The inhibition of peptidyl transferase activity by aminoacyl derivatives of some nucleoside aliphatic analogues

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Abstract

Aminoacyl (Phe,Gly) derivatives of nucleoside aliphatic analogues bearing a hydroxyalkyl chain have been prepared by the condensation of the alcohols with N-benzyloxycarbonyl-amino acid in the presence of DCC followed by hydrogenolysis in methanol. These compounds inhibit peptidyl transferase activity and binding of acceptor substrate to E. coli ribosomes. The inhibitory activity is not much affected by the nature of either the aminoacyl or the heterocyclic base residue. In the transfer reaction, no peptide bond formation occurs with the above compounds as acceptors.

Introduction

Recently, aliphatic analogues of nucleosides and nucleotides have attracted an increased attention and have been investigated both from chemical and biochemical points of view. Especially, the 2,3-dihydroxypropyl derivatives of naturally occurring pyrimidine and purine bases have been investigated in detail in this and other laboratories (cf.1,2 and references therein). It has been demonstrated2 that these compounds might assume a conformation similar to that of ribonucleosides. Only the (S)-enantiomeric derivatives are substrates for some nucleolytic enzymes1.

In this connection we have studied aminoacyl derivatives of nucleoside aliphatic analogues as potential substrates or inhibitors of peptidyl transferase. It has been shown that aminoacyl esters (particularly the Phe esters) of nucleosides, especially in adenine series show a pronounced "puromycin-like" activity both by inhibition of the amino-acid incorporation into proteins3 and by acting as acceptor substrates of the peptide chain during the peptide bond formation4. The acceptor activity of aminoacyl nucleosides
depends on the heterocyclic part, aminoacyl residue and sugar moiety: 2′(3′)-O-L-phenylalanyladenosine is as active acceptor as puromycin\(^4\),\(^5\) whereas 3′-O-L-phenylalanyl-2′-deoxyadenosine is a poor acceptor\(^4\),\(^5\) and 5′-O-aminoacyl derivatives\(^6\) and the 3′-O-aminoacyl ester of adenine arabinoside\(^7\) are inactive. The effect is evidently due to the presence of suitably oriented neighbouring hydroxylic function.

In the aliphatic series, the hydroxyl functions of 2,3-dihydroxypropyl derivatives (type III, IV) correspond to 2′- and 3′-positions of a ribonucleoside. The aminoacyl derivative of the type III or IV contains also a hydroxyl group in the neighbouring position. During the progress of our studies, a preliminary report has been published\(^8\) confirming the expected biological activity of such compounds. However, the examination was performed with the racemic derivative only. In this paper, we present results of our examinations of various aminoacyl derivatives of nucleoside aliphatic analogues upon the peptidyl transferase reaction.

\[
\begin{align*}
R_0\text{CH}_2\text{CH}_2\text{A} & \quad \text{CH}_3\text{CH}_2\text{A} & \quad R^1\text{OCH}_2\text{CH}_2\text{A} \\
\text{I} & \quad \text{II} & \quad \text{III} \\
\text{IV} & \quad \text{V} \\
R^1=R^2=\text{H} & \quad A = \text{adenin}-9\text{-yl} & \quad T = \text{thymin}-\text{yl} \\
R^1=\text{Z-Phe}, R^2=\text{H} & \quad \text{Phe} = \text{L-phenylalanyl} & \quad \text{Gly} = \text{glycyl} \\
R^1=R^2=\text{Z-Phe} & \quad \text{Z} = \text{benzoyloxycarbonyl residue} & \quad \text{Z} = \text{benzoyloxycarbonyl residue} \\
R^1=\text{Z-Gly}, R^2=\text{H} & \quad \text{Gly} = \text{glycyl} & \quad \text{Z} = \text{benzoyloxycarbonyl residue} \\
R^1=\text{Gly}, R^2=\text{H} & \quad \text{Z} = \text{benzoyloxycarbonyl residue} \\
\end{align*}
\]

\text{MATERIALS}

The starting aliphatic analogues of nucleosides, their racemates or (S)-enantiomers were prepared by condensation of the corresponding p-toluenesulfonyl derivatives with the sodium salt of the appropriate heterocyclic base (adenine,
thymine) by procedures described elsewhere\(^1,2\). \((R)-9-(2,3-
-dihydroxypropyl)adenine was synthesized from methyl 5-adenin-
-9-yl-5-deoxy-2,3-isopropylidene-D-ribofuranoside\(^10\) by acidic
hydrolysis followed by periodate oxidation and sodium boro-
ydride reduction\(^2\). All compounds were chemically and optical-
ly pure as checked by criteria described elsewhere\(^2\).

