17 Tsai, S. C., and Gaffney, E. V., Cancer Res. *46* (1986) 1471.
18 Heyns, A., Eldor, I., Vlodavsky, N., Kaiser, N., Fridman, R., and Panet, A., Exp. Cell Res. *161* (1985) 297.
19 Böhlen, P., Frater-Schröder, M., Michel, T., and Jiang, Z. P., in: Angiogenesis, p. 119. Eds D. Rifkin and M. Klagsbrun. Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor 1987.
20 Wright, T. C. Jr, Johnstone, T. V., Castellot, J. J., and Karnovsky, M. J., J. cell. Physiol. *125* (1985) 499.
21 Willems, C., Astaldi, G. C. B., DeGroot, P. G., Janssen, M. C., Gonsalvez, M. D., Zeijlemakerr, W. P., Van Mourik, J. A., and Van Aken, W. G., Exp. Cell Res. *139* (1982) 191.
22 Brown, M. T., and Clemmons, D. R., Proc. natl Acad. Sci. USA *83* (1986) 3321.
23 Brem, H., and Folkman, J., J. exp. Med. *141* (1975) 427.
24 Lutty, G. A., Thompson, D. C., Gallup, J. Y., Mello, R. J., Patz, A., and Fenselau, A., Invest. Ophthalm. vis. Sci. *23* (1983) 52.
25 Seyedin, S. M., Thomas, T. C., Thompson, A. Y., Rosen, D. M., and Piez, K. A., Proc. natl Acad. Sci. USA *82* (1985) 2267.
26 Williams, G. A., Eisenstein, R., Schumacher, B., Hsiao, K. C., and Grant, D., Am. J. Ophthalm. *97* (1984) 366.
27 Takigawa, M., Shirai, E., Enomoto, M., Hiraki, Y., Fukuya, M., Suzuki, F., Suiio, T., and Yugari, Y., Cell Biol. int. Reports *9* (1985) 619.
28 Schumacher, B., Grant, D., and Eisenstein, R., Arteriosclerosis *5* (1985) 110.
29 Homandberg, G., Williams, J., Grant, D., Schumacher, B., and Eisenstein, R., Am. J. Physiol. *120* (1985) 327.
30 Glaser, B. M., Campochiaro, P. A., Davis, J. L. Jr, and Sato, M., Archs Ophthalm. *103* (1985) 1870.
31 Lutty, G. A., Mello, R. J., Chandler, C., Fait, C., Bennett, A., and Patz, A., J. cell. Sci. *76* (1985) 53.
32 Lee, A., and Langer, R., Science *221* (1983) 1185.
33 Eisenstein, R., Goren, S. B., Schumacher, B., and Choromokos, E., Am. J. Ophthalm. *88* (1979) 1005.
34 Rastinejad, F., Polverini, P. J., and Bouck, N. P., Cell *56* (1989) 345.
35 Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D., and Sporn, M. B., J. biol. Chem. *258* (1983) 7155.
36 Bradford, M. M., Analyt. Biochem. *72* (1976) 248.
37 Gospodarowicz, D., Cheng, J., Lui, G. M., Baird, A., and Böhlen, P., Proc. natl Acad. Sci. USA *81* (1984) 6963.
38 Böhlen, P., Esch, F., Baird, A., and Gospodarowicz, D., EMBO J. *4* (1985) 1951.
39 Böhlen, P., Baird, A., Esch, F., Ling, N., and Gospodarowicz, D., Proc. natl Acad. Sci. USA *81* (1984) 5364.
40 Sharma, C. P., and Gehring, H., Biochem. biophys. Res. Commun. *39* (1986) 1243.
41 Koyama, H., Goodpasture, C., Miller, M. M., Teplitz, R. L., and Riggs, A. D., In Vitro *14* (1978) 239.
42 Danielpour, D., Dart, L. L., Flanders, K. C., Roberts, A. B., and Sporn, M. B., J. cell. Physiol. *138* (1989) 79.
43 Frolik, C. A., Wakefield, L. M., Smith, D. M., and Sporn, M. B., J. biol. Chem. *259* (1984) 10995.
44 Sporn, M. B., Roberts, A. B., Wakefield, L. M., and deCrombrugghe, B., J. Cell Biol. *105* (1987) 1039.
45 Anzano, M. A., Roberts, A. B., and Sporn, M. B., J. cell. Physiol. *126* (1986) 312.

