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Intramolecular proton transfer impact on antibacterial properties of ansamycin antibiotic rifampicin and its new amino analogues

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Figure 9S. ^{1}H-^{13}C HMBC spectrum of **1** in $CDCl_3$ in the range ^{1}H(1.5-2.4)-^{13}C (166-198) ppm.

Figure 10S. ^{1}H-^{13}C HMBC spectrum of **5** in $CDCl_3$.

Figure 11S. ^{1}H-^{13}C HMBC spectrum of **5** in $CDCl_3$ in the range ^{1}H(1.75-2.2)-^{13}C (168-190) ppm.

1. Experimental

X-ray measurements

All crystals of the studied compounds were solvated and unstable in the air. Therefore diffraction data were collected at 130 K and crystals were mounted on a loop with small amount of perfluoropolyether. The diffraction measurements for **1**-CH_3CCl_3 and **1**-CH_3OH-H_2O were carried out with a KumaCCD diffractometer using Mo-Kα radiation (λ=0.71073 Å). Data collection and reduction were performed with CrysAlis CCD[1S] and CrysAlis RED[1S], respectively. For **2**-CH_3OH-CH_2Cl_2 the measurements were performed with a SuperNova diffractometer using hi-flux micro-focus Nova Cu-Kα radiation (λ=1.54184 Å). Data collection and reduction were performed with the CrysAlis Pro software.[2S] The structures were solved by directed method using the SHELXS-97 program for **1**-CH_3CCl_3 and **1**-CH_3OH-H_2O[3S] and the Sir2004 program for **2**-CH_3OH-CH_2Cl_2.[4S] The structures were refined by full-matrix least-squares method on F^2 with SHELXL-97.[3S] All non-hydrogen atoms were refined with anisotropic displacement parameters. The hydrogen atom positions of O-H and N-H groups were located in difference Fourier maps. For further refinement positions of all hydrogen atoms were determined geometrically (N-H 0.90 Å, O-H 0.84 Å, C-H_3 0.98 Å, C-H_2 0.99 Å, C-H 1.00 Å, HC=CH 0.95 Å) and were refined in the riding-model approximation, with $U_{iso}(H) = 1.2\ U_{eq}(N,C)$ and $U_{iso}(H) = 1.5\ U_{eq}(O,C_{methyl})$. In **2**-$CH_3OH$-$CH_2Cl_2$ two carbon atoms (C28, C29) from rifampicin *ansa* chain and two methanol molecules are disordered over two position. The dichloromethane molecule is disordered at least over three positions. The crystal data and some details of data collection and structure refinement are given in **Table 2S.** The intra- and intermolecular hydrogen-bond parameters are given in **Tables 1S.**

1S. Oxford Diffraction, CrysAlis CCD and CrysAlis RED Ver. 1.171.31. Oxford Diffraction Ltd., Abingdon, Oxfordshire, England, 2006.

2S. Agilent Technologies, *CrysAlis^{Pro}*, Agilent Technologies, Yarnton, Oxfordshire, England, 2010.

3S. G.M. Sheldrick, *Acta Cryst.*, 2008, **A64**, 112-122.

4S. M. C. Burla, R. Caliandro, M. Camalli, B. Carrozzini, G. L. Cascarano, L. De Caro, C. Giacovazzo, G. Polidori and R. Spagna, *J. Appl. Cryst.*, 2005, **38**, 381-388.

1D and 2D NMR measurements

The NMR spectra of rifaldehyde and **1-9** (0.01-0.1 mol L^{-1}) were recorded in following anhydrous solvents: $CDCl_3$, CD_3CN, CD_3OD, DMSO-d_6 and py-d_5 and after addition of water drop using a Bruker Avance 600 M spectrometer at T = 253.0 K and T = 293.0 K. All spectra were locked to deuterium resonance of TMS.

The ^1H NMR measurements were carried out at the operating frequency of 600.001 M; flip angle, pw = 30^0; spectral width, swh = 6459.95 ; acquisition time, aq = 5.07 s; relaxation delay, d_1=1.0 s; using TMS as the internal standard. No window function or zero filling was used. Digital resolution was 0.2 per point. ^{13}C NMR spectra were recorded at the operating frequency 151.000 M; pw = 90^0; swh = 3594 ; aq = 0.9 s; d_1=2.0 s with TMS as the internal standard. Line broadening parameter was 1 .

The ^1H,^{13}C and ^{15}N NMR signals were assigned independently for each compound using two-dimensional ^1H-^1H COSY, ^1H-^{13}C HSQC, ^1H-^{15}N HSQC, ^1H-^{13}C HMBC, ^1H-^{15}N HMBC as well as ^1H-^1H NOESY spectra. Two-dimensional ^1H-^1H COSY and ^1H-^1H NOESY spectra were acquired in the magnitude mode with the gradient selection method and with spectral widths of 6562 for both dimensions. The ^1H-^1H COSY data were collected with 2048 points in t_2 and with 600 increments (4 scans per increment) in t_1 dimension. A relaxation delay of 1.0 s was applied between scans. The ^1H-^1H NOESY data were collected with 1024 points in t_2 and with 1024 increments (16 scans per increment) in t_1 dimension. A relaxation delay of 3.0 s was applied between scans. The data from ^1H-^{13}C HSQC and ^1H-^{13}C HMBC experiments were collected in the absolute-value mode using the gradient selection method and with spectral ranges of 6561 (^1H axis) and 33 557 (^{13}C axis). The ^1H-^{13}C HSQC data were collected with 1024 points in t_2 and with 1024 t_1 increments (4 scans per increment). A relaxation delay of 1.0 s was applied between scans. Before Fourier transformation, Gaussian window functions were applied to the data in both dimensions. The ^1H-^{13}C HMBC data were collected with 2048 data points in t_2 and 1024 t_1 increments (16 scans per increment) and with a delay of 1.0 s between scans. Prior to Fourier transformation, mixed-mode processing was used by applying to the data a sine-bell window function in t_2 dimension and a shifted Gaussian window function in t_1 dimension.

The data from ^1H-^{15}N HSQC and ^1H-^{15}N HMBC experiments were collected in the absolute-value mode using the gradient selection method and with spectral ranges of 9995 (^1H axis) and 48 732 (^{15}N axis). ^1H-^{15}N HMBC data were collected with 1024 points in t_2 and with 2048 t_1 increments (16 scans per increment). ^1H-^{15}N HSQC data were collected with 1024 points in t_2 and with 1024 t_1 increments (4 scans per increment). Acquisition time were

0.2048 s and 0.0026 s, respectively. A relaxation delay of 1.0 s was applied between scans. The detailed ^{1}H and ^{13}C NMR data were collected in **Tables 3S and 4S.** Exemplary ^{1}H-^{15}N HSQC of **1** and **2** as well as ^{1}H-^{13}C HSQC and HMBC of **5** and **8** spectra were shown in **Figures 1S-11S.**

MALDI-TOF measurements

The MALDI-TOF spectra of rifaldehyde and **1-9** were obtained on Water/Micromass (Manchester, UK) Q-TOF Premier mass spectrometer (software MassLynx V4.1, Manchester, UK) fitted with a 200 repetition rate Nd/YAG (λ = 355 nm, power density 107 W/cm^2). The compounds analyzed were solids and the matrix used was DHB.

PM5 modelling of interactions between 1 - zwitterionic form (phenolate form) and aminoacid residues of the binding site in the RNA polymerase (RNAP)

X-ray structure of **1**-CH_3OH-H_2O as phenolate form (gray color- **Figure 3a**) was docked on the naphthalene ring carbon atom coordinates of rifampicin (yellow color – **Figure 3a**) from the X-ray structure of Rifampicin-RNA polymerase complex according to Campbell et al.[3] The structure of RNAP enzyme was locked in earlier determined coordinates relative to rifampicin with exception of oxygen atoms of carboxylate group of E_{445}. The interactions between **1** molecule, docked as phenolate form, and the aminoacid residues at the binding site to RNAP were modelled *via* geometry calculation in MOPAC using PM5 parameters (Cache Work System Pro Version 7.5.085 – Fujitsu), with the energy gradient not exceeding 2 kcal mol^{-1} at one step (3678 steps). MOZYME algorithm suitable for large molecules was applied[5S]. To get insight into interactions between **2** and RNAP binding site, the crystal structure of **2**-CH_3OH-CH_2Cl_2 as zwitterion with the transferred proton from O_8-H to N(40) atom (grey color - **Figure 3b**) was docked at the coordinates of carbon atoms of naphthalene ring of rifampicin molecule according to Campbell et al.[3]

5S. Cache Work System Pro Version 7.5.085 UserGuide, Fujitsu, Beaverton, Oregon, USA, **2007**.

Antibacterial activity tests of 1-9 and ciprofloxacin (CIP)

The research of antibacterial activity performed for a series of Gram-positive, including reference and hospital strains. Microorganisms used in this study were as follows: standard strains of Gram-positive cocci: *Staphylococcus aureus* NCTC 4163, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228 and 20 hospital isolates of *Staphylococcus aureus* (10 methicillin-susceptible /MSSA/ and 10 methicillin-resistant/MRSA/). Clinical strains of tested bacteria were isolated from different biological materials of patients of the Warsaw Medical University Hospital. Other microorganisms used were obtained from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland. Minimal Inhibitory Concentration (MIC) was examined by the twofold serial dilution method using Mueller-Hinton II agar medium (Beckton Dickinson) according to CLSI guidelines.[6S] Concentrated solutions of **1-9** and **CIP** tested compounds were prepared in methanol, then diluted in water were performed to obtain the required concentration. Concentrations of tested agents in solid medium ranged from 8 to 0.002 µg mL^{-1} (Gram-positive bacteria). The final inoculum of all studied organisms were 104 CFU mL^{-1} (colony forming units per ml). Minimal inhibitory concentrations were read after 18h of incubation at 35 °C. The complete data of antibacterial tests were collected in **Table 5S.**

6S. Clinical and Laboratory Standards Institute Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A-7, Clinical and Laboratory Standards Institute, Wayne, Pa. USA, 2006.

2. General procedure of synthesis of rifaldehyde and new rifampicin analogs.

Synthesis of rifaldehyde (Ral)

To rifampicin (212.1 mg, 0.25 mmol) dissolved in diethyl ether (200 ml) 50 ml of 0.2 M HCl/H_2O was added and stirred for four days at room temperature. Next the organic layer was separated, twice washed with 100 ml of water and evaporated. To crude rifaldehyde 50 ml CH_2Cl_2 was added and extracted with 25 ml of brine and separated. Organic layer was evaporated to dryness yielding rifaldehyde as red powder (167.5 mg; Yield: 89%). The rifaldehyde was characterised by: Elemental analysis $C_{38}H_{47}NO_{13}$: calculated C=62.89%,

H=6.53%, N=1.93%; found C=62.83%, H=6.50%, N=1.87%; m.p.=180-185°C; HR-MALDI-TOF $[M+H]^+$ 726.3116; FT-IR (KBr, 1.5 mg): 3415 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3200 cm^{-1} $\nu(O_8\text{-}H\cdots O_1)+\nu(O_4\text{-}H\cdots O_{11})+\nu(O_1\text{-}H\cdots O_{15})$, 2550 cm^{-1} $\nu(N_{amide}\text{-}H)$, 1726 cm^{-1} $\nu(C_{35}=O)$, 1654 cm^{-1} $\nu(C_{38}=O)_{aldehyde}+\nu(C_{11}=O)_{ketone}$, 1642 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1575 cm^{-1} $\nu(C=C)_{napthalene}$, 1538 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1464 cm^{-1} $\nu(C=C)$, 1249 cm^{-1} $\nu(C\text{-}O)$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

Syntheses of 2-8

Rifaldehyde (181.4 mg, 0.25 mmol) was dissolved in 25 ml CH_2Cl_2 and the respective amine (0.25 mmol) in 5 ml of C_2H_5OH with 0.025mmol HCl was added. Mixture was stirred at 45°C for half an hour and after that a half of solvent volume was distilled off. To cooled reaction mixture (room temperature) reductant $NaBH_3CN$ (15.7 mg, 0.025 mmol) was added portionwise during 10 min. The reaction mixture was evaporated to dyness, dissolved in 30 ml of ethyl acetate and extracted twice with 30 ml of water and brine. The separated organic layer was evaporated and the respective synthesised analogs of rifampicin (compounds **2-8**) were purified by column chromatography with silica gel (25 cm × 1 cm, silica gel 60, 0.040-0.063 mm/230-400 mesh ASTM, Fluka) and ethyl acetate/methanol as eluent (from 100:0 to 15:1). TLC (10:1 ethyl acetate:methanol as eluent) was developed. Compounds **2-8** were obtained as orange-red solids.

