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TOTAL SYNTHESIS OF ADDA, THE UNIQUE C_{20} AMINO ACID OF CYANOBACTERIAL HEPATOTOXINS[1]

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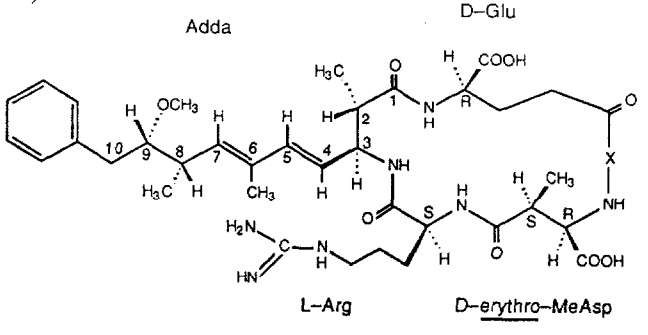
Abstract: Synthetic (2S,3R)-3-Amino-4-hydroxy-2-methylbutanoic acid, γ-lactone, and (4S,5S)-2,4-dimethyl-5-methoxy-6-phenyl-2-hexen-1-ol were oxidized and linked to give (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda).

Nodularin (**1**)[2] and microcystin-LR (**2**) are cyclic penta- and heptapeptides produced by cyanobacteria (Nodularia spumigena and Microcystis aeruginosa, respectively) whose unique C_{20} amino acid (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) seems to be important for their hepatotoxicity; hydrogenation or ozonolysis of the diene system gives an inactive compound. (By contrast, borohydride reduction of the dehydro amino acid unit or glutathione addition to it gives a derivative nearly as toxic as the parent.[3])



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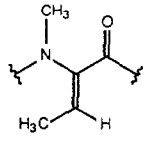

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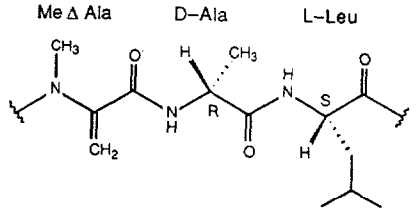
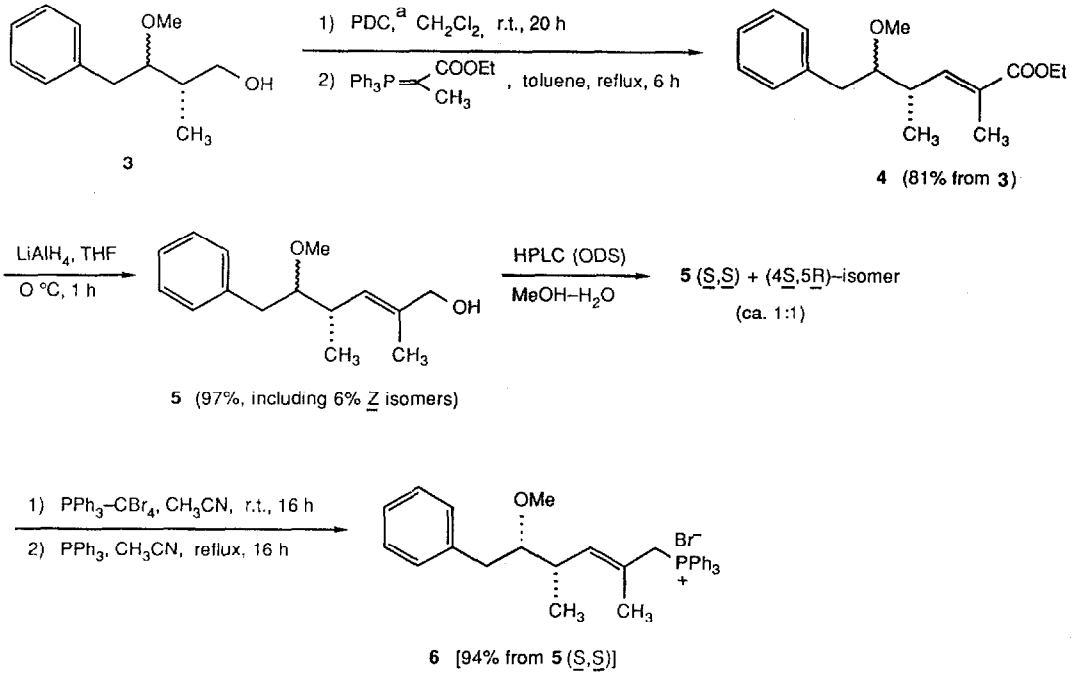