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Preferential hydrophobic interactions are responsible for a preference of D-amino acids in the aminoacylation of 5′-AMP with hydrophobic amino acids

J. C. Lacey Jr*, N. S. M. D. Wickramasinghe and R. S. Sabatini

Department of Biochemistry, University of Alabama at Birmingham, Birmingham (Alabama 35294, USA)
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Abstract. We have studied the chemistry of aminoacyl AMP to model reactions at the 3′ terminus of aminoacyl tRNA for the purpose of understanding the origin of protein synthesis. The present studies relate to the D, L preference in the esterification of 5′-AMP. All N-acetyl amino acids we studied showed faster reaction of the D-isomer, with a generally decreasing preference for D-isomer as the hydrophobicity of the amino acid decreased. The β-branched amino acids, Ile and Val, showed an extreme preference for D-isomer. Ac-Leu, the γ-branched amino acid, showed a slightly low D/L ratio relative to its hydrophobicity. The molecular basis for these preferences for D-isomer is understandable in the light of our previous studies and seems to be due to preferential hydrophobic interaction of the D-isomer with adenine. The preference for hydrophobic D-amino acids can be decreased by addition of an organic solvent to the reaction medium. Conversely, peptidylation with Ac-PhePhe shows a preference for the LL isomer over the DD isomer.
Key words. Aminoacylation and peptidylation of 5′-AMP; stereoselectivity.

We would like to understand how the process of protein synthesis came into existence. One of the most challenging aspects of that problem is that of chirality. Although chemical syntheses of amino acids generally proceed to give equal amounts (racemic mix) of both optical isomers (D and L), biological systems almost exclusively use L-amino acids in protein synthesis. This in spite of the fact that D-amino acids can participate in each step of protein synthesis [1–3]. It is our general assumption that the origin of a biochemical system is based on the set of possible chemical reactions and that by studying the relevant ones, we can understand the origin of that biochemical system. In the case of protein synthesis, the relevant chemical reactions concern 5′-AMP.

Each amino acid is first activated by ATP, yielding the aminoacyl adenylate anhydride, with the amino acid covalently attached to the phosphate of 5′-AMP. The amino acid is then passed to become an ester of the ribose of the AMP residue which is at the 3′ terminus of every tRNA. The amino group of the aminoacyl tRNA then

attacks the carboxyl group of the growing peptide which is esterified to the ribose of the terminal AMP residue of an adjacent tRNA. The resulting peptide now has one additional amino acid and the peptide is still esterified to an AMP residue at the end of a tRNA. So every reaction of the amino acid (or peptide) in this sequence involves covalent attachment to AMP. Consequently we have studied formation of and reactions of aminoacyl AMP, both the anhydrides, in which the amino acid is attached to the phosphate, and the esters in which the amino acid is attached to the ribose.

Those previous studies had shown pronounced differences in the esterification rates of different N-acetyl amino acids with 5′-AMP[4] and Poly A[5] but we had not studied differences in the rates of esterification with D- and L-amino acids. Usher and his associates[6–9] had, however, in a series of papers, addressed this problem and had found that tert-butoxycarbonyl alanine (tBOC-Ala) in esterifying 2′ OH groups of dinucleoside monophosphates, showed a preference for the L-isomer. Without the bulky blocking group, alanine showed, as we report here, a preference for D-isomer. Leucine, without a blocking group showed a greater preference for the D-isomer than did alanine when esterifying the 2′ OH groups on poly adenylic acid (poly A). They noted no stereoselectivity in the reactions of these compounds with the 2′–3′ terminal positions of diinosine monophosphate (IpI) nor did they investigate stereoselectivity in the esterification of monoribonucleotides. Weber[10] had, however, reported a slight preference for D-serine in the esterification of the monoribonucleotide, adenosine-5-O-methyl phosphate. These studies by Weber[10], Usher and his co-workers[6–9], and by us, are aimed at understanding the molecular basis for the origin of the process of protein synthesis, including the preference for L-amino acids.

