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Synthesis of the Cytostatic Cyclic Tetrapeptide, Chlamydocin

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Summary. Chlamydocin has been synthesized by a route utilizing allylic hydroxylation and epoxidation of the DL-2-amino-9-decenoic acid residue to form the 2-amino-8-oxo-9,10-epoxydecanoic acid residue.

Chlamydocin, cyclo-(α-aminoisobutyryl-L-phenylalanyl-D-prolyl-L-2-amino-8-oxo-(9S)-9,10-epoxydecanoyl) (1), is a cytostatic[1] cyclic tetrapeptide[2] isolated from Diheterospora chlamydosporia, and is related to two other biologically active cyclic tetrapeptides which contain Aoe, the phytotoxins Cyl-2 (2)[3] and HC-toxin (3)[4]. The synthesis of Chlamydocin is described herein via a route in which the epoxy-ketone amino acid (Aoe) is formed by successive oxidations of 2-amino-9-decenoic acid (Ade) after the cyclic tetrapeptide ring system has been closed stereoselectively.[21] The successful use of these oxidation reactions on peptide systems should permit the synthesis of other Aoe-containing peptides.

(Aib-L-Phe-D-Pro-L-Aoe) 1

(Aoe-D-Tyr(OMe)-L-Ile-L-Pip) 2

(L-Ala-D-Ala-L-Aoe-D-Pro) 3

Racemic 2-amino-9-decenoic acid (Ade) was synthesized as shown in Scheme I. Elimination of 1,8-dibromooctane 4 (1.3 eq. KO^{t}Bu, THF, reflux) gave bromooctene 5 (45% yield) which was reacted with benzylidene glycine ethyl ester 6[5] to give the imine 7. Hydrolysis of 7 (1.1 eq. aqueous $KHSO_4$ 0°C, 1 hr) gave, after extraction with ethyl acetate, H-Ade-OEt (8) which was converted to Boc-DL-Ade-OEt 9[6] using di-tert-butyl dicarbonate (71% yield after MPLC purification based on 6).

The cyclic tetrapeptide, cyclo-(L-Ade-Aib-L-Phe-D-Pro) (16), was synthesized as shown in Scheme II. Boc-DL-Ade-OEt was saponified (1.1 eq. 1 N NaOH in acetone for 1.5 hr) to give acid 10 which was coupled with tripeptide 12[6,7] via the mixed anhydride 11[8] to give the protected linear tetrapeptide, Boc-DL-Ade-Aib-L-Phe-D-Pro-OMe (13) (91% yield after MPLC purification, 4:1,Skellysolve B: acetone).[6,9] Tetrapeptide 13 was saponified (1.1 eq. 1N NaOH,

Scheme I

$Br\text{-}CH_2(CH_2)_5\text{-}R$

4 R = CH_2CH_2Br

5 R = $CH{=}CH_2$

$C_6H_5CH{=}NCHRCO_2Et$

6 R = H

7 R = $(CH_2)_5\text{-}CH_2CH{=}CH_2$

$Boc\text{-}NH\text{-}CH(\text{-}(CH_2)_5\text{-}CH_2CH{=}CH_2)\text{-}CO_2R$

9 R = Et

10 R = H

11 R = O-CO-O^{i}Bu

H-Aib-L-Phe-D-Pro-OMe (12)

acetone, 1.5 h, 96%) and the corresponding free acid was converted to the N-hydroxy succinimide ester 14 (N-hydroxysuccinimide, 1.1 eq. DCC,CH_2Cl_2, 2 h at 0°C, 4 h at room temp). The Boc group was cleaved with TFA-CH_2Cl_2 (1:1) (0°C, 20 min). The reaction mixture was worked up as usual[10] and the resulting TFA salt 15 was cyclized (< 1 mM in pyridine, room temp).[10] Cyclic tetrapeptide, cyclo-(L-Ade-Aib-L-Phe-D-Pro) (16)[6,11] was isolated in 12.0% overall yield (24% yield based on the amount of L-Ade diastereomer present in the starting material). Consistent with the results of model studies[12] only trace amounts of the corresponding D-Ade diastereomer of 16 were detected.[6,13] The configuration of Ade was assigned by comparing the chemical shifts and coupling constants in both Ade peptides with the corresponding resonances in model peptides of established configurations.[19,20] Allylic oxidation of the terminal olefin 16 (1.0 equiv. SeO_2, 4 eq. TBHP, room temp., 48 hrs) followed by column chromatography (silica gel-60, Skellysolve B: acetone, 3:2) gave allylic alcohol (17)[6,15] (56% yield plus 31% of recovered 16 and 5% of the enone 18). All modifications designed to increase the yield of allylic alcohol 17 produced higher yields of enone 18.

