Supporting Information

Computer-assisted ^{1H} NMR Analysis of the anti-Tuberculosis Drug Lead Ecumicin

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PMS file of ecumicin

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* NEW: the lines beginning by * are comment lines !
* To keep all the chemical shifts fixed during iteration
* replace "CHEMICAL SHIFTS(HZ):" by "..SHIFTS(HZ): fixed"
* The couplings can be fixed in the same way

NMR-data: C:\Users\jgnapo_2\Desktop\Wei_H14_XRays\H14_withoutGO
#$œ  Date  2. 8.2015;  Time 15:36:18     perch.10t

CHEMICAL SHIFTS(PPM):
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 10Trp_H8/ 1     6.981697  1*1*1  STAT=Y  PRED= 7.318 RANGE= 0.199 WIDTH(Y)= 1.202 RESP(Y)= 0.2709 HSQC= C74
 01ValH45/ 1     2.312742  1*1*6  STAT=Y  PRED= 2.370 RANGE= 0.307 WIDTH(Y)= 1.540 RESP(Y)= 0.2343 HSQC= C1_2
 01Val_H2/ 1     2.671555  1*1*1  STAT=Y  PRED= 4.169 RANGE= 0.639 WIDTH(Y)= 1.546 RESP(Y)= 0.2308 HSQC= C4
 01Val_H3/ 1     2.042422  1*1*1  STAT=Y  PRED= 1.952 RANGE= 0.819 WIDTH(Y)= 1.500 RESP(Y)= 0.1894 HSQC= C5
 01Val_H6/ 1     0.847129  1*1*3  STAT=Y  PRED= 0.509 RANGE= 0.869 WIDTH(Y)= 1.594 RESP(Y)= 0.2601 HSQC= C6
 01Val_H7/ 1     0.978224  1*1*3  STAT=Y  PRED= 0.818 RANGE= 0.359 WIDTH(Y)= 1.729 RESP(Y)= 0.2412 HSQC= C7
 02Val_H2/ 1     4.669049  1*1*1  STAT=Y  PRED= 4.508 RANGE= 0.809 WIDTH(Y)= 1.460 RESP(Y)= 0.2285 HSQC= C10
 02Val_H3/ 1     2.101392  1*1*1  STAT=Y  PRED= 2.155 RANGE= 0.779 WIDTH(Y)= 1.538 RESP(Y)= 0.2196 HSQC= C12
 02Val_H4/ 1     0.992211  1*1*3  STAT=Y  PRED= 1.038 RANGE= 0.279 WIDTH(Y)= 1.609 RESP(Y)= 0.2098 HSQC= C13
 02Val_H5/ 1     1.055395  1*1*3  STAT=Y  PRED= 0.943 RANGE= 0.249 WIDTH(Y)= 1.527 RESP(Y)= 0.2420 HSQC= C14
 03Ile_H7/ 1     3.233919  1*1*3  STAT=Y  PRED= 3.196 RANGE= 0.369 WIDTH(Y)= 1.517 RESP(Y)= 0.2365 HSQC= C15
 03Ile_H2/ 1     4.918278  1*1*1  STAT=Y  PRED= 4.617 RANGE= 1.628 WIDTH(Y)= 1.484 RESP(Y)= 0.2320 HSQC= C17
 03Ile_H3/ 1     1.957453  1*1*1  STAT=Y  PRED= 2.032 RANGE= 0.706 WIDTH(Y)= 1.597 RESP(Y)= 0.1798 HSQC= C19
 03IleH4A/ 1     0.988898  1*1*1  STAT=Y  PRED= 1.465 RANGE= 0.699 WIDTH(Y)= 3.116 RESP(Y)= 0.2201 HSQC= C20
 03IleH4B/ 1     1.246429  1*1*1  STAT=Y  PRED= 1.175 RANGE= 0.589 WIDTH(Y)= 1.972 RESP(Y)= 0.2221 HSQC= C20
 03Ile_H6/ 1     0.753937  1*1*3  STAT=Y  PRED= 0.723 RANGE= 0.398 WIDTH(Y)= 1.603 RESP(Y)= 0.2304 HSQC= C21
 03Ile_H5/ 1     0.743882  1*1*3  STAT=Y  PRED= 0.880 RANGE= 0.219 WIDTH(Y)= 1.382 RESP(Y)= 0.1977 HSQC= C22
 04Thr_H2/ 1     5.166931  1*1*1  STAT=Y  PRED= 4.668 RANGE= 1.016 WIDTH(Y)= 1.377 RESP(Y)= 0.2104 HSQC= C24
 04Thr_H3/ 1     5.783152  1*1*1  STAT=Y  PRED= 4.010 RANGE= 0.479 WIDTH(Y)= 1.946 RESP(Y)= 0.2161 HSQC= C25
 04Thr_H4/ 1     1.313137  1*1*3  STAT=Y  PRED= 1.270 RANGE= 0.499 WIDTH(Y)= 1.802 RESP(Y)= 0.2391 HSQC= C27
 05Thr_H5/ 1     3.329505  1*1*3  STAT=Y  PRED= 3.257 RANGE= 0.399 WIDTH(Y)= 1.449 RESP(Y)= 0.2122 HSQC= C29
 05Thr_H2/ 1     5.017576  1*1*1  STAT=Y  PRED= 4.099 RANGE= 1.025 WIDTH(Y)= 1.510 RESP(Y)= 0.2313 HSQC= C31
 05Thr_H3/ 1     4.457472  1*1*1  STAT=Y  PRED= 4.345 RANGE= 0.719 WIDTH(Y)= 1.566 RESP(Y)= 0.2173 HSQC= C32
 05Thr_H4/ 1     0.911822  1*1*3  STAT=Y  PRED= 1.252 RANGE= 0.299 WIDTH(Y)= 1.985 RESP(Y)= 0.2332 HSQC= C34
 06Val_H2/ 1     4.842219  1*1*1  STAT=Y  PRED= 4.525 RANGE= 1.409 WIDTH(Y)= 1.387 RESP(Y)= 0.1816 HSQC= C37
 06Val_H3/ 1     2.350365  1*1*1  STAT=Y  PRED= 1.975 RANGE= 1.019 WIDTH(Y)= 1.997 RESP(Y)= 0.2211 HSQC= C38
 06Val_H4/ 1     1.092249  1*1*3  STAT=Y  PRED= 0.938 RANGE= 0.219 WIDTH(Y)= 1.857 RESP(Y)= 0.2201 HSQC= C39
 06Val_H5/ 1     0.979237  1*1*3  STAT=Y  PRED= 1.094 RANGE= 0.389 WIDTH(Y)= 2.253 RESP(Y)= 0.2262 HSQC= C40
 07Leu_H7/ 1     3.258849  1*1*3  STAT=Y  PRED= 3.110 RANGE= 0.376 WIDTH(Y)= 1.427 RESP(Y)= 0.2063 HSQC= C42
 07Leu_H2/ 1     5.110367  1*1*1  STAT=Y  PRED= 5.342 RANGE= 1.085 WIDTH(Y)= 2.148 RESP(Y)= 0.2041 HSQC= C44
