(iodomethyl)-*p*-dioxane[9] in 49.5 g. (0.57 mole) of morpholine was refluxed for 1 hour. On cooling to −8°, 10 g. of light tan solid (morpholine hydroiodide) precipitated and was removed by filtration. Concentration of the filtrate at 50 mm. left 7 g. of dark crystalline 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane which, after recrystallization from ethanol, exhibited a m.p. and mixed m.p. with the product from A (above) of 109.5–111.5°.

C. From 4-(2,3-Epoxypropyl)-morpholine and HCl.—Hydrochloric acid (42 ml. of 10 *N* solution) was added with stirring and cooling to 4-(2,3-epoxypropyl)-morpholine during 20 minutes. The solution was then allowed to stand at room temp. for 3 days, after which sodium hydroxide (56 g. of 30% soln.) was added. The white precipitate which formed (20 g., 34%) was recrystallized from ethanol to give 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane, m.p. and mixed m.p. with the product from A (above), 107–110°.

4-(2,3-Epoxypropyl)-morpholine.—The reaction was performed exactly as in method A for the preparation of 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane above, except that the sodium hydroxide solution was added at 30° 1 hour after the initial exothermic reaction subsided. Fractionation of the residue after removal of the ether gave 231 g. (54%) of 4-(2,3-epoxypropyl)-morpholine, b.p. 93–95° (12 mm.), n^{30}D 1.4651, lit.[5] b.p. 95–97° (12 mm.).

Anal. Calcd. for $C_7H_{13}O_2N$: C, 58.72; H, 9.15; mol. wt., 143; neut. equiv., 143. Found: C, 59.33; H, 9.53; mol. wt., 148; neut. equiv., 143.

A crystalline residue of 43 g. (10%) of 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane remained after the distillation.

4-(2,3-Epoxypropyl)-2,6-dimethylmorpholine was isolated in 71–77% yield when 2,6-dimethylmorpholine (b.p. 58° (30 mm.), n^{30}D 1.4414) was treated with epichlorohydrin in the manner described in method A for 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane, and had the following properties: b.p. 71–73° (3 mm.), n^{30}D 1.4560.

Anal. Calcd. for $C_9H_{17}O_2N$: C, 63.13; H, 10.00; N, 8.19; neut. equiv., 171. Found: C, 62.94; H, 10.09; N, 8.48; neut. equiv., 170.8.

2,5-Bis-[4-(2,6-dimethylmorpholinyl)-methyl]-*p*-dioxane was prepared from *trans*-2,5-bis-(iodomethyl)-*p*-dioxane and 2,6-dimethylmorpholine in the manner described above for 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane by method B; yield 74%, m.p. 108–111° (recrystallized from diisopropyl ether). A mixed m.p. of this product and 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane was 93–104°.

Anal. Calcd. for $C_{15}H_{34}O_4N_2$: C, 63.13; H, 10.00; mol. wt., 342; neut. equiv., 171. Found: C, 62.57; H, 9.89; mol. wt., 341; neut. equiv., 167.7.

2,5-Bis-(1-piperidinylmethyl)-*p*-dioxane was prepared from piperidine and epichlorohydrin as in method A for 2,5-bis-(4-morpholinylmethyl)-*p*-dioxane (42% yield), and from piperidine and 2,5-bis-(iodomethyl)-*p*-dioxane as in method B (44% yield); m.p.'s (ligroin) and mixed m.p. of the two products were 101–104°. Binovic reported this compound as a viscous, non-distillable residue.[3]

Anal. Calcd. for $C_{16}H_{30}O_2N_2$: C, 68.04; H, 10.71; N, 9.93; mol. wt., 282; neut. equiv., 141. Found: C, 68.06; H, 10.87; N, 10.24; mol. wt., 296; neut. equiv., 139.6.

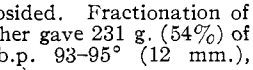
2,5-Bis-(1-pyrrolidinylmethyl)-*p*-dioxane was prepared in 28% yield from pyrrolidine and epichlorohydrin as in method A for the morpholine analog above. Attempts to prepare it from *trans*-2,5-bis-(iodomethyl)-*p*-dioxane and pyrrolidine were unsuccessful. The product had b.p. 131–138° (2 mm.), m.p. 107.5–109.5° (ligroin).

Anal. Calcd. for $C_{14}H_{26}O_2N_2$: C, 66.10; H, 10.30; N, 11.06: mol. wt., 254; equiv. wt., 127. Found: C, 67.23; H, 10.66; N, 10.06; mol. wt., 263; neut. equiv., 126.9.

Preparations of N-2,3-Epoxypropyldialkylamines.—The first two compounds listed in Table I were prepared by reaction of the dialkylamine and epichlorohydrin in a manner similar to the preparation of 4-(2,3-epoxypropyl)-morpholine (above). The last two were prepared as described for 4-(2,3-epoxypropyl)-2,6-dimethylmorpholine; *i.e.*, a longer reaction time did not afford the dioxane product.

