

## The Crystal and Molecular Structure of 2'-Acetyluridine-3',5'-cyclophosphate Benzyl Triester, a Pyrimidine Nucleotide with *syn* Conformation

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2'-Acetyluridine-3',5'-cyclophosphate benzyl triester crystallizes in the monoclinic space group  $P2_1$ ,  $Z = 2$ ,  $a = 6.507$  (1),  $b = 7.565$  (1),  $c = 19.694$  (4) Å,  $\beta = 90.72$  (5)°. The structure was solved by direct methods and refined to a final  $R$  value of 0.055 for 2021 unique reflexions including unobserved reflexions. Bond lengths and angles are in the usual range. The dioxophosphorinane ring has the chair conformation, while the ribose exhibits the  $E_4$  conformation. The benzyl group is equatorial to the dioxophosphorinane ring. The most striking feature is the *syn* conformation of the nucleotide. The torsion angle  $O(1')-C(1')-N(1)-C(6)$  is  $-108.6^\circ$ . Symmetry-related molecules are linked by  $N(3)-H(3)\cdots O(212)$  hydrogen bonds, where  $O(212)$  is the O of the acetyl group attached to  $O(2')$ , forming helices running along the  $b$  axis.

### Introduction

Because of their importance in the RNA-breakdown and as energy-rich mediators for cell processes the interest in the cyclic phosphates of the ribonucleosides, especially *cAMP*,\* has been increasing in recent years. Derivatives in large numbers have been synthesized (Simon, Shuman & Robins, 1973) and the hydrophilic cyclophosphates were converted to neutral triesters by esterifying the remaining OH group. This has been done by Cotton *et al.* (1975) for *cAMP* and by Engels & Pfeiderer (1975*a,b*) for *cUMP*. Recently, the natural occurrence of *cUMP* has been reported (Bloch, 1975) and Coulter (1969) published the results of the structure determination of the triethylammonium salt of uridine-3',5'-cyclophosphate. By the 3',5'-O-P-O cyclization the P becomes a new centre of chirality and diastereoisomerism is possible. It was of importance to establish whether the benzyl group is axial or equatorial to the dioxophosphorinane ring in 2'-acetyluridine-3',5'-cyclophosphate benzyl triester (hereinafter called BUMP), because this cannot be established by any other method.

### Experimental

Crystals of BUMP have been recrystallized from a mixture of water and methanol. A crystal approximately  $0.5 \times 0.2 \times 0.2$  mm was selected for the X-ray measurements. The prismatic crystal was mounted along its long axis, which turned out to be the  $b$  axis. Preliminary information about cell dimensions and symmetry was obtained from Weissenberg and precession photographs. The Laue group was  $2/m$  and from systematic extinctions,  $0k0$  with  $k = 2n + 1$ , space groups  $P2_1$  and  $P2_1/m$  were possible. Taking the optical activity into account, the only possible space group was  $P2_1$ . More accurate cell dimensions were obtained from a least-squares fit to the  $\sin^2 \theta$  values of 19 high-angle reflexions centred on a Siemens diffractometer. Crystal data are shown in Table 1. The intensities of reflexions up to  $\theta = 72^\circ$  were measured by a five-value measurement technique (Hoppe, 1965) with Ni-filtered  $Cu K\alpha$  radiation at room temperature, yielding 2021 unique reflexions of the 2200 possible in the  $Cu$

Table 1. Crystal data

\* Abbreviations used: *cGMP*: guanosine-3',5'-cyclomonophosphate (Chwang & Sundaralingam, 1974); 3',5'-*UMP*: triethylammonium salt of uridine-3',5'-cyclophosphate (Coulter, 1969); Et-*cAMP*: P-O ethyl ester of adenosine 3',5'-cyclomonophosphate (Cotton *et al.*, 1975); BUMP: 2'-acetyluridine 3',5'-cyclophosphate benzyl triester (this work); *cUMP*: uridine-3',5'-cyclomonophosphate; *cAMP*: adenosine-3',5'-cyclomonophosphate.

