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*Note*

# $^1$H NMR spectroscopic studies of the moenomycins

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ABSTRACT: Highly resolved $^1$H NMR spectra of the moenomycin antibiotics can be obtained in $D_2O$ solution when the concentration is below the critical micelle concentration. The 600 MHz spectra are structurally highly informative. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; $^1$H NMR; moenomycin A, $A_{12}$, $C_1$, $C_3$ and $C_4$; aggregates; critical micelle concentration

## INTRODUCTION

The moenomycins (Fig. 1) are a group of antibiotics with a unique mechanism of action. They have been shown to interfere with the enzyme(s) that catalyse the transglycosylation step of bacterial peptidoglycan biosynthesis. Most probably, their activity is related to their close structural similarities with the substrate(s) of the enzyme, and it is assumed (although not proven) that they are competitive inhibitors.[1] These antibiotics define a new target for antibacterials, an area of great interest in the light of the growing resistance against classical antibiotics.[2]

Until recently, structural work on moenomycin A and related antibiotics was based mainly on mass and $^{13}$C NMR spectroscopy,[3] whereas it was impossible to obtain good quality $^1$H NMR spectra. In view of ongoing work on the mode of action of these antibiotics, this was a real drawback since it would be highly desirable to obtain information on the solution conformation. Slowly the physicochemical properties of the moenomycins became better understood[4] and it appears that the $^1$H NMR problems, which are mainly associated with the amphiphilic nature of the moenomycins, can be solved. Recently, for the first time, we were able to obtain well resolved $^1$H NMR spectra of a

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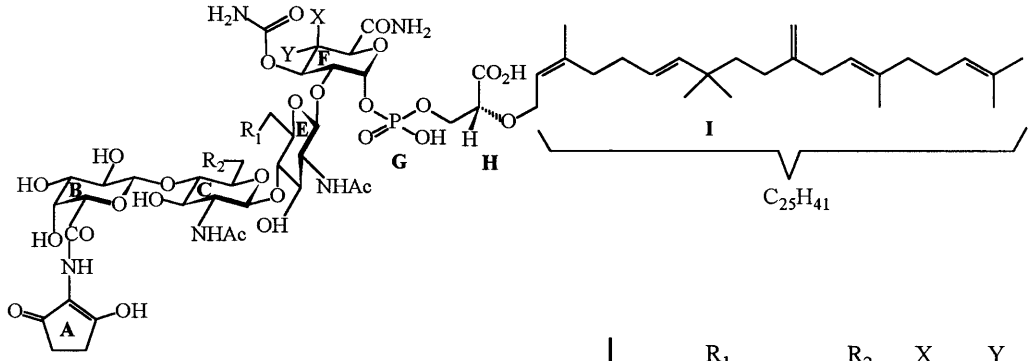


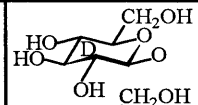
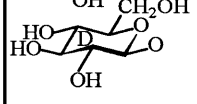
| | $R_1$ | $R_2$ | X | Y |
|---|---|---|---|---|
| **Moenomycin A** |  | H | $CH_3$ | OH |
| **Moenomycin A 12** |  | H | OH | H |
| **Moenomycin C 1** | H | H | OH | H |
| **Moenomycin C 3** | H | H | $CH_3$ | OH |
| **Moenomycin C 4** | OH | H | $CH_3$ | OH |

**Figure 1.** Structures of the moenomycins.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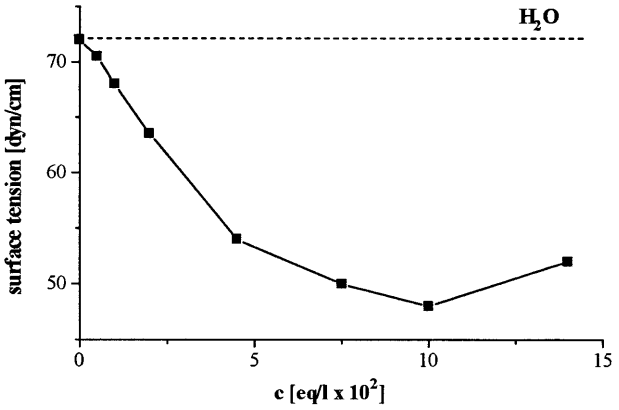


**Figure 2.** Surface tension of moenomycin (adapted from Ref. 6).

microemulsion consisting of moenomycin A, $D_2O$ and $C_6D_6$. Also, when moenomycin A was incorporated into SDS-$d_{25}$ micelles, good $^1H$ NMR spectra could be measured. These observations allowed us to assign the resonances of nearly all non-exchangeable protons of moenomycin A (with the exception of 3-$H^E$, 4-$H^E$ and 5-$H^E$) by means of TOCSY, HMQC, HMBC and COSY 45.[5] However, the microemulsion method lacks generality and the SDS-$d_{25}$ method is too expensive for everyday work.

