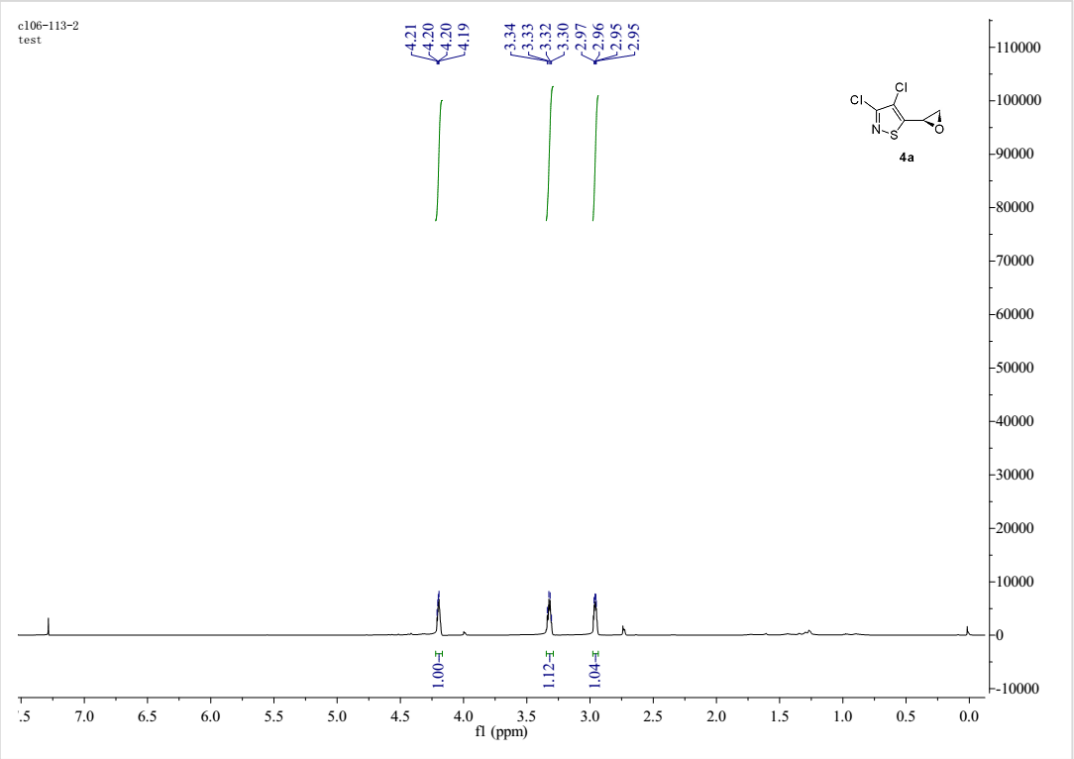

Figure 15. The ^{13}C NMR (101 MHz, $CDCl_3$) of **4a**.

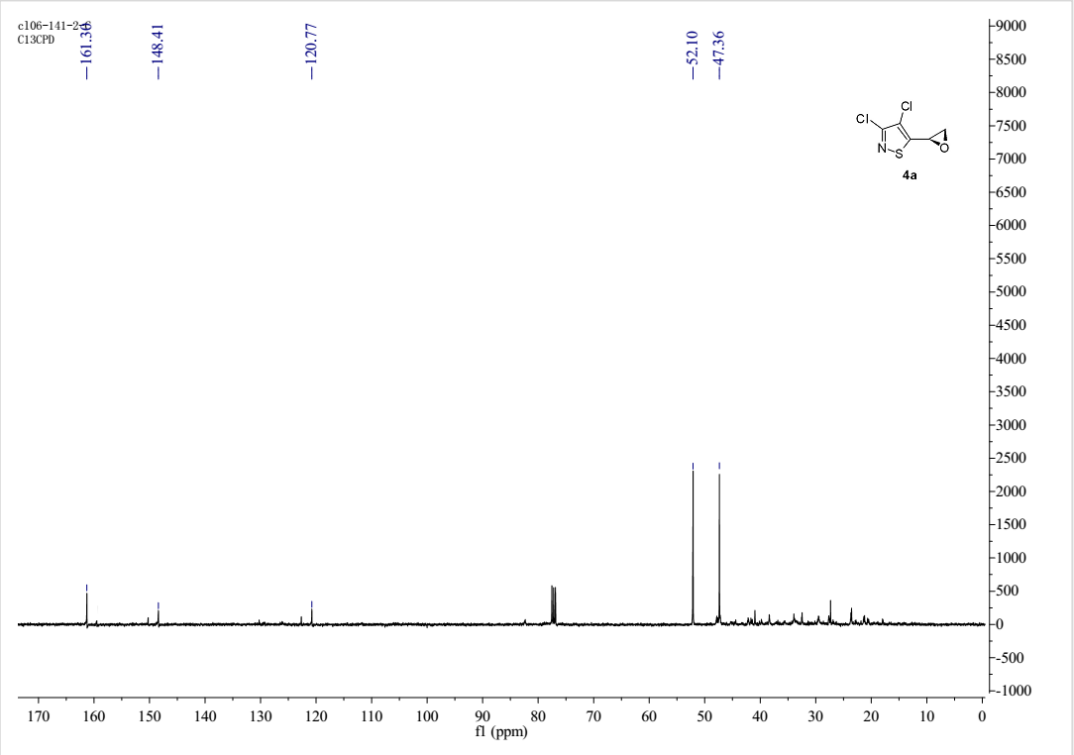

Figure 16. The ^{1}H NMR (400 MHz, $CDCl_3$) of **4b.**

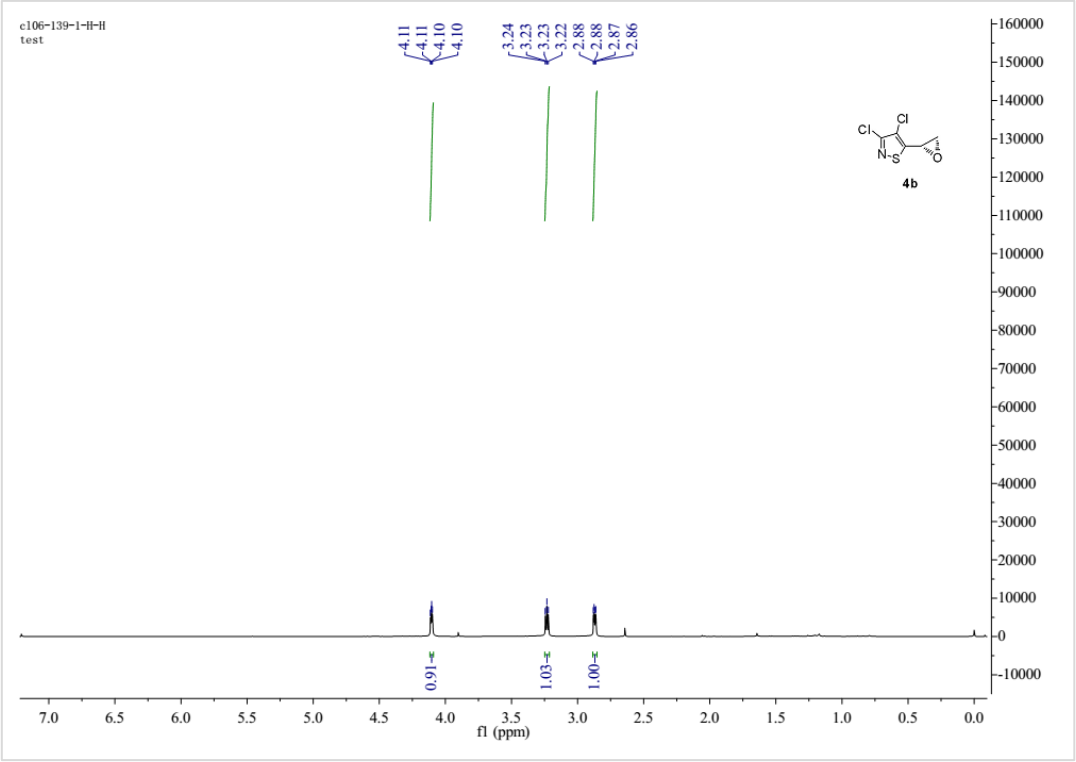

Figure 17. The ^{13}C NMR (101 MHz, $CDCl_3$) of **4b.**

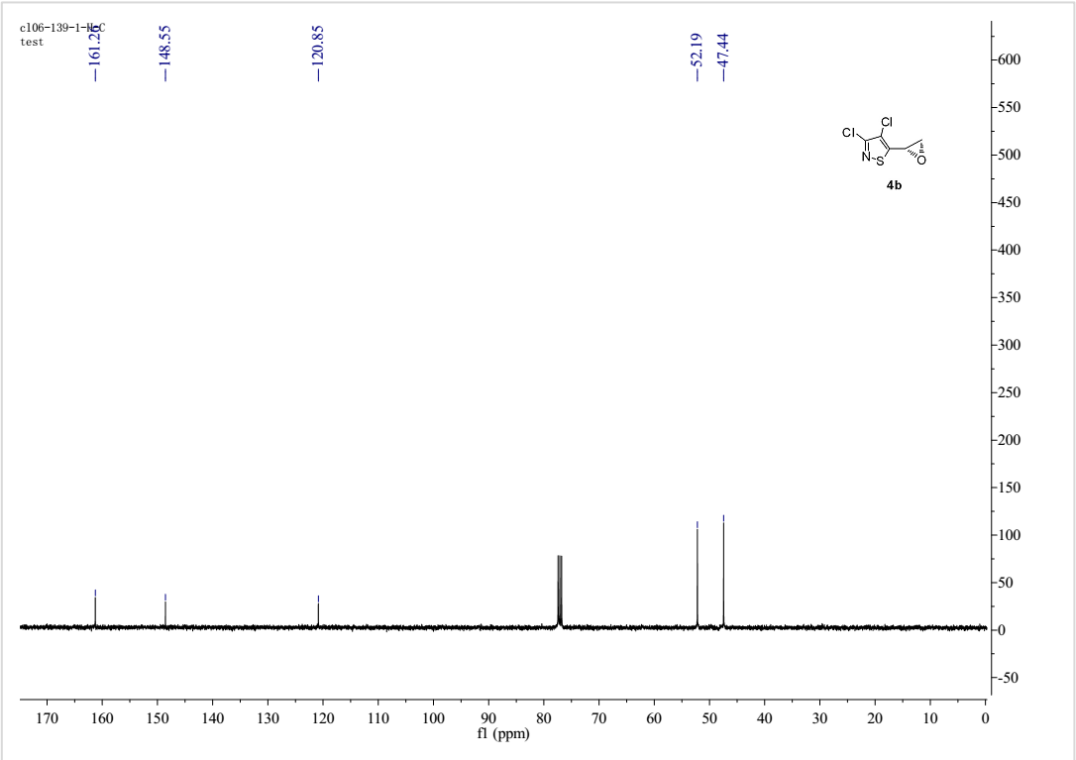

Figure 18. The ^{1}H NMR (400 MHz, DMSO-d_6) of **5a**.

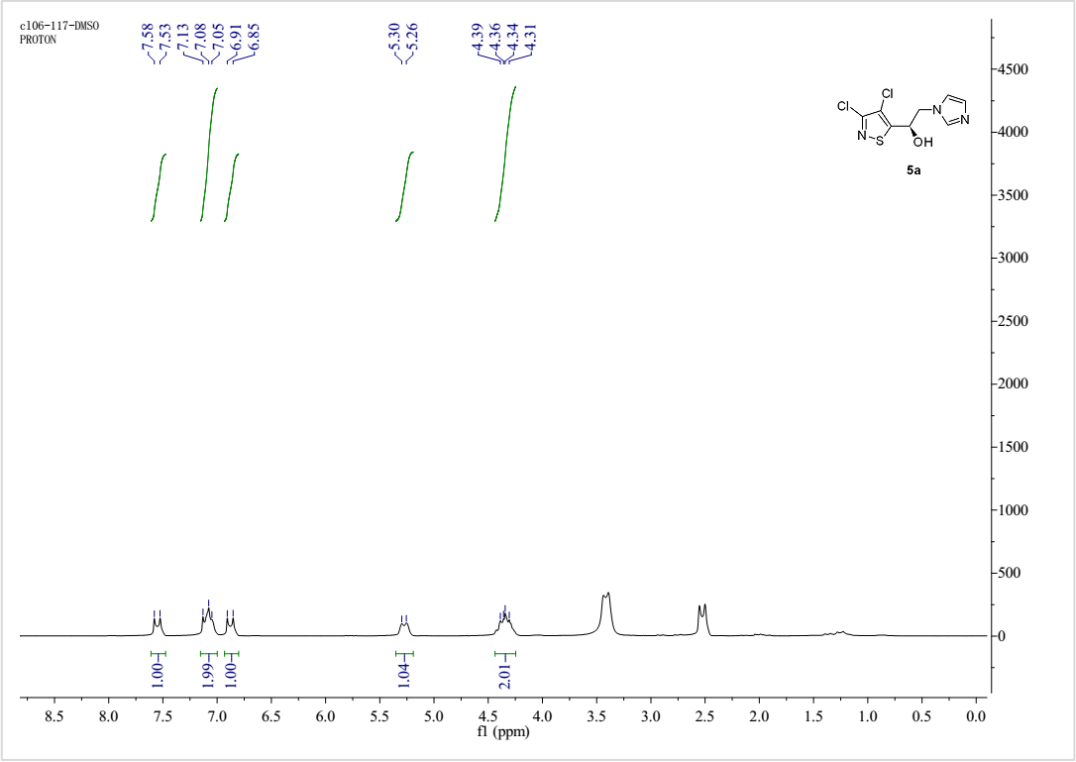

Figure 19. The ^{13}C NMR (101 MHz, DMSO-d_6) of **5a**.

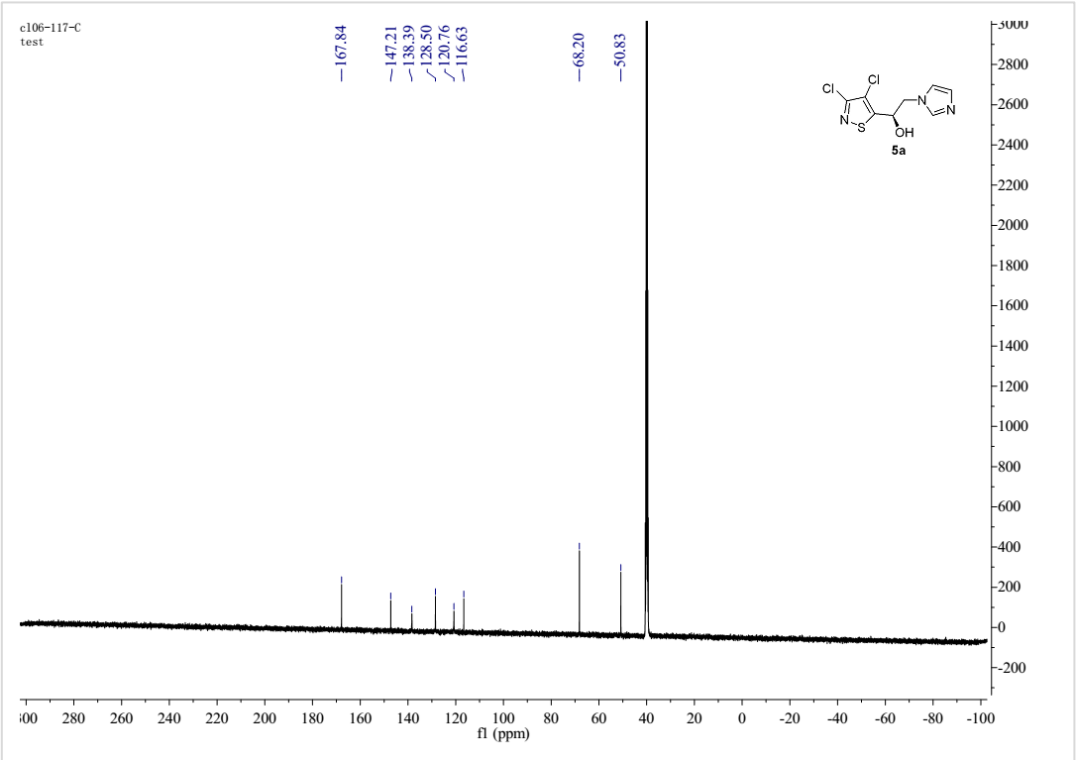

Figure 20. The ^{1}H NMR (400 MHz, DMSO-d_6) of **5b**.

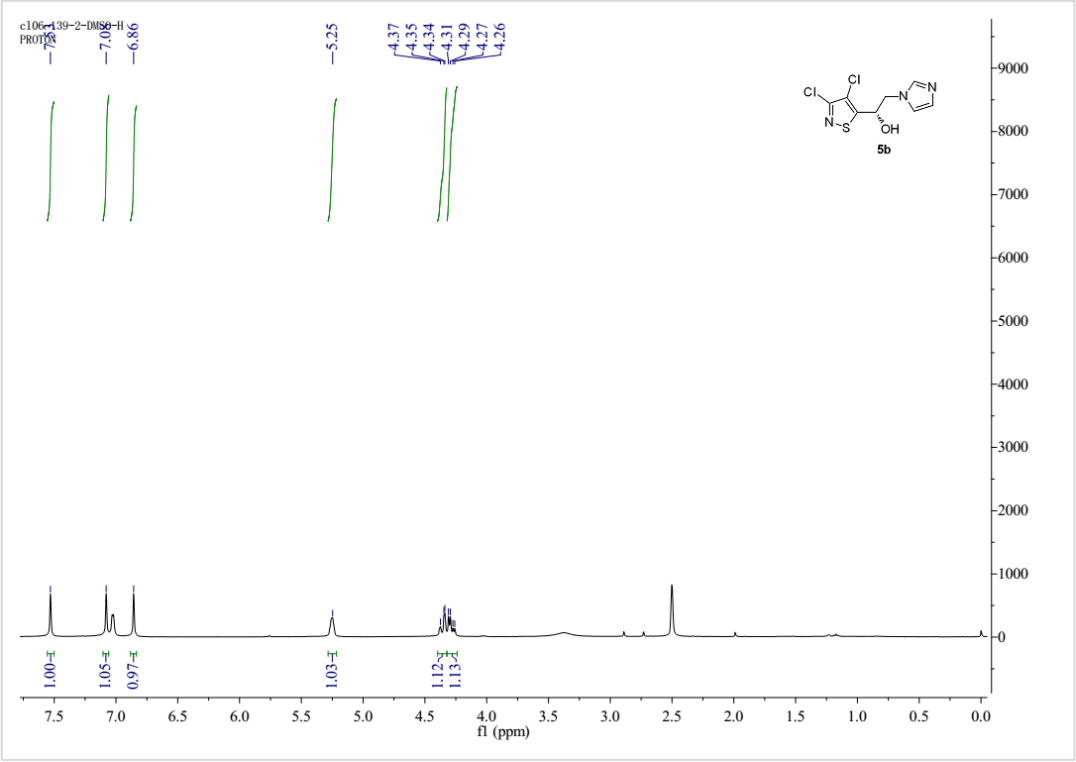

Figure 21. The ^{13}C NMR (101 MHz, DMSO-d_6) of **5b**.