 07LeuH3A/ 1     1.452290  1*1*1  STAT=Y  PRED= 1.444 RANGE= 0.429 WIDTH(Y)= 2.191 RESP(Y)= 0.1625 HSQC= C45
 07LeuH3B/ 1     1.238553  1*1*1  STAT=Y  PRED= 1.914 RANGE= 0.369 WIDTH(Y)= 2.174 RESP(Y)= 0.1754 HSQC= C45
 07Leu_H4/ 1     0.956881  1*1*1  STAT=Y  PRED= 1.630 RANGE= 0.627 WIDTH(Y)= 1.885 RESP(Y)= 0.2043 HSQC= C46
 07Leu_H5/ 1     0.330640  1*1*3  STAT=Y  PRED= 0.972 RANGE= 0.316 WIDTH(Y)= 1.951 RESP(Y)= 0.1929 HSQC= C47
 07Leu_H6/ 1     0.168232  1*1*3  STAT=Y  PRED= 0.972 RANGE= 0.149 WIDTH(Y)= 1.879 RESP(Y)= 0.1826 HSQC= C48
 08Val_H2/ 1     4.594360  1*1*1  STAT=Y  PRED= 4.489 RANGE= 0.899 WIDTH(Y)= 1.577 RESP(Y)= 0.2235 HSQC= C51
 08Val_H3/ 1     2.026606  1*1*1  STAT=Y  PRED= 1.995 RANGE= 0.486 WIDTH(Y)= 1.987 RESP(Y)= 0.1988 HSQC= C52
 08Val_H4/ 1     0.915094  1*1*3  STAT=Y  PRED= 1.060 RANGE= 0.237 WIDTH(Y)= 1.744 RESP(Y)= 0.2213 HSQC= C53
 08Val_H5/ 1     0.858567  1*1*3  STAT=Y  PRED= 0.999 RANGE= 0.229 WIDTH(Y)= 2.065 RESP(Y)= 0.2242 HSQC= C54
 09Val_H6/ 1     3.139915  1*1*3  STAT=Y  PRED= 3.169 RANGE= 0.466 WIDTH(Y)= 1.935 RESP(Y)= 0.2020 HSQC= C56
 09Val_H2/ 1     3.065437  1*1*1  STAT=Y  PRED= 5.153 RANGE= 0.719 WIDTH(Y)= 2.564 RESP(Y)= 0.1945 HSQC= C58
 09Val_H3/ 1     2.582934  1*1*1  STAT=Y  PRED= 2.524 RANGE= 0.407 WIDTH(Y)= 1.688 RESP(Y)= 0.1785 HSQC= C59
 09Val_H4/ 1     1.093789  1*1*3  STAT=Y  PRED= 1.319 RANGE= 0.227 WIDTH(Y)= 1.760 RESP(Y)= 0.2367 HSQC= C60
 09Val_H5/ 1     0.975923  1*1*3  STAT=Y  PRED= 1.075 RANGE= 0.239 WIDTH(Y)= 2.293 RESP(Y)= 0.2267 HSQC= C61
 10TrpH13/ 1     2.157313  1*1*3  STAT=Y  PRED= 2.936 RANGE= 0.895 WIDTH(Y)= 1.868 RESP(Y)= 0.2038 HSQC= C63
 10Trp_H2/ 1     4.102008  1*1*1  STAT=Y  PRED= 5.043 RANGE= 1.765 WIDTH(Y)= 2.033 RESP(Y)= 0.1981 HSQC= C65
 10TrpH3A/ 1     3.544880  1*1*1  STAT=Y  PRED= 3.613 RANGE= 0.469 WIDTH(Y)= 1.497 RESP(Y)= 0.2060 HSQC= C66
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 10TrpH3B/ 1     3.693816  1*1*1  STAT=Y  PRED= 3.367 RANGE= 0.629 WIDTH(Y)= 2.091 RESP(Y)= 0.2077 HSQC= C66
 10Trp_H5/ 1     6.698366  1*1*1  STAT=Y  PRED= 6.772 RANGE= 0.276 WIDTH(Y)= 1.646 RESP(Y)= 0.2387 HSQC= C68
 10Trp_H9/ 1     6.441818  1*1*1  STAT=Y  PRED= 7.089 RANGE= 0.219 WIDTH(Y)= 1.124 RESP(Y)= 0.2512 HSQC= C73
 10Trp_H7/ 1     6.918046  1*1*1  STAT=Y  PRED= 7.333 RANGE= 0.309 WIDTH(Y)= 0.720 RESP(Y)= 0.2360 HSQC= C75
 10TrpH12/ 1     3.825512  1*1*3  STAT=Y  PRED= 4.072 RANGE= 0.249 WIDTH(Y)= 1.239 RESP(Y)= 0.2230 HSQC= C77
 11Val_H2/ 1     4.528574  1*1*1  STAT=Y  PRED= 4.303 RANGE= 1.449 WIDTH(Y)= 1.881 RESP(Y)= 0.2193 HSQC= C80
 11Val_H3/ 1     2.200073  1*1*1  STAT=Y  PRED= 2.094 RANGE= 0.589 WIDTH(Y)= 1.787 RESP(Y)= 0.2038 HSQC= C81
 11Val_H4/ 1     1.028232  1*1*3  STAT=Y  PRED= 1.094 RANGE= 0.249 WIDTH(Y)= 2.114 RESP(Y)= 0.2186 HSQC= C82
 11Val_H5/ 1     0.989110  1*1*3  STAT=Y  PRED= 1.080 RANGE= 0.299 WIDTH(Y)= 1.810 RESP(Y)= 0.1543 HSQC= C83
 12Phe_H2/ 1     4.853685  1*1*1  STAT=Y  PRED= 4.499 RANGE= 0.989 WIDTH(Y)= 1.248 RESP(Y)= 0.1976 HSQC= C86
 12Phe_H3/ 1     5.343836  1*1*1  STAT=Y  PRED= 5.208 RANGE= 0.489 WIDTH(Y)= 1.873 RESP(Y)= 0.2247 HSQC= C87
 12Phe_H5/ 1     7.239580  1*2*1  STAT=Y  PRED= 7.524 RANGE= 0.309 WIDTH(Y)= 2.159 RESP(Y)= 0.2433 HSQC= C89_93
 12Phe_H6/ 1     7.255476  1*2*1  STAT=Y  PRED= 7.372 RANGE= 0.579 WIDTH(Y)= 1.167 RESP(Y)= 0.2391 HSQC= C90_92
 12Phe_H7/ 1     7.200628  1*1*1  STAT=Y  PRED= 7.281 RANGE= 0.309 WIDTH(Y)= 1.552 RESP(Y)= 0.2565 HSQC= C91
 13Val_H2/ 1     4.396075  1*1*1  STAT=Y  PRED= 4.169 RANGE= 0.669 WIDTH(Y)= 1.198 RESP(Y)= 0.2136 HSQC= C97
 13Val_H3/ 1     1.967770  1*1*1  STAT=Y  PRED= 1.935 RANGE= 0.869 WIDTH(Y)= 1.937 RESP(Y)= 0.2420 HSQC= C99
 13Val_H4/ 1     0.934957  1*1*3  STAT=Y  PRED= 1.277 RANGE= 0.219 WIDTH(Y)= 2.639 RESP(Y)= 0.2817 HSQC= C100
 13Val_H5/ 1     0.919351  1*1*3  STAT=Y  PRED= 0.933 RANGE= 0.278 WIDTH(Y)= 2.019 RESP(Y)= 0.2636 HSQC= C101

