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Solid-phase synthesis of an RNA nucleopeptide fragment from the nucleoprotein of poliovirus

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ABSTRACT

The naturally occurring RNA-nucleopeptide H-Ala-Tyr[5′-pUUAAAAC-3′]-NH_2 is prepared *via* a solid-phase phosphite triester approach using *N*-SiOMB/*O*-TBDMS-protected nucleosides. Preliminary ^{1}H-NMR studies show that the peptidyl unit has a remarkable effect on the conformational behaviour of the RNA moiety in the nucleopeptide.

INTRODUCTION

In the process of gene expression nucleic acid-protein interactions constitute a very important and multifarious stage. Practically all these interactions are of a noncovalent binding character. In the late 1950's it was discovered[1] that many RNA's and DNA's may be covalently linked to specific proteins. Later on it was established that the covalent attachment of proteins to nucleic acids forms an important step in the viral genome replication[2]. For example, the genome RNA of the poliovirus, isolated from virions, contains a covalently linked virus specific protein[3] (designated VPg for '*V*iral *P*rotein *G*enome-Linked'). Chemical and enzymatic degradation studies[4] revealed the presence of a phosphate diester bond between the 5′-hydroxyl of an uridine residue of the viral RNA and the phenolic hydroxy group of a tyrosine residue in the VPg.

As part of our continuous efforts[5] in optimizing the synthesis of nucleopeptides, we recently reported[6] that the protection of exocyclic amino functions in nucleobases (*i.e.* adenine, cytosine or guanine) with the 2-(*tert*-butyldiphenylsilyloxymethyl)benzoyl (SiOMB) group was a promising alternative, due to its smooth removal with fluoride ion under virtually neutral conditions, for the well-established *N*-acyl protecting group strategy. The successful application of the SiOMB together with the protection of 2′-hydroxyl function of ribonucleosides with *tert*-butyldimethylsilyl (TBDMS) group was nicely demonstrated[7] in a solid-phase synthesis of an RNA fragment.

In order to widen the scope of the SiOMB-TBDMS protecting group strategy, we report here that the same strategy can be adopted for a solid-phase synthesis of the RNA nucleopeptide fragment H-Ala-Tyr[5′-pUUAAAAC-3′]-NH_2 (**14**) of the nucleoprotein from the poliovirus.

EXPERIMENTAL

Acetonitrile, dioxane, dichloromethane, pyridine and *N,N*-diisopropylethylamine were dried by refluxing with calcium hydride (5 g/L), distilled and stored over molecular sieves (4Å). Dioxane was redistilled over lithium aluminium hydride (5 g/L) before use. *N,N*-dimethylformamide was stirred with calcium hydride (5 g/L) and distilled under reduced pressure. Triethylammonium hydrogen carbonate buffer (2 M) was prepared by passing a stream of carbon dioxide through a cooled (0°C) solution of triethylamine in water until a neutral solution was obtained. (*N,N*-Diisopropyl) 2-cyanoethyl chloro phosphoramidite (**3**)[8], bis(*N,N*-diisopropyl) 2-cyanoethyl phosphoramidite (**8**)[10] and N^{α}-*o*-nitrophenylsulfenylalanine[15] dicyclohexylammonium salt were prepared according to published procedures. 1*H*-Tetrazole was purchased from Pharmacia. 5′-*O*-4,4′-Dimethoxytrityl-2′-*O*-(*tert*-butyldimethylsilyl)uridinyl-3′-*O*-(*N,N*-diisopropyl) 2-cyanoethyl phosphoramidite (**4**, B=uridine) and tyrosine amide were purchased from Synorchem and Novabiochem, respectively. TLC analysis was performed on Schleicher and Schüll Fertigfolien F1500 LS254 with solvents systems: A (dichloromethane/methanol, 37/3, v/v); B (dichloromethane/methanol, 19/1, v/v); C (ethyl acetate/triethylamine, 39/1, v/v). Short column chromatography was performed on Kieselgel 60, 230–400 mesh (Merck). ^{1}H-NMR spectra were measured at 600 MHz, using a Bruker AM-600 spectrometer, equipped with an ASPECT-3000, operating in the Fourier transform mode. Chemical shifts are given in ppm (δ) relative to TMS ($CDCl_3$) or DSS (D_2O). ^{31}P-NMR and ^{13}C-NMR spectra were measured at 80.7 MHz and 50.3 MHz, respectively, using a JEOL JNM-FX 200 spectrometer. Chemical shifts are given in ppm (δ) relative to 85% H_3PO_4 (^{31}P-NMR) as external standard. FPLC analysis was carried out on a Pharmacia LCC-500 liquid chromatograph using a MonoQ HR 5/5 column (anion-exchange) or a ProRPC HR 5/10 column (reversed-phase). Gradient elution was performed at 20°C by building up a gradient starting with buffer A (0.01 M sodium hydroxide (anion-exchange); 0.1 M triethylammonium acetate in water pH 7 (reversed-phase) and applying buffer B (0.01 M sodium hydroxide and 1.2 M sodium chloride (anion-exchange); 0.1 M triethylammonium acetate in water/acetonitrile, 1/9, v/v, pH 7 (reversed-phase) at a flow rate of 2.0 mL/min (anion-exchange) and 0.75 mL/min (reversed-phase). Gel filtration was carried out