Many years ago, the surface tension of aqueous solutions as a function of added moenomycin was determined, approaching a value of 48 dyn $cm^{-1}$ at 0.1% of moenomycin in water (Fig. 2).[6] Although the conditions of this experiment are not very well defined, one may calculate the critical micelle concentration (cmc) of moenomycin to be in the region of $5 \times 10^{-4}$ mol $l^{-1}$. Work has been performed to describe the aggregation behaviour of moenomycin in water in more detail.[7] We reasoned that at concentrations below the cmc it should be possible to obtain $^1H$ NMR spectra of moenomycins of good quality. These expectations were confirmed by experiment, and the results obtained in these investigations are the subject of the present paper.

## RESULTS AND DISCUSSION

Figure 3 shows the 200 MHz $^1H$ NMR spectra of moenomycin A at three different concentrations in $D_2O$, $1 \times 10^{-3}$, $7.5 \times 10^{-4}$ and $5 \times 10^{-4}$ mol $l^{-1}$, i.e. in the concentration range of the cmc as discussed above. Whereas from the $1 \times 10^{-3}$ mol $l^{-1}$ solution only the usual broad and structurally non-informative spectrum was obtained, at $7.5 \times 10^{-4}$ mol $l^{-1}$ and even more at $5 \times 10^{-4}$ mol $l^{-1}$ spectra of dramatically improved quality could be measured. Although the samples were lyophilized several times from $D_2O$ solutions prior to the NMR measurements, water suppression at these very low concentrations was important and was performed by decoupler presaturation.

Figure 4 shows a 600 MHz spectrum of moenomycin A ($5 \times 10^{-4}$ mol $l^{-1}$ in $D_2O$) under optimized conditions, demonstrating the great versatility of this approach. Similar results were obtained for all other moenomycins. Based on the previous work, all resonances of moenomycin A and the other moenomycins could be assigned and the results are given in Table 1. Since details of the assignment procedure for moenomycin A have already been described,[5] in the current investigation the spectra of moenomycin $A_{12}$, $C_1$, $C_3$ and $C_4$ were assigned solely from 1D data, making use of the earlier assignments and restricting the discussion to the differences between the spectra of the various moenomycins.

### Moenomycin $A_{12}$

Moenomycin $A_{12}$ differs structurally from moenomycin A in ring F. The 4-C-methyl group is absent and ring F has a D-*galacto* rather than a D-*gluco* configuration. In the $^1H$ NMR spectrum, the methyl signal at $\delta = 1.01$ is absent and the signals of 3-$H^F$ and 5-$H^F$ are clearly different from those found in moenomycin A owing to the coupling with 4-$H^F$: Whereas 3-$H^F$ in moenomycin A gives a doublet, the corresponding signal in moenomycin $A_{12}$ is a doublet of doublets. The 5-$H^F$ signal of moenomycin $A_{12}$ is a doublet rather than a singlet as in moenomycin A. The coupling constants $J_{3,4} = 3.1$ Hz and $J_{4,5} = 2.6$ Hz prove the D-*galacto* configuration of unit F.

### Moenomycin $C_1$

Moenomycin $C_1$ belongs to the moenomycin $A_{12}$ series (D-galacturonamide-derived unit F). Unit D is absent and unit E is derived from D-quinovosamine rather than from D-glucosamine. This leads to a methyl doublet at $\delta = 1.08$, and the 5-$H^E$ and 4-$H^E$ signals ($\delta = 3.32$ and 3.12, respectively, assigned by H,H COSY at 400 MHz) appear at higher field than in moenomycin $A_{12}$. The spectral data demonstrate for unit F the same substitution pattern and configuration as in moenomycin $A_{12}$.