TABLE I

PREPARATION OF N-2,3-EPOXYPROPYLDIALKYLAMINES,



$R_2NCH_2CHCH_2$ (I)

R_2	Formula	B.p. °C.	Mm.	n^{30}D	Yield, %
$-(CH_2)_5-$	$C_8H_{15}ON$	77–78	12[a]	1.4637	77
$-(CH_2)_4-$	$C_7H_{13}ON$	63–64	12[b]	1.4567	43
$(C_2H_5)_2=$	$C_7H_{15}ON$	58–60	20[c]	1.4277	20[e]
$(n\text{-}C_3H_7)_2=$	$C_9H_{19}ON$	75–76	12[d]	1.4307	63

[a] Reported: 86.5–88° (15 mm.),[10] 98–101° (25 mm.),[5] 85–86° (19 mm.), n^{18}D 1.4690.[4] [b] Reported: 73–75° (18 mm.),[3] n^{23}D 1.4620. This compound decomposed on standing and a satisfactory analysis could not be obtained; neut. equiv., 129.5, 128.9 (theor., 127). [c] Reported: 60–63° (20 mm.),[5] 62–65° (20 mm.),[7] 55–60° (15 mm.),[10] 42–43° (7 mm.), n^{18}D 1.4386.[3] [d] Reported: 83° (20 mm.), n^{18}D 1.4375.[3] [e] In some preparations a small amount (3%) of what was probably dioxane product (b.p. 110–112° (1.5 mm.), 96–98° (1 mm.), n^{30}D 1.4548) was isolated. (Rothstein and Binovic[3] reported b.p. 135–137° (5 mm.), n^{20}D 1.5850.) *Anal.* Calcd. for $C_{14}H_{30}O_2N_2$: C, 65.07; H, 11.70; N, 10.85; mol. wt., 258. Found: C, 65.63; H, 11.92; N, 9.51; mol. wt., 251. Another by-product (8%) was 1,3-bis-(diethylamino)-propanol-2, b.p. 104–108° (9 mm.), n^{30}D 1.4452, lit.[8] b.p. 114° (9 mm.).

SOUTH CHARLESTON, W. VA.

COMMUNICATIONS TO THE EDITOR

POLY-β-BENZYL ASPARTATES: OPTICAL ROTATION AND THE SENSE OF THE HELIX[1]

Sir:

The synthesis of high molecular weight polypeptides with asymmetric carbon atoms has stimulated both theoretical[2] and experimental[3] investigations of their optical activities. When in the helical configuration,[4] these polypeptides do not obey a single-term Drude equation but, instead, may be fitted to a phenomenological equation proposed by Moffitt and Yang.[3b] Although Moffitt's theoretical evaluation of certain parameters in

(1) This paper is Polypeptides. XX. For the previous paper in this series see E. R. Blout and G. D. Fasman in "Recent Advances in Gelatin and Glue Research," Pergamon Press, London, 1957, p. 122.

(2) (a) D. D. Fitts and J. G. Kirkwood, *Proc. Nat. Acad. Sci.*, **42**, 33 (1956); (b) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956); (c) W. Moffitt, *Proc. Nat. Acad. Sci.*, **42**, 736 (1956); (d) D. D. Fitts and J. G. Kirkwood, THIS JOURNAL, **78**, 2650 (1956).

(3) (a) P. Doty and J. T. Yang, *ibid.*, **78**, 498 (1956); (b) W. Moffitt and J. T. Yang, *Proc. Nat. Acad. Sci.*, **42**, 596 (1956); (c) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Poly. Sci.*, **XXIII**, 851 (1957); (d) J. T. Yang and P. Doty, THIS JOURNAL, **79**, 761 (1957).

(4) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 235 (1951).

TABLE I

Compound	Sample no.	$(\eta_{sp}/c)^a$	MW_w[b]	CHCl₃ soln. b_0[c]	CHCl₃ soln. $[\alpha]^{22}_{546}$	DCA soln. b_0[c]	DCA soln. $[\alpha]^{22}_{546}$
L-PBA	1354	0.09	4,000	+124	− 84		
	1145	.26	30,000	+411	−168		−18
D-PBA	1335	.16	19,000	−363	165		
	1355	.30	35,000	−425	174		+19
L-PBG	2-10	.21	24,000	−363	+ 14	0	−15
D-PBG	1322	.89	140,000	+482	− 18	0	+17

[a] c = 0.2 in DCA solution. [b] Estimated from the viscosity using the molecular weight (MW_w) calibration furnished by J. C. Mitchell, A. E. Woodward and P. Doty, THIS JOURNAL, **79**, 3955 (1957). [c] The optical rotations were measured between 365 and 578 mμ using a Rudolph high precision photoelectric polarimeter. From these data, b_0 was calculated using λ_0 = 212 mμ as in ref. 3b.

the equation is now known to be incorrect,[5] the equation may still be used empirically. In particular the quantity b_0 has been used as a measure of helix content of proteins[6,7] and polypeptides,[3b] and the *negative values of b_0 for* L-*isomers* of a few polypeptides have been taken to indicate the presence of a definite screw sense of the helical configurations of these molecules. It is the purpose of this communication to describe synthetic polypeptides having *positive b_0 values for* L-*isomers* and negative b_0 values for D-isomers.