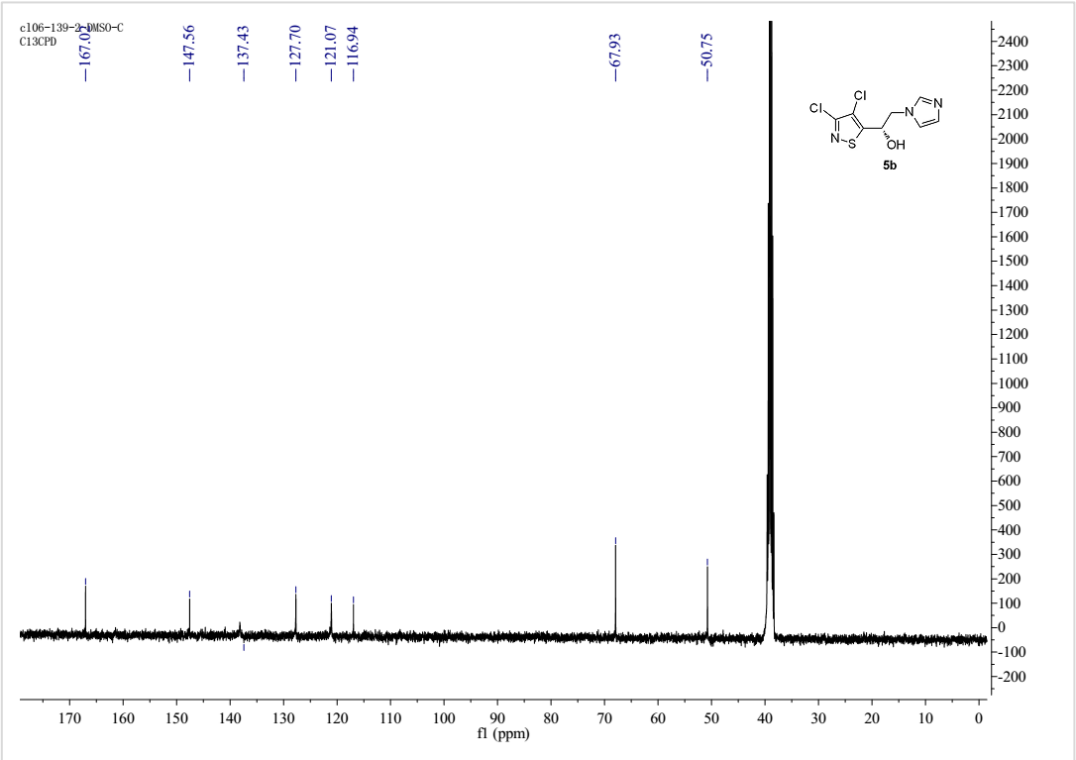

Figure 22. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-1**.

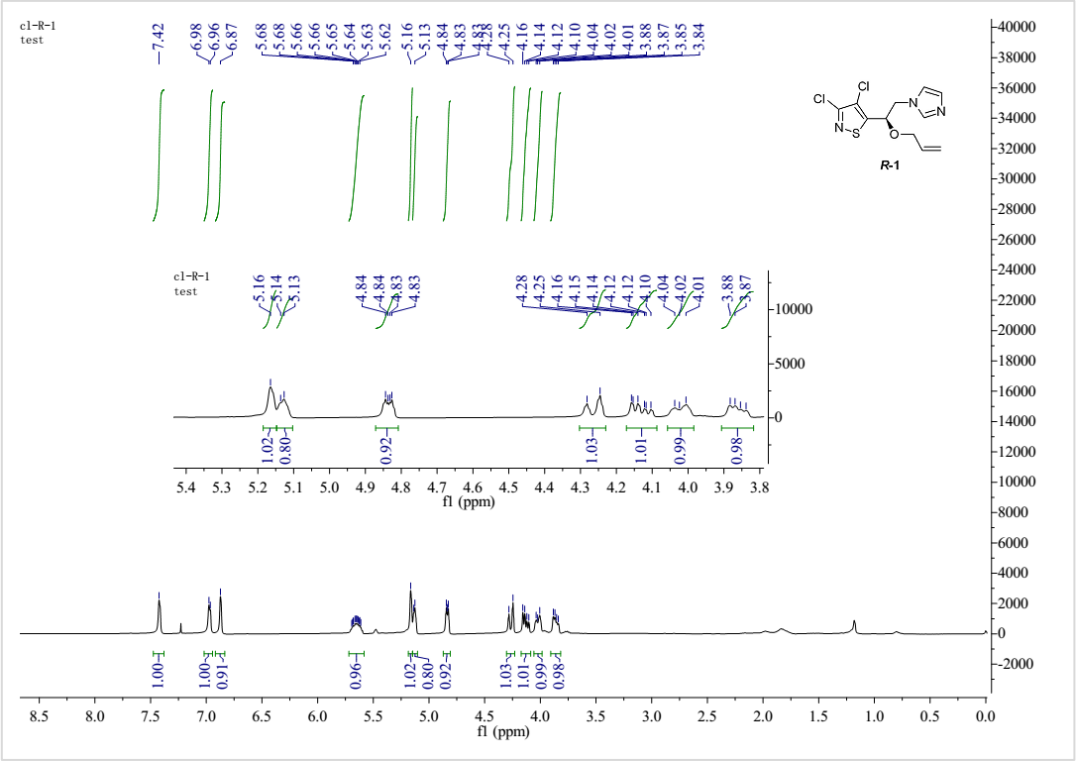

Figure 23. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-1**.

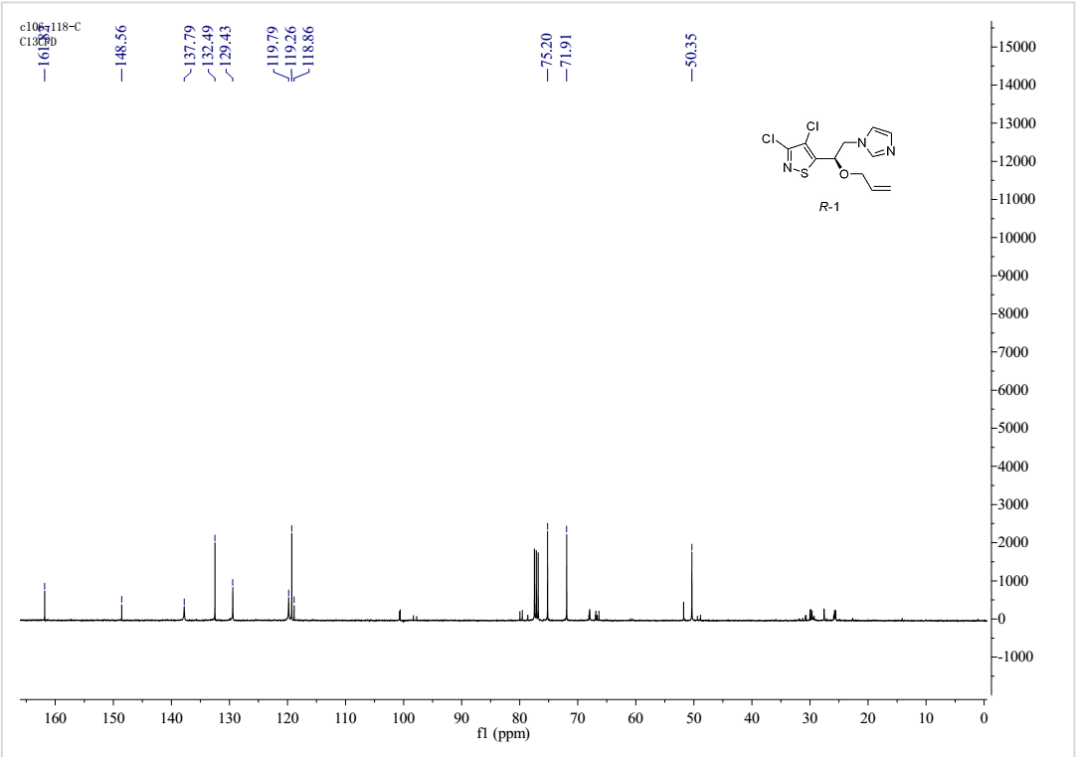

Figure 24. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-2**.

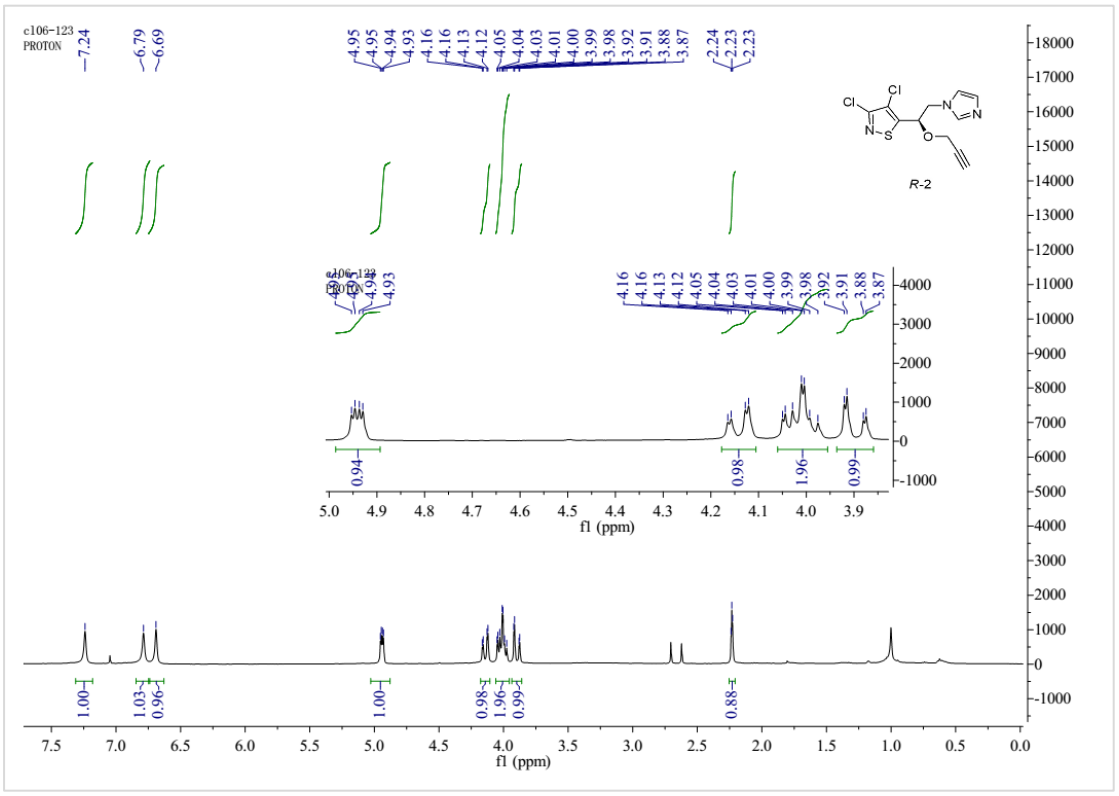

Figure 25. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-2**

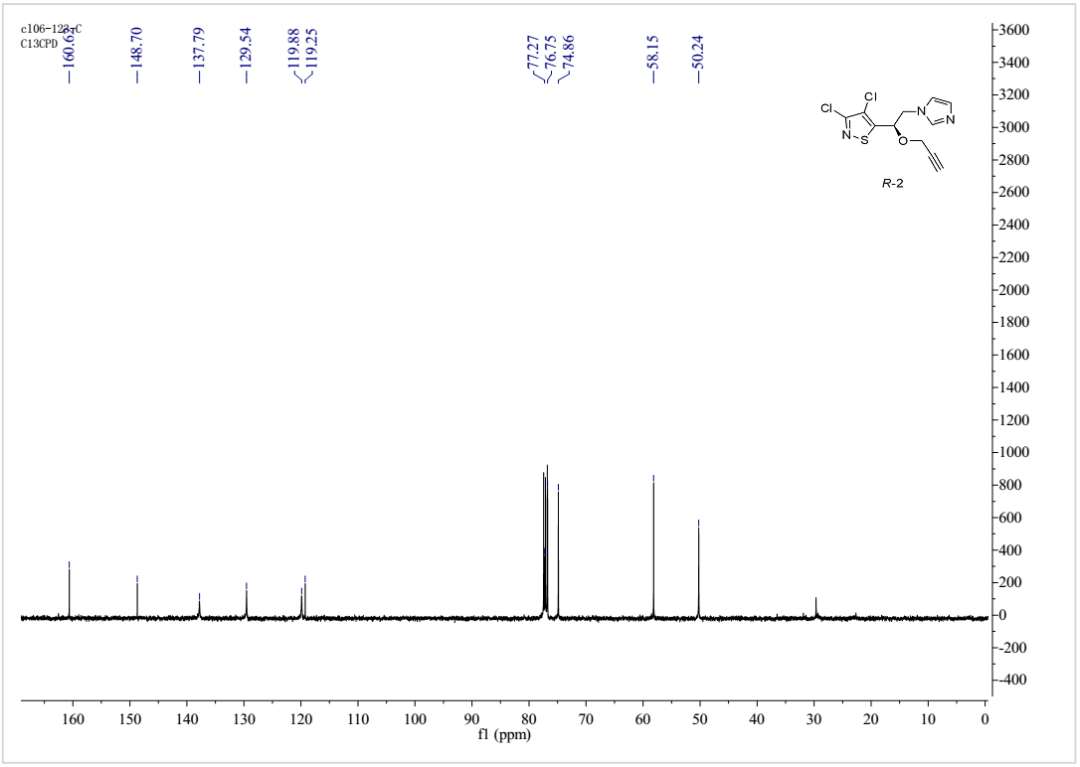

Figure 26. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-3**.

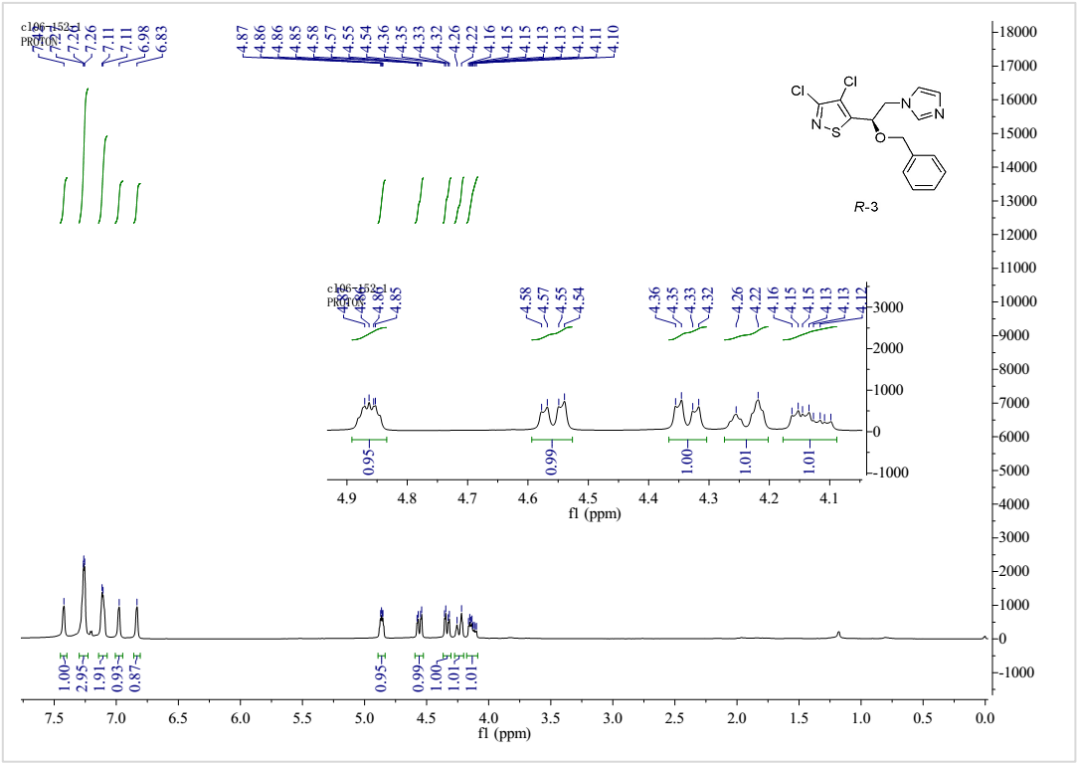

Figure 27. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-3**

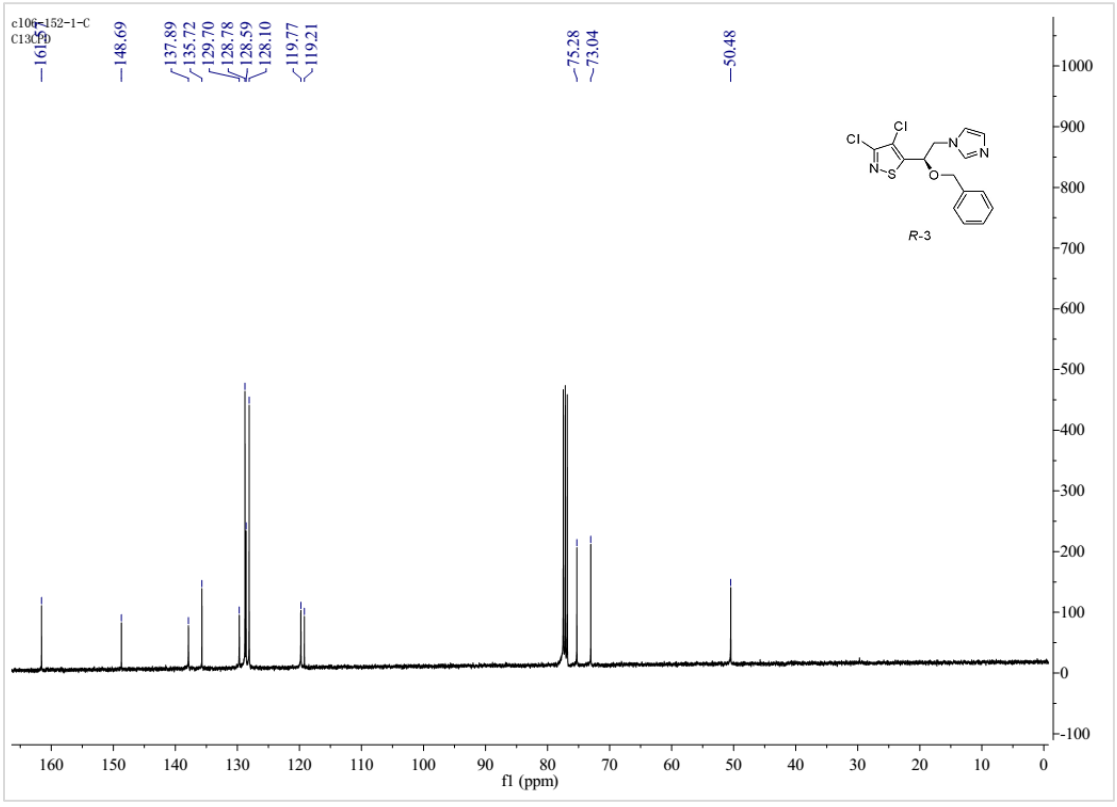

Figure 28. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-4**.

