Crystal Structure of Neotame Anhydrate Polymorph G

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Received June 11, 2002; accepted July 2, 2002

Purpose. To determine the crystal structure of the neotame anhydrate polymorph G and to evaluate X-ray powder diffractometry (XRPD) with molecular modeling as an alternative method for determining the crystal structure of this conformationally flexible dipeptide.

Methods. The crystal structure of polymorph G was determined by single crystal X-ray crystallography (SCXRD) and also from the X-ray powder diffraction (XRPD) pattern using molecular modeling (Cerius^{2,} ™, Powder Solve module).

Results. From SCXRD, polymorph G crystals are orthorhombic with space group of $P2_12_12_1$ with $Z = 4$, unit cell constants: $a = 5.5999(4)$, $b = 11.8921(8)$, $c = 30.917(2)$ Å, and one neotame molecule per asymmetric unit. The XRPD pattern of polymorph G, analyzed by Cerius^{2,} ™ software, led to the same $P2_12_12_1$ space group and almost identical unit cell dimensions. However, with 13 rigid bodies defined, Cerius^{2,} ™ gives a conformation of the neotame molecule, which is different from that determined by SCXRD.

Conclusions. For neotame anhydrate polymorph G, the unit cell dimensions calculated from XRPD were almost identical to those determined by SCXRD. However, the crystal structure determined by XRPD closely resembled that determined by SCXRD, only when the correct conformation of the neotame molecule had been chosen before detailed analysis of the XRPD pattern.

KEY WORDS: conformation; crystal structure; molecular modeling; neotame; X-ray powder diffractometry; X-ray crystallography.

INTRODUCTION

Neotame (*N*-[3,3-dimethylbutyl]-L-α-aspartyl-L-phenylalanine 1-methyl ester) is an alkylated derivative of a dipeptide sweetener, aspartame, and has intense sweetening potency. Discovered by Nofri and Tinti, neotame is currently being developed by The NutraSweet Company (Mount Prospect, IL, USA) (1). Neotame has recently been approved by the U.S. Food and Drug Administration as a general-use sweetener in food and beverages. Neotame has been found to exist in several solid forms, including neotame monohydrate (2), methanol solvate (3), neotame anhydrate polymorphs A-G (4).

The established method to determine a crystal structure is single crystal X-ray crystallography (SCXRD) (5). However, this approach requires a single crystal with an adequate quality and size. In some cases, it may be difficult, or even impossible, to grow a suitable single crystal, so that only a powder is available. If, in this situation, a good X-ray powder diffraction (XRPD) pattern is available, powder indexing becomes a promising approach. The Cerius^{2,} ™ program of Accelrys (San Diego, CA, USA) includes the Powder Indexing, Powder Fit, and Powder Solve modules that incorporate this function. This approach directly compares the XRPD patterns calculated from trial crystal structures with the experimental XRPD patterns and applies a Monte-Carlo search algorithm to calculate the energetically stable molecular conformation. The term, weighted Rietveld parameter (R_{wp}), is used to evaluate the similarity of the calculated XRPD pattern, based on the proposed crystal structure by Cerius^{2,} ™, to the experimental XRPD pattern. This approach has been tested on hydrates, hydrochloride salts, and metastable polymorphs (6). The present work tests this method on the conformationally flexible dipeptide, neotame anhydrate polymorph G.

MATERIALS AND METHODS

Materials

Neotame monohydrate with water content of 4.64% was supplied by The NutraSweet Company. Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and it was of analytical grade and dried by molecular sieves (3 or 4 Å) before use.

Form G crystallized within 2 days from a freshly prepared suspension or solution of polymorph A or amorphous neotame anhydrate in acetonitrile at room temperature. For this purpose, polymorph A can be obtained by the dehydration of neotame monohydrate (7), whereas amorphous anhydrate can be prepared by the desolvation of neotame methanol solvate, which can be produced from neotame monohydrate by crystallization in methanol (3).