Several of our earlier studies have shown differences in the behavior of D- and L-amino acids in regards to 5′-AMP[11–14]. Because several reactions did show preferences for the L-isomer, and knowing that the evolutionary process has brought us to a system which almost exclusively uses L-amino acids, it was then somewhat surprising in preliminary experiments, with racemic Ac-D, D-Lhe imidazolide esterification of 5′-AMP[15], to find in a 5-min esterification reaction at room temperature, a 1.8/1 ratio of Ac-D-Phe AMP monoester to Ac-L-Phe AMP monoester. The research in this paper was carried out to understand that preference for the D-amino acid but also to discover how the D/L ratio varied in the esterification of 5′-AMP as a function of hydrophobicity in a series of N-acetyl amino acids. N-acetyl amino acids were used in these studies because the resulting esters are more stable than free amino acid esters and also to prevent the formation of peptides. Furthermore, because Usher[6–9] had found amino acids with bulky blocking groups showed selectivity for L-isomer in reactions with nucleotides, we investigated the peptidylation of 5′-AMP for stereoselectivity also, with the thinking that a peptide like Ac-PhePhe is in fact an amino acid with a bulky blocking group. Furthermore, because the D-preference seemed to be due to hydrophobic interactions, we studied the effect of adding dimethylformamide (DMF), which should interfere with hydrophobic interactions, on the D-preference.

Materials and methods

The N-acetyl amino acids, 5′-AMP, dimethyl formamide and carbonyl diimidazole were purchased from Sigma Chemical Company and used without further purification. All esterifications were done in triplicate with racemic N-acetyl amino acids and by the imidazolide method of Gottikh et al.[15] at 0 °C.

Early results of these studies with acetyl amino acids gave unduly high ratios of Ac-D-amino acid monoesters of 5′-AMP to Ac-L-amino acid monoesters of 5′-AMP because when the ratio of reactant amino acid to AMP is high (10/1), the monoester rapidly converts to bis 2′, 3′ AMP esters (diesters)[12]. The L-monoester converts to diester much faster than the D-monoester does. Therefore formation of diester depletes the amount of L-monoester and gives us an elevated D/L ratio for the monoesters. Only by using both a short reaction time (10 s) combined with an excess of 5′-AMP (2/1 AMP to amino acid) could we get reproducible and reasonable D/L values. This is because both factors favor the formation of monoester and virtually no diester was detected in the products from reactions under these conditions. The results in the discussion section on 2′, 3′ aminoacylation of 5′-AMP, which explicitly models the aminoacylation of tRNA, are in general agreement with and extend the observations made by Usher, Profy and Needels[6–9], regarding the effect of side chains on D-L selectivity in esterifying internal 2′ OH groups of RNAs.

The present data show a decided preference for the non biological D-isomer, and this coupled with the fact that Usher and his associates[6–9] found the tBOC-blocking group caused a preference for the L-isomer made us wonder whether peptides (which are really blocked amino acids) would show a preference for the L-isomer. To test this idea, we obtained pure samples of Ac-L-Phe-L-Phe and Ac-D-Phe-D-Phe using preparative HPLC with 40% CH_3OH, 0.05 M phosphoric acid at pH 2 with C_{18} reverse phase. The column was 19 mm × 30 cm with 5 μ particles and the flow rate was 4 ml/min. The purified peptides displayed only one peak in analytical HPLC.

We had earlier found that Ac-Phe in the form of the imidazolide completely racemizes in 4 h at room temperature. We therefore checked these peptides, Ac-L-Phe-L-Phe and Ac-D-Phe-D-Phe, for racemization under the same conditions but found none. We had also found that Ac-Phe will readily form bis 2′, 3′ esters of 5′-AMP. However, we found no evidence of such diester formation with these peptides. Presumably there is too much steric hindrance to allow esterification with second peptide.

Furthermore, these peptides react very much slower to form the monoesters than do Ac-L-Phe and Ac-D-Phe. Consequently we had to react for 5 min at room temperature. Otherwise, the preparation of peptidyl imidazolide and formation of the ester was the same as for the acetyl amino acids described earlier. The products were analyzed with reverse phase HPLC (5 μ C_{18} particles Phenomenex) using 40% CH_3OH in 0.05 M phosphoric acid, pH 2. In these cases the 2′ and 3′ monoesters (14 min and 15.5 min for LL and 15 and 19 for DD) elute before the peptides, which both elute at about 30–32 min.

The yields of these peptidyl monoesters were very low, in the range of 1%, so we found the most reproducible way of comparing the yields of ester was to express the ratio of HPLC ester integration counts to peptide integration counts. On this basis the comparative yields are shown in table 2 for quadruplicate runs.