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on a Hiload Sephacryl 75 HR 16/120 by elution with 0.15 M triethylammonium hydrogen carbonate buffer at a flow rate of 1.5 mL/min. Solid-phase synthesis was performed on a Gene Assembler (Pharmacia). The synthesis was conducted on a 10 μmol scale using derivatized Monobeads (Pharmacia). Solvents were HPLC-grade and dried over molecular sieves (3Å, Fluka) before use. All manipulations during and after deprotection of the nucleopeptide were performed under sterile conditions. Glassware was rinsed with sterile water and heated at 120°C for 16 h before use. Gloves were used when necessary.

5'-*O*-4,4'-Dimethoxytrityl-2'/3'-*O*-*tert*-butyldimethylsilyl-*N*-2(*tert*-butyldiphenylsilyloxymethyl) benzoyl nucleosides 1

Compounds **1** (B=A^{SiOMB}, C^{SiOMB}) were prepared according to a published procedure[7].

5'-*O*-4,4'-Dimethoxytrityl-2'-*O*-(*tert*-butyldimethylsilyl)-*N*-2(*tert*-butyldiphenylsilyloxymethyl) benzoyl adenosyl/cytidyl-3'-*O*-(*N,N*-diisopropyl) 2-cyanoethyl phosphoramidite (4)

Compounds **4** (B=A^{SiOMB}, C^{SiOMB}) were prepared from compounds **1** by phosphitylation with reagent **3** according to published procedures[7].

5'-*O*-4,4'-Dimethoxytrityl-3'-*O*-(*tert*-butyldimethylsilyl)-2'-*O*-succinoyl-4-*N*-2-(*tert*-butyldiphenylsilyloxymethyl)benzoyl cytidine (5)

To a solution of **2** (B=C^{SiOMB}) (1.03 g, 1.0 mmol) in pyridine (5 mL) was added succinic anhydride (0.30 g, 3.0 mmol) and 4-dimethylaminopyridine (0.02 g, 0.20 mmol). After the reaction mixture has been stirred for 5 h at 50°C, the solvent was evaporated, the residue dissolved in dichloromethane (75 mL) and washed with water (2×20 mL). The organic layer was dried over magnesium sulphate and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (15 g, dichloromethane/methanol, 1/0 to 97/3, v/v) to give **5** (0.84 g, 74% yield) as a foam.

R_f 0.67 (system A); ^{13}C-NMR ($CDCl_3$) δ 17.7 (C(CH_3), TBDMS), 19.2 (C(CH_3), SiOMB), 25.4 (C(CH_3), TBDMS), 26.7 (C(CH_3), SiOMB), 29.1 (CH_2, succinoyl), 55.0 (OCH_3, DMT), 61.4 (C-5'), 63.7 (CH_2OSi), 69.4 (C-2'), 75.7 (C-3'), 83.8 (C-4'), 87.1 (Cq, DMT), 88.5 (C-1'), 97.0 (C-5), 113.1−135.4 (CH, aromatic), 133.0 (CSi, phenyl), 134.9, 140.9, 143.6 and 144.6 (Cq, SiOMB, DMT), 154.5 (C-2), 158.8 (Cq, DMT), 162.7 (C-4), 168.1 (C=O, SiOMB), 170.6 and 176.6 (C=O, succinoyl).