COUPLING CONSTANTS(HZ):
 J115_217    7.7949   J 10Trp_H8 10Trp_H9   STAT=Y  PRED= 8.100 RANGE= 0.890
 J115_218    8.1831   J 10Trp_H8 10Trp_H7   STAT=Y  PRED= 7.850 RANGE= 0.800
 J122_123    9.1830   J 01Val_H2 01Val_H3   STAT=Y  PRED= 12.510 RANGE= 4.000
 J123_124    6.5874   J 01Val_H3 01Val_H6   STAT=Y  PRED= 6.640 RANGE= 0.200
 J123_127    6.6158   J 01Val_H3 01Val_H7   STAT=Y  PRED= 6.640 RANGE= 0.200
 J131_132    8.7551   J 02Val_H2 02Val_H3   STAT=Y  PRED= 12.600 RANGE= 6.000
 J132_133    6.7883   J 02Val_H3 02Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J132_136    6.7203   J 02Val_H3 02Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J142_143   11.2368   J 03Ile_H2 03Ile_H3   STAT=Y  PRED= 13.150 RANGE= 4.000
 J143_144    1.5555   J 03Ile_H3 03IleH4A   STAT=Y  PRED= 1.950 RANGE= 3.000
 J143_145    3.3192   J 03Ile_H3 03IleH4B   STAT=Y  PRED= 12.660 RANGE= 2.800
 J143_146    6.6322   J 03Ile_H3 03Ile_H6   STAT=Y  PRED= 6.640 RANGE= 0.200
 J144_145  -12.9087   J 03IleH4A 03IleH4B   STAT=Y  PRED= -13.400 RANGE= 0.600
 J144_149    7.2572   J 03IleH4A 03Ile_H5   STAT=Y  PRED= 7.440 RANGE= 0.150
 J145_149    7.6370   J 03IleH4B 03Ile_H5   STAT=Y  PRED= 7.440 RANGE= 0.150
 J153_154    2.3441   J 04Thr_H2 04Thr_H3   STAT=Y  PRED= 2.690 RANGE= 6.600
 J154_155    6.5142   J 04Thr_H3 04Thr_H4   STAT=Y  PRED= 6.250 RANGE= 0.600
 J161_162    3.7286   J 05Thr_H2 05Thr_H3   STAT=Y  PRED= 2.440 RANGE= 4.400
 J162_164    6.4627   J 05Thr_H3 05Thr_H4   STAT=Y  PRED= 6.250 RANGE= 0.600
 J168_169    8.9241   J 06Val_H2 06Val_H3   STAT=Y  PRED= 2.980 RANGE= 6.600
 J169_170    6.6943   J 06Val_H3 06Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J169_173    7.0127   J 06Val_H3 06Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J179_180    8.5788   J 07Leu_H2 07LeuH3A   STAT=Y  PRED= 3.040 RANGE= 6.000
 J179_181    6.5941   J 07Leu_H2 07LeuH3B   STAT=Y  PRED= 13.950 RANGE= 4.000
 J180_181  -13.5581   J 07LeuH3A 07LeuH3B   STAT=Y  PRED= -14.710 RANGE= 1.600
 J180_182    5.4402   J 07LeuH3A 07Leu_H4   STAT=Y  PRED= 12.990 RANGE= 2.560
 J181_182    7.9864   J 07LeuH3B 07Leu_H4   STAT=Y  PRED= 2.270 RANGE= 3.000
 J182_183    6.5528   J 07Leu_H4 07Leu_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J182_186    6.5909   J 07Leu_H4 07Leu_H6   STAT=Y  PRED= 6.640 RANGE= 0.200
 J190_191    8.8837   J 08Val_H2 08Val_H3   STAT=Y  PRED= 12.880 RANGE= 6.000
 J191_192    6.5495   J 08Val_H3 08Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J191_195    6.7835   J 08Val_H3 08Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J201_202    7.6228   J 09Val_H2 09Val_H3   STAT=Y  PRED= 12.960 RANGE= 4.000
 J202_203    6.4970   J 09Val_H3 09Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J202_206    6.8287   J 09Val_H3 09Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J212_213   11.1639   J 10Trp_H2 10TrpH3A   STAT=Y  PRED= 12.890 RANGE= 4.000
 J212_214    4.7143   J 10Trp_H2 10TrpH3B   STAT=Y  PRED= 1.900 RANGE= 4.400
 J213_214  -13.7268   J 10TrpH3A 10TrpH3B   STAT=Y  PRED= -16.760 RANGE= 2.560
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 J215_218    0.4848   J 10Trp_H5 10Trp_H7   STAT=Y  PRED= -0.300 RANGE= 0.500
 J217_218    0.6721   J 10Trp_H9 10Trp_H7   STAT=Y  PRED= 1.020 RANGE= 1.200
 J223_224    7.9107   J 11Val_H2 11Val_H3   STAT=Y  PRED= 3.450 RANGE= 9.000
 J224_225    6.7500   J 11Val_H3 11Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J224_228    6.8020   J 11Val_H3 11Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200
 J232_233    1.8995   J 12Phe_H2 12Phe_H3   STAT=Y  PRED= 1.900 RANGE= 6.600
 J234_238    1.3331   J 12Phe_H5 12Phe_H5   STAT=Y  PRED= 1.980 RANGE= 1.200
 J234_235    7.6508   J 12Phe_H5 12Phe_H6   STAT=Y  PRED= 7.660 RANGE= 0.340
 J234_237    0.7591   J 12Phe_H6 12Phe_H5   STAT=Y  PRED= 0.550 RANGE= 0.750
 J234_236    1.2534   J 12Phe_H5 12Phe_H7   STAT=Y  PRED= 1.260 RANGE= 1.200
 J235_237    1.7067   J 12Phe_H6 12Phe_H6   STAT=Y  PRED= 1.480 RANGE= 1.200
 J235_236    7.4723   J 12Phe_H6 12Phe_H7   STAT=Y  PRED= 7.400 RANGE= 0.240
 J241_242    8.8995   J 13Val_H2 13Val_H3   STAT=Y  PRED= 12.320 RANGE= 6.000
 J242_243    6.8280   J 13Val_H3 13Val_H4   STAT=Y  PRED= 6.640 RANGE= 0.200
 J242_246    6.4124   J 13Val_H3 13Val_H5   STAT=Y  PRED= 6.640 RANGE= 0.200