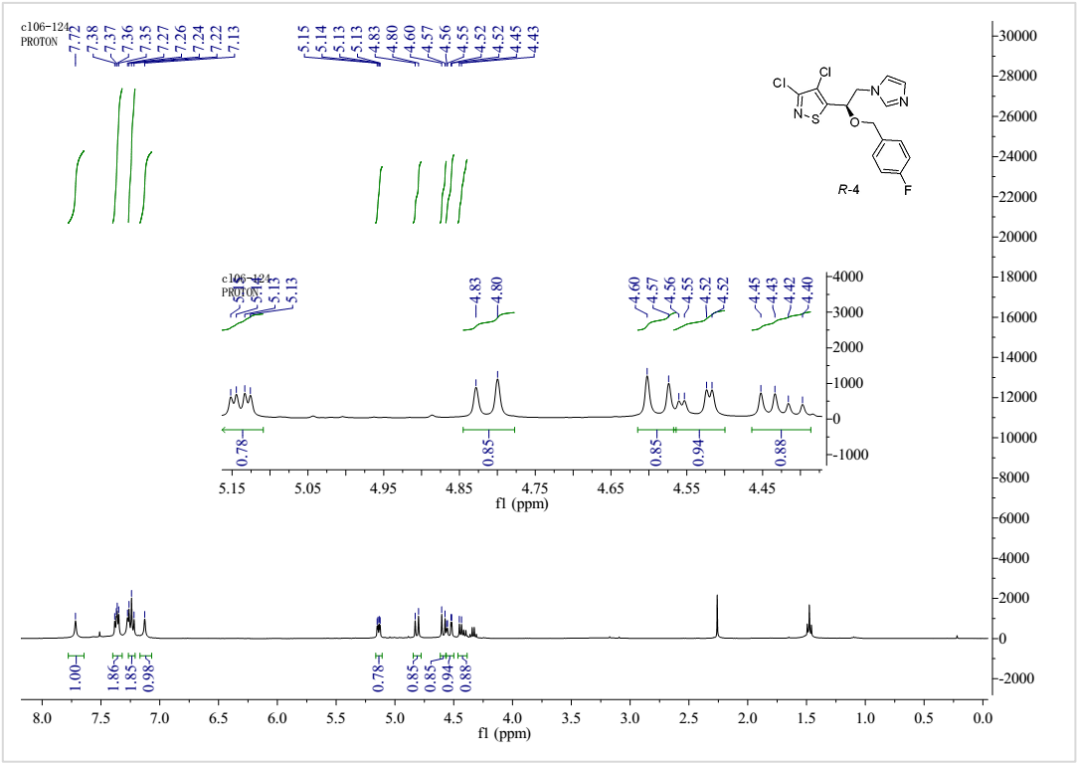

Figure 29. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-4**

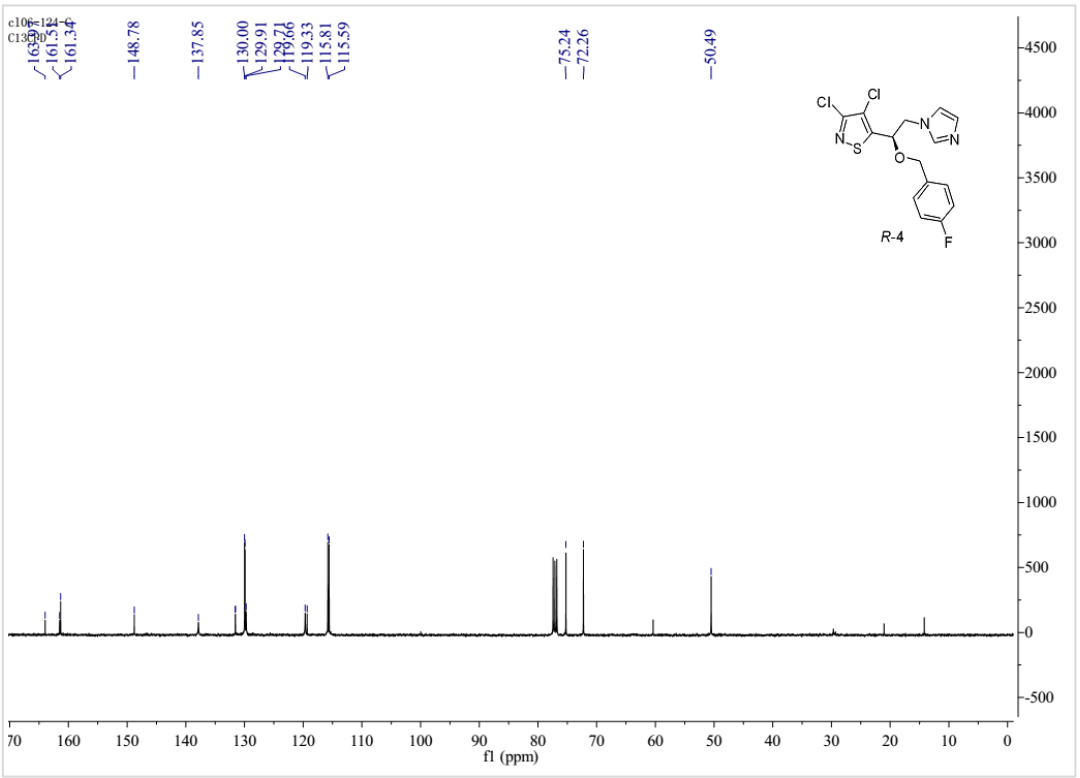

Figure 30. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-5**.

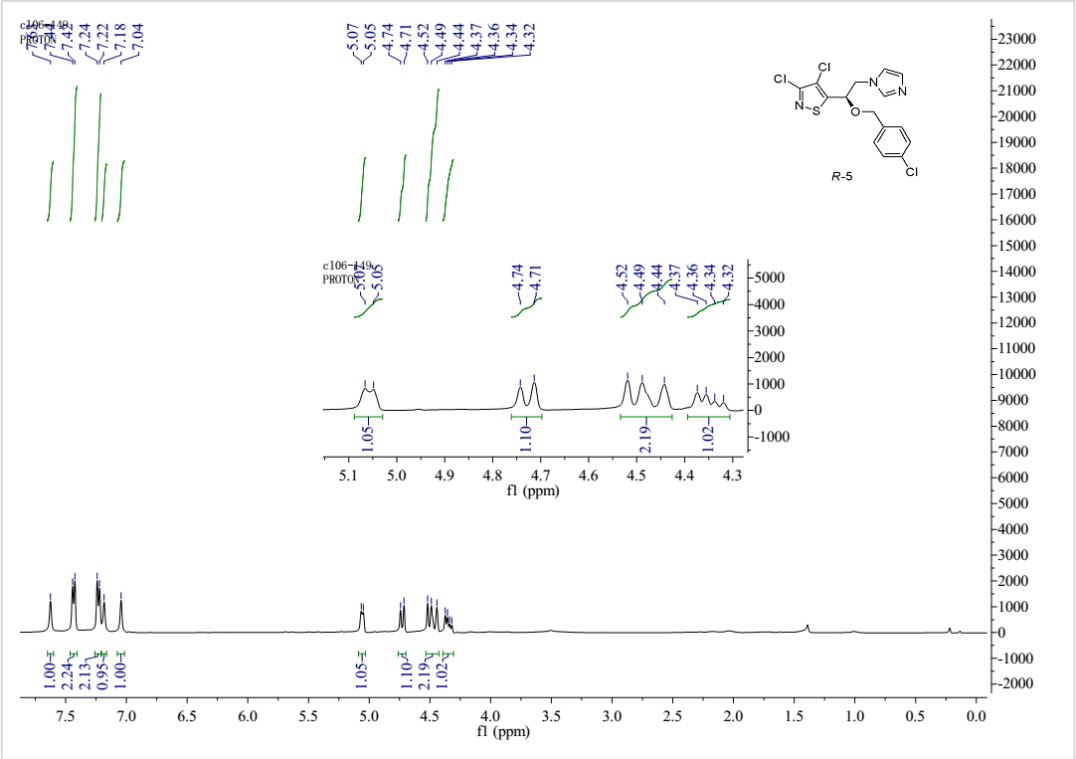

Figure 31. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-5.**

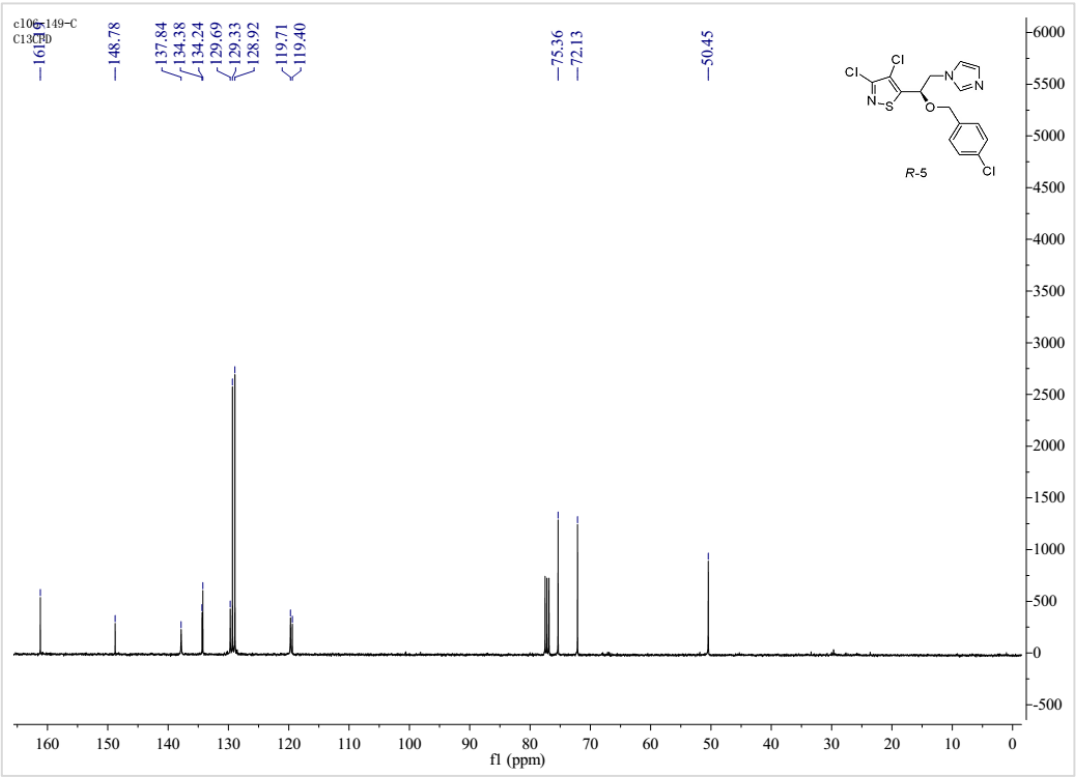

Figure 32. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-6**.

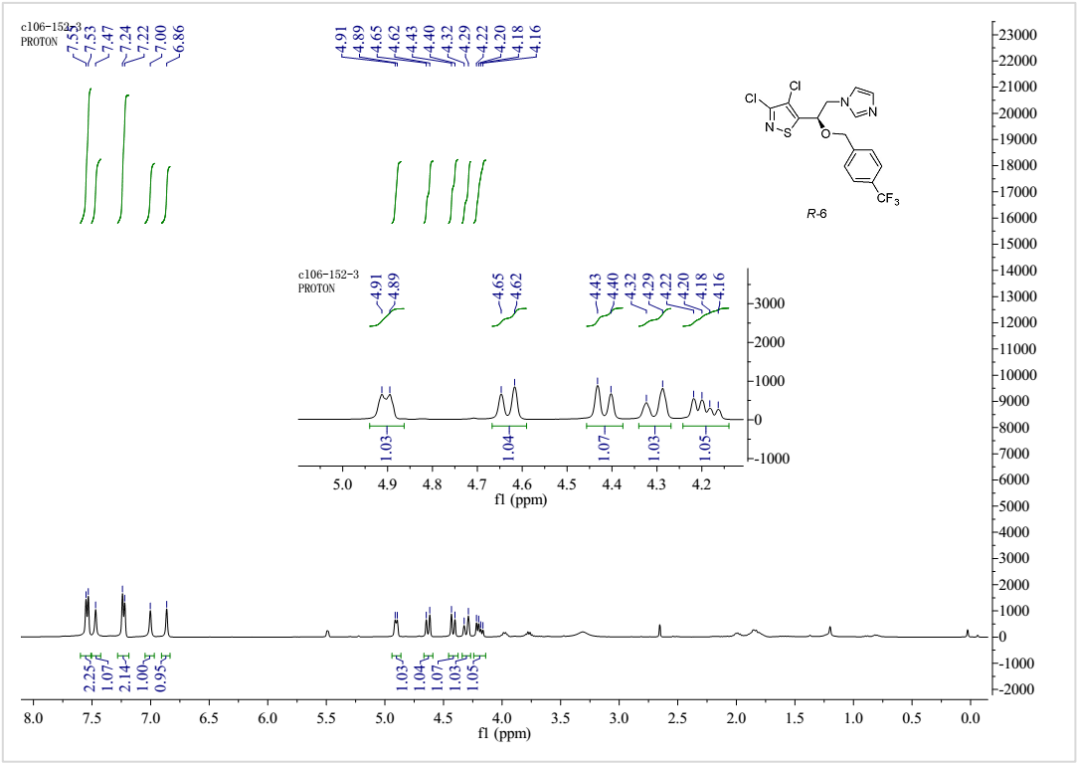

Figure 33. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-6.**

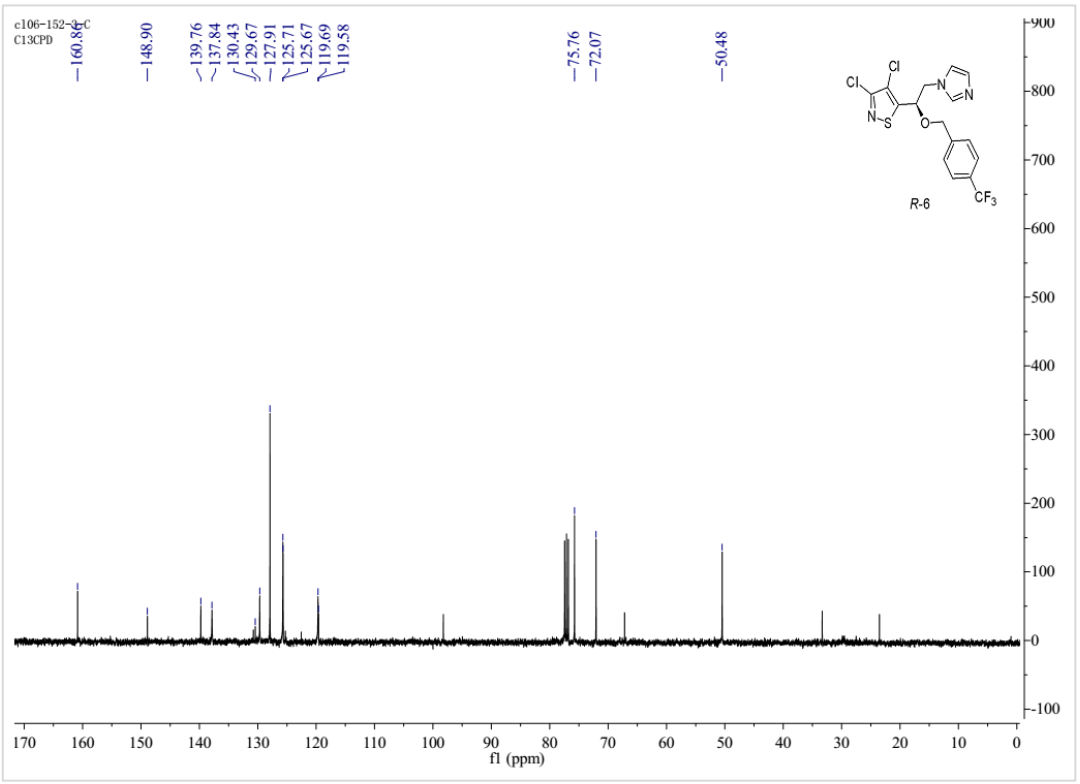

Figure 34. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-7**.