Functionalisation of the solid support

To a solution of the succinoyl derivative **5** (0.80 g, 0.70 mmol) in dioxane (10 mL) was added 1-hydroxybenzotriazole (0.14 g, 1.05 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.22 g, 1.05 mmol). After 6 h at 20°C the precipitate was removed by filtration. The filtrate, N-methylimidazole (0.28 mL, 3.50 mmol) and diisopropylethylamine (0.64 mL, 3.50 mmol) were added to the solid support (1.00 g, Monobeads). After the reaction mixture was shaken for 70 h at 20°C, the solvents were removed by filtration and the support was thoroughly rinsed with dioxane (25 mL), dichloromethane (25 mL), methanol (25 mL) and diethyl ether (10 mL). The extent of loading (73 μmol/g) was determined according to a published procedure[9].

Table 1. Chemical steps involved in each elongation cycle.

Step	Manipulation	Solvents and reagents[a]	Time (min)
1	Detritylation	2% trichloroacetic acid in 1,2-dichloroethane	2.5
2	Coupling	4[b] or 9[b], 1*H*-tetrazole[c], CH_3CN	90.0
3	Oxidation	0.02 M I_2 in CH_3CN/2,4,6-collidine/H_2O (10/1/5, v/v/v)	2.5
4	Capping	0.25 M 4-dimethylaminopyridine in Ac_2O/2,4,6-collidine/CH_3CN (1/1/8, v/v/v)	2.5

[a] Reactions were performed on 150 mg (10 μmol) of resin **6**. [b] 0.1 M in CH_3CN (0.6 mL). [c] 0.5 M 1*H*-tetrazole (1.1 mL) in CH_3CN.

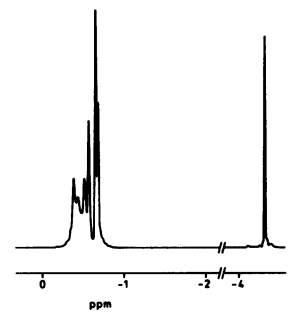


Figure 1. ^{31}P-NMR spectrum (200 MHz) of the RNA-nucleopeptide **14**.

N^{α}-*o*-nitrophenylsulfenylalanyltyrosine amide (7)

N^{α}-*o*-Nitrophenylsulfenylalanine dicyclohexylammonium salt (0.23 g, 5.5 mmol) was added to a mixture of ethyl acetate/water (100 ml, 1/1, v/v) at 0°C. Sulphuric acid (5.5 mL, 1 N) was added and the organic phase was separated, washed with an aqueous sodium chloride solution (2×25 mL, saturated) and dried over magnesium sulphate. The solvent was evaporated and the residue was redissolved in dimethylformamide (25 mL). The solution was cooled (−5°C), tyrosine amide (0.90 g, 5.0 mmol), 1-hydroxybenzotriazole (0.75 g, 5.5 mmol) and *N,N'*-dicyclohexylcarbodiimide were added. The reaction mixture was stirred for 16 h at 20°C. The precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in ethyl acetate (100 mL) and washed with potassium dihydrogen phosphate buffer (2×25 mL, 1 M), water (25 mL), aqueous sodium hydrogen carbonate (2×25 mL, 1 M) and water (25 mL). The organic layer was dried over magnesium sulphate and concentrated to a small volume (30 mL). The dipeptide (1.75 g, 85%, yellow crystals) crystallized after the addition of diisopropyl ether (20 mL).