### Moenomycin $C_3$

Between moenomycin $C_3$ and moenomycin A there exists the same structural relationship as between moenomycin $C_1$ and moenomycin $A_{12}$, i.e. unit D is missing and unit E is derived from D-quinovosamine. This leads to the spectral features for unit E as discussed for moenomycin $C_1$. The signal of 5-$H^F$ is, as expected, a singlet at $\delta = 4.24$.

### Moenomycin $C_4$

In this antibiotic, unit E is derived from D-glucosamine. Moenomycin $C_4$ is, therefore, the de-glucose derivative

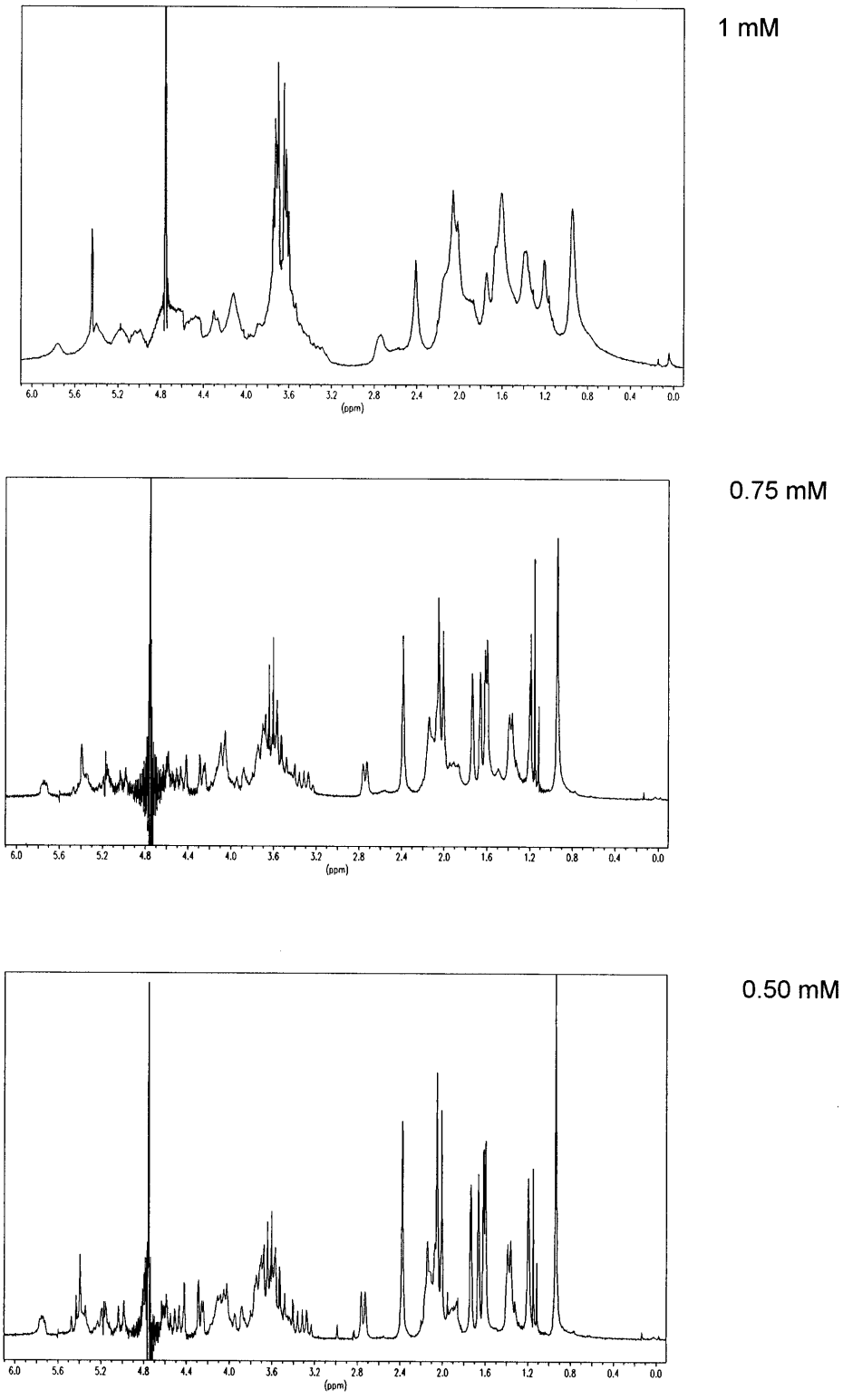


**Figure 3.** 200 MHz $^1$H NMR spectra of moenomycin A at concentrations of $1 \times 10^{-3}$, $7.5 \times 10^{-4}$ and $5 \times 10^{-4}$ mol $l^{-1}$ in $D_2O$.

of moenomycine A. The spectrum displays the expected features as discussed above (see Table 1).

