J. Mol. Biol. (1978) **123**, 607–630

Crystal Structure of Yeast Phenylalanine Transfer RNA

I. Crystallographic Refinement

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(Received 25 January 1978)

We present the results of the *final* stage of the X-ray crystallographic studies of yeast phenylalanine transfer RNA in an orthorhombic crystal form. The crystal structure of the transfer RNA has been refined by a least-squares procedure to minimize the difference between the observed (F_o) and calculated (F_c) structure factors from X-ray diffraction patterns. The final crystallographic discrepancy index, $R = \sum|F_o - F_c|/\sum F_o$, is 0·198, based upon 8426 structure factors with magnitudes over twice the estimated standard deviation, corresponding to 96·4% of the complete set of data with resolutions up to 2·7 Å.

During the refinement, bond lengths and angles within each phosphate group and each nucleoside (base plus sugar) were *constrained* exactly to their appropriate standard values, while those for the linkages between the nucleosides and phosphates were elastically *restrained* close to their standard values. The details of the application of the constraint–restraint least-squares (CORELS) refinement method to the crystal structure of yeast phenylalanine tRNA are described in this paper. A complete list of atomic co-ordinates and the rigid group thermal factors are presented.

The stereochemical details of this structure and their functional implications are described in the following paper.

1. Introduction

The crystal structure of yeast phenylalanine transfer RNA ($tRNA^{Phe}$) made a considerable contribution to our understanding of the central role played by tRNAs in protein synthesis and in their interaction with other molecules. The structure also helped to focus, in more detail, questions about the structure–function relationship of this class of RNA.

Currently, yeast $tRNA^{Phe}$ is the only example of a nucleic acid whose three-dimensional structure has been determined in detail by single-crystal X-ray diffraction analysis. Since this structure serves as a model for the general structure of all tRNAs (Kim *et al.*, 1974*a*) and provides a basis for formulating principles of nucleic acid conformation and interaction, it is necessary to extract the maximum amount of

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0022-2836/78/1234–0730 $02.00/0

information from the available X-ray data. We describe here the final results of the crystallographic refinement of a crystal structure of yeast $tRNA^{Phe}$ in an orthorhombic lattice *versus* the diffraction data to 2·7 Å resolution, beyond which point the intensities are too weak to be of *actual* value for the refinement. Intermediate results of the crystallographic studies of this structure have been published previously for the orthorhombic crystal form (Quigley *et al.*, 1975; Sussman & Kim, 1976*a*) and for a monoclinic form (Ladner *et al.*, 1975; Jack *et al.*, 1976; Stout *et al.*, 1976).

The crystals used for the X-ray diffraction data collection were grown by the vapor phase diffusion method described earlier (Kim *et al.*, 1971). These crystals have an average size of about 0·70 mm × 0·40 mm × 0·10 mm. The space group is $P2_122_1$ with unit cell parameters of $a = 33{\cdot}31$ Å (0·03), $b = 56{\cdot}22$ Å (0·08) and $c = 161{\cdot}62$ Å (0·19), where the numbers in parentheses are estimated errors in Å. A *complete* set of diffraction data up to 2·7 Å resolution was collected at 4°C on a Picker FACS-I diffractometer using an omega step scan mode. Of the theoretically possible 8741 reflections, 8426 reflections (96·4%) have $F_o > 2\sigma$, where σ is the estimated standard deviation. This corresponds to 98·44% of the data with resolution between 3·0 Å and infinity, plus 90·56% of those with resolutions between 3·0 Å and 2·7 Å.

The backbone structure of this molecule was determined at 4 Å resolution by the multiple isomorphous replacement method (Kim *et al.*, 1973), and the tertiary hydrogen-bonding scheme between the bases has since been described for this molecule in two different crystalline lattices based on the electron density maps at 3 Å resolution (Kim *et al.*, 1974*b*; Robertus *et al.*, 1974). Preliminary refinement of the structure at 2·7 Å resolution (Sussman & Kim, 1976*a*) was carried out by fitting skeletal models to successively improved electron density maps obtained by the partial structure Fourier method (Sussman & Kim, 1976*b*), aided by an interactive computer graphics system at the University of North Carolina, Chapel Hill. This iterative fitting into successively improved electron density maps followed by stereochemical idealization of the molecules yielded a set of atomic co-ordinates, which became the starting point for the reciprocal space refinement described in this paper.

Our specific goals for the reciprocal space refinement were to improve the phases and model co-ordinates so as to allow: (a) fitting of previously ill-defined portions of the molecule, (b) location of bound metal ions, spermines and water molecules, (c) more definitive interpretation of the tertiary hydrogen-bonding interactions, (d) conformational analysis of the backbone structure, (e) reliable estimates of thermal vibration, and (f) estimates of standard deviation in the atomic positions.

In this paper we report the course and results of our refinement procedure and evaluate accuracies of the refined structure by electron densities. The following paper describes the details of the structural features.

2. Experimental Procedure

(a) *Strategies and method of the reciprocal-space refinement*

During the course of our preliminary refinement of the structure, we followed a procedure somewhat similar to that of Freer *et al.* (1975), where 2 steps, electron density fitting and idealization of the resulting model, were iterated alternately. Model idealization in the second step (Hermans & McQueen, 1974) involved variation of all atomic co-ordinates, which were elastically restrained to target co-ordinates in the electron density map, plus restraint of bond lengths, bond angles, and non-bonded contacts to given canonical values. The degree of satisfaction of these various restraints could be controlled

by appropriate weighting factors. However, we often found that the planarity of the aromatic moieties was difficult to maintain and a serious error in the model could be absorbed either by small errors in the bond angles and distances distributed throughout the atoms near the region of distortion, or by shifting that portion out of the electron density in an attempt to improve the stereochemistry. To overcome these difficulties, we developed a least-squares refinement procedure which combines the 2 steps mentioned above into a single step, a procedure commonly used for small molecules (Scheringer, 1963; Doedens, 1970; Waser, 1963). In this approach, a macromolecular structure is subdivided into many *constrained* groups (e.g., nucleosides and phosphates), each of which is elastically *restrained* to their connected, neighboring constrained groups. The essence of this procedure, the constraint–restraint structure-factor least-squares (CORELS) method, is described below.

The quantity to be minimized, Q, in the least-squares procedure consists of the sum of 3 terms:

$$Q = w_F DF + w_D DD + w_T DT, \tag{1}$$

where w_F, w_D and w_T are overall weights for each term. The first term, DF, represents the usual structure factor differences summed over all or part of the reflections, h:

$$DF = \sum_h w_h(|F_{o,h}| - |F_{c,h}|)^2, \tag{2}$$

where $F_{o,h}$ and $F_{c,h}$ are the observed and calculated structure factors of index h. The second term restrains distances between 2 constrained groups, and is the sum over all such distances, d:

$$DD = \sum_d w_d(D_{o,d} - D_{c,d})^2, \tag{3}$$

where $D_{o,d}$ is the canonical distance between a specified pair of atoms (which may be a bond length, a distance between atoms facing a bond angle, a hydrogen-bond length, or a non-bonded contact distance), and $D_{c,d}$ is the corresponding distance calculated from the current model. The third term restrains the structure from moving away from a specified set of predetermined target co-ordinates. Here the sum is over all atoms, i, and over the 3 axial components, j, of each atom:

$$DT = \sum_i w_i \sum_j (X_{T,i,j} - X_{i,j})^2, \tag{4}$$

where $X_{T,i,j}$ is the axial co-ordinate (orthogonal and in Å) of the target atom, while $X_{i,j}$ is the corresponding co-ordinate of the current model. w_h, w_d, and w_i are weights.

For CORELS structure refinement, we set $w_T = 0$, while for model idealization we set $w_F = 0$. The detailed description of the CORELS refinement method has been published by Sussman *et al.* (1977), and the programs are available from the authors on request.

For the refinement of yeast $tRNA^{Phe}$, each nucleoside was considered as a constrained group with 1 rotatable bond, the glycosyl bond, and 2 restrained bonds, C5′—O5′ and 03′—P (see Fig. 1). Each constrained nucleoside group is thus composed of 2 rigid "sub-groups", a base and a ribose. In addition, each phosphate group was considered as a constrained rigid group. *A constrained* group is defined as a molecular moiety where all the bond distances and bond angles have been fixed to respective canonical values, but which can have any number of rotatable bonds. A *restrained distance* is defined as a distance (corresponding to a bond distance, bond angle, hydrogen bond, torsion angle or non-bonded contact) that is allowed to vary from its respective canonical value within a specified range of error. By thus classifying over 85% of bond distances and angles in the structure as constrained, the number of parameters to be refined is greatly reduced. At the same time, the influence of the linked neighbors on a positional shift of a group is also reduced by restraining, rather than constraining, bonds between groups. Initially, for the stem regions of tRNA, 2 nucleosides that are base-paired were considered as a constrained "super-group", as shown at the top of Fig. 1, to reduce further the number of refinable parameters. At a later stage of the refinement, the rotatable "bond" between 2 bases of each "super-group" was removed to allow each nucleoside to be refined independently

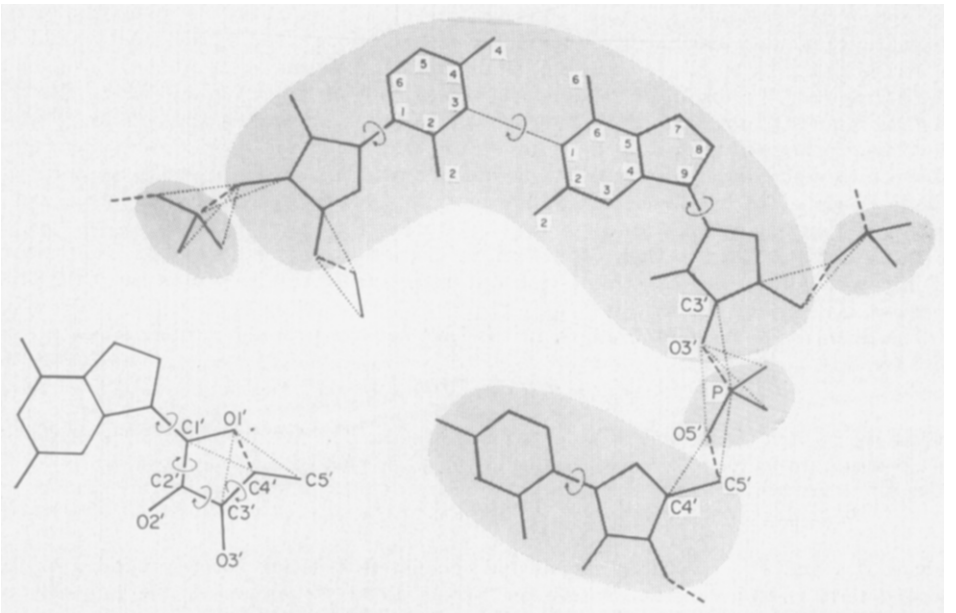


FIG. 1. Three types of constrained groups are shown shaded. The largest constrained supergroup is composed of base-paired nucleosides. The smallest constrained group is a phosphate group. Restrained distances corresponding to bond distances are shown as thick broken lines, and restrained distances corresponding to bond angles are shown as dotted lines. The numbering system used for nucleic acids is also shown. The smooth change of ribose conformation was achieved by allowing 3 torsion angles to rotate while restraining 4 distances as indicated by dotted and broken lines in the lower left of the Figure. The rotation of C1′—C2′ bond moves atoms O2′, C3′, O3′, C4′ and C5′, the rotation of C2′—C3′ bond moves atoms O3′, C4′ and C5′, and the rotation of C3′—C4′ bond moves atom C5′.

as a constrained group. Sugar conformation was also allowed to vary smoothly at a later stage by introducing restraints and variable torsion angles as shown at the bottom left of Fig. 1. Non-bonded contacts have been "restrained" to be equal to or greater than the sum of respective van der Waals' radii. The torsional freedom of the ribose-phosphate backbone has *not* been restricted except to prevent unacceptably short non-bonded contacts.

The relative weights for restraint terms involving bond distances, bond angles and non-bonded contacts were set arbitrarily at 6·0, 5·0 and 3·0, respectively. The relative overall weights for structure factors, w_F, and distance restraints, w_D, used in the least-squares refinement vary depending on the unweighted sums DF and DD in equations (2) and (3) (Konnert, 1976), and the values used for the refinement of yeast $tRNA^{Phe}$ were 1·0 for w_F and $0{\cdot}05 \sim 1{\cdot}0 \times 10^6$ for w_D. To each "rigid" group (ribose, base or phosphate), a single thermal parameter was assigned and refined. At the final stage, the thermal parameters for each atom were refined.

The least-squares matrix was routinely solved by the conjugate gradient iterative method using a "sparse matrix". Such a sparse matrix contains only blocks of intra-group cross derivatives and off-diagonal blocks of cross-derivative terms between groups sharing one or more restraint conditions (bond length, angle, H-bond or non-bonded contact). This matrix is only about 5% of the size of the corresponding full matrix, thus allowing the whole molecule to be treated as a single block. For the special purpose of calculating estimated standard deviations of the group parameters and atomic positions, we have used a standard full matrix inversion technique.

The procedure we used is different from the methods developed by Diamond (1971) or as used by Freer *et al.* (1975) in that their procedures use electron density to fit the model, while we use structure factors directly. In this sense, our procedure is similar to

that used by Watenpaugh *et al.* (1973) or that developed by Konnert (1976) with the major difference being that, in our procedure, most (85% of the total in the yeast phenylalanine tRNA structure) of the bond distances and angles in the model are fixed as parts of constrained groups so that the number of parameters to be refined is greatly reduced.

(b) *Starting model co-ordinates and data*

We have previously reported atomic co-ordinates for yeast $tRNA^{Phe}$ at 2·7 Å resolution derived from manual fitting of multiple isomorphous replacement and partial Fourier maps (Sussman & Kim, 1976*a*). These co-ordinates were "idealized" by the restraint method (Hermans & McQueen, 1974) and are referred to as "target" co-ordinates below. The R factor,

$$R = \frac{\sum|F_o - F_c|}{\sum|F_o|},$$

for 8426 data to 2·7 Å resolution, calculated from these co-ordinates, was 0·39.

In order to ensure *strictly* ideal stereochemistry within constrained groups in our starting model, we fitted canonical nucleosides, base-pairs and phosphates (Arnott *et al.*, 1972) to our published model by minimizing the distances between the constrained groups and corresponding target co-ordinates, while simultaneously allowing the glycosyl bond to rotate and restraining distances which specify connections between constrained groups. This was achieved by setting $w_F = 0$ in equation (1). The new model thus fitted maintained strictly canonical bond distances and angles within the constrained groups. The root-mean-square (r.m.s.) deviation from canonical bond distances for the restrained bonds was 0·037 Å (max. dev. = 0·1 Å) and the r.m.s. deviation in angles was 4° (max. dev. 20°). The r.m.s. difference between these "canonical" co-ordinates and the reported co-ordinates was 0·197 Å (max. = 1·0 Å). These newly generated atomic co-ordinates were considered as the starting model co-ordinates for the refinement.

For the preliminary stages of refinement we decided to use only 6153 data with resolution between 3·0 Å and 10 Å. We excluded the high-resolution data to reduce computation time and increase convergence range (Scheringer, 1963), and the low-order data below 10 Å resolution as these are intense reflections containing large solvent contributions and tend to dominate the refinement. The initial R factor for the "starting" model for 6153 data was 0·422.