Mp 136−142°C; R_f 0.15 (system B); $[\alpha]_D^{20}$ −23.2° (c=1, methanol); ^{1}H-NMR ($CDCl_3/CD_3OD$, 1/1, v/v) δ 1.35 (d, 3H, Cβ, $J_{\beta,\alpha}$ 7.2 Hz, Ala), 2.93 (dd, 1H, Hβa, $J_{\beta a,\beta a,\beta b}$ 14.0 Hz, $J_{\beta a,\alpha}$ 7.0 Hz, Tyr), 3.04 (dd, 1H, Hβb, $J_{\beta b,\alpha}$ 7.0 Hz, Tyr), 3.45 (m, 1H, Hα, Tyr), 4.60 (q, 1H, Hα, Ala), 6.74 (d, 2H, aromatic H, $J_{\gamma,\delta}$ 8.5 Hz, Tyr), 7.05 (d, 2H, aromatic H, Tyr), 7.29−8.28 (m, 4H, aromatic H, NPS); ^{13}C-NMR ($CDCl_3/CD_3OD$, 1/1, v/v) δ 19.4 (Cβ, Ala), 37.7 (Cβ, Tyr),

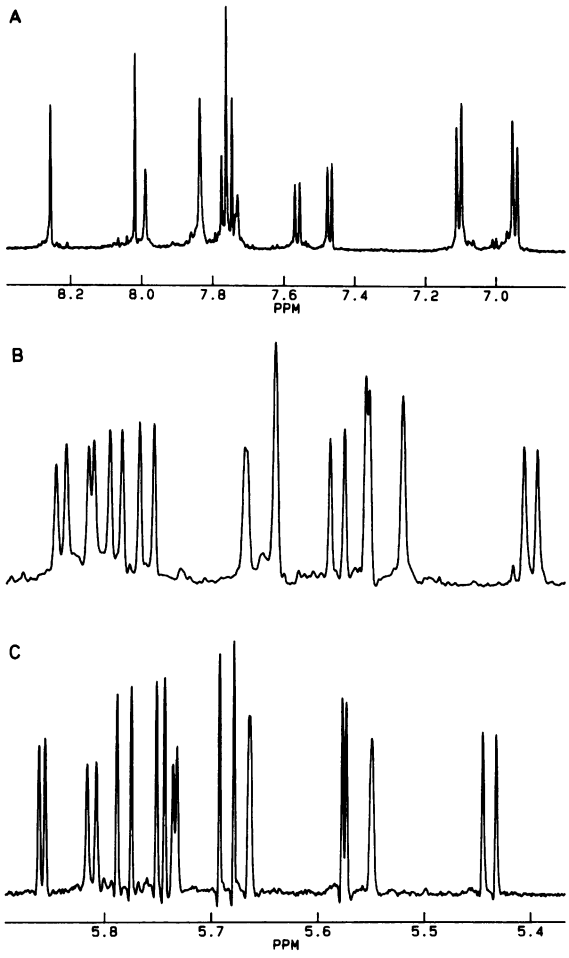


Figure 2. A:Aromatic region of the ^{1}H-NMR spectrum (600 MHz) of the RNA-nucleopeptide **14**. B:H-1′ and H-5 (*i.e.* of the U-1, U-2 and C-7 base residues) region of the ^{1}H-NMR spectrum (600 MHz) of the RNA-nucleopeptide **14**. C:H-1′ and H-5 (*i.e.* of the U-1, U-2 and C-7 base residues) region of the ^{1}H-NMR spectrum (600 MHz) of the native RNA fragment **15**. Chemical shifts (δ) are in ppm relative to DSS.

54.6 (Cα, Tyr), 60.2 (Cα, Ala), 115.8−134.6 (CH, aromatic), 127.9 (Cq, Tyr), 143.3 and 145.3 (Cq, NPS), 156.4 (C-OH, Tyr), 175.1 and 175.5 (2×C=O).

(N^{α}-*o*-nitrophenylsulfenylalanyltyrosine amide) (*N*,*N*-diisopropyl) 2-cyanoethyl phosphoramidite (9)

To a solution of dipeptide **7** (0.20 g, 0.5 mmol), which was dried by evaporation with acetonitrile, and 1H-tetrazole (44 mg, 0.63 mmol) in dry acetonitrile (3 mL) was added **8** (1.25 mL, 0.5 M in CH_2Cl_2). After stirring for 10 min, the reaction mixture was diluted with dichloromethane (50 mL), washed with aqueous sodium hydrogen carbonate (20 mL, 0.5 M) and aqueous sodium chloride (20 mL, saturated). The organic phase was dried over magnesium sulphate and concentrated *in vacuo* in the presence of toluene (5 mL). The crude product was purified by silica gel column chromatography (10 g, hexane/ethyl acetate/triethylamine, 20/79/1 to 0/99/1, v/v/v) to give **9** (0.27 g, 88% yield) as a yellow foam. R_f 0.74 (system C); ^{31}P-NMR (CH_2Cl_2) δ 147.0.

