Supplementary information

New insight on the structural features of the common cytotoxic auristatins MMAE and MMAF revealed by combined NMR spectroscopy and quantum chemical modelling

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NMR spectroscopic characterisation of MMAE

The sole aromatic residues in 1A (cis-conformer) and 1B (trans-conformer) were selected as suitable starting points for the NMR spectroscopic characterisation of MMAE. In the carbon spectrum, the C-1 (1) and C-1’ (1’) signals are well-resolved and appear at 144.1 (1A) and 143.9 (1B) ppm. In the HMBC spectrum (Figure 5), the cross peaks from C-1 (1) to H-3 (1) and H-5 (1) at 7.34 ppm, H-7 (1) at 4.52 ppm and H-8 (1) at 4.24 ppm were visible. There were no HMBC correlations between C-1 (1) and H-2 (1) or H-6 (1). The conventional use of edHSQC (Figure 3) and COSY (not shown) resulted in the identification of all signals in residue (1). The signals of residue (1’) were identified and assigned in a similar fashion. It should be noted that all of the $^1$H- and $^{13}$C chemical shifts in residues (1) and (1’) are similar. In fact, they differ by less than 0.1 ppm in the $^1$H-NMR spectrum and 1.0 ppm in the $^{13}$C-NMR spectrum.

The norephedrine-dolaproine amide bonds (C-1 (2) at 175.5 ppm and C-1’ (2’) at 175.7 ppm) and the protons close to them (H-8 (1) at 4.24 ppm, H-2 (2) at 2.12 ppm, 2-CH$_3$ (2) at 1.18 ppm and H-8’ (1’) at 4.20 ppm, H-2’ (2’) at 2.22 ppm, 2’-CH$_3$ (2’) at 1.12 ppm) were identified based on the cross peaks in the HMBC spectrum. The other signals in dolaproine residues (2) and (2’) could be identified by an iteration of this approach. The edHSQC-method was found to be a good tool for further verification of the assignments since CH/CH$_3$-protons and CH$_2$-protons appear in separate phases. In the dolaproine residues, many of the chemical shifts were similar, however, positions 3, 3’, 4, 4’, 7 and 7’ were found to differ significantly. The chemical shift difference was found to be greatest for position 3 (H-3 (2) at 3.42 ppm, C-3 (2) at 86.6 ppm) and 3’ (H-3’ (2’) at 3.87 ppm, C-3’ (2’) at 83.5 ppm). The deviation for H-4 (2) and H-4’ (2’) was identical id.est. 0.44 ppm, however, the corresponding carbon signals C-4 (2) and C-4’ (2’) appeared at a similar frequency. The H-7’a (2’) and H-7’b (2’) signals were separated by 0.17 ppm while the H-7a (2) and H-7b (2) were separated by 0.49 ppm.

With the chemical shifts of residues (2) and (2’) obtained, the next step was to identify the dolaproine-dolaisoleuine amide bonds (C-1 (3), C-1’ (3’)) and the other signals on residues (3) and (3’). The HMBC correlations of H-4 (2) and H-4’ (2’) were weak and the H-4’ (2’)/C-1’ (3’) cross peak could barely be observed while the H-4 (2)/C-1 (3) correlation was absent. The HMBC correlations between H-7a (2) (3.68 ppm)/H-7b (2) (3.19 ppm)/C-1 (3) (171.8 ppm) were utilized to acquire a starting point for residue (3). A similar protocol was utilized to confirm the chemical shift of C-1’ (3’) at 171.7 ppm. From this point forward, all of the $^1$H- and $^{13}$C-chemical shifts in residues (3) and (3’) could be identified and assigned by analyzing the spectra acquired with the various NMR spectroscopic techniques.

Continuing on the NMR spectroscopic characterisation, we examined the HMBC correlations of H-4 (3) (4.88 ppm) and H-4’ (3’) (4.76 ppm). The corresponding HMBC correlations to C-1 (4) (174.5 ppm) and C-1’ (4’) (174.7 ppm) were identified. In addition, the HMBC cross peaks between C-1 (4)/(3)/(4)-N-CH$_3$ and C-4 (3)/(3’)/(4)-N-CH$_3$ were visible and could be utilized to confirm the assignment up to this point (in both rotamers). Assigning the remaining signals in residues (4) and (4’) was accomplished by the use of the standard protocol featuring HMBC, edHSQC, COSY, TOCSY, HSQC-TOCSY.
As expected the remaining signals in the $^1$H- and $^{13}$C-NMR spectra could be assigned to residues (5) and (5$'$). For example, H-2 (5) at 3.70 ppm and H-2 (4) at 4.80 ppm had HMBC correlations to C-1 (5) at 167.4 ppm thus assuring that all residues had been assigned correctly (the same patterns were identified in (5$'$)). The chemical shifts for the H-2 (5) (3.70 ppm) and H-2$'$ (5$'$) (3.68 ppm) protons were significantly different than those reported for the corresponding protons in dolastatin 10 (2.65 ppm and 2.39 ppm).

While not mentioned previously, the commercial MMAE utilized in this study was supplied as a TFA-salt. The signals from TFA are not listed in the tables but appeared in the carbon spectrum as a quartet at 162.3 ppm ($J_{C,F}$ = 35.8 Hz) and a quartet at 117.9 ppm ($J_{C,F}$ = 291.8 Hz). In addition to these peaks, there was an unidentified signal at 101.4 ppm without edHSQC and HMBC correlations.

**NMR spectroscopic characterisation of MMAF**

The NMR spectroscopic characterisation of MMAF will not be discussed in detail since the guidelines provided above are directly applicable to MMAF. Instead, the chemical shift values of 2A (cis-conformer) and 2B (trans-conformer) will be compared to each other and those observed for MMAE in order to uncover common trends for this class of compounds. For this comparison to be possible, the spectra of MMAF were also measured in deuterated methanol. It should be noted that MMAF was supplied as a TFA-salt. The chemical shifts of TFA are not listed in tables 3 and 4. In the $^{13}$C-spectrum of MMAF, there are two different sets of signals for TFA which might reflect the existence of the two well-known isomers, two deviating salt forms or alternatively free TFA in addition to the salt. These signals appear at 167.7 ppm (q, $J_{C,F}$ = 35 Hz), 158.9 ppm (q, $J_{C,F}$ = 42 Hz), 118.0 ppm (q, $J_{C,F}$ = 292.4 Hz) and 116.0 ppm (q, $J_{C,F}$ = 284.4 Hz). In the spectra of MMAF, there is also an unknown signal at 101.4 ppm without HMBC or HSQC cross peaks.