Experimental Methods

Collection of the X-Ray Powder Diffraction Pattern

The X-ray powder diffraction (XRPD) pattern of Form G was collected at room temperature using an X-ray diffractometer (Siemens, model D-5005; Karlsruhe, Germany) with Cu Kα radiation at 40 mA, 45 kV. The sample was packed into a plastic holder of zero background and was scanned from 2° to 40° 2θ at a step size 0.05° with a dwell time 1 s. The XRPD pattern of Form G was saved in text format, then transformed and loaded on Cerius^{2,} ™ as a graph file.

Single Crystal X-Ray Diffraction (SCXRD) Data Collection

A single crystal of Form G was attached to a glass fiber and mounted on the Siemens SMART system for the data collection at 173(2) K. An initial set of cell constants was calculated from reflections harvested from three sets of 20

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frames. These initial sets of frames were oriented such that orthogonal wedges of reciprocal space were surveyed. This produces orientation matrices determined from 300 reflections. Final cell constants were calculated from the actual data collection using a set of 8192 strong reflections.

Structure Solution and Refinement

The space group $P2_12_12_1$ was determined based on systematic absences and intensity statistics (8). A successful direct-methods solution was calculated that provided most non-hydrogen atoms from the E-map. Several full-matrix least squared/difference Fourier cycles were performed that located the remainder of the non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters unless stated otherwise. All hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters.

Structure Solution of Form G from Its X-Ray Powder Diffraction Pattern

To determine the crystal structure of polymorph G from its XRPD pattern, the following three steps were employed. First, the cell constants and lattice class were initially estimated by the Powder Indexing module. After a XRPD pattern was loaded, about 10–20 peaks were found and edited in the low two-theta angle region. TREOR90 was chosen to index the XRPD pattern to produce the initial estimate of the cell constants and crystal lattice system. Second, the lattice parameters were refined and a Pawley analysis of the peak profile and background parameters were performed by the Powder Fit module. Based on the rough cell constants and crystal lattice found in the first step, various space groups were explored to fit the background, cell parameters, peak profile, and intensities of the XRPD pattern. Third, by the Powder Solve module, possible arrangements and conformations of the molecules in the unit cell were sought and the crystal structure whose simulated XRPD pattern matched the experimental XRPD pattern was proposed. In this step, the number of degrees of freedom of the structure depends on the number of defined rigid bodies and the torsions connecting them. Two approaches, i and ii, were employed to define rigid bodies: (i) To test the capability of Cerius$^{2,\ TM}$ to calculate the correct molecular conformation and atomic coordinates, a total of 13 rigid bodies were defined, including the hydrogen atoms on the carbon or nitrogen atoms: $C^1O^1O^2$, C^2, C^3, C^4O^3, C^5, C^6O^4, O^5, C^7, C^8 - C^{13}, C^{14}, C^{15} - C^{20}, N^1, N^2. The total number of degrees of freedom is 18. (ii) The whole neotame molecule with the same conformation as the one determined by SCXRD was defined as one rigid body, with six degrees of freedom. Five cycles were used in the calculations for the two approaches and the number of steps was proposed by the software. The crystal lattice energy of Form G is 622 kJ/mol, estimated from the SCXRD structure by Cerius$^{2,\ TM}$ with Dreiding 2.21 force field (9). All computations were performed using the Powder Solve module, which is fully integrated within the Cerius$^{2,\ TM}$ molecular modeling environment on a 300 MHz SGI O2 (Silicon Graphics) workstation with 128 Mbytes of memory at the University of Minnesota Supercomputing Institute.

RESULTS AND DISCUSSION

Structure Solution by Single Crystal X-Ray Diffractometry

Table I lists the crystallographic data of the structure of polymorph G determined by SCXRD. The crystal of polymorph G is needle-shaped, and it belongs to $P2_12_12_1$ space group. Fig. 1a shows the conformation of the neotame molecule in the crystal structure of Form G determined by SCXRD, while Fig. 2a shows the corresponding crystal packing pattern looking down the *a* axis.

