Supporting Information for World Wide Web Edition

Experiments for optimizing the conditions to derivatize 2'-amino-RNA with **pyr3-NHS** and **pyr1-NHS** are shown in Figure 1. A radiolabelled 13-mer 2'-amino-oligonucleotide (same as the 15-mer reported in the manuscript, except without the initial GG) was reacted with **pyr3-NHS** or **pyr1-NHS** under various conditions, and the products were examined by 20% PAGE. In Figure 1A, trace 13-mer was reacted with 50 mM **pyr3-NHS** in 100 mM Na phosphate, pH 8.0, 0.1 mM EDTA, and 60–90% DMF at 37 °C or 60 °C for the indicated times, and aliquots were quenched onto formamide stop solution and electrophoresed. The arrows indicate the underivatized 13-mer and the pyr3-derivatized 13-mer; the asterisk indicates a very minor side product, possibly formed by further reaction of the pyr3-labelled product, although this was not investigated further. DMF concentrations > 60% prevented efficient derivatization, and the yield was higher at 60 °C than at 37 °C. Test reactions of the radiolabelled 13-mer with **pyr3-NHS** and **pyr1-NHS** in 50% DMF at 60 °C are shown in Figure 1B and 1C, along with control derivatizations of the unmodified 13-mer (no 2'-amino). The side product (*) is formed in lesser amounts with the **pyr1-NHS** reagent. We performed preparative-scale derivatizations with both reagents in 50% DMF at 60 °C for 4 hr, although the yields may be even higher and/or shorter reaction times could be used with 60% DMF.

To assay the pyr3-labelled 15-mer oligonucleotide product for free 2'-amino groups, we reacted the gel-purified material with a previously reported pyridyl disulfide NHS ester shown to react rapidly and selectively with 2'-amino groups under very mild conditions (37 °C, 10% DMF, 15 min) (*34*) (Figure 2). Any oligonucleotide labelled with pyr3 at a site other than the 2'-amino group should have a free 2'-amino group and thus be reactive towards the NHS ester. A control reaction of unmodified 15-mer (no 2'-amino) showed no detectable change (reaction A), while a control with unlabelled 2'-amino-oligonucleotide showed that a significant portion of the

material reacted during the test conditions (reaction B). Reaction of pyr3-labelled 15-mer showed no new detectable band (reaction C), and by quantitating the bands we conservatively estimate that the pyr3-labelled 15-mer is > 90% pure. A control experiment in which the labelled and unlabelled 2'-amino-oligonucleotides were mixed before reaction gave the expected result (reaction D), showing that the pyr3-labelled oligonucleotide does not inhibit reaction with the NHS ester reagent.

Titration of P4-P6-wt-U107(pyr3) with Na^+ and dilution with water are shown in Figure 3; very little effect is seen. Titration of P4-P6-wt-U107(pyr3) with Mg^{2+} in 10 mM Tris, pH 8.0 + 200 mM NaCl and in 200 mM Tris, pH 8.0 are also shown in Figure 3. The titration data and curve fit parameters were very similar to those for titration with Mg^{2+} in 200 mM Tris, pH 8.0 + 200 mM NaCl (manuscript Figure 5C and Table 1).

The effects of annealing on the Mg^{2+} titrations of P4-P6-wt-U107(pyr3) and the labelled double-stranded oligonucleotide are shown in Figure 4 (see legend for annealing conditions). For the P4-P6 samples, annealing lowered the magnitude of the fluorescence intensity increase, while for the duplex, annealing raised the intensity increase. In all cases tested, the Mg^{2+} dependence of the titration curve was approximately unchanged upon annealing.

Supporting Information Figure Legends

Figure 1: Optimizing reaction conditions for derivatization of 2'-amino-RNA with **pyr3-NHS** and **pyr1-NHS**. See text for details.