Because hydrophobic interactions seem to be playing a role in the preference for the D-isomer, we carried out experiments with increasing concentrations of dimethylformamide (DMF) in the reaction mix to see if the organic solvent would interfere with those interactions and decrease the D/L ratio. Reaction conditions were the same as above except that reaction times were increased gradually as the DMF concentration increased (15% DMF, 30 s; 30% DMF, 1 min; 50% DMF, 2 min; 60% DMF, 2 min). The results showing the effect of DMF on the D/L ratio are in figure 2 for Ac-PHhe and Ac-Val.

Table 1. Ratio of Ac-D-amino acid AMP monoester to Ac-L-amino acid AMP monoester in reaction products from the esterification of 5′-AMP

Amino acid	HPLC Ret. time (min)**	Calc. relative hydrophobicity	D/L ratio*	HPLC column and % CH_3OH
Ac-Trp	45.5	1.0	2.22 ± 0.02	C_{18}, 20
Ac-Phe	31.0	0.68	1.63 ± 0.03	Phenyl, 9
Ac-Norleu	24.4	0.54	1.56 ± 0.04	C_{18}, 7
Ac-Norval	10.0	0.22	1.46 ± 0.03	C_{18}, 0
Ac-Met	8.8	0.19	1.30 ± 0.01	C_{18}, 7
Ac-Ala	4.0	0.09	1.18 ± 0.03	C_{18}, 0
Ac-Ile	18.5	0.41	6.03 ± 0.18	C_{18}, 9
Ac-Val	8.5	0.19	6.10 ± 0.27	C_{18}, 9
Ac-Leu	20.2	0.44	1.15 ± 0.03	C_{18}, 9

* Standard deviations are given after the averages. ** HPLC conditions in text. The reactions, by the method of Gottikh et al.[15], at 0 °C for 10 s (with 2/1 AMP/amino acid) were done in triplicate and separately assayed. At the end of the reaction period, the reactions were stopped by addition of 4 N HCl to a pH of 2. This 'freezes' the 2′/3′ distribution. HPLC separations, also at pH 2, were all in a Waters' reverse phase column 3.9 mm × 30 cm, either C_{18} or phenyl as indicated at the far right. All eluents were in 0.05 phosphoric acid, with the percent methanol indicated[11].

Table 2. Relative yields of peptidyl monoesters of 5′-AMP

Ac-peptide	Ratio of ester to peptide * with S.D.	% Yield based on peptide**
LL	0.56 ± 0.02	1.1
DD	0.29 ± 0.04	0.55

*HPLC integration counts; **Assuming the ester $\epsilon_{260} = 15{,}700$ i.e. AMP $\epsilon_{260} = 15{,}400$, peptide $\epsilon_{260} = 300$. Esterifications were as described in table 1 only at room temperature and for 5 min.

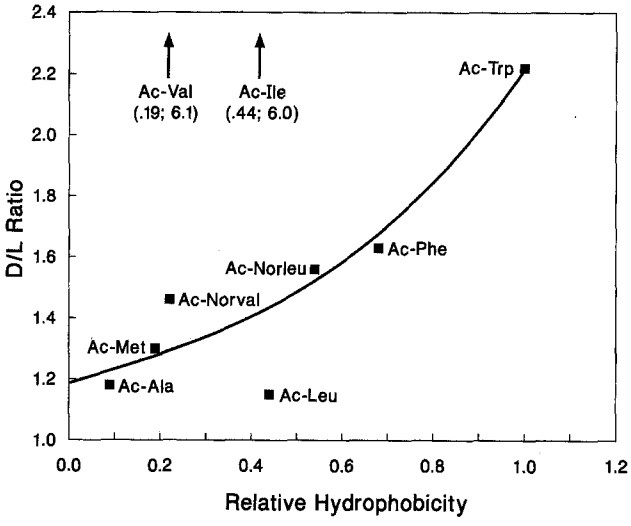

Figure 1. A plot of the D/L ratio in esterification of 5′-AMP as a function of the hydrophobicity of the amino acid as determined by HPLC (see text). Ac-Leu, Ac-Ile and Ac-Val, the branched amino acids, obviously do not fit this correlation, with Ac-Ile and Ac-Val showing extremely high D/L ratios and Ac-Leu showing a somewhat low D/L ratio.