We recently deduced the stereochemistry of Adda and assigned the structure of nodularin.[2] We describe here a total synthesis of Adda, which can be retro-synthetically divided into an amino acid part (C-1 to C-4); C-5, C-6; and an aromatic part (C-7 to C-10); the first and third parts correspond to degradation products from **2**.[2]

Synthesis of the aromatic C-7 to C-10 part of Adda (Scheme I) proceeded as described in our stereochemistry study[2] to the diastereometric mixture **3**, which was oxidized to the aldehyde, then treated immediately with the required stable ylide to give **4** ($C_{17}H_{24}O_3$, M^+, Δ0.3 mmu).[4a,b] Reduction of **4** gave alcohol **5** ($C_{15}H_{23}O_2$, M + H, Δ0.3 mmu),[4a,c] showing two spots (the E and Z geometrical isomers) on TLC (benzene–EtOAc, 7:3). These were separated (94:6 ratio) on a gravity silica gel column eluted with benzene–EtOAc (85:15),[5] and the required E-isomer (**5**) showed two peaks (ca. 1:1 ratio) on an ODS HPLC column (MeOH–H_2O). The (4S,5S)-isomer [**5**(S,S), $C_{15}H_{23}O_2$, M + H, Δ0.3 mmu][4a,c] was separated by preparative HPLC and converted successively to the bromide (96%, $C_{15}H_{22}BrO$, M + H, Δ0.3 mmu)[4a,d] and the triphenylphosphonium bromide **6** (98%, $C_{33}H_{36}OP$, M - Br, Δ1.4 mmu).[4c]

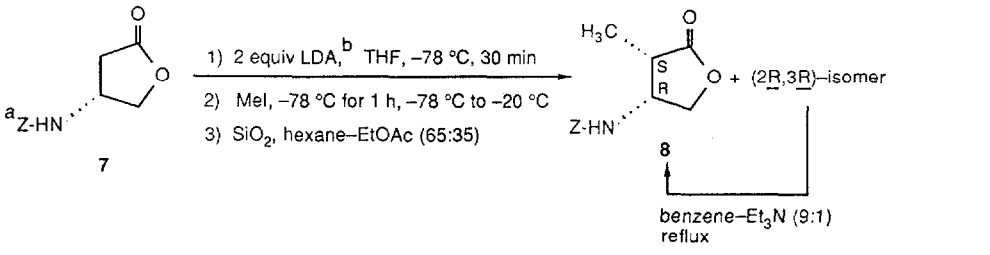


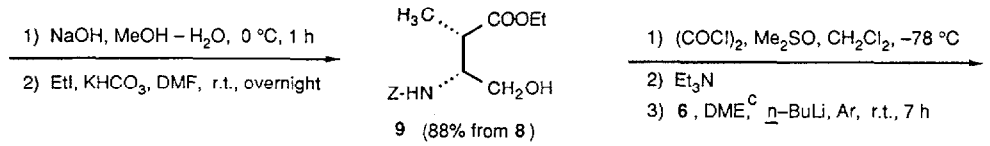
Scheme I. [a]PDC = pyridinium dichromate.

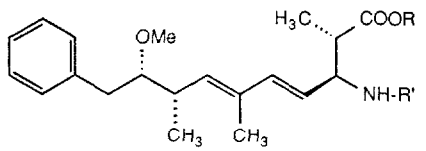
Synthesis of the amino acid part of Adda (C-1 to C-4) proceeded through the γ-butyrolactone **7** obtained earlier (Scheme II).[2] The diastereomeric mixture obtained (68%) by methylation of **7** was chromatographed[6] to give the (2R,3R)-isomer ($C_{13}H_{16}NO_4$, M + H, Δ0.5 mmu)[4a,c] and the required (2S,3R)-isomer (**8**, $C_{13}H_{16}NO_4$, M + H, Δ0.5 mmu)[4a,c] in about a 4:1 ratio; both gave colorless needles from benzene–hexane.[7] The (2R,3R)-isomer could be most efficiently converted to **8** by heating for 2 days to give **8** and recovered (2R,3R)-isomer in about a 1:2 ratio. Compound **8** on saponification afforded the hydroxy acid ($C_{13}H_{18}NO_5$, M + H, Δ0.3 mmu),[4a,c] which was converted to the ethyl ester **9** ($C_{15}H_{22}NO_5$, M + H, Δ0.5 mmu).[4a,c]