The C-1 (1) (138.7 ppm) and C-1$'$ (1$'$) (138.7 ppm) were the starting points utilized in the NMR spectroscopic characterisation of MMAF (HMBC and edHSQC spectra showed in Figure 12 and 13). In the phenylalanine residue, the chemical shifts were fairly similar for both 2A and 2B. A direct comparison to the chemical shifts of MMAE is not warranted since this residue is different. In the dolaprine residues (2) and (2$'$), the chemical shifts followed a similar pattern as reported for MMAE above id. est. C-3 (2) (86.8 ppm) appeared 3.5 ppm downfield from C-3$'$ (2$'$) (83.3 ppm) and H-3 (2) (3.41 ppm) 0.44 ppm upfield from H-3$'$ (2$'$) (3.85 ppm). A similar pattern was observed for H-4 (2)/H-4$'$ (2$'$) although the effect was reduced ($\Delta \delta = 0.31$ ppm) when compared to the values of H-3 (2)/H-3$'$ (2$'$). The previously described pattern concerning the chemical shift difference between H-7a (2) and H-7b (2)/H-7a$'$ (2$'$) and H-7b$'$ (2$'$) was also observed in the spectra of MMAF ($\Delta \delta = 0.46$ ppm in (2), 0.17 ppm in (2$'$)). Since these signals show a similar pattern in both auristatins and the previously reported data on dolastatin 10, it can be concluded that these are general NMR spectroscopic trends for this class of compounds. Apart from these signals, the remaining chemical shifts in (2) and (2$'$) were similar. In residues (3) and (3$'$), the H-2 (3) protons were split into one d and one dd, while the H-2$'$ (3$'$) protons appeared together as a d (as also observed for MMAE). Apart from these minor deviations, all of the remaining signals in 2A and 2B appeared at similar chemical shifts. It should be noted that the H-2 (5) and H-2$'$ (5$'$) protons appear at a similar chemical shift (~3.70 ppm) in deuterated methanol in both MMAE and MMAF. This shift is considerably different than the values
reported for dolastatin 10 in CD$_2$Cl$_2$ (2.39 ppm), DMSO-d6 (2.65 ppm) and CD$_3$OD (3.05 ppm) thus reflecting a deviation in the structural features of auristatins when compared to dolastatin 10.

Supplementary Table 1. Summary of the NMR results of 1A (cis-conformer) measured at 22 °C in CD$_3$OD with a Bruker 850 MHz instrument. Chemical shifts are expressed in ppm using solvent residual peaks as an internal reference (3.31 ppm $\delta$ $^1$H and 49.0 ppm $\delta$ $^{13}$C). Coupling patterns are given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.

| POSITION | $\delta$ $^{13}$C | $\delta$ $^1$H (J, Hz) | HMBC (H $\rightarrow$ C)-CORRELATIONS | ROESY CORRELATIONS$^A$ |
|----------|----------------|----------------|--------------------------------|----------------------|
| 1 (1)    | 144.1          | -              | C-3 (1), C-5 (1)               | H-7 (1), H-8 (1)     |
| 2 (1)    | 128.1          | 7.39           |                               |                      |
|          | (dd, $J_{2,4} = 1.6$, $J_{2,3} = 7.8$ Hz) |                 |                               |                      |
| 3 (1)    | 129.8          | 7.34           | C-1 (1), C-2 (1), C-4 (1), C-6 (1) | not determined      |
| 4 (1)    | 128.6          | 7.22           | C-2 (1), C-6 (1)               | not determined       |
| 5 (1)    | 129.8          | 7.34           | C-1 (1), C-2 (1), C-4 (1), C-6 (1) | not determined      |
| 6 (1)    | 128.1          | 7.39           | C-3 (1), C-5 (1)               | H-7 (1), H-8 (1)     |
| 7 (1)    | 77.5           | 4.52           | C-1 (1), C-6 (1), 8-CH$_3$ (1) | H-2 (1), H-6 (1), H-8 (1), 8-CH$_3$ (1) |
| 8 (1)    | 50.7           | 4.24           | C-1 (1), C-7 (1), 8-CH$_3$ (1), C-1 (2) | H-2 (1), H-6 (1), H-7 (1), 8-CH$_3$ (1) |
| 8-CH$_3$(1) | 17.0         | 1.20           | C-7 (1), C-8 (1)               | H-7 (1), H-8 (1)     |
| 1 (2)    | 175.5          | -              |                               |                      |
| 2 (2)    | 45.4           | 2.12           | C-1 (2), C-3 (2), 2-CH$_3$ (2) | H-5a (2), 2-CH$_3$ (2) |
|          | (dq, $J_{3,2-CH_3} = 6.8$ Hz, $J_{2,3} = 10.0$ Hz) |                 |                               |                      |
| 2-CH$_3$ (2) | 15.8         | 1.18           | C-1 (2), C-2 (2), C-3 (2)      | H-2 (2), H-3 (2)     |
| 3 (2)    | 86.6           | 3.42           | C-2 (2), 2-CH$_3$ (2), 3-OCH$_3$ (2), C-4 (2), C-5 (2) | H-2 (2), 2-CH$_3$ (2), H-2a (3) |
|          | (dd, $J_{3,4} = 1.2$ Hz) |                 |                               |                      |
| 3-OCH$_3$ (2) | 62.0         | 3.34           | C-3 (2)                       | 2-CH$_3$ (2)        |
| 4 (2)    | 60.6           | 3.27           | C-3 (2) weak, C-5 (2) weak, C-6 (2) weak, C-7 (2) weak | H-2b (3), H-5b (2)   |
| 5 (2)    | 26.6           | 1.78 (5a)      | C-3 (2), C-4 (2), C-6 (2), C-7 (2) | H-5a: H-2 (2), H-5b (2) |
|          | (m) 1.37 (5b)  |               |                               | H-5b: H-4 (2), H-5a (2) |
| 6 (2)    | 24.4           | 1.88 (6a)      | C-4 (2), C-5 (2), C-7 (2)     | H-6a: H-6b (2), H-7b (2) |
|          | (m) 1.57 (6b)  |               |                               | H-6b: H-6a (2)      |
| 7 (2) | 48.1 | 3.68 (7a) (m) | C-4 (2), C-5 (2), C-6 (2), C-1 (3) | H-7a: H-7b (2), H-7b: H-6a (2), H-7a (2) |
|-------|-------|--------------|----------------------------------|-------------------------------------------|
|       |       | 3.19 (7b) (m) |                                  |                                           |
| 1 (3) | 171.8 | -            | -                                |                                           |
| 2 (3) | 36.7  | 2.53 (2a) (d, $J_{2a,2b} = -15.8$ Hz) | C-1 (3), C-3 (3), C-4 (3) | H-2a: H-3 (2), H-3 (3), H-5 (3), aliphatic region H-2b: not determined |
|       |       | 2.46 (2b) (dd, $J_{2b,3} = 10.4$ Hz) |                                  |                                           |
| 3 (3) | 78.8  | 4.19 (m)     | C-1 (3), C-2 (3), 3-OCH$_3$ (3) | H-2a (3), 3-OCH$_3$ (3), aliphatic region |
| 3-OCH$_3$ (3) | 58.6 | 3.36 (s)     | C-3 (3)                          | H-3 (3), H-4 (3)                          |
| 4 (3) | 57.8  | 4.88 (m)     | C-2 (3), C-3 (3), C-5 (3), C-6 (3), 5-CH$_3$ (3), C-1 (4) | not observed |
| 5 (3) | 33.6  | 1.90 (m)     | C-4 (3), 5-CH$_3$ (3), C-7 (3)   | H-2a (3), (3)/(4)-N-CH$_3$, aliphatic region |
| 5-CH$_3$ (3) | 15.9 | 0.98 (d, $J_{5-CH_3} = 6.5$ Hz) | C-4 (3), C-5 (3), C-6 (3), C-7 (3) | H-3 (3), H-4 (3), H-5 (3) |
| 6 (3) | 27.1  | 1.38 (6a) (m) | C-4 (3), C-5 (3), 5-CH$_3$ (3), C-7 (3) | not determined |
|       |       | 1.02 (6b) (m) |                                  |                                           |
| 7 (3) | 10.9  | 0.88 (t, $J_{7,8} = 7.5$ Hz) | C-5 (3), C-6 (3) | H-5 (3) weak, H-6a (3) |
| (3)/(4)-N-CH$_3$ | 33.2 | 3.29 (s) | C-4 (3), C-1 (4) | H-2 (1) and/or H-6 (1), H-2a and/or H-2b (3), H-5 (3), H-2 (4) |
| 1 (4) | 174.5 | -            | -                                |                                           |
| 2 (4) | 56.7  | 4.80 (d, $J_{2a,3} = 8.4$ Hz) | C-1 (4), C-3 (4), 3-CH$_3$ (4), C-4 (4), C-1 (5) | (3)/(4)-N-CH$_3$, H-3 (4), aliphatic region |
| 3 (4) | 31.5  | 2.15 (dqq, $J_{3,3-CH_3} = 6.7$, $J_{3,4} = 6.8$ Hz) | C-1 (4), C-2 (4), 3-CH$_3$ (4), C-4 (4) | not determined |
| 3-CH$_3$ (4) | 18.9 | 0.99 (d) | C-2 (4), C-3 (4), C-4 (4) | H-2 (4), H-3 (4), aliphatic region |
| 4 (4) | 19.6  | 1.05 (d)    | C-2 (4), C-3 (4), 3-CH$_3$ (4)  | not determined |
| 1 (5) | 167.4 | -            | -                                |                                           |
| 2 (5) | 68.1  | 3.70 (d, $J_{2a,3} = 5.4$ Hz) | C-1 (5), C-3 (5), 3-CH$_3$ (5), C-4 (5), (5)-N-CH$_3$ | H-3 (5), H-4 (5), (5)-N-CH$_3$ |
| 3 (5) | 31.5  | 2.20 (dqq, $J_{3,3-CH_3} = 6.5$, $J_{3,4} = 6.9$ Hz) | C-1 (5), C-2 (5), 3-CH$_3$ (5), C-4 (5) | H-2 (5), aliphatic region |
| 3-CH$_3$ (5) | 18.3 | 1.04 (d) | C-2 (5), C-3 (5), C-4 (5) | not determined |
are provided only once when first encountered.