$C_{18}H_{19}O_8N_2P$	Space group $P2_1$
FW 438.31	$V = 969.4 \text{ \AA}^3$
$a = 6.507$ (1) Å	$D_x = 1.50 \text{ g cm}^{-3}$ with $Z = 2$
$b = 7.565$ (1)	$D_m = 1.51$
$c = 19.694$ (4)	$\mu(Cu K\alpha) = 17.8 \text{ cm}^{-1}$
$\beta = 90.72$ (5)°	$\lambda = 1.5418 \text{ \AA}$
Systematic extinctions: $0k0$ with $k = 2n + 1$	

$K\alpha$  sphere. Of these reflexions, 60 with  $I < 3\sigma(I)$  were classed as unobserved. A reference reflexion was measured after every 20 reflexions and showed no decrease in intensity during the measurement. The data were corrected as usual but no absorption correction was made. The programs used for the data reduction were written by J. Eck.

### Structure determination and refinement

The structure was solved by the multi-solution tangent-refinement method with the program *MULTAN* (Germain, Main & Woolfson, 1971). The  $E$  map of the best set showed the uracil ring, the P vicinity and the sugar ring in part. Neither the benzyl nor the acetyl group could be seen because of the higher thermal motion, as became evident after the completion of the structure refinement. By standard Fourier and least-squares methods all non-hydrogen atoms could be located. After isotropic and anisotropic refinement of the non-hydrogen atoms all H atoms but the methyl could be seen on a difference Fourier map.

Table 2. Final positional parameters ( $\times 10^4$ ) for the non-hydrogen atoms, with standard deviations in parentheses

The  $y$  coordinate of P was held invariant.

	$x$	$y$	$z$
N(1)	7677 (4)	-1165 (4)	776 (1)
C(2)	7730 (4)	295 (4)	351 (2)
N(3)	7466 (4)	107 (4)	9669 (1)
C(4)	7203 (4)	-1771 (5)	9386 (2)
C(5)	7179 (5)	-3202 (5)	9859 (2)
C(6)	7405 (4)	-2868 (4)	528 (2)
O(2)	7953 (4)	1804 (3)	558 (1)
O(4)	7068 (4)	-1893 (4)	8764 (1)
C(11)	7945 (4)	-927 (4)	1503 (1)
C(21)	9835 (4)	229 (4)	1734 (1)
C(31)	8745 (4)	1738 (4)	2083 (1)
C(41)	6824 (4)	855 (4)	2351 (1)
O(11)	6142 (3)	-126 (4)	1776 (1)
O(21)	1029 (3)	-721 (3)	2226 (1)
O(31)	9868 (3)	2579 (3)	2627 (1)
C(51)	5310 (4)	2206 (5)	2600 (2)
O(51)	6398 (3)	3184 (4)	3129 (1)
P	8528 (1)	4056	2975 (1)
O(6)	8398 (4)	5729 (3)	2602 (1)
O(7)	9572 (4)	4330 (3)	3685 (1)
C(73)	9848 (5)	2835 (5)	4144 (2)
C(93)	971 (3)	2942 (3)	5361 (1)
C(103)	2398 (3)	3352 (3)	5872 (1)
C(113)	4241 (3)	4181 (3)	5708 (1)
C(123)	4658 (3)	4601 (3)	5034 (1)
C(133)	3231 (3)	4191 (3)	4523 (1)
C(83)	1387 (3)	3361 (3)	4687 (1)
C(212)	2128 (4)	-2072 (4)	1970 (2)
O(212)	2089 (4)	-2398 (4)	1362 (2)
C(312)	3261 (5)	-3063 (5)	2492 (3)

The structure was refined by full-matrix least squares minimizing  $\Sigma w(\Delta F)^2$  with anisotropic temperature factors for C, N, O and P and an invariant isotropic temperature factor for H ( $U = 0.038 \text{ \AA}^2$ ). The phenyl was refined as a rigid group and only tertiary H positions were refined, all other H atoms were constrained on geometrically ideal positions. Unobserved reflexions were included and weights were  $w = [\sigma^2(F) + 0.02F^2]^{-1}$ . Complex neutral-atom scattering factors were employed (*International Tables for X-ray Crystallography*, 1974). The final  $R$  value [ $R = \Sigma w^{1/2}(F_o - F_c) / \Sigma w^{1/2}|F_o|$ ] was 0.062 with a corresponding unweighted  $R$  of 0.055 for 2021 reflexions and 319 parameters.\* For non-hydrogen atoms the maximal shift/e.s.d. was 0.14.

