Solvent and Salt Effects on Mononucleotides

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A Thesis Describing Chemical Research in Ch 80

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Research Report

Solvent Structure Effects on Nucleotide Conformation

Important solvent effects on oligonucleotides, particularly those such as ribonuclease can be dependant in two ways upon the structure of the solvent: either the intermolecular and intramolecular stacking interactions themselves[1] can be changed, or the conformations of the individual nucleotides can be changed, leading to changes in the intermolecular binding forces. An investigation of the effects of solvent structure on mononucleotides will help to prove or disprove the latter. pH is also a biologically important factor, and the effects of protonation on mononucleotides and their structure should be revealing in predicting their intermolecular interactions.

The effects of various salts on the conformation of mononucleotides in aqueous solutions has previously been shown to probably be for reasons other than specific binding to the nucleotide.[2] Changes effected in the structure of mononucleotides by the addition of salts to or the change of pH in their solution can be monitored through proton magnetic resonance spectroscopy of several appropriate and easily identifiable resonances. Since the mononucleotides have already been shown to interact with each other in solution, their concentration should be kept low enough that this type of interaction will be minimal, and still allow good spectra.

pH Effects on 3' AMP

Experimental:

3' AMP from Schwarz Bioresearch, Inc. was dried in vacuo over P_2O_5 and used. Solutions of approximately .003 M were prepared by w weight in 99.7% D_2O from Columbia Organic Chemicals. pH was adjusted by use of 1 N solutions of DCl or NaOD of reagent grade. The pH of the solutions was measured with a Leeds and Northrup 7401 pH meter equipped with miniature electrodes.

All spectra were run on a Varian HA 100 spectrometer in frequency sweep mode, using a C1024 time averaging computer to inhance signal to noise ratio. The lock signal was a TMS capillary and shifts were measured relative to this.

Results:

The peaks monitored during the changes in pH were the H_2 and H_8 peaks of the adenine ring, and the H_1' peak, which is split into a doublet by the H_2' of the ribose ring. As the pH was slowly lowered from about eleven to four, the chemical shifts of these peaks, and the coupling constant o f the H_1' were studied. Plots of these are shown in Fig. 1,2,3, and 4. All three protons show the characteristic shift toward lower fields as the pH was lowered, and there was no change in the coupling constant for the H_1'. Overall shift noted was 3.5 to 4.0 cy in the pH range studied.

Discussion:

The observed shifts for the H_2 and H_8 protons, particularly in the region around pH 6 ∅ (the pK of 3' AMP for the secondary phosphate protonation is 5.9), indicate that the

protonation of the phosphate has little effect on the conformation of the molecule. This is to be expected, since the phosphate is physically farther removed from the adenine ring than in 5" AMP as was previously investigated.[2] The shift at lower pH is expected to be due to the protonation of the adenine base itself.

The fact that the H_1' proton also undergoes about the same shift with no change in the coupling constant indicates that the conformation of this part of the molecule, the ribose ring, has not changed significantly.

The above results, while revealing little in the way of positive results, do confirm that the previous pH effects observed in 5' AMP[2] were mostly steric, and due to the close proximity of the phosphate to the adenine ring. Movement of the phosphate to a much more isolated position should then have a much smaller effect on the protons studied, and then overall conformation, which, from these results, appears to happen.

Salt Effects on 3' UMP

Experimental:

3' UMP was obtained from P. L. Biochemicals, Inc. and dried in vacuum over P_2O_5 at room temperature. A solution of approximately .03 M was prepared in 99.7% D_2O as with the AMP. The salt used was a 1 molal solution of $Mg(ClO_4)_2$ in D_2O prepared by J. H. Prestegard. Solutions were prepared by weight, and all spectra were run at room temperature. Equipment used was the same as that used with the 3' AMP, and the pH was kept near constant at 7.5 $\pm$ .1 .

Results:

The peaks observed were the H_5 proton, which is split into a doublet by the H_6 proton, the H_1' proton, which is split into a doublet by the H_2', and the H_6 proton, which is split into a doublet by the H_5. The coupling constants of all the protons were observed, but didn't change, indicating that the electronic structure of these regions of the molecule didn't change.(See Fig. 5). The Chemical shift results are plotted in Fig. 6,7, and 8. These results show that while the H_6 shift seemm to change at a constant rate with salt concentration (after allowing for bulk susceptability), the other two protons show no change at all.