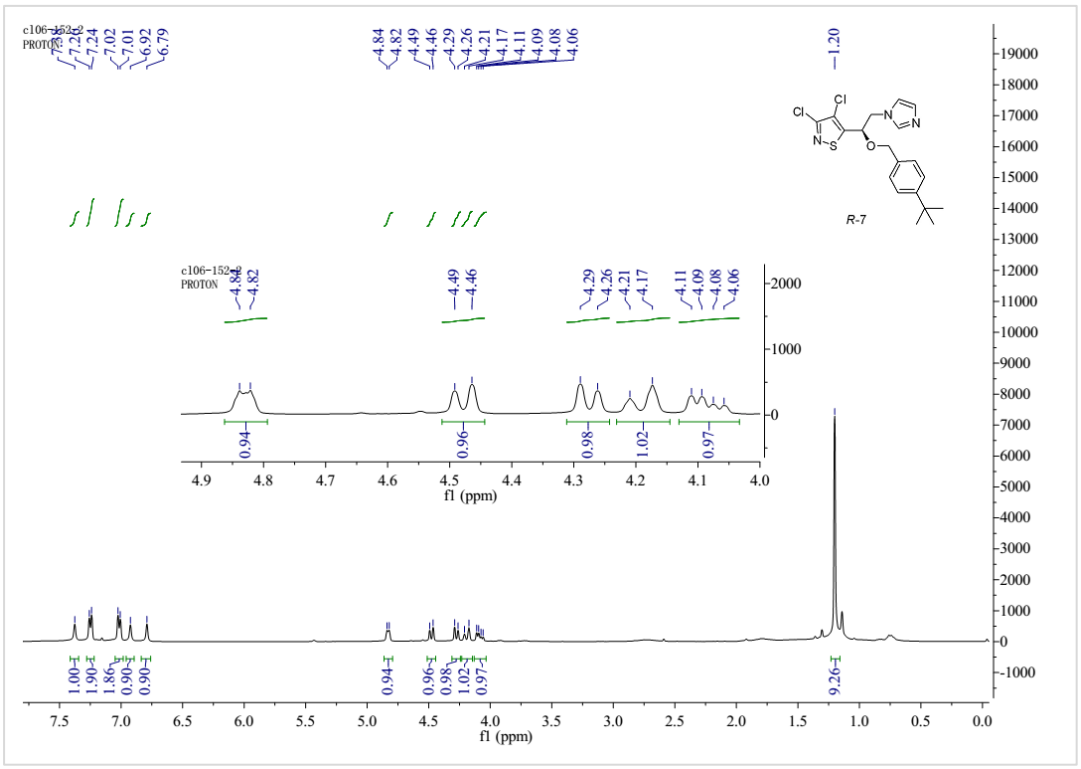

Figure 35. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-7.**

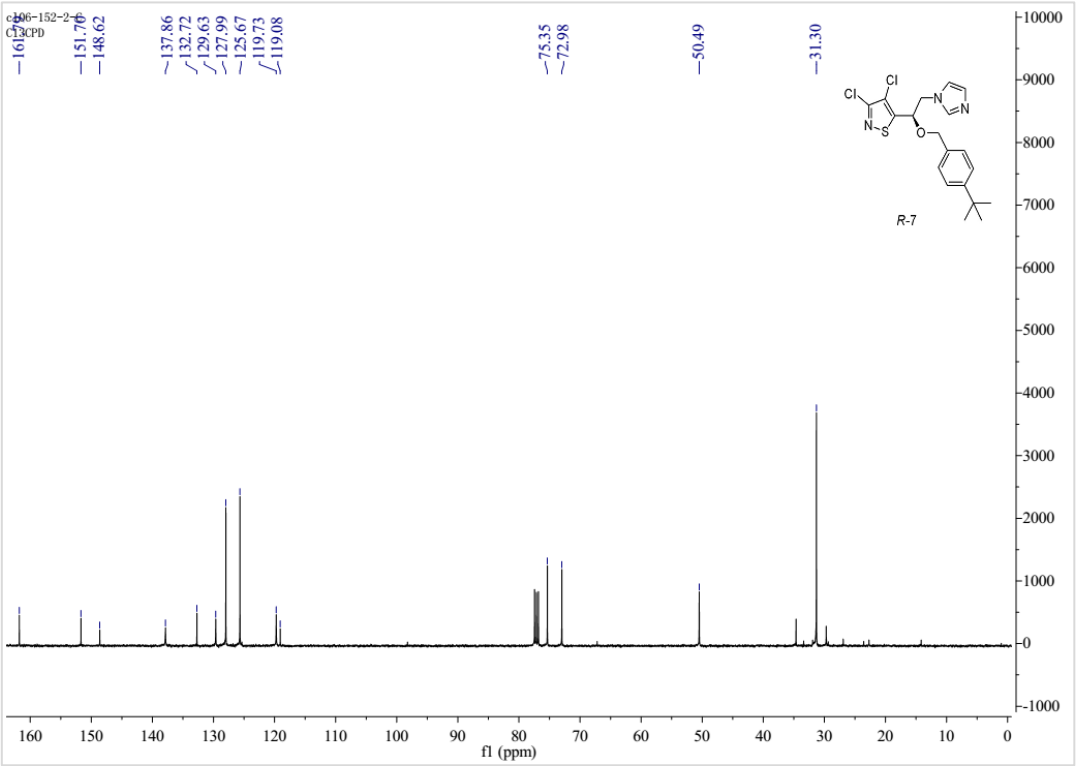

Figure 36. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-8**.

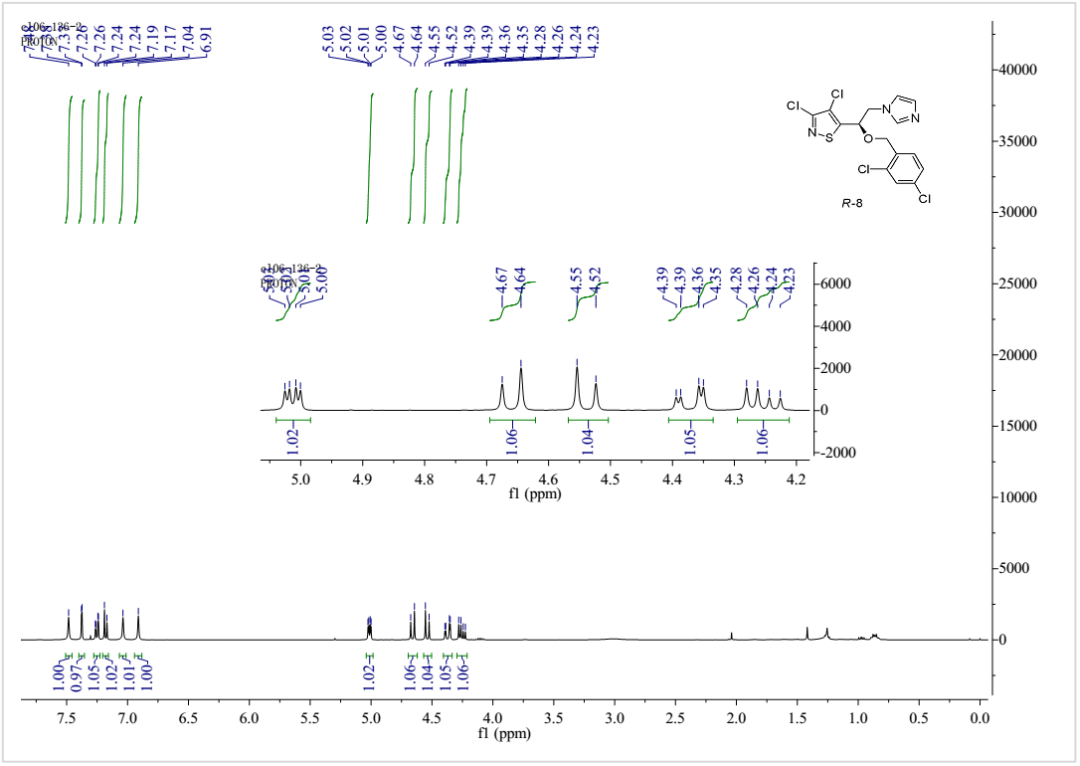

Figure 37. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-8**

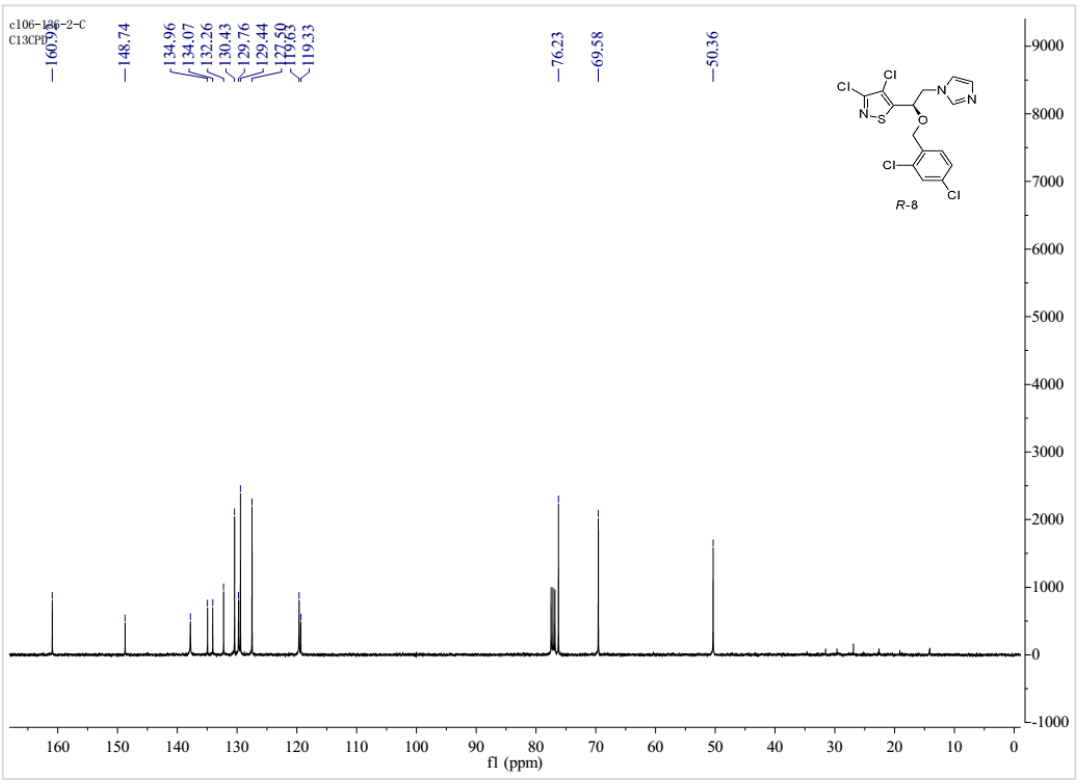

Figure 38. The ^{1}H NMR (400MHz, $CDCl_3$) of ***R*-9**.

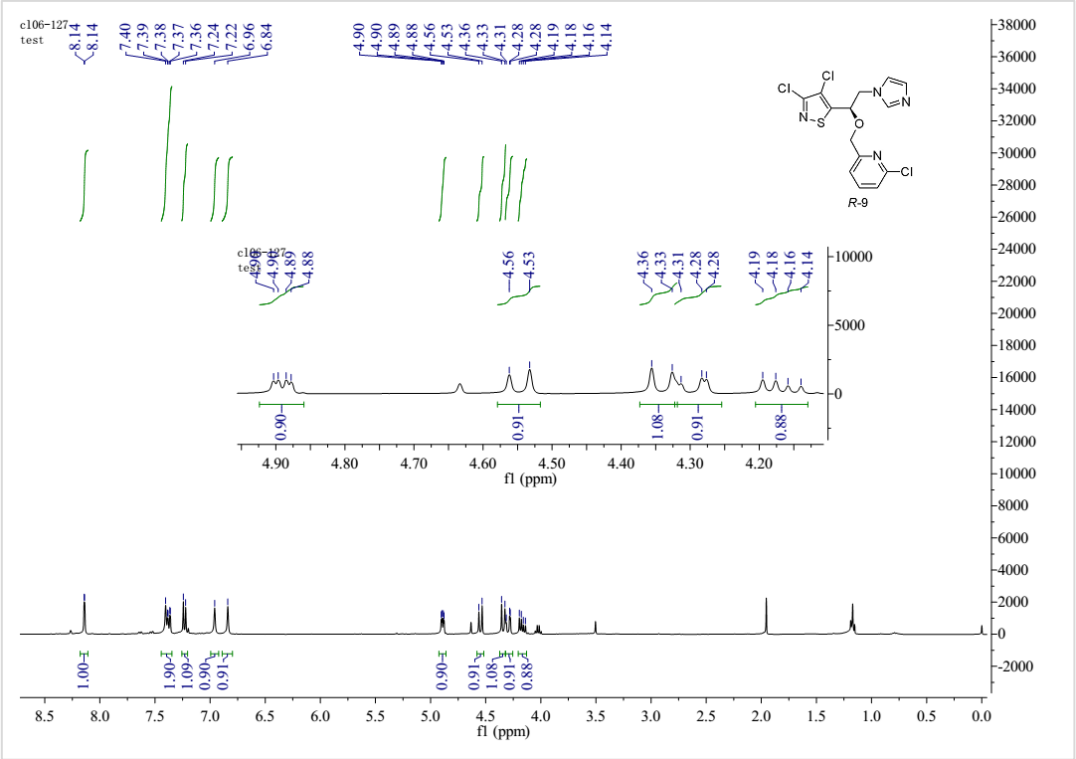

Figure 39. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-9.**

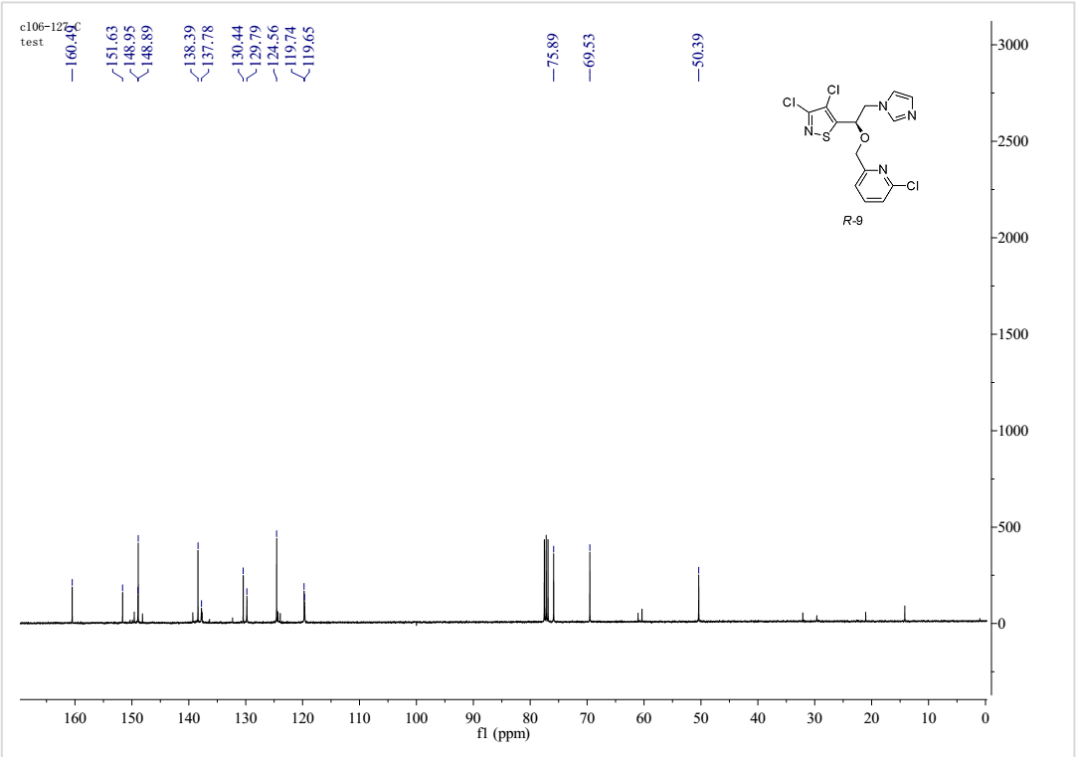

Figure 40. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-10**.

