Tetrahedron Letters Vol. 22, pp 765 - 768 0040-4039/81/0215-0765$02.00/0

SOLID PHASE SYNTHESIS OF RIBO-OLIGONUCLEOTIDES ON A POLYACRYLMORPHOLIDE SUPPORT

E. Ohtsuka, H. Takashima and M. Ikehara*

Faculty of Pharmaceutical Sciences, Osaka University, Suita 565 Japan

A riboheptanucleotides G-C-A-A-C-C-A which has a sequence of the 3'-end of E. coli formylmethionine tRNA has been synthesized on a polyacrylmorpholide resin by the triester approach using dinucleotide blocks.

Recently syntheses of deoxyoligonucleotides on polyacylamide resins have been reported by several investigators.[1] Yields of condensations and purity of products have been improved by using deoxyoligonucleotide blocks having protections at the internucleotide linkages.[1d,e,2] We wish to report in this paper the synthesis of a riboheptanucleotide G-C-A-A-C-C-A which corresponds to the 3'-terminal sequence of E. coli $tRNA_f^{Met}$ by the phosphotriester solid phase method using dinucleotide blocks. Crosslinked polyacrylmorpholide (Enzacryl Gel K2 from Aldrich) was treated with propylenediamine to introduce amino functions[1a] (0.188 mmol/g) as shown in Chart 1. 5'-O-Monomethoxytrityl-3'-O-(o-nitrobenzyl)N-benzoyladenosine[3] was succinylated and 2 (281.8 mg, 250 umol) was linked to the resin (1) (301.9 mg, 55 umol) as the 3'-terminal unit using either DCC or pentachlorophenol. After 23 hr reaction the resin was treated with acetic anhydride to block the unreacted amino groups. The 5'-monomethoxytrityl group was removed by treatment with 5% benzenesulfonic acid for 30 min and estimated spectrophotometrically by measuring absorbance in 3:2 60% $HClO_4$-EtOH (57.9 μmol, using ε_{473} of 5.33×10^4). The derivatized resine (3) was then condensed with the protected dinucleotide (4) which was prepared by a rapid method using 2'-O-(o-nitrobenzyl) nucleosides.[4] The conditions for the coupling reaction are summarized in Table I and the yield is listed in Table II together with those of later steps. The yield was estimated by spectrophotometric measurement of the amount of monomethoxytritanol except for the last step. The 5'-deblocked trimer on the resin (5) was then reacted with the dinucloetide (6) using mesitylenesulfonyl 3-nitrotriazolide[5] (MSNT) in the same way and further elongated by condensation with another dimer (8) to yield the resin linked heptamer.

Deblocking was performed by the following procedure. An aliquot of the resin (101.4 mg, 5 μmol) was treated with 0.5 M pyridinaldoxime-tetramethylguanidine[6] in 1:1 dioxane-water (10 ml) for 67 hr. The resin was filtered and washed with aqueous pyridine. The combined solutions were washed with ethylacetate,back extracted, passed through a column of Dowex 50X2 (pyridinium form) to remove the strong base and concentrated. The residue was treated with concentrated ammonia

