Table 1S. The pK_a values at 25° C for sugar 2'- hydroxyl dissociation in ^{13}C-labeled adenosine, uridine and cytidine obtained from the chemical shift values of C1', C2' and C3' (see Fig. 3S, 4S).

Adenosine		Uridine		Cytidine	
pK_a for 2'-OH from δC1'		pK_a for 2'-OH from δC2'		pK_a for 2'-OH from δC3'	
Titration curves	Calculated from Eq.2	Titration curves	Calculated from Eq.2	Titration curves	Calculated from Eq.2
12.27±0.05	12.31±0.03	12.46±0.04	12.55±0.02	12.43±0.05	12.42±0.02
12.25±0.03	12.29±0.04	12.60±0.03	12.67±0.02	12.48±0.02	12.49±0.02
12.25±0.03	12.26±0.03	12.68±0.03	12.71±0.02	12.54±0.02	12.48±0.02
Overall average pK_a		Overall average pK_a		Overall average pK_a	
12.27±0.02		12.63±0.03		12.52±0.03	

Legends to the Figures

Figure 1S. Chemical shift titration curves for H1', H2' and H3' proton resonances adjacent to 2'-OH group in **1-18**. The sigmoidal curves are the result of the least-squares best fit of the pH-dependent (*i.e.* 18-28 pHs in the range 7.0 – 13.6) experimental values of H1', H2' and H3' chemical shift (ppm) to the Henderson-Hasselbalch equation. The pK_a values of 2'-OH for **1, 3-12, 14, 16, 17** were also confirmed by calculating procedure.[4b] The pK_a value and the correlation coefficient (R) for each titration for each compound are shown on the graphs. For **11** and **12,** signals of H2' and H3' under pDs ranging from 13.46 to 13.97 (H2' of **11**), 12.18 to 13.18 (H3' of **11**), 13.26 to 13.81 (H2' of **12**), 12.02 to 13.00 (H3' of **12**) were isochronous with HDO signal what made the extraction of the precise chemical shift values impossible.

Figure 2S. Graphical determination of plateau chemical shift values (δ_h) for **1, 3-12, 14, 16, 17** for the further calculation of pK_a values by Eq. 2. The plots of chemical shift as a function of $(\delta_l - \delta_{obs})\cdot aH^+\cdot 10^{14}$ at pH values from the slope region of the titration curves show a straight line ($R > 0.9$) intercepting the ordinate axis at the δ_h.

Figure 3S. Chemical shift titration curves for C1', C2' and C3' carbon resonances for carbon labeled adenosine, uridine and cytidine. The sigmoidal curves are the result of the least-squares best fit of the pH-dependent (*i.e.* 18-21 pHs in the range 7.0 – 13.6) experimental values of C1', C2'and C3' chemical shift (ppm) to the Henderson-Hasselbalch equation.

Figure 4S. Graphical determination of plateau chemical shift values (δ_h) for carbon labeled adenosine, uridine and cytidine for the further calculation of pK_a values by Eq. 2. The plots of chemical shift as a function of $(\delta_{obs} - \delta_l) \cdot aH^+ \cdot 10^{14}$ at pH values from the slope region of the titration curves show a straight line ($R > 0.9$) intercepting the ordinate axis at the δ_h.

Figure 5S. The correlation between ^1H chemical shift change of H1' and ^{13}C chemical shift change of C1', C2' or C3' in ^{13}C-labeled adenosine, uridine, cytidine at pHs ranging from 7 to 13.6. The correlation coefficient (R) is shown on the graphs.