CONTROL PARAMETERS:
       Solvent =  none (def. 99% enriched)
         1.000 =  Concentration (vol%, def=1.0%)
    0.00100000 =  Minimum line-intensity
    0.00100000 =  Diagonalization criterium (not in use)
   11.63298470 =  Left frequency (ppm)
   -4.33373638 =  Right frequency (ppm)
        10.000 =  Acquisition time (s, for QMTLS)
         0.000 =  Line-width (for modes D, P & T, 0=use defaults)
   0.103379714 =  Data-point resolution (Hz)
        27.781 =  GAUSSIAN (%, 0=use default from INF)
        -9.448 =  Dispersion contribution (%, 0=use default from INF)
    0.00000000 =  Decoupling frequency (for DORES)

CONSTRAINTS (in equations X0 = 1.0)...use no empty lines
 EQUAL 01Val_H2 = 13Val_H2
 EQUAL 06Val_H5 = 11Val_H4
 EQUAL 07Leu_H5 = 07Leu_H6
 IGNORE(HZ):   940.350 to   936.356
 IGNORE(HZ):  2062.779 to  2027.293
 IGNORE(HZ):  1175.285 to  1149.842
 IGNORE(HZ):  1232.768 to  1209.210
 IGNORE(HZ):  5188.061 to  5161.182
 IGNORE(HZ):  4803.141 to  4787.420
 IGNORE(HZ):   812.060 to   780.231

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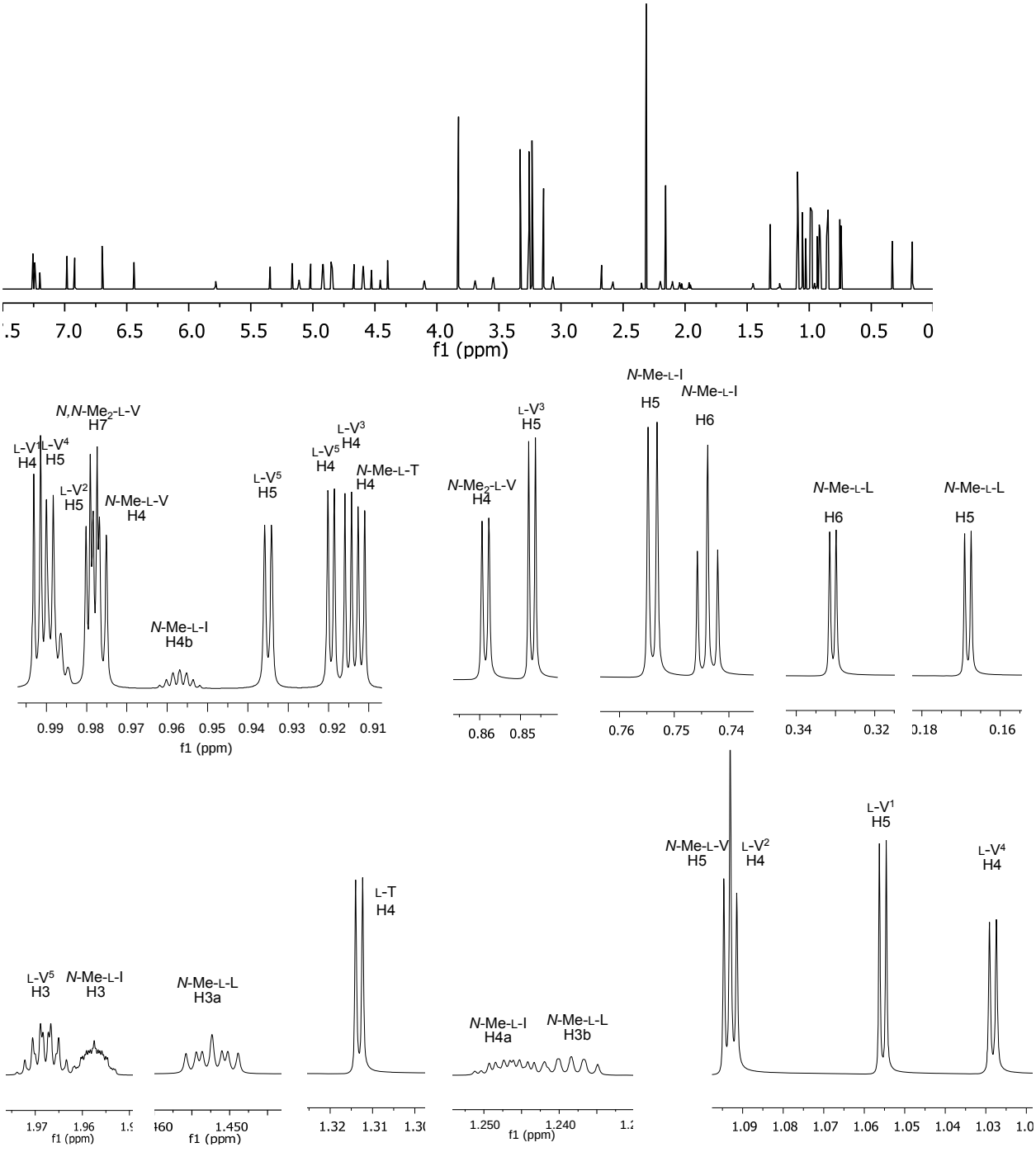
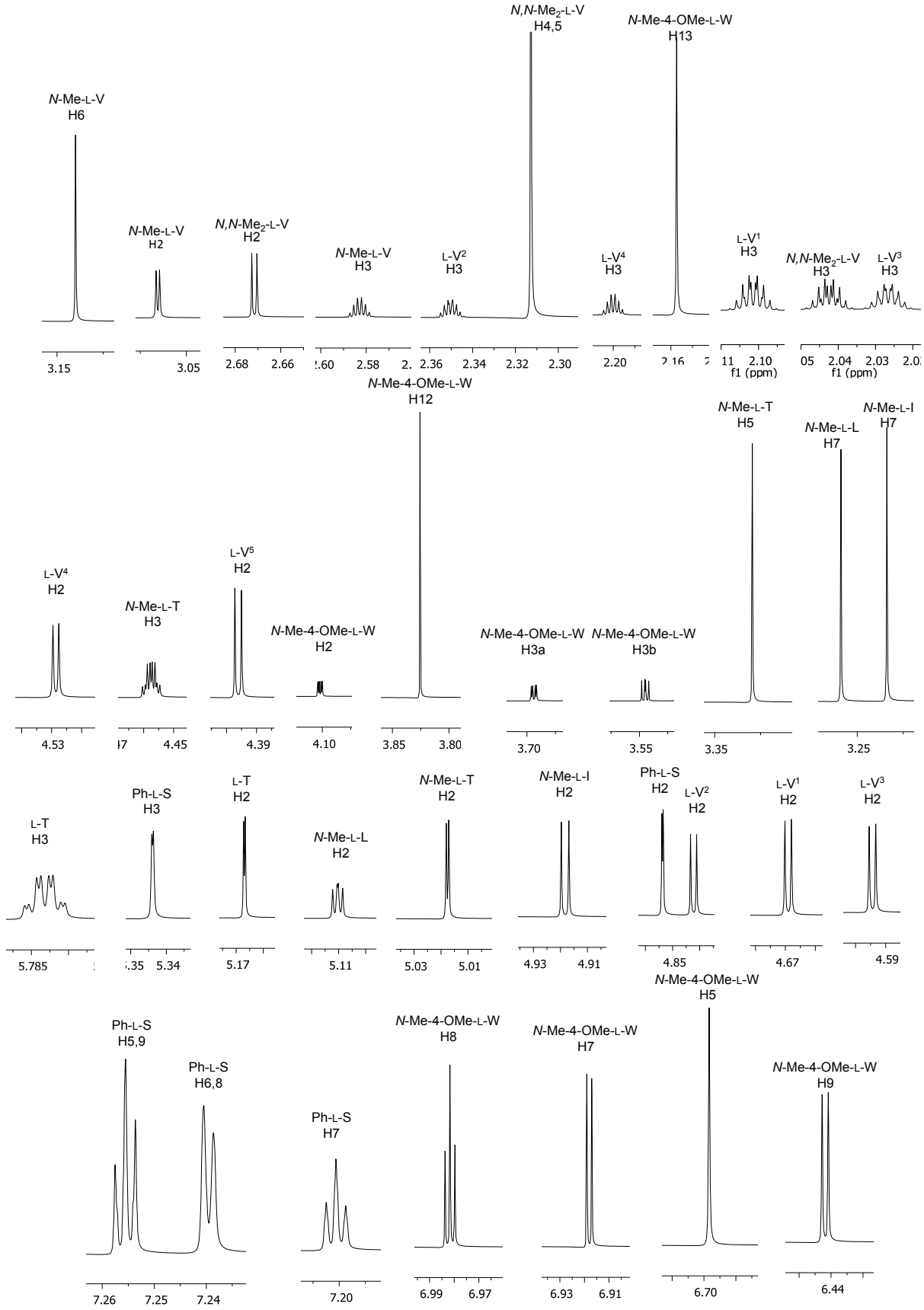
Supporting Figure 1. The HiFSA fingerprint of ecumicin (**1**) calculated at 4 GHz demonstrates that presently unattainable NMR magnetic fields will be required to achieve a near first order ^{1}H NMR spectrum, suitable for visual analysis of this oligopeptide.