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\text{patterns are given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.}
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Supplementary Table 2. Summary of the NMR results of compound 1B (trans-conformer) measured at 22 °C in CD$_3$OD with a Bruker 850 MHz instrument. Chemical shifts are expressed in ppm using solvent residual peaks as an internal reference (3.31 ppm δ $^1$H and 49.0 ppm δ $^{13}$C). Coupling patterns are given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.

| POSITION | δ $^{13}$C | δ $^1$H (J, Hz) | HMBC (H → C)-CORRELATIONS | ROESY CORRELATIONS$^a$ |
|----------|------------|----------------|---------------------------|------------------------|
| 1’ (1’)  | 143.9      | -              | -                         | -                      |
| 2’ (1’)  | 127.9      | 7.39           | C-3’ (1’), C-5’ (1’)      | H-7’ (1’), H-8’ (1’)  |
| 3’ (1’)  | 129.5      | 7.29           | C-1’ (1’), C-2’ (1’), C-4’ (1’), C-6’ (1’) | not determined |
| 4’ (1’)  | 128.4      | 7.21           | C-2’ (1’), C-6’ (1’)      | not determined         |
| 5’ (1’)  | 129.5      | 7.29           | C-1’ (1’), C-2’ (1’), C-4’ (1’), C-6’ (1’) | not determined |
| 6’ (1’)  | 127.9      | 7.39           | C-3’ (1’), C-5’ (1’)      | H-7’ (1’), H-8’ (1’)  |
| 7’ (1’)  | 77.3       | 4.61           | C-1’ (1’), C-2’ (1’), C-6’ (1’), C-8’ (1’), 8’-CH$_3$ (1’) | H-2’ (1’), H-6’ (1’), 8’-CH$_3$ (1’) |
| 8’ (1’)  | 51.4       | 4.20           | C-1’ (1’), C-7’ (1’), 8’-CH$_3$ (1’), C-1’ (2’), 8’-CH$_3$ (1’) | H-2’ (1’), H-6’ (1’), 8’-CH$_3$ (1’) |
| 8’-CH$_3$ (1’) | 16.0 | 1.15           | C-7’ (1’), C-8’ (1’)      | H-7’ (1’), H-8 (1’)   |
| 1’ (2’)  | 175.7      | -              | -                         | -                      |
| 2’ (2’)  | 45.9       | 2.22           | C-1’ (2’), C-3’ (2’), C-4’ (2’), 2’-CH$_3$ (2’) | H-3’ (2’), H-4’ (2’) |
| 2’-CH$_3$ (2’) | 15.1 | 1.12           | C-1’ (2’), C-2’ (2’), C-3’ (2’), 3’-OC$_3$ (2’) | H-2’ (2’), H-3’ (2’), 3’-OC$_3$ (2’) |
| 3’ (2’)  | 83.5       | 3.87           | C-1’ (2’), C-2’ (2’), 2’-CH$_3$ (2’), C-4’ (2’), 3’-OCH$_3$ (2’) | 3’-OCH$_3$ (2’), H-2’ (2’), 2’-CH$_3$ (2’) |
| 3’-OCH$_3$ (2’) | 61.5 | 3.35           | C-3’ (2’)                  | not determined        |

