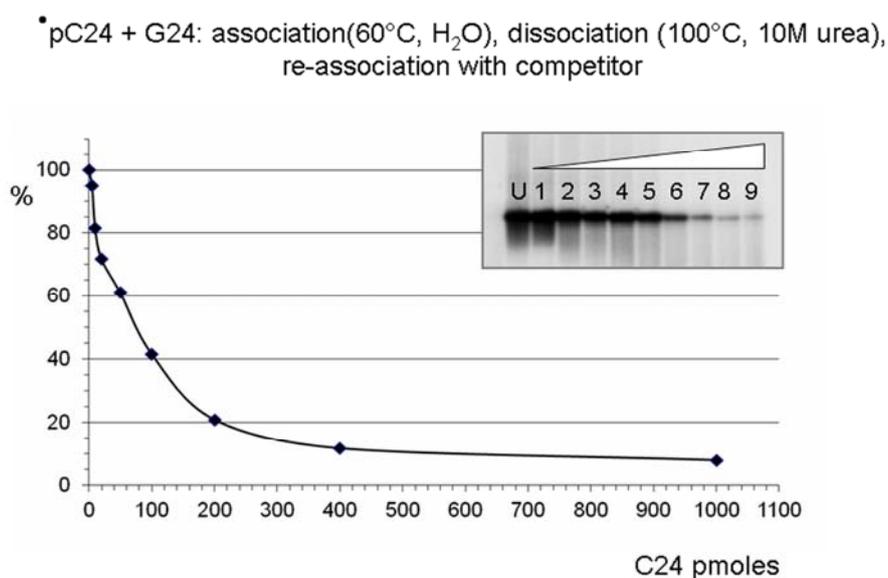


## Supplementary Material

**Item #1.** Determination of the Amount of Terminally Ligated Sequence-Complementary Oligonucleotides in the  $\bullet$ pC24 + G24 System.

2 pmoles of phosphorylated  $^{32}\text{P}$  labelled C24 were reacted with 7.5 pmoles of unlabelled G24 in 15  $\mu\text{L}$  MilliQ water (30 min, 60  $^{\circ}\text{C}$ ). The samples were precipitated at  $-20^{\circ}\text{C}$  (75  $\mu\text{L}$   $\text{H}_2\text{O}$  + 3M 10  $\mu\text{L}$  sodium acetate + 300  $\mu\text{L}$  96% ethanol + 20  $\mu\text{g}$  glycogen). The pellets were resuspended in 10  $\mu\text{L}$  of 10M urea and treated at 100  $^{\circ}\text{C}$  for 10 min. The samples were then added with the indicated amounts (abscissa) of unlabelled C24 (final volume = 20  $\mu\text{L}$ ) dissolved in 10 M urea. After 5 min at 100  $^{\circ}\text{C}$ , the samples were cooled at 25  $^{\circ}\text{C}$  and after 30 min were precipitated and analyzed. The figure shows the amount of 48mer remaining after this treatment, as % (ordinate) of the untreated sample (=U). This assay shows the determination of the amount of terminally ligated sequence-complementary oligonucleotides. In this combination of oligomers the ligated G24 $\bullet$ pC24 dimer is about 10%.

**Figure S1.** Determination of covalently ligated dimers.



**Item #2.** MALDI ToF Analysis of C24 and G9.

The MALDI ToF analysis of G9 and C24 was performed as described in Methods on 100 ng of samples.

Panels A and B show the profiles obtained for G9. A is the overall view for the  $m/z$  area between 1800 and 6000. The  $\text{Na}^+$  and  $\text{K}^+$  adducts are indicated in the blow-up panel B. No phosphorylated forms are present.

Panel C. Same for C24. The overall profile is shown between 3000 and 10,000, along with a blow-up of one sub-family as example (the 11 mer) and of the full-length 24 mer. The phosphorylated and non-phosphorylated forms are indicated along with their  $\text{K}^+$  adducts.

The cleavage pattern observed for C24 is probably not simply due to hydrolysis in water but is caused by active intramolecular cleavage by its own 3' OH extremity. Hydrolysis affords phosphated

extremities, while self-cleavage is expected to yield 3' OH extremities. This observation suggests that both hydrolytic degradation and splicing paths are occurring (to be detailed elsewhere).