2: Yield: 70%; Elemental analysis $C_{41}H_{54}N_2O_{12}$: calculated C=64.21%, H=7.10%, N=3.65%; found C=64.17%, H=7.11%, N=3.62%; m.p.=178-183°C; HR-MALDI-TOF $[M+H]^+$ 767.3741; FT-IR ($CHCl_3$, *c*=0.05M): 3471cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3384 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3200 cm^{-1} $\nu(O_4\text{-}H\cdots O_{11})$, 2750 cm^{-1} $\nu(N_{38}{}^+\text{-}H\cdots O_{15})$, 2500 cm^{-1} $\nu(O_1\text{-}H\cdots O_8{}^-)$, 1716 cm^{-1} $\nu(C_{35}=O)$, 1647 cm^{-1} $\nu(C_{15}=O)_{amide\ I}+\nu(C=C)_{allyl}$, 1600 cm^{-1} $\nu(C=C)_{napthalene}$, 1538 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1500 and 1480 cm^{-1} $\nu(C=C)$, 1250 and 1260 cm^{-1} $\nu(C\text{-}O)$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

3: Yield: 46%; Elemental analysis $C_{40}H_{54}N_2O_{13}$: calculated C=62.32%, H=7.06%, N=3.63%; found C=62.27%, H=7.00%, N=3.59%; m.p.=195-198°C; HR-MALDI-TOF $[M+H]^+$ 771.3699; FT-IR (KBr, 1.5 mg): 3429 and 3402 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)+\nu(O_{40}\text{-}H)$, 3350 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3141 cm^{-1} $\nu(O_4\text{-}H\cdots O_{11})$, 2746 cm^{-1} $\nu(N_{38}{}^+\text{-}H\cdots O_{15})$, 2460 cm^{-1} $\nu(O_1\text{-}H\cdots O_8{}^-)$, 1721 cm^{-1} $\nu(C_{35}=O)$, 1648 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1599 cm^{-1} $\nu(C=C)_{napthalene}$, 1562 cm^{-1} $\nu(C=C)$, 1539 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1453 and 1445 cm^{-1} $\nu(C=C)$, 1249 and 1238 cm^{-1} $\nu(C\text{-}O)$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

4: Yield: 56%; Elemental analysis $C_{42}H_{58}N_2O_{13}$: calculated C=63.14%, H=7.32%, N=3.51%; found C=63.07%, H=7.29%, N=3.48%; m.p.=168-176°C; HR-MALDI-TOF $[M+H]^+$ 799.4006; FT-IR (KBr, 1.5 mg): 3430 and 3400 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)+\nu(O_{42}\text{-}H)$, 3348 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3140 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2744 cm^{-1} $\nu(N_{38}^{+}\text{-}H^{\cdots}O_{15})$, 2462 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1720 cm^{-1} $\nu(C_{35}=O)$, 1645 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1596 cm^{-1} $\nu(C=C)_{napthalene}$, 1561 cm^{-1} $\nu(C=C)$, 1538 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1452 and 1444 cm^{-1} $\nu(C=C)$, 1247 and 1236 cm^{-1} $\nu(C\text{-}O)$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

5: Yield: 60%; Elemental analysis $C_{44}H_{62}N_2O_{13}$: calculated C=63.90%, H=7.56%, N=3.39%; found C=63.89%, H=7.54%, N=3.36%; m.p.=152-158°C; HR-MALDI-TOF $[M+H]^+$ 827.4321; FT-IR (KBr, 1.5 mg): 3434 and 3402 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)+\nu(O_{44}\text{-}H)$, 3350 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3144 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2740 cm^{-1} $\nu(N_{38}^{+}\text{-}H^{\cdots}O_{15})$, 2460 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1719 cm^{-1} $\nu(C_{35}=O)$, 1649 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1598 cm^{-1} $\nu(C=C)_{napthalene}$, 1560 cm^{-1} $\nu(C=C)$, 1536 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1450 and 1441 cm^{-1} $\nu(C=C)$, 1245 and 1235 cm^{-1} $\nu(C\text{-}O)$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

6: Yield: 62%; Elemental analysis $C_{46}H_{66}N_2O_{14}$: calculated C=63.43%, H=7.64%, N=3.22%; found C=63.40%, H=7.61%, N=3.18%; m.p.=190-193°C; HR-MALDI-TOF $[M+H]^+$ 871.4581; FT-IR (KBr, 1.5 mg): 3427 and 3390 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3342 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3100 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2745 cm^{-1} $\nu(N_{38}^{+}\text{-}H^{\cdots}O_{15})$, 2444 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1716 cm^{-1} $\nu(C_{35}=O)$, 1650 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1599 cm^{-1} $\nu(C=C)_{napthalene}$, 1574 cm^{-1} $\nu(C=C)$, 1539 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1458 and 1441 cm^{-1} $\nu(C=C)$, 1246 cm^{-1} $\nu(C\text{-}O)$, 1100 and 1082 cm^{-1} $\nu(C\text{-}O)_{oxaalkyl\ chain}$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

7: Yield: 59%; Elemental analysis $C_{44}H_{58}N_4O_{12}$: calculated C=63.29%, H=7.00%, N=6.71%; found C=63.27%, H=6.96%, N=6.65%; m.p.=183-186°C; HR-MALDI-TOF $[M+H]^+$ 835.4120; FT-IR (KBr, 1.5 mg): 3428 and 3394 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3345 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3105 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2740 cm^{-1} $\nu(N_{38}^{+}\text{-}H^{\cdots}O_{15})$, 2440 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1718 cm^{-1} $\nu(C_{35}=O)$, 1652 cm^{-1} $\nu(C_{15}=O)_{amide\ I}$, 1619 cm^{-1} $\nu(C=N)_{imidazole\ ring}$, 1598 cm^{-1} $\nu(C=C)_{napthalene}$, 1570 cm^{-1} $\nu(C=C)$, 1538 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1460 and 1443 cm^{-1} $\nu(C=C)$, 1250 cm^{-1} $\nu(C\text{-}O)$, 662 cm^{-1} $\gamma(C\text{-}H)_{imidazole\ ring}$; ^1H and ^{13}C NMR (**Table 3S** and **4S**).

8: Yield: 68%; Elemental analysis $C_{44}H_{56}N_2O_{12}S$: calculated C=63.14%, H=6.74%, N=3.35%; found C=63.10%, H=6.71%, N=3.33%; m.p.=187-192°C; HR-MALDI-TOF $[M+H]^+$ 837.3627; FT-IR (KBr, 1.5 mg): 3430 and 3390 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3349 cm^{-1}

$\nu(N_{amide}\text{-}H)$, 3109 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2736 cm^{-1} $\nu(N_{38}^{+}\text{-}H^{\cdots}O_{15})$, 2445 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1717 cm^{-1} $\nu(C_{35}{=}O)$, 1650 cm^{-1} $\nu(C_{15}{=}O)_{amide\ I}$, 1595 cm^{-1} $\nu(C{=}C)_{napthalene}$, 1566 cm^{-1} $\nu(C{=}C)$, 1538 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1461 and 1441 cm^{-1} $\nu(C{=}C)$, 1249 and 1237 cm^{-1} $\nu(C\text{-}O)$, 699 cm^{-1} $\gamma(C\text{-}H)_{thiophene\ ring}$; ^{1}H and ^{13}C NMR (**Table 3S** and **4S**).

Synthesis of 9

Rifampicin (212.1 mg, 0.25 mmol) was dissolved in 10 ml C_2H_5OH with 0.025mmol HCl. To reaction mixture (room temperature) reductant $NaBH_3CN$ (15.7 mg, 0.025 mmol) was added portionwise during 20 min. The reaction mixture was evaporated to dyness, dissolved in 30 ml of ethyl acetate and extracted twice with 30 ml of water and brine. The separated organic layer was evaporated and the reduced rifampicin (compound **9**) were purified by column chromatography with silica gel (25 cm × 1 cm, silica gel 60, 0.040-0.063 mm/230-400 mesh ASTM, Fluka) and ethyl acetate/methanol as eluent (40:1). TLC (10:1 ethyl acetate:methanol as eluent) was developed. Compound **9** was obtained as orange solid.

9: Yield: 75%; Elemental analysis $C_{43}H_{60}N_4O_{12}$: calculated C=62.60%, H=7.33%, N=6.79%; found C=62.52%, H=7.30%, N=6.74%; m.p.= 180-186°C; HR-MALDI-TOF $[M+H]^{+}$ 825.4273; FT-IR (KBr, 1.5 mg): 3440 and 3392 cm^{-1} $\nu(O_{21}\text{-}H)+\nu(O_{23}\text{-}H)$, 3345 cm^{-1} $\nu(N_{amide}\text{-}H)$, 3230 cm^{-1} $\nu(N_{40}^{+}\text{-}H)$, 3070 cm^{-1} $\nu(O_4\text{-}H^{\cdots}O_{11})$, 2450 cm^{-1} $\nu(O_1\text{-}H^{\cdots}O_8^{-})$, 1723 cm^{-1} $\nu(C_{35}{=}O)$, 1648 cm^{-1} $\nu(C_{15}{=}O)_{amide\ I}$, 1584 cm^{-1} $\nu(C{=}C)_{napthalene}$, 1561 cm^{-1} $\nu(C{=}C)$, 1536 cm^{-1} $\nu(C\text{-}N)_{amide\ II}$, 1457 and 1437 cm^{-1} $\nu(C{=}C)$, 1253 and 1236 cm^{-1} $\nu(C\text{-}O)$;^{1}H and ^{13}C NMR (**Table 3S** and **4S**).