### Results and discussion

Final positional parameters for non-hydrogen atoms are shown in Table 2. Tables 3 and 4 show positional parameters for H atoms, and least-squares plane calculation results for the uracil, the ribose and the dioxophosphorinane ring. Fig. 1 is an *ORTEP* plot (Johnson, 1965) of the molecule and schematic drawings of the molecule with bond lengths, bond angles and torsion angles are shown in Fig. 2(a)–(c). The average C–H bond length involving refined H atoms is 1.07 (1) Å.

\* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32487 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 3. Final positional parameters for H atoms ( $\times 10^3$ ), with standard deviations in parentheses for the refined H atoms only

	$x$	$y$	$z$
H(5)	712 (1)	-431 (1)	16 (1)
H(6)	719 (1)	-418 (1)	33 (1)
H(11)	823 (1)	-222 (1)	168 (1)
H(21)	137 (1)	-2 (1)	155 (1)
H(31)	848 (1)	281 (1)	172 (1)
H(41)	707 (1)	2 (1)	279 (1)
H(511)	397	156	280
H(512)	485	308	219
H(731)	128	214	421
H(732)	886	257	457
H(93)	-46	230	549
H(103)	208	303	639
H(113)	535	450	610
H(123)	609	524	491
H(133)	355	452	400
H(311)	411	-412	226
H(312)	219	-361	285
H(313)	432	-219	275
H(3)	767 (1)	86 (1)	946 (1)

Table 4. Displacements (Å) from the least-squares planes calculated for the uracil, the dioxophosphorinane and the ribose moiety

Planes are defined by atoms marked with an asterisk.

Equations of planes:

$$\text{I: } 6.453x - 0.743y - 1.896z - 4.894$$

$$\text{II: } -0.471x + 6.484y - 10.027z = -1.4006$$

$$\text{III: } 0.579x + 3.810y + 16.900z = 3.3836$$

where  $x$ ,  $y$ ,  $z$  are fractional coordinates of a point within the respective plane.

I Uracil		II Dioxophosphorinane		III Ribose	
N(1)*	-0.001	C(31)*	0.027	C(11)*	-0.031
C(2)*	0.005	O(31)*	-0.026	C(21)*	0.029
N(3)*	-0.006	C(51)*	-0.026	C(31)*	-0.020
C(4)*	0.002	O(51)*	0.026	C(41)	0.659
C(5)*	0.003	C(41)	-0.724	O(11)*	0.021
C(6)*	-0.003	P	0.646	O(21)	1.291
O(2)	-0.002	O(6)	2.110	O(31)	0.644
O(4)	0.042	O(7)	0.062	C(51)	0.477
C(11)	0.016	O(11)	-0.751	N(1)	-1.184
C(21)	1.106	O(21)	-1.819	O(2)	-2.668
C(31)	0.225	C(11)	-1.082	C(6)	-0.970
C(41)	-1.000	R.m.s.d.	0.0264	R.m.s.d.	0.0255
O(11)	-1.258				
R.m.s.d.	0.0037				

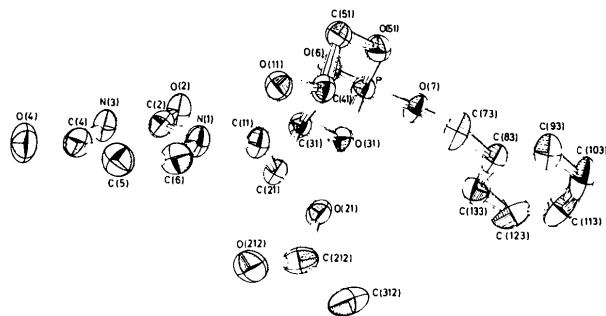


Fig. 1. ORTEP plot of BUMP, showing the atom numbering.