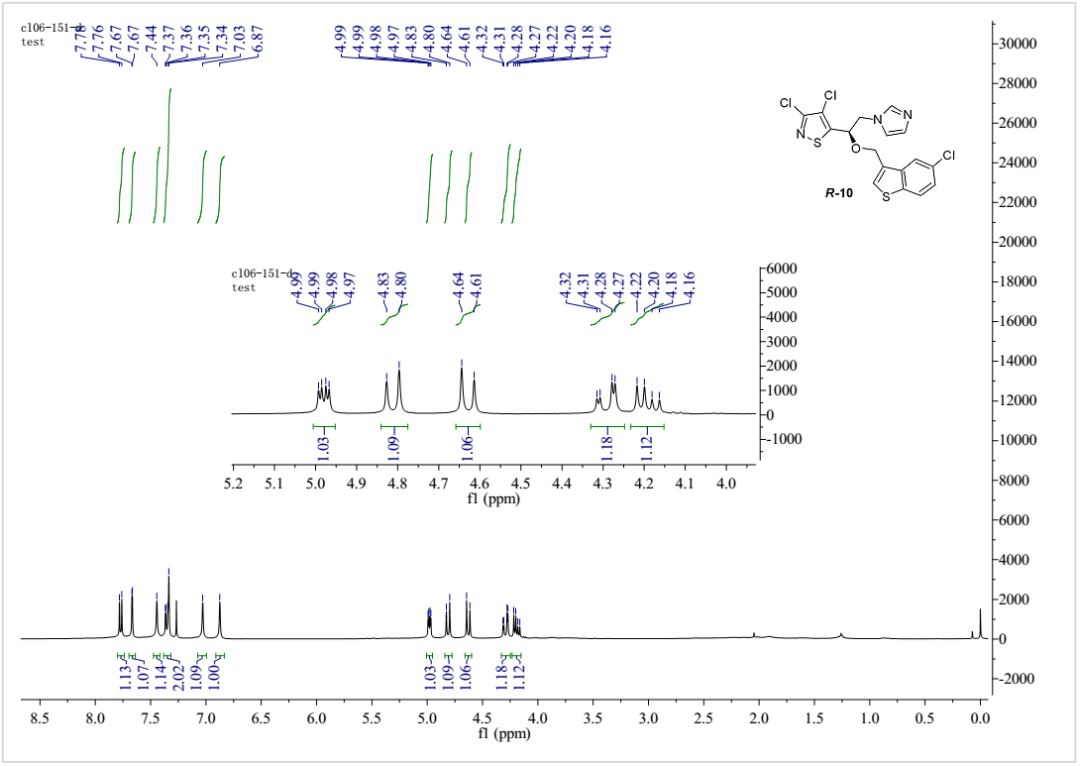

Figure 41. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-10.**

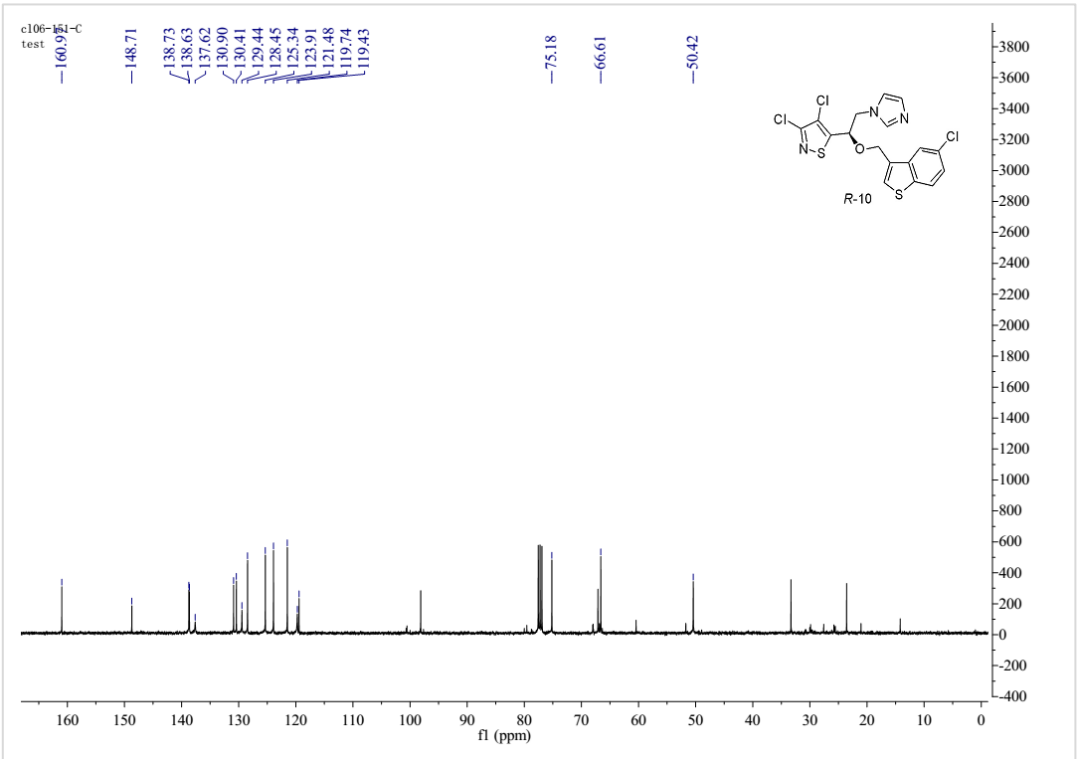

Figure 42. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-11**.

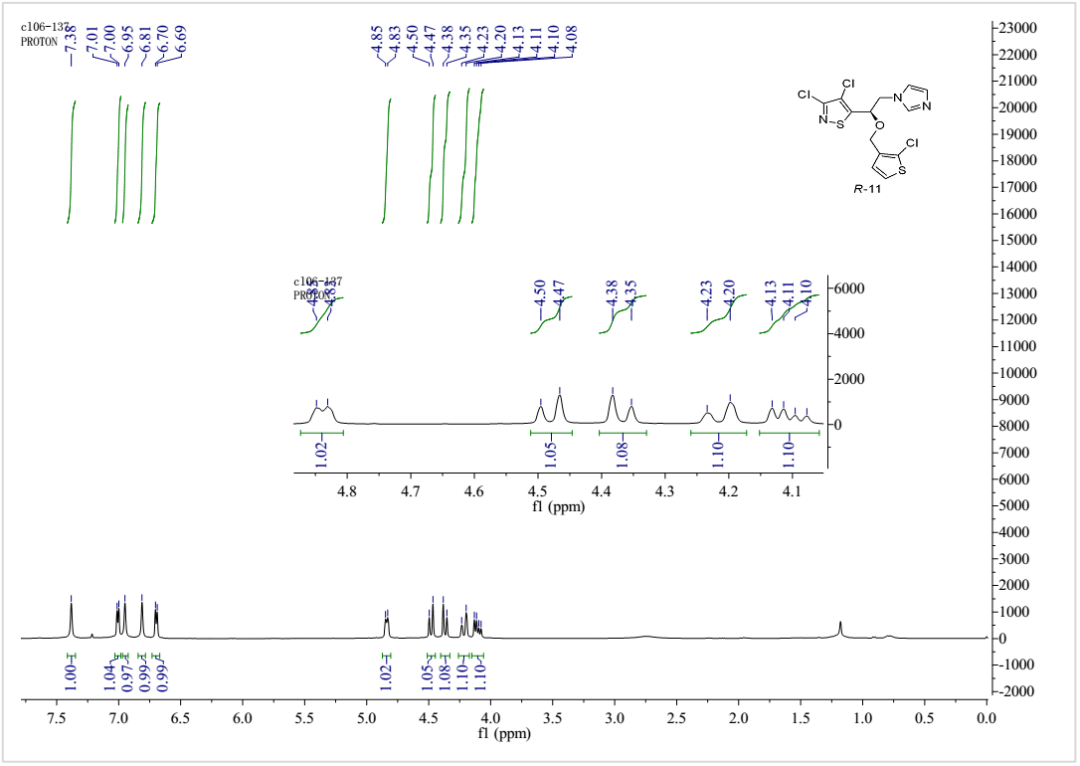

Figure 43. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-11.**

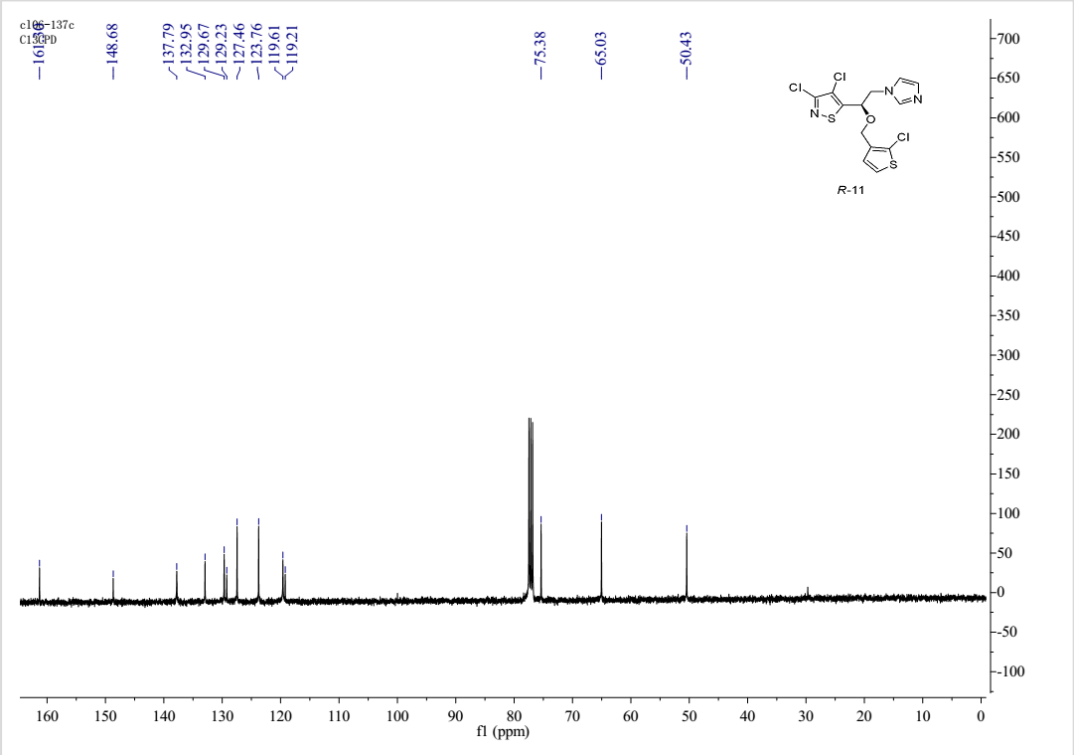

Figure 44. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***R*-12**.

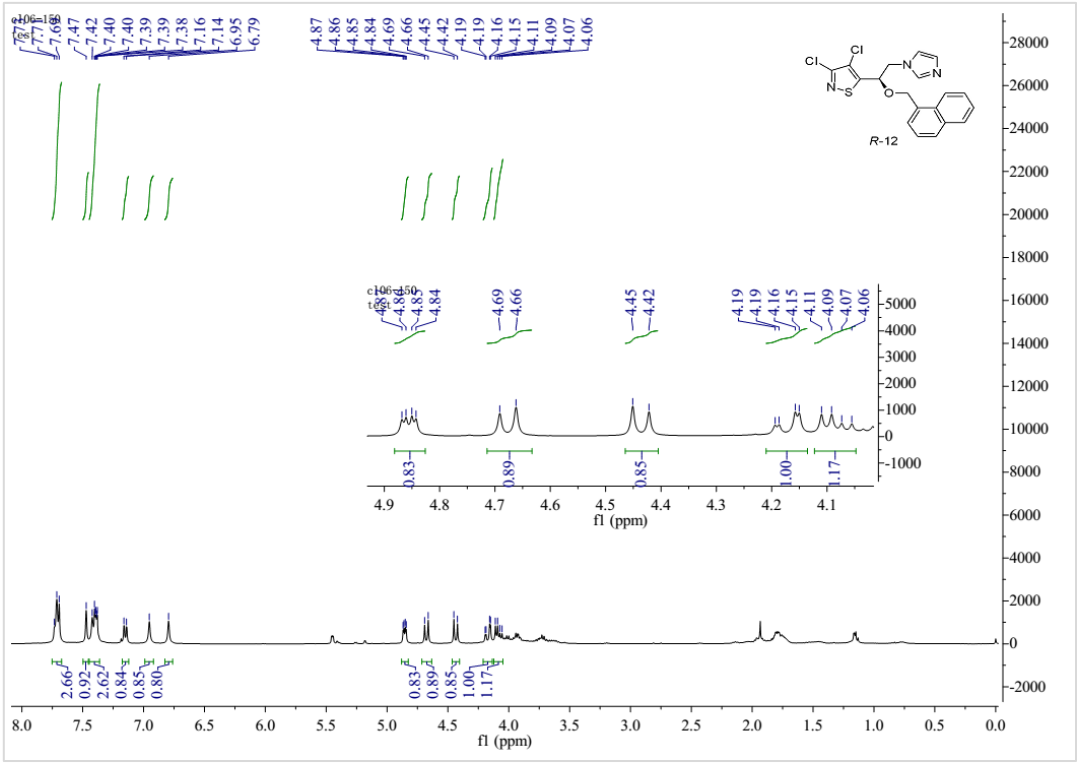

Figure 45. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***R*-12.**

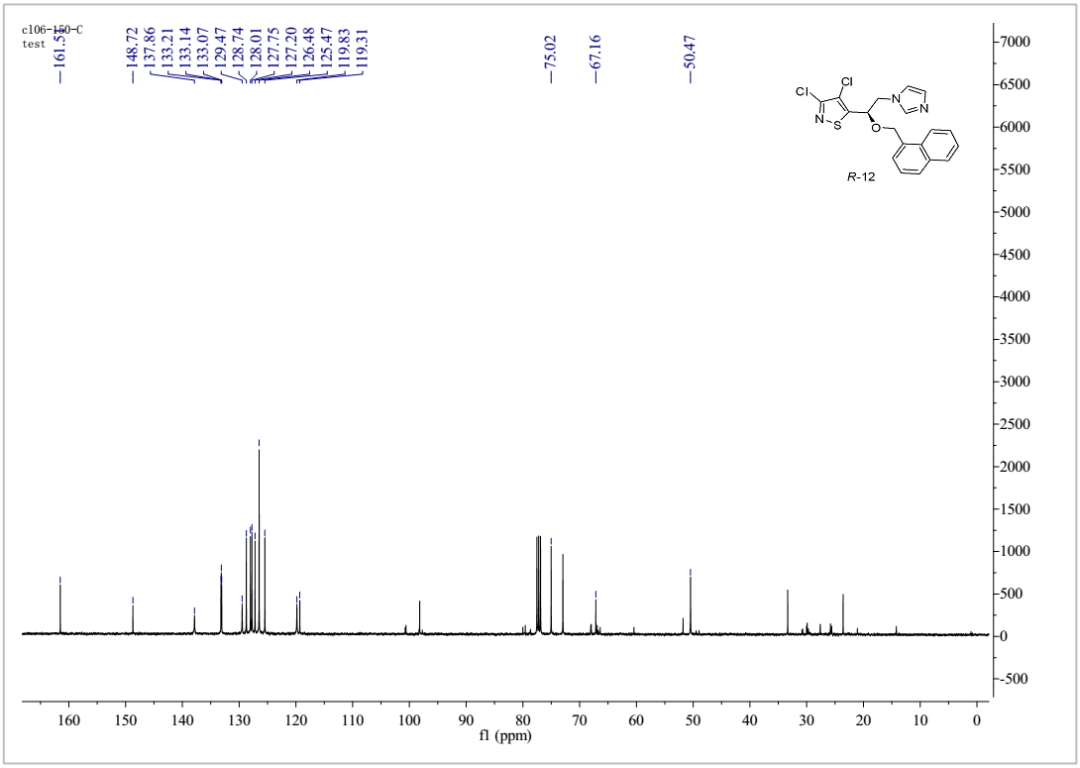

Figure 46. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***S*-1**.

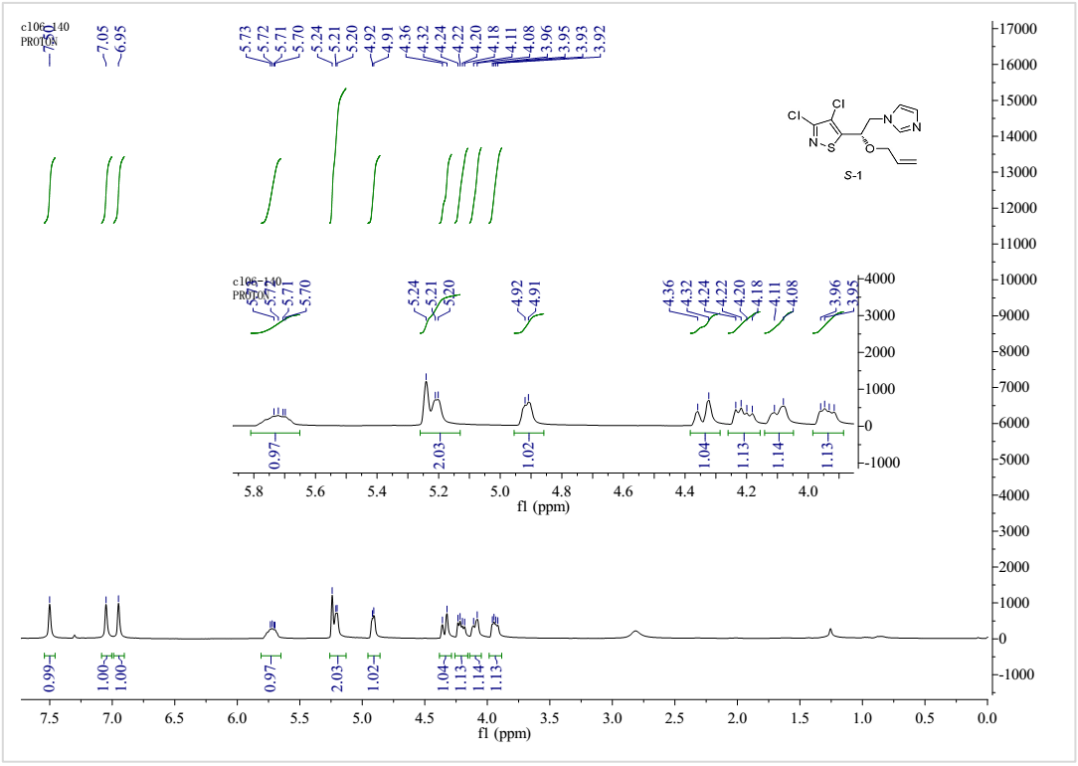

Figure 47. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***S*-1.**

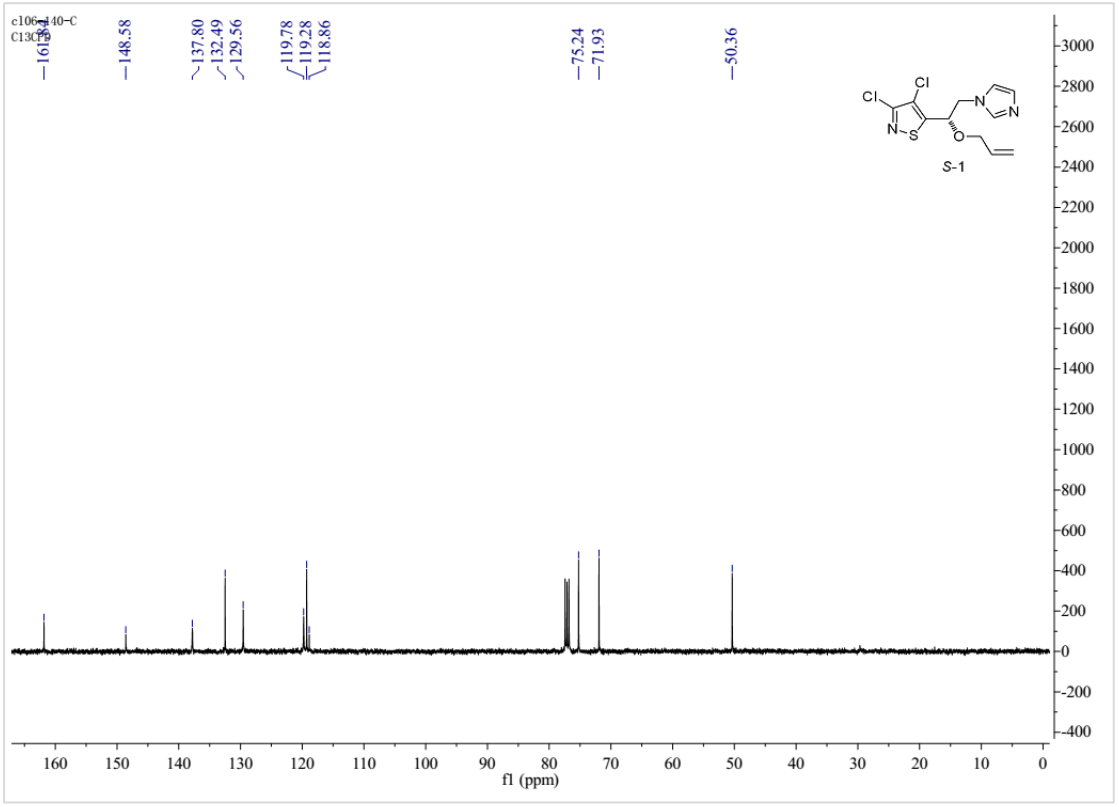

Figure 48. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***S*-11**.