(c) *Course of the refinement*

A stepwise description of the progress of structure refinement of yeast $tRNA^{Phe}$ is given in Table 1 and Fig. 2. In retrospect, the refinement may have been done in a more concise and possibly more efficient way; however, we were in part experimenting with a new refinement procedure and not initially aware of the particular characteristics and advantages of the method. Not counting the refinement of a few selected residues, a total of 8 cycles of positional parameter refinement and 7 cycles of group thermal parameter refinement (see below) completed the structure refinement. Our current version of the CORELS program greatly simplifies and facilitates the refinement process as tested in the recent refinement of 2 proteins, demetallized concanavalin A and triclinic lysozyme (Shoham, Herzberg, Sussman *et al.*, unpublished results).

The use of an interactive computer graphics system at the University of North Carolina (Wright, 1977) has been of great value in allowing visual inspection of and manual fitting to Fourier maps throughout the refinement. Such intervention is absolutely necessary to move grossly misplaced residues which may be simply shifted to an incorrect local minimum by the least-squares method. The computer graphics system has also been useful for assignment of electron density as bound cations or water (although this was at least partially automated). Manual fitting of misplaced residues was necessary in regions where the multiple isomorphous replacement electron density was less clear, e.g., the dihydrouracils 16 and 17, the anticodon loop and Y base tail, parts of the variable and T loops, and residues 75 to 76. At the end of the refinement, these residues could be identified as having high thermal parameters. Partial Fourier maps using both $F_o - F_c$ and $2F_o - F_c$ as amplitudes have been examined for density fitting, where F_c values and phases were

TABLE 1

Detailed course of CORELS refinement of yeast $tRNA^{Phe}$ crystal structure at 2·7 Å resolution.

Steps		Refinement operation	R (%)	No. of data	No. of variables	Comments
I	a	Generate starting co-ordinates by fitting ideal groups to multiple isomorphous replacement model	42·2	6153	—	Data 10 Å to 3 Å
	b	Refinement of positional parameters: 1 cycle	35·5	6153	966	Set overall B = 18 and 11 scale factors as function of sin θ/λ; structure divided into 9 overlapping blocks
	c	Refinement of positional parameters and scale factors: 1 cycle	31·9	6153	977	As above
	d	Exclude 193 data	30·7	5960	—	Data with $F_o < 2\sigma$ excluded
	e	Refinement of positional parameters: 1 cycle	30·1	5960	888	Whole structure refined simultaneously without breaking the structure into several blocks as done previously
	f	Include 2247 data	31·7	8207	—	F_o values for 3 to 2·7 Å data scaled to F_c values, include 4 additional scales for this data; R factor for the additional 2247 alone was 39·9%
	g	Refinement of positional parameters: 1 cycle	31·4	8207	888	Data (10 Å to 2·7 Å)
	h	Refinement of *group thermal parameters* only. Followed by scale factor refinement: 2 cycles	28·6	8207	243	One thermal parameter for each phosphate, ribose and base
II	a	Manual fitting using $F_o - F_c$ and $2F_o - F_c$ maps on computer graphics. Residues 15, 16, 18, 37, 47 and 58 refitted.	30·1	8207	—	After fitting, refine distance restraints for new co-ordinates. Also select 14 peaks as bound ligands (metal ions or waters). Finally update structure factors

	b	Refinement of positional and group thermal parameters of refitted groups: 2 cycles	28·2	8207	143	In addition, thermal parameters for the ligands refined
	c	Refinement of all positional and group thermal parameters: 1 cycle	27·3	8207	1133	No positional parameters of the ligands refined
	d	Refinement of residues 16, 17, 33–38, 54, 57, 58 and 13 ligands: 1 cycle	27·2	8207	213	One ligand removed; positional and thermal parameters refined
	e	Refinement of positional parameters allowing ribose pucker to vary for first time: 1 cycle	26·7	8207	1155	No B values refined
III	a	Fit using $F_o - F_c$ maps on computer graphics. Residues 6, 7, 31–33, 35–39, 45–47, 56–58, 67, 68	27·1	8207	—	Idealize distance restraints before CORELS structure factor refinement
	b	Refinement of positional parameters: 1 cycle	26·4	8207	1155	No B values refined
IV	a	Fitting to map of residues 31–40, 57–58	26·7	8207	—	Bond distance and angle restraints idealized after fitting. Structure factors updated
	b	Refinement of positional parameters for refitted groups: 2 cycles	26·3	8207	175	No B values refined
V	a	Fitting of residues 17, 47 to map	26·3	8207	—	Idealize distance restraints, then update structure factors
	b	Refinement of group thermal parameters: 2 cycles	25·7	8207	241	All phosphate, ribose bases and 13 ligands
	c	Refinement of positional parameters: 1 cycle	25·7	8207	1163	Torsion angles of Y base 'tail' refined
	d	Refinement of positional parameters for residues 2, 16, 17, 18, 29, 33, 34, 36, 37, 38, 76: 1 cycle	25·7	8207	132	Refine positional parameters not previously converged
	e	Remove tertiary base–base restraints and refine positional parameters: 2 cycles	25·7	8207	394	Positional parameters of 23 residues involved in tertiary H-bonding
	f	Refinement of positional parameters for residues 21, 22, 24, 26, 44 for 4 cycles	25·7	8207	82	Remove 3 ligands from calculated structure factors

TABLE 1—*continued*

Steps		Refinement operation	R (%)	No. of data	No. of variables	Comments
	g	Refinement of positional parameters of residues 26 and 44	25·7	8207	21	Allow dimethyl group to rotate
	h	Raise all group B values by 12 Å^2 and rescale. Then refine 2 cycles on all group thermal parameters including 10 ligands	25·4	8207	238	Allow all group B values to converge to non-negative values
	i	Refinement of group thermal parameters for residues 2, 16, 75, 76: 2 cycles	25·4	8207	9	Allow these group B values to converge
VI	a	Based on difference map, change ribose pucker of residues 21 and 75	25·7	8207	—	Fit sugars with opposite pucker by use of distance restraints and targetting
	b	Refinement of positional parameters of 21 and 75: 4 cycles	25·4	8207	44	Allow sugar pucker to vary
VII	a	Refit 75, 76 manually. Accept 15 new and remove 2 old ligands: total 23 ligands	25·0	8207	—	Very weak density for 76; idealize distance restraints after fitting
	b	Refinement of positional and group thermal parameters for residue 76 and 2 cycles for B values of new ligands	24·9	8207	29	Lower thermal parameter indicating better fit of 76 to density
	c	Calculate and scale 219 low-resolution data (> 10Å)	24·7	8426	1	Add 16th scale factor ($R = 22{\cdot}7\%$ for the additional 219 data only)
	d	Accept 33 ligands based on density and stereochemistry. Refine ligand thermal parameters: 2 cycles	24·2	8426	33	Use automatic peaksearch of difference Fourier
	e	Accept 26 more ligands and refine their thermal parameters: 2 cycles: total 82 ligands	23·8	8426	26	Again selected by automatic peaksearch of map

f	Delete 12 ligands having largest thermal parameters	23·9	8426	—	Total of 70 ligands, 4 of which are denoted as Mg^{2+}-hydrates
g	Four ligands identified as Mg^{2+} hydrates. Replace these four by $[Mg(H_2O)_n]^{2+}$ and refine group B values: 4 cycles	23·9 R_w† = 30·1	8426	4	Orient hydrates by restraints to molecular ligands as interpreted from density
h	Refine *all* group parameters in 37 non-overlapping *blocks, using full matrix and weighted structure* factors	23·3 R_w = 29·6	8426	1423	Two ligands removed. Obtained estimates of errors *from inverse matrix*
i	Idealize stereochemistry as specified by distance restraints while minimizing movement from target co-ordinates of previous step	23·8 R_w = 29·8	8426	—	Update structure factors of idealized model
j	Remove secondary structure constraints. Refine all positional parameters: 1 cycle. Refine additional cycle for 42 residues which had not converged	22·8 R_w = 28·8	8426	1224	Use group thermal parameters from above. Continue to use weighted structure factors
k	Refine group thermal parameters: 2 cycles. Then idealize stereochemistry as in VIIi for this "less constrained" model	23·1 R_w = 29·3	8426	296 (thermal)	Update structure factors after model idealization
l	Refine atomic thermal parameters of tRNA molecule	19·8 R_w = 25·3	8426	1740	Six cycles of atomic thermal parameters refinement after VIIk. These parameters were restricted to vary within an arbitrary range of $1 \sim 250$ Å^2.

Roman numerals represent stages at which some residues were refitted to a new electron density map manually using an interactive computer graphics system.

† R_w, weighted R factor.

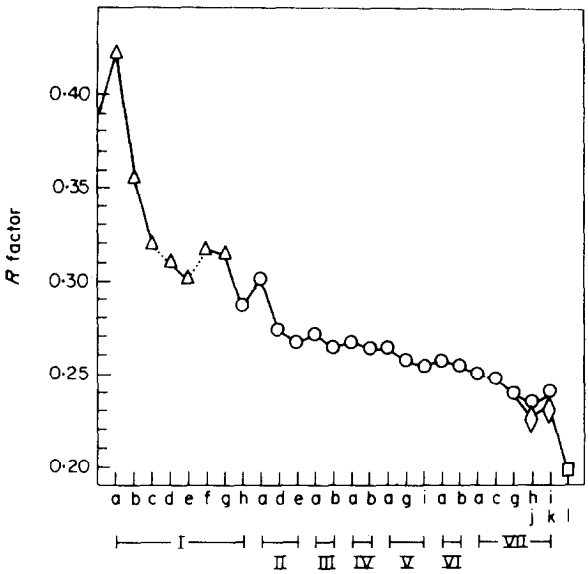


FIG. 2. A graph showing the course of structure refinement as followed with R factor. The ordinate is R factor in fractions, and the abscissa corresponds to the steps and the substeps described in Table 1. (△) The refinement step where the overall temperature factor was refined; and (○) the refinement using rigid group thermal parameters. (◇) The refinement without base-pairing constraints; and (□) the refinement using atomic thermal parameters. The dotted lines indicate the steps where the number of data points were changed.

calculated from the current atomic co-ordinates *minus* those for the residues to be re-examined.

As may be readily noticed by inspection of Table 1, we have used *local refinement* for portions of tRNA which had been manually fitted or which had not completely converged in previous cycles. Total calculated real (A) and imaginary (B) components of the structure factors are stored with the amplitudes and updated with each local refinement by subtracting partial A and B values calculated from beginning co-ordinates of the local residues and then adding partials calculated from the least-squares shifted co-ordinates (i.e. $A^{\text{New}}_{\text{Total}} = A^{\text{Old}}_{\text{Total}} - A^{\text{Old}}_{\text{Partial}} + A^{\text{New}}_{\text{Partial}}$). The derivatives required for the least-squares equations are calculated only for the groups being refined. This procedure eliminates refining previously converged portions of the structures and is valid due to the low correlation in shift between spatially distant residues of a molecule of this size. Stereochemistry between the locally refined groups and the remainder of the molecule is maintained adequately by including restraints to the fixed groups in the least-squares matrix.

To reduce the number of parameters refined in any one cycle (and hence the size of the least-squares matrix), we often delayed the refinement of rigid group thermal parameters until positional parameter shifts had converged. This procedure also allows more meaningful shifts in the thermal parameters to be calculated. Derivatives are calculated only for the positional or thermal parameters if just one set is varied in a given cycle.

Although we have attempted refinment of atomic thermal parameters, we regard the results of the rigid group thermal parameter refinement as more realistic and will therefore associate these with our final co-ordinate set. The atomic thermal parameters were restricted to vary within the range between B = 1 and 250 Å^2. Several of them attempted to exceed these limits, and fluctuated unpredictably. It is not clear whether the reduction in R from 23·1% to 19·8% by introducing refinable atomic thermal parameters implies an improved model. It is difficult to employ the Hamilton (1965) significance test due to the problem of counting parameters when refining with many restrained conditions. We suspect that refinement of individual atomic thermal parameters is not warranted at this

resolution (2·7 Å) unless one introduces some arbitrary restraint conditions among them.

As mentioned earlier, we defined "super-groups" (see Fig. 1) to reduce the number of refinable parameters. Such constraints kept the bases that are paired in the stems at proper distances and angles. When we removed these base-pair constraints as described in step VII of Table 1, the distances and angles involving the base-pair hydrogen bonds varied less than 0·1 Å and 4° on average, respectively (see also Table 4 of the following paper).

Through most of the tRNA refinement, the structure factors were all weighted equally. Analysis of the ΔF values (Fig. 3) in ranges as a function of sin θ/λ (or F_o) showed that the high-resolution (or weaker reflection) data were being weighted too low relative to the low-resolution (or stronger reflection) data. We therefore applied a weighting function to the F_o values such that

$$w_i = \frac{1}{\sigma_i^2} = \frac{1}{[63{\cdot}95 + (0{\cdot}075)F_o]},$$

where the constants 63·95 and 0·075 are the intercept and slope of a plot of $\Delta\bar{F}$ *versus* $\bar{F}$. This empirical weighting scheme tended to equalize the contributions of all structure factors to the least-squares equations.

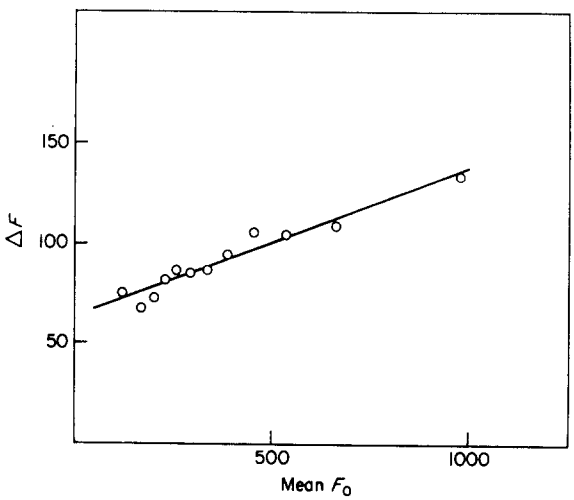


FIG. 3. The average differences between observed and calculated structure factors plotted against the average magnitude of observed structure factors for groups of reflections of similar magnitude. This curve was used to obtain the weighting function used in the final stages of refinement. The straight line in the graph represents the least-squares fitted straight line.

