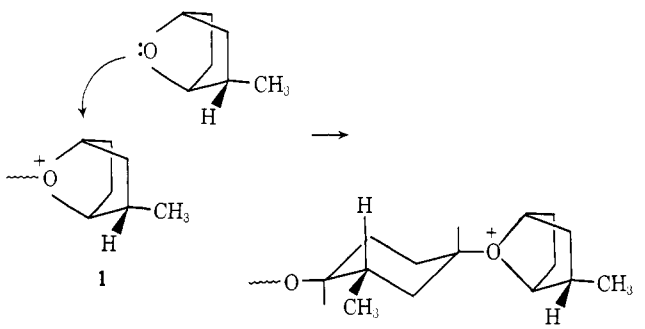

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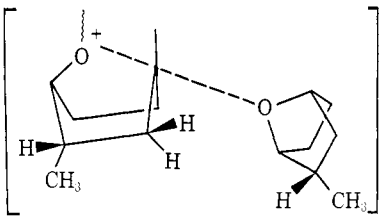
the attack of monomer on C-4. Table I shows that the k_p value of endo monomer is higher than that of exo monomer, and that the difference in this kinetic reactivity is ascribed mainly to the activation energy rather than the frequency factor.

From the heat of formation, Bedfoad, *et al.*,[10] reported that *endo*-MOBH is less stable (more strained) than *exo*-MOBH due to more severe interaction of the C-2 methyl group with the hydrogen atoms at C-3, C-5, and C-6. Therefore, it is not unreasonable to assume that the cyclic oxonium derived from *endo*-MOBH in the ground state is more strained than that derived from *exo*-MOBH. In the transition state **3** of the SN2 propagation, the steric interaction of C-2 methyl



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group with the neighboring hydrogen atoms will be relieved due to the stretch of C-4–O-7 bond. Thus, the increased strain energy in the ground state serves to decrease the activation energy, $\Delta E_p^{\ddagger}$.

(10) A. F. Bedfoad, A. F. Beezer, C. T. Moritimer, and H. D. Sppingall, *J. Chem. Soc.*, 3823 (1963).

Communications to the Editor

Stereoregularity of Polystyrene Determined by Carbon-13 Nuclear Magnetic Resonance Spectroscopy

We wish to report the preparation of polystyrenes with various tacticities, especially with random (atactic) configurations, and the determination of the stereoregularity by ^{13}C nmr spectroscopy. To our knowledge, all the reports on the stereoregularity of polystyrene have been concerned with isotactic- and syndiotactic-rich polymers.

The determination of the stereoregularity of polystyrene was first attempted by Brownstein, *et al.*,[1] using proton nmr spectroscopy. The three peaks in the methine proton spectrum of poly(styrene-β,β-d_2) were assigned to isotactic, heterotactic, and syndiotactic triads from a lower magnetic field. The authors concluded that polymers prepared by usual methods have syndiotactic-rich configurations. Bovey and coworkers studied in detail the proton nmr spectra of isotactic polystyrene.[2,3]

Recently, Segre and coworkers[4] observed six peaks in the methine proton spectrum of poly(styrene-d_7) and assigned the second peak from the lower magnetic field (216 Hz from TMS) to the *mmmm* pentad. They suggested the possibility of the presence of different heptads.

The ^{13}C nmr spectrum of polystyrene was first measured by Bovey, et al.,[5] revealing that the aromatic C-1 spectrum is similar to the α-methine proton spectrum. Recently, Nishioka and coworkers[6] determined ^{13}C nmr spectra of radically polymerized polystyrene. The aromatic C-1 spectra were analyzed in terms of triads, and they found that the observed triads did not obey Bernoullian statistics.

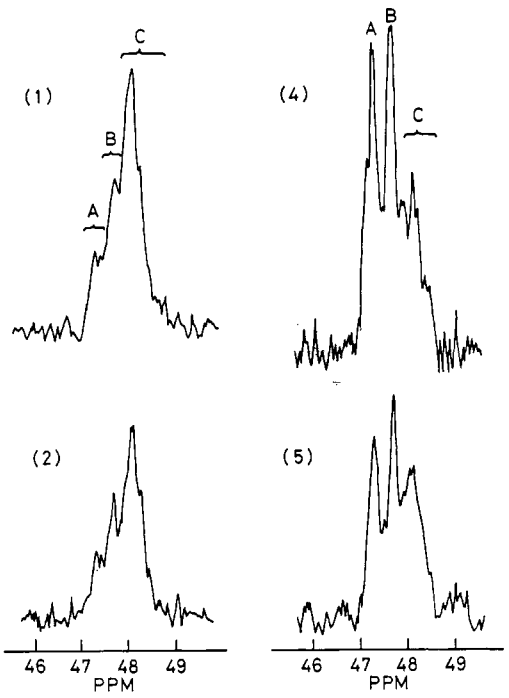

Figure 1. ^{13}C nmr spectra of aromatic C-1 of polystyrenes prepared with (1) BPO in toluene at 80° (256 scans), (2) *n*-butyllithium in *n*-heptane at 40° (400 scans), (4) BF_3 etherate in toluene at 30° (256 scans), and (5) BF_3 etherate in toluene at 0° (256 scans) ($^{13}CS_2$ as reference zero).