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L-V2 H3
L-V4 H3
L-V1 H3
N,N-Me2-L-V H3
L-V3 H3
N-Me-4-OMe-L-W H12
N-Me-L-T H5
N-Me-L-L H7
N-Me-L-I H7
L-V4 H2
N-Me-L-T H3
L-V5 H2
N-Me-4-OMe-L-W H2
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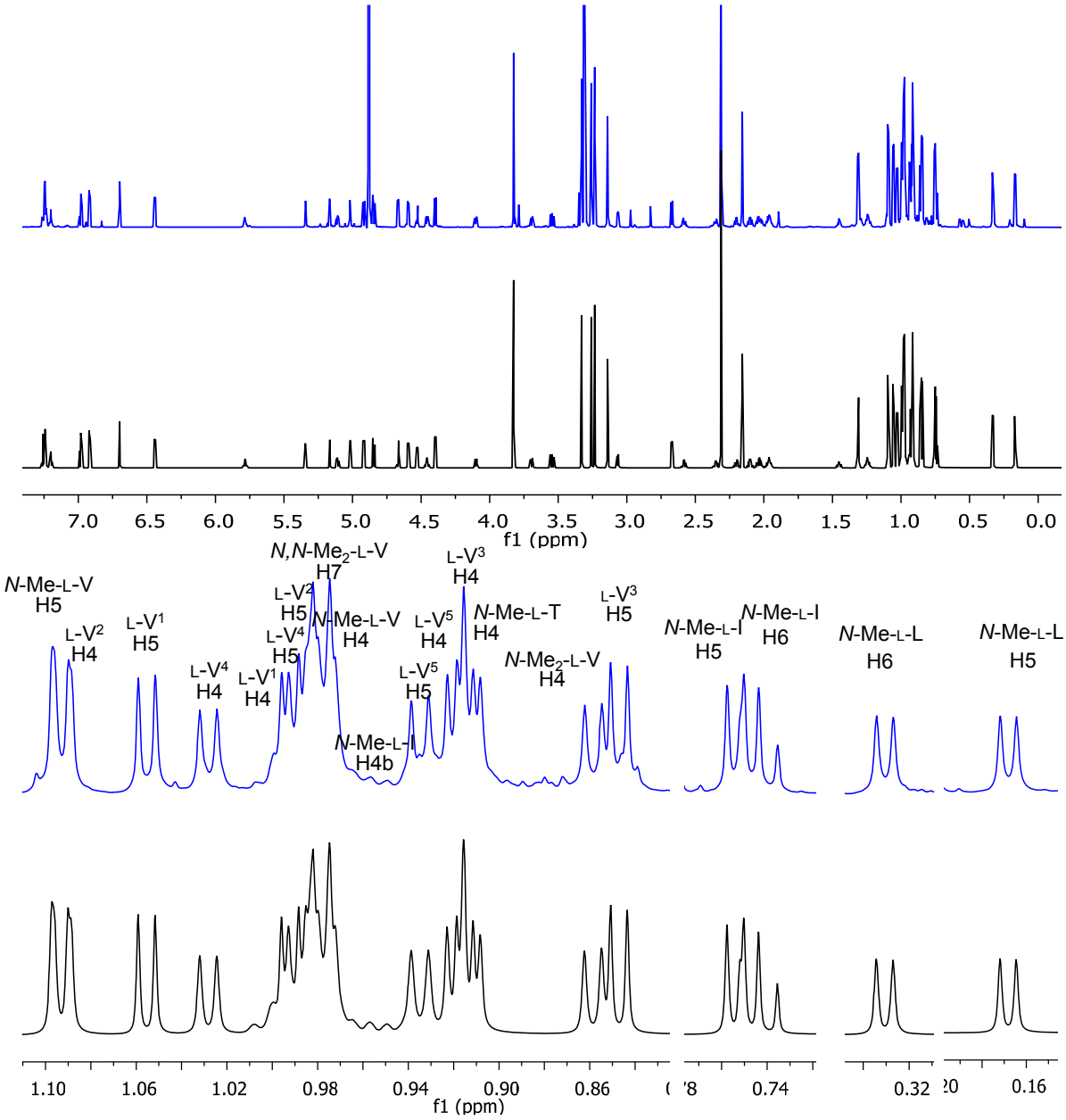
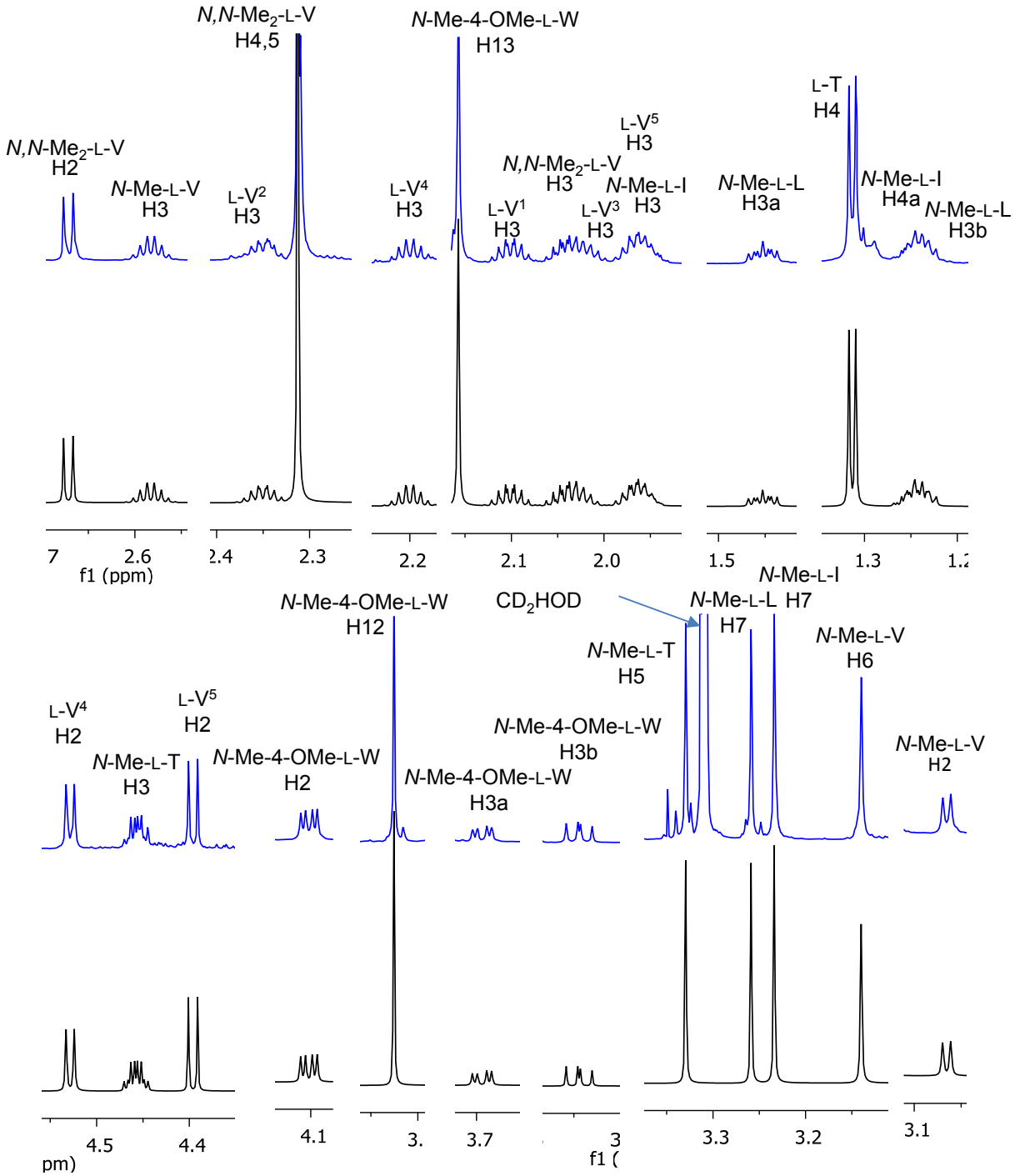
N-Me-4-OMe-L-W H3b
L-T H3
Ph-L-S H3
L-T H2
N-Me-L-L H2
N-Me-L-T H2
N-Me-L-I H2
Ph-L-S H2
L-V2 H2
L-V1 H2
L-V3 H2
N-Me-4-OMe-L-W H5
Ph-L-S H5,9
Ph-L-S H6,8
Ph-L-S H7
N-Me-4-OMe-L-W H8
N-Me-4-OMe-L-W H7
N-Me-4-OMe-L-W H9
f1 (ppm)
f1 (ppm)