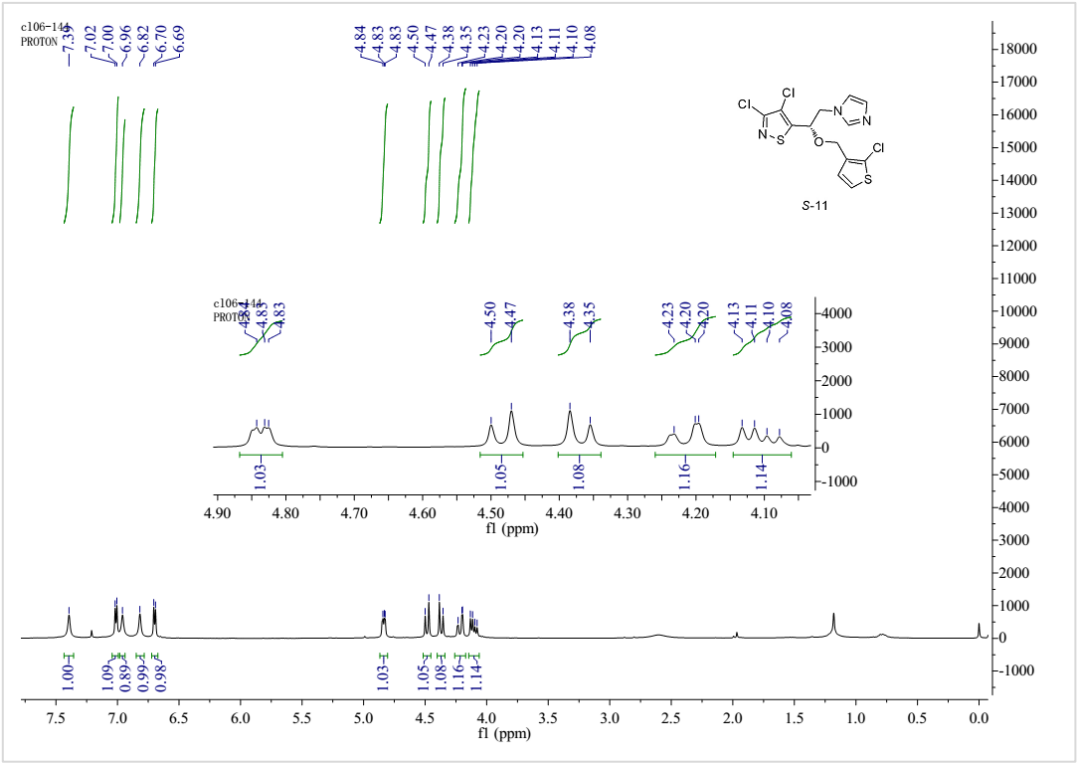

Figure 49. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***S*-11.**

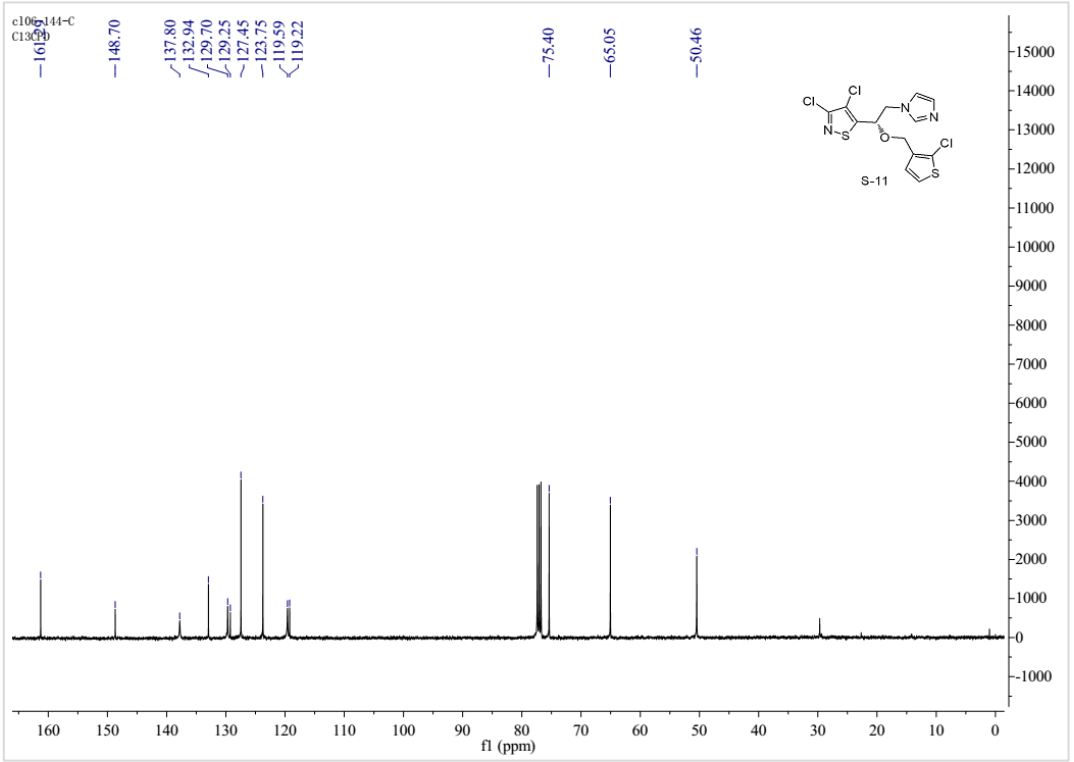

Figure 50. The ^{1}H NMR (400 MHz, $CDCl_3$) of ***S*-12**.