$^a$ The ROEs were not determined in crowded areas of the spectrum where the uncertainty was high, if these ROE-correlations were in the aliphatic region it is mentioned in the table.
| 4’ (2’) | 60.8 | 3.71 (m) | C-3’ (2’), C-5’ (2’) weak, C-6’ (2’) weak, C-7’ (2’) weak, C-1’ (3’) weak | H-5’b (2’) |
| 5’ (2’) | 25.6 | 1.84 (5’a) (m) | C-3’ (2’), C-4’ (2’), C-6’ (2’), C-7’ (2’) | H-5’a: not determined |
| 6’ (2’) | 25.9 | 1.95 (6’a) (m) | C-4’ (2’), C-5’ (2’), C-7’ (2’) | H-6’a: H-6’b (2’), H-7’b (2’) |
| 7’ (2’) | 48.4 | 3.56 (7’a) (m) | C-4’ (2’), C-5’ (2’), C-6’ (2’), C-1’ (3’) | H-7’a: H-6’b (2’), H-2’ (3’), H-7’b (2’) |
| 1’ (3’) | 171.7 | - | - | - |
| 2’ (3’) | 38.2 | 2.47 (d, J2,3 = 6.2 Hz) | C-1’ (3’), C-3’ (3’) | H-7’a (2’), H-3’ (3’), H-5’ (3’), (3’)/(4’)-N-CH3, aliphatic region |
| 3’ (3’) | 79.7 | 4.08 (m) | C-1’ (3’), C-2’ (3’), 3’-OCH3 (3’) | H-2’ (3’), 3’-OCH3 (3’), aliphatic region |
| 3’-OCH3 (3’) | 58.3 | 3.30 (s) | C-3’ (3’) | H-3’ (3’) |
| 4’ (3’) | 58.4 | 4.76 (m) | not determined | not determined |
| 5’ (3’) | 33.7 | 1.79 (m) | C-4’ (3’), 5’-CH3 (3’), C-7’ (3’) | H-2’ (3’), (3’)/(4’)-N-CH3 |
| 5’-CH3 (3’) | 16.3 | 1.01 (d, J5’,CH3,5’ = 6.8 Hz) | C-4’ (3’), C-5’ (3’), C-6’ (3’) | not determined |
| 6’ (3’) | 27.1 | 1.42 (6’a) (m) | C-4’ (3’), 5’-CH3 (3’), C-7’ (3’) | not determined |
| 7’ (3’) | 10.9 | 0.86 (t, J7,6 = 7.5 Hz) | C-5’ (3’), C-6’ (3’) | H-5’ (3’), H-6’a (3’) |
| (3’)/(4’)-N-CH3 | 33.0 | 3.14 (s) | C-4’ (3’), C-1’ (4’) | H-2’ (3’), H-3’ (3’), H-5’ (3’), H-6’a (3’), H-2’ (4’), aliphatic region |
| 1’ (4’) | 174.7 | - | - | - |
| 2’ (4’) | 56.9 | 4.70 (d, J2,3 = 8.8 Hz) | C-1’ (4’), C-3’ (4’), 3’-CH3 (4’), C-4’ (4’), C-1’ (5’) | (3’)/(4’)-N-CH3, H-3’ (4’), aliphatic region |
| 3’ (4’) | 31.9 | 2.08 (dqq, J3,3,CH3 = 6.7, J3,4 = 6.8 Hz) | C-2’ (4’), 3’-CH3 (4’), C-4’ (4’) | not determined |
| 3’-CH3 (4’) | 19.2 | 1.05 (d) | C-3’ (4’), C-4’ (4’) | not determined |
The ROEs were not determined in crowded areas of the spectrum where the uncertainty was high, if these ROE-correlations were in the aliphatic region it is mentioned in the table.

Supplementary Table 3. Summary of the NMR results of compound 2A (cis-conformer) measured at 22 °C in CD$_3$OD with a Bruker 850 MHz instrument. Chemical shifts are expressed in ppm using solvent residual peaks as an internal reference (3.31 ppm δ $^1$H and 49.0 ppm δ $^{13}$C). Coupling patterns are given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.

| POSITION | δ $^{13}$C | δ $^1$H (J, Hz) | HMBC (H → C)-CORRELATIONS | ROEY CORRELATIONS$^A$ |
|----------|-------------|-----------------|---------------------------|-----------------------|
| 1 (1)    | 138.7       | -               | -                         | -                     |
| 2 (1)    | 130.0       | 7.29–7.23 (m)   | C-3 (1), C-4 (1), C-5 (1), C-7 (1) | not determined         |
| 3 (1)    | 129.6       | 7.29–7.23 (m)   | not determined             | not determined         |
| 4 (1)    | 127.8       | 7.19            | C-2 (1), C-3 (1), C-5 (1), C-6 (1) | not determined         |
| 5 (1)    | 129.6       | 7.29–7.23 (m, identical to H-3(1)) | not determined | not determined |
| 6 (1)    | 130.0       | 7.29–7.23 (m, identical to H-2 (1)) | C-3 (1), C-4 (1), C-5 (1), C-7 (1) | not determined |
| 7 (1)    | 37.9        | 3.33 (H-7a) (dd) | C-1 (1), C-2 (1), C-6 (1), C-8 (1), 8-CO$_2$H (1) | H-7a: H-7b (1), aromatic region |
| 8 (1)    | 53.7        | 4.79 (dd, J = 4.3, 11.7 Hz) | C-1 (1), C-7 (1), 8-CO$_2$H (1) (1), C-1 (2) | not determined |
| 8-CO$_2$H (1) | 174.8 | - | - | - |
| 1 (2) | 176.6 | - | - | - |
| 2 (2)    | 45.3        | 2.25 (dq, J$_{2,3-CO_3}$ = 6.8, J$_{2,3}$ = 9.7 Hz) | C-1 (2), 2-CH$_3$ (2), C-3 (2), C-4 (2) | H-3 (2), H-5a (2) |
| 2-CH$_3$ (2) | 15.6 | 1.20 | C-2 (2), C-3 (2) | H-2 (2), H-3 (2) |
| Compound | δ (ppm) | J (Hz) | Assignment |
|----------|---------|--------|------------|
| 3 (2)    | 86.8    | 3.41 (br d) | C-2 (2), 2-CH₃ (2), C-(4), C-5 (2) |
| 3-OCH₃ (2) | 62.1    | 3.35 (s) | C-3 (2) not determined |
| 4 (2)    | 60.6    | 3.35 (m) | C-5 (2) not determined |
| 5 (2)    | 26.5    | 1.77 (H-5a) (m) | C-3 (2), C-4 (2), C-6 (2), C-7 (2) |
|          |         | 1.27 (H-5b) (m) | H-5a: H-2 (2), H-4 (2), H-5b (2) H-5b: not determined |
| 6 (2)    | 24.5    | 1.87 (H-6a) (m) | C-4 (2), C-5 (2), C-7 (2) not determined |
|          |         | 1.54 (H-6b) (m) | |
| 7 (2)    | 48.0    | 3.66 (H-7a) (m) | C-5 (2), C-6 (2), C-1 (3) |
|          |         | 3.20 (H-7b) (m) | H-7a: H-7b (2) H-7b: H-7a (2) |
| 1 (3)    | 172.1   | - | C-1 (3), C-3 (3) H-2a: H-5 (3) H-2b: H-4 (2), (3)/(4)-N-CH₃ |
| 2 (3)    | 36.7    | 2.47 (H-2a) (d, J₂₃,₂₄ = -15.2 Hz) | C-1 (3), C-3 (3) |
|          |         | 2.42 (H-2b) (dd, J₂₃,₂₄ = 10.5 Hz) | H-2a: H-5 (3) H-2b: H-4 (2), (3)/(4)-N-CH₃ |
| 3 (3)    | 79.1    | 4.15 (m) | C-1 (3), C-2 (3), C-4 (3) |
|          |         | 3.34 (s) | H-2 (3), H-4 (3), aliphatic region |
| 3-OCH₃ (3) | 58.5    | 3.34 (s) | C-3 (3) not determined |
| 4 (3)    | 57.9    | 4.83 (m) | C-2 (3), C-3 (3), C-5 (3), 5-CH₃ (3), C-6 (3), C-1 (4) not determined |
| 5 (3)    | 33.5    | 1.87 (m) | C-4 (3), 5-CH₃ (3), C-7 (3) H-3 (3) |
| 5-CH₃ (3) | 15.9    | 0.98 (d, J₅-OCH₃ = 6.6 Hz) | C-4 (3), C-5 (3), C-6 (3) not determined |
| 6 (3)    | 27.0    | 1.40 (H-6a) (m) | C-5 (3), 5-CH₃ (3), C-7 (3) not determined |
|          |         | 1.00 (H-6b) (m) | |
| 7 (3)    | 10.9    | 0.87 (t, J₆,₇ = 7.4 Hz) | C-5 (3), C-6 (3) not determined |
| (3)/(4)-N-CH₃ | 33.1    | 3.23 (s) | C-4 (3), C-1 (4) H-2 (4), H-2b (3), aromatic region |
| 1 (4)    | 174.6   | - | - |
| 2 (4)    | 56.7    | 4.82 (d, J₂₃ = 8.0 Hz) | C-1 (4), C-3 (4), C-4 (4), C-1 (5) |
| 3 (4)    | 31.7    | 2.18 (dqq, J₃₄ = 6.7, J₃₃-OCH₃ = 6.8 Hz) | C-1 (4), C-2 (4), C-4 (4) not determined |
| 3-CH₃ (4) | 18.9    | 1.05 (d) | C-2 (4), C-3 (4), C-4 (4) not determined |
Given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.