Discussion:

If the shift of the H_6 were due to specific binding of the magnesium ion to the molecule, then one would expect a classieal saturation curve, leveling off at about .1 molal salt concentration. This expectation is reinforced by the fact that the binding constant of magnesium to UMP is on the order of 100. In this case, however, the salt is still showing an effedt at 1 molal concentrations, and shows no signs of saturation.

Since the phosphate is now shifted away from the base, steric and spatial charge effects from the specific binding of magnesium to the phosphate should be reduced to almost nothing as is the case. In addition, coupling constants should remain constant, since there is nothing perturbing the electronic structure directly.

Since the shift observed for the H_6 can't be the result

of specific binding, as explained above, it must be due to somethin such as solvent structure. The upfield shift means that the proton is becoming more shielded, as the structure of the solvent is "broken" by the magnesium salt. There is little evidence for a conformational change in the part of the ribose ring near H_1' and H_2' since the coupling constant doesn't change at all. This seems to contradict previous predictions based on 5' UMP[2], but may be insignificant, because of the new phosphate position. The conformation of the nucleotide should, however, be changed in some way to explain the shift of the H_6, since solvent effects alone should also change the H_5. Further work should be done, both with 3 and 5' AMP as well as UMP in order to try to correlate these results.

References

1. Lumry, Smith, Glantz, J. Am. Chem. Soc., 73, p. 4330, (1951).
2. J. H. Prestegard, unpublished results.

FIG. 1

3' AMP H_1' CHEM SHIFT

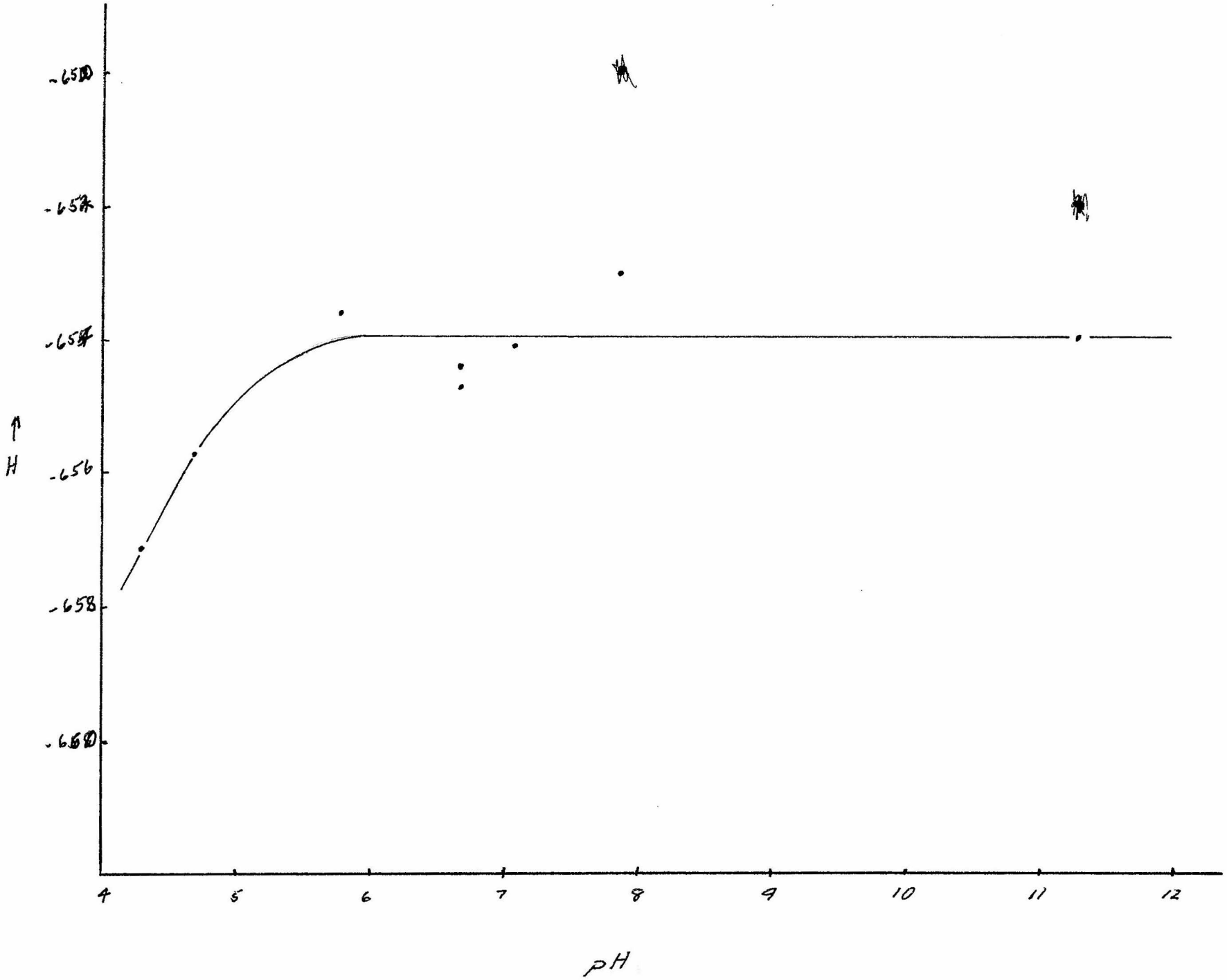

FIG 2

3' AMP H_2 SHIFT

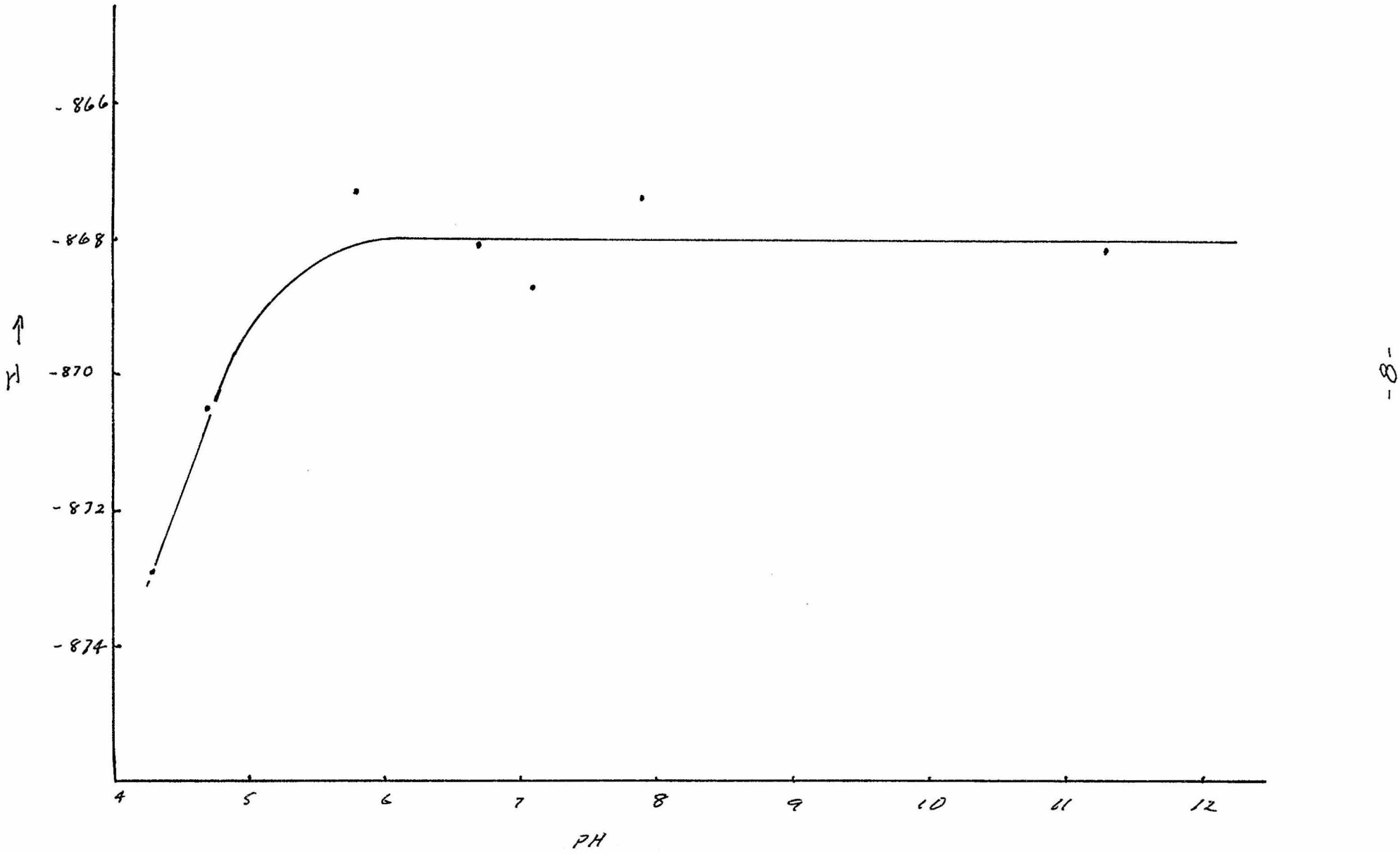

FIG 3

3' AMP H_8 SHIFT