Table 2. Conformational parameters[a] of RNA-nucleopeptide **14**[b] and the native RNA fragment (**15**)[b].

Fragment	Residue	U(1)	U(2)	A(3)	A(4)	A(5)	A(6)	C(7)
14	δ^c *H-1′*	5.80	5.85	5.82	5.68	5.65	5.53	5.60
	$J_{1',2'}{}^d$	6.8	5.7	3.0	1.6	1.0	1.0	1.9
	$\%N^e$	16	32	71	92	100	100	87
15	δ^c *H-1′*	5.80	5.74	5.85	5.72	5.65	5.54	5.57
	$J_{1',2'}{}^d$	5.2	4.6	3.4	2.3	1.0	0.9	2.2
	$\%N^e$	40	48	66	82	100	100	83

[a] ^{1}H-NMR spectra were measured at 600 MHz at 284 K. [b] D_2O, pD 6.9, 2.5 mM (**14**) 5.0 mM (**15**). [c] Chemical shifts (δ) in ppm are relative to DSS. [d] Coupling constants (*J*) are in Hz. [e] derived from the coupling constants[13].

Deprotection and purification of nucleopeptide 11−14

The solid support containing immobilized **11** was removed from the column and treated with dry ammonia methanol (10.0 mL, saturated) for 1 h at 20°C. The solid support was removed by filtration and rinsed with dichloromethane/methanol (3×15 mL, 1/1, v/v). The filtrate, containing **12**, was concentrated *in vacuo* and the residue was evaporated with tetrahydrofuran (3 mL). To a solution of compound **12** in tetrahydrofuran (1 mL) was added 2-mercaptopyridine (12 mg, 0.1 mmol). After 16 h at 20°C, the reaction mixture was concentrated and purified by gel filtration on an LH20 column (3×20 cm) suspended in dichloromethane/methanol (1/2, v/v). To a solution of **13** in tetrahydrofuran (0.5 mL) was added tetrabutylammonium fluoride (1.0 mL, 1.0 M in THF). After 16 h at 20°C, sterile water (20 mL) was added and the mixture was extracted with diethyl ether (2×10 mL). The aqueous phase was concentrated *in vacuo* and redissolved in triethylammonium hydrogen carbonate buffer (1.5 mL, 0.15 M, CH_3OH/H_2O, 1/9, v/v). The product was purified by gel filtration on a Sephacryl column suspended in the same buffer to give **14** (3.9 mg). The aqueous phase was lyophilized twice from deuterium oxide (2×0.5 mL, 99.9%) to give **14** as a white solid. The NMR sample was prepared by dissolving **14** (3.9 mg) in deuterium oxide (0.6 mL, 99.9%) and a sodium phosphate buffer (20 μL, pD 6.9, 25 mM).

