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A new approach to the epimeric analogue of cyclic peptides: epimerisation via oxazoles of RA-VII, an anti-tumour bicyclic hexapeptide from *Rubia* plants

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Abstract—[D-Ala-4]RA-VII (**9**) and [D-Ala-2]RA-VII (**10**) were prepared from RA-VII (**1**), an anti-tumour bicyclic hexapeptide from *Rubia* plants, by conversion of its thioamide **3** into oxazoles **4** and **5** and subsequent acid-catalysed hydrolysis.

Substitution of a D-amino acid for a particular L-amino acid or glycine in bioactive peptides produces analogues of parent peptides having modified characters such as enhanced resistance to enzymatic degradation,[1] which may also be useful for identification of the active conformation of the parent peptides.[2] In the case of linear peptides, such analogues are prepared in the usual manner. However, for naturally-occurring cyclic peptides, preparation of such analogues is not so easy since these peptides often include unusual amino acids which are difficult to prepare, and since cyclic peptide synthesis inevitably requires an elusive macrocyclisation reaction. We considered that the preparation of D-amino acid-containing analogues of cyclic peptides might be feasible by selective epimerisation of certain L-amino acid residues of cyclic peptides via oxazole intermediates. The present paper describes preparation of D-alanine containing analogues of RA-VII (**1**),[3,4] a bouvardin-related[5] anti-tumour bicyclic hexapeptide from *Rubia* plants (Fig. 1).

Treatment of RA-VII (**1**) with a thionating reagent, Lawesson's reagent [2,4-bis-(4-methoxyphenyl)-1,3,2λ^5,4λ^5-dithiadiphosphetane-2,4-dithione (**2**)] in dioxane at room temperature afforded a thionopeptide, [Tyr-3-Ψ(CS-NH)-Ala-4]RA-VII (**3**) in 80% yield.[6] The thionopeptide had a modified backbone in which an amido oxygen atom is replaced by a sulphur atom. The location of thionation was apparently determined by the constrains of their conformations.[7]

Then thioamide **3** was treated with 2 mole equiv. of thiophilic reagent, $AgBF_4$ or $Hg(OAc)_2$, in THF, 1,2-dimethoxyethane (DME), MeCN or $CHCl_3$ under an atmosphere of argon at room temperature for 20 h, and the product was separated by preparative HPLC. Condensation of the thioamide with an adjacent peptide bond occurred to produce an oxazole ring. As shown in Table 1, the desired oxazoles **4** and **5** were formed in moderate yields. Different thiophilic reagents gave different results. Reaction of thioamide **3** with $AgBF_4$ in THF gave oxazole **4** in 24% yield, whereas that with $Hg(OAc)_2$ under the same conditions afforded oxazole **5** as a major product (45%), along with acetimide **6** (14%) and oxazole **4** (3.9%). Oxazole **5** might be produced via spirocyclic intermediate **7** as shown in Scheme 1. The reaction was also greatly influenced by the solvent used. When $AgBF_4$ was used, the reaction proceeded efficiently in the ether-type solvents to give desired oxazole **4**, whereas a significant amount of the starting material, **3**, was recovered when MeCN or $CHCl_3$ was used.

When thioamide **3** was converted into imidothioate **8** by the reaction with iodomethane and potassium carbonate in acetone at room temperature, and then **8** was treated with $AgBF_4$ in THF and in DME, the yield of oxazole **4** from **3** was considerably improved to 67% in THF and 87% in DME. The structure of **4** was con-

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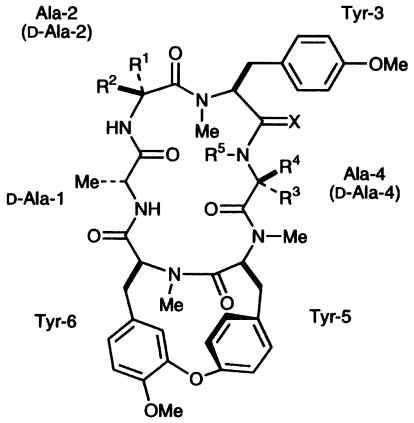
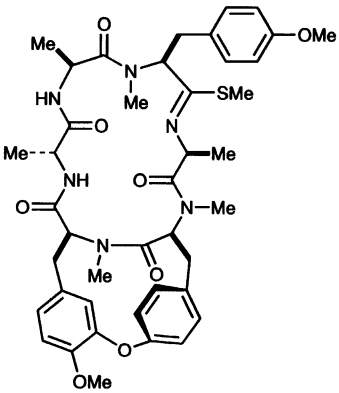


	R^1	R^2	R^3	R^4	R^5	X
1	H	Me	H	Me	H	O
3	H	Me	H	Me	H	S
6	H	Me	H	Me	Ac	O
9	H	Me	Me	H	H	O
10	Me	H	H	Me	H	O



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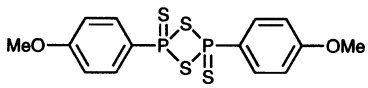

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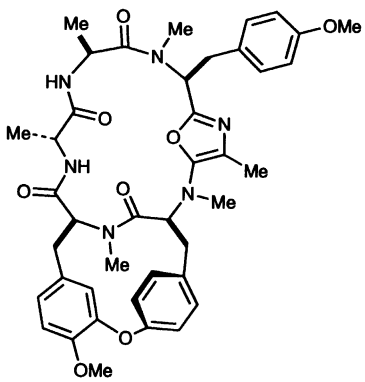