**Figure S2.** MALDI ToF profiles of G9 and G24 oligomers.

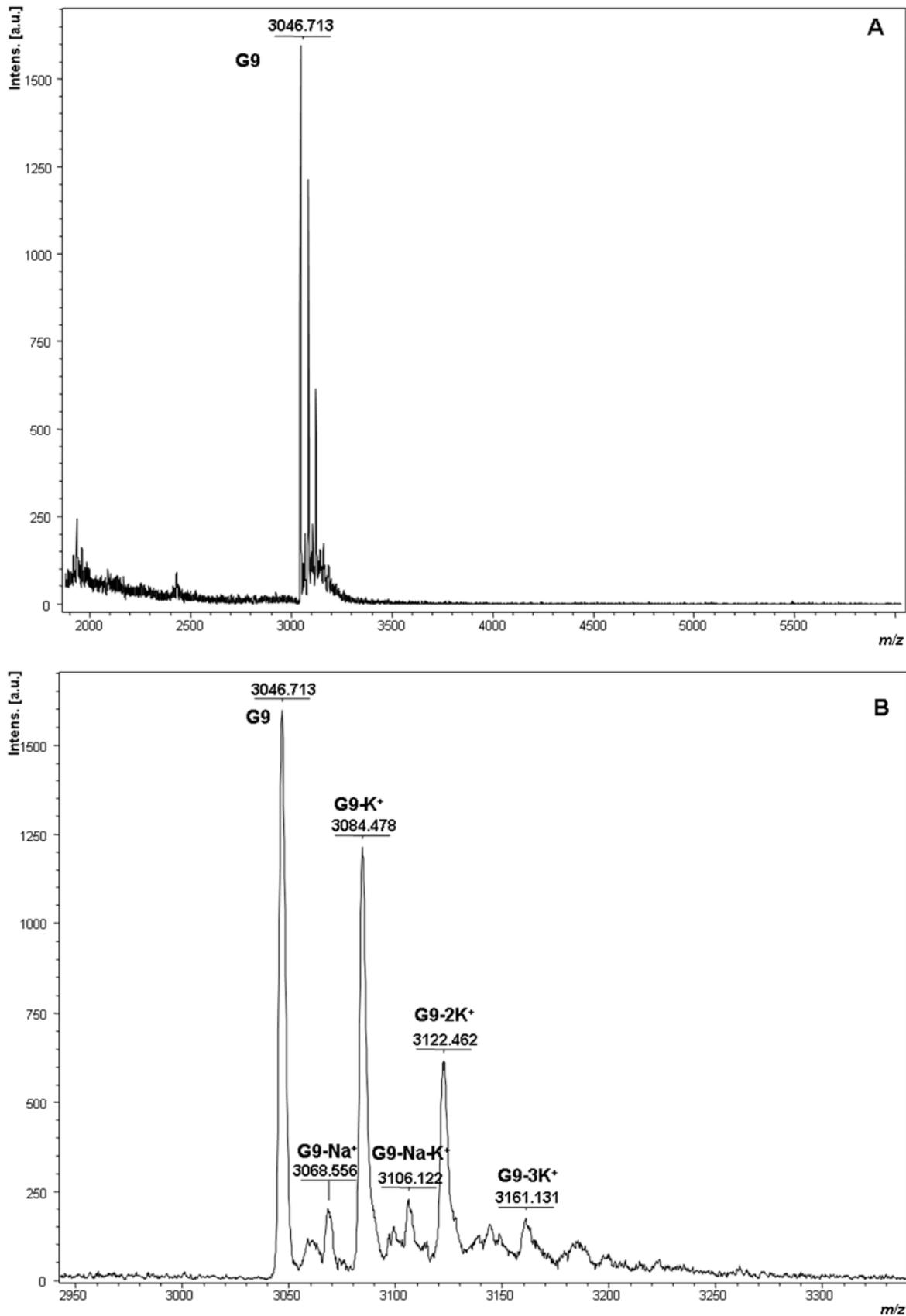
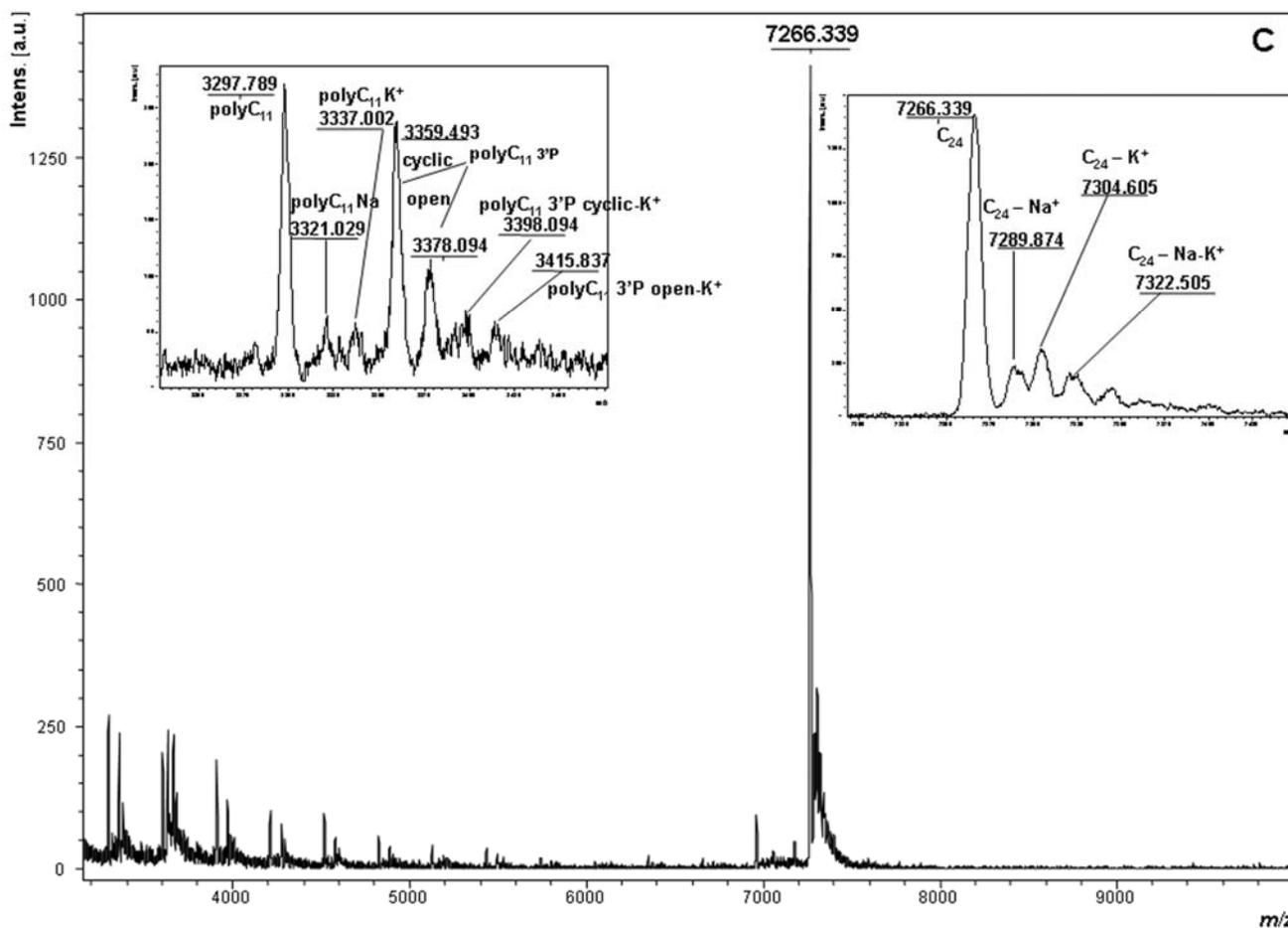