The aminoacyl derivatives were prepared by condensation of
the free nucleoside analogue with N-benzyloxycarbonyl-L-phenyl-
alanine or -glycine in the presence of N,N'-dicyclohexylcarbo-
diimide in pyridine. The N-benzyloxycarbonylaminoacyl deriva-
tive thus obtained was isolated by chromatography on a loose
layer of silica and crystallized. The purity of the product
was checked by thin-layer chromatography, analysis and IR
spectra as well as by amino-acid analysis after acidic hydro-
lysis (6 N HCl, 20 h at 110\(^0\)C). The reaction afforded mono-
aminoacyl derivatives as the main products. The preparation
of the bis-N-benzyloxycarbonylaminoacyl derivatives was per-
formed analogously with the excess of N-benzyloxycarbonylamino
acid. With dihydroxyalkyl derivatives, the reaction was ex-
pected to take place predominantly on the primary hydroxylic
function; no special attention was paid to the isomeric purity
of such mono-aminoacyl derivatives, owing to the expected easy
migration of the free aminoacyl derivatives (cf.\(^3\)).

Particular care was given to the removal of the protecting
groups from the N-benzyloxycarbonyl derivatives, owing to the
lability of the resulting aminoacyl esters in alkaline and
neutral media. As the most suitable alternative, a hydrogeno-
lysis in absolute methanol in the presence of palladium-on-
-charcoal catalyst and trifluoroacetic acid was used generally;
after the reaction had been completed, the acid was neutrali-
zied with pyridine and the aminoacyl derivative isolated and
freed from pyridinium trifluoroacetate by precipitation with
ether. The product obtained was dissolved in 1M acetic acid,
the concentration estimated spectrophotometrically and the
purity checked by paper chromatography and electrophoresis in
1M acetic acid. The aminoacyl esters thus obtained were essen-
tially pure and contained the product as the only ultraviolet-
-absorbing and ninhydrin-positive component.
The procedures described are demonstrated by the following general procedures:

**The preparation of N-benzyloxy carbonyl aminoacyl derivatives of nucleoside aliphatic analogues:** The nucleoside analogue (4 mmol) and the N-benzyloxy carbonyl amino acid (5 mmol) in pyridine (20 ml) were stirred for 15 min and N,N'-dicyclohexyl carbodiimide (2.5 g) was added afterwards. The mixture was stirred overnight at room temperature with the exclusion of moisture and afterwards water (5 ml) was added. After additional 1 h of stirring, water (100 ml) was added, the whole extracted with chloroform (three 25 ml portions), the extract washed with water (50 ml), dried over magnesium sulfate, evaporated at 40°C/15 Torr and freed from pyridine by repeated codistillations with toluene (three 25 ml portions) under the above conditions. The residue was applied to two loose layers of silica with fluorescent indicator (produced by Sevice Laboratories of the Institute) (40x16x0.3 cm) and developed in ethanol-chloroform mixture (5:95). The bands of the product were eluted with methanol (200 ml), filtered and evaporated in vacuo (see above); the residue was precipitated from methanol (5 ml) with ether (200 ml), the product collected with suction and dried at 0.1 Torr over phosphorus pentoxide. The yields and characteristics of the products thus obtained are summarized in Table I.

**The preparation of bis-N-benzyloxy carbonyl aminoacyl derivatives of nucleoside aliphatic analogues:** The reaction was performed analogously as above, with the nucleoside analogue (2 mmol), N-benzyloxy carbonyl amino acid (10 mmol), pyridine (10 ml) and N,N'-dicyclohexyl carbodiimide (2.5 g) for 24 h at room temperature. The yields and characteristics of products thus obtained are given in Table I.