Solvent residual peaks

Patterns are g

RA

OE

The ROEs were not determined in crowded areas of the spectrum where the uncertainty was high, if these ROE-correlations were in the aliphatic region it is mentioned in the table.

Supplementary Table 4. Summary of the NMR results of compound 2B (trans-conformer) measured at 22 °C in CD3OD with a Bruker 850 MHz instrument. Chemical shifts are expressed in ppm using solvent residual peaks as an internal reference (3.31 ppm δ1H and 49.0 ppm δ13C). Coupling patterns are given as d (doublet), t (triplet), q (quartet), m (multiplet) etc. and coupling constants are provided only once when first encountered.

| POSITION | δ13C | δ1H (J, Hz) | HMBC (H → C)-CORRELATIONS | ROEY CORRELATIONS4 |
|----------|------|-------------|---------------------------|-------------------|
| 1'(1')   | 138.7| -           | C-3' (1'), C-4' (1'), C-5'  |
|          |      |             | (1'), C-7' (1')            | not determined    |
| 2' (1')  | 130.1| 7.29 – 7.23 | C-3' (1'), C-4' (1'), C-5'  |
|          |      | (m)         | (1'), C-7' (1')            | not determined    |
| 3' (1')  | 129.5| 7.29 – 7.23 | not determined             | not determined    |
|          |      | (m)         |                           |                   |
| 4' (1')  | 127.7| 4.17        | C-2' (1'), C-3' (1'), C-5'  |
|          |      | (tt, Jx,x = 1.6, Jy,y = 7.1 Hz) | (1'), C-6' (1')      | not determined    |
| 5' (1')  | 129.5| 7.29 – 7.23 | not determined             | not determined    |
|          |      | (m, identical to H-3' (1')) |                           |                   |
| 6' (1')  | 130.1| 7.29 – 7.23 | C-3' (1'), C-4' (1'), C-5'  |
|          |      | (m, identical to H-2' (1')) | (1'), C-7' (1')      | not determined    |
| 7' (1')  | 37.9 | 3.28 (H-7'a) | C-1' (1'), C-2' (1'), C-6'  |
|          |      | (dd)        | (1'), C-8' (1'), 8'-CO2H    |
|          |      | 2.93 (H-7'b)| (1')                       | (1')              | not determined    |
| 8' (1')  | 54.5 | 4.72        | C-1' (1'), C-7' (1'), 8'-   |
|          |      | (dd, J = 4.4, 11.0 Hz) | CO2H (1'), C-1' (2')      | not determined    |
| 8'-CO2H(1)| 174.8| -           |                           |                   |
| 1' (2')  | 176.7| -           |                           |                   |
| 2' (2')  | 45.3 | 2.31        | C-1' (2'), C-3' (2'), C-4'  |
|          |      | (dq, Jx,x,CH3 = 6.8, Jy,y = 8.9 Hz) | (2'), 2'-CH3 (2')      | not determined    |
|          |      |             | 2'-CH3 (2'), H-3' (2'),    |                   |
|          |      |             | H-5'a (2')                 |                   |
|                | 14.9 | 1.15 | C-1' (2'), C-2' (2'), C-3' (2') |
|----------------|------|------|-----------------------------|
| 2' (3')         | 1.15 |      | C-2' (2'), 2'-CH₃ (2'), C-4' (2'), C-5' (2'), 3'-OCH₃ (2') |
| 3' (2')         | 61.4 | 3.30 | C-3' (2')                   |
| 3'-OCH₃ (2')    |      |      | not determined | 2'-CH₃ (2') |
| 4' (2')         | 60.6 | 3.66 | C-3' (2'), C-5' (2'), C-1' (3') |
| 5' (2')         | 25.5 | 1.73 | C-3' (2'), C-4' (2'), C-6' (2'), C-7' (2') |
| 6' (2')         | 25.8 | 1.89 | C-4' (2'), C-5' (2'), C-7' (2') |
| 7' (2')         | 49.0 | 3.52 | C-4' (2'), C-5' (2'), C-6' (2'), C-1' (3') |
| 1' (3')         | 171.6| -    | -                           |
| 2' (3')         | 38.2 | 2.46 | C-1' (3'), C-3' (3'), C-4' (3') |
| 3' (3')         | 79.6 | 4.07 | C-1' (3'), C-2' (3'), C-4' (3') |
| 3'-OCH₃ (3')    | 58.3 | 3.29 | C-3' (3')                   |
| 4' (3')         | 58.3 | 4.76 | C-2' (3')                   |
| 5' (3')         | 33.7 | 1.79 | not determined              |
| 5'-CH₃ (3')     | 16.2 | 1.01 | C-4' (3'), C-5' (3'), C-6' (3') |
| 6' (3')         | 27.1 | 1.40 | not determined              |
| 7' (3')         | 10.8 | 0.86 | C-5' (3'), C-6' (3')        |
| (3')/(4')-N-CH₃| 33.1 | 3.13 | C-4' (3'), C-1' (4')        |
| 1' (4')         | 175.0| -    | -                           |
| 2' (4')         | 56.9 | 4.70 | C-1' (4'), C-3' (4'), C-4' (4'), 3'-CH₃ (4'), C-1' (5') |
| 3' (4')         | 31.8 | 2.08 | C-1' (4'), C-2' (4'), C-4' (4'), 3'-CH₃ (4') |
| 3'-CH₃ (4')     | 19.2 | 1.03 | C-2' (4'), C-3' (4')        |

Not determined
|          | 19.1  | 1.05  | C-2’ (4’), C-3’ (4’) | not determined |
|----------|-------|-------|----------------------|----------------|
| 4’ (4’)  |       |       |                      |                |
| 1’ (5’)  | 167.6 | -     | C-1’ (5’), C-3’ (5’), C-4’ (5’), 3’-CH₃ (5’), (5’)-N-CH₃ |                |
| 2’ (5’)  | 68.0  | 3.68  |                      |                |
|          |       |       | (d, \(J=5.2\) Hz)   |                |
| 3’ (5’)  | 31.5  | 2.18  | C-1’ (5’), C-2’ (5’), C-4’ (5’), 3’-CH₃ (5’), (5’)-N-CH₃ | not determined |
|          |       |       |                      |                |
| 3’-CH₃ (5’) | 18.3 | 1.01  | C-2’ (5’), C-3’ (5’), C-4’ (5’), 3’-CH₃ (5’) | not determined |
|          |       |       |                      |                |
| 4’ (5’)  | 18.7  | 1.05  | C-2’ (5’), C-3’ (5’), 3’-CH₃ (5’) | not determined |
| (5’)-N-CH₃ | 33.1 | 2.65  | C-2’ (5’) | H-2’ (5’) |

\(^{a}\) The ROEs were not determined in crowded areas of the spectrum where the uncertainty was high, if these ROE-correlations were in the aliphatic region it is mentioned in the table.
Supplementary Figure 1. $^1$H-NMR spectrum of MMAE in CD$_3$OD recorded at 22 °C (850 MHz).