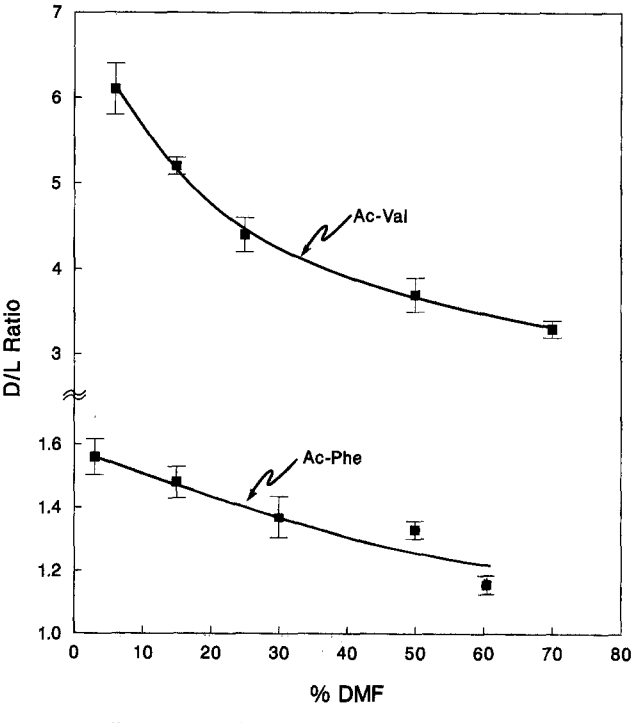

Figure 2. Effect of increasing concentrations of DMF in the reaction medium on the ratio of D/L with Ac-Val (upper) and Ac-Phe (lower) N-acetylaminoacyl esters of 5′-AMP (see text for reaction conditions).

We wished to correlate the D/L ratio with amino acid hydrophobicity but had no hydrophobicity data for Ac-Norleu and Ac-Norval so in order to include them we determined the retention time of the N-acetylamino acids in the study on a hydrophobic (C_{18}) reverse phase column (Phenomenex) 3.9 mm × 30 cm using 15% CH_3OH in 0.05 M phosphoric acid as eluent at 1.0 ml/

min. From the retention times we calculated relative hydrophobicity dividing the retention time of each amino acid by the retention time of the most hydrophobic amino acid Ac-Trp (table 1).

Results and discussion

Acetylaminoacylation. For the acetyl amino acids, excepting the branched amino acids Ac-Leu, Ac-Ile and Ac-Val, it is quite clear that as the hydrophobicity of the amino acid declines, (decreasing from top of table 1 to the bottom) the D/L ratio in the product ester does also. We believe this general trend is explained by two factors which we have previously reported. First, in a study of the four possible bis 2′, 3′ Ac-Phe esters of 5′-AMP (i.e. all combinations of 2′ and 3′ and D- and L-amino acid), we found by NMR studies that when the D-amino acid is in the 2′ position there is a much closer association between the phenyl ring and the adenine ring[12]. We believe the interactions involved are predominantly hydrophobic because we have previously documented the interaction of aliphatic, as well as aromatic, chains with adenine[16], the more hydrophobic side chains showing a stronger interaction. Secondly, esterifications take place predominantly at the 2′ position[13]. These observations would suggest that the transition intermediate of the esterification at the 2′ position can be stabilized by hydrophobic interaction of the amino acid side chains with the adenine ring. As mentioned above this happens more readily with the D-isomer than with the L-isomer. Because the interaction is hydrophobic, the more hydrophobic amino acids would show this effect to a greater extent than the less hydrophobic ones. Reuben and Polk[17] have also observed that the binding of amino acids to 5′-AMP varies directly as the hydrophobicity of the amino acid. If our above speculation that the transition intermediate is stabilized by interaction of the amino acid with the adenine ring, then we should see some correlation between the D/L ratio and the hydrophobicity of the amino acids with 5′-AMP. In figure 1 we have plotted the D/L ratio of monoesters in the esterification products as a function of the hydrophobicity of these amino acids. There is a relationship here which suggests our above speculation is correct. As mentioned before, Ac-Ile and Ac-Val do not correlate at all, the β-branching seeming to cause an extreme favoring of the D-isomer. Furthermore Ac-Leu, which is γ-branched, does not quite fit the curve for the linear amino acids. In fact its D/L ratio is a little low. The reason for the non-correlation of these branched amino acids is not immediately obvious to us. Furthermore, even Ac-Trp favors the D-isomer more than would seem tolerable by a system which eventually utilizes only L-Trp.