**The preparation of mono- and bis-aminoacyl derivatives of nucleoside aliphatic analogues:** The appropriate protected aminoacyl derivative (see above) (1 mmol) was dissolved in dry methanol (25 ml), trifluoroacetic acid (0.2 ml) was added followed by 10% Pd/C catalyst (0.5 g, Koch-Light) and the whole hydrogenated at room temperature under stirring in
Table I. Preparation and Properties of Aminoacyl Derivatives of Aliphatic Nucleoside Analogue

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield, %</th>
<th>Amino acid base</th>
<th>$S_1^a$</th>
<th>$R_F$</th>
<th>$S_2^b$</th>
<th>$E^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib</td>
<td>62.8</td>
<td>0.90 Phe/A</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(RS)-IId</td>
<td>25.0</td>
<td>0.94 Phe/A</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIIb</td>
<td>64.0</td>
<td>0.86 Phe/A</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(R)-IIIb</td>
<td>69.0</td>
<td>0.90 Phe/A</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>44.0</td>
<td>1.92 Phe/A</td>
<td>0.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>44.0</td>
<td>0.98 Gly/A</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IVb</td>
<td>68.0</td>
<td>0.87 Phe/T</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IVc</td>
<td>42.2</td>
<td>1.76 Phe/T</td>
<td>0.58</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>(RS)-Vb</td>
<td>65.0</td>
<td>0.88 Phe/A</td>
<td>0.20</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>(RS)-Vc</td>
<td>42.8</td>
<td>1.82 Phe/A</td>
<td>0.47</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Id</td>
<td>72.0</td>
<td>1.12 Phe/A</td>
<td>-</td>
<td>0.65</td>
<td>1.11</td>
<td>-</td>
</tr>
<tr>
<td>(RS)-IId</td>
<td>54.0</td>
<td>1.02 Phe/A</td>
<td>-</td>
<td>0.64</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>44.3</td>
<td>0.93 Phe/A</td>
<td>-</td>
<td>0.72</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>(R)-IIId</td>
<td>45.5</td>
<td>0.90 Phe/A</td>
<td>-</td>
<td>0.72</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>62.0</td>
<td>2.12 Phe/A</td>
<td>-</td>
<td>0.77</td>
<td>1.28</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>55.5</td>
<td>1.04 Gly/A</td>
<td>-</td>
<td>0.64</td>
<td>1.54</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IVd</td>
<td>67.0</td>
<td>0.97 Phe/T</td>
<td>-</td>
<td>0.69</td>
<td>0.75</td>
<td>-</td>
</tr>
<tr>
<td>(S)-IVe</td>
<td>40.5</td>
<td>2.20 Phe/T</td>
<td>-</td>
<td>0.72</td>
<td>1.04</td>
<td>-</td>
</tr>
<tr>
<td>(RS)-Vd</td>
<td>62.5</td>
<td>1.17 Phe/A</td>
<td>-</td>
<td>0.75</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>(RS)-Ve</td>
<td>54.5</td>
<td>1.87 Phe/A</td>
<td>-</td>
<td>0.82</td>
<td>1.30</td>
<td>-</td>
</tr>
<tr>
<td>Z-Phe</td>
<td>-</td>
<td>-</td>
<td>0.65</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z-Gly</td>
<td>-</td>
<td>-</td>
<td>0.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.72</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td>Gly</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.65</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ TLC on plates of silica (Silufol UV$_{254}$) in chloroform-ethanol (95:5); $^b$ paper chromatography in 1-butanol-acetic acid-water (5:2:3, v/v); $^c$ paper electrophoresis in 1M acetic acid (referred to adenine); $^d$ (R) and (S) designate an absolute configuration, (RS) a racemic compound.
hydrogen atmosphere (1 atm) for 1-4 h. The progress of the reaction was followed by TLC in ethanol–chloroform mixture (1:9) till the starting compound disappeared. Afterwards, pyridine (1 ml) was added, the whole filtered through a cellite layer and the filtrate evaporated to dryness. The residual foam was precipitated from methanol (5 ml) with ether (100 ml), the product collected by filtration and washed with ether (200 ml). The residue was dried in vacuo, dissolved in 1M acetic acid (10 ml), filtered through paper (Schleicher/Schuell) filter from a small amount of insoluble material and the concentration estimated spectrophotometrically at pH 2.