Structure Solution by Molecular Modeling

Table II lists the 15 peaks in the low two-theta region that were used in the powder indexing. After powder indexing and powder fit with Cerius$^{2,\ TM}$, XRPD led to the orthorhombic space group, $P2_12_12_1$, with unit cell dimensions, a = 30.9172, b = 11.8919, c = 5.5994 Å (Table III), which are almost identical (after interchanging the *a* and *c* axes) with those from SCXRD, a = 30.917(2), b = 11.8921(8), c = 5.5999(4) Å (Table I), suggesting an almost perfect match (R_{wp} = 2.33%) between the simulated XRPD pattern (after powder indexing and powder fit) and the experimental XRPD pattern. Considering that the SCXRD data were collected at 173(2) K, whereas the XRPD data were collected at room temperature, these similarities in cell dimensions show that the thermal expansivity of neotame anhydrate Form G is

Table I. Crystal Data and Structural Refinement of Neotame Anhydrate Polymorph G

Empirical formula	$C_{20}H_{30}N_2O_5$
Color/shape	colorless/needle
Crystal dimensions	0.45 × 0.18 × 0.18 mm
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.5999(4) Å b = 11.8921(8) Å c = 30.917(2) Å
Volume	2058.9(2) Å^3
Z	4
Formula weight	378.46
Density (calculated at 173(2) K)	1.221 Mg/m^3
Density (measured at 300 K)[a]	1.181 ± 0.008 Mg/m^3 (n = 5)
Absorption coefficient	0.088 mm^{-1}
F(000)	816
Diffractometer	Siemens SMART platform CCD
Wavelength of the X-rays	0.71073 Å
Temperature	173(2) K
θ range for data collection	1.83 to 25.05°
Reflection collected	12845
Independent reflections	3638 (R_{int} = 0.0344)
Absorption correction	SADABS (10)
T_{max}/T_{min}	1.000/0.775
Absolute structure parameter	–0.4(9)
Data/restraints/parameters	3638/0/248
R indices (I > 2σ(I) = 3131)[b]	$R1$ = 0.0342, $wR2$ = 0.0797
R indices (all data)[c]	$R1$ = 0.0429, $wR2$ = 0.0848
Goodness-of-fit[d] on F^2	1.040

[a] From helium pycnometry on the powder, reference 4.
[b] $R_1 = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$.
[c] $wR2 = [\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]]^{1/2}$, where $w = q / [\sigma^2(F_o^2) + (aP)^2 + bP + d + e\sin(\theta)]$.
[d] Goodness-of-fit = $S = [\Sigma[w(F_o^2 - F_c^2)^2] / (n - p)]^{1/2}$.

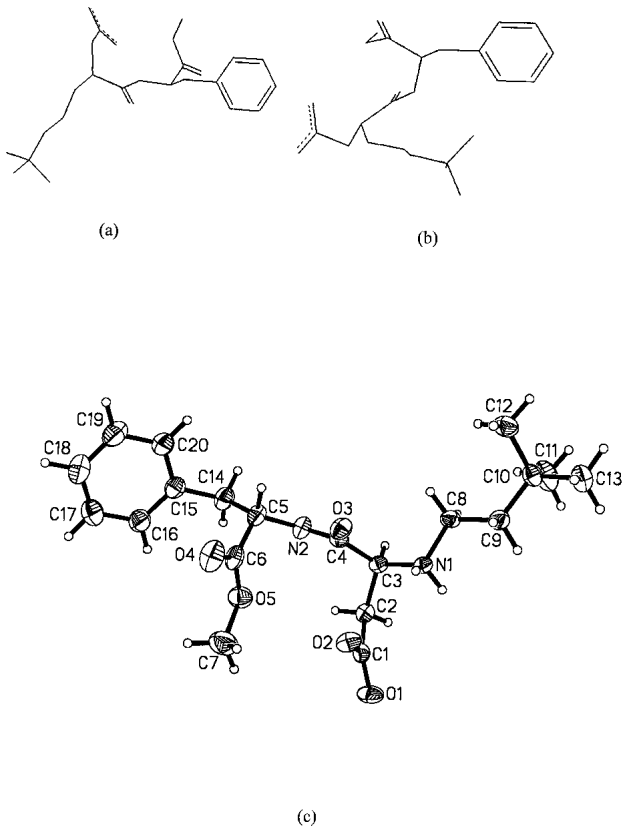

Fig. 1. Molecular conformations of neotame molecules in neotame anhydrate polymorph G determined by: (a) single crystal X-ray diffractometry (SCXRD); (b) Cerius$^{2,\ TM}$ (Accelrys) assuming that the number of rigid bodies is 13. In (a) and (b) the hydrogen atoms are omitted; (c) thermal ellipsoid diagram of the neotame molecule from singal crystal X-ray diffractometry (SCXRD).