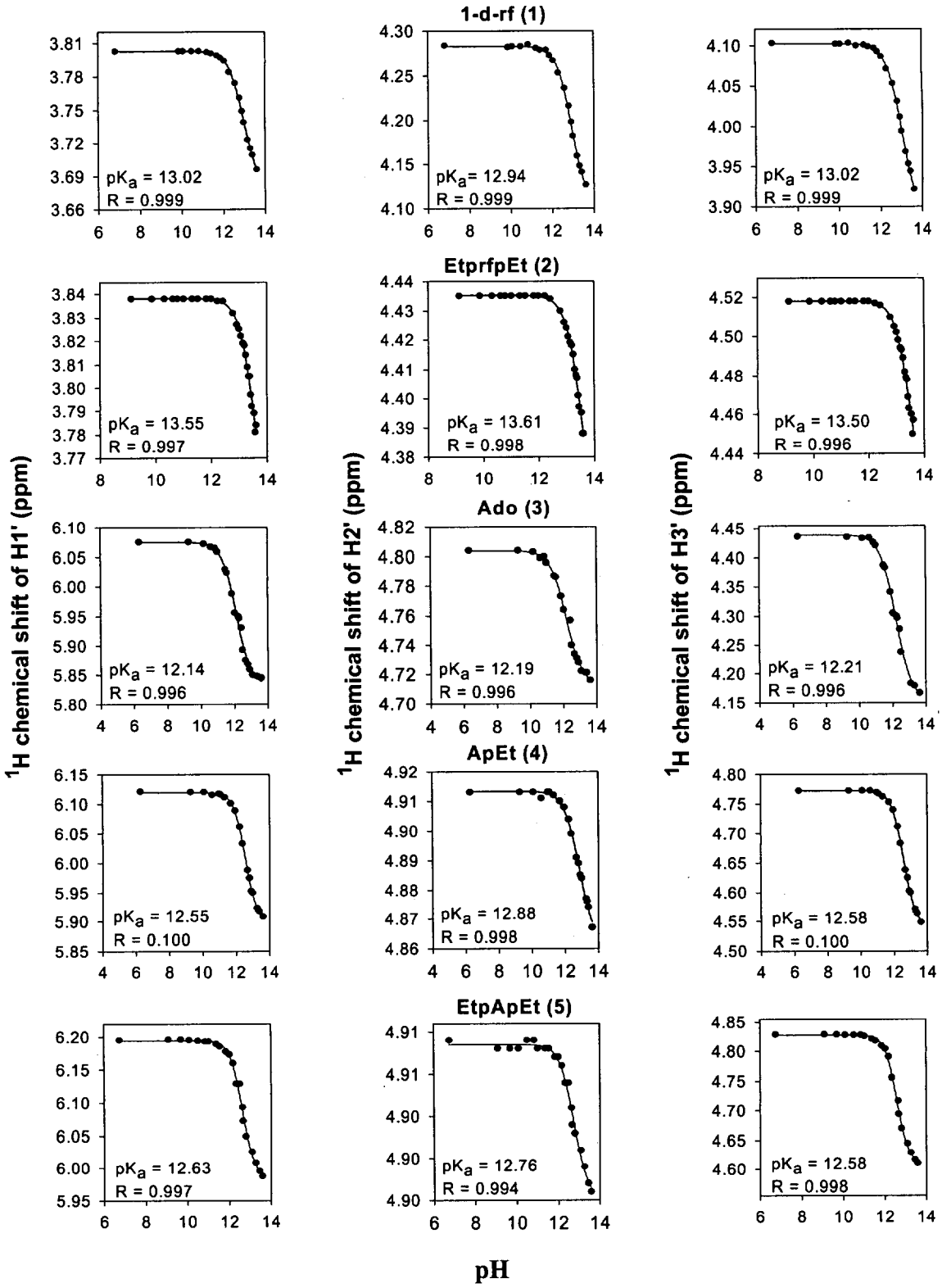
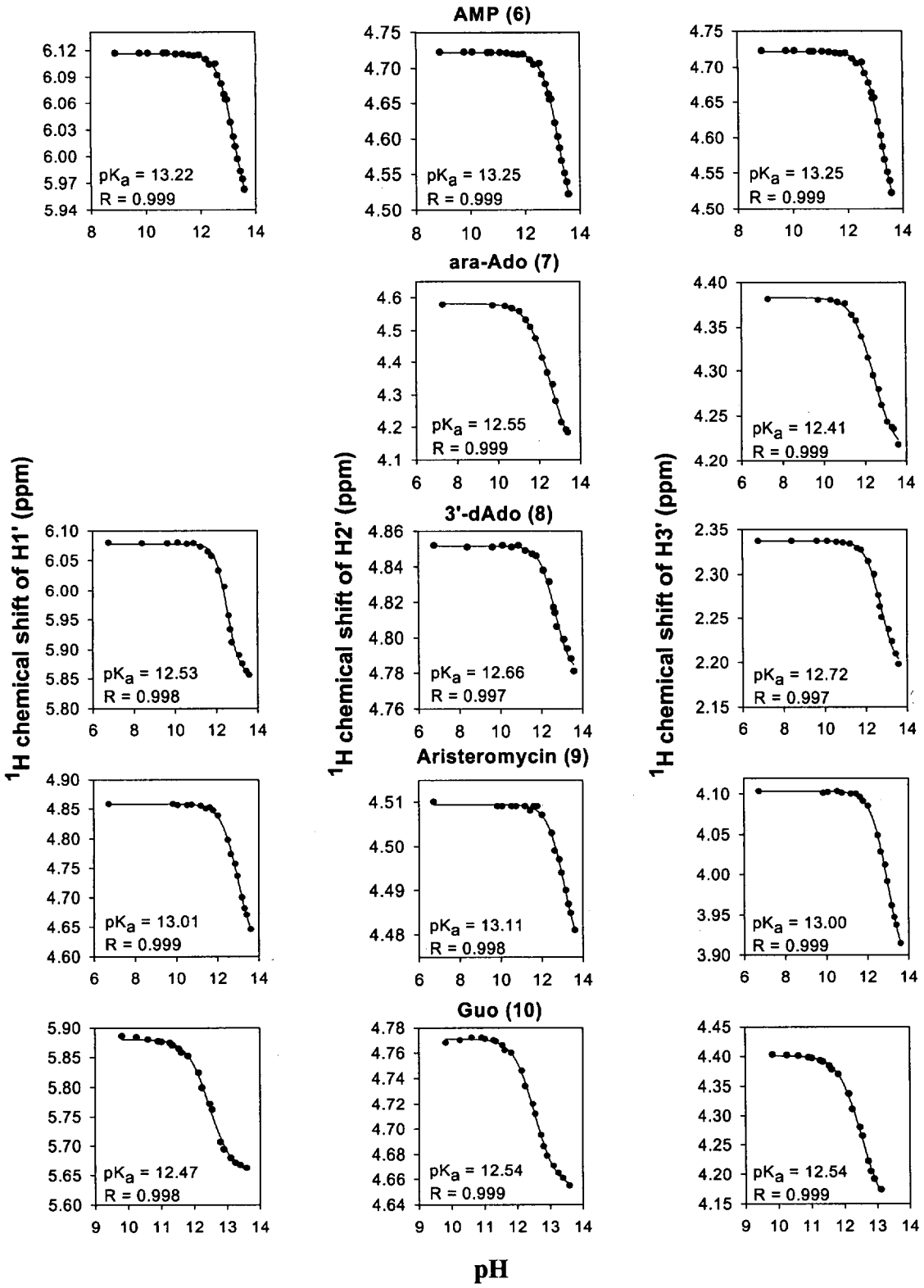


Figure 1S (Continued)



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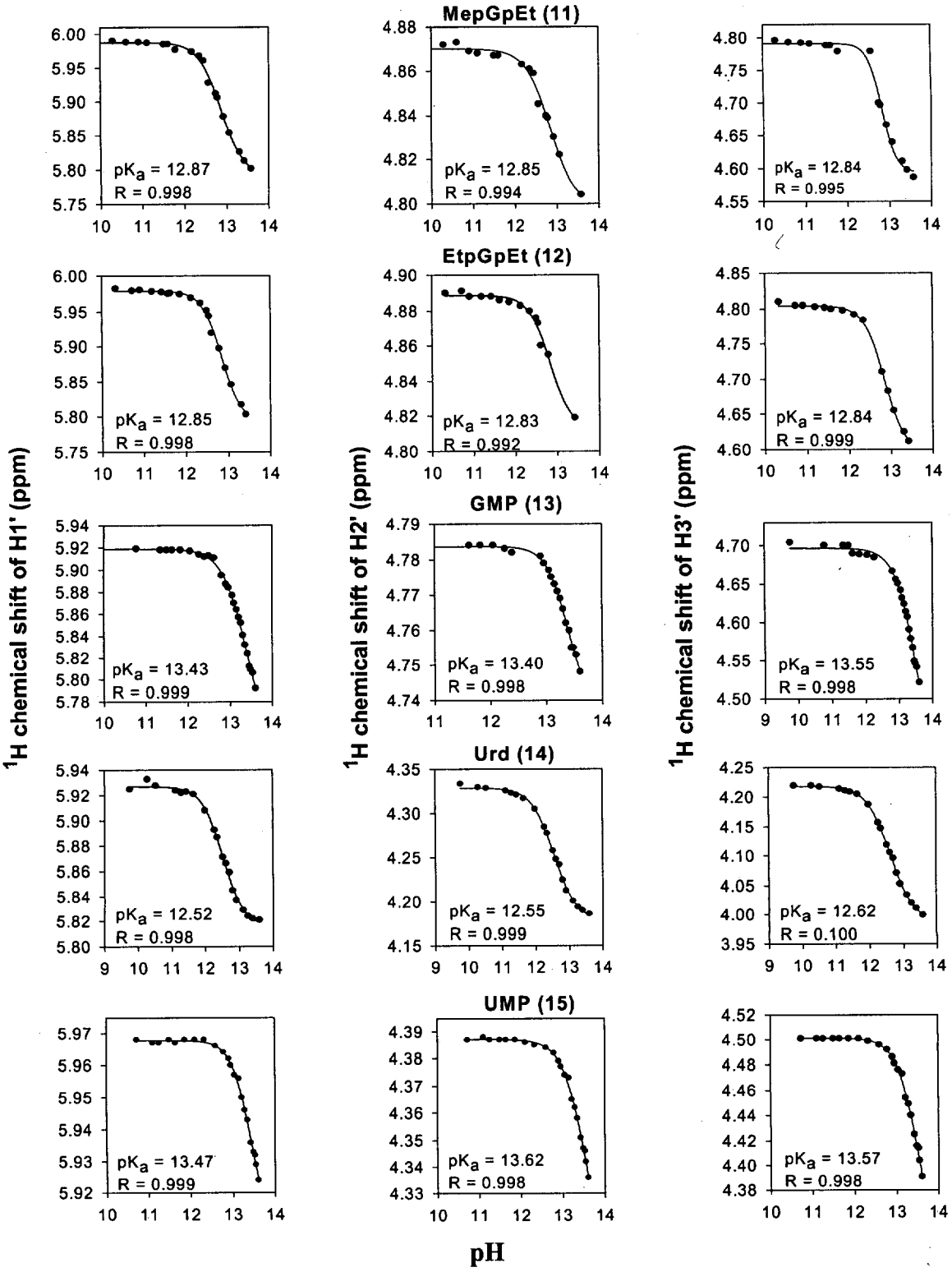