3. Results and Discussion

The refined atomic co-ordinates of the crystal structure of yeast $tRNA^{Phe}$ are listed in Table 2 and rigid group thermal parameters in Table 3. These and the atomic co-ordinates of the tightly bound magnesium hydrate ions and bound water or other cations are deposited in the Brookhaven Data Bank (write to Dr. T. F. Koetzle, Chemistry Dept., Brookhaven National Laboratory, Upton, N.Y.). The positions of the latter are less well-defined than the magnesium hydrate ions. The locations and the description of the environments of four site-specifically bound magnesium ions have been presented earlier (Holbrook *et al.*, 1977). There are two locations in the electron density map which we could interpret as the sites for the bound spermines: one in the deep groove of the double helix formed by the acceptor and T stems, and the other

TABLE 2

Atomic co-ordinates of the yeast tRNA^{Phe} crystal structure in an orthogonal co-ordinate system (X, Y, Z) in Å units

1 GUANOSINE			
O	27.5	24.0	69.1
P	27.5	24.9	70.4
O1P	28.6	25.9	70.3
O2P	26.2	25.6	70.5
O5'	27.8	23.9	71.6
C5'	29.0	23.2	71.6
C4'	28.9	22.1	72.6
O3'	30.7	20.4	72.2
C3'	29.3	20.7	72.1
O2'	29.0	19.6	74.3
C2'	28.4	19.8	73.0
C1'	27.2	20.6	73.2
O1'	27.6	22.0	73.2
N9	26.2	20.4	72.1
C8	25.8	21.3	71.1
N7	24.9	20.8	70.3
C5	24.6	19.6	70.8
C6	23.7	18.6	70.4
O6	22.9	18.6	69.5
N1	23.7	17.5	71.2
C2	24.5	17.3	72.3
N2	24.4	16.1	73.0
N3	25.4	18.2	72.7
C4	25.4	19.3	71.9
2 CYTIDINE			
P	31.7	20.5	71.0
O1P	31.0	21.1	69.8
O2P	33.0	21.0	71.4
O5'	31.8	18.9	70.7
C5'	30.6	18.2	70.3
C4'	30.5	16.9	71.1
O3'	31.7	15.0	70.3
C3'	30.5	15.6	70.4
O2'	30.0	14.2	72.3
C2'	29.4	14.8	71.1
C1'	28.5	15.9	71.6
O1'	29.3	17.1	71.9
N1	27.5	16.3	70.6
C6	27.4	17.7	70.2
C5	26.4	18.0	69.3
C4	25.6	17.0	68.8
N4	24.7	17.3	67.9
N3	25.7	15.7	69.1
C2	26.7	15.4	70.1
O2	26.8	14.2	70.4
3 GUANOSINE			
P	32.6	15.1	69.0
O1P	32.2	16.3	68.2
O2P	34.0	15.0	69.3
O5'	32.2	13.8	68.2
C5'	32.2	12.5	68.9
C4'	31.0	11.7	68.6
O3'	31.6	10.1	66.8
C3'	30.8	11.3	67.1
O2'	29.0	9.7	67.7
C2'	29.3	11.0	67.1
C1'	28.7	12.1	67.9
O1'	29.8	12.5	68.8
N9	28.3	13.2	67.2
C8	28.7	14.5	67.1
N7	28.1	15.3	66.3
C5	27.1	14.5	65.8
C6	26.0	14.8	64.9
O6	25.8	15.9	64.3
N1	25.2	13.7	64.6
C2	25.4	12.5	65.2
N2	24.5	11.5	64.8
N3	26.4	12.2	66.0
C4	27.2	13.2	66.3
4 GUANOSINE			
P	32.4	10.1	65.4
O1P	32.5	11.5	64.9
O2P	33.7	9.3	65.5
O5'	31.3	9.4	64.5
C5'	31.2	9.8	63.1
C4'	30.0	9.2	62.5
O3'	30.3	8.7	60.1
C3'	29.7	9.6	61.0
O2'	27.7	8.2	60.9
C2'	28.2	9.5	61.0
C1'	27.8	10.0	62.4
O1'	28.8	9.6	63.3
N9	27.7	11.5	62.5
C8	28.4	12.4	63.2
N7	28.1	13.6	63.0
C5	27.0	13.6	62.2
C6	26.2	14.6	61.6
O6	26.3	15.8	61.8
N1	25.2	14.1	60.8
C2	25.0	12.8	60.5
N2	24.1	12.5	59.6
N3	25.8	11.8	61.0
C4	26.8	12.3	61.8
5 ADENOSINE			
P	31.4	9.4	59.1
O1P	31.9	10.6	59.7
O2P	32.3	8.4	58.6
O5'	30.4	9.8	57.9
C5'	29.7	8.8	57.1
C4'	28.4	9.3	56.6
O3'	28.6	9.9	54.3
C3'	28.4	10.4	55.6
O2'	26.0	10.3	55.2
C2'	27.1	11.1	55.7
C1'	26.9	11.1	57.3
O1'	27.6	9.9	57.8
N9	27.4	12.3	57.9
C8	28.4	12.4	58.9
N7	28.6	13.6	59.3
C5	27.7	14.4	58.6
C6	27.5	15.7	58.5
N6	28.1	16.6	59.3
N1	26.5	16.2	57.7
C2	25.9	15.3	56.9
N3	26.0	14.0	56.9
C4	27.0	13.6	57.7
6 URIDINE			
P	30.1	10.3	53.7
O1P	31.1	10.5	54.8
O2P	30.5	9.3	52.6
O5'	29.8	11.7	53.0
C5'	28.8	11.6	51.8
C4'	27.8	12.8	51.9
O3'	28.3	14.1	49.9
C3'	28.3	14.1	51.3
O2'	26.1	15.0	51.4
C2'	27.4	15.1	52.0
C1'	27.2	14.5	53.4
O1'	27.6	13.1	53.3
N1	28.1	15.1	54.4
C6	29.0	14.3	55.1
C5	29.8	14.9	56.0
C4	29.8	16.3	56.3
O4	30.5	16.9	57.1
N3	28.9	17.0	55.6
C2	28.0	16.4	54.6
O2	27.3	17.1	54.0
7 URIDINE			
P	29.7	14.1	49.2
O1P	30.7	13.6	50.1
O2P	29.6	13.4	47.9
O5'	29.9	15.6	48.9
C5'	29.0	16.3	48.1
C4'	28.8	17.8	48.6
O3'	29.2	19.9	47.4
C3'	29.8	18.7	47.9
O2'	31.5	20.2	49.1
C2'	30.7	19.0	49.1
C1'	29.8	19.0	50.3
O1'	28.9	17.9	50.0
N1	30.3	18.8	51.6
C6	30.6	17.5	52.1
C5	31.1	17.4	53.3
C4	31.5	18.5	54.1
O4	32.0	18.4	55.3
N3	31.2	19.7	53.6
C2	30.6	19.9	52.3
O2	30.4	21.1	51.9
8 URIDINE			
P	28.8	19.8	45.9
O1P	27.6	18.8	45.7
O2P	28.5	21.2	45.3
O5'	30.1	19.2	45.2
C5'	31.2	20.0	45.0
C4'	31.8	19.8	43.6
O3'	30.6	21.1	42.0
C3'	30.9	19.8	42.4
O2'	32.7	19.7	40.8
C2'	31.6	19.0	41.4
C1'	32.3	17.9	42.3
O1'	32.5	18.5	43.6
N1	31.5	16.7	42.4
C6	30.7	16.4	43.6
C5	30.0	15.3	43.7
C4	30.0	14.3	42.7
O4	29.3	13.3	42.7
N3	30.8	14.6	41.6
C2	31.5	15.8	41.4
O2	32.2	15.9	40.4
9 ADENOSINE			
P	29.2	21.3	41.2
O1P	28.4	20.0	41.2
O2P	28.4	22.5	41.8
O5'	29.7	21.7	39.7
C5'	29.9	23.1	39.4
C4'	29.4	23.4	38.1
O3'	27.4	24.6	37.2
C3'	27.9	23.5	38.0
O2'	26.3	22.1	36.8
C2'	27.5	22.2	37.4
C1'	28.7	22.0	36.4
O1'	29.8	22.4	37.1
N9	28.9	20.6	35.9
C8	28.4	19.4	36.5
N7	28.8	18.4	35.8
C5	29.5	18.9	34.8
C6	30.2	18.2	33.7
N6	30.2	16.9	33.5
N1	30.9	19.0	32.9
C2	30.9	20.3	33.0
N3	30.3	21.0	34.0
C4	29.6	20.2	34.8
10 2M-GUANOSINE			
P	27.2	26.0	37.9
O1P	28.1	26.2	39.0
O2P	27.3	27.1	36.8
O5'	25.7	25.9	38.4
C5'	24.9	27.1	38.3
C4'	23.4	26.7	38.3
O3'	22.9	25.5	40.4
C3'	23.1	25.4	39.0
O2'	20.7	25.6	38.7
C2'	21.9	24.9	38.2
C1'	22.2	25.5	36.8
O1'	22.9	26.7	37.0
N9	23.0	24.6	35.9
C8	24.3	24.8	35.5
N7	24.7	23.8	34.7
C5	23.6	22.9	34.6
C6	23.5	21.7	34.0
O6	24.3	21.1	33.3
N1	22.2	21.2	34.1
C2	21.2	21.7	34.9
N2	20.1	21.0	34.9
C2M	19.0	21.6	35.7
N3	21.3	22.9	35.5
C4	22.6	23.4	35.4
11 CYTIDINE			
P	22.5	24.2	41.2
O1P	23.7	23.3	41.3
O2P	22.0	24.6	42.5
O5'	21.4	23.5	40.3
C5'	20.0	23.6	40.8
C4'	19.4	22.2	40.7
O3'	19.7	20.9	42.7
C3'	20.1	21.1	41.4
O2'	18.3	19.5	40.9
C2'	19.6	19.9	40.5
C1'	19.6	20.5	39.1
O1'	19.2	21.9	39.3
N1	20.8	20.4	38.4
C6	21.7	21.5	38.3
C5	22.8	21.4	37.6
C4	23.1	20.2	36.9
N4	24.2	20.0	36.2
N3	22.3	19.1	37.0
C2	21.1	19.2	37.7
O2	20.3	18.3	37.8
12 URIDINE			
P	20.2	19.7	43.6
O1P	21.7	19.7	43.7
O2P	19.5	19.7	44.9
O5'	19.8	18.4	42.7
C5'	19.1	17.3	43.3
C4'	19.4	16.0	42.5
O3'	20.2	14.4	44.2
C3'	20.5	15.2	43.0
O2'	19.8	13.3	41.6
C2'	20.8	14.3	41.8
C1'	20.6	15.3	40.6
O1'	19.7	16.4	41.2
N1	21.8	16.0	40.2
C6	22.2	17.2	40.6
C5	23.3	17.8	40.2
C4	24.2	17.2	39.3
O4	25.2	17.7	38.8
N3	23.8	15.9	38.9
C2	22.6	15.3	39.3
O2	22.3	14.2	38.9
13 CYTIDINE			
P	21.3	14.2	45.3
O1P	21.5	15.5	46.0
O2P	20.9	13.0	46.1
O5'	22.5	13.8	44.4
C5'	23.2	12.6	44.6
C4'	23.8	12.0	43.3
O3'	25.3	10.2	43.9
C3'	25.2	11.5	43.3
O2'	24.9	10.3	41.2
C2'	25.5	11.4	41.8
C1'	24.8	12.7	41.3
O1'	23.8	13.0	42.2
N1	25.7	13.9	41.1
C6	25.6	15.0	41.9
C5	26.4	16.1	41.7
C4	27.4	16.0	40.7
N4	28.2	17.0	40.5
N3	27.6	14.9	40.0
C2	26.7	13.8	40.2
O2	26.8	12.8	39.5
14 ADENOSINE			
P	26.7	9.7	44.4
O1P	27.5	10.9	44.9
O2P	26.5	8.6	45.4
O5'	27.3	9.1	43.1
C5'	28.7	8.7	43.1
C4'	29.0	8.1	41.7
O3'	30.8	6.4	42.1
C3'	30.5	7.7	41.5
O2'	30.0	6.6	39.4
C2'	30.6	7.8	40.0
C1'	29.7	9.0	39.7
O1'	28.7	9.0	40.7
N9	30.4	10.3	39.7
C8	30.2	11.3	40.5
N7	31.0	12.3	40.3
C5	31.8	11.9	39.2
C6	32.8	12.5	38.4
N6	33.2	13.8	38.7
N1	33.3	11.8	37.4
C2	32.9	10.6	37.2
N3	31.9	9.9	37.8
C4	31.4	10.7	38.8
15 GUANOSINE			
P	31.7	6.4	43.4
O1P	31.3	7.5	44.3
O2P	31.8	5.1	43.9
O5'	33.1	6.8	42.7
C5'	33.3	6.5	41.3
C4'	34.6	7.2	40.7
O3'	36.4	6.6	42.2
C3'	35.6	7.7	41.8
O2'	37.1	8.3	40.1
C2'	36.2	8.8	41.1
C1'	35.1	9.5	40.3
O1'	34.1	8.4	40.0
N9	34.4	10.5	41.1
C8	33.3	10.4	42.0
N7	32.9	11.5	42.5
C5	33.8	12.5	42.0
C6	33.9	13.8	42.2
O6	33.2	14.6	43.0
N1	35.0	14.4	41.5
C2	35.9	13.7	40.7
N2	36.8	14.4	40.1
N3	35.8	12.4	40.5
C4	34.7	11.9	41.2
16 DIHYDROURIDINE			
P	37.0	6.8	43.7
O1P	38.1	7.7	43.7
O2P	35.8	7.1	44.6
O5'	37.5	5.3	44.0
C5'	38.2	4.6	43.0
C4'	39.2	3.7	43.6
O3'	40.8	2.0	42.7
C3'	39.4	2.4	42.8
O2'	38.9	0.0	43.4
C2'	38.6	1.4	43.6
C1'	38.8	1.8	45.0
O1'	38.8	3.2	44.9
N1	37.9	1.3	46.0
C6	37.0	0.2	45.7
O6	35.7	0.3	46.4
C5	36.0	0.5	47.9
C4	35.2	0.2	48.8
N3	37.1	1.2	48.2
C2	38.0	1.7	47.3
O2	38.9	2.5	47.7
17 DIHYDROURIDINE			
P	41.8	3.1	42.0
O1P	41.5	3.0	40.5
O2P	41.6	4.4	42.6
O5'	43.2	2.5	42.3
C5'	43.9	2.9	43.4
C4'	45.2	3.6	43.1
O3'	45.8	6.0	43.4
C3'	45.0	5.1	42.6
O2'	45.7	6.2	40.5
C2'	45.3	5.0	41.1
C1'	46.4	4.0	41.1
O1'	45.9	3.0	42.0
N1	46.7	3.4	39.9
C6	48.0	2.7	39.7
C5	48.4	2.7	38.3
C4	47.3	2.3	37.3
O4	47.5	1.8	36.2
N3	46.1	2.6	37.8
C2	45.7	3.2	39.0
O2	44.6	3.5	39.1
18 GUANOSINE			
P	45.4	6.3	44.9
O1P	44.0	6.0	45.1
O2P	46.4	5.7	45.8
O5'	45.6	7.9	44.9
C5'	44.7	8.7	44.0
C4'	45.5	9.8	43.3
O3'	46.6	10.0	41.1
C3'	46.5	9.2	42.3
O2'	49.0	9.1	42.4
C2'	47.8	9.2	43.1
C1'	47.7	10.5	43.9
O1'	46.3	10.5	44.3
N9	48.6	10.7	45.0
C8	49.0	9.8	46.0
N7	49.8	10.3	46.8
C5	50.0	11.6	46.5
C6	50.7	12.7	47.0
O6	51.5	12.6	48.0
N1	50.6	13.9	46.3
C2	49.8	14.0	45.2
N2	49.8	15.3	44.7
N3	49.1	13.1	44.7
C4	49.2	11.9	45.3
19 GUANOSINE			
P	45.9	9.7	39.7
O1P	46.4	8.4	39.2
O2P	44.5	9.9	39.9
O5'	46.5	10.9	38.9
C5'	46.1	12.2	38.9
C4'	46.9	13.2	38.1
O3'	47.0	14.1	35.8
C3'	46.8	12.9	36.6
O2'	48.4	11.6	35.2
C2'	47.9	11.9	36.5
C1'	49.0	12.5	37.4
O1'	48.3	13.2	38.4
N9	49.9	11.5	38.0
C8	49.8	10.2	38.3
N7	50.8	9.6	38.8
C5	51.7	10.7	39.0
C6	53.1	10.7	39.5
O6	53.7	9.8	40.0
N1	53.6	12.0	39.4
C2	53.0	13.1	38.9
N2	53.7	14.2	38.9
N3	51.8	13.1	38.4
C4	51.2	11.9	38.5
20 GUANOSINE			
P	45.7	15.0	35.6
O1P	44.9	14.8	36.8
O2P	46.0	16.4	35.3
O5'	45.1	14.3	34.3
C5'	45.5	14.9	33.0
C4'	44.4	14.6	32.1
O3'	43.1	16.7	31.8
C3'	43.1	15.4	32.4
O2'	42.0	14.5	30.4
C2'	42.0	14.5	31.8
C1'	42.6	13.1	32.2
O1'	44.0	13.2	32.3
N9	42.0	12.5	33.4
C8	42.7	12.1	34.5
N7	41.9	11.7	35.5
C5	40.6	11.8	35.0
C6	39.4	11.5	35.6
O6	39.1	11.1	36.7
N1	38.3	11.8	34.7
C2	38.5	12.4	33.4
N2	37.4	12.6	32.7
N3	39.7	12.6	32.9
C4	40.7	12.3	33.7
21 ADENOSINE			
P	43.2	17.9	32.8
O1P	44.5	17.9	33.4
O2P	42.8	19.2	32.0
O5'	42.1	17.6	33.8
C5'	40.7	18.1	33.7
C4'	39.7	17.1	34.1
O3'	38.3	16.6	32.2
C3'	38.3	17.2	33.5
O2'	37.5	15.1	34.4
C2'	37.5	16.5	34.5
C1'	38.2	16.9	35.8
O1'	39.6	17.1	35.5
N9	37.7	18.1	36.5
C8	38.1	19.4	36.3
N7	37.5	20.2	37.0
C5	36.6	19.5	37.7
C6	35.6	19.8	38.7