## CONCLUSION

A method is now at hand which enables one to obtain high-quality $^1$H NMR spectra of the moenomycins. Work is in progress to make use of these results in approaching the problem of the solution conformation of these antibiotics.

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a Gemini 200 (Varian, 199.98 MHz, processed by Bruker WINNMR

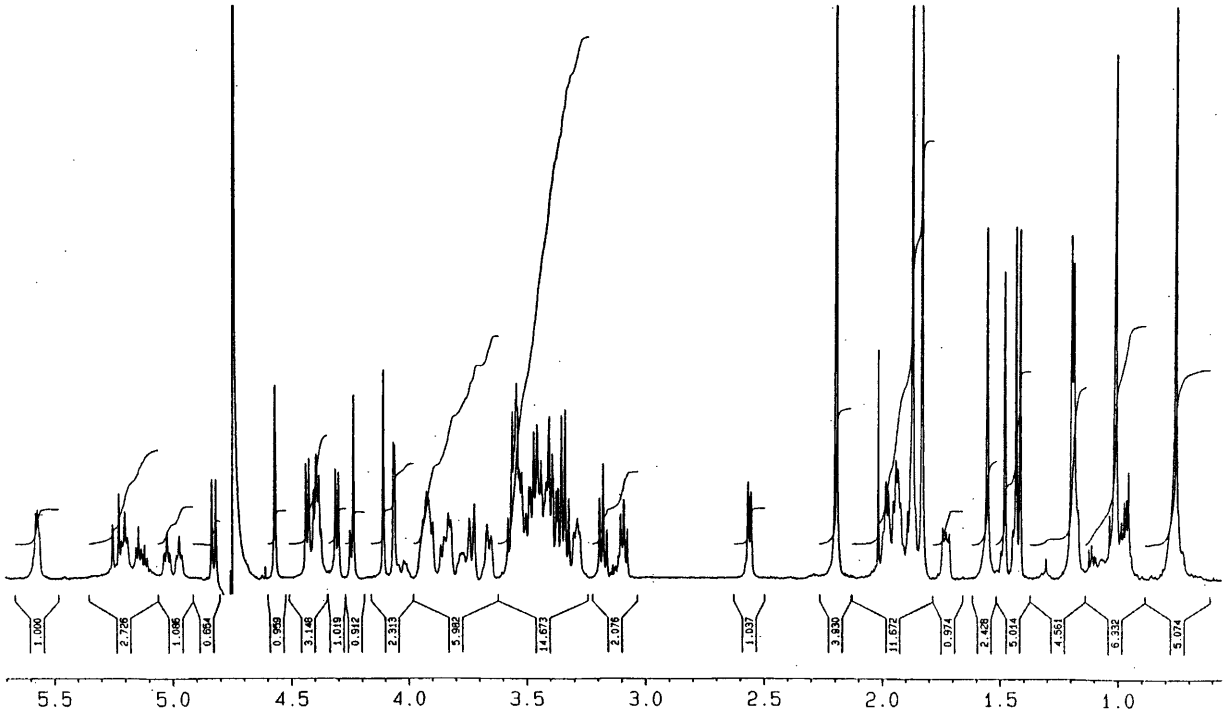


**Figure 4.** 600 MHz $^1$H NMR spectrum of moenomycin A ($5 \times 10^{-4}$ mol $l^{-1}$ in $D_2O$ at 285 K).

**Table 1.** $^1$H NMR spectra of moenomycin A, $A_{12}$, $C_1$, $C_3$ and $C_4$

| | Moenomycin | | | | |
|---|---|---|---|---|---|
| Assignment | A | $A_{12}$ | $C_1$ | $C_3$ | $C_4$ |
| $CH_3$-23$^I$,24$^I$ | 0.76 s | 0.76 s | 0.76 s | 0.76 s | 0.76 s |
| 4$^F$-$CH_3$ | 1.01 s | — | — | 1.01 s | 1.01 s |
| $CH_3$-6$^E$ | — | — | 1.08 d ($J_{5,6}$ = 6.1 Hz) | 1.07 d ($J_{5,6}$ = 6.1 Hz) | — |
| $CH_2$-9$^I$ | 1.18 m | 1.18 m | 1.18 m | 1.18 m | 1.18 m |
| $CH_3$-6$^C$ | 1.19 d ($J_{5,6}$ = 6.0 Hz) | 1.19 d ($J_{5,6}$ = 5.8 Hz) | 1.19 d ($J_{5,6}$ = 5.8 Hz) | 1.19 d ($J_{5,6}$ = 5.9 Hz) | 1.19 d ($J_{5,6}$ = 6.1 Hz) |
| $CH_3$-20$^I$ | 1.42 s | 1.42 s | 1.42 s | 1.42 s | 1.42 s |