Polystyrenes were prepared under various conditions by using a high-vacuum technique. The results are shown in Table I. The 25.14-MHz ^{13}C nmr spectra were obtained at 60° with a JEOL PS-100 spectrometer equipped with an IS-100 proton irradiation sweep unit and an SD-HC heterospin decoupler. Polymers were dissolved in *p*-xylene–chloroform (2:3 v/v) or benzene–chloroform (1:4 v/v) to give about 20–40% solutions. The spectra obtained were the same in both solvent mixtures.

(1) S. Brownstein, S. Bywater, and P. J. Worsfold, *J. Phys. Chem.*, **66**, 2067 (1962).
(2) F. A. Bovey, F. P. Hood, E. W. Anderson, and L. C. Snyder, *J. Chem. Phys.*, **42**, 3900 (1965).
(3) F. Heatley and F. A. Bovey, *Macromolecules*, **1**, 301 (1968).
(4) A. L. Segre, P. Ferruti, E. Toja, and F. Danusso, *ibid.*, **2**, 35 (1969).
(5) L. F. Johnson, F. Heatley, and F. A. Bovey, *ibid.*, **3**, 175 (1970).
(6) Y. Inoue, A. Nishioka, and R. Chujo, Preprints of the 26th Annual Meeting of the Chemical Society of Japan, April 1972, p 1899.

TABLE I
POLYMERIZATIONS OF STYRENE[a]

No.	Polymerization conditions: Catalyst	[Catalyst], (mol %)	Solvent	Temp, °C	Time, hr	Conversion, %	$\bar{M}_n$[b]
1[c]	Benzoyl peroxide	2	Toluene	80	25	95.1	
2[d]	*n*-Butyllithium		*n*-Heptane	40	4	100	
3	$BF_3 \cdot O(C_2H_5)_2$	5	Toluene	40	24	58.0	
4	$BF_3 \cdot O(C_2H_5)_2$	5	Toluene	30	24	63.0	5.2×10^3
5[e]	$BF_3 \cdot O(C_2H_5)_2$	10	Toluene	0	22	80.7	
6	$BF_3 \cdot O(C_2H_5)_2$	5	Toluene	−40	48	45.9	1.96×10^4
7	$BF_3 \cdot O(C_2H_5)_2$	5	CCl_4	30	24	85.9	
8	$BF_3 \cdot O(C_2H_5)_2$	5	CH_2Cl_2	−25	20	83.5	1.08×10^5
9	PF_5	2	CH_2Cl_2	30	20	83.2	
10	PF_5	1	CH_2Cl_2	0	24	89.7	2.94×10^4
11	PF_5	2	CH_2Cl_2	−78	1	91.5	7.62×10^4

[a] Styrene, 2.7 g; solvent, 10 ml. [b] Molecular weights were measured by means of vapor pressure and membrane osmometers. [c] Styrene, 4.5 g. [d] Styrene, 40 g; *n*-heptane, 200 ml. [e] Toluene, 5 ml.

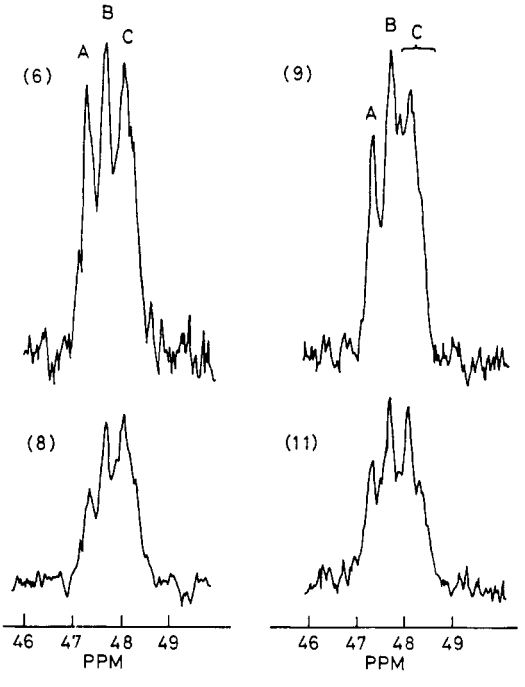

Figure 2. ^{13}C nmr spectra of aromatic C-1 of polystyrenes prepared with (6) BF_3 etherate in toluene at −40° (128 scans), (8) BF_3 etherate in CH_2Cl_2 at −25° (128 scans), (9) PF_5 in CH_2Cl_2 at 30° (256 scans), and (11) PF_5 in CH_2Cl_2 at −78° (196 scans) ($^{13}CS_2$ as reference zero).