Table 1S. Selected hydrogen-bond parameters.

a) intramolecular hydrogen bonds

D—H···*A*	*D*—H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*—H···*A* (°)
1-CH_3CCl_3				
O1—H1···O15	0.84	1.64	2.457 (6)	164
O8—H8···O1	0.84	1.76	2.510 (6)	148
O21—H21···O23	0.84	2.01	2.744 (6)	146
O4—H4O···O11	0.84	1.75	2.567 (6)	164
N2—H2N···N38	0.90	1.88	2.670 (7)	145
1-CH_3OH-H_2O				
O1—H1···O8	0.84	1.75	2.497 (3)	148
O21—H21O···O23	0.84	1.99	2.732 (3)	147
O4—H4O···O11	0.84	1.71	2.553 (3)	178
N2—H2N···N38	0.90	2.00	2.701 (3)	134
2-CH_3OH-CH_2Cl_2				
O1—H1···O8	0.84	1.76	2.507 (3)	146
O23—H23O···O21	0.84	2.04	2.752 (3)	141
O4—H4O···O11	0.84	1.74	2.582 (3)	178
N38—H2N3···O15	0.90	2.08	2.893 (3)	150

b) intermolecular hydrogen bonds

D—H···*A*	*D*—H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*—H···*A* (°)
1-CH_3CCl_3				
O23—H23O···N40^{i}	0.84	1.98	2.806 (6)	170
1- CH_3OH-H_2O				
O1A—H1OA···O15	0.84	1.95	2.763 (3)	162
O1D—H2OD···O11	0.84	1.85	2.678 (3)	169
O1E—H1OE···O21	0.84	1.95	2.786 (3)	175
O1C—H2OC···O1E^{ii}	0.84	1.94	2.691 (4)	147
O23—H23O···O4^{iii}	0.84	2.00	2.828 (3)	170
O1B—H1OB···O1D	0.84	2.04	2.874 (4)	173
O1D—H1OD···O8^{iv}	0.84	1.98	2.796 (3)	165

O1E—H2OE···O1D^{iii}	0.84	2.05	2.888 (3)	176
O1C—H1OC···O27	0.84	1.95	2.772 (4)	166
N40—H40N···O1C^{v}	0.90	1.79	2.668 (4)	165
2-CH_2Cl_2-CH_3OH				
O1C—H1C···O11	0.84	1.88	2.654 (18)	153
O1C'—H1C'···O11	0.84	1.82	2.65 (3)	172
O1D—H1D···O15	0.84	2.09	2.872 (5)	155
O1D'—H1D'···O1	0.84	2.26	3.056 (15)	159
N2—H1N2···O1A	0.90	1.99	2.850 (4)	160
O1A—H1A···O21	0.84	1.92	2.756 (3)	173
O21—H21O···O1C^{vi}	0.84	1.86	2.677 (19)	163
O21—H21O···O1C'^{vi}	0.84	1.94	2.77 (3)	169
N38—H1N3···O23^{vii}	0.90	1.95	2.828 (3)	164
N38—H2N3···O35^{vii}	0.90	2.34	2.813 (3)	112

Symmetry code(s): (i) *x*+1, *y*, *z*; (ii) *x*-1, *y*, *z*; (iii) -*x*+3/2, -*y*+1, *z*-1/2; (iv) -*x*+1, *y*+1/2, -*z*+3/2; (v) -*x*+3/2, -*y*+1, *z*+1/2; (vi) -*x*+1, *y*-1/2, -*z*+1; (vii) -*x*+1, *y*+1/2, -*z*+1.

Table 2S. Crystal data and details of structure refinement

Compound reference	**1**-CH_3CCl_3	**1**-CH_3OH-H_2O	**2**-CH_3OH-CH_2Cl_2
Chemical formula	$C_{43}H_{58}N_4O_{12}\cdot 2(C_2H_3Cl_3)$	$C_{43}H_{58}N_4O_{12}\cdot 2(CH_3OH)\cdot 3(H_2O)$	$(C_{41}H_{54}N_2O_{12})\cdot 3(CH_3OH)\cdot (CH_2Cl_2)$
Formula Mass	1089.72	941.07	947.91
Crystal system	Monoclinic	Orthorhombic	Monoclinic
a/Å	10.9716 (4)	13.8494 (6)	9.2035 (1)
b/ Å	18.7546 (6)	17.4243 (7)	20.0206 (1)
c/ Å	13.2194 (4)	20.0324 (9)	13.8049 (1)
α/°	90	90	90
β/°	101.595 (3)	90	103.152 (1)
γ/°	90	90	90
Unit cell volume/$Å^3$	2664.62 (15)	4834.1 (4)	2476.96 (3)
Temperature/K	130	130	130
Space group	$P2_1$	$P2_12_12_1$	$P2_1$
No. of formula units per unit cell, Z	2	4	2
Radiation type	Mo *K*α	Mo *K*α	Cu *K*α
Absorption coefficient, μ/mm^{-1}	0.38	0.10	1.73
No. of reflections measured	21144	18270	40851
No. of independent reflections	4837	4875	5207
R_{int}	0.036	0.042	0.030
Final R_1 values ($I > 2\sigma(I)$)	0.054	0.040	0.047
Final wR(F^2) values ($I>2\sigma(I)$)	0.139	0.095	0.138
Final R_1 values (all data)	0.080	0.048	0.047
Final wR(F^2) values (all data)	0.165	0.100	0.138
Goodness of fit on F^2	1.05	1.05	1.06

Table 3S. Total assignment of ^{1}H NMR δ [ppm] chemical shifts and coupling constants J[Hz] for **1-9** and **Ral** (rifaldehyde) in $CDCl_3$.

Atom number	**Ral**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	---	---	---	---	---	---	---	---	---	---
2	---	---	---	---	---	---	---	---	---	---
3	---	---	---	---	---	---	---	---	---	---
4	---	---	---	---	---	---	---	---	---	---
5	---	---	---	---	---	---	---	---	---	---
6	---	---	---	---	---	---	---	---	---	---
7	---	---	---	---	---	---	---	---	---	---
8	---	---	---	---	---	---	---	---	---	---
9	---	---	---	---	---	---	---	---	---	---
10	---	---	---	---	---	---	---	---	---	---
11	---	---	---	---	---	---	---	---	---	---
12	---	---	---	---	---	---	---	---	---	---
13	3H 1.82 (s)	3H 1.81 (s)	3H 1.74 (s)	3H 1.79 (s)	3H 1.78 (s)	3H 1.77 (s)	3H 1.78 (s)	3H 1.74 (s)	3H 1.76 (s)	3H 1.78(s)
14	3H 2.27 (s)	3H 2.21 (s)	3H 2.03 (s)	3H 2.04 (s)	3H 2.01 (s)*	3H 2.02 (s)*	3H 2.05 (s)	3H 2.00 (s)*	3H 2.03 (s)	3H 2.21 (s)
15	---	---	---	---	---	---	---	---	---	---
16	---	---	---	---	---	---	---	---	---	---
17	1H 6.51 (d) $^{3}J_{H17\text{-}H18}$=11.4	1H 6.39 (d) $^{3}J_{H17\text{-}H18}$=11.1	1H 6.27 (d) $^{3}J_{H17\text{-}H18}$=10.8	1H 6.24 (m)	1H 6.28 (d) $^{3}J_{H17\text{-}H18}$=10.4	1H 6.28 (d) $^{3}J_{H17\text{-}H18}$=10.6	1H 6.28 (d) $^{3}J_{H17\text{-}H18}$=10.8	1H 6.23 (m)	1H 6.26 (d) $^{3}J_{H17\text{-}H18}$=10.1	1H 6.27 (d) $^{3}J_{H17\text{-}H18}$=10.9
18	1H 6.57 (dd) $^{3}J_{H18\text{-}H19}$=14.9	1H 6.58 (dd) $^{3}J_{H18\text{-}H19}$=15.2	1H 6.55 (dd) $^{3}J_{H18\text{-}H19}$=15.0	1H 6.48 (m)	1H 6.55 (dd) $^{3}J_{H18\text{-}H19}$=15.5	1H 6.56 (dd) $^{3}J_{H18\text{-}H19}$=15.8	1H 6.50 (dd) $^{3}J_{H18\text{-}H19}$=15.1	1H 6.52 (m)	1H 6.48 (dd) $^{3}J_{H18\text{-}H19}$=14.9	1H 6.46 (d) $^{3}J_{H18\text{-}H19}$=15.4
19	1H 6.07 (dd) $^{3}J_{H19\text{-}H20}$=5.0	1H 5.93 (dd) $^{3}J_{H19\text{-}H20}$=4.9	1H 6.11 (dd) $^{3}J_{H19\text{-}H20}$=6.0	1H 6.04 (m)	1H 6.11 (dd) $^{3}J_{H19\text{-}H20}$=6.7	1H 6.10 (dd) $^{3}J_{H19\text{-}H20}$=6.7	1H 6.15 (dd) $^{3}J_{H19\text{-}H20}$=5.9	1H 6.07 (m)	1H 6.07 (m)	1H 5.97 (m)
20	1H 2.43 (m)	1H 2.38 (m)	1H 2.38 (m)	1H 2.38 (m)	1H 2.38 (m)	1H 2.40 (m)	1H 2.39 (m)	1H 2.33 (m)	1H 2.37 (m)	1H 2.42 (m)
21	1H 3.78 (d) $^{3}J_{H16\text{-}H17}$=9.5	1H 3.78 (d) $^{3}J_{H16\text{-}H17}$=9.5	1H 3.71 (d) $^{3}J_{H16\text{-}H17}$=9.1	1H 3.73 (m)	1H 3.71 (d) $^{3}J_{H16\text{-}H17}$=9.4	1H 3.73 (d) $^{3}J_{H16\text{-}H17}$=9.8	1H 3.71 (d) $^{3}J_{H16\text{-}H17}$=8.9	1H 3.72 (m)	1H 3.72 (d) $^{3}J_{H16\text{-}H17}$=8.6	1H 3.85 (m)
22	1H 1.76 (m)	1H 1.71 (m)	1H 1.71 (m)	1H 1.75 (m)	1H 1.75 (m)	1H 1.75 (m)	1H 1.76 (m)	1H 1.75 (m)	1H 1.73 (m)	1H 1.81 (m)
23	1H 3.05 (m)*	1H 3.02 (m)*	1H 2.97 (m)*	1H 2.97 (m)	1H 2.97 (m)	1H 2.98 (m)	1H 3.00 (m)	1H 2.95 (m)	1H 2.97 (m)	1H 3.03 (m)
24	1H 1.54 (qd) $^{3}J_{H24\text{-}H33}$=6.8 $^{3}J_{H23\text{-}H24}$=14.0	1H 1.53 (qd) $^{3}J_{H24\text{-}H33}$=6.8 $^{3}J_{H23\text{-}H24}$=14.0	1H 1.43 (m)	1H 1.39 (m)	1H 1.44 (m)	1H 1.45 (m)	1H 1.45 (m)	1H 1.43 (m)	1H 1.39 (m)	1H 1.56 (m)

