Figure S1. Lack of a 5'-terminal triphosphate group is not responsible for the stronger binding of G to the L-16 *Sca*I ribozyme. (A) The dependence of the reaction of E•S on G concentration for the L-21 *Sca*I (●), L-21 *Sca*I^{OH} (○), or L-16 *Sca*I (■) ribozymes. Non-linear least square fitting to a single binding site model yielded binding constants of 94, 87 and 34 μM, respectively. Data were obtained at pH 7.2 with 10 mM MgCl$_2$ as described in the Materials and Methods, and rate constants were normalized for comparison. (B) Dissociation of CCUCdT from the L-16 *Sca*I (●) and the L-21 *Sca*I^{OH} (○) ribozymes in native gel pulse-chase experiments. Non-linear least square fitting yielded dissociation rate constants of 0.023 min^{-1} and 0.00088 min^{-1}, respectively. Data were obtained at 10 mM MgCl$_2$, pH 6.0 as described in the Materials and Methods. Note that the small burst for dissociation from the L-16 *Sca*I ribozyme presumably represents dissociation from a truncated molecule.

Table S1: Dissociation Rate Constants of Different Substrates at 50 and 30 °C

Substrates	50 °C		30 °C	
	k_{off} (min^{-1})	k_{rel}	k_{off} (min^{-1})	k_{rel}
(C)CCUCdTA	6.0	(1)	1.9 [a]	(1)
(C)CCUCdTAAACC	$1.6 \cdot 10^{-3}$	$3.4 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$ [a]	$1.5 \cdot 10^{-4}$
(C)CCUCdTAAAAA	0.58	0.1	0.091 [a]	0.05

Dissociation constants of full-length or –6C$^{\Delta}$ substrates (Chart 1) from the E•S complex were determined at pH 6.0 and 50 °C or 30 °C, respectively, as described in the Materials and Methods and Table 1. Values are averages of two or more measurements. [a] Data from Table 1 are included for comparison.

Figure S1