Supplementary Figure 2. $^{13}$C-NMR spectrum of MMAE in CD$_3$OD recorded at 22 °C (213 MHz).
Supplementary Figure 3. EdHSQC spectrum of MMAE in CD$_3$OD recorded at 22 °C (850 MHz).

Supplementary Figure 4. 2D TOCSY spectrum of MMAE in CD$_3$OD recorded at 22 °C (180 ms spinlocktime) (850 MHz).
Supplementary Figure 5. 2D HSQC-TOCSY spectrum of MMAE in CD$_3$OD recorded at 22 °C (850 MHz).

Supplementary Figure 6. HMBC spectrum of MMAE in CD$_3$OD recorded at 22 °C (850 MHz).
Supplementary Figure 7. 2D ROESY spectrum of MMAE in CD$_3$OD recorded at 22 °C (0.8 s mixing time) (850 MHz).

Supplementary Figure 8. $^1$H-NMR spectrum of MMAF in CD$_3$OD recorded at 22 °C (850 MHz).
Supplementary Figure 9. $^{13}$C-NMR spectrum of MMAF in CD$_3$OD recorded at 22 °C (213 MHz).

Supplementary Figure 10. EdHSQC spectrum of MMAF in CD$_3$OD recorded at 22 °C (850 MHz).
Supplementary Figure 11. 2D TOCSY spectrum of MMAF in CD$_3$OD recorded at 22 °C (180 ms spinlocktime) (850 MHz).

Supplementary Figure 12. 2D HSQC-TOCSY spectrum of MMAE in CD$_3$OD recorded at 22 °C (850 MHz).
Supplementary Figure 13. HMBC spectrum of MMAF in CD$_3$OD recorded at 22 °C (850 MHz).

Supplementary Figure 14. 2D ROESY spectrum of MMAF in CD$_3$OD recorded at 22 °C (0.8 s mixing time) (850 MHz).
Supplementary Figure 15. $^1$H-NMR spectrum of MMAE in D$_2$O recorded at 22 °C (600 MHz).

Supplementary Figure 16. $^1$H-NMR spectrum of MMAF in D$_2$O recorded at 22 °C (600 MHz).
Molecular coordinates

Below we tabulate the molecular coordinates for the isomers and transition states of the studied species, in standard XYZ format (Ångström units). All geometries computed at the TPSSh-D3(BJ) density functional theory level, with the COSMO solvation model simulating a methanol environment, using the dielectric constant $\varepsilon=32.6$. The stable points on the potential energy surface were optimised with the def2-TZVPP basis set, and the transition states using the def2-SVP basis set.

1A: cis-MMAE

|  |  |  |  |
|---|---|---|---|
| N | -0.6117916 | 58.5490463 | 21.6998763 |
| C | 0.3083166 | 58.5785729 | 22.8348631 |
| C | -0.6318824 | 57.2529442 | 21.0345989 |
| C | -1.9350678 | 55.9360898 | 19.4578757 |
| C | -1.7525471 | 58.2176508 | 18.9381413 |
| O | 0.7041157 | 57.0079901 | 20.3358654 |
| O | 1.2849313 | 56.8679099 | 19.6730705 |
| N | 1.1791018 | 55.7301898 | 20.4835501 |
| C | 2.3089915 | 55.2362416 | 19.7324678 |
| C | 1.8177829 | 53.9400086 | 19.0829806 |
| C | 1.2295990 | 53.1302833 | 18.8154087 |
| C | 3.5346179 | 54.9479751 | 20.6248784 |
| C | 4.7166768 | 54.4813729 | 19.7767776 |
| C | 3.9045064 | 56.1821170 | 21.4475379 |
| N | 2.0956234 | 55.7812088 | 17.7665407 |
| C | 0.6053241 | 52.5781372 | 16.0634477 |
| C | 1.6384834 | 53.4309131 | 17.1964921 |
| C | 3.5330830 | 51.9645029 | 15.4806211 |
| N | 3.8661978 | 51.6299874 | 17.7976609 |
| C | 2.7282086 | 49.6742303 | 15.3128351 |
| C | -0.6178002 | 53.3687578 | 16.5427768 |
| C | -1.5524588 | 53.7831456 | 15.4088451 |
| C | 0.2132951 | 51.2000811 | 15.2562010 |
| C | 2.7230461 | 54.6703463 | 16.9000598 |
| C | 3.5874029 | 50.8328230 | 18.9386960 |
| C | 6.0978784 | 50.8491079 | 11.8429871 |
| C | 1.0989459 | 53.7887726 | 10.9906554 |
| C | 2.5226567 | 53.6784131 | 11.5426666 |
| C | 1.6348515 | 50.2481702 | 11.8193787 |
| C | 2.7310527 | 54.7354222 | 12.6122555 |
| C | 4.4203440 | 50.9471031 | 13.4509680 |
| C | 4.2218031 | 52.1835492 | 12.6793914 |
| C | 5.2171460 | 52.0586705 | 11.5061097 |
| C | 5.1896852 | 49.9638956 | 12.6899115 |
| C | 2.7689908 | 52.3125073 | 12.1973049 |
| O | 2.4620661 | 51.2719428 | 11.2739701 |
| O | 2.0763522 | 54.7099912 | 13.6618268 |
| O | 5.6154438 | 58.0833484 | 14.2866009 |
| C | 3.2752462 | 58.0751400 | 12.7163714 |
| N | 3.6598971 | 55.6761499 | 12.3547426 |
| C | 3.9218836 | 56.7967133 | 13.2460007 |
| C | 5.4395160 | 56.9418172 | 13.4413699 |
| C | 5.8414081 | 55.3797350 | 15.3807351 |
| C | 6.8706097 | 58.0751400 | 13.2689570 |
| C | 4.003459 | 54.2332086 | 13.9336868 |
| C | 7.4360590 | 53.7221224 | 13.8205538 |
| C | 6.0628648 | 55.7025382 | 14.0405593 |
| C | 7.1946533 | 53.3964425 | 15.1510864 |
| H | -1.5526881 | 58.7450103 | 22.0260059 |
| H | 1.3350312 | 58.5080993 | 22.4689299 |
| O | 0.1440771 | 57.7558636 | 23.5474770 |
| H | 0.2004144 | 59.5256557 | 23.3634212 |
| H | -0.7681173 | 56.4366557 | 21.7696154 |
| H | -2.7050423 | 57.3581662 | 20.6528849 |
| H | -1.9700455 | 54.9970767 | 20.2399123 |
-1.0876133 55.5317490 18.807234
-2.8438151 55.6743859 18.8585742
-0.9036925 50.0368030 19.3524267
-1.6481983 59.2207365 19.3417356
-2.6672515 58.1908700 18.3417356
0.6347428 57.029914 20.9952431
2.5734757 56.0114229 19.0186742
5.0465149 52.8388266 19.1105933
4.4637782 53.6121987 19.1105933
5.5618252 54.2135298 20.4150728
4.0067565 52.6460747 15.6797142
2.5734757 52.5117492 14.8363505
-0.2700101 54.2698678 17.0522160
-1.1607425 52.7816441 17.2914043
-2.0052748 52.922112 14.9124079
-1.0059656 54.3543703 14.6533157
-2.3630851 54.4103756 15.7860397
-0.1639944 50.5614413 16.3292261
1.0553967 50.6865073 15.0522160
-0.5675675 58.5490463 21.6998763
3.8003455 54.5165201 16.4989575
2.4926919 54.6958946 17.1419315
2.3952883 54.5233460 15.8460005
3.4057621 54.7948604 18.6398906
2.7186352 52.164013 19.4810155
4.4612717 50.8762801 19.5867676
6.9560196 51.572914 12.4421496
6.4659948 50.3402691 10.9532712
0.9466718 54.7588280 10.5144366
0.3750360 53.6830682 11.8008859
0.9236147 53.005953 10.2543035
3.2427298 53.6224647 17.7325201
0.6851670 50.6625877 12.1722466
2.1308463 49.7341961 12.6480184
1.4450424 49.5381471 11.0673069
4.4680957 53.0423035 13.3072131
4.6676371 51.8763123 10.5830606
5.7945059 52.9730547 11.3859835
5.7161703 49.3079943 13.3774069
4.5219958 49.3608143 12.0671565
2.0894786 52.2364875 13.0590156
6.5585722 58.1659633 14.4766983
2.2000299 57.9248035 12.6086030
3.6896951 58.3408044 11.7402944
3.4474070 58.9026320 13.4035355
4.1030700 55.6680624 14.4768688
3.4758653 56.5244317 14.026462
5.9015881 57.1302431 12.4630369
5.2386251 56.0415711 15.9907506
7.061126 55.1219753 12.2317037
6.2189176 53.9869444 16.9731900
8.0706479 53.0886268 13.2135838
7.6274490 52.5015600 15.5808742