| $CH_3$-21$^I$ | 1.43 s | 1.43 s | 1.43 s | 1.43 s | 1.43 s |
| $CH_3$-19$^I$ | 1.48 s | 1.48 s | 1.48 s | 1.48 s | 1.48 s |
| $CH_3$-25$^I$ | 1.56 s | 1.55 s | 1.55 s | 1.55 s | 1.55 s |
| $CH_2$-10$^I$ | 1.73 m | 1.73 m | 1.73 m | 1.73 m | 1.73 m |
| $CH_3CONH^C$ | 1.83 s | 1.81 s | 1.82 s | 1.83 s | 1.83 s |
| $CH_3CONH^E$ | 1.87 s | 1.87 s | 1.83 s | 1.83 s | 1.85 s |
| $CH_2$-15$^I$ | 1.87 m | 1.87 m | 1.87 m | 1.87 m | 1.87 m |
| $CH_2$-5$^I$, $CH_2$-16$^I$ | 1.94 m | 1.95 m | 1.95 m | 1.94 m | 1.94 m |
| $CH_2$-4$^I$ | 1.98 m | 1.98 m | 1.98 m | 1.99 m | 1.99 m |
| $CH_2$-4$^A$, $CH_2$-5$^A$ | 2.19 s | 2.19 s | 2.19 s | 2.20 s | 2.19 s |
| $CH_2$-12$^I$ | 2.56 d ($J_{12,13}$ = 7.4 Hz) | 2.56 d ($J_{12,13}$ = 7.4 Hz) | 2.56 d ($J_{12,13}$ = 7.4 Hz) | 2.56 d ($J_{12,13}$ = 7.4 Hz) | 2.56 d ($J_{12,13}$ = 7.4 Hz) |
| 2-H$^D$ | 3.09 dd ($J_{2,3}$ = 8.1 Hz) | 3.10 dd ($J_{2,3}$ = 8.2 Hz) | — | — | — |
| 4-H$^D$ | 3.18 dd ($J_{4,5}$ = 9.5 Hz) | 3.20 dd ($J_{4,5}$ = 9.4 Hz) | — | — | — |
| 5-H$^D$ | 3.29 m | 3.29 m | — | — | — |
| 3-H$^D$ | 3.33 dd ($J_{3,4}$ = 9.4 Hz) | 3.34 dd ($J_{3,4}$ = 9.2 Hz) | — | — | — |
| 4-H$^C$ | 3.35–3.43 | 3.35–3.42 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |