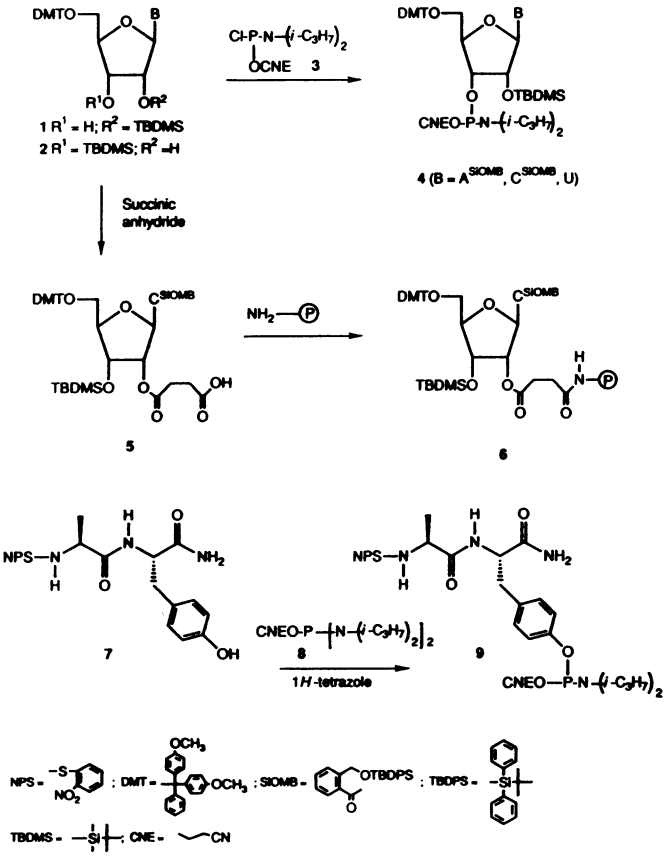
FPLC analysis: Rt 5.3 min (anion-exchange), 8.8 min (reversed-phase); Rt Sephacryl 172.5 min; ^{31}P-NMR (D_2O, pD 6.9, 2.5 mM) δ −4.31, −0.68, −0.65, −0.56, −0.51, −0.44, −0.38; ^{1}H-NMR (D_2O, 2.5 mM) δ 1.41 (d, 3H, Hβ, Ala, $J_{\beta,\alpha}$ 6.6 Hz), 2.85 (m, 1H, Hβa, Tyr), 2.99 (m, 1H, Hβb, Tyr), 3.95−4.70 (m, 37H, H-2′, H-3′, H-4′, H-5′, H-5″, 2×Hα), 5.41 (d, 1H, H-5, C(7), $J_{5,6}$ 7.5 Hz), 5.53 (bs, 1H, H-1′, A(6)), 5.56 (d, 1H, H-1′, C(7), $J_{1',2'}$ 1.9 Hz), 5.59 (d, 1H, H-5, U(1), $J_{5,6}$ 8.1 Hz), 5.65 (bs, 1H, H-1′, A(5)), 5.68 (d, 1H, H-1′, A(4), $J_{1',2'}$ 1.6 Hz), 5.77 (d, 1H, H-5, U(2), $J_{5,6}$ 8.0 Hz), 5.80 (d, 1H, H-1′, U(1), $J_{1',2'}$ 6.8 Hz), 5.82 (d, 1H, H-1′, A(3), $J_{1',2'}$ 3.0 Hz), 5.85 (d, 1H, H-1′, U(2), $J_{1',2'}$ 5.7 Hz), 6.95 (d, 2H, aromatic H, Tyr, J 8.4 Hz), 7.11 (d, 2H, aromatic H, Tyr), 7.48 (d, 1H, H-6, C(7)), 7.57 (d, 1H, H-6, U(1)), 7.74 (s, 1H, H-2, A(3)), 7.75 (s, 1H, H-2, A(4)), 7.77 (s, 1H, H-8, A(6)), 7.77 (d, 1H, H-6, U(2)), 7.84 (s, 2H, H-8 and H-2, A(5)), 7.99 (s, 1H, H-8, A(4)), 8.02 (s, 1H, H-2, A(6)), 8.26 (s, 1H, H-8, A(3)).

Oligonucleotide: 5′-UUAAAAC-3′(15)

UUAAAAC was prepared according to a published procedure[7]. FPLC analysis: Rt 5.4 min (anion-exchange); ^{1}H-NMR (D_2O, pD 6.9, 5 mM) δ 3.95−4.70 (m, 35H, H-2′, H-3′, H-4′, H-5′,