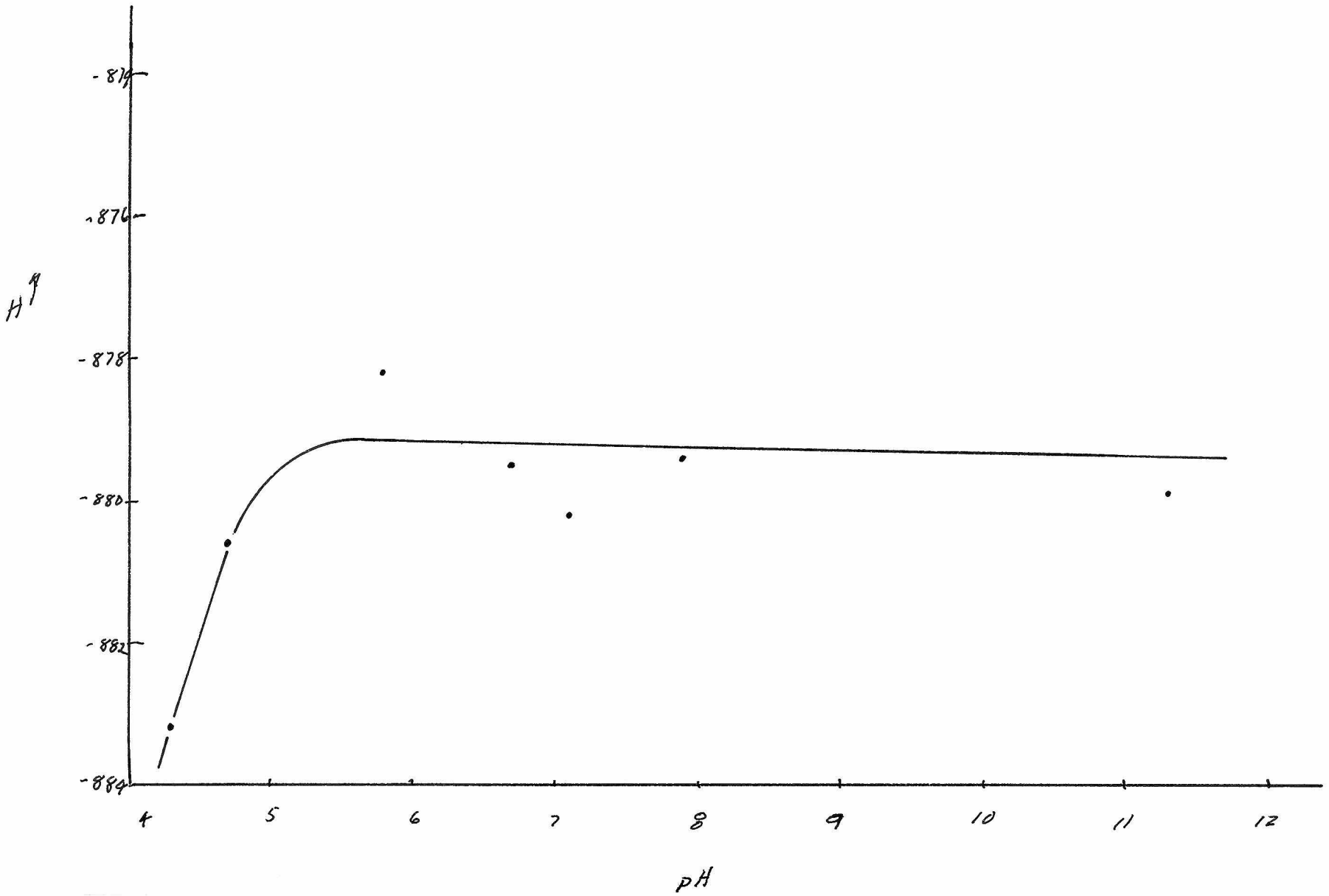

Fig 4

3' AMP H_1' Coupling

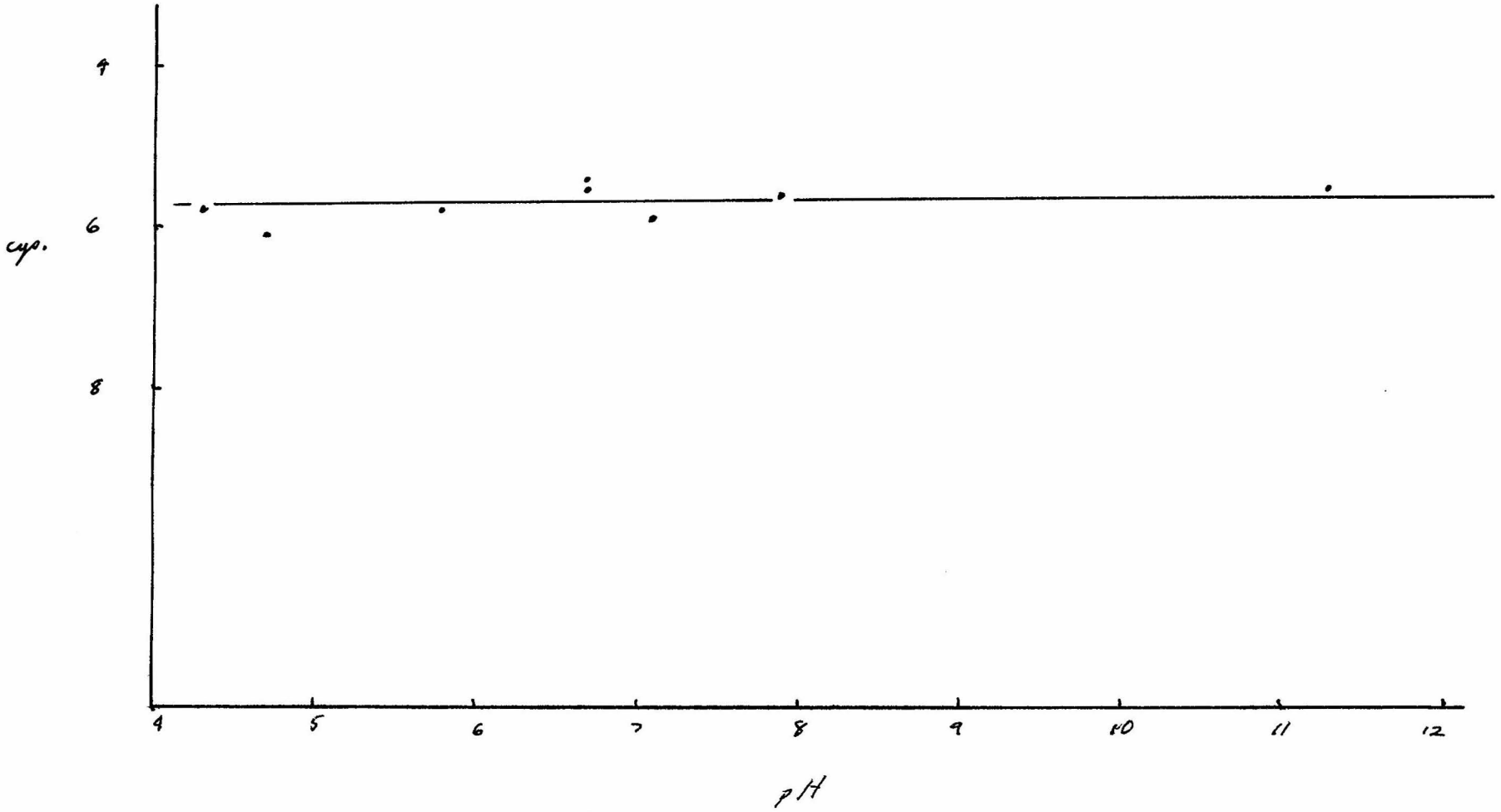

FIG 5

3' UMP COUPLING

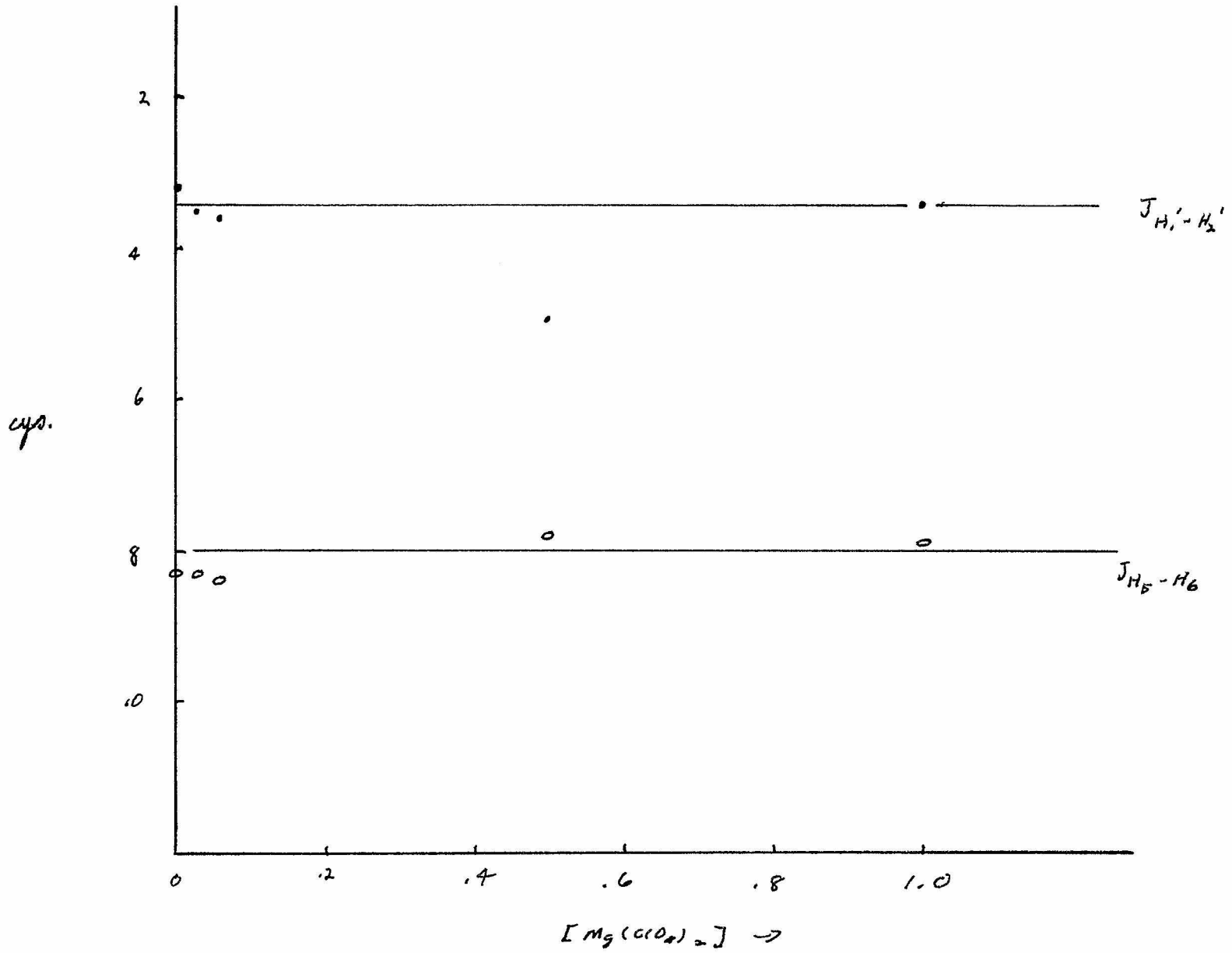

FIG 6

3' UMP H_1' SHIFT

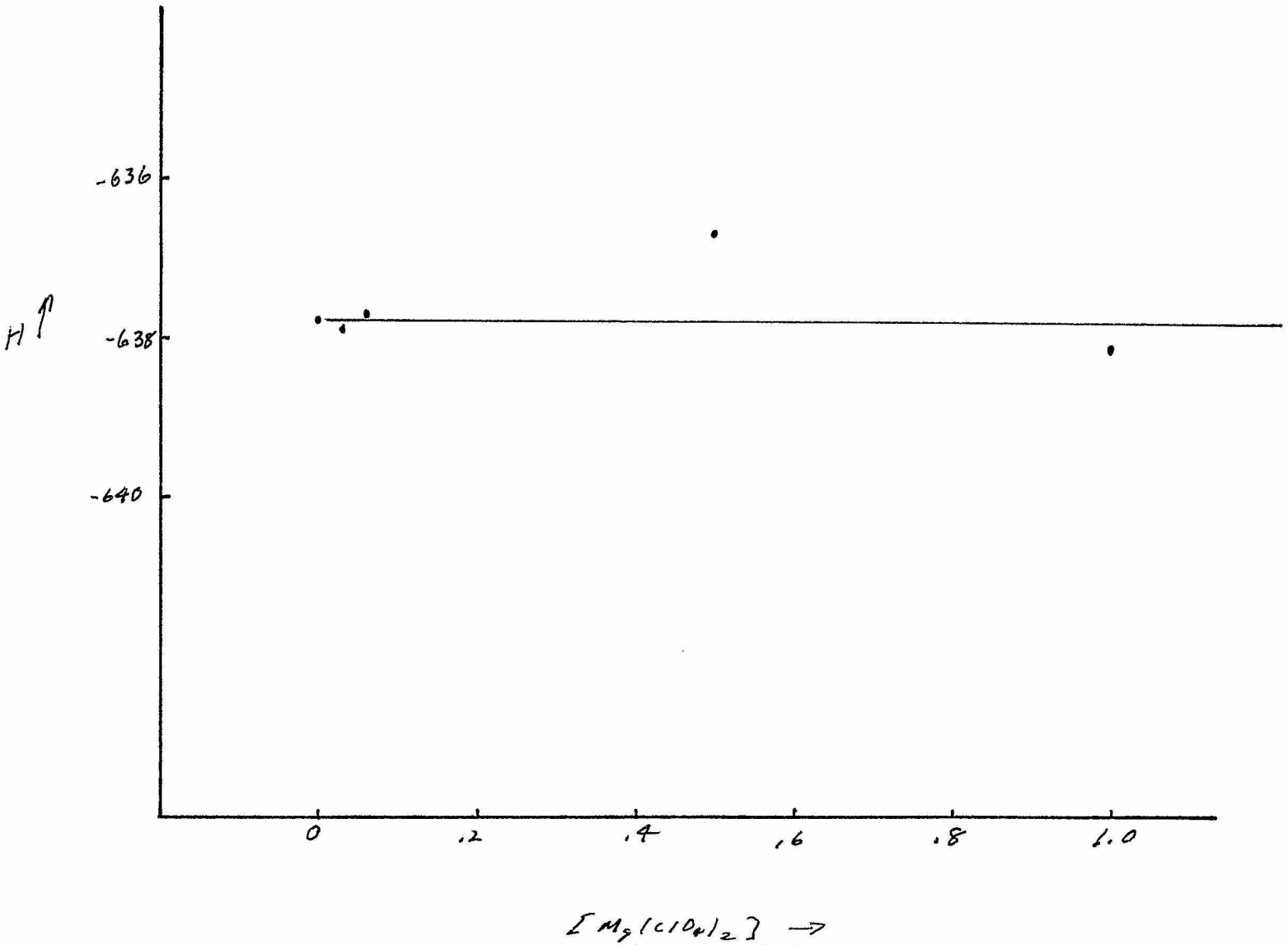

FIG 7

3' UMP H_5 SHIFT

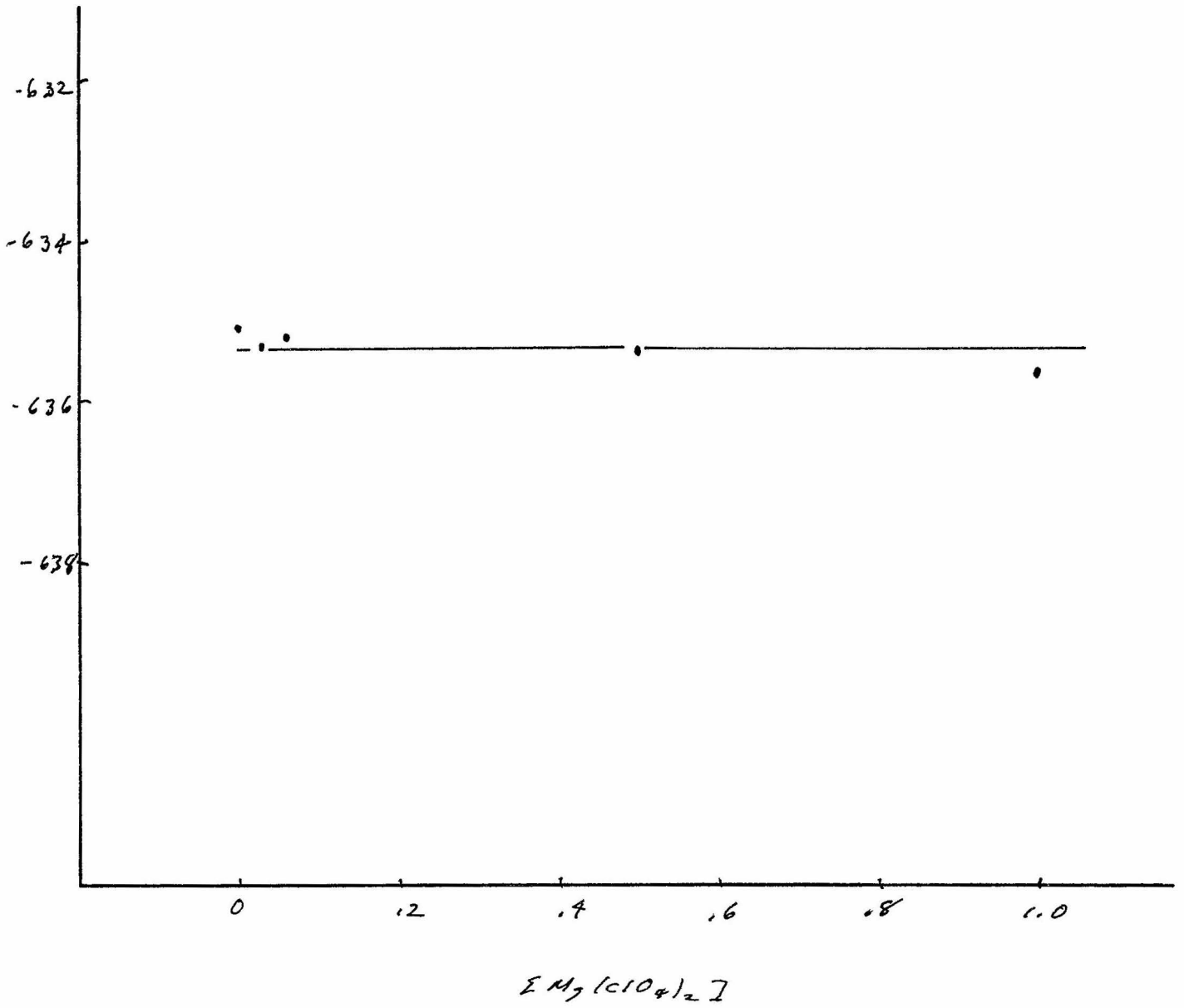

FIG 8
3' UMP H_6 SHIFT