Figure 2: Assaying the level of free 2'-amino groups in a pyr3-labelled 2'-amino-oligonucleotide. All tested olignucleotides were 13-mers with the same sequence as used in the manuscript to ligate to the rest of P4-P6, except missing the first two Gs. A, negative control of unmodified oligonucleotide (all wild-type residues). B, positive control of 2'-amino-oligonucleotide. C, experimental test of pyr3-labelled oligonucleotide. D, 1:1 mixture of B and C. Radiolabelled oligos were reacted with 50 mM pyridyl disulfide NHS ester in 200 mM Na borate, pH 8.0, 100 mM NaCl, 0.1 mM EDTA containing 10% DMF for 0–60 min as indicated, then quenched onto stop solution and electrophoresed on 20% PAGE. Lanes marked with letters are standard samples of the corresponding radiolabelled oligo quenched before exposure to the NHS ester. The lane between A and B is a 1:1 mixture of the A and B standard samples. Note that samples were gel-purified after radiolabelling, so for the pyr3-labelled 15-mer any unlabelled 15-mer contaminant (~8% by HPLC; see manuscript) was removed, although any side products labelled with pyr3 at sites other than the 2'-amino should be retained.

Figure 3: Equilibrium fluorescence titrations of P4-P6-wt-U107(pyr3). The bottom two data sets are control titrations with Na^+ and with water in 10 mM Tris, pH 8.0. For the water dilution experiment, volumes of water equal to those of appropriate metal ion stock solution for the other titrations were added; the data points were placed on the concentration axis along with the analogous metal ion data points. The top two data sets are titrations with Mg^{2+}. In 200 mM Tris, pH 8.0, curve fit values (n_A=3, n_B=1) were $[Mg^{2+}]_{1/2}$ = 2.5 ± 0.1 mM (component A), 38 ± 8 mM (component B); I_{init} = 1.0; I_A = 2.7; I_B = 4.0. In 10 mM Tris, pH 8.0 + 200 mM NaCl, curve fit

values (n_A=3, n_B=1) were $[Mg^{2+}]_{1/2}$ = 2.2 ± 0.1 mM (component A), 19 ± 3 mM (component B); I_{init} = 1.0; I_A = 1.8; I_B = 3.3.

Figure 4: Testing the effect of annealing on the fluorescence titrations. Samples were heated to 90 °C for 3 min, then cooled slowly to room temperature, before fluorescence titration as described in the manuscript. For all cases, titration of an unannealed sample is shown for comparison. (A) P4-P6-wt-U107(pyr3) in 10 mM Tris, pH 8.0. (B) P4-P6-wt-U107(pyr3) in 1x TB. (C) Double-stranded pyr3-labelled 15-mer oligonucleotide in 1x TB. See manuscript Table 1 for curve fit parameters for unannealed samples. Curve fit parameters (manuscript eq. 4) for annealed samples were as follows. Panel A: (n_A=2, n_B=1) $[Mg^{2+}]_{1/2}$ = 0.27 ± 0.01 mM (component A), 125 ± 34 mM (component B); I_{init} = 1.1; I_A = 2.7; I_B = 3.6. Panel B: (n_A=2, n_B=1) $[Mg^{2+}]_{1/2}$ = 0.73 ± 0.05 mM (component A), 11 ± 4 mM (component B); I_{init} = 1.0; I_A = 1.7; I_B = 2.1. Panel C: (n_A=1, n_B=1) $[Mg^{2+}]_{1/2}$ = 0.31 ± 0.02 mM (component A); I_A = 3.9.