25	1H 4.94 (d) $^3J_{H25-H26}$=10.0	1H 4.95 (d) $^3J_{H25-H26}$=10.6	1H 4.90 (d) $^3J_{H25-H26}$=10.5	1H 4.89 (bs)	1H 4.87 (d) $^3J_{H25-H26}$=10.6	1H 4.87 (d) $^3J_{H25-H26}$=10.6	1H 4.88 (d) $^3J_{H25-H26}$=10.8	1H 4.91 (bs)	1H 4.89 (d) $^3J_{H25-H26}$=10.4	1H 4.92 (d) $^3J_{H25-H26}$=10.4
26	1H 1.37 (m)	1H 1.35 (m)	1H 1.32 (m)	1H 1.45 (m)	1H 1.37 (m)	1H 1.29 (m)	1H 1.45 (m)	1H 1.39 (m)	1H 1.38 (m)	1H 1.43 (m)
27	1H 3.51 (d) $^3J_{H27-H28}$=7.0	1H 3.51 (d) $^3J_{H27-H28}$=6.7	1H 3.34 (d) $^3J_{H27-H28}$=6.6	1H 3.34 (m)	1H 3.37 (d) $^3J_{H27-H28}$=6.9	1H 3.38 (d) $^3J_{H27-H28}$=6.6	1H 3.39 (d) $^3J_{H27-H28}$=7.0	1H 3.36 (m)	1H 3.31 (m)	1H 3.51 (d) $^3J_{H27-H28}$=6.9
28	1H 5.12 (dd) $^3J_{H28-H29}$=12.7	1H 5.10 (dd) $^3J_{H28-H29}$=12.6	1H 5.06 (dd) $^3J_{H28-H29}$=12.5	1H 5.07 (m)	1H 5.05 (dd) $^3J_{H28-H29}$=12.5	1H 5.05 (dd) $^3J_{H28-H29}$=12.6	1H 5.08 (dd) $^3J_{H28-H29}$=12.5	1H 5.02 (m)	1H 5.11 (dd) $^3J_{H27-H28}$=6.8 ^{3} $J_{H28-H29}$=12.3	1H 5.07 (dd) $^3J_{H27-H28}$=7.0 ^{3} $J_{H28-H29}$=12.6
29	1H 6.24 (d)	1H 6.20 (d)	1H 6.03 (d)	1H 6.02 (m)	1H 6.01 (d)	1H 6.03 (d)	1H 6.12 (d)	1H 6.02 (m)	1H 6.02 (d)	1H 6.23 (d)
30	3H 2.07 (s)*	3H 2.09 (s)	3H 2.08 (s)	3H 2.05 (s)	3H 2.09 (s)	3H 2.08 (s)	3H 2.07 (s)	3H 2.02 (s)	3H 2.06 (s)	3H 2.11 (s)
31	3H 0.91 (d) $^3J_{H20-H31}$=7.0	3H 0.88 (d) $^3J_{H20-H31}$=7.0	3H 0.84 (d) $^3J_{H20-H31}$=6.9	3H 0.84 (bs)	3H 0.85 (d) $^3J_{H20-H31}$=7.1	3H 0.86 (d) $^3J_{H20-H31}$=6.9	3H 0.86 (d) $^3J_{H20-H31}$=6.8	3H 0.81 (m)	3H 0.84 (d) $^3J_{H20-H31}$=6.7	3H 0.93 (d) $^3J_{H20-H31}$=7.1
32	3H 1.03 (d) $^3J_{H22-H32}$=7.0	3H 1.01 (d) $^3J_{H22-H32}$=7.0	3H 0.96 (d) $^3J_{H22-H32}$=6.9	3H 1.00 (m)	3H 0.99 (d) $^3J_{H22-H32}$=6.8	3H 0.98 (d) $^3J_{H22-H32}$=7.1	3H 1.00 (d) $^3J_{H22-H32}$=6.9	3H 0.96 (m)	3H 0.99 (d) $^3J_{H22-H32}$=6.4	3H 1.03 (d) $^3J_{H22-H32}$=7.1
33	3H 0.67 (d) $^3J_{H24-H33}$=6.9	3H 0.59 (d) $^3J_{H24-H33}$=6.9	3H 0.50 (d) $^3J_{H24-H33}$=6.6	3H 0.55 (bs)	3H 0.52 (d) $^3J_{H24-H33}$=6.8	3H 0.49 (d) $^3J_{H24-H33}$=6.8	3H 0.56 (d) $^3J_{H24-H33}$=6.8	3H 0.50 (d) $^3J_{H24-H33}$=6.6	3H 0.50 (d) $^3J_{H24-H33}$=6.8	3H 0.70 (d) $^3J_{H24-H33}$=6.9
34	3H -0.30 (d) $^3J_{H26-H36}$=6.9	3H -0.31 (d) $^3J_{H26-H36}$=6.9	3H -0.29 (d) $^3J_{H26-H36}$=6.5	3H -0.30 (bs)	3H -0.27 (d) $^3J_{H26-H36}$=7.1	3H -0.25 (d) $^3J_{H26-H36}$=6.7	3H -0.23 (d) $^3J_{H26-H36}$=6.9	3H -0.30 (bs)	3H -0.33 (d) $^3J_{H26-H36}$=6.1	3H -0.33 (d) $^3J_{H26-H36}$=6.9
35	---	---	---	---	---	---	---	---	---	---
36	3H 2.06 (s)*	3H 2.06 (s)	3H 2.01 (s)	3H 2.00 (s)	3H 2.01 (s)*	3H 2.01 (s)*	3H 2.03 (s)	3H 2.01 (s)	3H 1.99 (s)	3H 2.07 (s)
37	3H 3.05 (s)	3H 3.04 (s)*	3H 2.98 (s)*	3H 3.01 (s)*	3H 2.99 (s)*	3H 2.99 (s)*	3H 3.03 (s)*	3H 2.97 (s)	3H 2.99 (s)*	3H 3.04 (s)
38	1H 10.65 (s)	1H 8.25 (s)	1H 4.18 (d) ^2J=11.6	1H 4.29 (bs)	1H 4.20 (d) ^2J=11.5	1H 4.27 (d) ^2J=11.9	1H 4.36 (d) ^2J=11.6	1H 4.27 (bs)	1H 4.25 (d) ^2J=10.8	1H 4.49 (d) ^2J=13.3
			1H 3.82 (d)	1H 3.93 (bs)	1H 3.86 (m)	1H 3.77 (m)	1H 3.89 (d)	1H 3.77 (m)	1H 3.91 (m)	1H 3.99 (d)
HO-1	1H 12.30 (s)	1H 13.19 (s)	1H 15.96 (bs)	1H 16.40 (bs)	1H 16.15 (bs)	1H 16.05 (bs)	1H 16.07 (bs)	1H 15.93 (bs)	1H 16.20 (bs)	1H 13.20 (s)
HO-4	1H 12.65 (s)	1H 11.98 (s)	1H 12.73 (s)	1H 12.68 (s)	1H 12.69 (s)	1H 12.85 (s)	1H 12.92 (s)	1H 12.70 (s)	1H 12.59 (s)	1H 12.02 (s)

HO-8	1H 13.16 (s)	1H 13.19 (s)	---	---	---	---	---	---	---	1H 12.02 (s)
HO-21	1H 3.55 (bs)	1H 3.48 (bs)	1H 3.09 (bs)	1H 3.10 (bs)	1H 3.20 (bs)	1H 3.15 (bs)	1H 3.35 (bs)	1H 3.15 (bs)	1H 3.05 (bs)	1H 3.58 (bs)
HO-23	1H 3.63 (bs)	1H 3.69 (bs)	1H 3.70 (bs)	1H 3.84 (bs)	1H 3.86 (bs)	1H 4.04 (bs)	1H 3.81 (bs)	1H 4.01 (bs)	1H 3.80 (bs)	1H 3.75 (bs)
HO-40	---	---	---	1.99 (bs)	---	---	---	---	---	---
HO-42	---	---	---	---	2.03 (bs)	---	---	---	---	---
HO-44	---	---	---	---	---	2.08 (bs)	---	---	---	---
NH (amid)	13.75 (s)	1H 13.30 (vbs)	1H 8.20 (s)	1H 8.38 (s)	1H 8.26 (s)	1H 8.79 (s)	1H 8.30 (s)	1H 8.76 (s)	1H 8.00 (s)	1H 8.29 (s)
N^+H (non-hydrogen bonded)	---	---	1H 3.99 (d) ^2J =16.8	1H 3.92 (bs)	1H 3.90 (bs)	1H 3.96 (bs)	1H 3.92 (bs)	1H 4.16 (bs)	1H 3.95 (m)	---
N^+H (hydrogen bonded)	---	---	1H 8.29 (vbs)	1H 8.21 (vbs)	1H 9.00 (vbs)	1H 8.71 (vbs)	1H 8.25 (vbs)	1H 8.93 (vbs)	1H 8.41 (vbs)	---
NH	---	---	---	---	---	---	---	---	---	1H 2.94 (bs)
39	---	1H 3.17 (m) 1H 2.53 (m)	2H 3.77 (m)	2H 3.43 (m)	2H 3.09 (m)	2H 3.13 (m)	2H 3.74 (m)	2H 3.10 (m)	2H 3.43 (m)	1H 3.16 (m) 1H 3.09 (m)
40	---	1H 3.09 (m) 1H 2.58 (m)	1H 5.98 (m)	2H 4.95 (m)	2H 1.30 (m)	2H 1.83 (m)	2H 3.89 (m)	2H 2.29 (m)	2H 3.41 (m) 3.33 (m)	1H 2.57 (m) 1H 2.53 (m)
41	---	1H 3.09 (m) 1H 2.58 (m)	1H 5.56 (d) $^3J_{H40\text{-}H41}$=16.8	---	2H 1.73 (m)	2H 1.50 (m)	2H 3.89 (m)	2H 3.71 (m)	---	1H 2.57 (m) 1H 2.53 (m)
42	---	1H 3.17 (m) 1H 2.53 (m)	1H 5.51 (d) $^3J_{H40\text{-}H41}$=10.2	---	2H 3.82 (t) $^3J_{H41\text{-}H42}$=5.0	2H 1.43 (m)	2H 3.63 (m)	1H 7.75 (s)	1H 7.22 (d) $^3J_{H42\text{-}H43}$=4.9	1H 3.16 (m) 1H 3.09 (m)
43	---	3H 2.34 (s)	---	---	---	2H 1.54 (m)	2H 3.63 (m)	1H 7.01 (m)	1H 6.98 (dd) $^3J_{H43\text{-}H44}$=3.2	3H 2.29 (s)

44	---	---	---	---	---	2H 3.60 (t) $^{3}J_{H41-H42}$=5.0	2H 1.52 (m)	1H 6.98 (m)	1H 7.00 (bs)	---
45	---	---	---	---	---	---	2H 1.31 (m)	---	---	---
46	---	---	---	---	---	---	3H 0.89 (t) $^{3}J_{H45-H46}$=7.4	---	---	---

Table 4S. Total assignment of ^{13}C NMR δ [ppm] chemical shifts for **1-9** and **Ral** (rifaldehyde) in $CDCl_3$.