H-5''), 5.43 (d, 1H, H-5, C(7), $J_{5,6}$ 7,5 Hz), 5.54 (bs, 1H, H-1', A(6)), 5.57 (d, 1H, H-1', C(7), $J_{1',2'}$ 2.2 Hz), 5.65 (bs, 1H, H-1', A(5)), 5.68 (d, 1H, H-5, U(2), $J_{5,6}$ 8.1 Hz), 5.72 (d, 1H, H-1', A(4), $J_{1',2'}$ 2.3 Hz), 5.74 (d, 1H, H-1', U(1), $J_{1',2'}$ 4.6 Hz), 5.77 (d, 1H, H-5, U(1), $J_{5,6}$ 8.1 Hz), 5.80 (d, 1H, H-1', U(1), $J_{1',2'}$ 5.2 Hz), 5.85 (d, 1H, H-1', A(3), $J_{1',2'}$ 3.4 Hz), 7.51 (d, 1H, H-6, C(7)), 7.74 (d, 1H, H-6, U(2)), 7.77 (bs, 3H, H-8, A(6), H-2, A(3) and A(4)), 7.78 (d, 1H, H-6, U(1)), 7.84 (bs, 2H, H-2, A(5), H-8, A(5)), 8.03 (bs, 2H, H-2, A(6), H-8, A(4)), 8.23 (s, 1H, H-8, A(3)).

Scheme 1



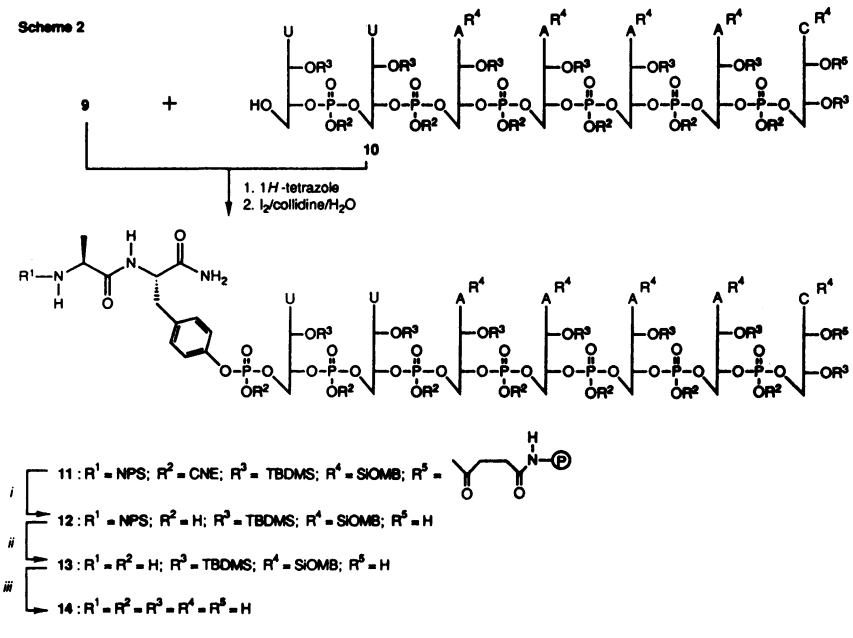
RESULTS AND DISCUSSION

The required incoming ribonucleoside synthons **4** (Scheme 1) are readily accesible by phosphitylation of the known[7] 5'-*O*-DMT-2'-*O*-TBDMS-*N*-SiOMB protected nucleosides **1** with (*N*,*N*-diisopropyl) 2-cyanoethyl chloro phosphoramidite (**3**)[8] in the presence of *N*,*N*-diisopropylethylamine.

The ribonucleoside derivative **2** (B=C^{SiOMB}), a side-product isolated in the early stage of the synthesis of **1** (B=C^{SiOMB}), was applied for the functionalisation of the solid support. Thus, treatment of **2** (B=C^{SiOMB}) with succinic anhydride and 4-dimethylaminopyridine in pyridine at elevated temperature yielded the succinoyl derivative **5**. Subsequent condensation of **5** with the amino groups on the resin (Monobeads, Pharmacia) could be effected with *N*,*N'*-dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole. The latter condensation reaction afforded immobilized cytosine **6** loaded with 70 μmol nucleoside/g support as gauged[9] from the spectroscopic determination of the 'trityl-color'.