In Figures 1 and 2 are shown ^{13}C nmr spectra of the aromatic C-1 carbon of polystyrenes. Three main peaks with small peaks and shoulders are seen in each figure. The estimated areas of the peaks A, B, and C are given in Table II. If the three main peaks are assigned to isotactic, heterotactic, and syndiotactic triads from the lower magnetic field, the triads obtained do not obey Bernoullian statistics; *e.g.*, polymer 4 has too small a heterotactic triad. Now, when the following assumption based on pentad sequences is made, it is seen that all the samples very nearly obey Bernoullian statistics; peak A includes *mmmm*, *mmmr*, *rmmr*, and *mrmm*, peak B includes *rmrm*, *rrmm*, and *mrrm*, and peak C includes *rrmr*, *rrrm*, and *rrrr*.

In Table II are also shown peak areas calculated on the assumption that Bernoullian statistics hold. The calculated values agree well with the observed. It is known that the polymerization of α-methylstyrene in a homogeneous phase

TABLE II
STEREOREGULARITY OF POLYSTYRENES FROM ^{13}C NMR SPECTRA

Polymer no.		Peak proportion: A	B	C	Racemic dyad, *r*
1	Obsd	0.12	0.21	0.67	
	Calcd	0.12	0.21	0.67	0.71
2	Obsd	0.13	0.24	0.63	
	Calcd	0.14	0.23	0.63	0.69
3	Obsd	0.39	0.28	0.33	
	Calcd	0.38	0.31	0.31	0.50
4	Obsd	0.39	0.29	0.32	
	Calcd	0.38	0.31	0.31	0.50
5	Obsd	0.29	0.30	0.41	
	Calcd	0.29	0.30	0.41	0.56
6	Obsd	0.26	0.28	0.46	
	Calcd	0.25	0.29	0.46	0.59
7	Obsd	0.32	0.32	0.36	
	Calcd	0.32	0.31	0.37	0.54
8	Obsd	0.20	0.26	0.54	
	Calcd	0.20	0.27	0.53	0.63
9	Obsd	0.22	0.29	0.49	
	Calcd	0.23	0.28	0.49	0.61
10	Obsd	0.21	0.31	0.48	
	Calcd	0.23	0.28	0.49	0.61
11	Obsd	0.25	0.32	0.43	
	Calcd	0.26	0.30	0.44	0.58

obeys Bernoullian statistics.[7] It seems reasonable that the polymerizations of styrene and α-methylstyrene proceed without a penultimate effect, since, for nonpolar monomers, interactions between side groups and catalyst may be too weak to cause the penultimate effect.

Polymers 1 and 2, which were radically and anionically polymerized, respectively, have syndiotactic-rich structures (69–71% racemic dyads); while polymers 3 and 4, which were polymerized with BF_3 etherate in toluene at 40 and 30°, respectively, have atactic structures containing 50% racemic dyads. Polymer 6 prepared at −40° has a configuration of 59% racemic dyads. In the case of cationic polymerization in a nonpolar solvent such as toluene, the syndiotacticity of polystyrene increased with decreasing polymerization temperature.

Polymerizations with PF_5 catalyst in methylene chloride solution gave a configuration of 61–58% racemic dyads in

(7) Y. Ohsumi, T. Higashimura, and S. Okamura, *J. Polym. Sci., Part A-1*, 3, 3729 (1965).

the temperature range 30 to −78°, as the spectra given in Figure 2 show.

The determination of stereoregularity of styrene-β,β-d_2 polymers, including the atactic polymer, using proton nmr spectroscopy, is in progress. It shows good agreement with the determination by ^{13}C nmr spectroscopy, and will be reported in the near future.

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Received June 5, 1972

The γ Turn. Evidence for a New Folded Conformation in Proteins

As the number of known protein structures has increased, it has become apparent that "hairpin bends," in which the direction of the polypeptide chain changes by 180°, occur relatively frequently.[1,2] It has also been suggested[1,2] that these bends may be important in determining the folding of the polypeptide chain. Venkatachalam[3] studied the allowed conformations of tripeptides and concluded that chain reversal could be achieved by four classes of bend which he designated types I, I′, II, and II′. The conformations of these bends has been discussed in detail elsewhere,[1-5] and need not be reviewed here, except to restate the conclusion that a reversal of the polypeptide chain direction could be achieved through a bend containing *at least four* α-carbon atoms. These so-called β bends have been commonly observed in globular proteins, notably at points where the polypeptide chain folds back and forth to form an extended antiparallel β structure.

