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THE ABSOLUTE CONFIGURATIONS AT 8 AND 9-CARBONS OF ADDA, AN AMINO ACID COMPONENT OF A HEPATOTOXIN, CYANOVIRIDIN RR

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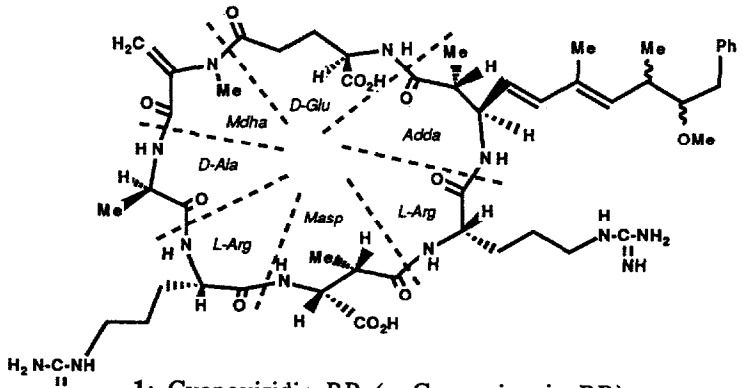
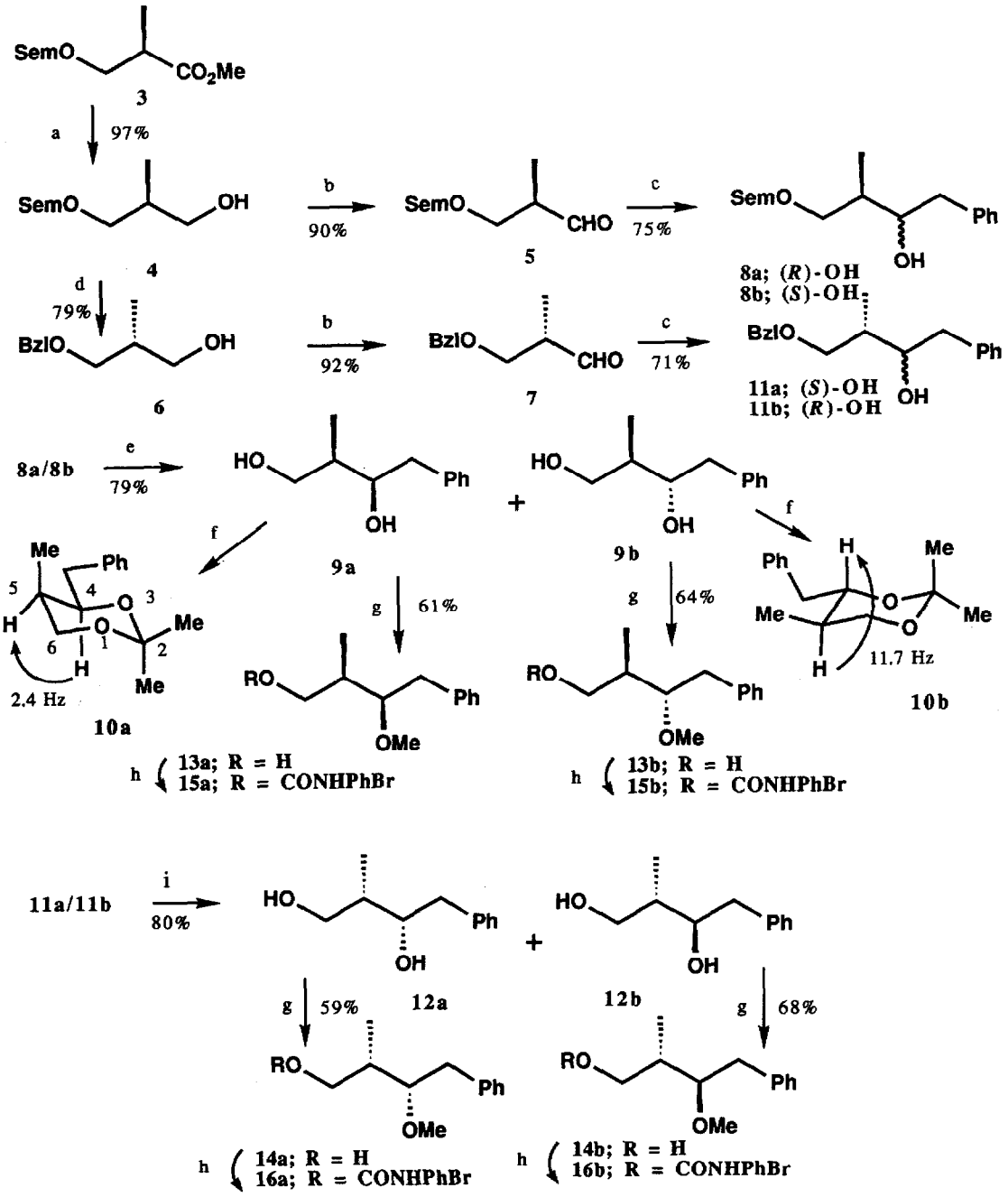
Abstract---The absolute configurations at 8 and 9-positions of Adda (2), a component of cyanoviridin RR isolated from Microcystis species (Cyanobacteria), have been synthetically determined as S, S.