Supporting Figure 2. Comparison of the HiFSA fingerprint of ecumicin (**1**) calculated at 900 MHz (black) to the 900MHz experimental spectrum (blue) with expansions. RMS = 0.104%.

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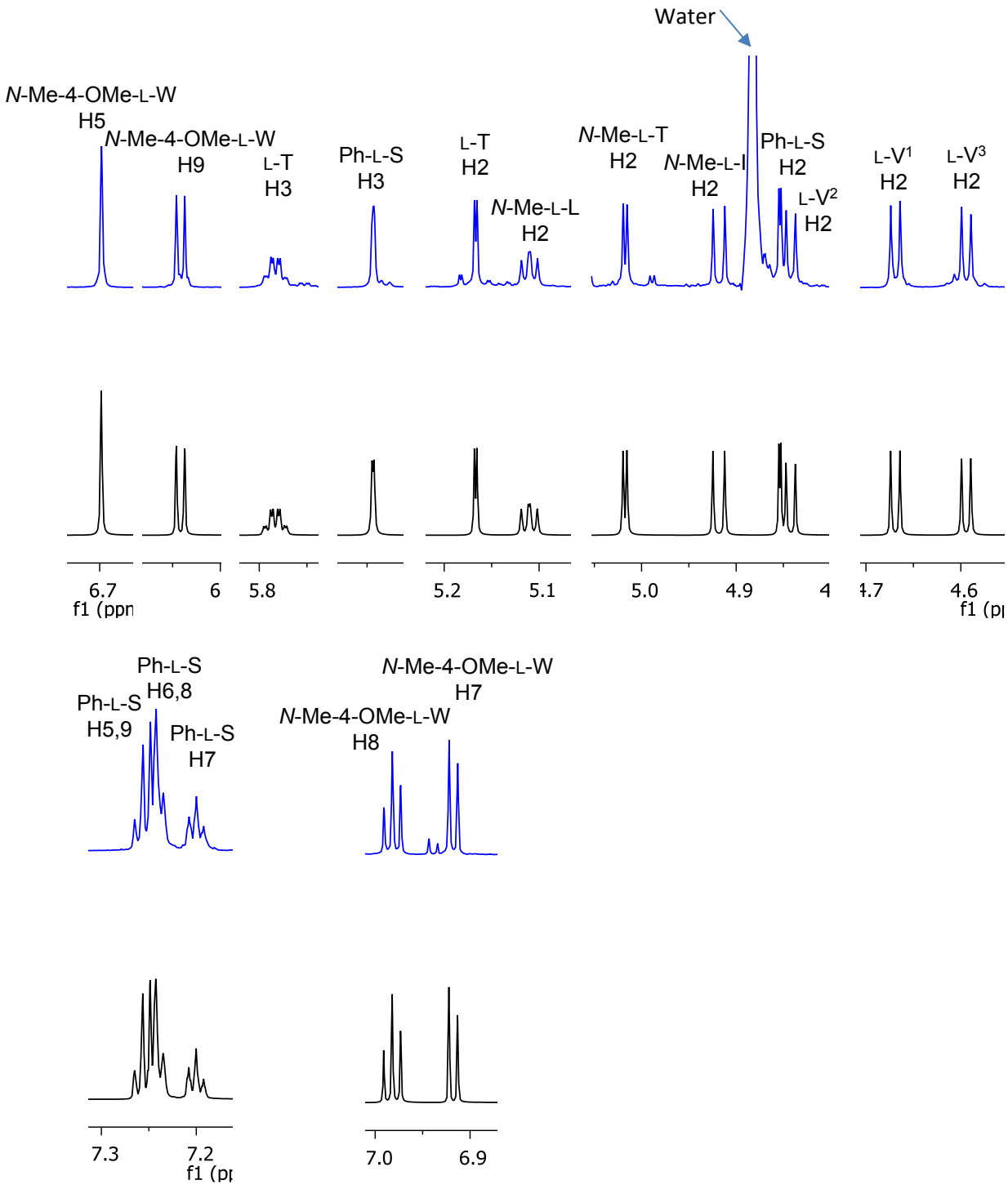
L-T H4
L-V5 H3
N,N-Me2-L-V H2
N-Me-L-V H3
L-V2 H3
L-V4 H3
N,N-Me2-L-V H3
L-V1 H3
L-V3 H3
N-Me-L-I H3
N-Me-L-L H3a
N-Me-L-I H4a
N-Me-L-L H3b
f1 (ppm)
N-Me-L-I
N-Me-4-OMe-L-W H12
CD2HOD
N-Me-L-L H7
N-Me-L-T H5
N-Me-L-V H6
L-V4 H2
L-V5 H2
N-Me-L-T H3
N-Me-4-OMe-L-W H2
N-Me-4-OMe-L-W H3b
N-Me-4-OMe-L-W H3a
N-Me-L-V H2


Water
N-Me-4-OMe-L-W H5
N-Me-4-OMe-L-W H9
L-T H3
Ph-L-S H3
L-T H2
N-Me-L-L H2
N-Me-L-T H2
N-Me-L-I H2
Ph-L-S H2
L-V2 H2
L-V1 H2
L-V3 H2
6.7
6
5.8
5.2
5.1
5.0
4.9
4.7
4.6
f1 (ppm
Ph-L-S H6,8
Ph-L-S H5,9
Ph-L-S H7
N-Me-4-OMe-L-W H7
N-Me-4-OMe-L-W H8
7.3
7.2
7.0
6.9

Supporting Table 1. Comparison of the final HiFSA-based ^{1}H chemical shifts (δ_H, in ppm) and multiplicities (Mult.) of ecumicin (1), the chemical shifts of previously reported free amino acids (FAA)[7]d, and the PERCH predicted values.