**Table 1**—*Continued*

| Assignment | Moenomycin A | Moenomycin $A_{12}$ | Moenomycin $C_1$ | Moenomycin $C_3$ | Moenomycin $C_4$ |
|---|---|---|---|---|---|
| 3-H$^C$ | 3.35–3.43 | 3.35–3.42 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |
| 2-H$^B$ | 3.35–3.43 | 3.35–3.42 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |
| 5-H$^C$ | 3.43–3.50 | 3.43–3.51 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |
| 2-H$^E$ | 3.43–3.50 | 3.43–3.51 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |
| 4-H$^E$ | 3.43–3.50 | 3.43–3.51 | 3.12 dd ($J_{4,5}$ = 9.0 Hz) ($J_{3,4}$ = 8.4 Hz) | 3.12 dd ($J_{4,5}$ = 8.9 Hz) ($J_{3,4}$ = 8.6 Hz) | 3.34–3.49 |
| 3-H$^E$ | 3.43–3.50 | 3.43–3.51 | 3.35–3.49 | 3.34–3.49 | 3.34–3.49 |
| 5-H$^E$ | 3.43–3.50 | 3.43–3.51 | 3.32 dd ($J_{4,5}$ = 9.2 Hz) ($J_{5,6}$ = 6.1 Hz) | 3.27 dd ($J_{4,5}$ = 9.2 Hz) ($J_{5,6}$ = 6.1 Hz) | 3.22 m |
| 2-H$^C$ | 3.50–3.59 | 3.51–3.59 | 3.52–3.61 | 3.53–3.63 | 3.53–3.63 |
| 3-H$^B$ | 3.50–3.59 | 3.51–3.59 | 3.52–3.61 | 3.53–3.63 | 3.53–3.63 |
| 6-H$_y^E$ | 3.50–3.59 | 3.51–3.59 | – | – | 3.34–3.49 |
| 6-H$_y^D$ | 3.50–3.59 | 3.51–3.59 | – | – | – |
| 4-H$^F$ | – | 3.51–3.59 | 3.52–3.61 | – | – |
| 2-H$^F$ | 3.66 bd ($J \sim$ 10.5 Hz) | 3.87–3.97 | 3.75–3.85 | 3.53–3.63 | 3.53–3.63 |
| 6-H$_x^D$ | 3.73 d ($J_{A,B}$ = 12.5 Hz) | 3.73 d ($J_{A,B}$ = 10.8 Hz) | – | – | – |
| 1-H$_y^I$ | 3.78–3.85 | 3.74–3.80 | 3.75–3.85 | 3.74–3.84 | 3.72–3.83 |
| 2-H$^H$ | 3.78–3.85 | 3.74–3.80 | 3.75–3.85 | 3.74–3.84 | 3.72–3.83 |
| 3-H$_y^H$ | 3.78–3.85 | 3.74–3.80 | 3.75–3.85 | 3.74–3.84 | 3.72–3.83 |
| 1-H$_x^I$ | 3.89–3.97 | 3.87–3.97 | 3.89–3.96 | 3.90–3.96 | 3.89–3.94 |
| 3-H$_x^H$ | 3.89–3.97 | 3.87–3.97 | 3.89–3.96 | 3.90–3.96 | 3.89–3.94 |
| 6-H$_x^E$ | 3.89–3.97 | 3.87–3.97 | – | – | 3.89–3.94 |
| 4-H$^B$ | 4.07 bd ($J_{3,4}$ = 3.0 Hz) | 4.07 bd ($J_{3,4}$ = 3.0 Hz) | 4.07 bd ($J_{3,4}$ = 2.8 Hz) | 4.07 bd ($J_{3,4}$ = 3.0 Hz) | 4.07 bd ($J_{3,4}$ = 3.1 Hz) |
| 5-H$^B$ | 4.11 d ($J_{4,5}$ < 1 Hz) | 4.11 d ($J_{4,5}$ < 1 Hz) | 4.11 d ($J_{4,5}$ < 1 Hz) | 4.11 d ($J_{4,5}$ < 1 Hz) | 4.11 d ($J_{4,5}$ < 1 Hz) |
| 5-H$^F$ | 4.24 s | 4.25 d ($J_{4,5}$ = 2.6 Hz) | 4.25 d ($J_{4,5}$ = 2.5 Hz) | 4.24 s | 4.23 s |
| 1-H$^D$ | 4.31 d ($J_{1,2}$ = 8.0 Hz) | 4.31 d ($J_{1,2}$ = 8.0 Hz) | – | – | – |
| 2-H$^C$ | 4.38 d ($J_{1,2}$ = 8.5 Hz) | 4.40 d ($J_{1,2}$ = 8.5 Hz) | 4.41 d ($J_{1,2}$ = 8.0 Hz) | 4.42 d ($J_{1,2}$ = 8.6 Hz) | 4.34 d ($J_{1,2}$ = 8.5 Hz) |