Figure S2. Cont.



**Item #3.** Molecule **d**, Product of LIC Reaction, is Made of Cs. Hybridization Competition Assay of the  $\bullet$ pG24/C24 Products.

In the experiment reported in Figure S3  $\bullet$ pG24 (**a**) (lane 2) was reacted (60 °C, 30 min) with C24, resulting (lane 3) in the formation of C24/ $\bullet$ pG24 dimers (**c**) and of the C24 $\bullet$ pG terminally grown molecule **d**. The sample was then dissociated by bringing it to 100 °C in 10M urea (15 min) (lanes 4–12), then cooled to reannealing temperature (60 °C) in the presence of increasing amounts (lanes 5–12) of unlabeled G24. At sufficiently high concentrations of unlabeled G24 (lane 7–12) the oligo C24 $\bullet$ pG **d** base-pairs with G24, forms a dimer and becomes part of the **c** band. Upon 10 M urea/100 °C treatment, the  $\bullet$ pG24 molecules are released from the 48mer, are substituted by the excess unlabeled G24mer and reappear as free  $\bullet$ pG24 **a** (lanes 10–12).

Not all the dimers **c** are dissociated. A part remains as  $^{32}$ P-containing covalently linked 48mer molecules even after 15 min boiling in 10 M urea and addition of 380fold excess of G24 competitor (lane 12) because of the described terminal ligation [26], as quantified in Supplementary Item # 1. This assay shows that C24 $\bullet$ pG **d** hybridizes with G oligos, thus being made of C residues, and not of G residues.