Epoxidation of 17 (1.2 eq. of mCPBA in CH_2Cl_2, 2 h at 0°C, 2 h at room temp) gave epoxy alcohol 19 which was oxidized *in situ*[16] (1.5 eq. mCPBA, 0.02 equiv. 2,2,6,6-tetramethylpiperidine hydrochloride, 12 h at room temp) to 1a,b. After purification by preparative TLC, synthetic chlamydocin 1a,b was isolated in 69% yield based on 17 along with 23% of the epoxy alcohol 19. The synthetic material was identical to natural chlamydocin on the basis of 270 MHz ^{1}H-NMR, ^{13}C-NMR, TLC and microanalysis.[6,17] The synthetic material inhibited ^{3}H-thymidine uptake in PHA induced bovine lymphocytes at a concentration of 2.3 ng/mL compared to 1 ng/mL for natural chlamydocin, suggesting the (9R)-Aoe diastereomer 1b is much less potent that the natural (9S)-Aoe-diastereomer 1a. It should be noted that the synthetic chlamydocin obtained here is assumed to be a mixture of (9RS)-Aoe diastereomers since the configuration of the epoxide was not controlled. A chiral synthesis of the epoxide in chlamydocin may be possible from 17 if Sharpless' procedures for chiral epoxidation of allylic alcohols[18] work on peptide systems. This possibility as well as the effects of ring conformation[19,20] on biological activity are currently being explored.

Scheme II

11+12 → R_1-DL-Ade-Aib-L-Phe-D-Pro-R_2

cyclo(L-NH-CH($(CH_2)_5$-X)-CO-Aib-L-Phe-D-Pro)

13 R_1 = Boc; R_2 = OMe

14 R_1 = Boc; R_2 = OSu

15 R_1 = H•TFA; R_2 = OSu

16 X = $CH_2CH{=}CH_2$

17 X = $CH(OH)CH{=}CH_2$

18 X = $CO{-}CH{=}CH_2$

19 X = $CH(OH){-}CH{-}CH_2$ (epoxide)

1a,b X = $C(=O){-}CH{-}CH_2$ (epoxide)

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7. Compound 12 synthesized in 74% yield from D-Pro-OMe.
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9. Compound 13: Diastereomers did not separate on TLC in all solvent systems tried. ^{1}H-NMR ($CDCl_3$) δ 1.39 (s, 6H, Aib, β-methyls).
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11. Compound 16: ^{1}H-NMR ($CDCl_3$) δ 1.33 (s, 3H, Aib, β-methyl), 1.74 (s, 3H, Aib, β-methyl); ^{13}C-NMR ($CDCl_3$) for $-CH_2-CH=CH_2$ are 33.67, 139.01, and 114.25, respectively.
12. Kawai, M., Pastuszak, J., Gardner, J. H., Rich, D. H., unpublished experiments.
    HCl-L-Ala-Aib-L-Phe-D-Pro-OSu → cyclo(L-Ala-Aib-L-Phe-D-Pro), 45%
    HCl-D-Ala-Aib-L-Phe-D-Pro-OSu → cyclo(D-Ala-Aib-L-Phe-D-Pro), 5%
13. Cyclo(D-Ade-Aib-Phe-D-Pro) 16b was isolated in about 2% yield. TLC Rf=0.2 vs. 0.6 for the L-Ade peptide 16. The C-9 proton pattern was identical to that obtained for natural chlamydocin.
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