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An Efficient Backbone Amide Nitrogen Alkylation of RA-VII, an Antitumor Cyclic Hexapeptide[1)]

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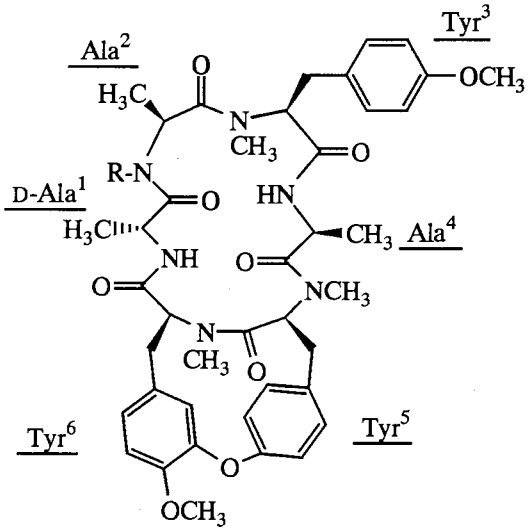
RA-VII, a potent antitumor cyclic hexapeptide, was effectively alkylated under the phase-transfer condition to afford [N-dialkylaminoethyl-Ala^2]RA-VII derivatives without racemization.

An antitumor cyclic hexapeptide, RA-VII (**1**), has been isolated from *Rubia akane* and *R. cordifolia* (Rubiaceae),[2)] and is now under clinical trial. On conducting clinical study, some difficulties were encountered in solubilizing **1** under the physiological conditions.

To obtain the derivatives which would show improved solubility in water and hopefully might express more promising biological properties, we planned to introduce a dialkylaminoethyl moiety to **1**. As **1** adopts rather rigid conformations in solution by internal hydrogen bonding between amide hydrogens and carbonyl oxygens, only Ala^2 NH could be alkylated.[3)]

We initially tried to carry out this alkylation under very mild conditions in order to avoid any potential problems associated with racemization. However, previously reported procedures for the N-alkylation of the Ala^2 residue with potassium fluoride on alumina in dimethoxyethane at room temperature [3,4)] or with potassium carbonate in refluxing acetone are only effective in the case of methylation with highly reactive and less bulky alkylating agents, such as iodomethane or dimethyl sulfate. Even under these mild conditions, some racemization was detected in the latter case.

After exhaustive and fruitless attempts to introduce aminoalkyl group at the Ala^2 residue of **1** including with sodium hydride in dimethyl formamide or tetrahydrofuran at room temperature to elevated temperatures, we found that use of dichloromethane (CH_2Cl_2)-50% sodium hydroxide (NaOH) system in the presence of phase transfer catalyst (tetra-n-butylammonium bromide, n-Bu_4NBr)[5)] with 1.5 equivalents of 2-chloroethyldialkylamine hydrochloride afforded corresponding [N-dialkylaminoethyl-Ala^2]RA-VII derivatives in good yields.[6)] In this manner, derivatives **2a-f** were prepared (Table 1). Under these conditions, no racemization was observed, which was confirmed by comparison of ^{1}H and ^{13}C-nuclear magnetic resonance (NMR) chemical shifts and/or coupling constants of the products



1 : R = H (RA-VII)
2 : R = $CH_2CH_2NR'R''$

with those of **1**, and by almost complete recovery (>97%) of the intact starting material after 24 hours period exposure of **1** under the conditions with no alkylating agent added. The observations that the reaction conditions had no influence on racemization nor caused decomposition of the substrate suggested that this protocol might be applicable to other base-sensitive cyclic peptides.

The [N-dialkylaminoethyl-Ala^2]RA-VII derivatives **2a-f** retain cytotoxicities against P388 and KB cells. Further biological evaluations of these derivatives are in progress and the results will be reported elsewhere.

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Table 1. Preparations and cytotoxicities of [N-dialkylaminoethyl-Ala^2]RA-VII derivatives

	Compound **2**		Time	Yield	Mp	$[\alpha]_D$/°	^{13}C-NMR	Cytotoxicity[a]	
	R'	R"	h	%	°C	($CHCl_3$)	(-$CH_2CH_2NR'R"$,$CDCl_3$,δ)	P388	KB
a	CH_3	CH_3	24	84	254-255	-163.0 (*c* 0.137)	59.6(t),45.7(q),42.2(t)	0.12	0.19
b	C_2H_5	C_2H_5	38	85	211-212	-146.0 (*c* 0.190)	59.1(t),47.6(t),43.0(t),11.9(q)	0.21	0.46
c	C_3H_7-i	C_3H_7-i	42	72	234-236	-143.4 (*c* 0.166)	49.4(d),45.7(t),44.9(t),21.5(q) 21.2(q)	0.25	0.40
d	Bn	Bn	45	44[b]	220-222	-146.1 (*c* 0.151)	139.4(s),129.0(d),128.4(d), 127.1(d),60.1(t),58.3(t),43.4(t)	0.19	0.48
e	-$(CH_2)_4$-		40	88	187-189	-144.2 (*c* 0.110)	56.1(t),54.3(t),42.5(t),23.4(t)	0.13	0.70
f	-$(CH_2)_5$-		40	87	178-179	-147.1 (*c* 0.101)	59.4(t),55.1(t),41.8(t),25.8(t), 24.1(t)	0.19	1.09

a) IC_{50}; μg/ml. b) 10 equivalents of N-(2-chloroethyl)dibenzylamine hydrochloride were used.

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6) A typical procedure is described for the preparation of **2a**: To a solution of **1** (29.5 mg, 0.0383 mmol), N-(2-chloroethyl)dimethylamine hydrochloride (8.8 mg, 0.060 mmol) and n-Bu_4NBr (6.3 mg, 0.019 mmol) in CH_2Cl_2 (2 ml) was added 50%NaOH (0.5 ml). After vigorous stirring for 24 h at room temperature, 29% NH_4OH (1 ml) was added and stirred over night. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2-MeOH, 10:1) to afford **2a** (27.0 mg, 84%) as a colorless powder.

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