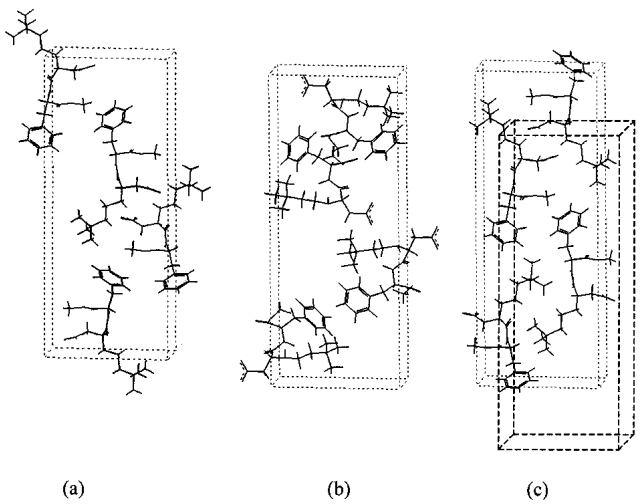

Fig. 2. Crystal packing patterns of neotame anhydrate polymorph G determined by: (a) single crystal X-ray diffractometry (SCXRD), looking down the *a* axis; (b) Cerius$^{2,\ TM}$ Powder Solve (Accelrys) assuming that the number of rigid bodies is 13, looking down the *c* axis; (c) Cerius$^{2,\ TM}$ Powder Solve, assuming that the number of rigid bodies is one, looking down the *c* axis. The unit cell after the translational operation (–1/4, –1/4, +1/4) is shown as a black-dashed line and is identical to the unit cell in (a).

Table II. X-Ray Powder Diffraction Peaks of Neotame Anhydrate Polymorph G Selected for Powder Indexing and Analysis by Cerius^{2,TM} Powder Solve (Accelrys)

2θ (degrees)	Relative intensity (%)	Miller indices	*d*-spacing *(Å)*
5.7048	89.96	200	15.479
7.9329	100.00	110	11.136
9.3913	45.36	210	9.4096
11.457	6.89	400	7.7173
13.645	3.55	410	6.4843
14.86	20.83	020	5.9568
15.144	23.06	120	5.8457
16.035	72.68	101	5.5228
17.21	47.59	600	5.1483
18.385	68.78	211	4.8219
18.749	30.87	610	4.7290
19.519	48.71	401	4.5442
20.937	26.97	411	4.2395
21.464	18.05	710	4.1366
22.638	55.96	511	3.9247

relatively small. However, Table I shows that the density calculated from SCXRD, 1.221 Mg/m^3 at 173(2) K, is 3.4% larger than the density of the powder, 1.181 ± 0.008 Mg/m^3 measured at 300 K by helium pycnometry (4). If thermal expansion is negligible, as deduced above, a possible explanation for this difference is the presence of appreciable concentrations of defects in the crystals of the powder that may reduce the measured density while not significantly influencing the density calculated from the SCXRD structure.

From Cerius$^{2,\ TM}$ Powder Solve, with 13 rigid bodies, the number of degrees of freedom is 18. The proposed number of steps is 36,800,000 for each cycle, with five cycles proposed altogether, which took about 11 days to complete the calculation. The final R_{wp} value, the lowest among the five cycles, is 25.0% (Table III). Fig. 1b shows the conformation of the neotame molecule and Fig. 2b shows the packing pattern of the crystal structure so determined. The two approaches, SCXRD and Powder Solve, give different conformations of the neotame molecule. It is expected that the molecular conformation determined by SCXRD is the actual molecular conformation. However, during the computation, Cerius$^{2,\ TM}$ Powder Solve also minimizes the conformational energy of the neotame molecule, in addition to calculating the cell constants and atomic coordinates by matching the calculated and experimental XRPD patterns. As determined by the molecu-