We have synthesized poly-β-benzyl-L-aspartate[8] (L-PBA) and poly-β-benzyl-D-aspartate[9] (D-PBA) by the polymerization of the corresponding amino acid-N-carboxyanhydrides in chloroform solution using sodium methoxide initiation.[10] That no inversion of configuration occurred during the synthesis was shown by hydrolysis of the polymers with 6 *N* HCl to the amino acids. L-PBA upon hydrolysis gave $[\alpha]^{22}_{578}$ +23.9 in 6 *N* HCl, reported for L-aspartic acid $[\alpha]^{25}_D$ +24.6; D-PBA upon hydrolysis gave $[\alpha]^{22}_{578}$ −23.0. The optical rotatory dispersions of the polybenzyl aspartates were measured in chloroform and dichloroacetic acid (DCA) solutions. Pertinent data are summarized in the table along with data for poly-γ-benzyl-D-glutamate (D-PBG) for comparison.

In DCA solutions both L-PBA and L-PBG show nearly identical negative values for $[\alpha]_{546}$; the D isomer values are positive and the same for both polypeptides. In this solvent b_0 = 0, for each glutamate polymer indicating the normal dispersion behavior characteristic of random configurations.

In chloroform solutions anomalous rotatory dispersion characteristic of helical configurations is observed, and b_0 is positive for L-PBA and negative for D-PBA.[11] This is the converse of the situation with the corresponding optical isomers of polybenzyl glutamate. Two possible explanations are offered for the observed b_0 values in the polybenzyl aspartates. First b_0 values may be sensitive to the nature of the side chain, and in particular a chromophoric group such as the ester group attached to the β carbon atom may influence the absorption and dispersion of the helix (as suggested for the phenol group in poly-L-tyrosine).[12] If this is so, the sense of twist of the helical peptide core could be the same in both the poly-L-aspartates and poly-L-glutamates. The second and alternative explanation is that the helix in L-PBA has a different sense of twist from that in L-PBG.[13] A change in sense of the helix may be caused by steric effects of the large benzyl ester groups which lie closer to the peptide core in L-PBA than in L-PBG. The unexpected results reported here indicate the necessity for both investigations of the optical rotatory properties of other polypeptides and studies of the relevance of their optical properties to similar data from proteins.[14]

(5) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Nat. Acad. Sci.*, **43**, 723 (1957).
(6) C. Cohen and A. Szent-Gyorgyi, THIS JOURNAL, **79**, 248 (1957).
(7) C. B. Kretschmer, *J. Phys. Chem.*, **61**, 1627 (1957).
(8) A. Berger and E. Katchalski, THIS JOURNAL, **73**, 4084 (1951).
(9) E. R. Blout and R. H. Karlson, to be published.
(10) E. R. Blout and R. H. Karlson, THIS JOURNAL, **78**, 941 (1956).
(11) It should be noted that the value of b_0 seems to depend on the molecular weight—increasing in either a negative or positive sense as the molecular weight increases.
(12) A. Elliott, W. Hanby and B. Malcolm, *Nature*, **180**, 1340 (1957).
(13) A third explanation of the data would be that b_0 is not a satisfactory measure of helical content of all polypeptides and proteins.
(14) We are pleased to acknowledge the support of this work by the Office of the Surgeon General, Department of the Army, Washington 25, D. C.

(15) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Massachusetts.

THE CHILDREN'S CANCER RESEARCH FOUNDATION
BOSTON 15, MASSACHUSETTS
E. R. BLOUT[15]
R. H. KARLSON

RECEIVED JANUARY 16, 1958

AMINO ACID SEQUENCE IN THE REGION OF DIISOPROPYL PHOSPHORYL BINDING IN DIP-TRYPSIN

Sir:

Trypsin has been treated with P^{32}-diisopropyl phosphorofluoridate and the enzymatically inactive, labeled protein degraded by means of performic acid oxidation followed by tryptic hydrolysis. As has been reported elsewhere,[1] several large radioactive peptides were obtained by fractionation of the complex tryptic hydrolyzate by a combination of high voltage ionophoresis[2] and paper chromatography. The smallest peptide contained 15 residues and the largest, from which it appears to be derived, 55 residues. We now wish to report the sequence of amino acids in the smallest labeled peptide which has the composition: $(CySO_3H)_2$, Asp_2, Glu, Ser_3, Gly_4, Val, Pro, Lys, DIP.

Sequential degradation by aminopeptidase[3] indicated that asparagine was the N-terminal residue, whereas application of Sanger's method to the peptide yielded DNP-aspartic acid. The kinetics

(1) H. Neurath and G. H. Dixon, *Fed. Proc.*, **16**, 791 (1957).
(2) H. Michl, *Naturwissenschaften*, **40**, 390 (1953).
(3) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955).