Atom Number	**Ral**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	137.8	138.5	144.9	144.9	144.9	144.8	144.9	144.9	144.7	138.6
2	120.6	120.4	118.2	118.1	118.1	118.4	118.4	118.3	118.1	119.0
3	109.7	110.8	112.1	112.5	112.2	112.4	111.5	112.4	112.1	110.6
4	156.0	147.9	147.8	147.4	147.9	148.0	148.0	147.9	147.9	148.0
5	105.5	104.5	99.3	99.1	99.4	99.3	99.2	99.5	99.7	105.4
6	168.6	169.2	173.3	173.1	173.4	173.2	173.2	173.2	173.5	169.3
7	109.5	106.0	103.4	103.3	103.2	103.3	103.3	103.4	103.5	106.2
8	174.8	174.4	183.0	182.5	182.1	182.6	183.0	182.7	181.7	174.2
9	117.4	112.8	115.3	114.9	115.0	115.2	115.4	115.3	115.0	112.6
10	119.1	117.9	115.8	115.6	115.6	115.6	115.6	115.6	115.8	117.0
11	196.6	195.4	187.3	187.7	189.8	187.5	187.7	187.6	188.5	195.3
12	109.2	108.7	108.5	108.3	108.4	108.4	108.6	108.5	108.4	108.9
13	21.5	21.4	21.3	21.0	20.9	21.1	21.4	21.1	21.2	21.7
14	7.8	7.5	7.3	7.1	7.2	7.3	7.1	7.4	7.3	7.4
15	170.4	169.6	171.6	171.3	172.0	171.5	171.6	171.7	171.8	171.5
16	127.8	129.3	130.9	130.5	131.1	130.7	131.1	130.9	131.0	130.6
17	136.9	135.1	133.6	134.0	133.3	133.6	133.6	133.6	133.5	133.6
18	122.6	123.2	124.6	124.5	124.2	124.8	124.4	124.7	124.3	123.5
19	143.7	142.7	141.5	141.6	141.9	141.3	141.6	141.3	141.7	141.5
20	38.6	38.5	37.8	37.7	37.8	37.6	37.8	37.8	38.0	38.2
21	70.7	70.6	73.3	73.2	71.9	73.3	71.4	73.3	73.0	70.9
22	33.3	33.3	33.3	33.2	33.2	33.1	33.3	33.1	33.3	33.3
23	77.0	76.8	76.9	77.0	76.8	76.9	76.8	76.8	76.8	76.8
24	37.6	37.5	37.5	37.3	37.5	37.4	37.4	37.5	37.6	37.5
25	74.2	74.4	74.0	74.0	73.8	74.1	74.1	73.9	73.8	74.4
26	39.6	39.5	39.0	38.7	38.4	39.0	38.8	38.9	38.7	39.6
27	76.5	76.7	78.1	78.2	78.8	78.1	78.9	79.0	78.8	76.7
28	119.4	118.6	116.8	116.2	116.3	116.7	116.2	116.6	116.8	118.1
29	142.8	142.6	142.6	142.5	142.9	142.4	134.1	142.1	142.8	143.0
30	20.5	20.5	20.3	20.5	20.4	20.5	20.3	20.4	20.4	20.6
31	16.9	17.8	17.8	17.7	17.8	17.8	17.9	17.7	17.7	17.3
32	10.9	10.8	11.0	11.1	11.1	11.1	11.0	11.1	11.1	10.9
33	8.6	8.4	8.6	8.7	8.7	8.7	8.7	8.8	8.8	9.0
34	9.1	8.9	9.1	9.1	9.5	9.0	9.3	9.1	9.4	9.1
35	172.1	172.0	171.9	172.0	172.6	172.1	172.3	172.9	171.8	172.2
36	20.7	20.5	20.8	20.8	20.7	20.8	20.8	20.8	20.9	20.7
37	57.1	57.0	56.7	56.8	56.8	56.8	56.7	56.8	56.7	57.0
38	194.1	134.4	44.5	45.5	44.8	45.2	45.3	44.5	45.5	43.4
39	---	50.2	50.4	49.1	50.2	48.1	47.6	46.1	49.3	50.2
40	---	53.9	127.3	25.2	74.3	25.5	65.7	29.7	27.1	53.8

41	---	53.9	124.6	29.8	---	25.9	70.2	46.7	137.3	53.8
42	---	50.2	---	61.7	---	24.8	70.2	137.0	125.1	50.2
43	---	45.9	---	---	---	31.7	70.7	128.1	127.6	45.5
44	---	---	---	---	---	62.0	31.5	120.7	126.8	---
45	---	---	---	---	---	---	19.2	---	---	---
46	---	---	---	---	---	---	13.8	---	---	

Table 5S. Antibacterial activity MIC (μg/mL) of **1-9** against Gram-(+) strains including methicillin-resistant /MRSA/ and methicillin-susceptible /MSSA/ ones.

Bacteria strain	1	2	3	4	5	6	7	8	9
S. aureus NCTC 4163	0.008	0.5	1	1	0.5	0.5	2	0.5	0.008
S. aureus ATCC 25923	0.016	1	2	2	2	1	2	1	0.016
S. aureus ATCC 6538	0.008	0.5	1	1	1	1	2	0.5	0.008
S. aureus ATCC 29213	0.008	0.5	1	1	2	1	2	0.5	0.008
S. epidermidis ATCC 12228	0.008	0.125	0.25	0.25	0.25	0.25	1	0.25	0.008
E. coli ATCC 10538	8	128	256	256	256	256	>256	>256	16
E. coli ATCC 25922	8	64	256	128	128	128	>256	>256	16
E. coli NCTC 8196	4	64	256	256	256	256	>256	>256	8
P. aeruginosa ATCC 15442	16	128	>256	256	>256	>256	>256	>256	32
P. aeruginosa NCTC 6749	32	64	256	256	>256	>256	>256	>256	32
P. aeruginosa ATCC 27853	16	64	256	256	256	>256	>256	>256	32
MSSA 440/11	0.016	1	2	1	1	1	0.25	0.5	0.03
MSSA 441/11	0.016	1	2	1	2	1	0.5	1	0.03
MSSA 442/11	0.016	0.5	2	1	1	0.5	0.25	1	0.03
MSSA 443/11	0.016	0.5	2	2	2	1	0.5	1	0.03
MSSA 444/11	0.016	1	4	2	4	1	0.5	1	0.03
MSSA 445/11	0.016	1	2	1	2	1	0.5	1	0.03
MSSA 446/11	0.016	1	4	2	2	1	0.5	1	0.03
MSSA 447/11	0.016	0.5	4	1	1	1	0.5	1	0.03
MSSA 448/11	0.008	0.5	2	1	2	1	0.5	0.5	0.016
MSSA 449/11	0.008	0.5	2	1	1	0.5	0.25	0.5	0.016
MRSA 389/10	0.008	0.5	0.5	0.5	1	0.5	1	0.25	0.008
MRSA 390/10	0.008	0.5	1	1	2	1	2	0.5	0.008
MRSA 391/10	0.008	0.5	1	1	2	1	2	0.5	0.008
MRSA 392/10	0.008	1	1	1	2	1	2	0.5	0.016
MRSA 393/10	0.008	0.5	0.5	0.5	2	1	2	1	0.008
MRSA 394/10	0.008	0.5	0.5	1	2	1	2	1	0.008
MRSA 399/10	0.008	0.5	0.5	1	1	1	2	0.5	0.008
MRSA 450/11	0.008	0.5	2	1	2	1	2	1	0.008
MRSA 451/11	0.008	0.5	1	0.5	2	1	2	1	0.008
MRSA 452/11	0.008	0.5	2	0.5	2	1	2	1	0.008

Figure 1S. ^{1}H-^{15}N HMBC spectrum of **1** in DMSO-d_6 after addition of water drop.

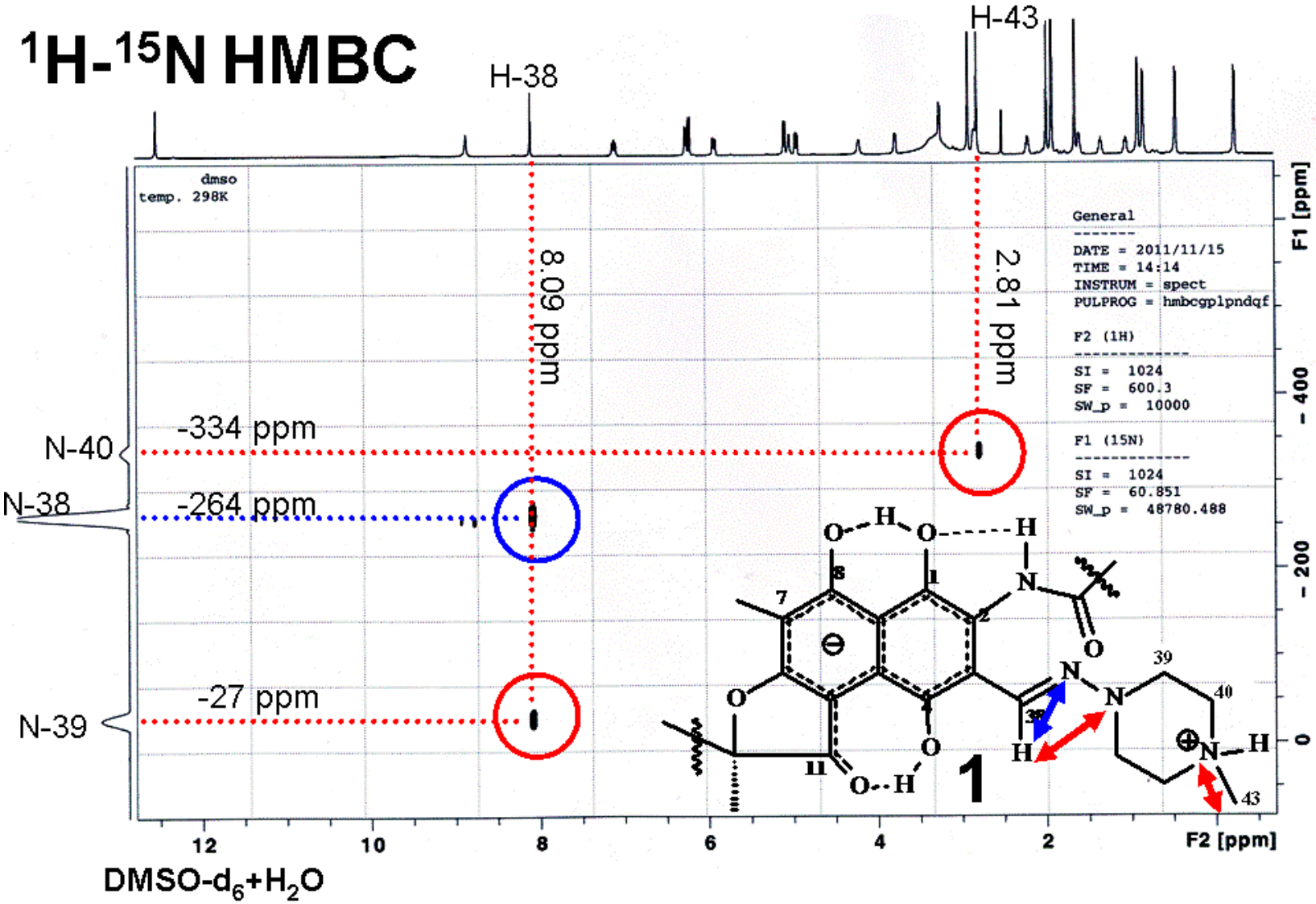

Figure 2S. ^{1}H-^{15}N HSQC spectrum of **1** in DMSO-d_6 after addition of water drop.