### The uracil ring

The dimensions of the uracil ring are comparable with those found in other pyrimidines. The bond lengths and internal angles of BUMP agree well with those of 3',5'-UMP (Coulter, 1969). In contrast with 3',5'-UMP the external angle C(11)–N(1)–C(2) exceeds C(11)–N(1)–C(6). The angle N(3)–C(4)–O(4) is about 8° smaller than C(5)–C(4)–O(4). This non-equivalence has been found by others [see Coulter (1969), Table 9 and the literature cited therein]. The planarity of the uracil ring is shown by the torsion angles (Fig. 2c) as well as by the least-squares plane shown in Table 4. Atoms O(4) and C(11) are slightly out of the plane. This is comparable with the results for 3',5'-UMP.

### The ribose

The bond lengths of the ribose ring agree well with those of 3',5'-UMP except that the bond lengths C(21)–C(31) and C(31)–C(41) are nearly equal. O(11)–C(11) is not significantly longer than O(11)–C(41). The angles are in the range of values found in other cyclic nucleotides (Coulter, 1969; Chwang & Sundaralingam, 1974; Cotton *et al.*, 1975). The torsion angles (Fig. 2c) show that the conformation of the ribose is well described by  $E_4$  (Carbohydrate Nomenclature Committee, 1972). The ideal envelope conformation would require the torsion angle O(11)–C(11)–C(21)–C(31) to be 0° as compared with –5.1° found in BUMP. In *c*GMP and the molecules *A* and *B* of 3',5'-UMP the ribose is more twisted, as is shown by the torsion angles –9.3, –10.4 and –5.5° respectively. From Table 4 it can be seen that C(41) is displaced 0.66 Å from the plane defined by atoms C(11), C(21), C(31) and O(11), while C(51) is displaced 0.48 Å from this plane. This means the displacement of C(41) is opposite to the configurational orientation of C(51). Therefore, the conformation can be described as C(41)-*exo*. Following Sundaralingam & Abola (1972) the reference atom for cyclic nucleotides should be the glycosyl N. Since N(1) is displaced –1.2 Å from the plane the puckering of the ribose would also be described as C(41)-*exo*.