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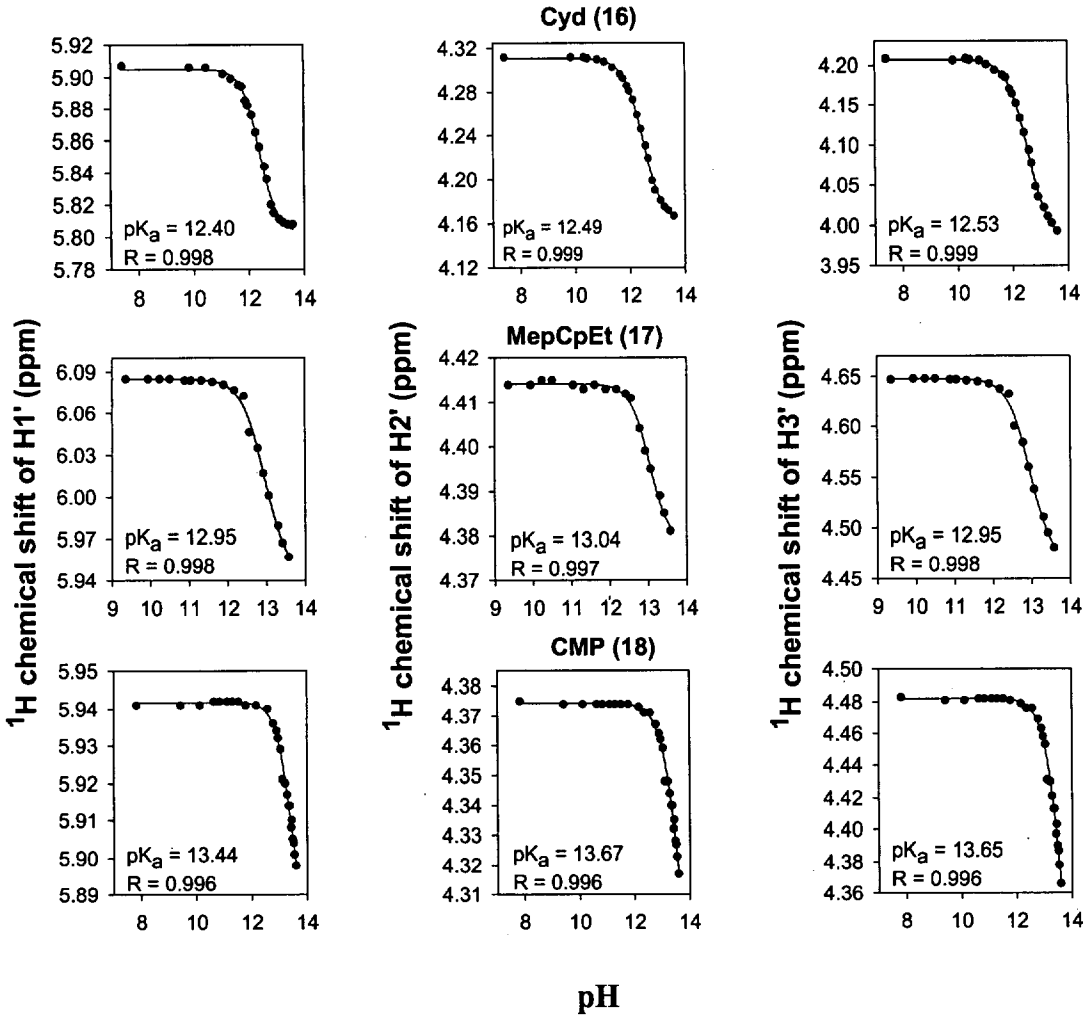
Cyd (16)
MepCpEt (17)
CMP (18)
^{1}H chemical shift of H1' (ppm)
^{1}H chemical shift of H2' (ppm)
^{1}H chemical shift of H3' (ppm)
pK$_a$ = 12.40
R = 0.998
pK$_a$ = 12.49
R = 0.999
pK$_a$ = 12.53
R = 0.999
pK$_a$ = 12.95
R = 0.998
pK$_a$ = 13.04
R = 0.997
pK$_a$ = 12.95
R = 0.998
pK$_a$ = 13.44
R = 0.996
pK$_a$ = 13.67
R = 0.996
pK$_a$ = 13.65
R = 0.996
pH