In the amino acid analysis, 10 μl samples of the stock solutions were treated with 6 N HCl under the usual conditions and the quantitative analysis performed on an automatic amino–acid analyzer. The data obtained on spectrophotometrical measurements and amino acid analysis differed by less than 10% (the phenylalanine absorption has not been taken into account).

Before use, the stock solutions were freeze-dried, re-dissolved in water, adjusted to pH = 7 with 1 N sodium hydroxide at 0°C and made up with water to an appropriate volume.

As checked by paper chromatography and electrophoresis in 1M acetic acid (20 V/cm, 1 h), the products thus obtained are homogeneous both in ultraviolet-absorbing and ninhydrine-positive components and are not contaminated either by starting nucleoside analogues or by free amino acid within the limits of experimental error. The ultraviolet absorption spectra resemble those of starting nucleoside analogues. The yields and properties of products thus obtained are summarized in Table I.

METHODS

Ribosomes were prepared from Escherichia coli B as described elsewhere.

Transfer assay. The transfer of acetylleucyl residue from CpApCpCpA-(ac[14C]Leu) fragment to puromycin was measured according to Monro et al. Assay of the CpApCpCpA-[3H]Phe substrate binding to the ac-
RESULTS AND DISCUSSION

The biological activity of the aminoacyl esters of aliphatic nucleoside analogues and related derivatives was examined with the use of a technique consisting in the transfer reaction of ac[14C]Leu residue from ac[14C]Leu-pentanucleotide (the terminal fragment of acLeu-tRNA) to puromycin in the presence of E. coli ribosomes. The results are summarized in Table II for the whole series investigated. The data suggest that the inhibitory activity is limited to the aminoacyl esters of aliphatic analogues whereas the parent compounds do not exhibit any significant activity upon the transfer reaction. In agreement with the data, on polyphenylalanine synthesis de novo, the phenylalanyl ester of 2,3-dihydroxypropyladenine (IIIId) was found to be an inhibitor of the transfer reaction. We have investigated the both enantiomers of this derivative; both of them show approximately the same activity. This observation is of considerable interest: only the (S)-enantiomer ((S)-IIIId) can assume the conformation of the D-ribonucleoside (or its aminoacyl derivative) whereas the (R)-compound is comparable either to the L-ribonucleoside or to the 2'-epimer, i.e. adenine arabinose. However, the aminoacyl derivative of the latter was inactive in the transfer reaction.

The activity of phenylalanyl derivatives in the adenine series is not much dependent upon the character of the aliphatic chain; the optimum effect may be observed with the molecule IIIId bearing the aminoacyl group at the position γ with respect to the adenine base. The replacement of phenylalanyl by glycyl, or of adenine by thymine residues results in a small decrease of the activity. Thereby, the aliphatic analogues differ most significantly from the corresponding interchanges in nucleoside series. Therein, replacement of Phe by glycine, or the replacement of adenine by thymine results in a considerable loss of activity (by 2-3 orders of magnitude). Similarly to the nucleoside series, the substitution of the dihydroxyalkyl chain by two Phe residues does not influence the activity of the analogue.
Table II. Effect of Aminoacyl Derivatives of Aliphatic Nucleoside Analogues upon Transfer Reaction and Binding of Acceptor Substrate (Conditions, see Methods)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Aminoacyl residue</th>
<th>Aliphatic residue</th>
<th>Base</th>
<th>50% Inhibition ($10^{-3}$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transfer reaction</td>
<td>Binding of CACCA-Phe</td>
</tr>
<tr>
<td>Id</td>
<td>Phe</td>
<td>2-Hydroxyethyl</td>
<td>Adenine</td>
<td>3</td>
</tr>
<tr>
<td>(RS)-IId</td>
<td>Phe</td>
<td>(RS)-2-Hydroxypropyl</td>
<td>Adenine</td>
<td>3a</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>Phe</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>1</td>
</tr>
<tr>
<td>(R)-IIId</td>
<td>Phe</td>
<td>(R)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>1</td>
</tr>
<tr>
<td>(S)-IIIe</td>
<td>Phe$_2$</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>1</td>
</tr>
<tr>
<td>(S)-IIIf</td>
<td>Gly</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>2</td>
</tr>
<tr>
<td>(S)-IVd</td>
<td>Phe</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Thymine</td>
<td>3a</td>
</tr>
<tr>
<td>(S)-IVe</td>
<td>Phe$_2$</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Thymine</td>
<td>2</td>
</tr>
<tr>
<td>(RS)-Vd</td>
<td>Phe</td>
<td>(RS)-3,4-Dihydroxybutyl</td>
<td>Adenine</td>
<td>2a</td>
</tr>
<tr>
<td>(RS)-Ve</td>
<td>Phe$_2$</td>
<td>(RS)-3,4-Dihydroxybutyl</td>
<td>Adenine</td>
<td>1a</td>
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<tr>
<td>(S)-IIib</td>
<td>Z-Phe</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>3a</td>
</tr>
<tr>
<td>(S)-IIIc</td>
<td>Z-Gly</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>2a</td>
</tr>
<tr>
<td>(S)-IIId</td>
<td>None</td>
<td>(S)-2,3-Dihydroxypropyl</td>
<td>Adenine</td>
<td>3a</td>
</tr>
</tbody>
</table>