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Supp Info
Figure 1

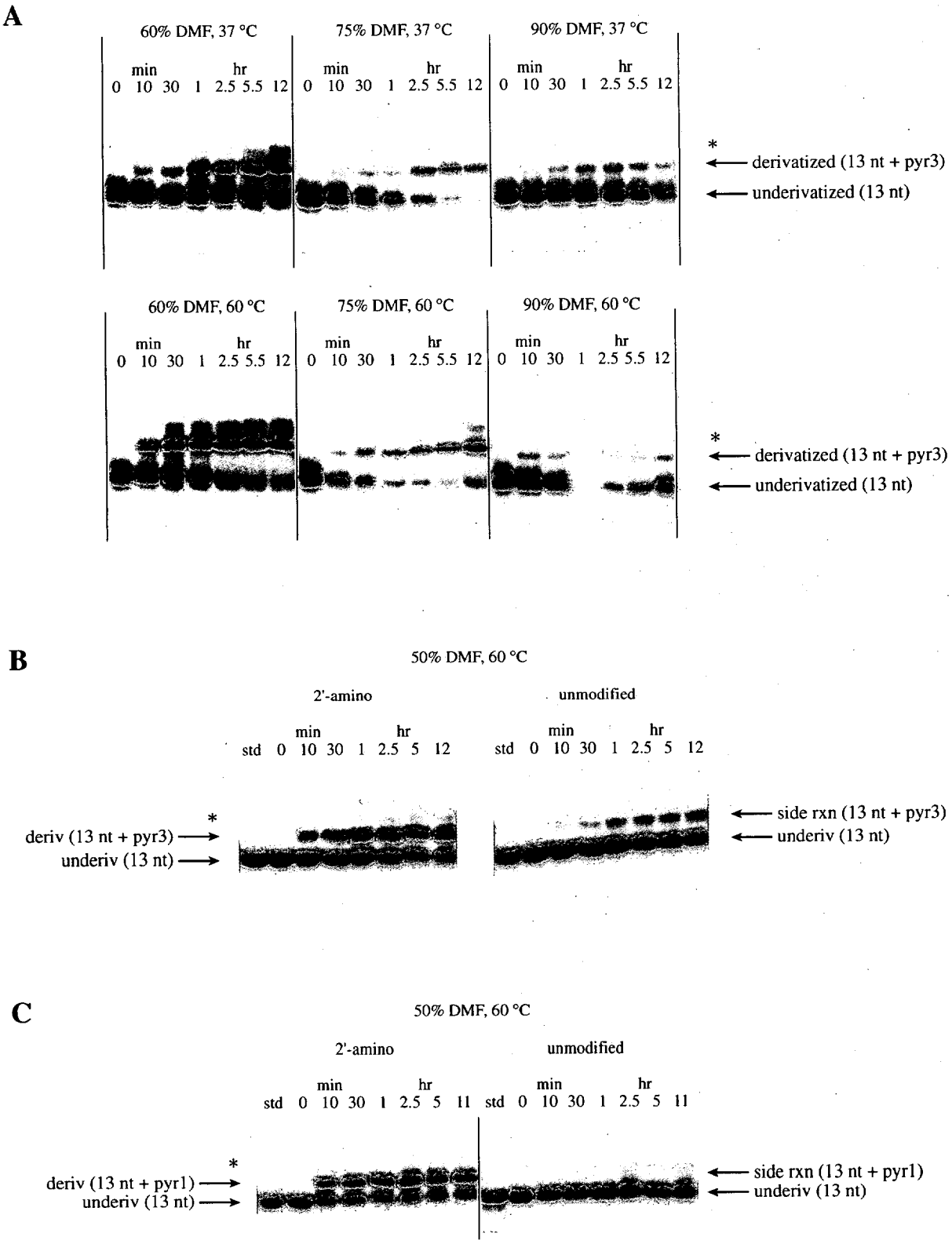

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Figure 2

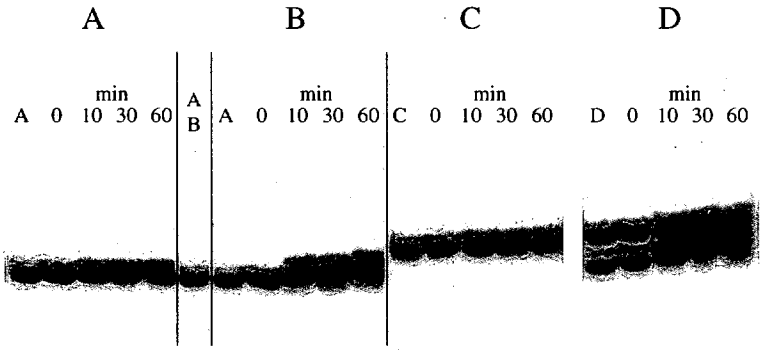

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Figure 3

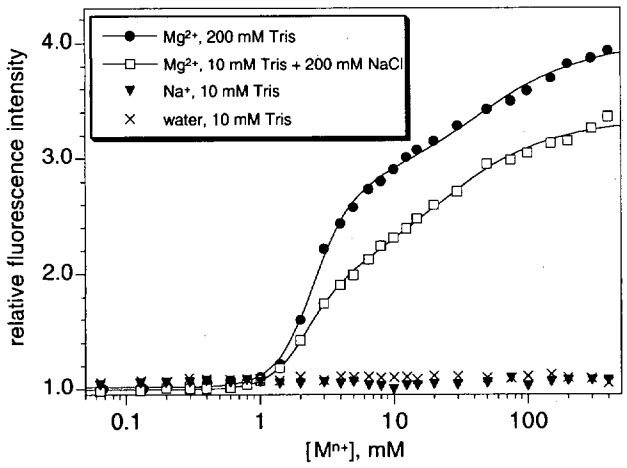

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Figure 4