Figure 1S

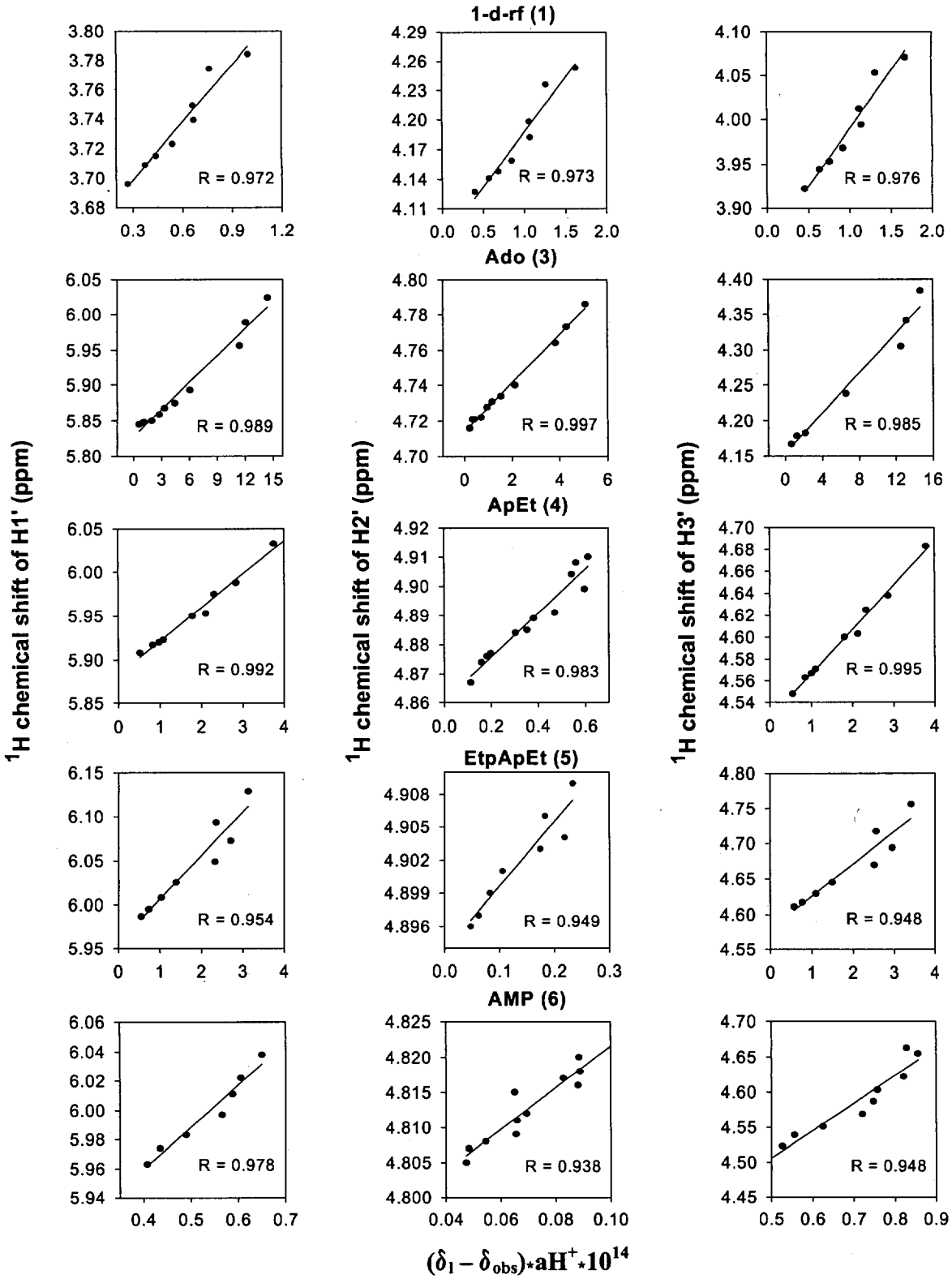

Figure 2S (Continued)

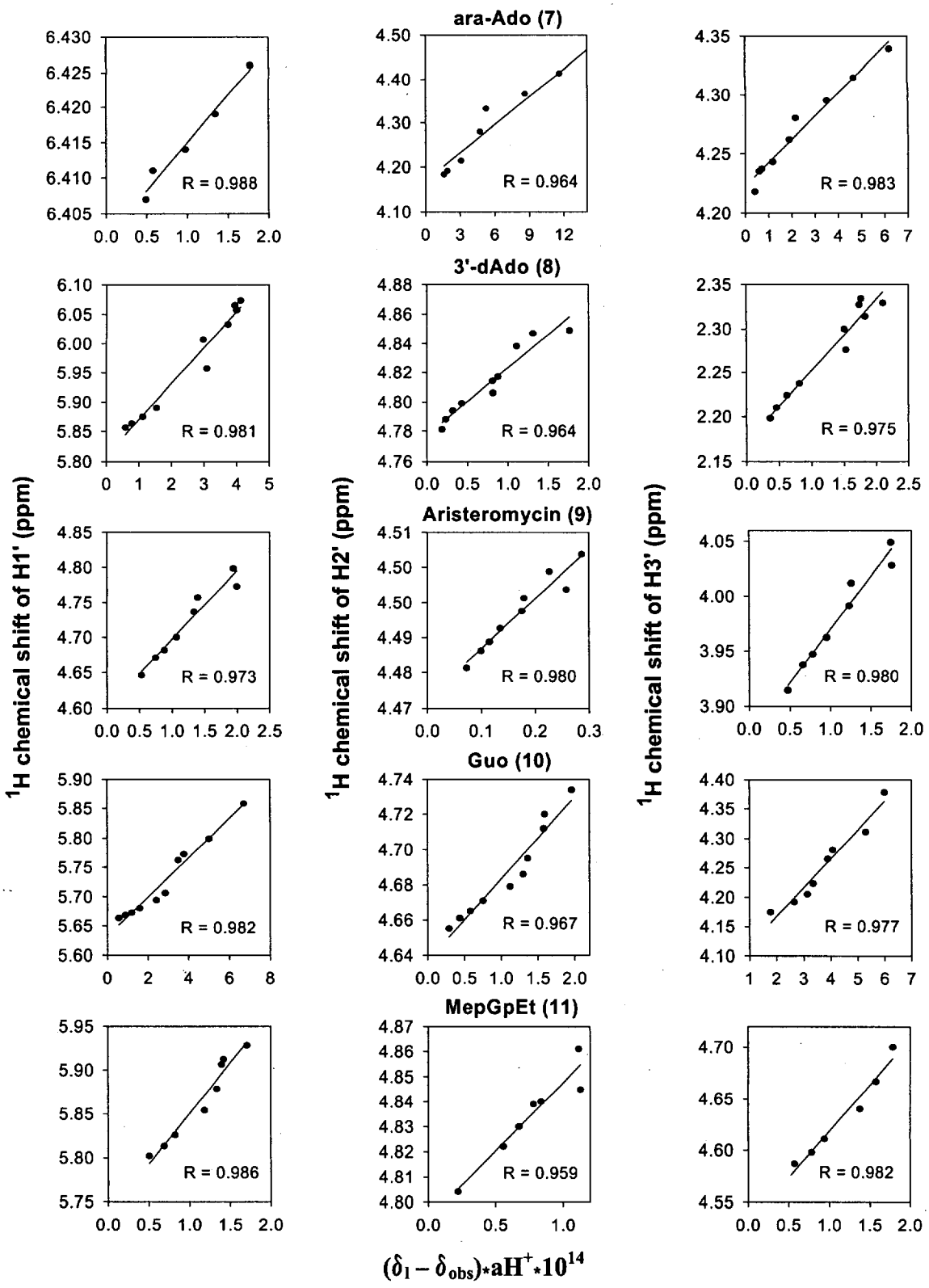

Figure 2S (Continued)