Table III. Crystal Structural Data of Neotame Anhydrate Polymorph G Determined by Cerius^{2,TM} Powder Solve (Accelrys) Assuming (a) 13 Rigid Bodies and (b) One Rigid Body

Number of rigid bodies	13	1
Number of degrees of freedom	18	6
Number of steps	36,800,000	7,000
R_{wp} (%)	25.0	17.2
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions, Å		
a	30.9172	30.9172
b	11.8919	11.8919
c	5.5994	5.5994

lar modeling, the conformational energies of the neotame molecule are 792.0 and 154.3 kJ/mol in the structures solved by SCXRD and Powder Solve, respectively, using the Dreiding 2.21 force field (9). The discrepancy arises from the fact that, when calculating the molecular conformation, Cerius$^{2,\ TM}$ Powder Solve does not take the influence of crystal packing into consideration, assuming the gaseous state of the molecule.

When only one rigid body is defined using the neotame conformation from the structure determined by SCXRD, the number of degrees of freedom is six and the proposed number of steps is 7,000 for each cycle of a total of five cycles proposed that took only about 30 min to perform the calculation. In this procedure, the crystal structure determined above by Cerius$^{2,\ TM}$ Powder Solve with 18 degrees of freedom was used at the starting structure. The final R_{wp} is 17.2%, which is lower than that with 13 rigid bodies (Table III). The better fit of the structure with one rigid body than that with 13 rigid bodies indicates that the molecular conformation determined by SCXRD is more appropriate than that produced by Cerius$^{2,\ TM}$ Powder Solve. In addition, the value of R_{wp}, 17.2%, suggests that, even the correct conformation may not necessarily lead to a perfect fit, which is the drawback of this software. Fig. 2c shows the packing pattern of the neotame molecules in the crystal structure determined by this approach.

Although the three crystal structures in Fig. 2 have almost the same unit cell dimensions, Fig. 2b clearly shows a different molecular packing pattern. After careful examination, Fig. 2c shows the same packing pattern as Fig. 2a, with Fig. 2c employing an alternate origin. The origin of Fig. 2c is related by a translational operation of (–1/4, –1/4, +1/4) to the origin of Fig. 2a. Thus, although Cerius$^{2,\ TM}$ Powder Solve can accurately calculate the cell constants and space group of neotame anhydrate polymorph G, it still cannot solve the crystal structure completely based solely on the XRPD pattern. The neotame molecule may simply be too large and too flexible for Cerius$^{2,\ TM}$ Powder Solve to handle reliably.

Fig. 3 presents the experimental XRPD pattern of neotame anhydrate polymorph G, and the XRPD patterns predicted by Cerius$^{2,\ TM}$, from the crystal structure by SCXRD and that by Cerius$^{2,\ TM}$ Powder Solve with the numbers of rigid bodies of 13 and one, respectively. The three predicted XRPD patterns show almost no differences, whereas the experimental XRPD pattern differs slightly from the predicted XRPD patterns in the relative intensities of some peaks, which may be attributed to preferred orientation in the sample with the needle-shaped crystals of polymorph G. The close similarity in the three predicted XRPD patterns suggests that Cerius$^{2,\ TM}$ assumes that there is little influence of the molecular conformational differences on the XRPD patterns, provided that the cell parameters and space group remain unchanged, or that the XRPD pattern contains insufficient information to distinguish conformational differences.