Recently, Némethy and Printz[5,6] have proposed a new class of bend, containing only *three* α-carbon atoms. They propose the name γ turn, or 1–3 turn, for this new conformation, and discuss its relation to the β turn.

The purpose of this note is to present evidence which shows rather convincingly that a "γ turn" exists in the thermostable protease thermolysin, whose atomic structure has recently been determined.[7-9]

The segment of the protein which is of interest in the present context is Ser(25)–Thr(26)–Tyr(27). The apparent secondary structure in the vicinity of these residues is shown in Figure 1. This figure is essentially part of Figure 10 of ref 9, but drawn in more detail. Recently we have calculated an improved electron density map of thermolysin, based on five isomorphous heavy-atom derivatives. Study of this map has confirmed our initial interpretation of the conformation of the polypeptide chain in the vicinity of residues 25–27. The conformational angles[10] and hydrogen-bond distances for these residues, measured from our current model of the thermolysin structure, are listed in Table I. As will be discussed

TABLE I

Conformational angles, deg							
—Observed for thermolysin[a]—				—Predicted for the γ turn[b]—			
	φ	ψ	ω[c]		φ	ψ	ω
Ser(25)	−148	92	(180)	Ala(1)	172	128	−170
Thr(26)	86	−57	(180)	Ala(2)	68	−61	172
Tyr(27)	−114	148	(180)	Ala(3)	−131	162	

Hydrogen-bond lengths (H···O), Å			
Observed		Predicted[b]	
$N_{25}H_{25}\cdots O_{27}C_{27}$	1.8	$N_1H_1\cdots O_3C_3$	1.82
$N_{27}H_{27}\cdots O_{25}C_{25}$	1.6	$N_3H_3\cdots O_1C_1$	1.78

[a] The observed conformational angles have an estimated standard error of ±15°. Both the angles and bond lengths are subject to refinement of the thermolysin crystal structure. [b] Reference 6. [c] Reference 20.

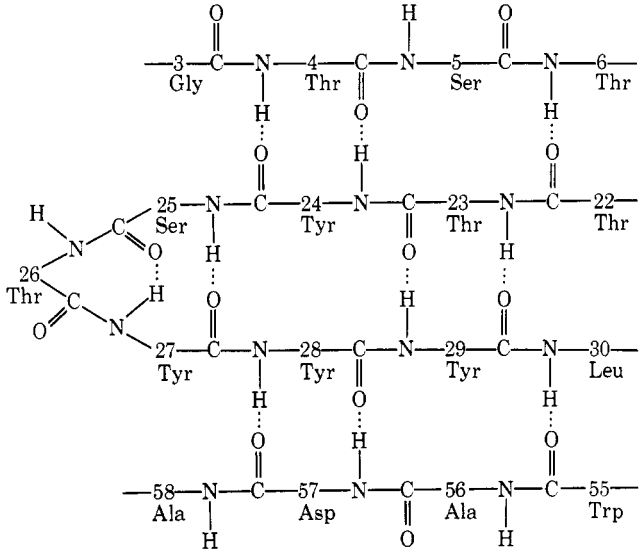

Figure 1. Secondary structure of thermolysin in the vicinity of the γ turn.

below, the proposed backbone conformation at Thr(26) is somewhat unusual, and we will therefore summarize the evidence that our interpretation of the electron density map at this point is essentially correct. First, the electron density in this part of the molecule is quite well defined, as evidenced by the fact that we were able to correctly identify[8,9] over 60% of the 80 amino-terminal residues of thermolysin, without reference to the chemically determined amino acid sequence.[11] In the vicinity of residues 25–27, density corresponding to all the backbone carbonyl groups and to the amino acid side chains can be seen. Secondly, the antiparallel β structure in the vicinity of these residues (Figure 1) tends to confirm their apparent conformation. For example, the apparent hydrogen bond (N_4—$H_4\cdots O_{24}$) (subscripts refer to residue numbers) tends to confirm the orientation of the peptide between C_{24}^{α} and C_{25}^{α}, and the hydrogen bond (N_{28}—$H_{28}\cdots O_{57}$) tends to confirm the orientation of the peptide between C_{27}^{α} and C_{28}^{α}. Finally, our confidence in interpretation of the "γ turn" is strengthened by the fact that it was made without foreknowledge of the prediction of Nemethy and Printz.[6]

The side chains of Ser(25), Thr(26), and Tyr(27) are all exposed to solvent; in fact, Thr(26) is at an "apex" of the molecule at the end of its longest diagonal. The hydrogen bond (N_{25}—$H_{25}\cdots O_{27}$) appears to be at least partly shielded

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(3) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(4) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 284 (1968).
(5) M. P. Printz, G. Némethy, and H. Bleich, *Nature (London), New Biol.*, **237**, 135 (1972).
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