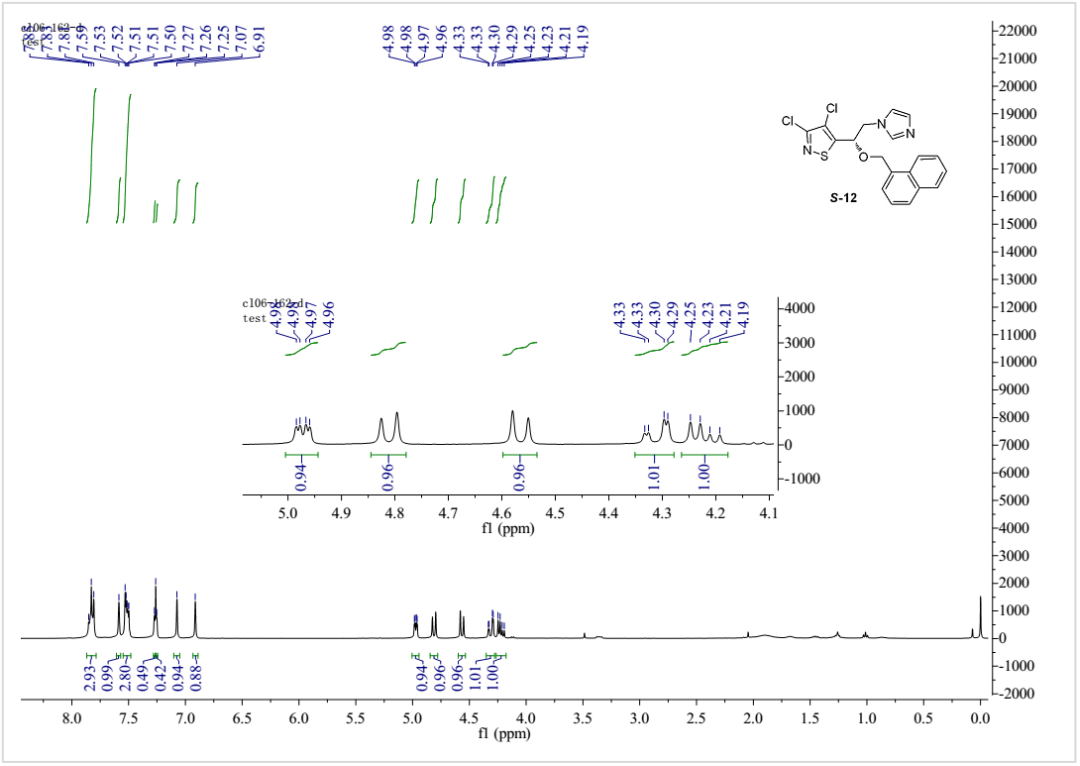

Figure 51. The ^{13}C NMR (101 MHz, $CDCl_3$) of ***S*-12.**

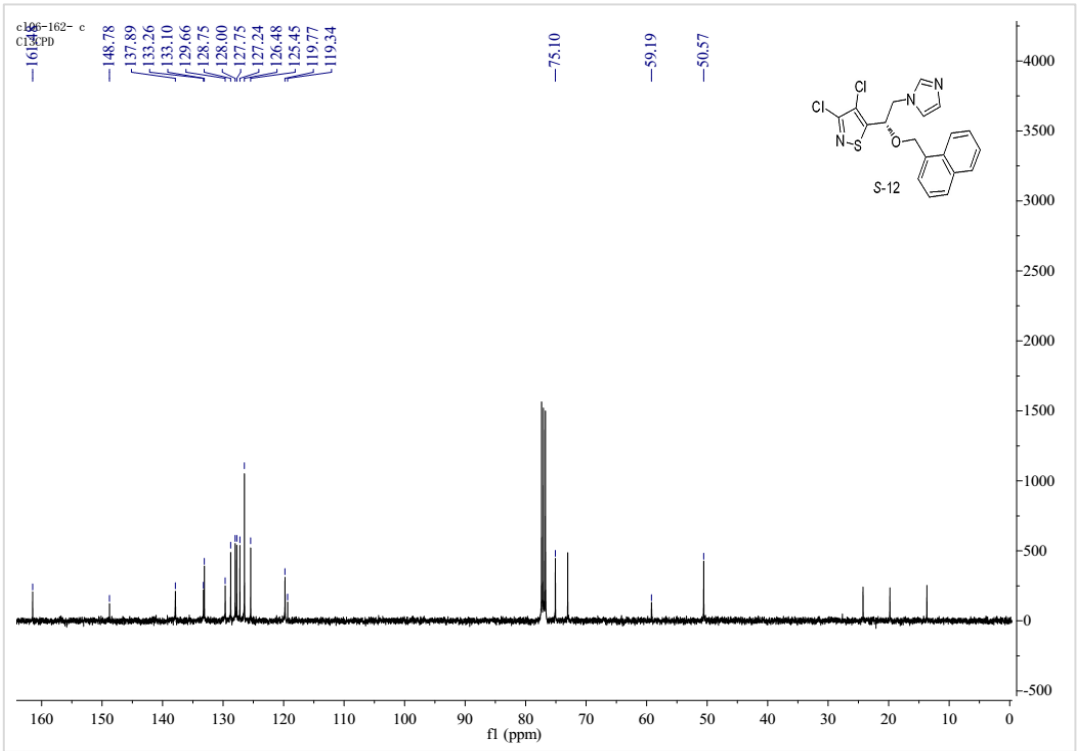

Figure 52. The UV spectra of ***R*-1.**

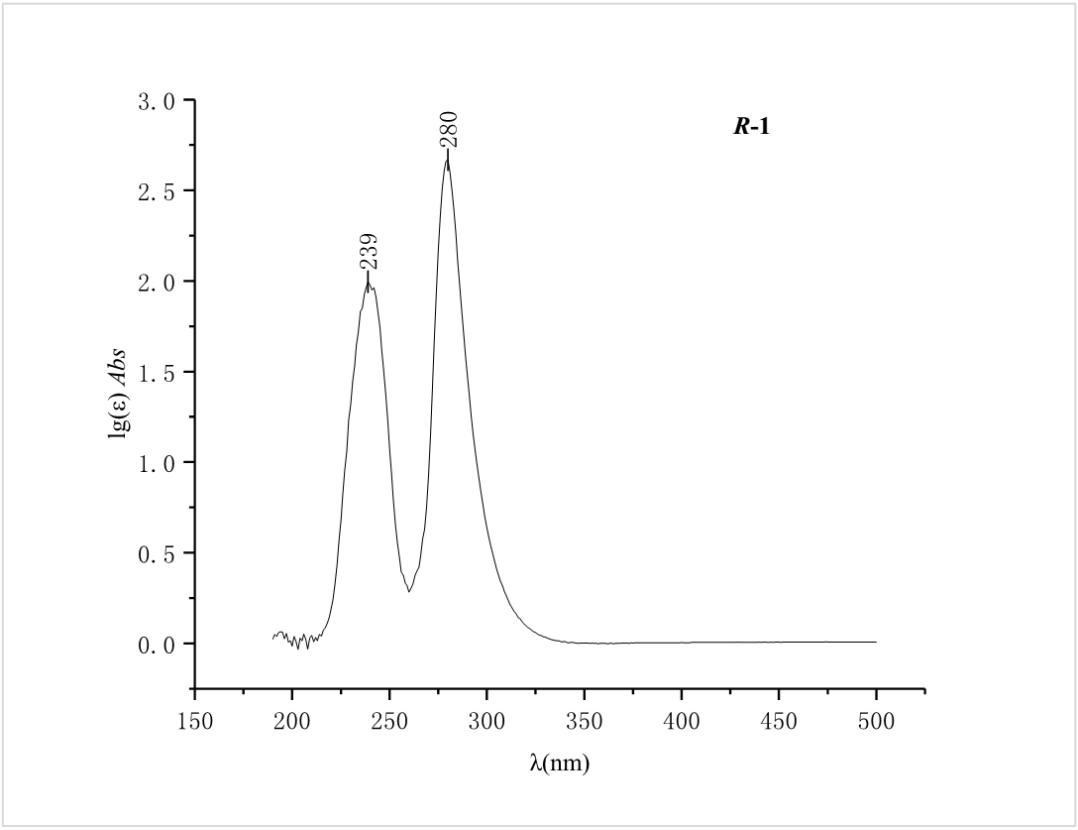

Figure 53. The UV spectra of ***R*-2.**

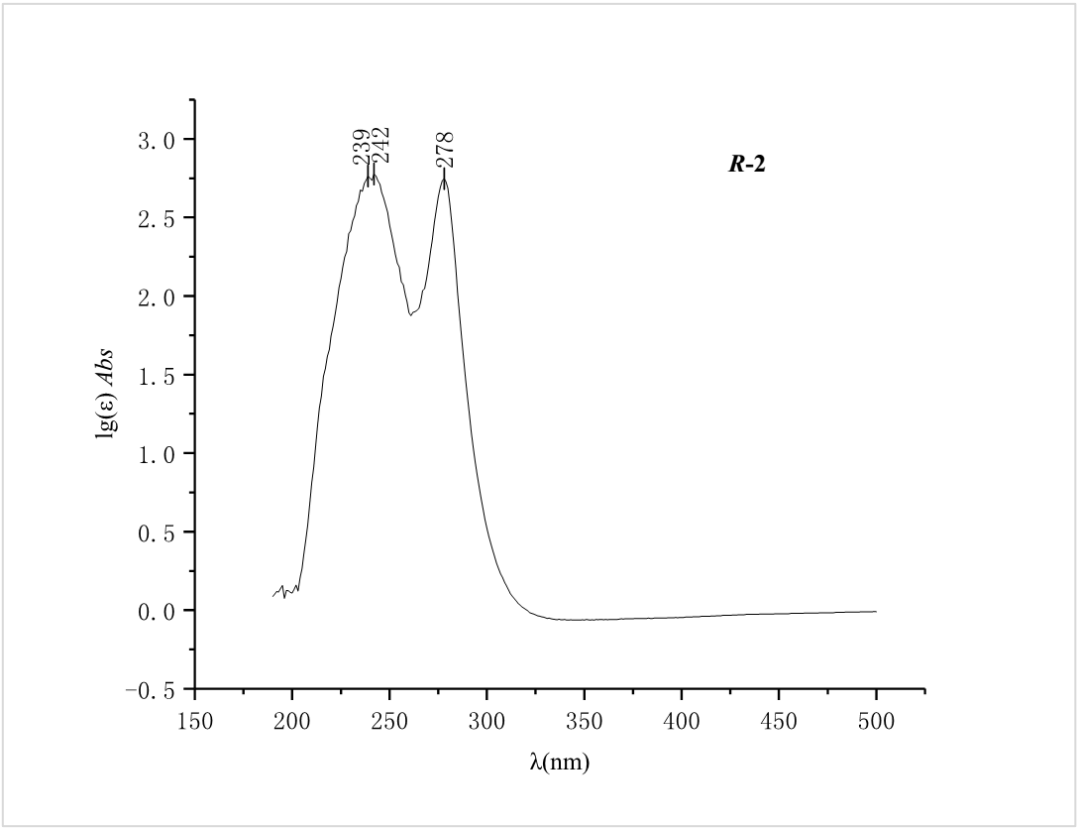

Figure 54. The UV spectra of ***R*-3.**

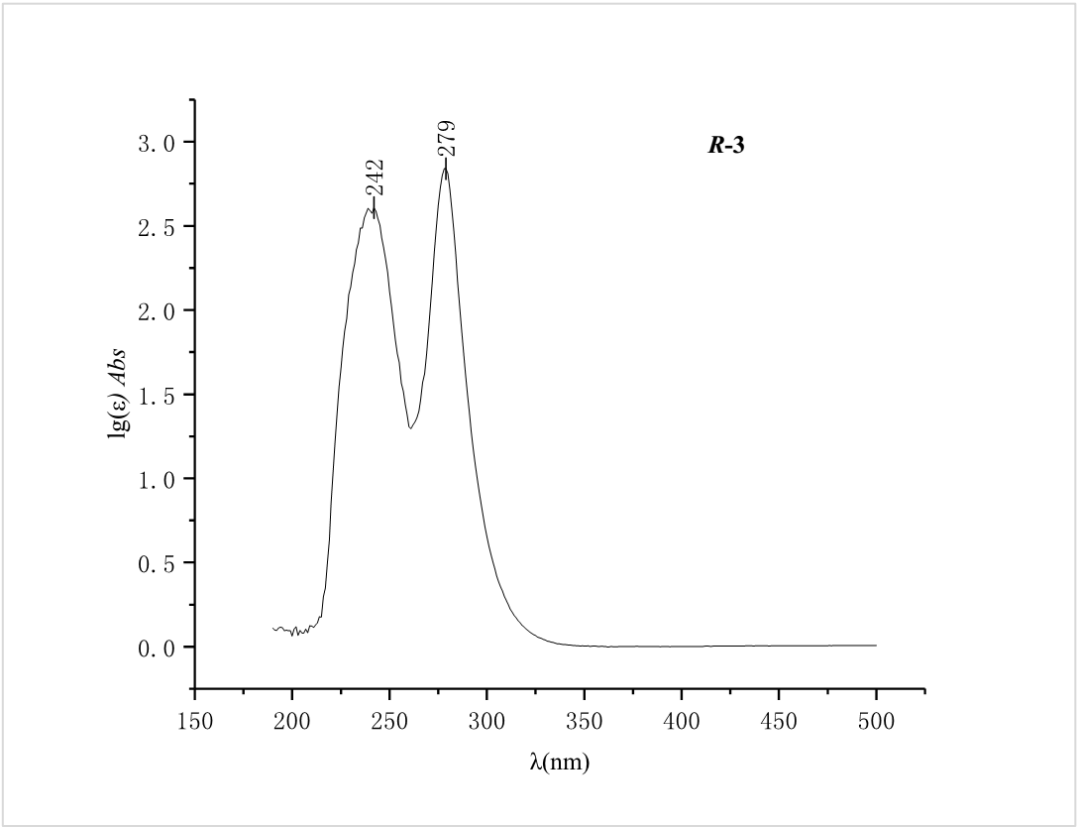

Figure 55. The UV spectra of ***R*-4.**

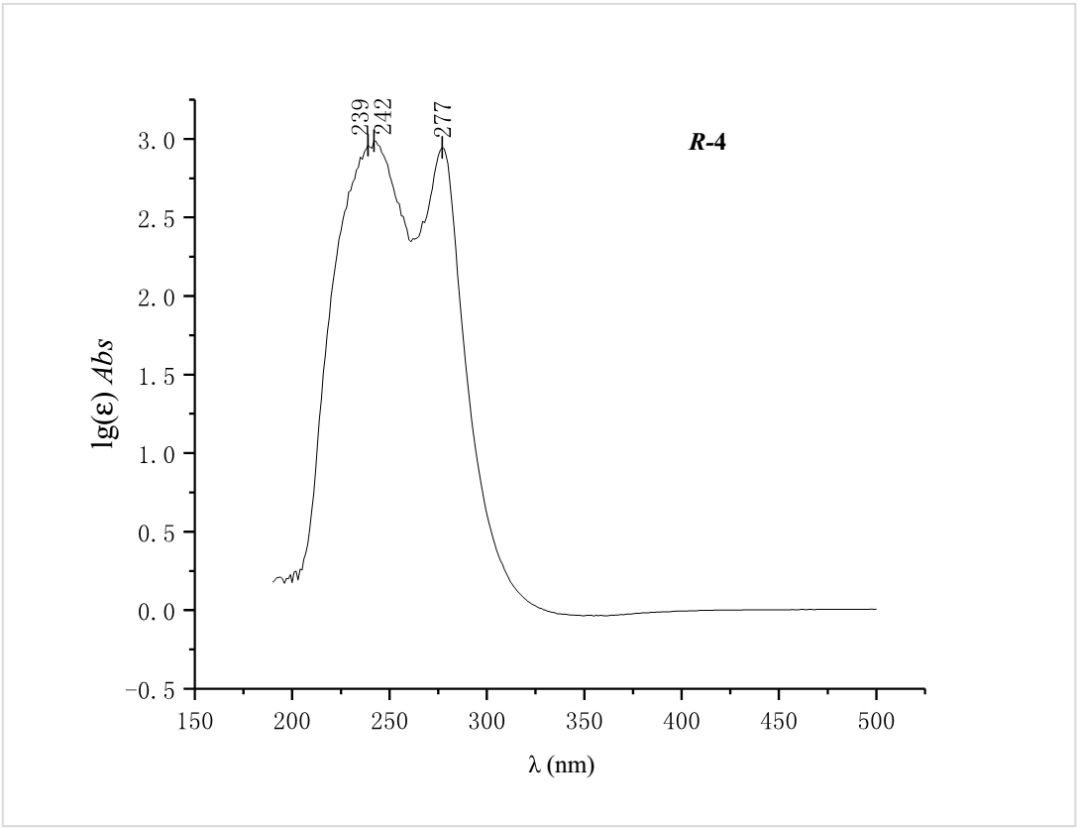

Figure 56. The UV spectra of ***R*-5.**

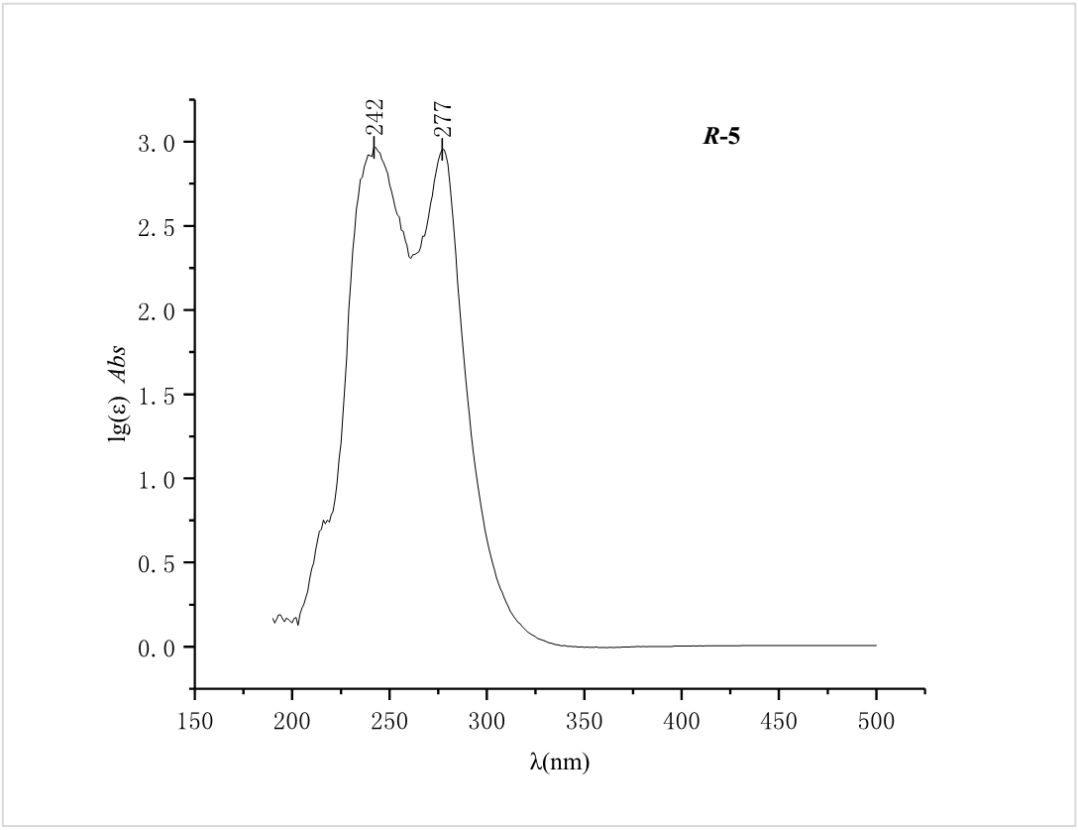

Figure 57. The UV spectra of ***R*-6.**

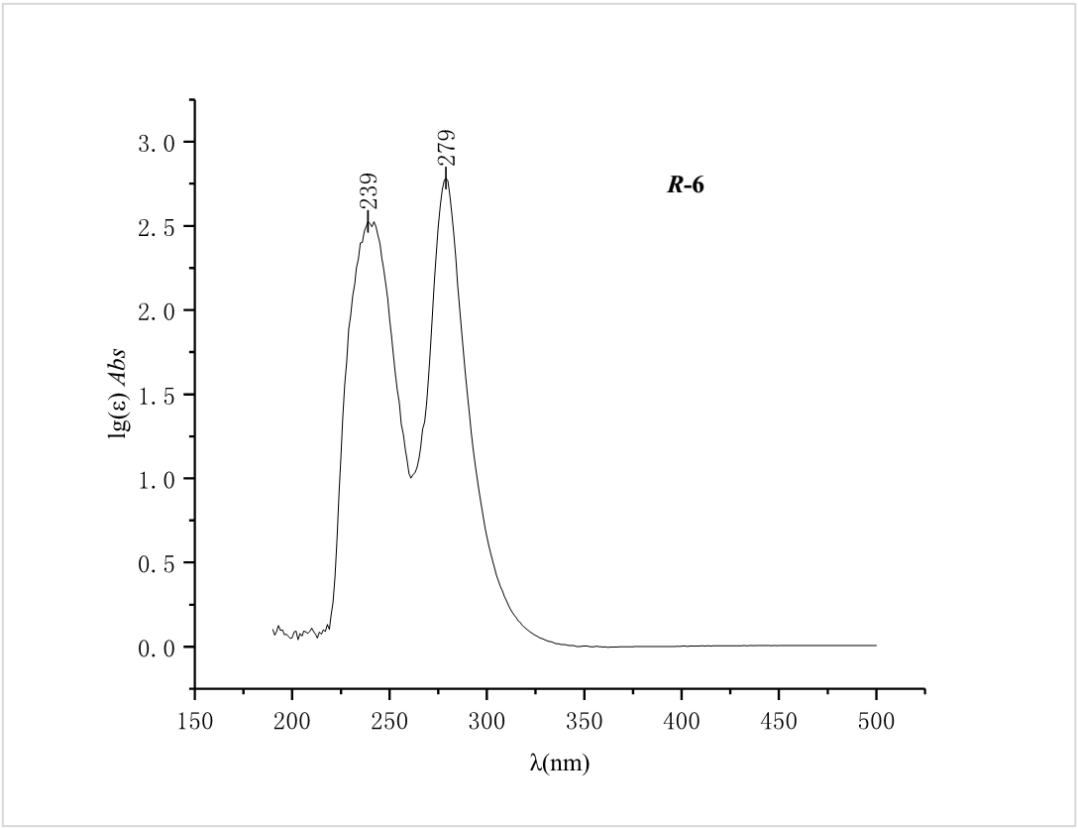

Figure 58. The UV spectra of ***R*-7.**