In our earlier studies on the origin of genetic coding, we had found that Ile and Trp were two of only four amino acids which did not show a correlation between their hydrophobicity rankings and the hydrophobicity rankings of their anticodon dinucleoside monophosphates[18]. Studies on the evolution of *E. coli* tRNAs for Ile[19] and Trp[20] suggested that they appeared late in evolution, perhaps after the bulk of the other assignments. The present data would suggest that the reason Trp and Ile, and perhaps Val as well were incorporated late is because of their high preference for D-isomer but also we found Ac-Ile and Ac-Val were less reactive than Ac-Phe and Ac-Leu in esterifying 5′-AMP[4] and poly A[5]. The high selectivity for D by Ac-Ile and Ac-Val in the present studies would certainly be additional factors against their early inclusion in the catalog of usable amino acids in any method of peptidyl synthesis which would eventually use only L-isomer. For, the other amino acids, however, the favoring of the D-amino acid in the formation of monoester would perhaps be offset by other factors favoring the L-isomer. Also once the system was established using L-amino acids, late amino acids may have only been accepted as L-isomers because the enzymatic systems involved were fashioned to accept only the L.

Effect of increasing concentrations of dimethylformamide on the D/L ratio. The results in figure 2 show that although in aqueous solution there is a great preference for Ac-D-Phe over Ac-L-Phe, the preference can be virtually eliminated by high concentrations of DMF. These results confirm our suggestion that stronger hydrophobic interactions between the hydrophobic D-amino acids and the adenine ring are responsible for the D-preference. The results with Ac-Val also show a decrease in the D/L ratio as the DMF increased; however, there seems a plateauing of the effect as if the extreme D preference for Ac-D-Val might be due to other factors in addition to hydrophobic interaction. What those factors might be is not readily apparent to us.

Peptidylation. The results of the peptidylation of 5′-AMP with Ac-L-Phe-L-Phe and Ac-D-Phe-D-Phe show, as the work of Usher et al.[6–9] would suggest, addition of a bulky blocking group reverses the isomeric preference. With the Ac-PhePhe peptides, the LL isomer is preferred about 2/1 over the DD isomer.

Origin of protein synthesis. The results presented here, we believe, have their own value simply because they show stereoselectivity in chemical reactions of biochemical molecules. Furthermore, the basis for the D-stereoselectivity does seem to be preferential hydrophobic interactions between reactants, and this D-preference can be considerably reduced by adding a water-miscible organic solvent to the reaction medium. While the results obviously do not explain how our contemporary system evolved to prefer L-amino acids, they do present us with an obvious difficulty that was somehow overcome along the way.

We need to discuss these results in relation to a model of ours for primitive protein synthesis. Based on our earlier experiments, we had proposed that 5′-AMP might serve to catalyze the formation of L-based peptides via a bis 2′, 3′ aminoacyl intermediate[21,22]. The present data show-

ing a preference for the D-isomer in the formation of monoester do not negate that model because the extreme favoring of D-isomer is only at the first step. Subsequent steps all involve bis 2′, 3′ esters and in the formation of diester, preexisting Ac-L-Phe monoester was shown to react 2.5 times as fast as preexisting Ac-D-Phe monoester[12]. During the formation of diester, the incoming D- and L-isomers reacted about the same, so the preference for D was lost at the formation of diester. However, the results showing that peptidylation can give an L-preference may require some rethinking of such models regarding the origin of protein synthesis. Because we know that evolution eventually devised a method of making only L-based peptides, the origin and evolution must have proceeded along lines which we see can favor the L-isomer given D-ribose nucleotides. The new insight furnished by previous and present data would suggest that intramolecular transfer of incoming amino acids from the adenylate anhydride to adenylate ester[14] and intermolecular transfer of the growing peptide might have been involved at some point. Both of these procedures favor the L-isomer. Furthermore, the favoring of the D-isomer may have been diminished if the reactions were carried out in a hydrophobic milieu perhaps at an oil-water interface as we have previously suggested[23].

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* To whom all correspondence should be addressed.

