Degradation of an antitumour bicyclic hexapeptide RA-VII into cycloisodityrosines

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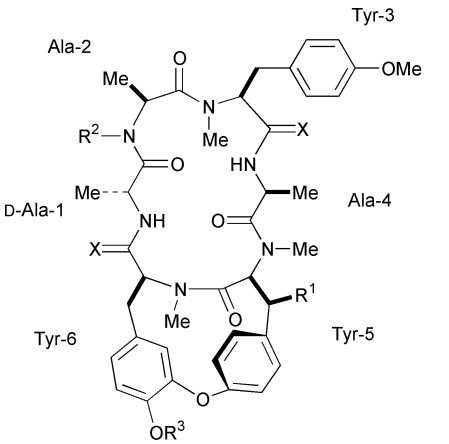
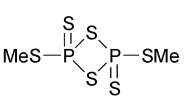
Degradation of an antitumour bicyclic hexapeptide, RA-VII 1, produced protected cycloisodityrosines in an efficient manner through bis(thioamide) intermediate 6.

RA-VII **1**[1] and bouvardin (NSC 259968) **2**[2] are a class of peptides originating from Rubiaceous plants. They show potent antitumour activity, and their mode of action is considered to be inhibition of protein synthesis through interaction with eukaryotic 80 S ribosomes.[3] While the cycloisodityrosine moiety is proposed to be the pharmacophore for this type of peptide,[4] the role of the 18-membered ring moiety for the activity is still an outstanding issue. Although several approaches towards the synthesis of cycloisodityrosines have been reported,[5–7] their multi-step synthesis inevitably requires construction of a strained diphenyl ether linkage, which is not easily accessible, using unusual amino acids. Also, the easily epimerisable properties of cycloisodityrosines[6d–f,7b] hamper ready access to the cycloisodityrosine unit needed for the synthesis of 18-membered ring modified analogues. We report herein an alternative practical approach towards cycloisodityrosines from RA-VII **1** which is the most abundant congener of RAs obtained from commercially available Rubiae Radix.[8]

To obtain cycloisodityrosines from peptide **1**, selective cleavage of the peptide bonds at specific positions was required. Previously, we reported that when RA-VII **1** was treated with 2,4-bis(methylthio)-1,3,2λ^5,4λ^5-dithiadiphosphetane-2,4-dithione **3** at rt, [Tyr-3-Ψ(CS-NH)-Ala-4; Tyr-6-Ψ(CS-NH)-D-Ala-1]RA-VII **4**, with accompanying monothioamides and other bis(thioamides), was obtained in 38% yield.[9] We deemed that bis(thioamide) **4** would be a suitable substrate for such degradation. Although some enhancement of the yield (~50%) of **4** has been made by modifying the reaction conditions, we found that when [*N*-methyl-Ala-2]RA-VII **5**, which is readily prepared from peptide **1** in 97% yield,[10] was thionated using the same reagent, bis(thioamide) **6** possessing thioamide bonds at the same positions as **4** was produced in 91% yield (Scheme 1). Compound **6** was converted into bis(imidothioate) **7** using





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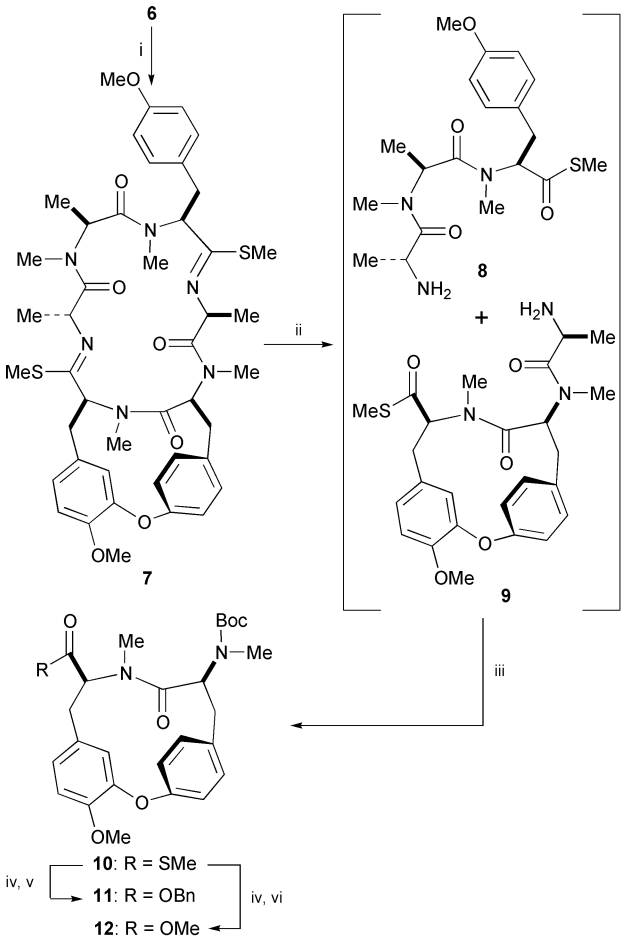
Scheme 1 *Reagents and conditions*: i, **3**, dioxane, rt, 4 d, 91%.