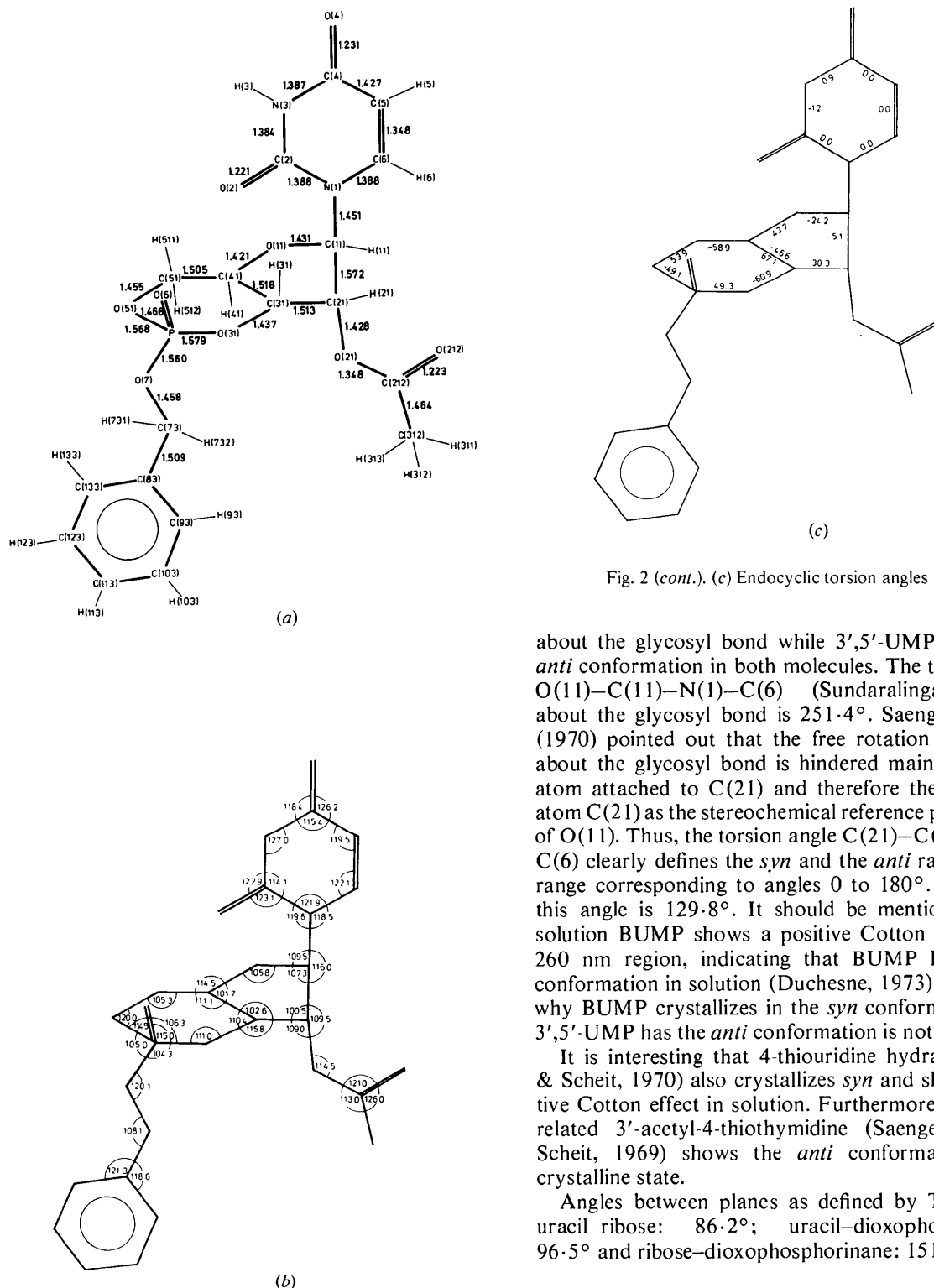
### The dioxophosphorinane ring

The bond distances in this ring are in good agreement with other cyclic nucleotides. Apparent deviations are within the range of the standard deviations and are not significant. The angle O(51)–P–O(31) is about 3° greater than those found in *c*GMP and 3',5'-UMP but 2° smaller than that in Et-*c*AMP. The torsion angles (Fig. 2c) show that this ring has a distorted chair conformation, as has been found with other cyclic nucleotides. Its conformation compares best with molecule *B* of 3',5'-UMP. As compared with *c*GMP and molecule *A* of 3',5'-UMP the disagreement between the torsion angles is up to 8°; nevertheless this conformation seems stable in all cyclic nucleotides. As usual with cyclic nucleotides the greatest puckering occurs in the C(31)–C(41) bond.

The bond lengths and angles show that the coordination of the phosphorus is no longer tetrahedral but almost trigonal pyramidal, as is shown by the O(6)–P–O angles which are in the range 110.9–115.0°. The benzyl group was found to be equatorial to the dioxophosphorinane ring.