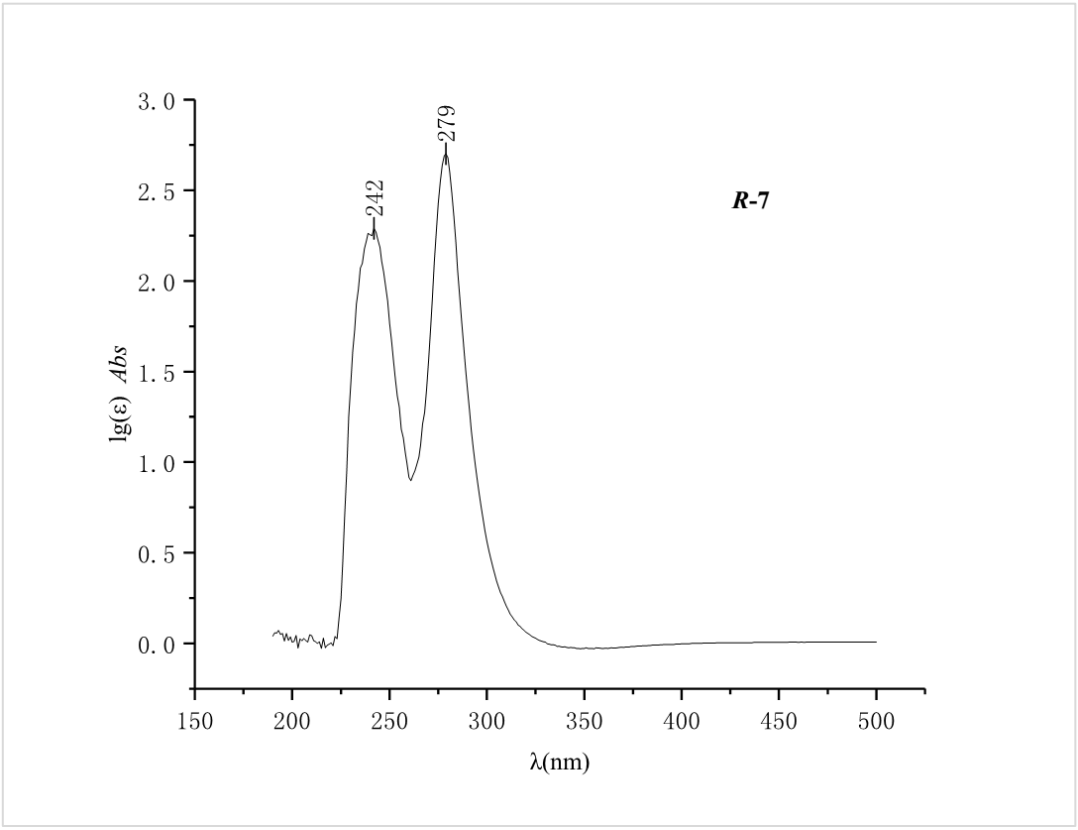

Figure 59. The UV spectra of ***R*-8.**

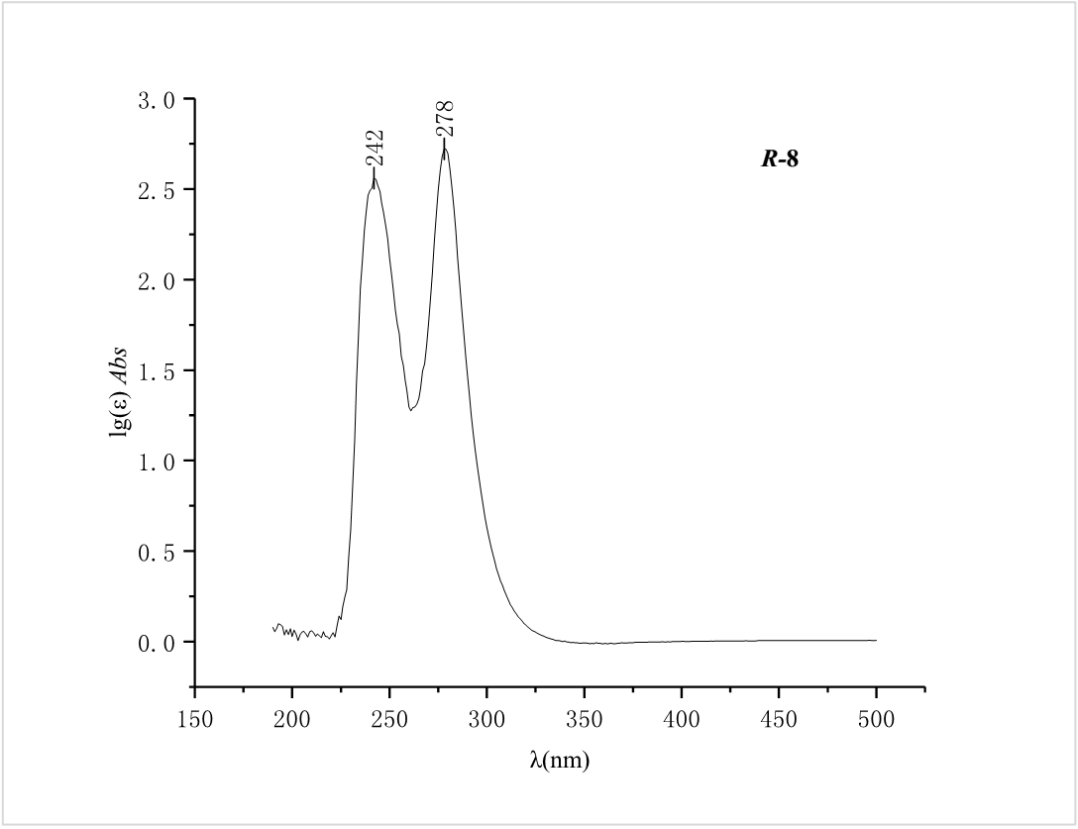

Figure 60. The UV spectra of ***R*-9.**

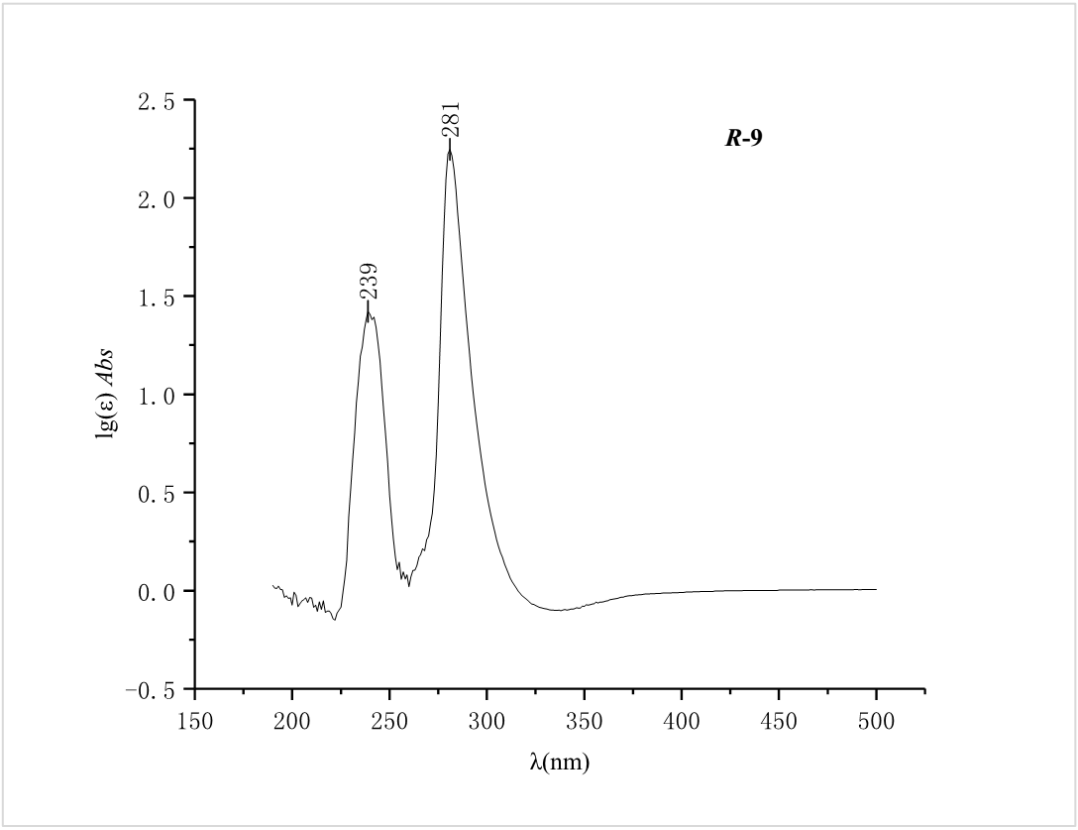

Figure 61. The UV spectra of ***R*-10.**

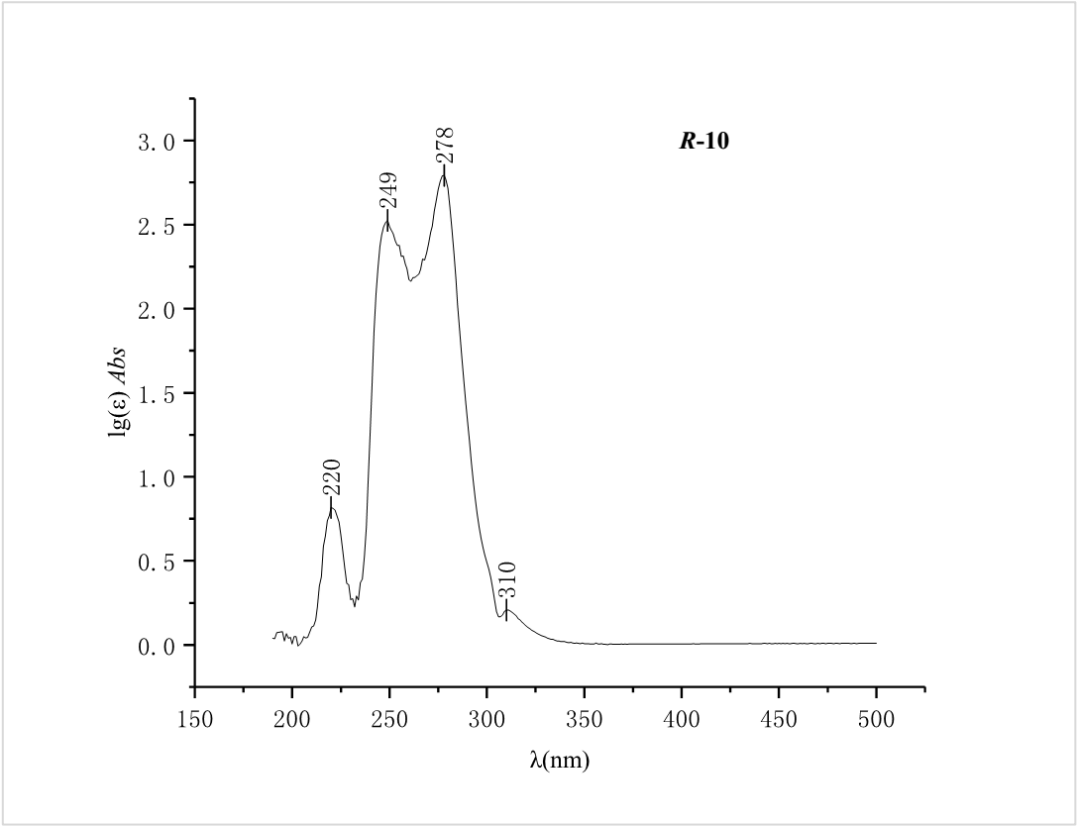

Figure 62. The UV spectra of ***R*-11.**

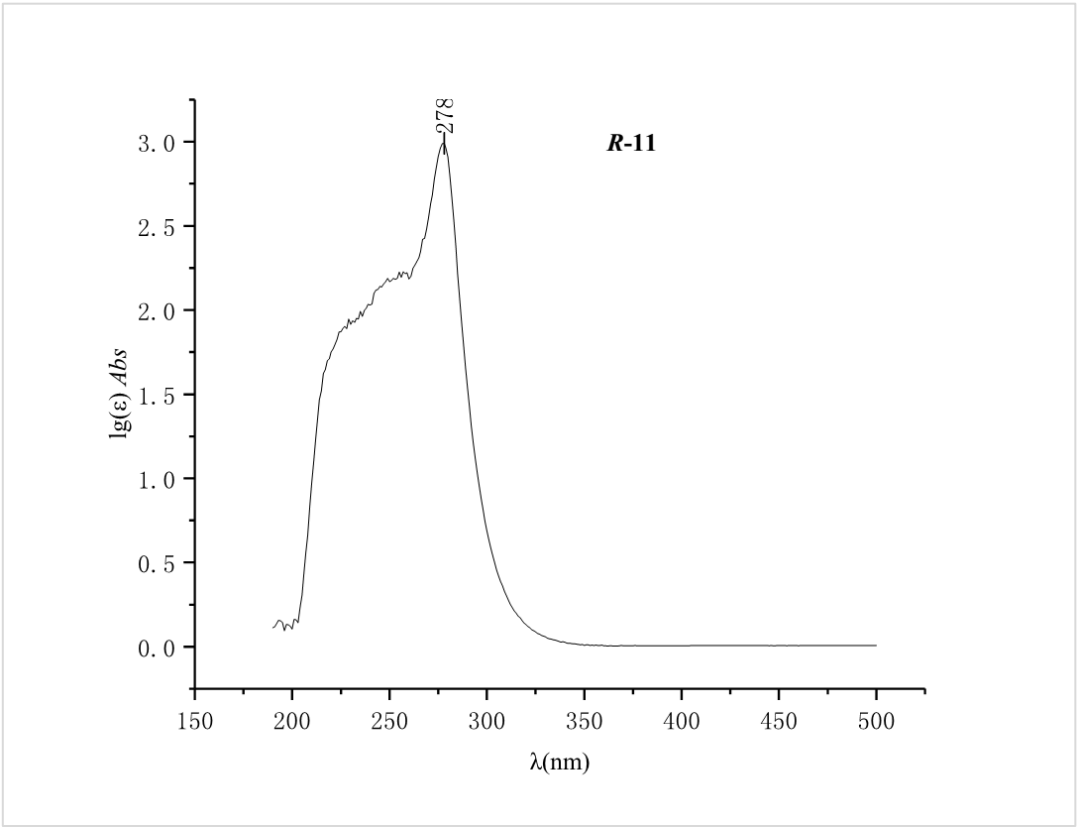

Figure 63. The UV spectra of ***R*-12.**

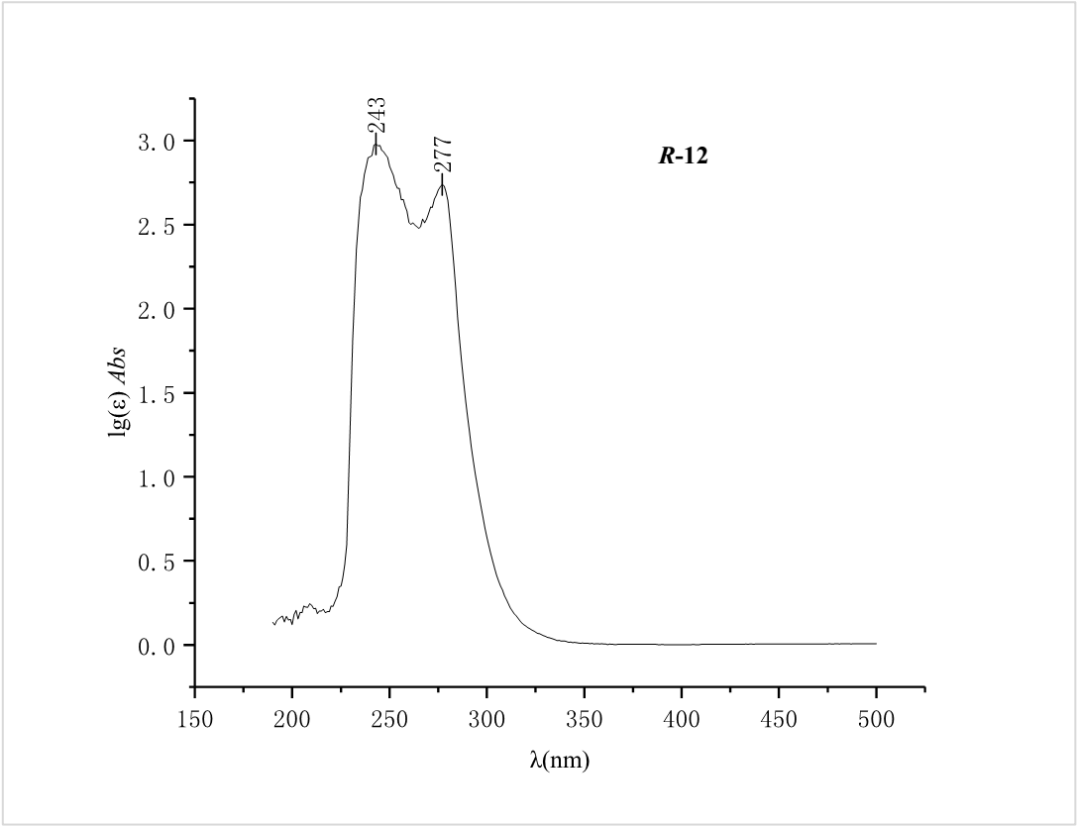

Figure 64. The UV spectra of ***S*-1.**

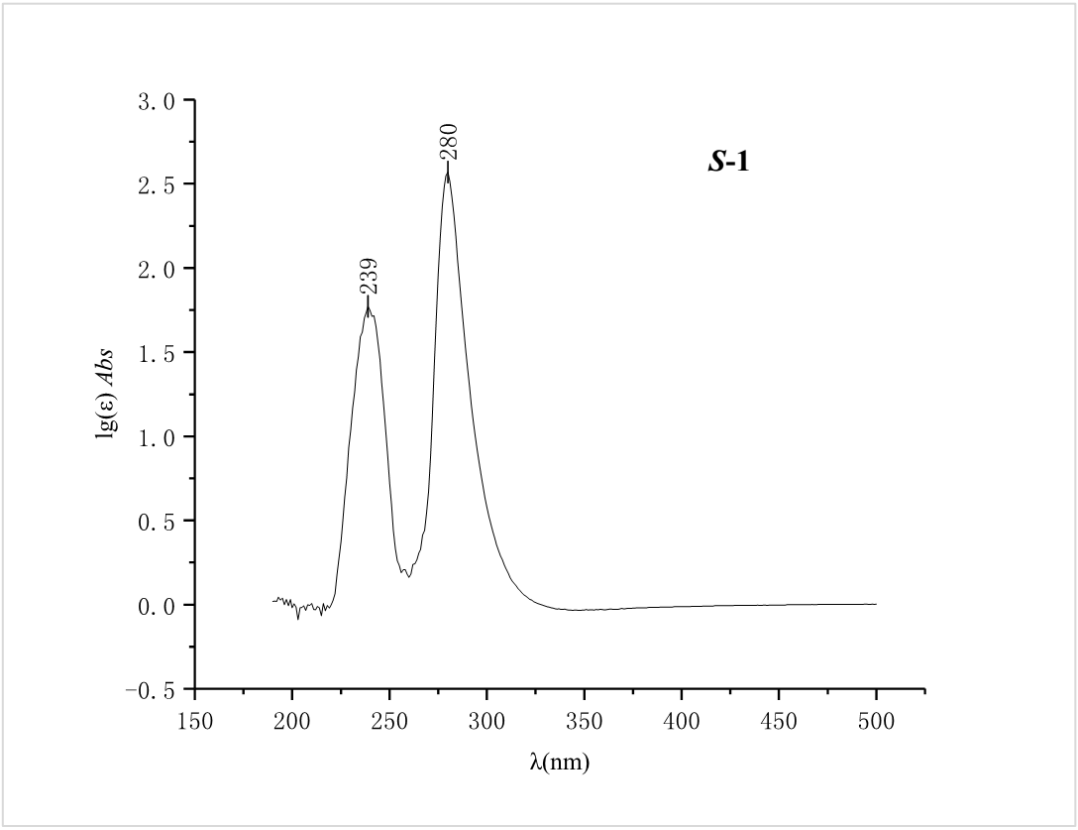

Figure 65. The UV spectra of ***S*-11.**

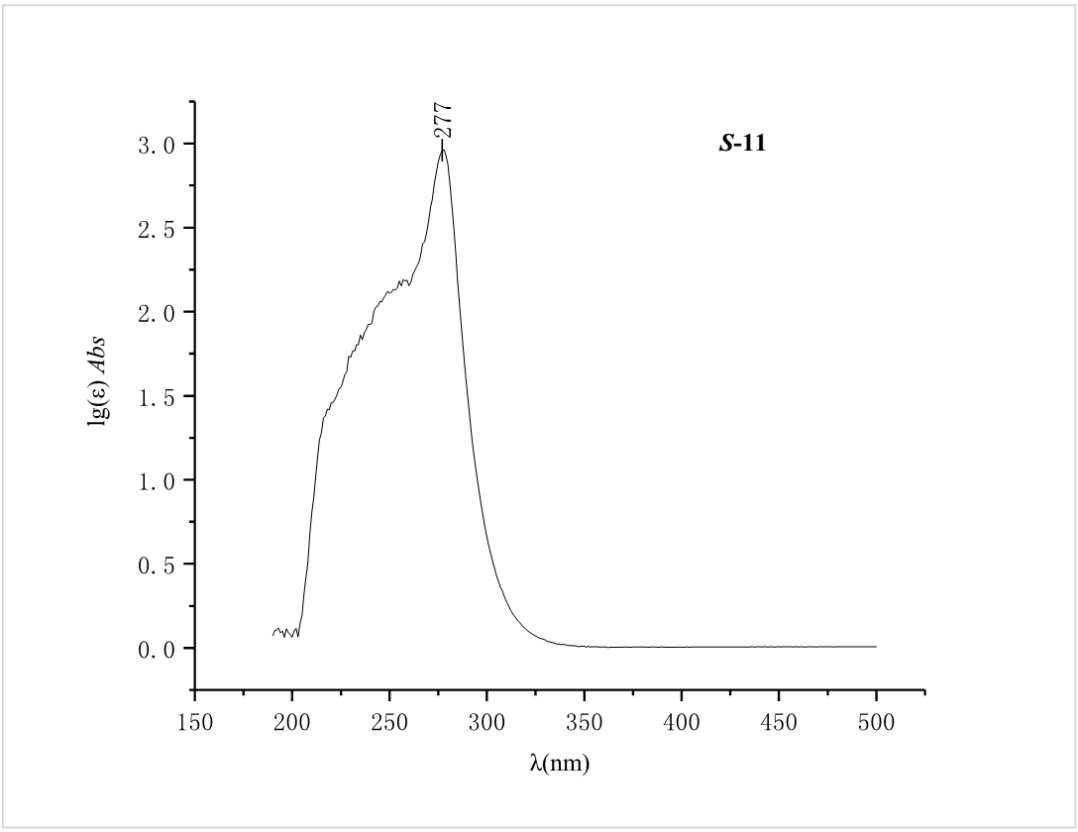

Figure 66. The UV spectra of ***S*-12.**