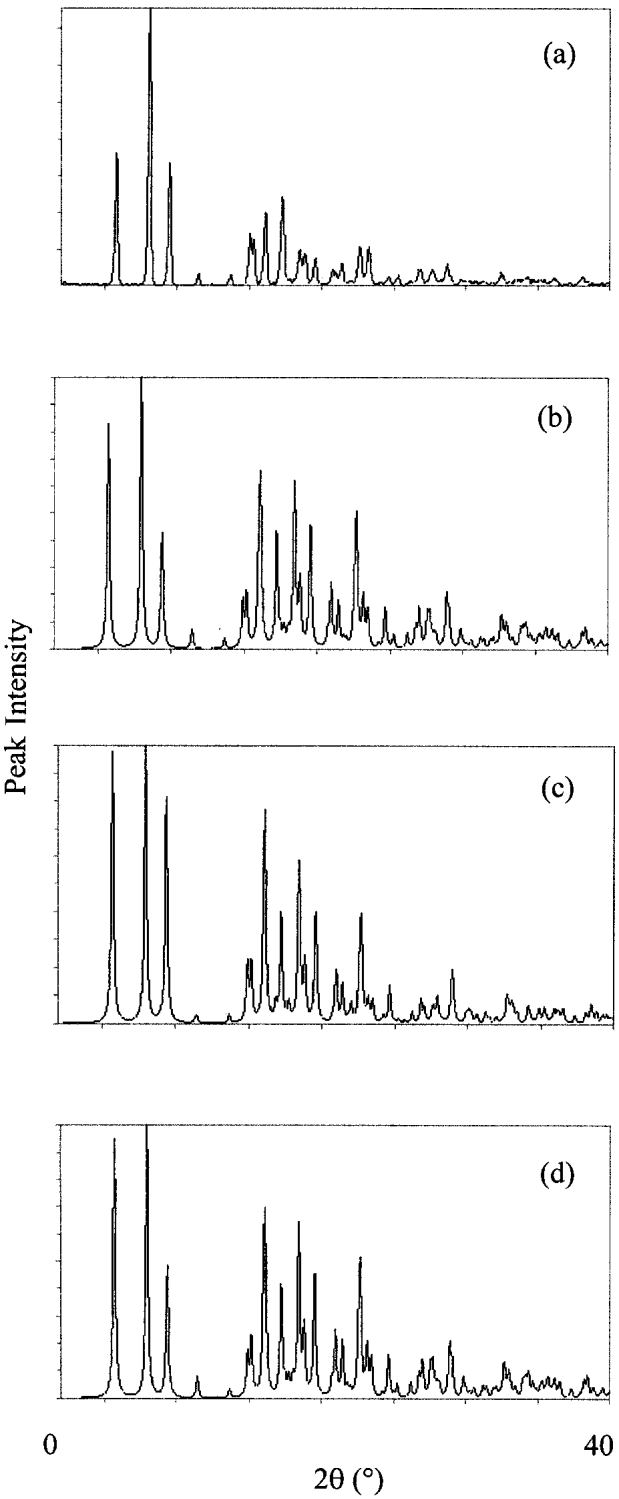

Fig. 3. The X-ray powder diffraction (XRPD) patterns of neotame anhydrate polymorph G: (a) experimental pattern; (b) pattern predicted by Cerius$^{2,\ TM}$ (Accelrys) from the crystal structure determined by single crystal X-ray diffractometry (SCXRD); (c) pattern predicted by Cerius$^{2,\ TM}$ from the crystal structure determined by Cerius$^{2,\ TM}$ Powder Solve, assuming that the number of rigid bodies is 13; (d) pattern predicted by Cerius$^{2,\ TM}$ from the crystal structure determined by Cerius$^{2,\ TM}$ Powder Solve, assuming that the number of rigid bodies is one.

CONCLUSIONS

The crystal structure of neotame anhydrate Form G was determined either by SCXRD or by applying Powder Solve in the Cerius$^{2,\ TM}$ molecular modeling program to the XRPD pattern. SCXRD determines a reliable crystal structure of Form G, which belongs to the orthorhombic space group, $P2_12_12_1$, with the unit cell constants of a = 5.5999(4), b =

11.8921(8), $c = 30.917(2)$ Å. Cerius^{2,} ™ Powder Solve leads to the same space group and almost identical unit cell dimensions. However, Cerius^{2,} ™ Powder Solve cannot determine the crystal structure completely without the correct molecular conformation, due to the conformational flexibility and relatively large size of the neotame molecule or due to the lack of sufficient information in the XRPD pattern. After the correct molecular conformation has been assumed, Cerius^{2,} ™ Powder Solve provides a crystal structure close to that determined by SCXRD.

ACKNOWLEDGMENTS

We thank The NutraSweet Company for financial support and the United States Pharmacopeial (USP) Convention for the award of a USP Fellowship in Drug Standards Research to Zedong Dong. We also thank Novartis Corporation for a Graduate Research Fellowship and Pharmacia Corporation for a Walter F. Enz award to Zedong Dong.

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