Potent hepatotoxins have been isolated from myceria of the cyanobacteria Microcystis species.[1] They are heptapeptides, and, among the seven amino acid units, Adda (2; 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) commonly occurs in the toxins, and seems to have an essential role to exhibit the toxicity. We have recently reported the isolation of cyanoviridin RR[2] (1; =cyanoginosin RR[3], probably identical with microcystin RR[2a]) from Microcystis viridis[2] and M. aeruginosa[3]. The configurations at the chiral centers of microcystin RR have been established[3,4] except for those at 8 and 9-carbons of Adda. We have elucidated the absolute configurations at the centers of Adda. Recently, Rinehart et al reported independently the determination of the stereochemistry of Adda part of microcystin LR,[5] which prompted us to report our results.



1; Cyanoviridin RR (= Cyanoginosin RR)

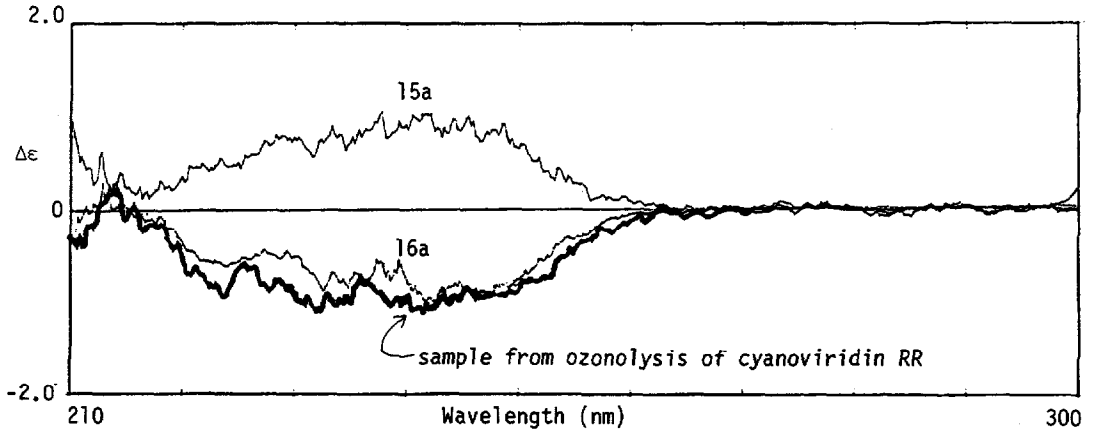
The ester 3, prepared from (R)-(-)-methyl 3-hydroxy-2-methylpropionate[6] was treated with $LiAlH_4$, and the resultant alcohol 4 was oxidized to afford



a) $LiAlH_4$/ether, b) DMSO, $(COCl)_2$, CH_2Cl_2, -60°C, then Et_3N, c) $PhCH_2MgBr$/ether, d) *i*) NaH, benzyl bromide, *ii*) Bu_4NF/THF, e) Bu_4NF/THF, f) CAS/acetone, g) *i*) TBSCl, imidazole/DMF, *ii*) NaH, MeI/ether, *iii*) *p*-TsOH, h) *p*-bromophenylisocyanate, pyridine, i) Pd-C, H_2

(R)-aldehyde **5**.[7,8] The (S)-aldehyde **7**[7,8] was also obtained from **4** via the alcohol **6**. The (R)-aldehyde **5** was allowed to react with benzylmagnesium bromide, producing a 1:6 mixture of diastereomers **8a** and **8b**, which were deprotected to give a separable mixture of **9a** and **9b**.[9] The stereochemistry of the diols was deduced by NMR spectroscopic analysis of the acetonides **10a** and **10b**;[9] 4-H of the acetonide derived from the minor diol **9a** exhibited an axial-equatorial coupling (J=2.4 Hz) to 5-H, while 4-H of the acetonide from the major diol **9b** an axial-axial coupling (J=11.7 Hz). The predominant formation of **8b** to **8a** is interpretable by β-alkoxy chelation control.[10]

Upon treatment with benzylmagnesium bromide followed by deprotection, the (S)-aldehyde **7** yielded a separable mixture of **12a** and **12b**,[9] the stereochemistry of which was determinable by comparing their spectroscopic properties with those of their enantiomers **9a** and **9b**. The diol **9a** was treated with tert-butyldimethylsilyl (TBS) chloride, the monosilylated product being methylated to give a methyl ether, which subsequently yielded **13a** by removal of the protection group. Finally, the hydroxy group of **13a** was converted into a urethane group, giving rise to **15a**.[9] By following the same procedure, the diols **9b**, **12a**, and **12b** were transformed to the urethanes **15b**, **16a**, and **16b**,[9] respectively.[11] In a separate experiment, cyanoginosin RR (=cyanovirigin RR) (20mg) was ozonized at -70°C in methanol and reduced with $NaBH_4$. The reaction product was extracted with ether. The ether extract (4.02mg) was treated with p-bromophenylisocyanate (5mg, CCl_4). The urethane was purified by preparative TLC (benzene:CH_2Cl_2=1:1) to a pure specimen (0.76mg), whose ^{1}H-NMR spectrum was identical with those of the urethanes **15a** and **16a**. Furthermore, the CD spectrum of "natural" urethane was identical with that of synthetic **16a** as shown in the figure, determining that the configuration at C-8 and C-9 of Adda are both S.