A mixture of the corresponding aldehyde (made from **9** by Swern oxidation), butyllithium, and **6** (1:1.2:1.2 equiv) was stirred for 7 h at room temperature under argon. The reaction was quenched with aqueous ammonium chloride, extracted with ethyl acetate, and separated by preparative TLC to give protected Adda (**10**).

The desired (2S,3S,8S,9S)-isomer (**10**, $[\alpha]_D^{26}$ -20.9° ($CHCl_3$), $C_{30}H_{40}NO_5$, M + H, Δ1.6 mmu)[4c] was purified by cyanated silica gel HPLC (hexane–2-propanol, 100:1).







10 (43% from **9**): R = Et, R' = Z
11: R = R' = H

Scheme II. [a] Z = benzyloxycarbonyl. [b] LDA = lithium diisopropylamide. [c] DME = dimethoxyethane.

The ^1H NMR spectrum (CD_3OD) of **10** is very similar to that of the Adda protons in nodularin, with chemical shifts within 0.03 ppm and coupling constants within 0.1 Hz for H-5 through the phenyl ring. The chemical shifts are quite close for H-3, H-4 and the 2-CH_3 protons (≤0.13 ppm), but their coupling constants differ (as does the chemical shift of H-2) because C-2 and C-3 of Adda are incorporated into the constrained peptide ring.

Deprotection of **10** gave Adda itself (**11**, $C_{20}H_{30}NO_3$, M + H, Δ0.0 mmu).[4c] Both **10** and **11** show little or no toxicity in the mouse liver assay.

Unfortunately, one cannot compare the synthetic product directly with natural Adda, because conditions vigorous enough to hydrolyze the peptide links of Adda in nodularin or microcystin lead to decomposition, with loss of methanol. The product of hydrolysis of either **1** or **2** is the same as that obtained by similar hydrolysis of **10**, with identical reversed-phase HPLC behavior (C-18, MeOH:0.1% TFA in H_2O : 85:15, co-elution) and essentially identical 500-MHz ^1H NMR spectra (1.05, 1.10, 1.30, 1.48, 1.58, 2.51, 2.93, 3.30, 3.63, 7.27, 7.33, 7.40, 7.58, 7.77, 8.24 ppm, $CDCl_3$). Particularly compelling are the identical collisionally induced metastable ion spectra (MS/MS, B/E scan, m/z 300 → 226, 209, 195, 181, 169, 155, 141, 128, 115, 102, 95, 84) obtained in our four-sector tandem mass spectrometer (VG 70-SE4F).

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(1) Reported in part: (a) at the IUPAC Symposium on the Chemistry of Natural Products, Kyoto, May 29-June 3, 1988; Abstract CLa6. (b) in Pure Appl. Chem. **1989**, 61, 525-528. (c) at the 197th ACS National Meeting, Dallas, TX, April 9-14, 1989, ORGN 23.

(2) Rinehart, K. L.; Harada, K.-I.; Namikoshi, M.; Chen, C.; Harvis, C. A.; Munro, M. H. G.; Blunt, J. W.; Mulligan, P. E.; Beasley, V. R.; Dahlem, A. M.; Carmichael, W. W. J. Am. Chem. Soc. **1988**, 110, 8557-8558.

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(4) (a) Satisfactory ^1H NMR data were obtained. (b) HREIMS. (c) HRFABMS. (d) HRCIMS.

(5) Geometries of these two isomers were determined by NOE experiments.

(6) Takahashi et al. made a diastereomeric mixture of 2-methylated aminolactones from L-Asp, but they did not separate these isomers; Takahashi, Y.; Hasegawa, S.; Izawa, T.; Kobayashi, S.; Ohno, M. Chem. Pharm. Bull. **1986**, 34, 3020-3024.

(7) The stereochemistry of these compounds is established from their ^1H NMR spectra with aid of NOE experiments.

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