| 1-H$^E$ | 4.40 d ($J_{1,2}$ = 8.5 Hz) | 4.43 d ($J_{1,2}$ = 10.0 Hz) | 4.42 d ($J_{1,2}$ = 10.5 Hz) | 4.38 d ($J_{1,2}$ = 8.0 Hz) | 4.43 d ($J_{1,2} \sim$ 8.5 Hz) |
| 1-H$^B$ | 4.43 d ($J_{1,2}$ = 7.8 Hz) | 4.43 d ($J_{1,2} \sim$ 8.5 Hz) | 4.44 d ($J_{1,2}$ = 7.9 Hz) | 4.44 d ($J_{1,2}$ = 7.9 Hz) | 4.43 d ($J_{1,2}$ = 7.9 Hz) |
| $CH_2$ = 22$^I$ | 4.57 d ($J_{gem}$ < 1 Hz) | 4.57 d ($J_{gem}$ < 1 Hz) | 4.57 d ($J_{gem}$ < 1 Hz) | 4.57 d ($J_{gem}$ < 1 Hz) | 4.57 d ($J_{gem}$ < 1 Hz) |
| 3-H$^F$ | 4.83 d ($J_{2,3}$ = 10.4 Hz) | 4.81 dd ($J_{2,3}$ = 10.5 Hz) ($J_{3,4}$ = 3.1 Hz) | 4.80 dd ($J_{2,3}$ = 10.4 Hz) ($J_{3,4}$ = 3.0 Hz) | 4.83 d ($J_{2,3}$ = 10.4 Hz) | 4.83 d ($J_{2,3}$ = 10.4 Hz) |
| 17-H$^I$ | 4.97 t ($J_{16,17}$ = 6.9 Hz) | 4.98 t ($J_{16,17}$ = 6.9 Hz) | 4.98 t ($J_{16,17}$ = 7.0 Hz) | 4.98 t ($J_{16,17}$ = 6.6 Hz) | 4.98 t ($J_{16,17}$ = 6.8 Hz) |
| 13-H$^I$ | 5.03 t ($J_{12,13}$ = 7.4 Hz) | 5.03 t ($J_{12,13}$ = 7.4 Hz) | 5.03 t ($J_{12,13}$ = 7.4 Hz) | 5.03 t ($J_{12,13}$ = 7.4 Hz) | 5.03 t ($J_{12,13}$ = 7.4 Hz) |
| 6-H$^I$ | 5.13 dt ($J_{6,7}$ = 15.8 Hz) ($J_{5,6}$ = 6.5 Hz) | 5.13 dt ($J_{6,7}$ = 15.7 Hz) ($J_{5,6}$ = 6.5 Hz) | 5.13 dt ($J_{6,7}$ = 15.7 Hz) ($J_{5,6}$ = 6.7 Hz) | 5.14 dt ($J_{6,7}$ = 15.7 Hz) ($J_{5,6}$ = 6.7 Hz) | 5.13 dt ($J_{6,7}$ = 15.8 Hz) ($J_{5,6}$ = 6.6 Hz) |
| 2-H$^I$ | 5.20 dd (2 *J*s ≈ 7.0 Hz) | 5.20 dd (2 *J*s ≈ 6.8 Hz) | 5.19 dd (2 *J*s ≈ 6.8 Hz) | 5.20 dd (2 *J*s ≈ 7.1 Hz) | 5.19 dd (2 *J*s ≈ 7.0 Hz) |
| 7-H$^I$ | 5.24 d ($J_{6,7}$ = 15.8 Hz) | 5.24 d ($J_{6,7}$ = 15.7 Hz) | 5.24 d ($J_{6,7}$ = 15.7 Hz) | 5.24 d ($J_{6,7}$ = 15.7 Hz) | 5.24 d ($J_{6,7}$ = 15.8 Hz) |
| 1-H$^F$ | 5.57 dd ($^3J_{1,P}$ = 6.0 Hz) ($J_{1,2}$ = 3.5 Hz) | 5.62 dd ($^3J_{1,P}$ = 6.1 Hz) ($J_{1,2}$ = 3.6 Hz) | 5.58 dd ($^3J_{1,P}$ = 6.5 Hz) ($J_{1,2}$ = 3.7 Hz) | 5.54 dd ($^3J_{1,P}$ = 6.1 Hz) ($J_{1,2}$ = 3.6 Hz) | 5.65 dd ($^3J_{1,P}$ = 7.0 Hz) ($J_{1,2}$ = 3.6 Hz) |

software) and a Bruker DMX 600 spectrometer (600.13 MHz, processed on an SGI O2 workstation using the X-WINNMR program). Samples consisted of moenomycin A (lyophilized three times from $D_2O$ solution) in $D_2O$, degassed. For the 600 MHz spectrum the following conditions were used: sample temperature 285 K; 1024 scans without spinning; relaxation delay 2 s; acquisition time 5.5 s; four steady state pulses; 64 K data points, no zero-filling; chemical shifts were calibrated with respect to internal HOD (assuming a chemical shift of 4.75 ppm at 285 K); very slight decouple presaturation of the remaining HOD peak.

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