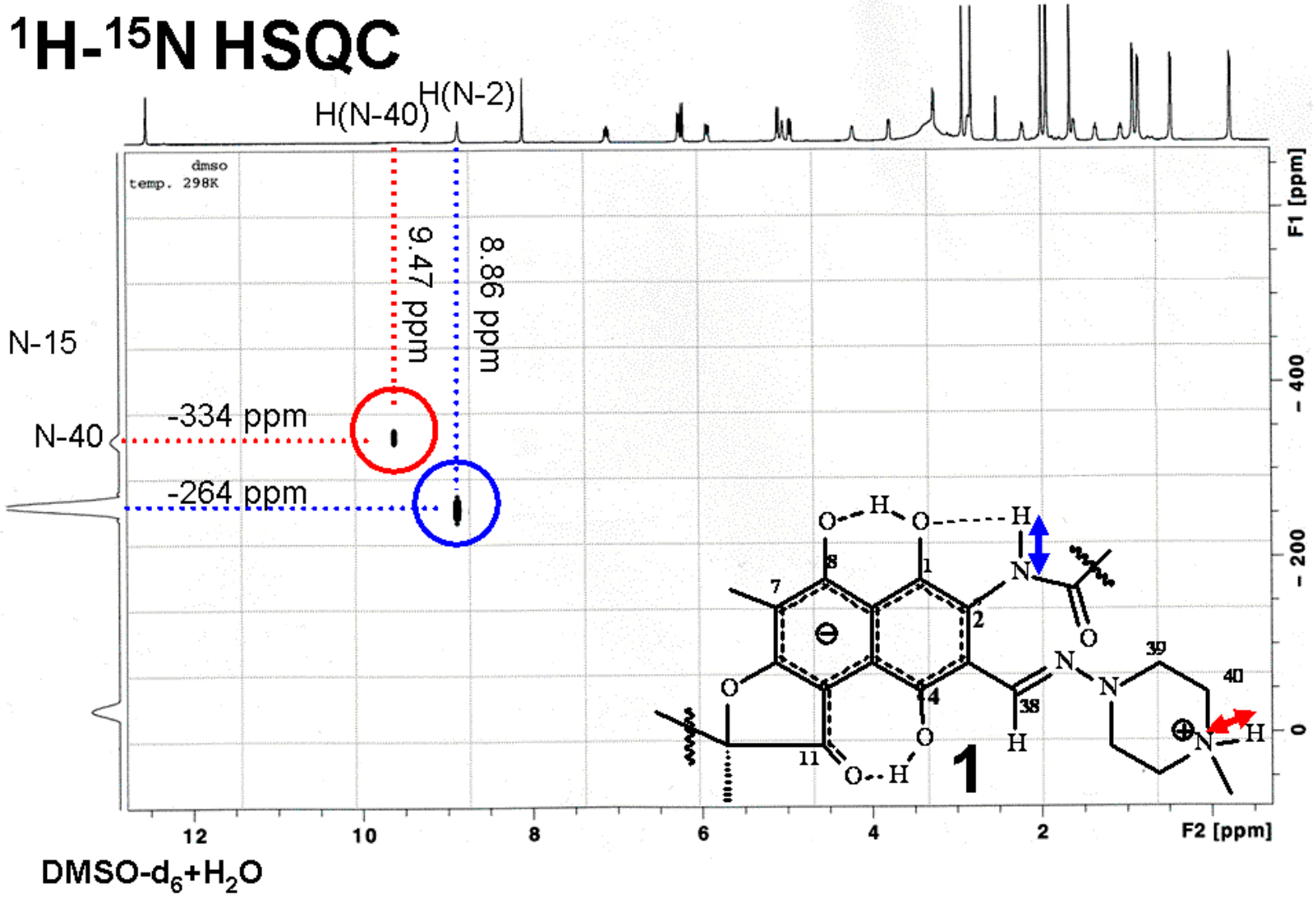

Figure 3S. ^{1}H-^{15}N HMBC spectrum of **2** in DMSO-d_6 after addition of water drop.

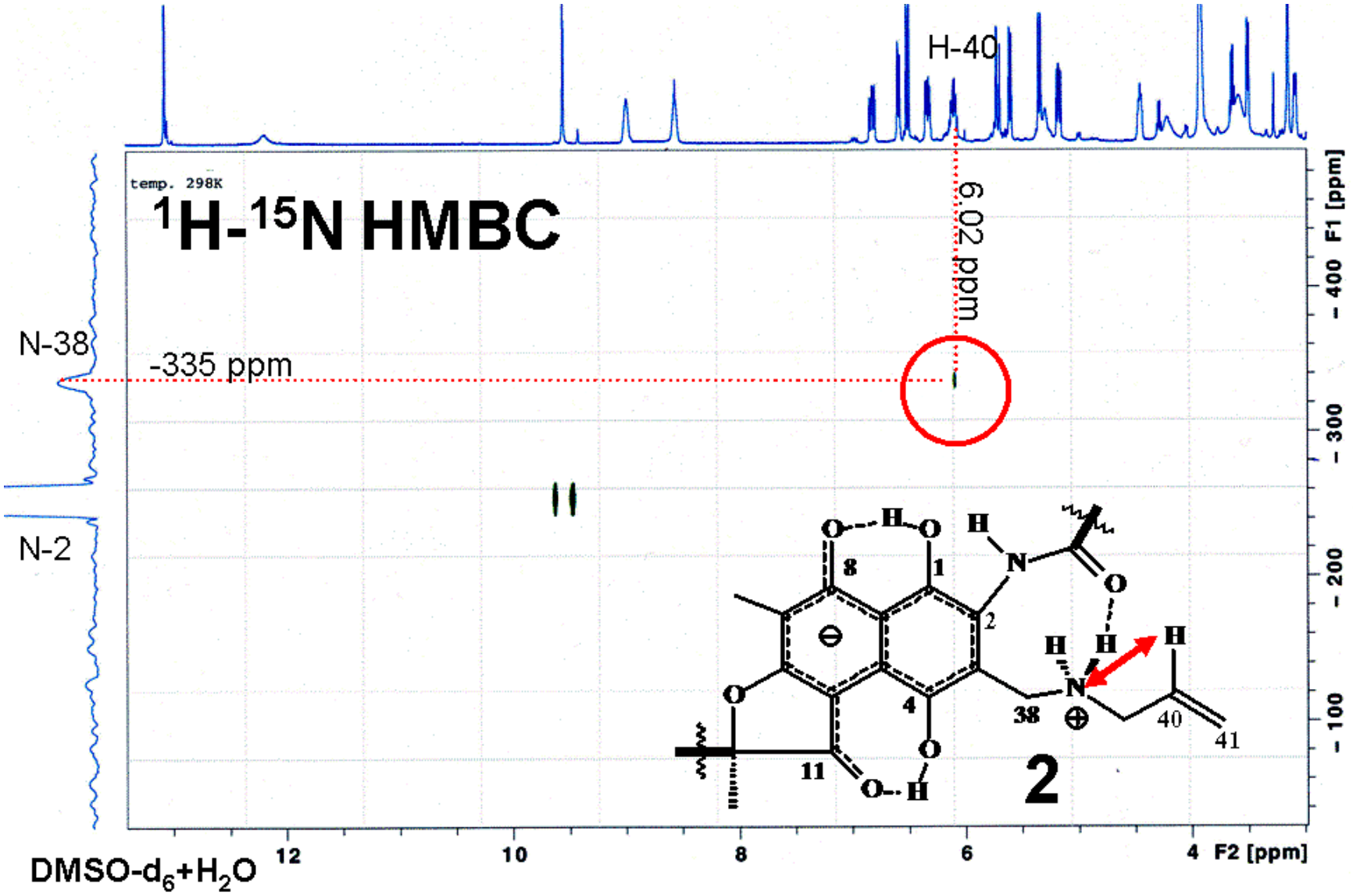

Figure 4S. ^{1}H-^{15}N HSQC spectrum of **2** in DMSO-d_6 after addition of water drop.

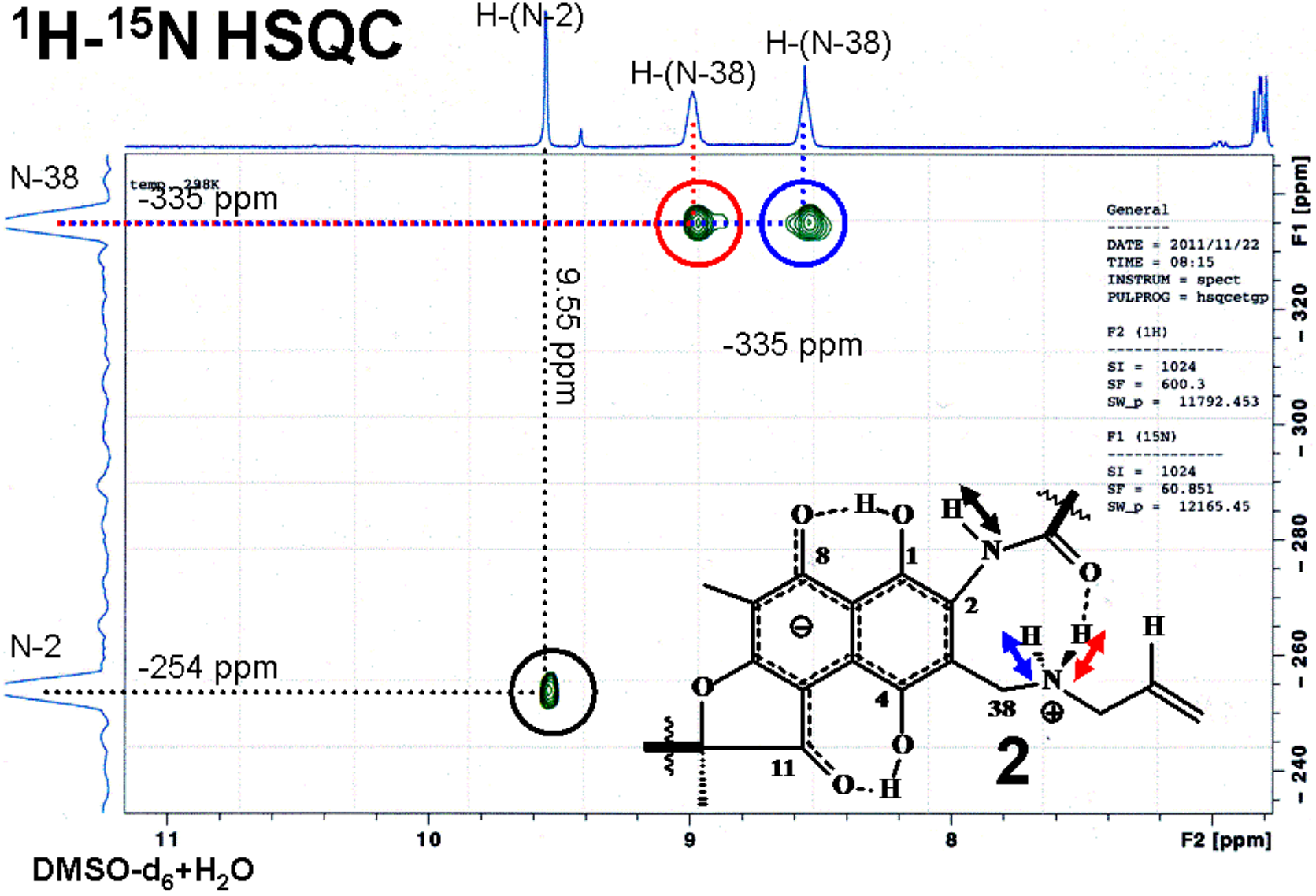

Figure 5S. ^{1}H-^{13}C HMBC spectrum of **1** in DMSO-d_6 after addition of water drop.