TABLE 2—*continued*

N6	35.3	21.0	39.1	N3	21.7	18.6	32.9	30 GUANOSINE				O5'	8.6	17.6	5.3	H20	8.2	8.7	11.7
N1	38.9	18.8	39.2	C2	20.6	17.9	33.1	P	20.5	25.5	13.7	C5'	8.2	18.5	4.3	C21	7.7	9.8	11.1
C2	35.1	17.5	38.8	O2	19.7	18.4	33.9	O1P	19.5	25.1	14.7	C4'	7.2	19.5	4.8	O22	7.1	10.6	11.8
N3	36.0	17.1	37.9					O2P	20.1	26.8	12.6	O3'	8.3	20.9	6.4	O23	7.7	9.9	9.8
C4	36.7	18.1	37.4	26 2,2DIM-GUANOSINE				O5'	21.2	24.2	13.1	C3'	7.3	19.9	6.2	C28M	6.7	10.7	9.1
				P	16.3	17.7	28.8	C5'	22.6	24.2	12.8	C2'M	4.4	22.3	6.3	C11	8.5	9.1	17.4
22 GUANOSINE				O1P	17.4	17.2	28.0	C4'	23.1	22.7	12.9	O2'	5.7	21.7	6.1	C11M	8.4	7.6	17.5
P	36.9	16.5	31.3	O2P	15.0	17.5	28.3	O3'	22.7	21.7	10.8	C2'	5.9	20.4	6.6	H12	8.1	10.0	18.4
O1P	36.3	17.8	31.3	O5'	16.6	19.2	29.3	C3'	22.4	21.7	12.1	C1'	5.1	19.4	5.8	C2	8.4	11.1	17.9
O2P	37.3	15.9	30.0	C5'	16.0	19.6	30.5	O2'	24.1	20.0	12.4	O1'	5.8	19.0	4.6	H3	8.1	12.3	18.5
O5'	36.0	15.5	32.2	C4'	16.6	21.0	31.0	C2'	22.8	20.8	12.8	H9	4.7	18.2	6.5	C3H	7.5	12.3	19.9
C5'	34.6	15.5	32.0	O3'	16.0	22.7	29.4	C1'	22.9	20.8	14.3	C8	5.1	16.8	6.3	C4	8.5	13.3	17.8
C4'	34.0	14.2	32.3	C3'	17.1	21.9	29.8	O1'	22.9	22.3	14.3	H7	4.6	16.0	7.1				
O3'	32.2	13.6	30.8	O2'	17.7	23.6	31.4	N9	21.8	20.4	15.1	C5	3.8	16.8	8.0	38 ADENOSINE			
C3'	32.5	14.0	32.1	C2'	18.2	22.6	30.5	C8	20.7	21.2	15.7	C6	3.1	16.4	9.2	P	11.3	16.3	22.4
O2'	32.5	11.7	32.8	C1'	18.9	21.5	31.4	N7	19.9	20.5	16.4	O6	2.9	15.3	9.6	O1P	12.3	16.6	21.3
C2'	32.2	13.0	33.2	O1'	17.8	20.7	31.8	C5	20.4	19.2	16.4	H1	2.5	17.5	9.8	O2P	11.6	16.8	23.7
C1'	33.1	13.4	34.3	N9	19.9	20.7	30.7	C6	19.9	18.0	17.0	C2	2.6	18.8	9.3	O5'	11.0	14.7	22.3
O1'	34.3	13.8	33.7	C8	19.8	19.5	30.2	O6	18.9	17.9	17.7	H2	2.0	19.7	10.0	C5'	12.1	13.9	22.7
N9	32.7	14.5	35.2	N7	20.9	19.0	29.6	N1	20.7	16.9	16.6	N3	3.3	19.2	8.3	C4'	11.7	12.4	22.7
C8	33.1	15.7	35.4	C5	21.8	20.1	29.8	C2	21.8	16.9	15.9	C4	3.9	18.1	7.7	O3'	13.4	10.8	23.4
N7	32.5	16.4	36.3	C6	23.2	20.2	29.4	N2	22.5	15.8	15.7					C3'	12.8	11.5	22.2
C5	31.5	15.6	36.7	O6	23.9	19.4	28.8	N3	22.3	18.1	15.3	35 ADENOSINE				O2'	11.3	9.6	22.2
C6	30.5	15.8	37.7	N1	23.7	21.5	29.8	C4	21.5	19.1	15.6	P	8.6	21.3	8.0	C2H	12.0	10.5	21.4
O6	30.2	16.8	38.3	C2	23.0	22.5	30.4					O1P	8.3	20.2	8.9	C1'	11.0	11.4	20.7
N1	29.7	14.6	37.8	N2	23.6	23.6	30.7	31 ADENOSINE				O2P	10.0	21.9	8.1	O1'	10.6	12.4	21.6
C2	29.8	13.4	37.1	C2M1	23.3	24.7	29.8	P	21.5	22.0	9.7	O5'	7.5	22.5	8.2	N9	11.6	12.1	19.5
N2	29.0	12.4	37.4	C2M2	24.6	23.7	31.8	O1P	21.1	23.4	9.7	C5'	7.7	23.4	9.2	C8	11.8	13.5	19.4
N3	30.8	13.2	36.2	N3	21.7	22.4	30.8	O2P	22.0	21.5	8.3	C4'	6.8	23.1	10.4	H7	12.3	13.8	18.2
C4	31.6	14.3	36.1	C4	21.2	21.1	30.4	O5'	20.4	21.1	10.2	O3'	8.1	23.5	12.4	C5	12.5	12.6	17.6
								C5'	19.9	20.0	9.3	C3'	7.4	22.5	11.7	C6	13.0	12.3	16.3
23 ADENOSINE				27 CYTIDINE				C4'	20.6	18.7	9.7	O2'	5.5	22.9	13.1	H6	13.4	13.2	15.4
P	30.8	14.2	30.1	P	15.9	23.0	27.8	O3'	19.7	16.8	8.5	C2'	6.3	21.9	12.4	H1	13.0	11.0	15.9
O1P	30.6	15.6	30.6	O1P	16.2	21.8	27.1	C3'	19.7	17.4	9.7	C1'	5.4	21.4	11.3	C2	12.5	10.0	16.8
O2P	30.9	14.1	28.7	O2P	14.5	23.5	27.6	O2'	21.5	15.9	10.4	O1'	5.9	22.0	10.1	H3	12.0	10.2	18.0
O5'	29.7	13.3	30.8	O5'	16.9	24.1	27.6	C2'	20.3	16.6	10.9	N9	5.5	19.9	11.1	C4	12.0	11.5	18.3
C5'	29.9	11.8	30.7	C5'	17.3	25.0	28.7	C1'	20.7	17.7	11.8	C8	6.1	19.2	10.1				
C4'	28.8	11.1	31.4	C4'	18.5	25.9	28.3	O1'	21.2	18.8	11.0	N7	6.0	17.9	10.2	39 PSEUDOURIDINE			
O3'	26.8	11.2	29.9	O3'	18.0	27.0	26.1	N9	19.7	18.2	12.8	C5	5.3	17.7	11.4	P	14.8	11.7	23.8
C3'	27.4	11.7	31.1	C3'	18.7	26.0	26.8	C8	19.2	19.4	13.0	C6	4.8	16.6	12.1	O1P	14.6	13.1	23.4
O2'	26.3	9.9	32.3	O2'	20.5	27.6	27.0	N7	18.3	19.5	13.9	N6	5.1	15.3	11.7	O2P	15.1	11.4	25.2
C2'	26.7	11.3	32.4	C2'	20.2	26.2	26.7	C5	18.1	18.2	14.2	N1	4.1	16.8	13.2	O5'	15.8	11.0	22.9
C1'	27.7	11.4	33.5	C1'	20.7	25.4	27.9	C6	17.2	17.6	15.2	C2	3.9	18.1	13.7	C5'	15.6	9.6	22.4
O1'	29.0	11.2	32.8	O1'	19.7	25.4	28.9	N6	16.3	18.3	15.9	N3	4.3	19.2	13.1	C4'	16.0	9.5	20.9
N9	27.8	12.7	34.1	N1	21.1	24.0	27.6	N1	17.3	16.2	15.3	C4	5.0	19.0	12.0	O3'	18.4	9.0	20.9
C8	28.7	13.8	33.9	C6	20.3	23.0	27.9	C2	18.1	15.6	14.6					C3'	17.4	9.9	20.5
N7	28.4	14.8	34.6	C5	20.6	21.7	27.6	N3	19.0	16.0	13.7	36 ADENOSINE				O2'	17.2	8.8	18.3
C5	27.3	14.5	35.3	C4	21.9	21.5	26.9	C4	18.9	17.3	13.6	P	9.5	23.1	13.1	C2'	17.2	10.1	19.0
C6	26.5	15.2	36.3	N4	22.3	20.3	26.6					O1P	10.6	23.2	12.1	C1'	15.8	10.7	18.9
N6	26.8	16.4	36.7	N3	22.7	22.5	26.6	32 2'OM-CYTIDINE				O2P	9.7	23.8	14.4	O1'	15.1	10.3	20.1
N1	25.5	14.5	36.8	C2	22.3	23.8	26.9	P	18.6	17.2	7.4	O5'	9.2	21.5	13.4	C5	15.8	12.1	18.7
C2	25.2	13.3	36.4	O2	23.0	24.8	26.7	O1P	18.1	18.5	7.6	C5'	9.7	21.0	14.6	C6	14.9	12.9	19.5
N3	25.8	12.5	35.6					O2P	19.1	16.9	6.0	C4'	8.5	20.6	15.5	H1	14.9	14.2	19.4
C4	26.9	13.2	35.0	28 CYTIDINE				O5'	17.4	16.1	7.7	O3'	9.4	20.3	17.8	C2	15.7	14.9	18.4
				P	18.0	27.1	24.6	C5'	17.9	14.8	8.2	C3'	8.8	19.7	16.7	O2	15.7	16.1	18.2
24 GUANOSINE				O1P	17.7	25.7	24.0	C4'	16.8	14.2	9.1	O2'	6.6	20.0	17.7	H3	16.5	14.1	17.6
P	26.1	12.2	28.9	O2P	17.1	28.2	24.1	O3'	14.8	13.9	7.7	C2'	7.4	19.1	17.0	C4	16.6	12.7	17.8
O1P	26.5	13.6	29.3	O5'	19.5	27.4	24.2	C3'	15.4	14.7	8.8	C1'	6.8	19.0	15.6	O4	17.3	12.0	17.1
O2P	26.5	11.9	27.5	C5'	19.7	28.6	23.4	C2'M	13.8	12.8	11.6	O1'	7.5	19.9	14.7				
O5'	24.6	12.0	29.2	C4'	21.0	28.5	22.6	O2'	14.4	13.2	10.4	N9	6.9	17.7	15.0	40 5M-CYTIDINE			
C5'	24.2	10.7	29.9	O3'	20.5	28.7	20.3	C2'	14.7	14.5	10.1	C8	7.5	17.3	13.8	P	19.5	9.3	22.0
C4'	23.0	11.0	30.8	C3'	20.9	27.8	21.3	C1'	15.8	15.0	11.1	N7	7.4	16.0	13.5	O1P	19.1	10.5	22.7
O3'	21.2	11.8	29.4	O2'	23.2	28.3	20.7	O1'	17.0	14.7	10.5	C5	6.7	15.5	14.6	O2P	19.7	8.1	22.8
C3'	22.1	12.2	30.4	C2'	22.3	27.2	21.1	N1	15.7	16.4	11.4	C6	6.2	14.2	14.9	O5'	20.7	9.7	21.0
O2'	20.4	11.6	32.1	C1'	22.7	26.8	22.5	C6	16.5	17.4	10.7	N6	6.5	13.2	14.1	C5'	21.2	8.6	20.2
C2'	21.5	12.6	31.7	O1'	21.9	27.5	23.4	C5	16.4	18.7	11.0	N1	5.5	14.1	16.0	C4'	21.7	9.2	18.8
C1'	22.6	12.3	32.7	N1	22.4	25.3	22.8	C4	15.6	19.1	12.0	C2	5.2	15.1	16.7	O3'	24.1	9.6	19.1
O1'	23.5	11.3	32.1	C6	21.3	24.9	23.5	N4	15.5	20.4	12.4	N3	5.6	16.4	16.6	C3'	22.8	10.2	18.9
N9	23.4	13.5	33.0	C5	21.1	23.6	23.7	N3	14.8	18.2	12.7	C4	6.3	16.5	15.5	O2'	23.2	10.1	16.5
C8	24.7	13.9	32.6	C4	22.1	22.7	23.2	C2	14.9	16.9	12.4					C2'	22.7	10.9	17.6
N7	25.1	15.0	33.0	N4	21.9	21.4	23.4	O2	14.2	16.0	13.0					C1'	21.2	11.0	17.4
C5	24.0	15.5	33.8	N3	23.1	23.1	22.5					37 Y				O1'	20.6	9.9	18.1
C6	23.9	16.7	34.5	C2	23.3	24.4	22.3	33 URIDINE				P	10.6	19.6	18.6	N1	20.5	12.2	17.9
C6	24.7	17.6	34.7	O2	24.3	24.8	21.7	P	14.2	14.7	6.5	O1P	11.1	18.5	17.8	C6	19.7	12.2	19.0
N1	22.7	16.8	35.2					O1P	15.1	15.7	6.0	O2P	11.5	20.6	19.0	C5	19.1	13.4	19.4
C2	21.7	15.8	35.1	29 ADENOSINE				O2P	13.8	13.7	5.5	O5'	9.7	19.0	19.8	C4	19.4	14.6	18.7
N2	20.6	16.1	35.8	P	19.3	28.2	19.3	O5'	12.9	15.4	7.1	C5'	8.3	18.7	19.6	N4	18.9	15.7	19.1
N3	21.8	14.7	34.4	O1P	18.3	27.5	20.2	C5'	11.6	15.0	6.7	C4'	8.1	17.3	20.3	H4	20.2	14.6	17.6
C4	23.0	14.6	33.8	O2P	18.8	29.3	18.5	C4'	10.5	15.1	7.8	O3'	9.9	16.9	21.9	C2	20.8	13.4	17.2
				C5'	20.0	27.1	18.4	O3'	8.4	15.6	6.7	C3'	9.3	16.5	20.7	O2	21.5	13.3	16.2
25 CYTIDINE				C5'	20.5	27.5	17.1	C3'	9.4	16.1	7.6	O2'	8.0	14.9	21.9	C5M	18.3	13.3	20.6
P	19.8	12.5	29.2	C4'	21.8	26.9	16.8	O2'	8.2	15.2	9.5	C2'	8.8	15.1	20.7				
O1P	19.4	12.6	27.8	O3'	21.8	26.1	14.5	C2'	8.9	16.3	9.0	C1'	7.8	15.1	19.5				
O2P	18.8	11.9	30.2	C3'	21.8	25.7	15.8	C1'	10.2	16.3	9.8	O1'	7.3	16.4	19.4	41 URIDINE			
O5'	20.1	14.0	29.7	O2'	24.2	25.7	15.7	O1'	11.2	15.6	9.0	N9	8.4	14.7	18.3	P	25.1	10.3	20.1
C5'	19.1	15.1	29.4	C2'	23.1	25.0	16.2	N1	10.8	17.7	10.1	C8	9.0	15.4	17.2	O1P	24.4	10.8	21.3
C4'	18.2	15.3	30.6	C1'	23.2	25.2	17.7	C6	11.8	18.2	9.3	N7	9.4	14.7	16.2	O2P	26.2	9.3	20.4
O3'	16.4	16.9	30.3	O1'	22.5	26.5	18.0	C5	12.3	19.4	9.5	C5	9.2	13.4	16.6	O5'	25.7	11.5	19.3
C3'	17.7	16.7	30.4	N9	22.5	24.2	18.5	C4	11.8	20.2	10.6	C6	9.4	12.2	15.9	C5'	25.7	11.4	17.8
O2'	16.7	16.3	32.9	C8	21.5	24.3	19.4	O4	12.2	21.4	10.9	O6	10.0	12.0	14.8	C4'	26.4	12.6	17.2
C2'	17.8	16.9	32.1	N7	21.1	23.1	19.9	N3	10.7	19.6	11.3	C10	9.1	9.7	16.4	O3'	28.4	13.1	18.5
C1'	19.0	16.1	32.6	C5	21.9	22.2	19.3	C2	10.2	18.4	11.1	C13	9.7	9.1	15.1	C3'	27.0	13.6	18.2
O1'	19.0	14.9	31.8	C6	22.0	20.8	19.4	O2	9.3	18.0	11.8	C14	9.0	9.7	13.9	O2'	27.9	14.9	16.4
N1	20.3	16.7	32.4	N6	21.2	20.1	20.2					C15	8.2	8.5	13.2	C2'	27.0	14.9	17.4
C6	21.3	16.2	31.6	N1	22.9	20.2	18.6	34 2'OM-GUANOSINE				C16	6.7	8.6	13.6	C1'	25.6	14.8	16.8
C5	22.5	16.9	31.4	C2	23.7	20.9	17.8	P	8.4	16.0	5.2	O17	6.3	9.6	14.2	O1'	25.2	13.4	16.7
C4	22.7	18.1	32.1	N3	23.7	22.2	17.6	O1P	9.6	15.5	4.5	O18	5.9	7.5	13.5	N1	24.5	15.5	17.6
N4	23.8	18.7	31.9	C4	22.8	22.8	18.4	O2P	7.1	15.7	4.6	C19M	6.0	6.4	14.3	C6	23.7	14.7	18.4
																C5	22.8	15.3	19.2