**1B: trans-MMAE**

-0.6117916 58.5490463 21.6998763
0.3083166 58.5785729 22.8348631
-0.6318824 57.2529442 12.395489
-1.8126252 57.1653532 20.0445970
-1.9350678 55.7596089 19.4578577
-1.7525471 58.2176508 18.9381413
0.7041157 57.0079901 20.3358654
1.2849313 57.8687909 19.6737053
1.1791018 55.7530189 20.4835501
2.3089915 55.2362416 19.7324678
3.8177829 53.9400086 19.0829806
1.2295990 53.1302833 19.8154087
1TS: MMAE 1A↔1B transition state

N     2.1753224    -5.4329896   -5.5170134
C     3.6082100    -5.4655663   -5.7770242
C     1.6019032    -4.0991504   -5.5548965
C     0.0528608    -4.1770271   -5.4806590
C     -0.5944796   -2.8069242   -5.7046083
C     -0.4462398   -4.8439773   -4.1952786
C     2.1626531   -3.2610305   -4.4004140
O     2.3070573   -3.7069584   -3.2603871
N     2.4722266   -1.9802869   -4.7284625
C     2.8286973   -0.9985816   -3.7166776
C     1.5418235   -0.2572155   -3.3227292
O     0.9305995    0.3514596   -4.2099281
C     3.8772720    0.0117200   -4.2232519
C     2.666846    0.9667498   -3.0883311
C     5.102463   -0.7017490   -4.8001576
N     1.1167953   -0.3270511   -2.0339471
C     0.3297260   -1.7378174   -0.8815317
C     -1.1637490   -3.3898163   -0.9830606
O     -0.0656264    0.4614627   -1.6625694
C     0.7779722    1.5259970    0.5583999
C     0.5803792    2.7479111    1.4240118
O     1.3735391    2.4456638   -1.5597871
O     0.0763600    3.7742631    1.0029384
C     -1.5535283   -1.5696137   -1.8971133
C     -2.3700975   -2.6559985   -1.1978228
C     -2.379386    0.4664222   -0.6068066
C     1.8397257   -1.0857222   -1.0132729
C     0.9432201    3.2658872   -2.6090582
C     2.1911362    2.4510939    4.7102489
C     -3.3379402    4.6115824    4.6331349
C     -2.3885289    3.4114141    4.5367764
C     -0.5114448    6.0367356    3.3067187
C     -3.0781121    2.2658789    3.7977922
N     0.9359480    2.5416614    2.7811241
C     -0.1048072    2.5809975    3.8263720
C     0.7205688    2.5939287    1.5391373
C     2.2081988    3.0392645    3.2993087
C     -1.1074469    3.7501382    3.7459237
O     -0.5120403    4.9515807    4.2200190
O     -3.2644183    2.3078434    2.5781652
O     -4.1816399   -2.3030305    3.9211445
C     -5.6511575    0.0796140    3.9792928
N     -3.4708279    1.2193576    4.5652829
C     -4.1629923    0.0579202    4.0284176
**2B: trans-MMAF**

**N**
-0.2406557 58.6041143 20.1379034
-0.9955919 59.2735267 20.5378677
-0.1284006 57.1545512 20.1914085
-1.5224353 56.4969032 20.0535083
-1.4570242 54.9943435 20.3279707
-1.9875255 54.7807619 18.7139347
0.8219094 56.6678962 19.0961421
0.8121040 57.1205348 17.9506729