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6) (R)-(-)-Methyl 3-hydroxy-2-methylpropionate was purchased from Aldrich Chemical Company.

7) This product was directly used for the next step without purification to avoid racemization.

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9) Physical properties of **9a**: NMR ($CDCl_3$, 90MHz) δ 0.98 (3H, d, J=7 Hz, Me), 1.2-1.5 (1H, m, H-2), 2.74 (2H, d, J=6.5 Hz, H-4), 3.70 (2H, d, J=5.5 Hz, H-1), 4.05 (1H, dt, J=3.0, 6.5 Hz, H-3), 7.30 (5H, s), $[\alpha]_D^{22}$= +28.6° (c=0.4, $CHCl_3$), **9b**: NMR δ 0.95 (3H, d, J=7.1 Hz, Me), 1.6-1.9 (1H, m, H-2), 2.60 (1H, dd, J=14.9, 8.2 Hz, H-4), 2.95 (1H, dd, J=14.9, 4.0 Hz, H-4), 3.6-3.9 (3H, m, H-1, H-3), 7.29 (5H, s), $[\alpha]_D^{22}$ = -69.2° (c=0.4, $CHCl_3$), **12a**: $[\alpha]_D^{22}$ = -28.3° (c=0.9, $CHCl_3$) **12b**: $[\alpha]_D^{22}$ = +70.1°,**10a**: NMR ($CDCl_3$, 500MHz) δ 1.05 (3H, d, 8.1 Hz, 5-Me), 1.40 (6H, s, 2-Me), 1.40 (1H, dddq, J=2.4, 2.7, 1.5, 8.1 Hz, H-5), 2.61 (1H, dd, J=6.9, 14.0 Hz, benzyl-H), 2.81 (1H, dd, 6.9, 14.0 Hz, benzyl-H), 3.56 (1H,dd,J=2.7, 14.1 Hz, H-6), 4.05 (1H, dd, 1.5, 14.1 Hz, H-6), 4.18 (1H, dt, J=2.4, 6.9 Hz, H-4), 7.30 (5H, s), **10b**: NMR δ 0.78 (3H, d, J=8.2 Hz, 5-Me), 1.36 (6H, s, 2-Me), 1.68 (1H, dddq, J=5.0, 11.7, 11.5, 8.2 Hz, H-5), 2.72 (1H, dd, 1.1, 14.5 Hz, benzyl-H), 2.91 (1H, dd, J=10.0, 14.5 Hz, benzyl-H), 3.51 (1H, dd, 5.0 Hz, J=11.5, H-6), 3.67 (1H, t, J=11.5 Hz, H-6), 3.71 (1H, ddd, J=1.1, 10.0, 11.7 Hz, H-4), 7.31 (5H,s), **15a**: NMR δ 1.02 (3H, d, J=7.0 Hz, Me), 1.97 (1H, d, sext, J=3.3, 7.0 Hz, H-2), 2.71 (1H, dd, J=13.6, 7.0 Hz, H-4), 2.93 (1H, dd, J=13.6, 7.0 Hz, H-4), 3.28 (3H, s, OMe), 3.45 (1H, dt, J=3.3, 7.0 Hz, H-3), 4.08 (1H, dd, J=7.0, 10.5 Hz, H-1), 4.18 (1H, dd, J=7.0, 10.5 Hz, H-1), 6.55 (1H, br., 1H, NH), 7.5-7.2 (9H, m), CD: Δε = +1.04 (242nm), **15b**: NMR: δ 1.03 (3H, d, J=7.0 Hz, Me), 2.05 (1H, m, H-2), 2.75 (1H, dd, J=14.1, 7.5 Hz, H-4), 2.88 (1H, dd, J=14.1, 4.5 Hz, H-4), 3.24 (3H, s, OMe), 3.34 (1H, m, H-3), 4.15 (1H, dd, J=10.7, 6.7 Hz, H-1), 4.30 (1H, dd, J=10.7, 5.3 Hz, H-1), 6.60 (1H, br., NH), 7.5-7.2 (9H, m), **16a**: CD: Δε =-1.05 (242 nm).

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11) The same alcohols **13a**, **13b**, **14a**, and **14b** are reported in the lietrature[5]. Comparison of the physical properties was, however, impossible because no spectral data are described in the literature.

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