TABLE 2—*continued*

C4	22.6	16.8	19.2
O4	21.7	17.4	19.8
N3	23.5	17.4	18.3
C2	24.5	16.9	17.6
O2	25.2	17.5	16.9

42 GUANOSINE

P	28.9	13.7	19.9
O1P	27.8	13.5	20.9
O2P	30.2	13.0	20.3
O5'	29.1	15.2	19.6
C5'	30.1	15.6	18.6
C4'	30.1	17.1	18.5
O3'	31.9	17.9	19.9
C3'	30.5	17.9	19.8
O2'	30.6	20.0	18.6
C2'	29.8	19.2	19.5
C1'	28.5	18.8	18.8
O1'	28.8	17.6	18.2
N9	27.4	18.6	19.8
C8	26.9	17.5	20.3
N7	25.9	17.7	21.1
C5	25.7	19.1	21.1
C6	24.7	19.9	21.7
O6	23.8	19.5	22.5
N1	24.9	21.2	21.4
C2	25.8	21.7	20.6
N2	25.8	23.0	20.4
N3	26.7	21.0	19.9
C4	26.6	19.7	20.2

43 GUANOSINE

P	32.4	18.5	21.3
O1P	32.0	17.7	22.5
O2P	33.9	18.8	21.2
O5'	31.7	19.9	21.4
C5'	32.4	21.1	21.1
C4'	31.6	22.4	21.3
O3'	32.6	23.7	23.0
C3'	31.5	23.0	22.7
O2'	30.4	25.0	21.9
C2'	30.2	23.8	22.6
C1'	29.3	22.9	21.8
O1'	30.1	22.1	20.9
N9	28.4	22.0	22.6
C8	28.5	20.6	22.7
N7	27.6	20.1	23.5
C5	26.9	21.2	23.9
C6	25.7	21.3	24.8
O6	25.2	20.4	25.3
N1	25.3	22.6	24.9
C2	25.8	23.7	24.3
N2	25.3	24.9	24.5
N3	26.9	23.6	23.5
C4	27.4	22.4	23.3

44 ADENOSINE

P	33.4	23.4	24.4
O1P	32.8	22.1	24.9
O2P	34.8	23.4	24.2
O5'	32.9	24.6	25.3
C5'	32.8	25.9	24.8
C4'	31.6	26.7	25.4
O3'	32.4	27.7	27.4
C3'	31.6	26.7	26.9
O2'	29.8	28.4	26.8
C2'	30.1	27.0	27.1
C1'	29.4	26.2	26.1
O1'	30.4	26.0	25.0
N9	29.0	24.8	26.5
C8	29.6	23.6	26.3
N7	28.9	22.6	26.8
C5	27.9	23.2	27.5
C6	26.8	22.7	28.3
N6	26.7	21.4	28.6
N1	25.9	23.6	28.8
C2	26.1	24.9	28.5
N3	27.0	25.5	27.8
C4	27.9	24.6	27.3

45 GUANOSINE

P	33.2	27.4	28.7
O1P	34.1	26.2	28.5
O2P	33.9	28.6	29.2
O5'	32.1	27.0	29.7
C5'	31.5	28.1	30.5
C4'	30.4	27.6	31.4
O3'	31.2	27.4	33.6
C3'	30.8	26.7	32.5
O2'	28.6	26.5	33.4
C2'	29.6	25.8	32.8
C1'	29.1	25.6	31.3
O1'	29.6	26.6	30.5
N9	29.6	24.3	30.7
C8	30.6	24.1	29.8
N7	30.8	22.8	29.5
C5	29.8	22.2	30.2
C6	29.6	20.8	30.3
O6	30.1	19.8	29.7
N1	28.5	20.5	31.2
C2	27.8	21.5	31.9
N2	26.9	21.0	32.7
N3	28.1	22.8	31.9
C4	29.1	23.0	31.0

46 7M-GUANOSINE

P	32.8	27.8	33.9
O1P	33.4	28.2	32.6
O2P	32.9	28.8	35.0
O5'	33.4	26.4	34.3
C5'	34.1	26.4	35.5
C4'	33.2	26.4	36.7
O3'	33.1	26.5	39.2
C3'	34.0	26.8	38.1
O2'	35.4	24.6	39.2
C2'	34.7	25.0	38.0
C1'	33.6	24.1	37.5
O1'	32.8	24.9	36.8
N9	34.1	23.0	36.7
C8	35.0	22.9	35.7
N7	35.2	21.8	35.2
C5	34.2	21.0	35.8
C6	33.9	19.6	35.6
O6	34.4	18.8	34.8
N1	32.8	19.2	36.5
C2	32.2	20.0	37.8
N2	31.2	19.5	38.1
N3	32.5	21.3	37.5
C4	33.5	21.7	36.7
C7M	36.1	21.4	34.1

47 URIDINE

P	33.0	27.9	39.9
O1P	33.2	29.0	38.9
O2P	31.7	27.9	40.6
O5'	34.2	27.9	40.9
C5'	35.5	28.3	40.5
C4'	36.5	27.9	41.5
O3'	37.5	25.8	42.4
C3'	36.3	26.6	42.2
O2'	35.5	26.1	44.5
C2'	35.6	27.0	43.5
C1'	36.3	28.3	43.8
O1'	36.5	28.9	42.6
N1	35.7	29.2	44.8
C6	34.3	29.4	44.7
C5	33.7	30.2	45.6
C4	34.5	30.9	46.7
O4	34.1	31.6	47.5
N3	35.9	30.5	46.7
C2	36.5	29.7	45.8
O2	37.7	29.5	45.8

48 CYTIDINE

P	37.6	24.3	42.0
O1P	37.4	24.1	40.6
O2P	38.8	23.7	42.7
O5'	36.2	23.7	42.7
C5'	36.4	22.9	43.9
C4'	35.5	21.7	43.9
O3'	34.6	20.1	45.6
C3'	35.8	20.7	45.1
O2'	36.9	18.5	45.1
C2'	36.7	19.7	44.4
C1'	36.1	19.6	43.1
O1'	35.8	20.9	42.7
N1	37.0	19.0	42.0
C6	38.0	19.7	41.5
C5	38.9	19.2	40.6
C4	38.6	17.9	40.2
N4	39.3	17.3	39.2
N3	37.6	17.2	40.7
C2	36.8	17.7	41.6
O2	35.8	17.1	42.1

49 5M-CYTIDINE

P	34.4	19.9	47.2
O1P	33.1	19.2	47.4
O2P	35.6	19.3	47.7
O5'	34.3	21.4	47.7
C5'	33.1	22.2	47.4
C4'	33.2	23.5	48.2
O3'	35.2	24.8	47.6
C3'	34.6	24.0	48.6
O2'	33.7	26.0	49.6
C2'	34.3	24.7	49.9
C1'	33.2	23.9	50.5
O1'	32.5	23.3	49.5
N1	33.7	22.8	51.5
C6	33.7	21.5	51.1
C5	34.1	20.6	51.9
C4	34.6	21.0	53.2
N4	35.1	20.1	54.1
N3	34.6	22.3	53.6
C2	34.1	23.2	52.7
O2	34.1	24.4	53.0
C5M	34.1	19.1	51.5

50 URIDINE

P	36.7	25.0	47.7
O1P	37.4	23.7	47.8
O2P	37.2	25.8	46.5
O5'	36.9	25.8	49.0
C5'	37.9	26.9	49.1
C4'	37.8	27.6	50.4
O3'	39.9	28.7	50.8
C3'	39.1	27.7	51.2
O2'	38.2	29.2	52.9
C2'	38.6	27.8	52.7
C1'	37.3	27.0	52.6
O1'	36.8	27.0	51.3
N1	37.5	25.6	53.0
C6	37.0	24.6	52.2
C5	37.2	23.3	52.5
C4	37.9	22.9	53.7
O4	38.1	21.8	54.1
N3	38.3	24.0	54.5
C2	38.1	25.3	54.2
O2	38.6	26.2	54.9

51 GUANOSINE

P	41.1	28.4	49.8
O1P	41.0	27.0	49.4
O2P	41.1	29.4	48.7
O5'	42.4	28.6	50.7
C5'	42.3	29.6	51.7
C4'	42.7	29.0	53.1
O3'	43.1	29.1	53.1
C3'	44.0	28.3	53.2
O2'	44.1	28.4	55.6
C2'	43.9	27.5	54.5
C1'	42.4	27.2	54.5
O1'	41.7	28.0	53.5
N9	42.1	25.8	54.3
C8	41.4	25.2	53.2
N7	41.3	23.9	53.3
C5	41.9	23.6	54.5
C6	42.1	22.3	55.1
O6	41.7	21.2	54.8
N1	42.8	22.5	56.4
C2	43.2	23.7	56.9
N2	43.8	23.6	58.1
N3	43.0	24.8	56.3
C4	42.4	24.7	55.1

52 URIDINE

P	46.4	28.6	52.2
O1P	46.0	27.9	51.0
O2P	47.3	29.8	52.0
O5'	47.0	27.6	53.2
C5'	47.4	28.2	54.2
C4'	48.2	27.2	55.4
O3'	50.5	26.5	55.1
C3'	49.2	26.1	55.0
O2'	49.3	25.4	57.3
C2'	48.8	25.0	56.0
C1'	47.3	25.1	56.1
O1'	46.9	26.4	55.7
N1	46.6	24.1	55.2
C6	45.9	24.6	54.1
C5	45.3	23.7	53.3
C4	45.3	22.3	53.6
O4	44.8	21.4	52.9
N3	46.0	22.0	54.7
C2	46.7	22.8	55.5
O2	47.3	22.4	56.5

53 GUANOSINE

P	51.4	26.1	53.7
O1P	50.5	26.2	52.5
O2P	52.6	26.9	53.7
O5'	51.7	24.6	54.0
C5'	52.5	24.3	55.2
C4'	52.1	22.9	55.7
O3'	54.0	21.5	55.3
C3'	52.7	21.7	55.0
O2'	52.1	20.2	56.7
C2'	51.7	20.6	55.4
C1'	50.4	21.3	55.5
O1'	50.7	22.7	55.6
N9	49.5	21.1	54.3
C8	49.0	22.0	53.4
N7	48.3	21.5	52.5
C5	48.3	20.1	52.8
C6	47.6	19.1	52.1
O6	46.9	19.1	51.1
N1	47.9	17.8	52.8
C2	48.6	17.7	53.9
N2	48.7	16.4	54.4
N3	49.3	18.7	54.5
C4	49.0	19.9	53.9

54 RIBOSYLTHYMINE

P	54.9	20.8	54.1
O1P	54.8	21.5	52.9
O2P	56.3	20.7	54.6
O5'	54.3	19.3	54.0
C5'	54.5	18.3	55.0
C4'	54.2	16.9	54.4
O3'	56.2	16.2	53.1
C3'	54.8	16.6	53.0
O2'	54.1	14.3	53.1
C2'	53.9	15.5	52.5
C1'	52.5	16.0	53.0
O1'	52.8	16.9	54.1
N1	51.7	16.8	52.0
C6	51.9	18.1	51.9
C5	51.2	18.8	50.9
C4	50.3	18.1	50.0
O4	49.6	18.7	49.2
N3	50.2	16.8	50.2
C2	50.9	16.0	51.2
O2	50.8	14.8	51.2
C5M	51.4	20.3	50.9

55 PSEUDOURIDINE

P	57.1	16.4	51.8
O1P	57.3	17.8	51.5
O2P	58.3	15.6	52.0
O5'	56.1	15.7	50.7
C5'	56.3	14.3	50.4
C4'	55.7	14.0	49.1
O3'	57.5	14.1	47.4
C3'	56.3	14.8	47.9
O2'	55.1	13.5	46.3
C2'	55.2	14.8	46.9
C1'	54.0	15.0	47.8
O1'	54.3	14.5	49.0
C5	53.6	16.4	47.9
C6	54.1	17.2	49.0
N1	53.8	18.5	49.1
C2	52.9	19.1	48.2
O2	52.5	20.3	48.3
N3	52.4	18.3	47.2
C4	52.8	17.0	47.0
O4	52.3	16.4	46.1

56 CYTIDINE

P	58.8	14.9	47.0
O1P	58.9	16.2	47.9
O2P	60.0	14.1	47.0
O5'	58.4	15.4	45.5
C5'	58.6	16.7	45.2
C4'	58.3	17.0	43.7
O3'	56.5	18.6	43.6
C3'	56.9	17.2	43.4
O2'	57.4	17.8	41.1
C2'	56.8	16.8	41.9
C1'	57.7	15.6	41.9
O1'	58.6	15.7	43.0
N1	57.0	14.3	42.0
C6	57.2	13.5	43.1
C5	56.5	12.3	43.2
C4	55.7	12.0	42.1
N4	55.0	10.8	42.1
N3	55.5	12.7	41.0
C2	56.2	13.9	41.0
O2	56.0	14.7	40.0