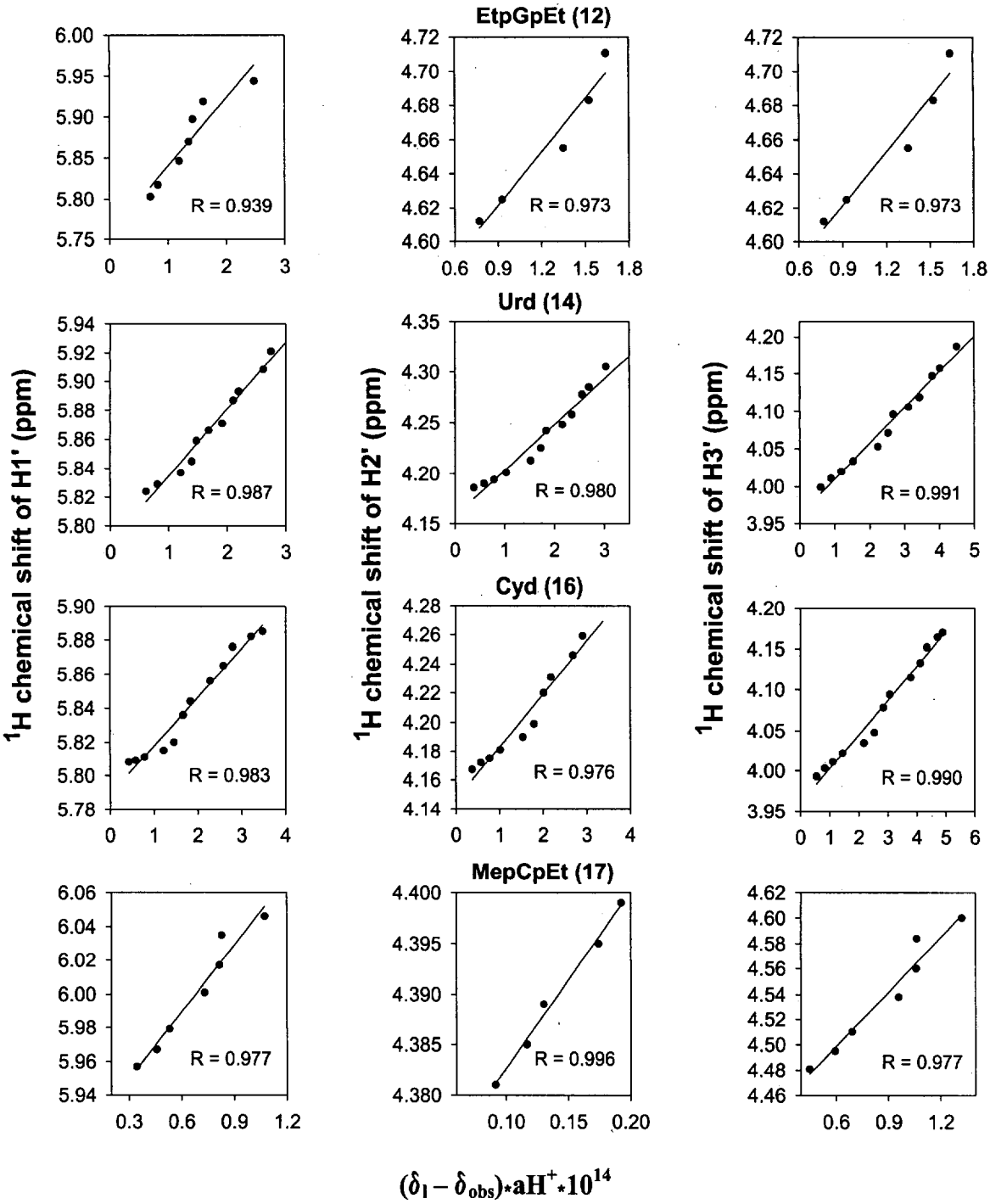
EtpGpEt (12)
Urd (14)
Cyd (16)
MepCpEt (17)
^{1}H chemical shift of H1' (ppm)
^{1}H chemical shift of H2' (ppm)
^{1}H chemical shift of H3' (ppm)
R = 0.939
R = 0.973
R = 0.973
R = 0.987
R = 0.980
R = 0.991
R = 0.983
R = 0.976
R = 0.990
R = 0.977
R = 0.996
R = 0.977
$(\delta_I - \delta_{obs})*aH^+*10^{14}$

