Supplementary Material (ESI) for Chemical Communications

Synthesis of 35-40 mers of RNA oligomers from unblocked monomers. A simple approach to the RNA world

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Experimental

To the filter compartment of a Pall centrifuge device (Nanosep MF 0.45 μm) was added 2 mg of Na^+-montmorillonite, prepared by the Banin procedure [21, 22], to a 100 μL of a mixture containing 0.2 M MOBS (pH 8), 0.4 M NaCl, 0. 15 M $MgCl_2$ (pH 8) was added 50 or 100 μL of 0.06 M solution of activated nucleotide(s) and RNAse free water as required to bring the total volume to 200 μL. The mixture was mixed and then allowed to stand at room temperature for various time periods. When additional activated monomers were added at longer reaction times, the reaction mixture was centrifuged at 14, 000 rpm and the filtrate discarded and a fresh mixture of buffer, salts and activated nucleotides was added. The reactions were terminated by centrifugation to remove the supernatant and the oligomers were eluted from the clay particles by washing with 2x 200 μL of 0.1 M pyrophosphate (pH 9). The combined pyrophosphate eluates were desalted using a Microcon YM-3 spin tube.

When RNA products were purified by HPLC analysis the reaction products of a 3-day reaction of 1-MeadpU and 1-MeadpA was worked up as described above. The combined filtrate and pyrophosphate eluates were passed through a HEMA IEC BIO Q anion exchange column (Melcor Technologies) using a gradient of (A) 2 mM Tris (pH 8) and (B) 2 mM Tris, 0.4 M $NaClO_4$, pH (8.0). Gradient: 98-0 % A and 2-100 % B in 98 min and the fraction containing 9 mers and greater was collected. The collected fraction

was rechromatographed on a Hydrocell NS 1,000, 50 x 4.6 mm ion exchange HPLC column (Biochrom**)** using a gradient of A 96 % - 4 % A and B 4% -100 % (Fig. 3b).

Hydrolysis of the 5'-phosphate group on the RNAs was accomplished with shrimp alkaline phosphatase (USB, 152 units/mL), the 3', 5'-phosphodiester bonds were cleaved with RNase T_2 (Sigma, 2.8 units/□L), and all phosphodiester bonds were cleaved with phosphodiesterase I (500 units/mL). The monomers formed by hydrolysis with phosphodiesterase I were separated on an Alltima reverse phase column (Alltech) using 100 % water for 10 min followed by the gradient A 100-0% water and 0-100% B (30 % acetonitrile in water) for 100 min. Phosphorylation of the 5-hydroxyl group of the RNAs was accomplished with T_4-polynucleotide kinase (New England Biolabs 690 units/mL) and [γ-^{32}P-] ATP (Amersham). Electrophoresis was performed on acrylamide-bisacrylamide 20 % denaturing gels on 16 x 26 cm plates and the bands were visualized by autoradiography.