57 GUANOSINE

P	55.6	18.9	44.8
O1P	55.7	18.0	45.9
O2P	55.8	20.4	45.2
O5'	54.1	18.7	44.2
C5'	54.0	17.7	43.2
C4'	52.9	18.0	42.2
O3'	50.9	19.2	42.8
C3'	51.5	17.9	42.7
O2'	50.4	17.7	40.6
C2'	50.8	17.0	41.7
C1'	52.0	16.1	41.2
O1'	53.1	17.0	41.1
N9	52.3	15.0	42.2
C8	53.2	15.0	43.2
N7	53.2	13.8	43.8
C5	52.3	13.1	43.2
C6	51.9	11.7	43.5
O6	52.3	11.0	44.3
N1	50.9	11.3	42.6
C2	50.3	12.1	41.6
N2	49.4	11.6	40.9
N3	50.7	13.4	41.3
C4	51.7	13.8	42.2

58 1M-ADENOSINE

P	50.0	19.6	44.0
O1P	50.8	19.4	45.3
O2P	49.4	20.9	43.8
O5'	48.9	18.4	44.0
C5'	47.8	18.5	43.1
C4'	46.6	17.7	43.5
O3'	46.3	18.2	44.0
C3'	45.6	18.5	44.3
O2'	45.0	18.3	46.7
C2'	46.0	18.1	45.7
C1'	46.3	16.6	45.5
O1'	47.1	16.6	44.3
N9	47.1	16.0	46.6
C8	48.1	16.5	47.4
N7	48.6	15.7	48.2
C5	47.9	14.5	48.0
C6	48.0	13.2	48.6
N6	48.8	12.9	49.6
N1	47.2	12.3	48.1
C2	46.3	12.6	47.1
N3	46.2	13.7	46.5
C4	47.0	14.6	47.0
C1M	47.2	10.9	48.6

59 URIDINE

P	43.5	19.0	42.8
O1P	44.2	20.2	42.5
O2P	43.3	18.1	41.7
O5'	42.1	19.3	43.5
C5'	41.0	18.6	43.2
C4'	40.2	18.1	44.4
O3'	41.5	17.6	46.4
C3'	40.9	17.1	45.3
O2'	39.0	16.6	46.7
C2'	39.8	16.1	45.6
C1'	38.9	16.2	44.3
O1'	38.9	17.5	43.9
N1	39.4	15.3	43.2
C6	40.2	15.8	42.3
C5	40.6	15.0	41.3
C4	40.2	13.7	41.1
O4	40.5	12.9	40.2
N3	39.3	13.3	42.1
C2	38.9	14.0	43.2
O2	38.1	13.6	44.0

60 CYTIDINE

P	42.2	16.8	47.5
O1P	43.5	16.3	47.0
O2P	42.3	17.6	48.8
O5'	41.2	15.5	47.7
C5'	41.4	14.7	48.9
C4'	41.1	13.3	48.6
O3'	41.7	11.1	49.6
C3'	42.2	12.3	49.1
O2'	43.9	11.1	47.8
C2'	43.0	12.2	47.8
C1'	41.9	12.1	46.7
O1'	41.0	13.0	47.1
N1	42.4	12.4	45.4
C6	42.9	13.6	45.1
C5	43.3	13.9	43.8
C4	43.2	12.9	42.8
N4	43.5	13.1	41.5
N3	42.6	11.7	43.1
C2	42.2	11.4	44.4
O2	41.7	10.4	44.7

61 CYTIDINE

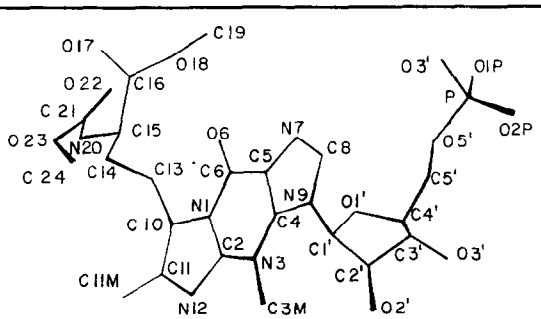
P	41.1	11.0	51.1
O1P	41.1	12.4	51.6
O2P	39.9	10.2	51.1
O5'	42.3	10.2	51.8
C5'	43.6	10.0	51.2
C4'	44.7	10.2	52.2
O3'	43.9	10.4	54.5
C3'	44.4	11.1	53.4
O2'	46.6	10.7	54.4
C2'	45.8	11.7	53.7
C1'	46.4	11.8	52.3
O1'	45.8	10.8	51.5
N1	46.1	13.1	51.6
C6	45.3	13.2	50.5
C5	45.1	14.4	49.9
C4	45.6	15.5	50.5
N4	45.4	16.7	49.9
N3	46.4	15.4	51.6
C2	46.6	14.2	52.2
O2	47.4	14.1	53.2

62 ADENOSINE

P	43.3	11.2	55.8
O1P	42.2	12.2	55.3
O2P	42.8	10.2	56.8
O5'	44.5	11.9	56.4
C5'	45.2	11.3	57.5
C4'	46.1	12.5	58.1
O3'	45.0	13.1	60.2
C3'	45.3	13.5	58.8
O2'	47.3	14.6	59.7
C2'	46.2	14.7	58.7
C1'	46.8	14.6	57.3
O1'	46.7	13.2	57.0
N9	46.1	15.3	56.3
C8	45.3	14.9	55.3

TABLE 2—*continued*

N7	44.8	15.8	54.6	O1'	37.7	25.1	61.4	C8'	22.2	23.0	55.4	O3'	17.8	9.4	68.2	O2P	18.3	15.5	80.0
C5	45.4	17.0	55.1	N9	36.9	24.1	59.5	O3'	20.1	22.1	56.2	C3'	18.7	10.1	67.3	O5'	18.8	17.1	78.0
C6	45.3	18.3	54.7	C8	37.2	22.8	60.0	C3'	21.5	22.0	56.3	O2'	20.5	8.7	68.0	C5'	19.4	17.9	79.0
N6	44.5	18.8	53.7	N7	36.9	21.8	59.2	O2'	21.5	20.3	54.5	C2'	20.2	10.0	67.6	C4'	19.6	19.3	78.4
N1	46.0	19.2	55.4	C5	36.4	22.5	58.0	C2'	22.0	20.7	55.8	C1'	20.8	10.3	66.3	O3'	18.2	20.9	79.7
C2	46.7	18.8	56.4	C6	35.9	22.0	56.8	C1'	23.5	21.0	55.6	O1'	20.0	9.9	65.3	C3'	18.4	20.2	78.5
N3	46.9	17.6	56.9	O6	35.8	20.8	56.4	O1'	23.6	22.4	55.5	N9	21.2	11.7	66.1	O2'	19.6	22.1	77.7
C4	46.2	16.7	56.1	N1	35.5	23.0	55.9	N1	24.4	20.5	56.6	C8	20.6	12.6	65.2	C2'	18.7	21.2	77.3
				C2	35.6	24.4	56.3	C6	24.9	21.4	57.6	N7	21.1	13.8	65.3	C1'	19.3	20.3	76.3
63	CYTIDINE			N2	35.2	25.2	55.3	C5	25.8	21.0	58.5	C5	22.1	13.7	66.3	O1'	19.9	19.2	77.0
P	43.6	13.5	60.8	N3	36.0	24.9	57.4	C4	26.1	19.6	58.6	C6	23.0	14.7	66.8	N1	18.4	19.7	75.3
O1P	42.5	13.4	59.9	C4	36.4	23.9	58.2	O4	26.9	19.1	59.5	O6	23.1	15.9	66.5	C6	18.0	18.4	75.3
O2P	43.4	12.8	62.1					N3	25.6	18.8	57.7	N1	23.8	16.2	67.8	C5	17.2	17.8	74.4
O5'	43.8	15.1	61.1	66	ADENOSINE			C2	24.7	19.2	56.7	C2	23.8	12.9	68.2	C4	16.6	18.7	73.4
C5'	44.8	15.4	62.1	P	33.3	26.0	63.1	O2	24.3	18.4	55.9	N2	24.7	12.6	69.1	N4	15.8	18.3	72.5
C4'	45.0	17.0	62.1	O1P	33.4	24.5	63.3					N3	23.0	11.9	67.7	N3	16.9	20.0	73.4
O3'	43.3	17.7	63.7	O2P	32.8	26.8	64.2	69	URIDINE			C4	22.1	12.5	66.8	C2	17.8	20.5	74.4
C3'	43.7	17.7	62.3	O5'	32.3	26.2	61.8	P	19.2	21.4	57.4					O2	18.1	21.7	74.4
O2'	44.9	19.8	62.6	C5'	32.3	27.6	61.3	O1P	19.5	22.1	58.7	72	CYTIDINE						
C2'	44.0	19.1	61.8	C4'	32.0	27.6	59.8	O2P	17.8	21.4	57.0	P	16.9	10.4	69.1	75	CYTIDINE		
C1'	44.8	18.7	60.5	O3'	29.6	27.7	59.6	O5'	19.8	19.9	57.4	O1P	16.6	11.7	68.4	P	17.1	20.4	80.7
O1'	45.3	17.4	60.7	C3'	30.7	26.9	59.4	C5'	19.4	19.0	56.4	O2P	15.7	9.7	69.7	O1P	17.1	18.9	80.8
N1	44.0	18.7	59.3	O2'	30.9	27.8	57.1	C4'	19.8	17.6	56.8	O5'	17.9	10.8	70.3	O2P	17.3	21.1	82.0
C6	43.5	17.6	58.8	C2'	30.9	26.6	57.9	O3'	17.9	16.7	58.2	C5'	18.8	9.6	70.6	O5'	15.8	20.9	80.0
C5	42.8	17.6	57.6	C1'	32.4	26.2	57.9	C3'	19.3	17.1	58.2	C4'	20.1	10.1	71.4	C5'	15.3	22.2	80.3
C4	42.6	18.8	57.0	O1'	33.0	26.7	59.1	O2'	19.9	14.8	57.8	O3'	19.4	10.5	73.7	C4'	14.6	22.9	79.2
N4	42.0	18.9	55.8	N9	32.6	24.7	58.0	C2'	20.3	16.0	58.5	C3'	19.8	11.1	72.5	O3'	12.3	23.3	80.0
N3	43.2	20.0	57.4	C8	33.2	23.9	59.0	C1'	21.6	16.5	57.9	O2'	22.1	10.8	73.3	C3'	13.1	22.5	79.1
C2	43.9	19.9	58.6	N7	33.2	22.7	58.7	O1'	21.2	17.4	56.8	C2'	21.2	11.7	72.7	O2'	12.5	24.1	77.3
O2	44.4	20.9	59.1	C5	32.7	22.6	57.5	N1	22.5	17.1	58.8	C1'	21.7	11.8	71.2	C2'	12.8	22.8	77.6
				C6	32.4	21.5	56.6	C6	22.5	18.5	58.9	O1'	20.9	10.8	70.5	C1'	14.1	22.3	76.9
64	ADENOSINE			N6	32.7	20.2	57.0	C5	23.3	19.1	59.8	N1	21.5	13.1	70.6	O1'	15.1	22.5	77.9
P	41.8	18.1	68.0	N1	31.9	21.7	55.4	C4	24.3	18.4	60.6	C6	20.5	13.4	69.8	N1	14.1	20.8	76.6
O1P	40.9	17.1	63.3	C2	31.5	23.0	55.1	O4	25.1	18.8	61.4	C5	20.3	14.6	69.2	C6	14.7	19.9	77.3
O2P	41.6	18.1	65.6	N3	31.7	24.1	55.8	N3	24.2	17.0	60.4	C4	21.2	15.6	69.6	C5	14.7	18.6	77.0
O5'	41.6	19.6	63.4	C4	32.3	23.8	57.0	C2	23.3	16.3	59.5	N4	21.1	16.9	69.1	C4	13.9	18.2	75.9
C5'	41.8	20.6	64.3					O2	23.3	15.1	59.5	N3	22.2	15.4	70.5	N4	13.9	16.9	75.5
C4'	41.8	22.0	63.6	67	ADENOSINE							C2	22.4	14.1	71.0	N3	13.3	19.1	75.1
O3'	39.8	23.1	64.5	P	28.4	27.0	60.4	70	CYTIDINE			O2	23.3	13.9	71.8	C2	13.3	20.4	75.5
C3'	40.4	22.5	63.3	O1P	29.0	25.9	61.3	P	17.2	16.1	59.4					O2	12.7	21.3	74.8
O2'	41.2	24.7	62.7	O2P	27.6	28.0	61.1	O1P	17.2	17.0	60.6	73	ADENOSINE						
C2'	40.6	23.5	62.2	O5'	27.6	26.4	59.3	O2P	15.9	15.6	59.0	P	18.3	11.3	74.6	76	ADENOSINE		
C1'	41.7	22.8	61.4	C5'	26.9	27.3	58.4	O5'	18.2	14.8	59.8	O1P	17.2	11.7	73.8	P	12.4	23.0	81.5
O1'	42.3	21.9	62.3	C4'	26.7	26.7	57.0	C5'	17.5	13.5	59.8	O2P	18.0	10.5	75.8	O1P	11.0	23.1	82.1
N9	41.2	22.1	60.2	O3'	24.3	26.0	56.8	C4'	18.4	12.5	60.5	O5'	19.2	12.6	75.0	O2P	13.1	21.8	81.8
C8	41.2	20.7	60.0	C3'	25.7	25.5	56.9	O3'	17.2	11.6	62.4	C5'	20.4	12.4	75.8	O5'	13.2	24.3	82.0
N7	40.7	20.4	58.8	O2'	25.8	25.4	54.5	C3'	18.3	12.3	62.0	C4'	21.0	13.7	76.2	C5'	14.6	24.1	82.4
C5	40.3	21.6	58.3	C2'	26.1	24.7	55.7	O2'	19.6	10.3	61.9	O3'	19.6	14.7	76.0	C4'	15.3	25.4	82.6
C6	39.6	21.9	57.1	C1'	27.7	24.8	55.9	C2'	19.7	11.6	62.3	C3'	20.0	14.8	76.6	O3'	17.7	25.1	82.8
N6	39.3	21.0	56.2	O1'	28.0	26.0	56.6	C1'	20.6	12.3	61.3	O2'	21.8	16.3	77.2	C3'	16.5	25.4	83.5
N1	39.4	23.2	56.9	N9	28.2	23.7	56.7	O1'	19.8	12.8	60.2	C2'	20.8	16.1	76.2	O2'	16.9	27.7	83.3
C2	39.7	24.1	57.8	C8	28.8	23.7	57.9	N1	21.3	13.5	61.9	C1'	21.5	15.7	75.0	C2'	16.5	26.7	84.2
N3	40.3	24.0	59.0	N7	29.2	22.6	58.8	C6	21.0	14.8	61.6	O1'	21.6	14.2	75.0	C1'	15.0	27.0	84.3
C4	40.6	22.7	59.1	C5	28.9	21.7	57.3	C5	21.7	15.8	62.2	N9	20.8	16.0	73.7	O1'	14.3	26.3	83.3
				C6	29.0	20.3	57.2	C4	22.8	15.5	63.0	C8	20.1	15.2	72.9	N9	14.4	26.4	85.6
65	GUANOSINE			N6	29.6	19.5	58.1	N4	23.5	16.5	63.6	N7	19.6	15.9	71.9	C8	14.7	26.8	86.9
P	38.2	22.7	64.7	N1	28.6	19.8	56.0	N3	23.1	14.2	63.3	C5	20.0	17.2	72.0	N7	14.0	26.1	87.8
O1P	38.0	21.2	64.4	C2	28.0	20.5	55.1	C2	22.4	13.2	62.8	C6	19.7	18.4	71.3	C5	13.2	25.3	87.1
O2P	37.7	23.2	66.0	N3	27.8	21.9	55.2	O2	22.7	12.0	63.0	N6	19.0	18.4	70.2	C6	12.3	24.3	87.4
O5'	37.5	23.5	63.5	C4	28.3	22.4	56.3					N1	20.3	19.5	71.8	N6	12.0	24.1	88.7
C5'	37.0	24.8	63.8					71	GUANOSINE			C2	21.0	19.5	72.9	N1	11.7	23.6	86.5
C4'	36.9	25.7	62.5	68	URIDINE			P	16.5	11.9	63.8	N3	21.3	18.4	73.7	C2	12.0	23.9	85.2
O3'	34.7	26.6	62.6	P	23.4	25.6	58.0	O1P	16.5	13.4	63.9	C4	20.7	17.3	73.2	N3	12.8	24.8	84.7
C3'	35.5	25.7	61.9	O1P	24.1	25.1	59.2	O2P	15.2	11.3	63.9					C4	13.4	25.5	85.7
O2'	36.1	27.5	60.4	O2P	22.4	26.7	58.3	O5'	17.5	11.3	64.8								
C2'	35.8	26.1	60.5	O5'	22.6	24.8	57.4	C5'	17.6	9.9	64.9								
C1'	37.1	25.4	60.2	C5'	22.3	24.4	56.0	C4'	18.7	9.4	65.9								