Chart 1

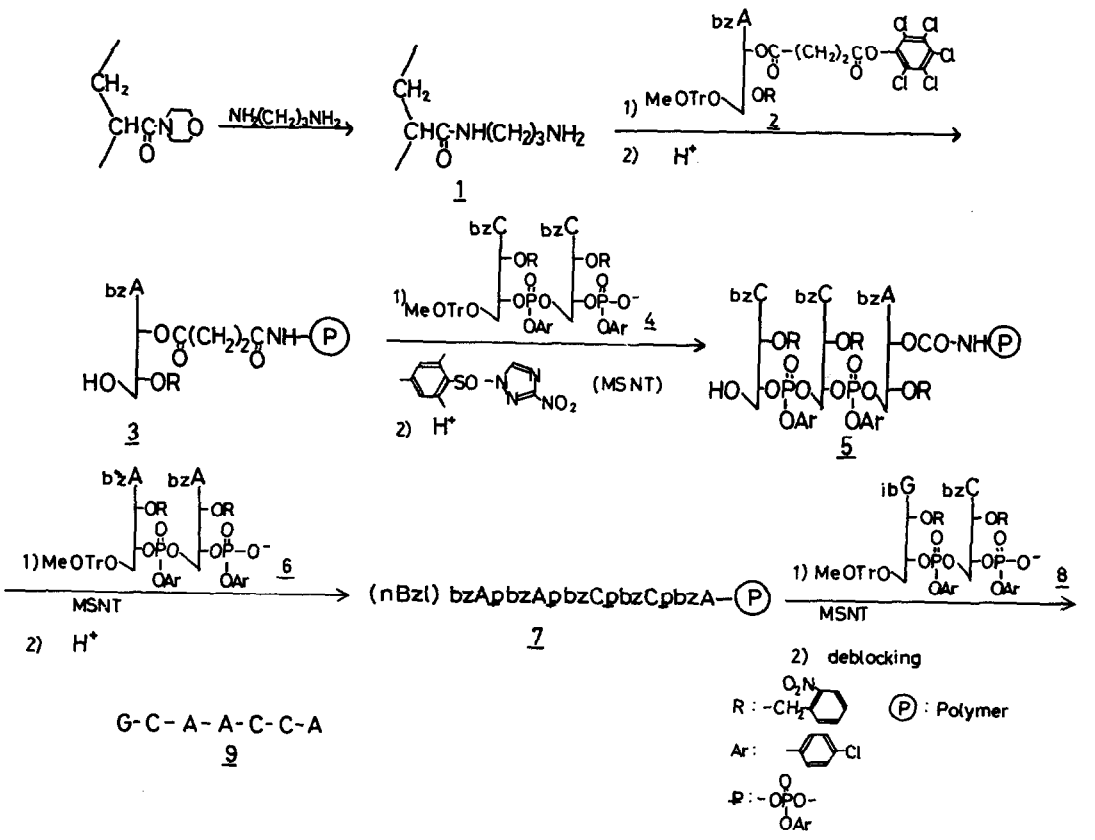

Table I. Conditions of coupling cycle

Step	Solvent or reagent	Amount	Time of shaking (min)	No. of operation
1	5% benzenesulfonic acid	10 ml	30	1
2	$CHCl_3$-MeOH (7:3)	10 ml	1	5
3	pyridine	10 ml	1	2
4	pyridine (coevaporation)	5 ml		3
5	dinucleotide block	5 eq.	120	1
	mesitylenesulfonyl nitrotriazolide	10 eq.		
	pyridine	6 ml		
6	pyridine	10 ml	1	2
7	Ac_2O-pyridine (3:7)	10 ml	30	1
8	pyridine	10 ml	1	2
9	$CHCl_3$-MeOH	10 ml	1	5

Table II. Summary of yields

Chain length	Sequence of linked oligonucleotide	MeOTrOH (A_{473})	Amount (μmol)	Yield(%)	Cal. resin wt. (mg)
1	HOA-	1.235	57.9		336.0
3	HOCCA-	1.118	52.4	90.5	406.5
5	HOAACCA-	0.528	24.8	47.3	442.0
7	MeOTrGAACCA-	0.295[a]	23.7	95.7	480.5

a, estimated from an aliquot of the resin

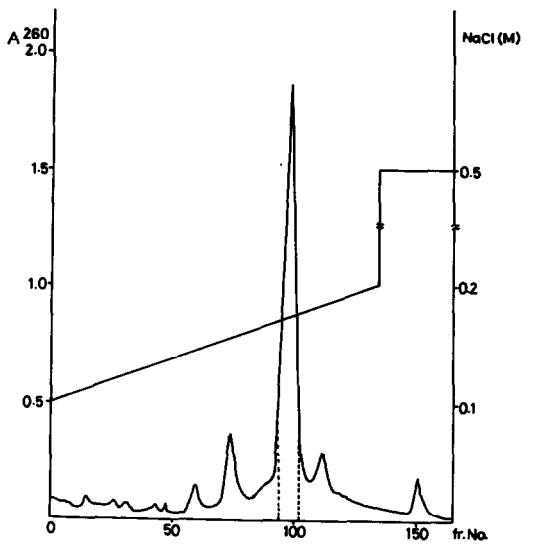

Fig. 1

Purification of G-C-A-A-C-C-A on a column (1.0 x 50 cm) of DEAE-cellulose. Elution was performed with a linear gradient of sodium chloride (0.1-0.2M, total 400 ml) in 7 M urea and 0.02 M tris-HCl, pH 7.5.