The immobilized and partially protected heptamer **10** (5'-OH) was now assembled by stepwise elongation of immobilized cytosine **6** (Scheme 2) using an automated Gene Assembler (Pharmacia). One elongation cycle comprises (see Table 1) the following consecutive steps; (1) acidolysis of the 5'-*O*-4,4'-dimethoxytrityl group with trichloroacetic acid; (2) 1*H*-tetrazole-mediated coupling of the appropriate ribonucleoside phosphoramidites **4**; (3) oxidation of the intermediate phosphite triester with iodine/water; (4) capping of unreacted 5'-hydroxyl groups with 4-dimethylaminopyridine/acetic anhydride. With respect to step 2 of the elongation cycle, it is of interest to note that the amount of nucleoside phosphoramidite **4** (*i.e.* 6 or 10

Scheme 2



Reagents: (*i*) NH_3/MeOH. (*ii*) 2-mercaptopyridine, MeOH (*iii*) 1.0 M (*n* - Bu)$_4$ NF in THF.

equivalents) displayed no profound effect on the coupling efficiency. On the other hand, prolonged coupling (*i.e.* 90 instead of 60 min) had a beneficial effect on the coupling efficiency (*i.e.* 98%).

At this stage, the phenolic hydroxyl function of tyrosine in the dipeptide **7** was phosphitylated with bis(*N,N*-diisopropyl) 2-cyanoethyl phosphordiamidite (**8**)[10] by the agency of 1*H*-tetrazole to give, after silica gel column chromatography, the homogeneous phosphoramidite **9** in 88% yield. Subsequent 1*H*-tetrazole-mediated coupling of **9** with the immobilized heptamer **10** (step 2 in Table 1) afforded, after oxidation (step 3) of the resulting phosphite triester, the fully protected and immobilized nucleopeptide **11**.

The nucleopeptide **11** was completely deprotected by the following three-step procedure. Ammonolysis (1 h at 20°C) of **11** effected the release of **12** from the solid support (R^3) and concomitant elimination of the 2-cyanoethyl groups (R^2). The *o*-nitrophenylsulfenyl (NPS, R^1) group of **12** was removed by thiolysis with 2-mercaptopyridine[11] resulting in partly deprotected **13**. Finally, removal of the *N*-SiOMB- (R^4) and 2′-/3′-*O*-TBDMS (R^5) groups of **13** with fluoride ion resulted in the isolation of fully deprotected **14**. Purification by gel filtration afforded the RNA-nucleopeptide **14**, the homogeneity and identity of which was firmly established by FPLC analysis and NMR spectroscopy. Thus, ^{31}P-NMR spectroscopy revealed (Figure 1) the presence of seven distinct resonances, one of which (*i.e.* resonance at −4.3 ppm) is characteristic for the presence of the phosphate diester between the phenolic hydroxyl group of tyrosine and the 5′-end of the RNA moiety. Further, high-resolution NMR spectroscopy showed that the RNA-nucleopeptide **14** was in accord with the proposed structure (see Figure 2A/B). Interestingly, additional conformational analysis of the RNA-nucleopeptide indicated that the uridines at the 5′-terminal of the RNA moiety adopted, in contrast with the same units in the native RNA fragment **15**[7], preferentially an S-type (C2′-*endo*)[12] conformation (see Table 2). Moreover, n.O.e.'s were observed between the Hδ protons of tyrosine and the individual 2′ and 3′ protons of the 5′-terminal uridine residue, denoting that the peptide moiety dramatically alters the conformation of the RNA in the nucleopeptide. More detailed studies dealing with the unexpected conformational behaviour of the nucleopeptide will be published elsewhere[14].

The synthesis of the nucleopeptide fragment H-Ala-Tyr[5′-pUUAAAAC-3′]-NH_2 presented in this paper indicates that a solid-phase approach, based on the *N*-SiOMB/*O*-TBDMS protecting group strategy, can be applied to assemble nucleopeptides containing RNA-(P-*O*-)tyrosine bonds. It has to be noted, however, that the efficiency of the key coupling step between the peptide derivative **9** and immobilised **10** (see Scheme 2) could not be determined due to the absence of a suitable reporter group in the incoming peptide moiety. At present we are studying in detail wether this shortcoming can be nullified by anchoring an appropriate tag on the incoming peptidyl unit.

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