The model has been rotated such that the X–Z plane is parallel, and the Y axis perpendicular to the molecular plane. The crystallographic co-ordinates (x, y, z) were converted to the values given here (X, Y, Z) by the relation:

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} 0{\cdot}8709 & -0{\cdot}4357 & 0{\cdot}2270 \\ 0{\cdot}4904 & 0{\cdot}8030 & -0{\cdot}3397 \\ -0{\cdot}0347 & 0{\cdot}4066 & 0{\cdot}9129 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 5{\cdot}514 \\ 7{\cdot}130 \\ -1{\cdot}278 \end{pmatrix}$$

The nomenclature used here is identical to that used by Sussman & Kim (1976*a*) and an example is given at the end of the Table. for the hypermodified Y nucleotide.

TABLE 3

Thermal parameters for each rigid group in Å² units

Residue	Phosphate	Ribose	Base	Residue	Phosphate	Ribose	Base
1	121	89	57	39	41	79	70
2	142	100	49	40	46	45	41
3	111	99	49	41	55	34	45
4	81	60	35	42	63	48	34
5	58	49	36	43	58	40	42
6	41	4	18	44	77	37	44
7	30	19	17	45	35	27	33
8	22	8	20	46	39	33	30
9	23	20	21	47	26	61	82
10	29	9	47	48	46	12	23
11	23	28	39	49	26	20	22
12	18	18	33	50	30	29	23
13	26	10	24	51	30	24	26
14	16	11	13	52	34	25	26
15	10	25	40	53	47	26	21
16	41	89	201	54	11	31	12
17	131	43	37	55	37	18	31
18	36	18	12	56	18	15	12
19	16	20	18	57	9	33	4
20	15	17	10	58	21	13	13
21	15	24	28	59	45	2	44
22	19	9	22	60	18	17	20
23	21	19	38	61	28	31	33
24	34	25	40	62	24	31	8
25	52	67	32	63	39	37	26
26	52	29	53	64	51	37	39
27	48	22	45	65	55	36	20
28	53	75	32	66	39	16	12
29	55	57	37	67	23	50	14
30	57	65	45	68	35	35	15
31	73	57	65	69	36	27	37
32	75	104	48	70	41	40	35
33	84	148	44	71	51	93	44
34	116	82	42	72	73	43	41
35	64	76	54	73	74	76	54
36	55	58	54	74	79	79	45
37	94	95	82	75	75	45	58
38	65	92	53	76	108	188	225

in the deep groove of the double helix formed by the D and the anticodon stems. The former is not as well-defined as the latter.

The final unweighted R factor with rigid group thermal parameters is 23·1%, and that with atomic thermal parameters is 19·8%. The correlation coefficient between F_o and F_c values, $\sum[(F_o - \bar{F}_o)(F_c - \bar{F}_c)]/[(\sum F_o - \bar{F}_o)^2 \times \sum(F_c - \bar{F}_c)^2]^{\frac{1}{2}}$, is 0·93, where $\bar{F}_o$ and $\bar{F}_c$ are the means of the F_o and F_c values, respectively. A correlation coefficient of 1·0 represents a complete correlation between two data sets and 0·0 no correlation.

An average co-ordinate error calculated from the inversion of the least-squares full matrix is about 0·1 Å. Three stereo views of the refined tRNA structure are shown in Figure 4. In this model, over 85% of total bond distances and angles are constrained, and therefore have canonical values. For the remainder, the average deviations from

canonical values in restrained bond distances, bond angles, and non-bonded contacts are 0·02 Å, 1·5° and 0·03 Å, respectively, as shown in Table 4. The corresponding average deviations for *all* bond distances and angles are 0·003 Å and 0·18°, respectively. The canonical values for the bond distances and angles are from Arnott *et al.* (1972) and those for the van der Waals' radii are from Ramachandran & Sasisekharan (1968).

TABLE 4

Deviations from "canonical" bond distances, bond angles and non-bonded contact distances for the restrained parameters

Type of restraint	No. of restraints	Average deviation (Å)	r.m.s. deviation (Å)	Max. deviation (Å)	Relative weight
Bond distances	227	0·011	0·016	−0·058 O3′(18)—P(19)	6·0
Bond angles	680	0·018 (1·1°)	0·023 (1·5°)	−0·095 (5·9°) C3′—C4′—O1′(46)	5·0
Non-bonded contacts (Restraint applied only to atoms within the specified van der Waals' contact distance)	89	0·044	0·050	−0·099 C2′(17)—O2(17)	3·0

The values of rigid group thermal parameters ranged between 2 and 225 Å^2, with no restrictions applied. The rigid group thermal parameters of the base, ribose and phosphate for each residue are illustrated in Figure 5, where the radius of each circle is proportional to the corresponding rigid group thermal parameters. There are two interesting trends noticeable in the "thermal" motion of the molecule. The first is that both extreme ends (and three protruding residues 16, 17 and 47) have higher "thermal" motion than the corner of the "L". The second is that the average thermal parameter for the bases (39 Å^2) is smaller than that for the riboses (44 Å^2), which in turn is smaller than that for the phosphates (48 Å^2). This trend is still more pronounced in the stem regions, i.e. 34 Å^2, 41 Å^2 and 48 Å^2 for the bases, the riboses and the phosphates, respectively. This can be interpreted in one of two ways: (a) each long helical arm of the "L" has flexing or precessing motion around the average helical axis of each long arm, or (b) each long double-helical arm is partially opening up (unwinding) and closing (winding) at the extreme end. The functional implication of this is discussed in the following paper. Since the magnitude of thermal motion of various parts of the molecule cannot be explained in terms of the lattice contacts, our interpretation is that they reveal the intrinsic flexibility of the molecule (Kim, 1978).

Between the starting model and the refined model, the average and the r.m.s. shifts in atomic co-ordinates *including* the shifts due to the manual refitting of residues were 0·9 Å and 1·3 Å, respectively.

The average change between the multiple isomorphous replacement phases and the phases calculated from the starting model was 66°, and between those from multiple isomorphous replacement and the final refined model was 61°. These and related phase

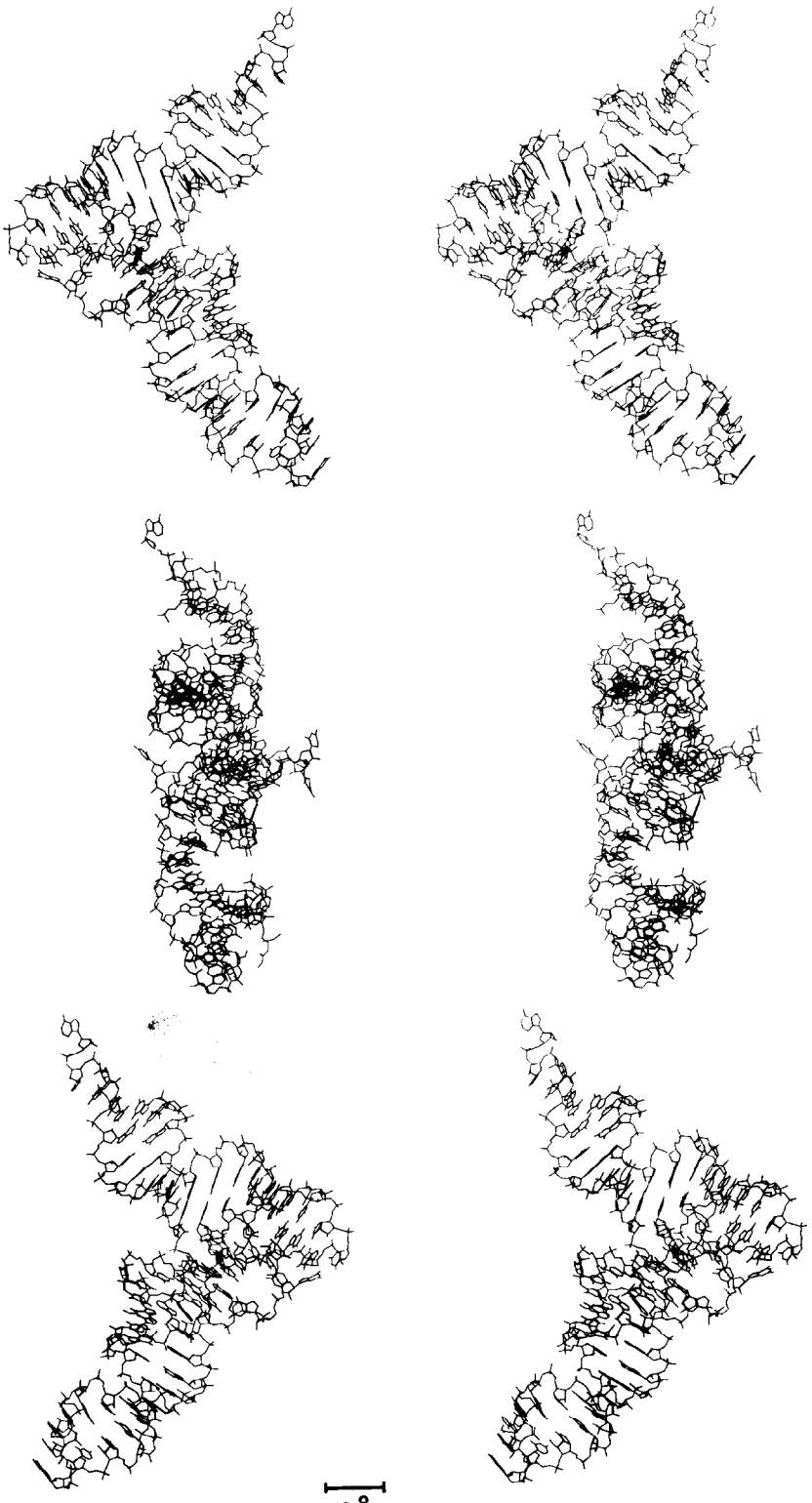


FIG. 4

changes are shown in Figure 6. An analysis of the R factors and average phase changes as a function of sin θ/λ and F_o is given in Figure 7. The abrupt increase of R factor beyond 3·0 Å resolution reflects the poorer quality of the diffraction data and rapid decrease of the intensity as the resolution approaches 2·7 Å.

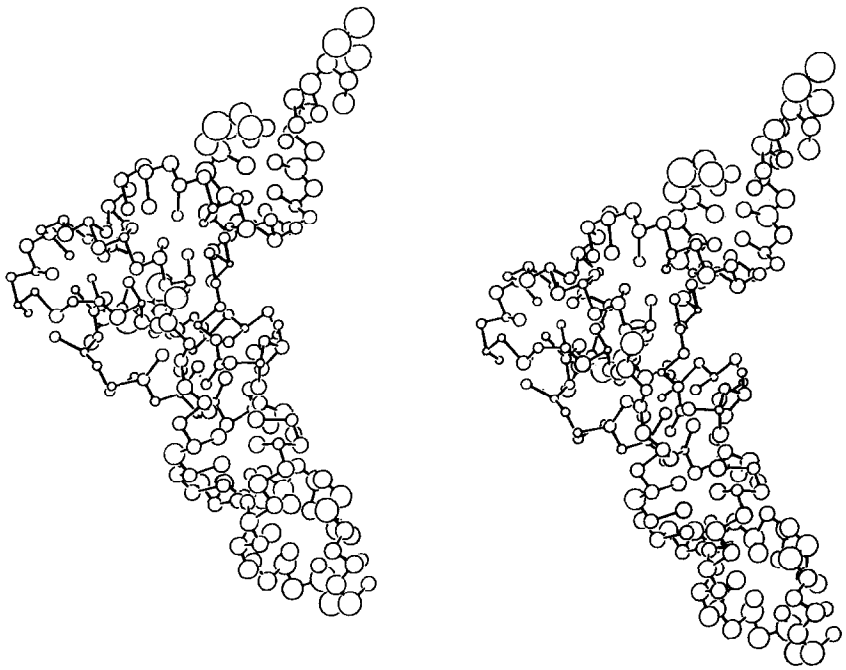


FIG. 5. The thermal parameters for the base, the ribose and the phosphate for each residue are represented as a circle for each group. The radius of each circle was drawn proportional to the magnitude of the group thermal parameter for each group.

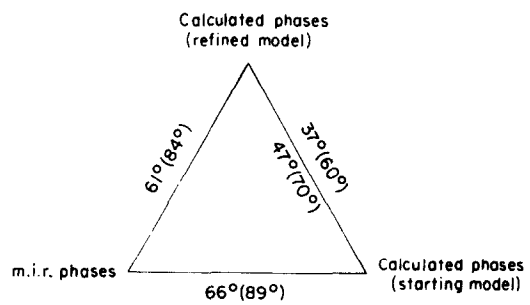


FIG. 6. Overall average phase changes among multiple isomorphous replacement (m.i.r.) phases, phases calculated from the starting model, and phases calculated from the refined model. The r.m.s. phase changes are indicated in parentheses. The numbers outside the triangle refer to the 4902 data common to all 3 sets. The numbers within the triangle refer to the 8426 data common to the phases calculated from the initial and the refined models.

FIG. 4. Three pairs of stereo photographs of the 3-dimensional structure of yeast phenylalanine transfer RNA. A complete structure is shown here. Each stereo pair is rotated 90° around the vertical axis from the previous stereo pair.