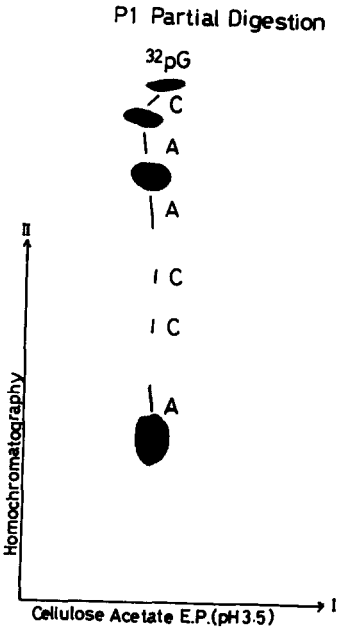

Fig. 2

Two dimensional homochromatography of G-C-A-A-C-C-A. Conditions were as described previously.[7]

at 60° for 6 hr and then with 80% acetic acid. The o-nitrobenzyl groups were then removed by irradiation of UV light with wave length longer than 280 nm.[7] The deblocked mixture (489 A_{259}) was analyzed by high pressure ion-exchange chromatography on Permaphase AAX, and one half was applied to paper chromatography (n-propanol-conc. ammonia-water 55:10:35, v/v) to remove faster travelling impurities. The eluted nucleotides were separated by ion-exchange chromatography on DEAE-cellulose in the presence of 7 M urea (Fig. 1) and the heptamer G-C-A-A-C-C-A (35.5 A_{260}) was obtained in a yield of 22% (0.55 μmol), assuming 15% hypochromicity. Aliquots (2 A_{260}) were completely hydrolyzed by treatment with nuclease P1 and RNase T2 to give correct ratios when analyzed by a Nucleic Acid Analyzer. Mobillity shift analysis of the partially digested 5'-labelled heptamer is shown in Fig. 2.

Thus ribo-oligonucleotides have been synthesized on the polyacrylmorpholide resin by the triester method. Dinucleotides[4] containing 2'-O-(o-nitrobenzyl) nucleosides have been proved useful in a solid phase synthesis. The 3'-O-(o-nitrobenzyl)adenosine was linked to the resin as the first nucleoside. This should avoid the attacking of the secondary hyroxyl group to the internucleotide phosphate during deblocking. Since ribonucleotides are more stable in acidic media than deoxyribonucleotides, elongation of the chain by removal of the monomethoxytrityl group in the 5'-direction is feasible in the ribo-series.

Acknowledgement

The authors thank Dr. A.F. Markham for personal communications. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan and by a grant from the Mitsubishi Foundation.

References

1. (a) C.K. Narang, K. Brunfeldt and K.E. Norris, Tetrahedron Lett. 1819-1822 (1977); (b) M.J. Gait, M. Singh, R.C. Sheppard, M.D. Edge, A.R. Greene, G.R. Heathcliffe, T.C. Atkinson, C.R. Newton and A.F. Markham, Nuceic Acids Res. 8, 1081-1096 (1980); (c) K. Miyoshi and K. Itakura, Tetrahedron Lett. 3635-3638 (1979); (d) Idem, Nucleic Acids Res. Symp. Ser. No.7, 281-291 (1980); (e) M.J. Gait, S.G. Popov, M. Singh and R.C. Titmas, ibid. 243-257 (1980)
2. A.F. Markham, personal communication.
3. E. Ohtsuka, T. Wakabayashi, S. Tanaka, T. Tanaka, K. Oshie, A. Hasegawa and M. Ikehara, Chem. Pharm. Bull. in press.
4. E. Ohtsuka, T. Wakabayashi and M. Ikehara, Chem. Pharm. Bull. in press.
5. J.F.M. de Rooij, G. Wille-Hazeleger, P.H. van Daursen, J. Serdijin and J.H. van Boom, Rec. Trav. Chim. Pays-Bas 98, 537-548 (1979).
6. C.B. Reese, R.C. Titmas and L. Yau, Tetrahedron Lett. 2727-2730 (1978)
7. E. Ohtsuka, S. Tanaka and M. Ikehara, J. Am. Chem. Soc., 100, 8210-8213 (1978).

(Received in Japan 17 November 1980)