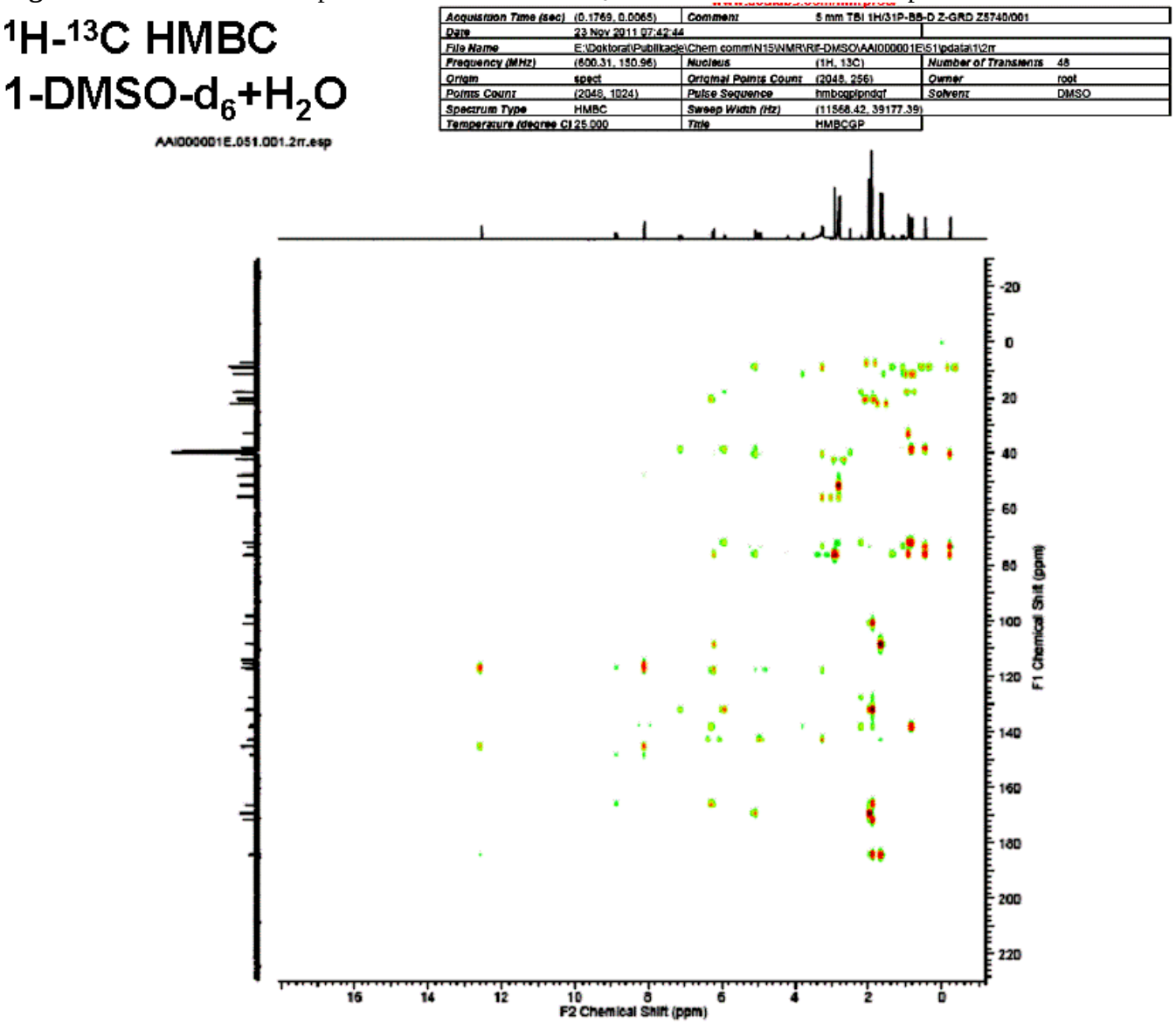

Figure 6S. ^{1}H-^{13}C HMBC spectrum of **1** in DMSO-d_6 after addition of water drop in the range ^{1}H(1.4-2.2)-^{13}C (164-188) ppm.

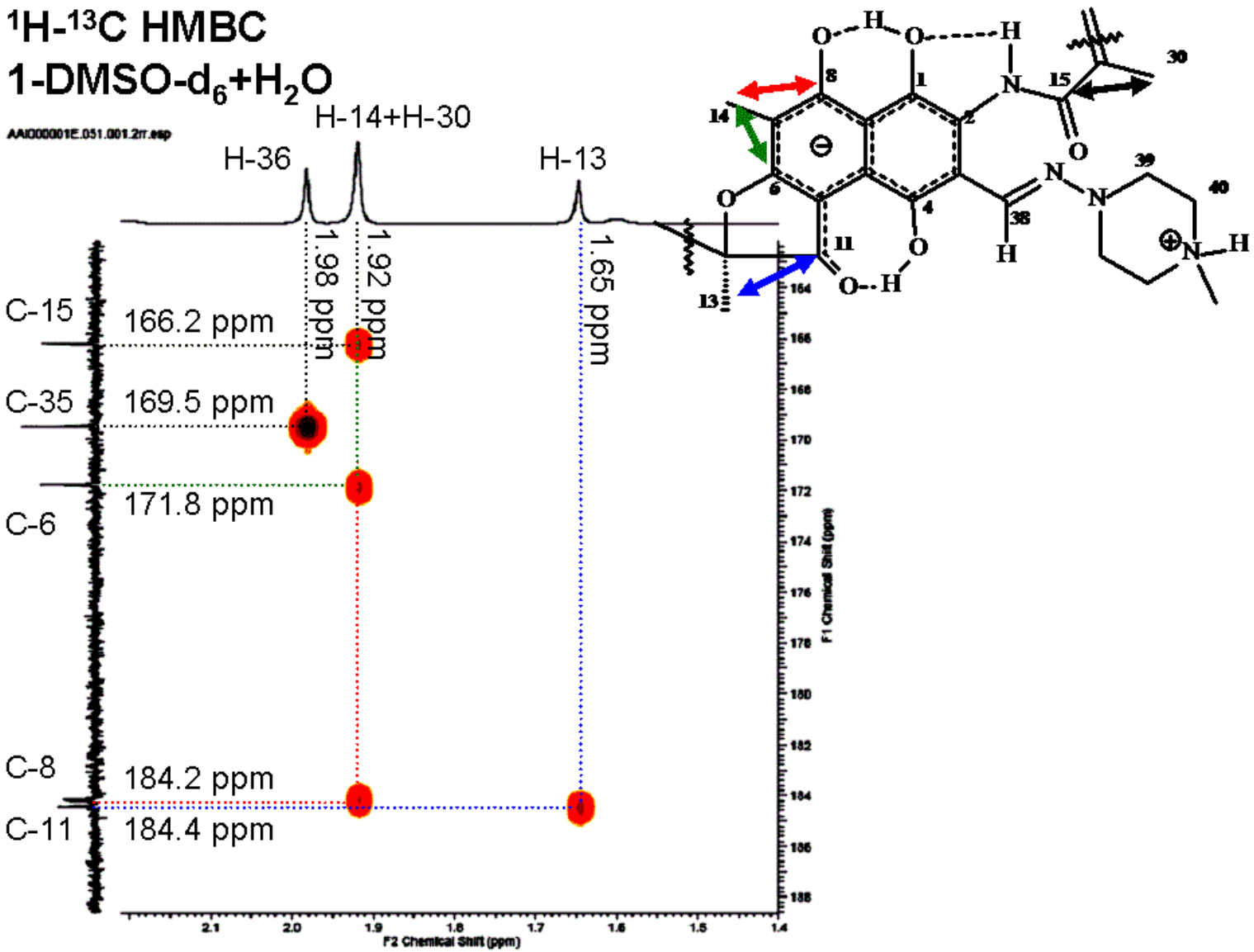

Figure 7S. ^{1}H-^{13}C HMBC spectrum of **1** in $CDCl_3$.

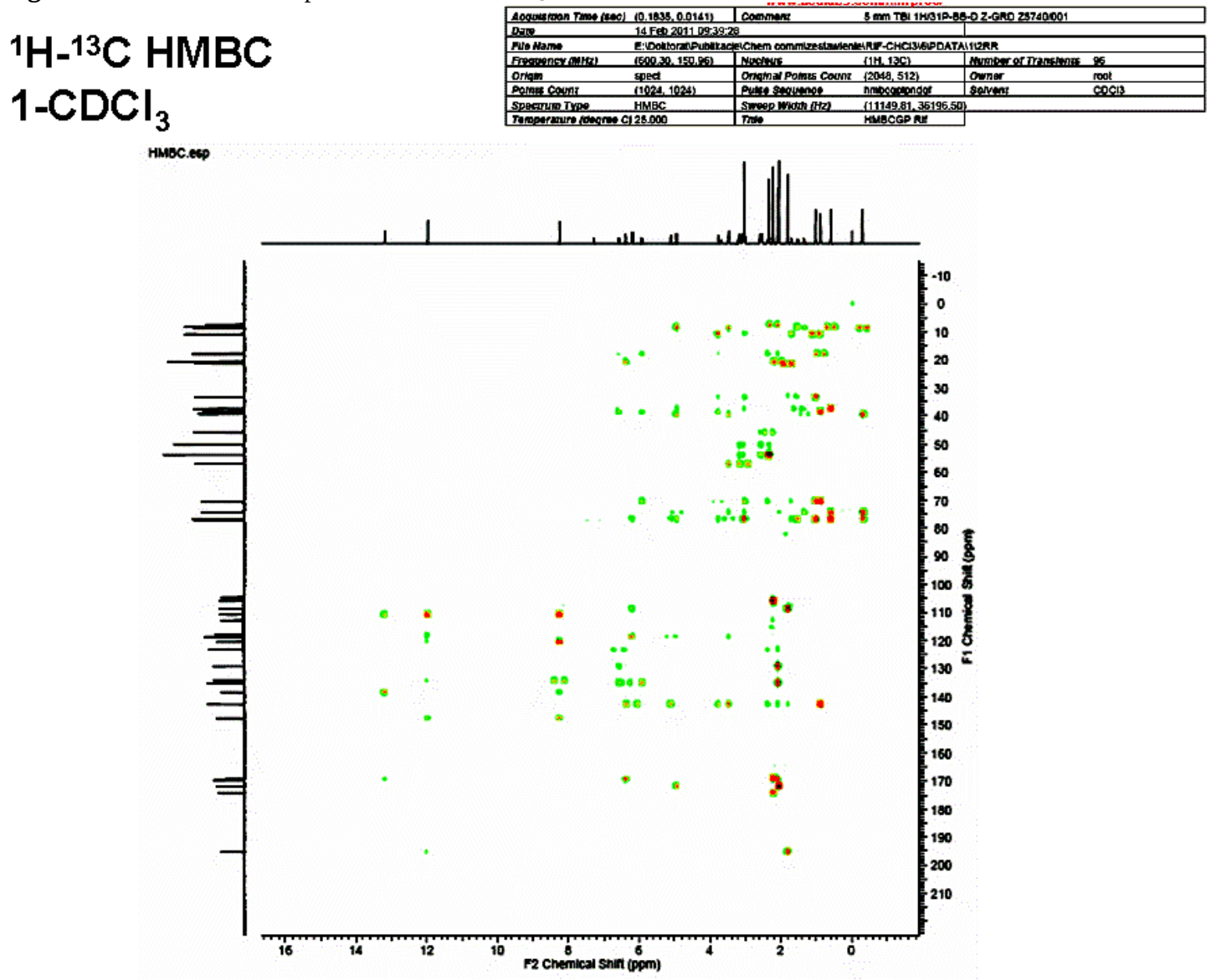

Figure 8S. ^{1}H-^{13}C HMBC spectrum of **1** in $CDCl_3$ in the range ^{1}H(11.5-14.5)-^{13}C (103-195) ppm.