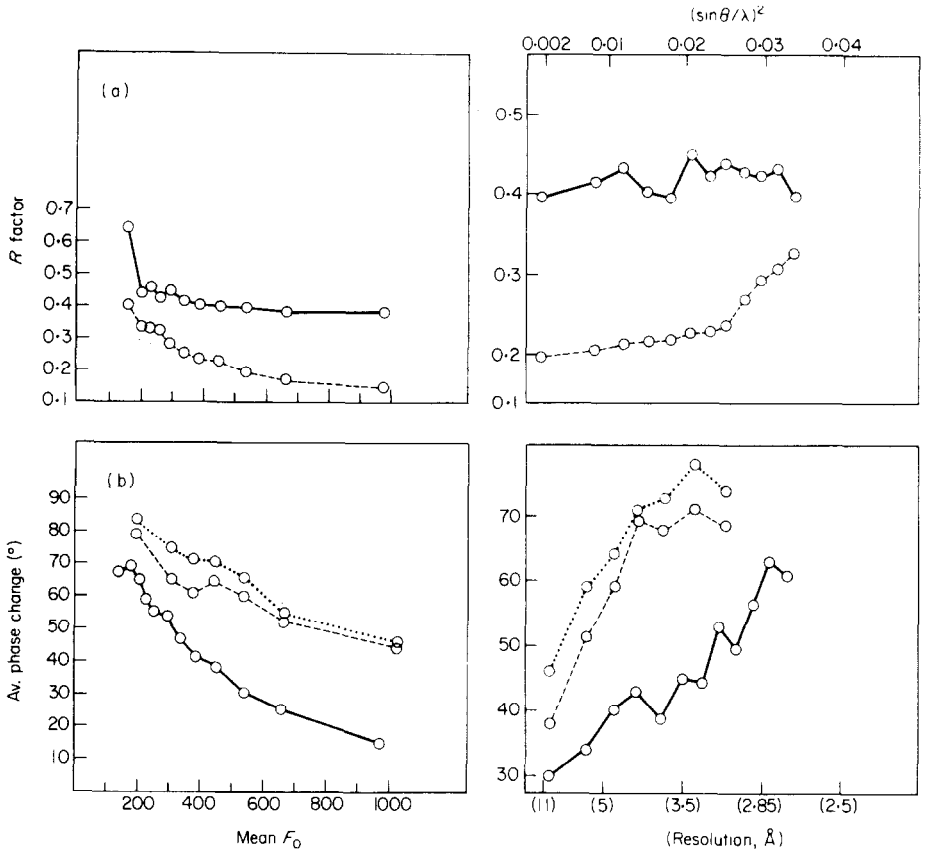


FIG. 7. (a) Structure factor analysis as represented by average R factor *versus* sin θ/λ and the magnitude of the observed structure factor (F_o). (————) Starting co-ordinates; (– – – –) final co-ordinates.

(b) Average phase changes *versus* sin θ/λ and observed structure factors. (————) Final *versus* initial; (· · · · ·) initial *versus* m.i.r.; (– – – –) final *versus* m.i.r.

The improved phases calculated from the refined structure yield Fourier maps of low noise and highly discernible detail. Examples of the model-fitting to the $2F_o - F_c$ electron density are shown in Figure 8. The top three stereo pairs of Figure 8 show the fitting of typical Watson–Crick pairs in the stem region and the fourth shows the anticodon loop fitting. The bottom two Figures show two of the poor electron density

FIG. 8. Model-fitting to the electron density map using $2F_o - F_c$ as amplitudes and phases calculated from the refined model of yeast phenylalanine tRNA. The contour lines are drawn at one single value for each case.

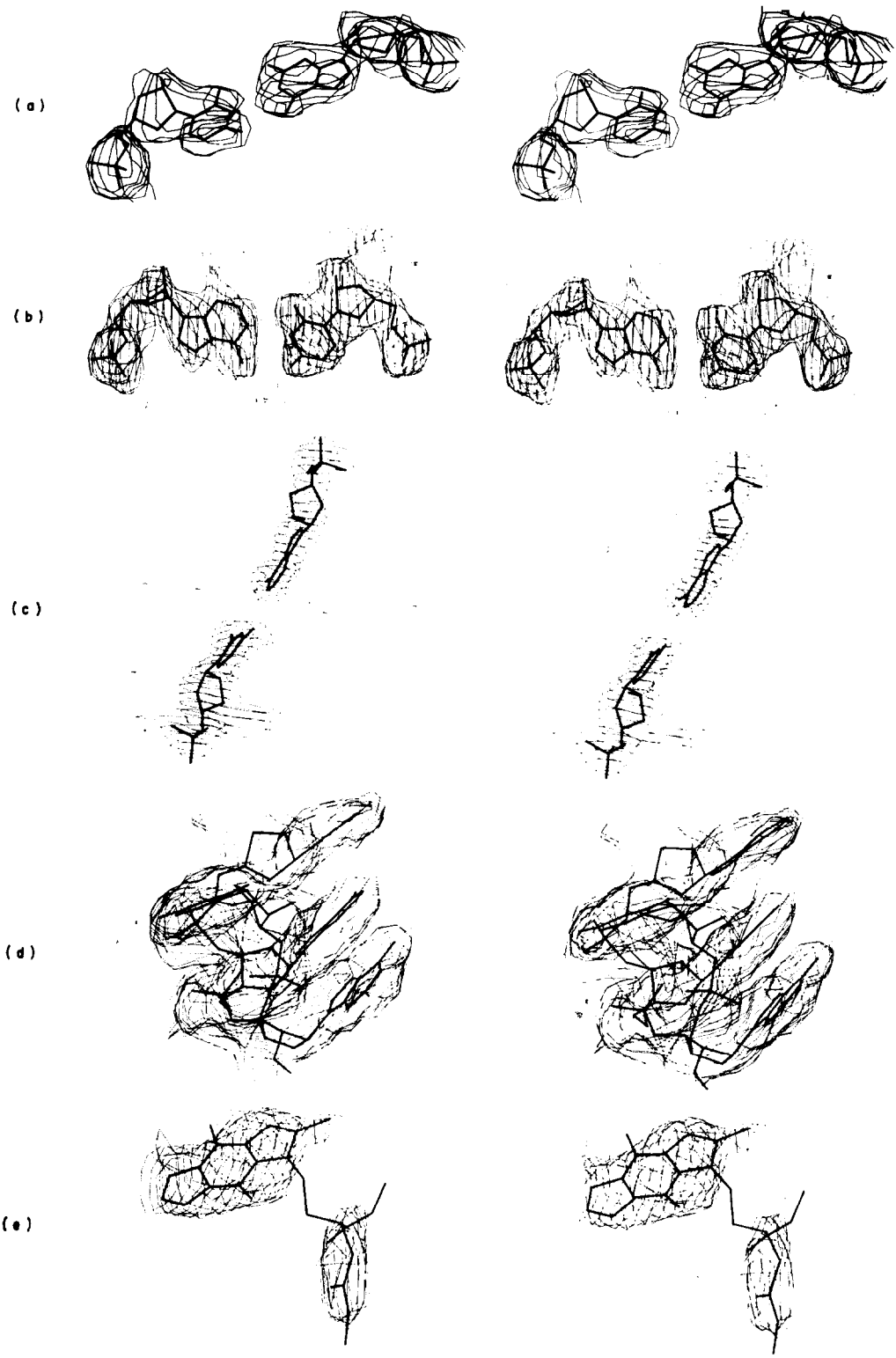
(a) A typical Watson–Crick C·G pair: G(51)·C(63).

(b) A typical Watson–Crick A·U pair: A(67)·U(6).

(c) The same A·U pair as in (b), but viewed approximately along the edge of the base-pair plane to illustrate the twist of the base-pair. Twisting of this kind is observed in all base-pairs in this structure. The electron density at bottom right is for the 3′ phosphate of U, which is not drawn in.

(d) Molecular fitting of a region of the anticodon loop containing the anticodon triplet. Electron densities in these regions are relatively weaker than most other parts of the molecule. The residues 33 to 36 are shown.

(e) Model-fitting of the hypermodified base of residue 37. Note the weak electron density for the side-chain of this hypermodified base.


(a)
(b)
(c)
(d)
(e)

regions, one around the anticodon and the other near the side-chain of the hypermodified base of residue 37. Electron density maps obtained using phases calculated from a model have the tendency to show the same input model.. To minimize this circular effect, we made "partial" electron density maps where structure factors and phases are calculated from atomic co-ordinates of the model *lacking* the regions of interest. We calculated such a "partial" electron density map from the refined model *excluding* residues 16, 17, 75 and 76 and bases of residues 4, 69, 19 and 56, i.e. about 8% of the structure was deleted. The model-fitting of two unusual base-pairs G(4)·U(69) and G(19)·C(56), in the "partial" $2F_o - F_c$ map are shown in Figure 9(a) and (b). The bottom two Figures are the two weakest electron density regions in the whole unit cell excluding the background. Figure 9(b) shows a G·C pair which is highly bent and twisted. A functional implication of this unusual feature was discussed earlier (Kim, 1978). There is a discontinuity of electron density between the phosphate and the ribose of residue 17 in Figure 9(c), suggesting that some or all of the tRNA molecules in the crystals have a cleaved bond at this site. This is consistent with the observations that a co-ordinated Mg^{2+} ion was found at this location (Holbrook *et al.*, 1977) and that the ester linkage between two dihydrouracil residues, 16 and 17, is vulnerable to metal ion mediated cleavage at pH 9·5 (Wintermeyer & Zachau, 1973). Although our crystals are at pH 6·0, it is possible that the cleavage may have taken place because of long storage at 4°C over several months and an extensive exposure to X-rays during X-ray diffraction data collection.

Crystallographically, the CORELS method refined the yeast $tRNA^{Phe}$ structure overwhelmingly better than "manual" electron density fitting on an interactive computer graphics system. The R factor of 0·198 (for 8426 independent reflections representing an almost complete set of data up to 2·7 Å resolution) is probably near the minimum one can achieve with the CORELS method without compromising stereochemistry. A structure of the same tRNA crystallized in a monoclinic form has been refined by a real space refinement method (Diamond, 1971) to an R factor of 30·7% for 6006 reflections representing an almost complete data set up to 3 Å resolution and the strongest 40% of the data between 3 Å and 2·5 Å resolution (Jack *et al.*, 1976).

4. Conclusion

The crystal structure of yeast phenylalanine tRNA refined by a structure-factor least-squares method using constrained *and* restrained parameters has provided us with the most accurate atomic co-ordinates for the structure we could obtain with the available diffraction data collected at 4°C. These atomic co-ordinates and thermal parameters serve as a source for all structural information such as distance between any two functionally important sites, details of hydrogen-bonding schemes, binding geometry of ligands, molecular "flexibility", and the helicity of each of the stems for this particular tRNA and possibly for all tRNAs. They also serve as a basis for formulating principles of nucleic acid conformation in general.

This research has been supported by grants from the National Institutes of Health (CA-15802, K04-CA-00352) and the National Science Foundation (PCM76-04248). We wish to thank and acknowledge many colleagues who participated in this project, especially Gary Quigley, F. L. Suddath, Alex McPherson and Alex Rich for a very fruitful collaboration at the earlier stages of the yeast $tRNA^{Phe}$ project. We thank John Konnert, Wayne Hendrickson and Charles Alden for helpful discussions.

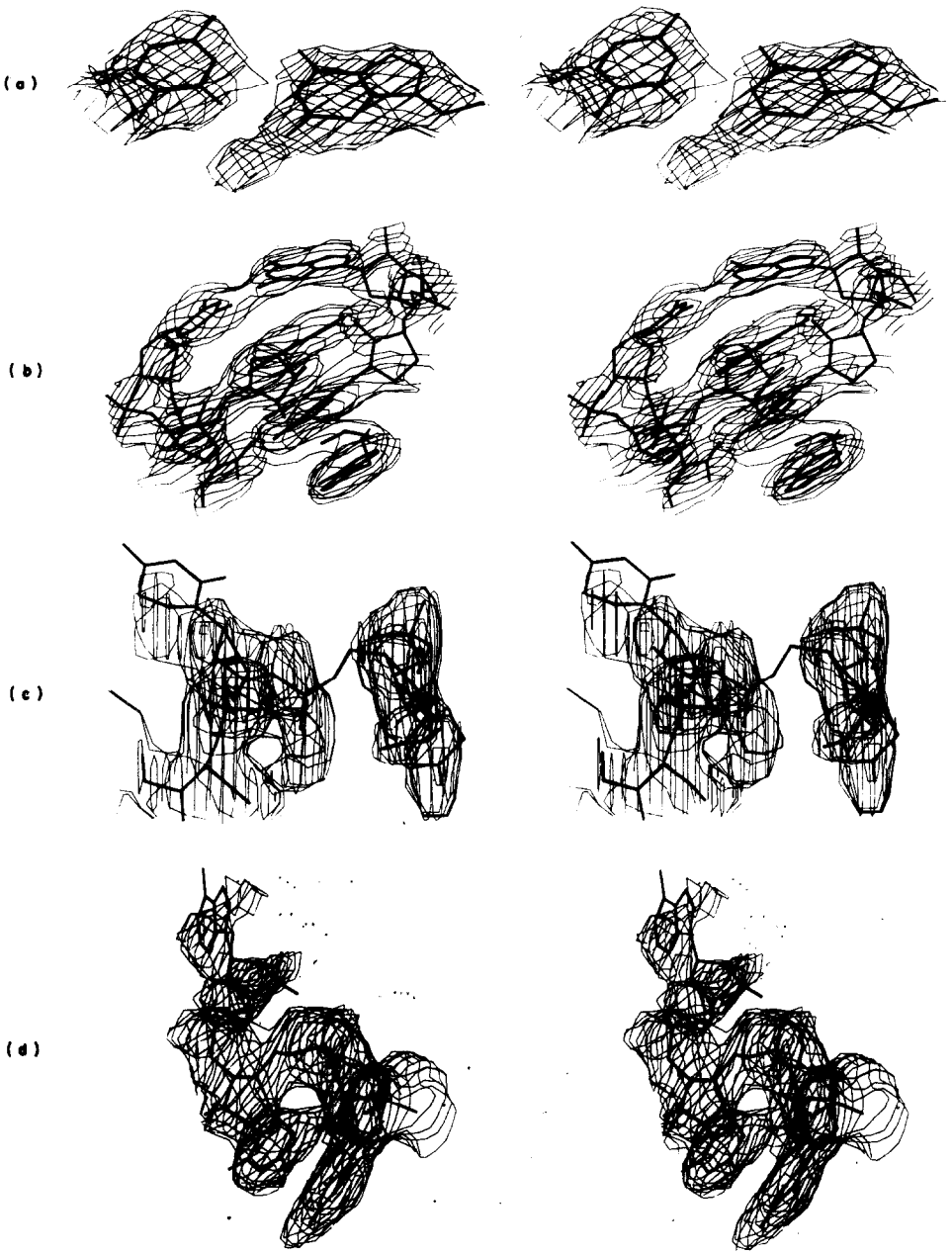


FIG. 9. Model-fitting of several regions of interest using a "partial" electron density map. The calculated structure factors and the phases are obtained from the refined model *excluding* residues 16, 17, 75, 76 and bases of residues 4, 69, 19 and 56.

(a) G(4)·U(69) "wobble" base-pair. A small electron density peak near N2 has been refined as a bound water.

(b) Corner of "L". The top of the Figure is base-pair G(19)·C(56). Note the highly bent and twisted nature of this base-pair.

(c) Residues 16 and 17. Note the discontinuity between phosphate 17 and ribose 17. This region is one of the weakest electron density regions.

(d) Residues 74, 75 and 76. The region around residue 76 is the weakest in the whole electron density map.

The model-fitting and some of the drawings were done with the aid of the GRIP-76 molecular graphics system built at the University of North Carolina Department of Computer Science. Major systems contributions were made by E. G. Britton, J. S. Lipscomb, M. E. Pique, J. E. McQueen, Jr, J. Hermans, W. V. Wright, W. Siddal and F. P. Brooks, Jr, and the systems development has been supported by N.I.H. (RR00898), N.S.F. (GJ34697), Atomic Energy Commission (AT-(40-1)-3917) and International Business Machine Corp. Some computing was done at Weizmann Institute Computer Center.

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