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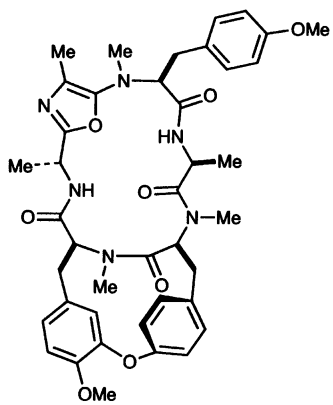

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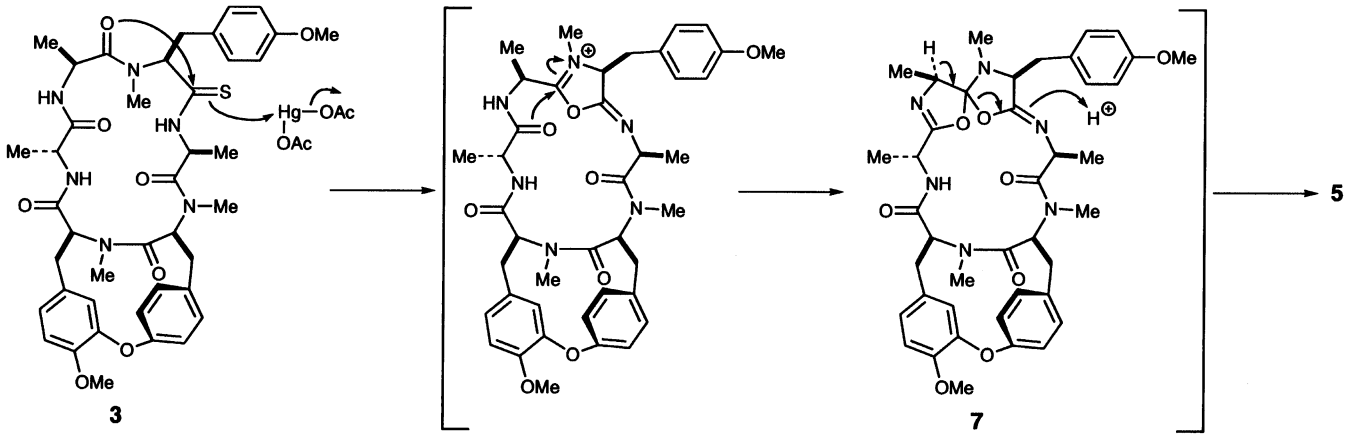
Figure 1.

Table 1. Reaction of thioamide **3** or imidothioate **8** with thiophilic reagents

Entry	Substrate	Reagent[a]	Solvent	Time (h)	Yield (%)				
					1	**4**	**5**	**6**	**3**[b]
1	**3**	A	THF	20	23	24	0	0	9.1
2	**3**	B	THF	20	9.3	3.9	45	14	0
3	**3**	A	DME	20	24	24	1.0	0	18
4	**3**	B	DME	20	14	8.7	42	8.2	0
5	**3**	A	MeCN	20	7.7	1.0	0	0	81
6	**3**	B	MeCN	20	22	26	32	4.2	0
7	**3**	A	$CHCl_3$	20	35	7.3	0	0	41
8	**3**	B	$CHCl_3$	20	29	4.1	44	6.2	0
9	**3** (via **8**)	A	THF	8	6.3	67	1.3	0	0
10	**3** (via **8**)	B	THF	8	8.3	2.2	40	29	0
11	**3** (via **8**)	A	DME	12	7.5	87	0.8	0	0
12	**3** (via **8**)	B	DME	12	7.6	1.6	41	18	0

[a] 2 mol. equiv. of reagent was used. A: $AgBF_4$; B: $Hg(OAc)_2$.
[b] Recovered starting material.



Scheme 1.

firmed by X-ray crystallography,[8] and those of **5** and **6** by the analysis of 2D NMR spectra.[9,10]

The oxazoles were hydrolysed under mild conditions, i.e. by treatment with boron trifluoride diethyl etherate in MeCN–H_2O (9:1) at 50°C. Oxazole **4** afforded **1** and [D-Ala-4]RA-VII (**9**)[11] in yields of 74 and 26%, respectively, and **5** afforded **1** and [D-Ala-2]RA-VII (**10**)[11] in yields of 60 and 24%, respectively.

Those D-alanine-containing analogues **9** and **10** showed a cytotoxic action on P-388 murine leukaemia cells (IC_{50} 8.7 and 9.0 μg/mL, respectively), which were much weaker than that of the parent cyclic peptide RA-VII (**1**) (0.0026 μg/mL).

There are a number of naturally-occurring cyclic peptides with a variety of interesting biological activities. This new approach provides a practical method for preparation of D-amino acid-containing analogues of those peptides, which may be of great value to peptide chemistry.

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8. Crystal data for **4**: $C_{41}H_{48}N_6O_8 \cdot 2H_2O$, $M=788.90$, 0.20×0.08×0.43 mm, monoclinic, $P2_1$ (no. 4), $a=7.941(1)$, $b=30.348(6)$, $c=8.998(1)$ Å, $\beta=112.14(1)°$, $V=2008.6(6)$ Å^3, $T=173$ K, $Z=2$, μ(Cu Kα)=14.09 cm^{-1}, 3302 reflections measured, 3047 unique reflections ($R_{int}=0.062$), $R=0.058$, $Rw=0.080$. The structure was solved by direct methods and expanded using Fourier techniques.
9. The amide configurations of all compounds are provisionally depicted in the structures.
10. We consider that this oxazole formation is not a specific reaction for substrate **3**, since oxazoles were also formed when [Tyr-6-Ψ(CS-NH)-D-Ala-1]RA-VII was treated in the same manner. Details of analogous experiments on different substrates including these will be reported in a full paper in due course.
11. Compound **9**: mp 234–237°C; **10**: mp 283–284°C.