**C**
1.6602767 55.6818311 19.4874000
2.4745923 54.9296839 18.5468941
1.7412484 53.6126132 18.2686524
1.4922013 52.8751589 19.2300620
3.8782038 54.8427705 19.1068446
4.6984409 53.8569907 18.0827664
4.5792473 55.9423985 19.4973209
3.8186777 53.3273884 16.9971669
1.7818256 51.0296797 16.1417219
-0.5537635 52.1396306 15.9383669
0.7580325 52.0206952 16.7318900
2.2577450 51.3456084 14.7341755
3.1032164 50.1971882 14.2089780
2.9594792 50.9896409 16.9457660
2.8320037 49.0243755 14.4848780
-1.5210615 53.0856283 16.6677672
-2.7647766 53.4521459 15.8635758
-1.1728496 50.7539928 15.7287917
1.6255928 54.2607217 15.8957735
2.8592764 50.1208236 18.0654008
| C     | X     | Y     | Z     |
|-------|-------|-------|-------|
| 5.1941170 | 51.5387951 | 11.6102859 |
| 7.6415451 | 47.148911 | 14.669585 |
| 6.8195929 | 47.7505973 | 14.669585 |
| 6.2006541 | 50.0964851 | 16.0581042 |
| 6.0045992 | 46.6628151 | 12.8565838 |
| 5.8661850 | 48.8396316 | 14.0522665 |
| 6.6340637 | 48.807482 | 14.7320480 |
| 5.2158874 | 49.904606 | 12.9180038 |
| 6.4505969 | 44.5195609 | 10.2632145 |
| 6.024825 | 46.5287775 | 11.5232810 |
| 5.177990 | 52.523705 | 10.7390005 |
| 7.247773 | 46.1251851 | 9.5736892 |
| 2.8182082 | 46.927446 | 10.3766821 |
| 3.7472089 | 44.416873 | 9.420321 |
| 9.198655 | 47.9109010 | 11.4406777 |
| 2.8373894 | 49.4210931 | 9.8107446 |
| 3.7458644 | 47.148911 | 10.0220843 |
| 1.9235035 | 45.199899 | 18.7901063 |
| -0.9894173 | 58.8821475 | 20.7506586 |
| 1.7678800 | 59.0818656 | 19.7895153 |
| 1.3747209 | 58.9348262 | 21.5141500 |
| 0.8275311 | 60.3493274 | 20.5836886 |
| 0.2895315 | 56.8228016 | 21.157091 |
| -2.1248910 | 50.9576273 | 20.8461553 |
| -1.0043028 | 54.7859308 | 21.3008939 |
| -0.8704288 | 54.4742568 | 19.5673847 |
| -2.4610756 | 54.5671801 | 20.321093 |
| -1.6722094 | 56.2943739 | 17.8945359 |
| -2.2166870 | 57.8572710 | 18.5060174 |
| -3.2263090 | 56.4161608 | 18.7306313 |
| 1.5421533 | 55.2903308 | 20.410638 |
| 5.605843 | 55.5533810 | 17.6616330 |
| 3.7466813 | 54.0212551 | 19.9989518 |
| 4.8728065 | 54.4614081 | 17.187177 |
| 4.1983451 | 52.9345100 | 17.7791533 |
| 5.6730440 | 53.5943383 | 18.4999147 |
| 4.0043458 | 56.5023265 | 20.2367437 |
| 4.7195478 | 56.5825446 | 18.6211881 |
| 5.5630666 | 55.7282663 | 19.9203377 |
| 1.3323069 | 50.0332151 | 16.1369717 |
| -0.3512734 | 52.5643912 | 14.9487964 |
| 0.5033529 | 51.6468003 | 17.7243231 |
| 2.8315845 | 52.2684549 | 14.7444690 |
| 1.4077902 | 51.4876532 | 14.0611012 |
| -0.9860476 | 54.004524 | 16.9199006 |
| -1.8087270 | 52.683238 | 17.6206216 |
| -3.899487 | 52.5826151 | 15.6501504 |
| -2.4882157 | 53.9091037 | 14.9093528 |
| -3.3768743 | 54.1708668 | 16.4128580 |
| -1.305933 | 50.2430541 | 16.6865269 |
| -0.5505711 | 50.1221729 | 15.0934867 |
| -2.1485039 | 50.8371147 | 15.2487749 |
| 2.6869671 | 54.3263637 | 15.699290 |
| 1.2511045 | 55.2536474 | 16.1432053 |
| 1.0436059 | 53.9065903 | 15.0181537 |
| 2.9911567 | 49.1103994 | 17.7398538 |
| 2.127233 | 50.4879795 | 18.7901063 |
| 3.8431957 | 50.1019468 | 18.5325035 |
| 4.4489539 | 51.3620499 | 10.8343370 |
| 5.845798 | 52.3489448 | 11.2858979 |
| 8.3456556 | 46.4026066 | 14.2835377 |
| 6.9840787 | 46.6510973 | 15.3887335 |
| 8.2022921 | 47.9248188 | 15.1787356 |
| 7.4956055 | 48.2046311 | 12.8019295 |
| 6.2894966 | 49.1954377 | 16.6745858 |
| 5.1621820 | 50.4481837 | 16.0831260 |
| 6.8574372 | 50.8694727 | 16.4565650 |
| 4.4620563 | 48.7254321 | 12.4273319 |
| 6.9138091 | 50.4776950 | 12.3774498 |
| 6.1346164 | 49.6534115 | 11.0194781 |
| 3.5825215 | 52.4435901 | 12.7713434 |
| 5.1433019 | 52.3883933 | 13.6177215 |
2TS: MMAF 2A↔2B transition state

...
2C: internally hydrogen bonded MMAF

N  0.5214915  -4.6214203  0.3975345
C  0.8564307  -4.5996631  1.8204589
C  1.4920284  -3.9205205  -0.4350293
C  1.0915586  -3.9532322  -1.9209015
C  0.7561541  -5.3662367  -2.3912174
C  2.1936173  -3.3296722  -2.7796630
C  1.6257646  -2.4595646  -0.0020987
O  0.6519899  -1.6945157  -0.0294624
N  2.3854464  -2.0603280  0.4388346
C  3.1187562  -0.6847671  0.7730734
C  4.0086420  -0.1016576  -0.3313202
O  4.8964242  -0.8118289  -0.8233156
C  3.7518221  -0.5222340  2.1793949
C  2.7288081  -0.9143395  3.2412777
C  5.0630003  -1.2891917  2.3456333
N  3.7857700  1.1716790  -0.7322691
C  1.3715150  1.6057585  -0.9324112
| Atom | X    | Y    | Z    |
|------|------|------|------|
| H    | 1.5251944 | 0.9304686 | -4.1560928 |
| H    | 0.8069766 | -0.0654082 | -2.8632826 |
| H    | -4.4448033 | 3.6174138 | 1.1404697 |
| H    | -4.3773016 | 1.8534622 | 1.3008836 |
| H    | -1.7055247 | -2.0485747 | 3.6970284 |
| H    | -1.5968773 | -2.7809751 | 2.1390959 |
| H    | -2.8727346 | -2.2350259 | 3.2398430 |
| H    | -0.8146032 | -0.4244082 | 1.8727816 |
| H    | -1.9768323 | 0.5020935 | 6.4148128 |
| H    | -3.4805272 | 0.6576945 | 5.4655224 |
| H    | -2.2660688 | 1.9626976 | 5.4439581 |
| H    | -1.5829623 | 2.2334011 | 3.3483580 |
| H    | -3.8772857 | 2.6122407 | 3.5810268 |
| H    | -2.8234057 | 3.8938692 | 2.9682111 |
| H    | -2.2513051 | 3.6910139 | 0.1567879 |
| H    | -2.7694887 | 2.0960155 | -0.4185057 |
| H    | -3.4107292 | 0.3571576 | 3.2385289 |
| H    | -1.4988367 | -1.4024797 | -0.1134513 |
| H    | -3.9591199 | -1.4175870 | -1.0271453 |
| H    | -1.4910408 | -1.0016809 | -2.7764459 |
| H    | -1.0961977 | 1.2315049 | -3.3469924 |
| H    | -5.0097480 | 0.1556959 | -1.9687049 |
| H    | -1.7875257 | 3.5895729 | -3.4795185 |
| H    | -5.7055813 | 2.5056115 | -2.1125480 |
| H    | -4.1039104 | 4.2440317 | -2.8648184 |
| O    | -3.4040160 | -3.6122595 | -2.4868628 |
| O    | -2.3565469 | -3.8700474 | -0.5200312 |
| H    | -3.3754086 | -4.5838789 | -2.4622681 |