Figure 2S

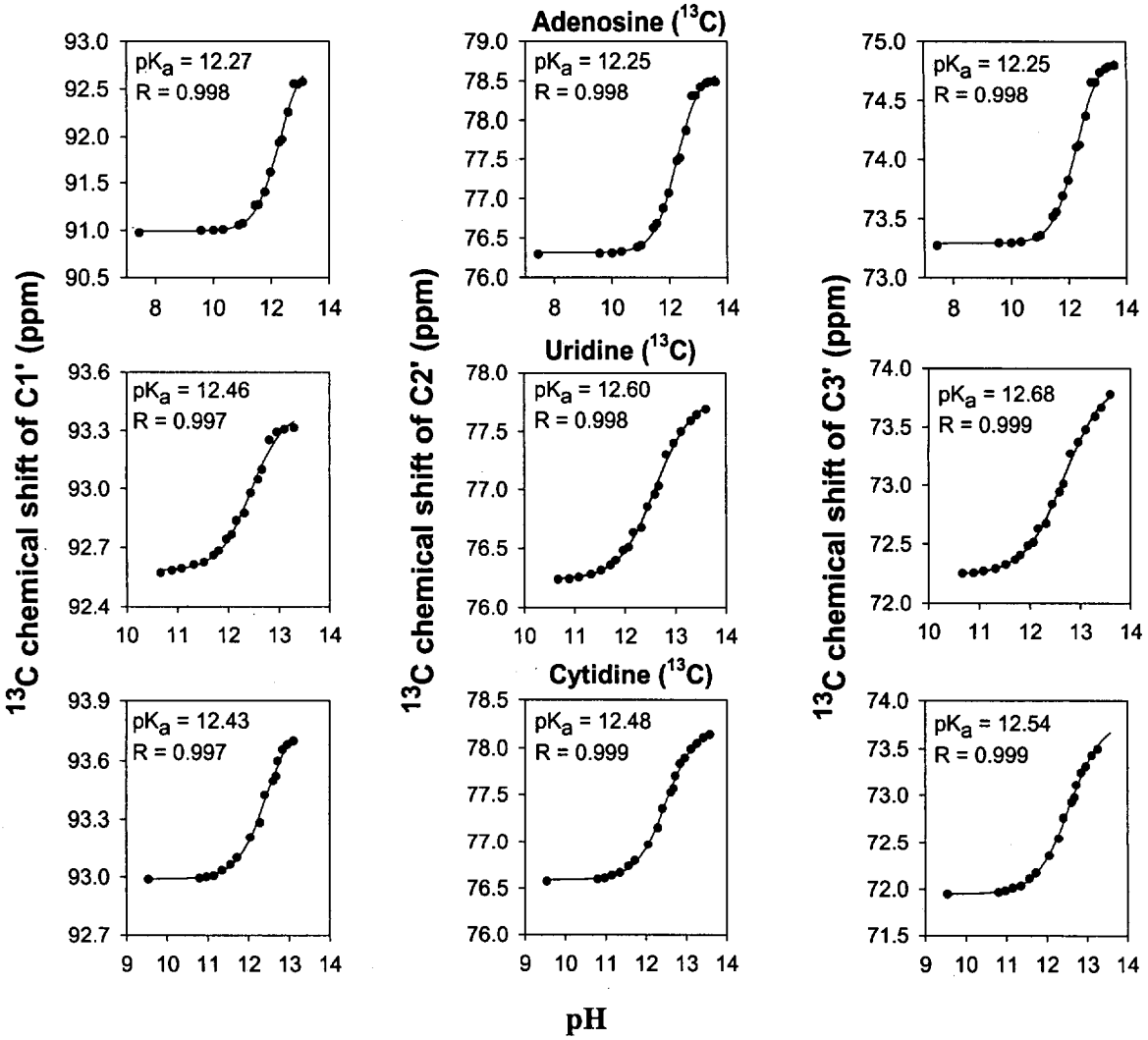
Adenosine (^{13}C)
Uridine (^{13}C)
Cytidine (^{13}C)
^{13}C chemical shift of C1' (ppm)
^{13}C chemical shift of C2' (ppm)
^{13}C chemical shift of C3' (ppm)
pK$_a$ = 12.27
R = 0.998
pK$_a$ = 12.25
R = 0.998
pK$_a$ = 12.25
R = 0.998
pK$_a$ = 12.46
R = 0.997
pK$_a$ = 12.60
R = 0.998
pK$_a$ = 12.68
R = 0.999
pK$_a$ = 12.43
R = 0.997
pK$_a$ = 12.48
R = 0.999
pK$_a$ = 12.54
R = 0.999
pH

Figure 3S

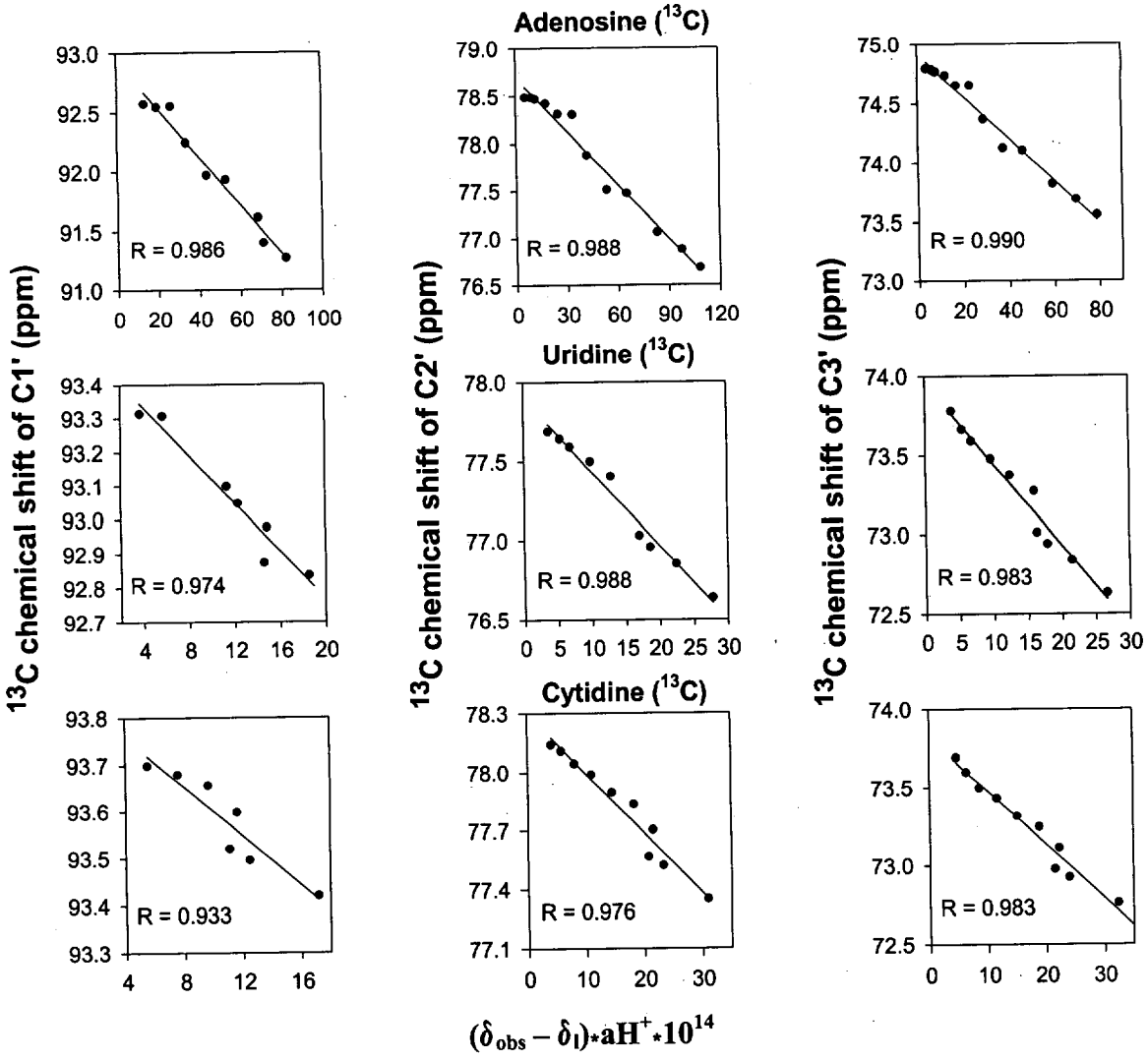
Adenosine (^{13}C)
Uridine (^{13}C)
Cytidine (^{13}C)
^{13}C chemical shift of C1' (ppm)
^{13}C chemical shift of C2' (ppm)
^{13}C chemical shift of C3' (ppm)
R = 0.986
R = 0.974
R = 0.933
R = 0.988
R = 0.988
R = 0.976
R = 0.990
R = 0.983
R = 0.983
$(\delta_{obs} - \delta_l)*aH^+*10^{14}$

