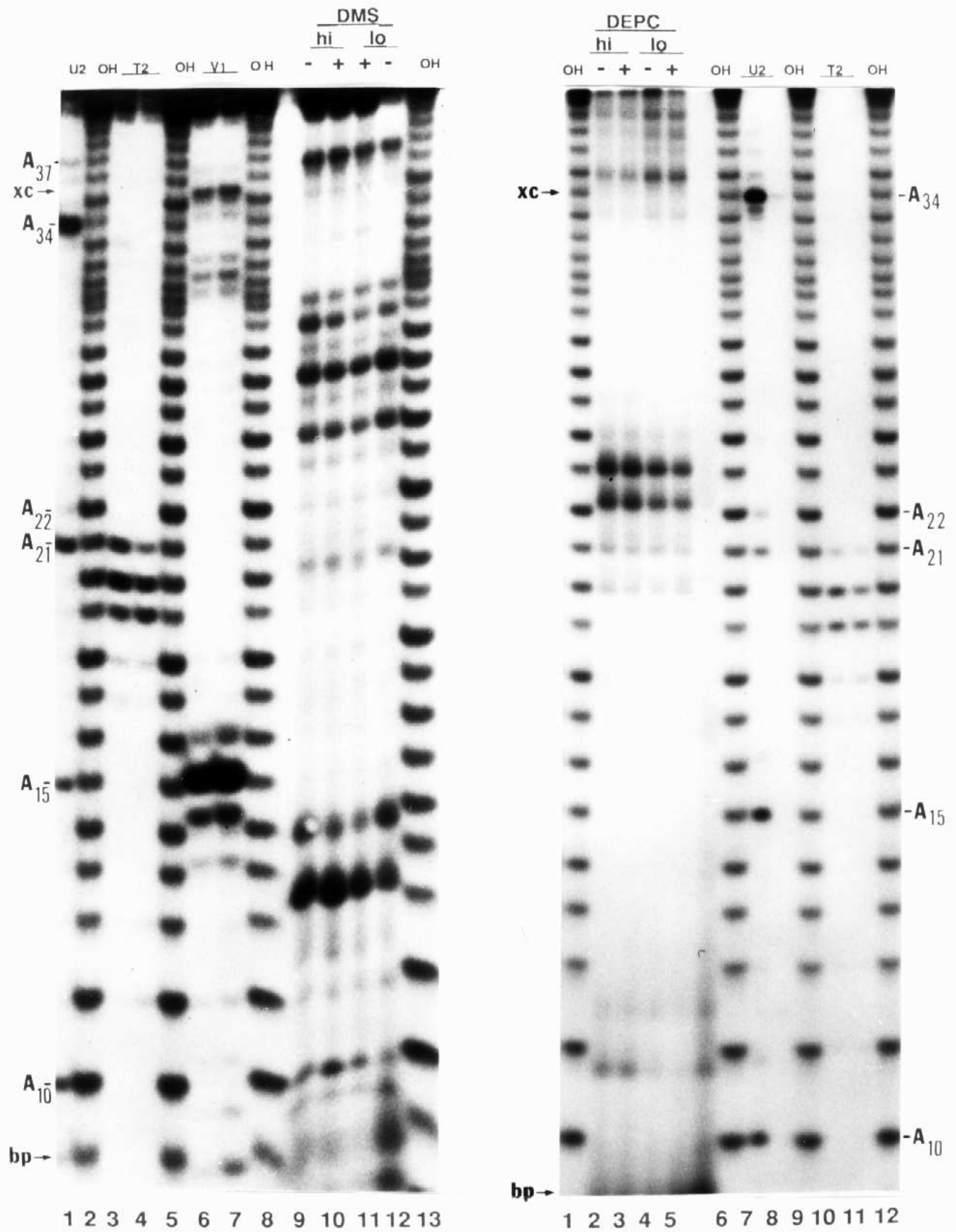


Figure 1.3.5: Chemical and enzymatic structure probing of R1.1[LSΔ6] RNA. Figure 1.3.6 summarizes the results. For structure–probing with RNase T2 or RNase V1, $^{32}P$–labeled RNA ($6 \times 10^4$ dpm) was incubated with enzyme at 30° for 15 minutes in 10 ml of cacodylate buffer (pH 7.5) containing 200 mM KCl

and 5 mM $MgCl_2$. Separate reactions (data not shown) omitted the KCl and/or $MgCl_2$. For DMS structure probing, $^{32}P$–labeled RNA (3x105 dpm) was treated with 0.5 ml DMS for 15 minutes at 30° in cacodylate buffer (200 ml), with or without KCl and/or $MgCl_2$. For DEPC structure probing, $^{32}P$–labeled RNA (3x105 dpm) was treated for 2 hours at 30° with 20 ml of DEPC in cacodylate buffer (200 ml), with or without KCl and/or $MgCl_2$. To generate the alkaline ladder, $^{32}P$–labeled RNA (2x$10^5$ dpm) in 10 ml of 0.25 M NaCO3 buffer (pH 9.2) was heated at 90° for 7 minutes in a sealed glass capillary tube. The adenine ladder was generated by reacting $^{32}P$–labeled RNA (6x$10^4$ dpm) with RNase U2 (1−2 units) at 55° for 15 minutes in 25 mM sodium citrate (pH 3.5), 7 M urea, 1 mM EDTA and 0.05% bromphenol blue and xylene cyanol dyes. A. Lane 1: RNase U2 reaction (U2) (1.2x$10^4$ dpm). Lanes 2,5,8,13: alkaline ladder (OH) (2.3x$10^4$ dpm/lane). Lanes 3,4: RNase T2 (T2) (10 and 15 units, respectively; $10^4$ dpm/lane). Lanes 6,7: RNase V1 (V1) (0.02 and 0.04 units, respectively; $10^4$ dpm/lane). Lanes 9−12: DMS reactions (1.9x$10^4$ dpm/lane). Lane 9, 200 mM KCl ("hi"), 0 mM MgCl2 (−); lane 10, 200 mM KCl, 5 mM $MgCl_2$ (+); lane 11, 0 mM KCl ("lo") , 5 mM $MgCl_2$; lane 12, 0 mM KCl, 0 mM $MgCl_2$. B. Lanes 1,6,9,12: alkaline ladder; lanes 2−5, DEPC reactions (4x$10^4$ dpm/lane); lane 2, 200 mM KCl; lane 3, 200 mM KCl, 5 mM $MgCl_2$; lane 4, 200 mM KCl, 0 mM $MgCl_2$; lane 5, 0 mM KCl, 0 M $MgCl_2$. Lanes 7,8: RNase U2 reactions (1 and 2 units, respectively). Lanes 10,11: RNase T2 reactions (10 and 15 units, respectively) in 200 mM KCl, 5 mM $MgCl_2$. The positions of the adenine residues are indicated. The DEPC and RNAse U2 cleavage reactions result in different 3′ terminal modifcations and thus in different mobilities; lines between lanes 5 and 6 connect fragments with the same number of nucleotides. ′xc′ and ′bp′ refer to the positions of the xylene cyanol and bromphenol blue dye markers, respectively.