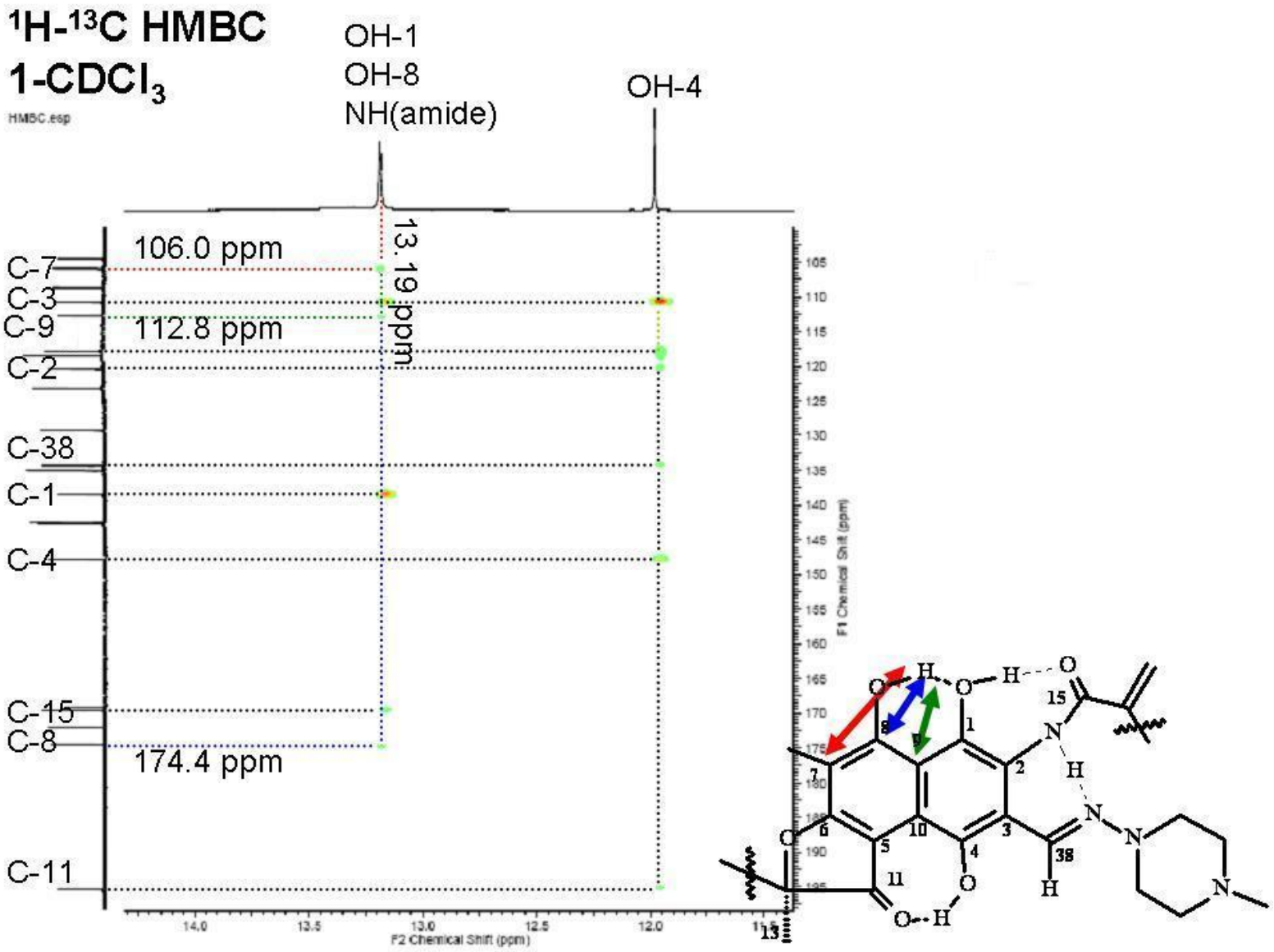

Figure 9S. ^{1}H-^{13}C HMBC spectrum of **1** in $CDCl_3$ in the range ^{1}H(1.5-2.4)-^{13}C (166-198) ppm.

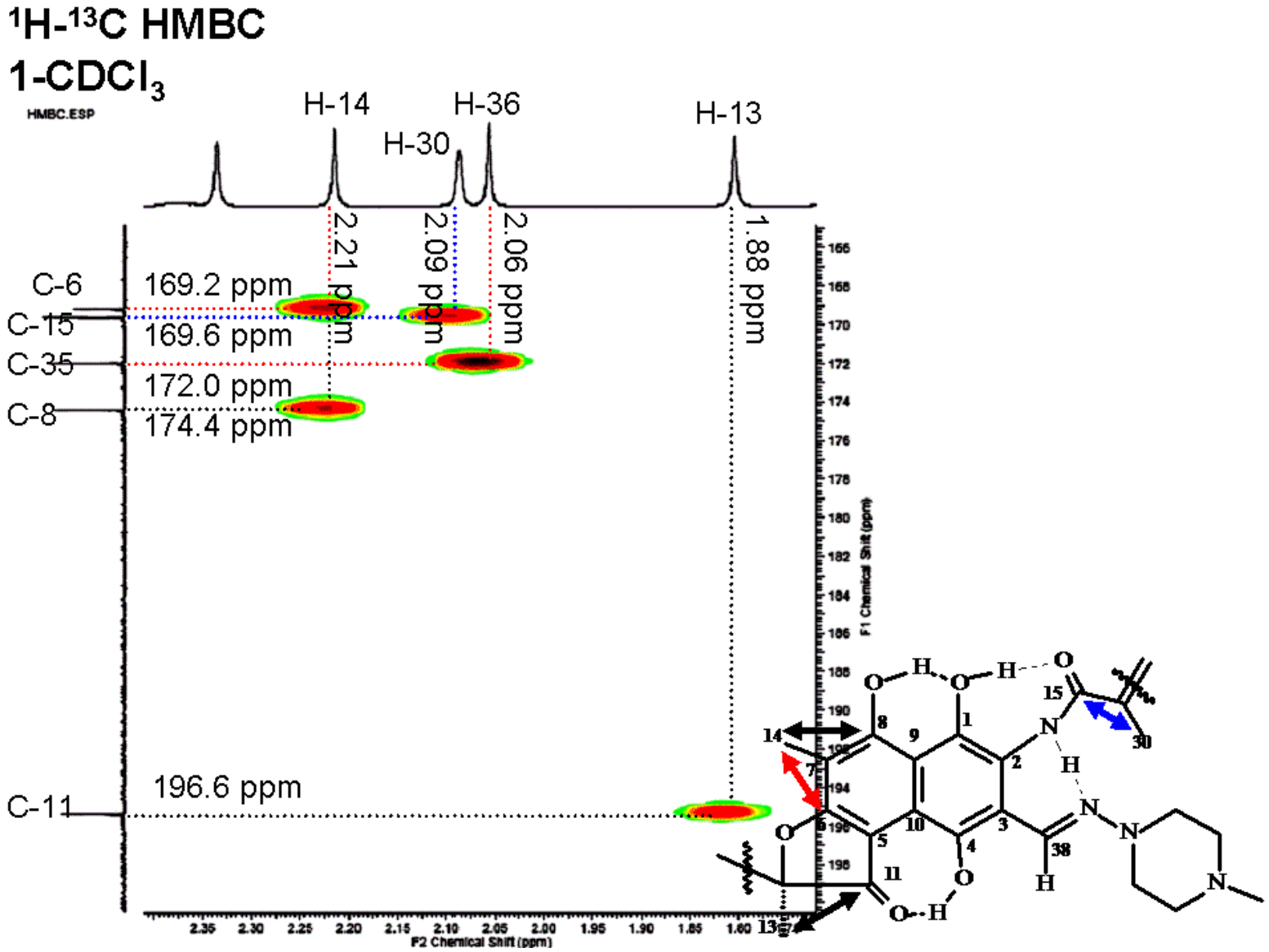

Figure 10S. ^{1}H-^{13}C HMBC spectrum of **5** in $CDCl_3$.

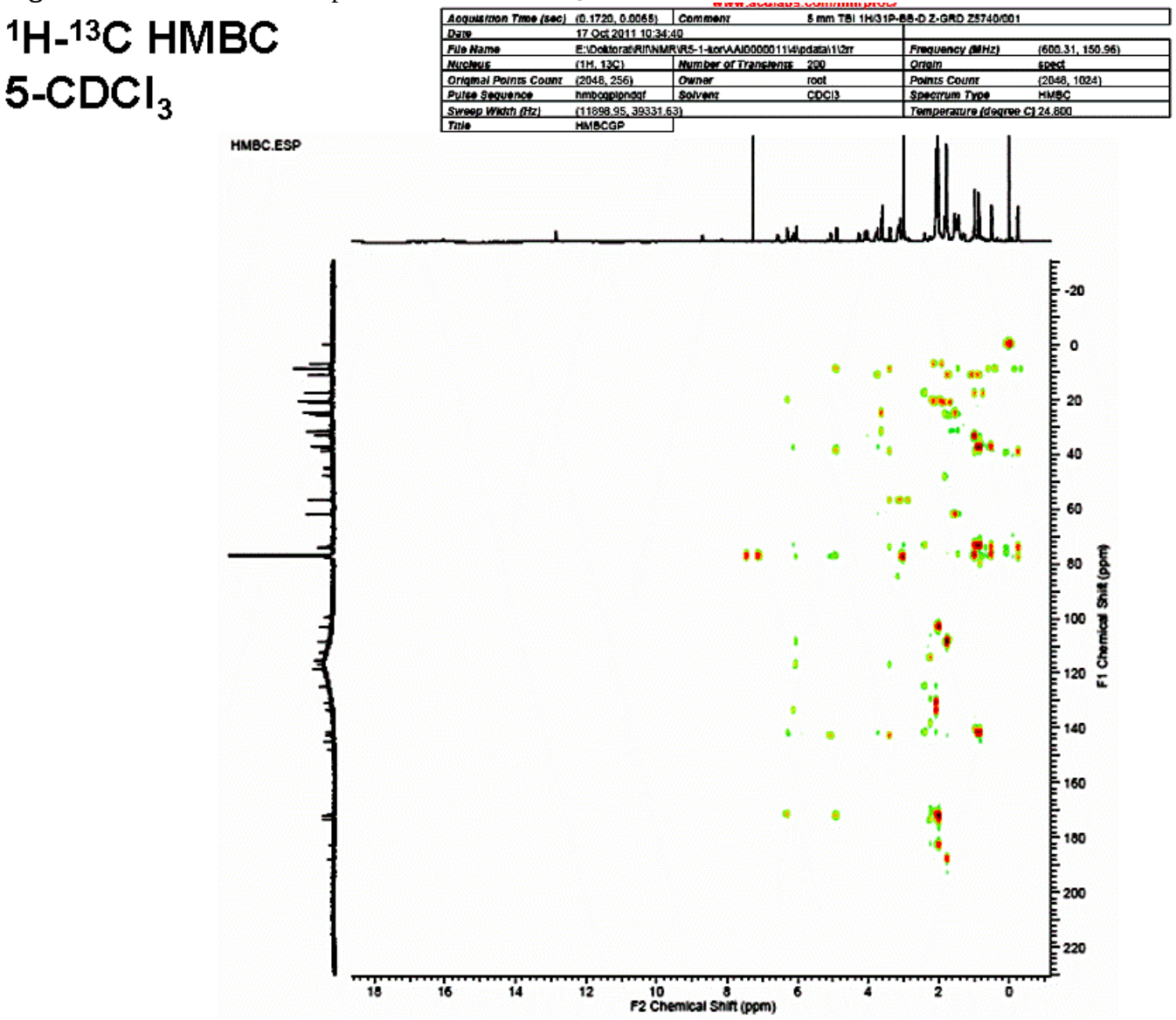

Figure 11S. ^{1}H-^{13}C HMBC spectrum of **5** in $CDCl_3$ in the range ^{1}H(1.75-2.2)-^{13}C (168-190) ppm.