1 Calendar, R., and Berg, P., J. molec. Biol. *26* (1967) 39.
2 Yamane, T., and Hopfield, J., Proc. natl Acad. Sci USA *74* (1977) 2246.
3 Yamane, T., Miller, D. L., and Hopfield, J. Biochemistry *20* (1981) 7059.
4 Mullins, D. W. Jr, and Lacey, J. C. Jr, J. molec. Evol. *23* (1986) 76.
5 Lacey, J. C. Jr, Hall, L. M., and Mullins, D. W. Jr, Orig. Life *16* (1985) 69.
6 Profy, A. T., and Usher, D. A., J. Am. chem. Soc. *105* (1984) 5030.
7 Profy, A. T., and Usher, D. A., J. molec. Evol. *20* (1984) 147.
8 Usher, D. A., and Needels, M. C., Adv. Space Res. *4* (1984) 163.
9 Usher, D. A., Profy, A. T., Nolstrum, S. A., Needels, M. C., Bulack, S. C., and Lo, K. M., Orig. Life *14* (1984) 621.
10 Weber, A. L., J. molec. Evol. *25* (1987) 7.
11 Lacey, J. C. Jr, Hawkins, A. F., Thomas, R. D., and Watkins, C. L., Proc. natl Acad. Sci. USA *85* (1988) 4996.
12 Lacey, J. C. Jr, Thomas, R. D., Staves, M. P., and Watkins, C. L., Biochim. biophys. Acta *1076* (1991) 395.
13 Lacey, J. C. Jr, Thomas, R. D., Wickramasinghe, N. S. M. D., and Watkins, C. L., J. molec. Evol. *31* (1990) 251.
14 Wickramasinghe, N. S. M. D., Staves, M. P., and Lacey, J. C. Jr, Biochemistry *30* (1991) 2768.
15 Gottikh, K. H., Krayevsky, A. A., Tarussova, N. B., Purygin, P. O., and Tsilevich, L. L., Tetrahedron *26* (1970) 4419.
16 Lacey, J. C. Jr, Mullins, D. W. Jr, and Watkins, C. L., J. biomol. Struct. Dynam. *3* (1986) 783.
17 Reuben, J., and Polk, F., J. molec. Evol. *15* (1980) 103.
18 Lacey, J. C. Jr, and Mullins, D. W. Jr, Orig. Life *13* (1983) 3.
19 Staves, M. P., Bloch, D. P., and Lacey, J. C. Jr, Z. Naturforsch. *42C* (1987) 129.
20 Staves, M. P., Bloch, D. P., and Lacey, J. C. Jr, Orig. Life Evol. Biosphere *18* (1988) 97.
21 Lacey, J. C. Jr, Staves, M. P., and Thomas, R. D., J. molec. Evol. *31* (1990) 244.
22 Lacey, J. C. Jr, Thomas, R. D., Staves, M. P., Minic, V., and Watkins, C. L., Orig. Life Evol. Biosphere *19* (1989) 332.
23 Lacey, J. C. Jr, in: Prebiological Self Organization of Matter, pp. 117–130. Eds C. Ponnamperuma and F. Eirich. A. Deepak Pub., Hampton, VA, USA 1990.

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Potential aldose reductase inhibitors: 1,2,4-triazolidine-3,5-diones and 2-(3,4,5-trimethoxybenzoyl)-4,4-diethyl-3,5-isoxazolidinedione

I. H. Hall, R. A. Izydore[a], R. Simlot and O. T. Wong

Division of Medicinal Chemistry and Natural Products, School of Pharmacy, CB # 7360 Beard Hall, University of North Carolina, Chapel Hill (North Carolina 27599, USA), and [a] Department of Chemistry, North Carolina Central University, Durham (North Carolina 27707, USA)
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Abstract. 1,2,4-Triazolidine-3,5-diones and the 3,5-isoxazolidinedione were observed to be potent inhibitors of rat lens aldose reductase activity. In vivo in streptozotocin-diabetic rats, selected agents at 20 mg/kg/day, orally for 21 days reduced significantly the sorbitol levels of rbc, lens and sciatic nerves, suggesting that these derivatives may have some usefulness to treat clinical complications of diabetes mellitus.
Key words. Aldose reductase inhibitors; 1,2,4-triazolidine-3,5-diones; 3,5-isoxazolidinediones; diabetes.

Long-term diabetes leads to complications in other tissues including neuropathy (peripheral nerve dysfunction), nephropathy (intracapillary glomerulo sclerosis), vascular complications (capillary basement membrane thickness, pericyte loss in capillaries, aneurysm), retinopathy, cataracts, and skin and bone disorders[1–4]. In the last decade there has been considerable interest in aldose reductase inhibitors to control aldose reductase dependent polyol accumulation which initiates sugar cataract formation. Accumulation of polyol in the lens