Scheme 2 *Reagents and conditions*: i, MeI, K_2CO_3, acetone, rt, 6 h; ii, 6 M HCl, MeCN, rt, 2 h; neutralised with 1 M K_2CO_3; iii, phenyl isothiocyanate, rt, 2 h; 6 M HCl, MeCN, rt, 5 h; neutralised with 1 M K_2CO_3; Boc_2O, rt, 4 h, 78% from **6**; iv, LiOH, H_2O_2, THF–H_2O, 0 °C, 20 min; v, benzyl alcohol, DEAD, Ph_3P, THF, 0 °C, 2 h, 90% from **10**; vi, (trimethylsily)diazomethane, MeCN–MeOH, rt, 3 h, 97% from **10**.

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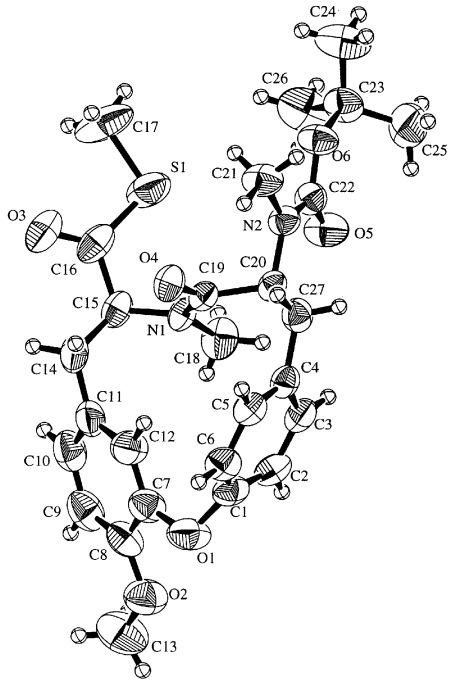

Fig. 1 The crystal structure of compound **10**.

iodomethane and potassium carbonate, and successive treatment with 6 M HCl in acetonitrile resulted in the cleavage of two imidothioate linkages to produce a mixture of two tripeptide segments **8** and **9** (Scheme 2). This mixture, without separation, was then subjected to Edman degradation, and *N*-protection using di-*tert*-butyl dicarbonate afforded cycloisodityrosine thioester **10** in 78% yield from bis(thioamide) **6**.† The structure of compound **10** was confirmed by X-ray crystallography (Fig. 1).‡ Compound **10** was converted into benzyl ester **11** and known methyl ester **12**[6f,7b] in yields of 90 and 97%, respectively.§ The spectroscopic data of **12** were in good agreement with those of **12** previously reported. The chemical conversion described here proceeds in an efficient manner; the overall yields of cycloisodityrosines **11** and **12** from RA-VII **1** were 62 and 67%, respectively. We are currently engaged in the design and synthesis of 18-membered ring modified analogues of RA-VII using **11** and **12**, and the results will be disclosed in due course.

Notes and references

† This key transformation (**6** → **10**) was most effectively carried out on a 0.15–0.2 mmol scale.

‡ *Crystal data* for **10**: $C_{27}H_{34}N_2O_6S$, M = 514.64, 0.30 × 0.10 × 0.40 mm, monoclinic, $P2_1$ (no. 4), a = 6.359(3), b = 21.465(5), c = 9.991(2) Å, β = 92.68(2)°, V = 1362.2(7) Å^3, T = 298(1) K, Z = 2, μ(Cu-Kα) = 14.09 cm^{-1}, 5470 reflections measured, 2494 unique reflections (R_{int} = 0.015), R = 0.036, Rw = 0.031. The structure was solved by direct methods and expanded using Fourier techniques. CCDC 182/1722. See http://www.rsc.org/suppdata/cc/b0/b004459h/ for crystallographic files in .cif format.

§ Compound **11**: $[\alpha]_D^{18}$ –202 (*c* 0.10, $CHCl_3$); compound **12**: $[\alpha]_D^{18}$ −198 (*c* 0.18, $CHCl_3$).

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