Figure 4S

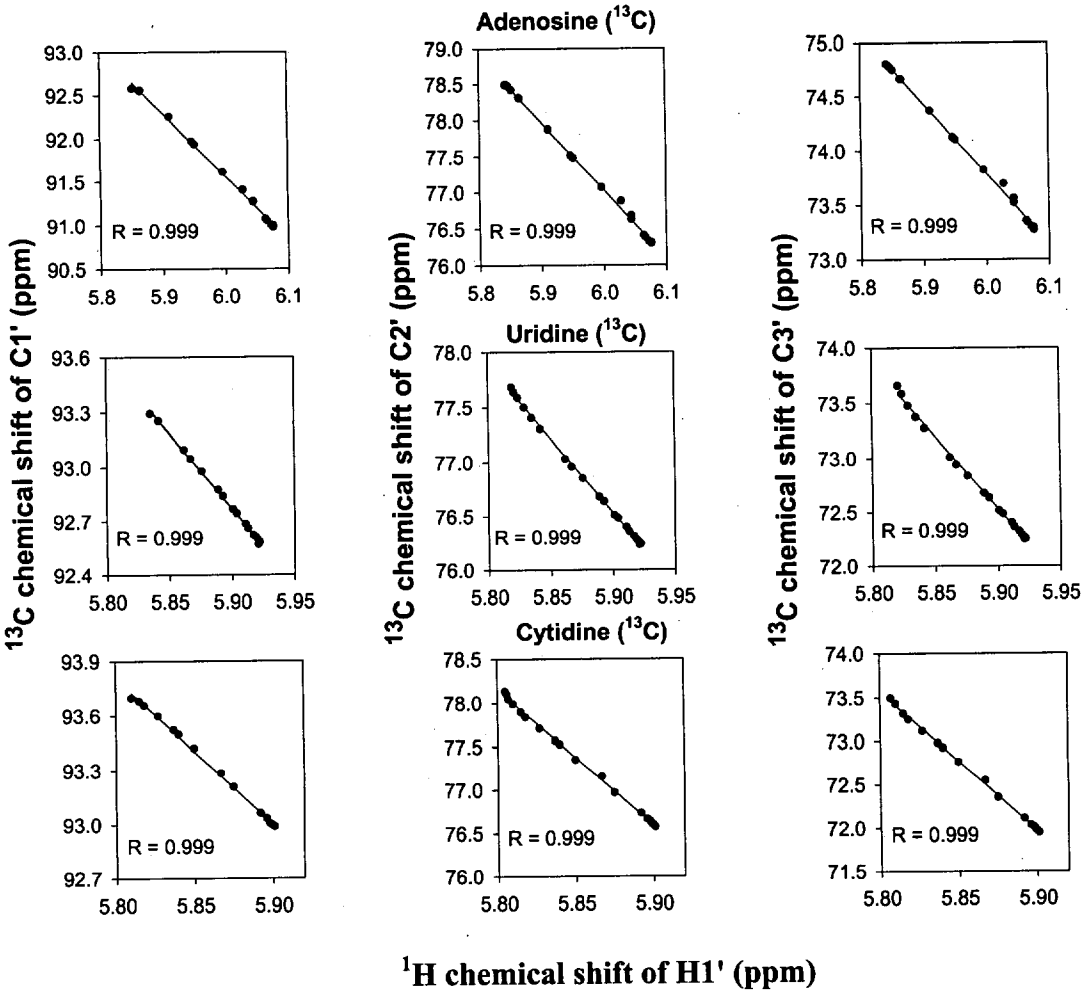

Figure 5S

Experimental Section

(A) Sample preparation. Solutions of **1**[3d], **8**[3c], **2,4,5,11,12,17**[3b] and **3,6,7,9,10,13-16,18**[3a] (Scheme 1) (~5 mM) were made up in D_2O and contained DSS (3-(Trimethylsilyl)-1-propane-sulfonic acid, sodium salt, δ = 0.015 ppm) as an internal standard. For pDs from 7.00 to 13.00, buffers (Robinson, R. A.; *Handbook of Chemistry ahd Physics*, CRC press, Inc.,Ohio, 1977; p D-134.) were used in the sample preparation to ensure that pD remained constant during the data acquisition. From pD 13.05 to 14.00, NaOD solutions covering the

concentration range 0.158-1M were standardized by titration with 0.5 M sulfuric acid in D_2O. All buffer solutions were freshly prepared at least once weekly, and stored (at 4°C) under a pure nitrogen atmosphere. Titrated solutions were prepared freshly for every experiment. D_2O was made free of dissolved carbon dioxide by flushing a stream of nitrogen through it and transferring to a stoppered bottle. Thus the chemical shift measurements were performed under 18-28 different pDs at 25°C to obtain the pK_as of compounds **1-18**.

(C) pD Measurement. The pD measurements were made on a Denver Instrument AP15pH/mV/FET meter with a Russell combination pH electrode. The electrode was standardized against standard buffers of pH = 7.0 and pH = 12.45 or 10.00. The pH was calculated using the empirical relationship pD = (pH + 0.40) (Force, R. K. et al. *Anal. Chem.* **1974,** Vol.46, # 13, p.2049.). Measurements of the pD of the sample were taken before and after each NMR experiment, but the latter measurements were used for plots. These values differed by less than 0.05 pD units (making a difference of 0.01 pK_a value, which is within the error limit of our study, Table 1) for most measurements.

(D) NMR Measurements. Proton and carbon NMR spectra were recorded at 25°C at 500 MHz (Bruker AMX 500) for **1-18** in D_2O solution. The ^1H chemical shifts were referenced to the internal standard DSS at 0.015 ppm, and the ^{13}C chemical shifts were referenced to an external standard of DSS at 0.00 ppm. The precision of the chemical shift measurements was estimated to be 0.001 ppm for ^1H experiments and 0.02 ppm for ^{13}C. Control experiments using a sample of DSS and acetonitrile (as a reference) showed minimal chemical shift variations (± 0.001 ppm) of the DSS signal with alterations in pD, indicating that chemical shift corrections for DSS were not required. (Primrose, W.U. *Samlpe Preparation*; Roberts, G.C.K., **1993,** Ed.; Oxford University press, pp7-34.)