Amino Acid	Position	δ_H (ppm)			Amino Acid	Position	δ_H (ppm)		
		HiFSA	FAA	Predicted			HiFSA	FAA	Predicted
N,N-Me$_2$-L-V	2	2.672	3.945	4.169	L-V[3]	2	4.594	3.945	4.489
	3[a]	2.042	2.358	1.952		3[a]	2.027	2.358	1.995
	4	0.847	1.041	0.509		4	0.915	1.066	1.060
	5	0.978	1.066	0.818		5	0.859	1.041	0.999
	6,7 (*N,N*-Me$_2$)	2.313		2.370					
						2	3.065	3.945	5.153
L-V[1]	2	4.669	3.945	4.508	*N*-Me-L-V	3[a]	2.583	2.358	20524
	3[a]	2.101	2.358	2.155		4	1.094	1.066	1.319
	4	0.992	1.041	1.038		5	0.976	1.041	1.075
	5	1.055	1.066	0.943		6 (*N*-Me)	3.140		3.160
	2	4.918	4.022	4.617		2	4.102	4.384	5.043
	3[a]	1.957	2.065	2.032		3a	3.545	3.460	3.613
	4a[a]	0.989	1.337	1.465		3b	3.694	3.535	3.367
N-Me-L-*allo*-I	4b[a]	1.246	1.508	1.175	*N*-Me-4-OMe-L-W	5[b]	6.698	7.337	6.772
	5	0.754	0.948	0.723		7[c]	6.918	7.698	7.333
	6	0.744	0.948	0.880		8	6.984	7.192	7.318
	7 (*N*-Me)	3.234		3.196		9	6.442	7.278	7.089
						12 (*O*-Me)	3.826		4.072
	2	5.167	3.989	4.668		13 (*N*-Me)	2.157		2.936
L-T	3	5.783	4.422	4.010					
	4	1.313	1.351	1.270		2	4.529	3.945	4.303
					L-V[4]	3[a]	2.200	2.358	2.094
	2	5.018	3.989	4.099		4	1.028	1.066	1.094
N-Me-L-T	3	4.457	4.422	4.345		5	0.989	1.041	1.080
	4	0.912	1.351	1.252					
	5 (*N*-Me)	3.330		3.257		2	4.854	4.334	4.499
						3	5.344	-	5.208
	2	4.842	3.945	4.525	Ph-L-*threo*-S	5 (o)[b]	7.240	7.332	7.524
L-V[2]	3[a]	2.350	2.358	1.975		6 (m)[b]	7.255	7.429	7.372
	4	1.092	1.066	0.938		7 (p)[b]	7.201	7.338	7.281
	5	0.979	1.041	1.094		8 (m)[b]	7.255	7.429	7.372
						9 (o)[b]	7.240	7.332	7.526
	2	5.110	4.061	5.342					
	3a[a]	1.453	1.857	1.444		2	4.396	3.945	4.169
N-Me-L-*allo*-L	3b[a]	1.239	1.744	1.914	L-V[5]	3[a]	1.968	2.358	1.935
	4[a]	0.957	1.766	1.630		4	0.935	1.066	1.277
	5	0.168	0.958	0.972		5	0.919	1.041	0.933
	6	0.331	0.970	0.972					
	7 (*N*-Me)	3.259		3.110					

[a] Defined multiplicities are assigned to signals previously reported as multiplets (m).[4]
[b] Defined multiplicities are assigned to signals previously reported as singlets (s) or broad singlets (br s).[4]
[c] HiFSA reveals the signal previously assigned as a doublet of doublets (dd)[4] to be a ddd.
[d.] The reported values are based on basic free amino acids with no structural modification, e.g. *N*-methylation.

Supporting Table 2. HiFSA-based $^{1}H,^{1}H$ spin-spin coupling constants (J_{HH}, in Hz) of ecumicin (1) compared with the chemical shifts of previously reported free amino acids (FAA) [7]b, and the PERCH predicted values.

Amino acid	Coupling	J_{HH} (Hz)			Amino acid	Coupling	J_{HH} (Hz)		
		HiFSA	FAA	Predicted			HiFSA	FAA	Predicted
N,N-Me$_2$-L-V	^{3}J (2,3)	9.18	4.44	12.51	L-V^{3}	^{3}J (2,3)	8.88	4.44	12.88
	^{3}J (3,4)	6.59	7.01	6.64		^{3}J (3,4)	6.55	7.01	6.64
	^{3}J (3,5)	6.62	7.02	6.64		^{3}J (3,5)	6.78	7.02	6.64
L-V^{1}	^{3}J (2,3)	8.75	4.44	12..6	*N*-Me-L-V	^{3}J (2,3)	7.62	4.44	12.96
	^{3}J (3,4)	6.79	7.01	6.64		^{3}J (3,4)	6.50	7.01	6.64
	^{3}J (3,5)	6.72	7.02	6.64		^{3}J (3,5)	6.82	7.02	6.64
N-Me-L-*allo*-I	^{3}J (2,3)	11.23	3.92	13.15	*N*-Me-4-OMe-L-W	^{3}J (2,3a)	11.16	7.33	12.89
	^{3}J (3,4a)[a]	1.56	7.00	1.95		^{3}J (2,3b)	4.71	5.30	1.90
	^{3}J (3,4b)[a]	3.32	9.00	12.66		^{2}J (3a,3b)	-13.73	-15.40	-16.76
	^{3}J (3,6)	6.63	5.27	6.64		^{5}J (5,7)[a]	0.48	-	-0.3
	^{2}J (4a,4b)[a]	-12.91	-13.68	-13.40		^{4}J (7,9)[a]	0.67	1.10	1.02
	^{3}J (4a,5)	7.26	7.40	7.44		^{3}J (8,9)	7.79	7.02	8.10
	^{3}J (4b,5)[a]	7.64	7.47	7.44		^{3}J (7,8)	8.18	8.05	7.85
L-T	^{3}J (2,3)	2.34	3.88	2.69	L-V^{4}	^{3}J (2,3)	7.91	4.44	3.45
	^{3}J (3,4)	6.51	6.64	6.25		^{3}J (3,4)	6.75	7.01	6.64
						^{3}J (3,5)	6.81	7.02	6.64
N-Me-L-T	^{3}J (2,3)	3.73	3.88	2.44					
	^{3}J (3,4)	6.46	6.64	6.25		^{3}J (2,3)	1.90		1.90
						^{4}J [5(o),9(o)][a]	1.33	2.06	1.98
L-V^{2}	^{3}J (2,3)	8.92	4.44	2.98		^{3}J [5(o),6(m)][a]	7.65	7.69	7.66
	^{3}J (3,4)	6.69	7.01	6.64		^{3}J [8(m),9(o)][a]	7.65	7.69	7.66
	^{3}J (3,5)	7.01	7.02	6.64		^{5}J [5(o),8(m)][a]	0.76	0.59	0.55
					Ph-L-*threo*-S	^{5}J [6(m),9(o)][a]	0.76	0.59	0.55
N-Me-L-L	^{3}J (2,3a)	8.58	8.36	3.04		^{4}J [5(o),7(p)][a]	1.25	1.25	1.26
	^{3}J (2,3b)	6.59	5.84	13.95		^{4}J [7(p),9(o)][a]	1.25	1.25	1.26
	^{2}J (3a,3b)[a]	-13.56	-14.40	-14.71		^{4}J [6(m),8(m)][a]	1.71	1.41	1.48
	^{3}J (3a,4)[a]	5.44	6.31	12.99		^{3}J [6(m),7(p)][a]	7.47	7.524	7.40
	^{3}J (3b,4)[a]	7.99	8.27	2.27		^{3}J [7(p),8(m)][a]	7.47	7.524	7.40
	^{3}J (4,5)	6.55	6.50	6.64					
	^{3}J (4,6)	6.59	6.62	6.64					
					L-V^{5}	^{3}J (2,3)	8.90	4.44	12.32
						^{3}J (3,4)	6.83	7.01	6.64
						^{3}J (3,5)	6.41	7.02	6.64

[a] Scalar coupling constants that were not reported previously due to extensive resonance overlap.[4]
[b.] The reported values are based on basic free amino acids with no structural modification, e.g. *N*-methylation.