### Glycosyl torsion angle

The most striking feature of the molecular structure of BUMP is the fact that it exhibits a *syn* conformation

Fig. 2 (cont.). (c) Endocyclic torsion angles ( $^{\circ}$ ).

about the glycosyl bond while 3',5'-UMP showed an *anti* conformation in both molecules. The torsion angle O(11)–C(11)–N(1)–C(6) (Sundaralingam, 1969) about the glycosyl bond is 251.4 $^{\circ}$ . Saenger & Scheit (1970) pointed out that the free rotation of the base about the glycosyl bond is hindered mainly by the H atom attached to C(21) and therefore they suggested atom C(21) as the stereochemical reference point instead of O(11). Thus, the torsion angle C(21)–C(11)–N(1)–C(6) clearly defines the *syn* and the *anti* range, the *syn* range corresponding to angles 0 to 180 $^{\circ}$ . For BUMP this angle is 129.8 $^{\circ}$ . It should be mentioned that in solution BUMP shows a positive Cotton effect in the 260 nm region, indicating that BUMP has an *anti* conformation in solution (Duchesne, 1973). The reason why BUMP crystallizes in the *syn* conformation while 3',5'-UMP has the *anti* conformation is not clear.

It is interesting that 4-thiouridine hydrate (Saenger & Scheit, 1970) also crystallizes *syn* and shows a positive Cotton effect in solution. Furthermore, the closely related 3'-acetyl-4-thiothymidine (Saenger, Suck & Scheit, 1969) shows the *anti* conformation in the crystalline state.

Angles between planes as defined by Table 4 are: uracil–ribose: 86.2 $^{\circ}$ ; uracil–dioxophosphorinane: 96.5 $^{\circ}$  and ribose–dioxophosphorinane: 151.2 $^{\circ}$ .

#### Hydrogen bonding and packing

As expected, the hydrogen attached to N(3) is involved in hydrogen-bond formation. Symmetry-related

Fig. 2. (a) Bond lengths of BUMP with atom numbering. The mean standard deviation is 0.004 Å throughout the molecule and 0.002 Å involving P. The phenyl was refined as a rigid group. (b) Bond angles. Standard deviations are 0.2 $^{\circ}$  except for the uracil and acetyl (0.3 $^{\circ}$ ) and at the phosphorus (0.1 $^{\circ}$ ).

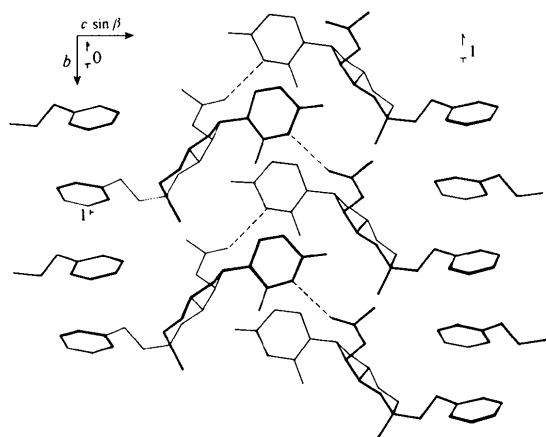


Fig. 3. Projection of the crystal structure down *a*, showing the channels running along the *b* axis.

Table 5. Intramolecular contacts (Å) between O(2) and some atoms of the ribose, compared with the values found for 4-thiouridine hydrate

	BUMP	4-Thiouridine hydrate
O(2)...C(31)	3.041	
O(2)...C(11)	2.781	
O(2)...O(11)	3.057	3.24
O(2)...C(21)	2.866	2.88

molecules are linked in a head-to-tail fashion by a hydrogen bond, N(3)—H(3)···O(212)'. The distance N(3)···O(212)' is 2.902 Å and H(3)···O(212)' is 2.095 Å, while N(3)—H(3) is 0.85 Å. The angle at the H is 158.3°. The hydrogen-bonded molecules form helices along the *b* axis, the preferred direction of crystal growth. The helices form channels in the structure defined by the bases, the acetyl group and the sugars of symmetry-related molecules. The remainder of the molecule points away from the channels (see Fig. 3). There are no other important intermolecular contacts. A point of interest, however, are the short intramolecular contacts between O(2) and the sugar

forced by the *syn* conformation. In Table 5 these contacts are compared with those of 4-thiouridine hydrate.

The calculations were performed at the Rechenzentrum der Universität Hamburg and at the Rechenzentrum der Universität Konstanz. The authors wish to thank Mrs E. Neumann for the drawings.

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