(E) pK_a Determination. The pK_a of the 2'-OH group in all 18 compounds was determined by proton NMR spectroscopy at 25°C. In addition, to prove the use of the proton chemical shift as a probe for the pK_a measurement, ^{13}C NMR spectroscopy was employed to obtain pK_a values from pH-dependent study of ^{13}C chemical shift of C1', C2' and C3' in ^{13}C-labeled (at the pentose sugar moiety only) adenosine, cytidine and uridine. A series of solutions with pD ranging from 7.00 to 14.00 were prepared, and the chemical shifts of a non-exchanging protons (H1', H2'and H3') near the ionizing group were plotted against pH. Determination of pK_a of each compound was based on the chemical shifts observed for 18-28

different solutions with different pDs. Between two successive solutions a pD difference of 0.05-0.3 was used. pK_a values were estimated both by fitting[4a] and calculating[4b] procedures. The latter was applied since the end point (or plateau) at high pH was not defined for all compounds clearly. The curves through the experimental points were fitted with the use of nonlinear least-squares fitting procedure[4a] to the Henderson-Hasselbalch (Eq. 1) (Atkins, P.W. *The Elements of Physical Chemistry*; Oxford, 1992)

$$pH = pK_a + \log [A^-]/[AH] = pK_a + (1-\alpha)/\alpha \qquad (1)$$

where α is a molar fraction of the protonated state. Typically, the single titration curves employed a Hill coefficient set close to unity. Standard errors are reported in the Table 1. The pK_a values obtained from curve fitting were confirmed by calculating procedure based on the Eq. 2:

$$pK_a = pH + \log (\delta_h - \delta_{obs})/(\delta_{obs} - \delta_l) \qquad (2)$$

where δ_h is the chemical shift at high pH; δ_l is the chemical shift at low pH; and δ_{obs} is chemical shift at a given pH. In cases where plateau at high alkaline pH was not defined δ_h was obtained from Eq. 3[4b], as follows:

$$\delta_{obs} = \delta_h + 1/K_a(\delta_l - \delta_{obs})aH^+ \qquad (3)$$

Eq. 3 gives a straight line with a slope $1/K_a$ and δ_h value at intercept (Figure 2S in the Supporting information). The standard error of the pK_a values determined in this way is given in Table 1.

Various techniques, such as UV (Darzynkiewicz, E. et al. *Acta Biochim. Polonica 21*, **1974**, 305-322.), potentiometric[2d], thermometric[2a] (Christensen, J.J. et al. *J. Am. Chem. Soc.* **1966**, 88, 5105-5106), Hplc[2f] have been so far used for the measurement of pK_a of 2'-OH group for ribo[2a-d], arabino (Darzynkiewicz, E. et al. *Z.Naturforsch.* **1975**, 30c, 565-570; Darzynkiewicz, E. et al. D. *Cancer Biochem. Biophys.* **1975**, 1, 85-88; Darzynkiewicz, E. et al. *Acta Biochim.*

Polonica 21, **1974**, 305-322; Remin, M. et al. *J. Am. Chem. Soc.* **1976**, 98, 367-376.) and xylo-nucleosides (Christensen, J.J. et al. *J. Am. Chem. Soc.* **1966**, 88, 5105-5106; Birnbaum, G. I. et al. *J. Am. Chem. Soc.* **1976**, 98, 4640-4644): (i) spectrophotometric titration (Darzynkiewicz, E. et al.,1974) in the ultraviolet has been employed to determine the pK_a values for dissociation of the sugar 2'-hydroxyl in the 3',5'-di-*O*-alkyl and 3'-*O*-alkyl derivatives of 9-β-D-arabinofuranosyladenine (*ara*-C) and 1-β-D-arabinofuranosyluracil (*ara*-U). In particular, the value of 12.4±0.1 was found for 2'-OH of ara-C. The pK_a values of 2'-OH in 3',5'-di-*O*-alkyl derivatives of *ara*-C and *ara*-U were about 0.7-0.9 units higher than in 3'-*O*-alkyl derivatives. The order of dissociation of the sugar hydroxyls in the arabinofuranose ring was 2'-OH > 3'-OH > 5'-OH. The higher acidity (lower pKa) of the 2'-OH was interpreted in terms of formation of an intramolecular 5'-O-H$^{\cdots}$O-2' hydrogen bond, and the accompanying changes in conformation of the arabinose ring. The value of 12.5 was found for 3'-OH in 2,2'-*O*-anhydro-1-α-D-xylofuranosyluracil by thermometric titration (Birnbaum, G. I. et al., **1976**). (ii) pK_a value of 12.34±0.04 was reported for 3'-OH group of 9-β-D-xylofuranosyladenine (Christensen, J.J. et al., 1966) on the basis of a thermometric titration. Izatt et al.[2a] also used this technique to determine the pK_a for 2'-OH group of adenosine (12.35±0.03), and the H-bonding between the vicinal 2'- and 3'-OH groups was used to explain this low acidity; they also showed that substitution of H for either the 2'- or 3'-OH group or OCH_3 for the 2'-OH group results in loss of this acidity. For the basic *cis*-tetrahydrofuran-4-ol-3-phenyl phosphate, a value of 13.9 has been reported for its 2'-OH[2d]. (iii) The pH-dependent kinetic[2f] studies on the alkaline hydrolysis have been used for the pK_a measurement of the 2'-OH (12.04 to 12.84) in dinucleoside(3'→ 5')phosphates, basing on the assumption that 2'-OH ionization takes place rapidly at the initial stage of hydrolysis of the internucleotidyl-phosphodiester group. (iv) Recently, theoretical estimates[2i] of pK_a value for the 2'-hydroxyl of ribose and phosphorylated ribose based on quantum chemistry calculations revealed the values of 13.14 and 14.9 respectively, which are rather high compared to the experimental one[2d].

(F) pK_a Determination for 2, 13, 15, 18 with incomplete ionization at pH = 13.6. For these compounds the δ_h values were calculated from the following Eq. 4

$$b = (pH_1 - pH_2)/\log((\delta_h - \delta_{pH1})*(\delta_{pH2} - \delta_l)/(\delta_{pH1} - \delta_l)*(\delta_h - \delta_{pH2})) \quad (4)$$

where b is the slope value of the titration curve, obtained by the fitting procedure, δ_{pH1} is the chemical shift at pH_1, δ_{pH2} is the chemical shift at pH_2. The difference between the δ_l and δ_h makes 100% ionization, so the percentage of the compound ionized at pH 13.6 can be found from the ratio $(\delta_l - \delta_{13.6})/ (\delta_l - \delta_h) * 100\%$.