*a* No inhibition at $10^{-2}$M
In order to localize further the site of action of the above compounds, we have studied their effect on the binding of substrates to the active site of peptidyl transferase. As indicated by the results in Table II, the analogues inhibiting the overall transfer reaction prevent the binding of the acceptor substrate to the acceptor site of peptidyl transferase. The inhibition of this binding was reached at a concentration higher by one order of magnitude than in the case of inhibition of the transfer reaction. However, mutual relation of inhibitory activities of individual derivatives remains preserved. Similar difference in the action on the transfer reaction and on the binding of acceptor substrate was observed also with gougerotin\textsuperscript{12-15}.

In contrast to the inhibition of the binding of the acceptor substrate, the analogues tested either do not exert any significant effect on the binding of the donor substrate (CACCA-acLeu) or even stimulate the binding slightly. These results indicate that the inhibition of peptidyl transferase by the above compounds can be explained by their inhibitory effect on the binding of acceptor substrate to peptidyl transferase. This interpretation is in agreement with the finding that both peptidyl transferase and binding of acceptor substrate are unaffected by Z-Phe and Z-Gly derivatives which have blocked α-amino group of the amino acid residue and therefore should not interact with the acceptor site.

When compared with other inhibitors acting on acceptor site of peptidyl transferase, the most active analogues, (S)-IIId, (R)-IIId, (S)-IIIe and (RS)-Ve, are less effective approximately by one or two orders of magnitude than chloramphenicol and gougerotin\textsuperscript{12-15}.

In spite of our effort, we were not able to find any acceptor activity of the compounds examined, which is typical for the derivatives of puromycin or aminocycl adenosines. In this respect our results differ from those of Gottikh et al.\textsuperscript{8} who described the acceptor activity with the racemic phenylalanine ester of 9-(2,3-dihydroxypropyl)adenine (IIIId) and also from the result of Chládek et al.\textsuperscript{17} who found the acceptor activity of another open chain adenosine analogue. The different be-
haviour of our compounds and the latter derivatives might be connected with the conformation of the molecule; whereas the 2-(Adenin-9-yl)-4-methylol-3-oxapentane-1, 5-diol molecule might still preserve a conformation similar to that of adenosine, the open-chain hydroxyalkyl analogues studied in this paper possess an increased freedom of rotation and, thereby, probably adopt a conformation different from that required for the acceptor molecule.

It should be mentioned in this connection that aminoacyl esters of aliphatic analogues of nucleosides are stable under the conditions of peptidyl transferase reaction. Compounds (S)-IIId and (S)-IIIf do not undergo any cleavage of the ester linkage during incubation with ribosomes (30 minutes, 37°C, 0.01M Tris pH 7.8, 0.01M MgCl₂, 0.03M NH₄Cl, ribosomes 3 mg/ml), as demonstrated by paper electrophoresis.

Thus, it may be concluded that the activity of the aminoacyl esters of nucleoside hydroxyalkyl analogues differs in principle from that of aminoacyl esters of nucleosides or that of puromycin. It follows from the fact that (1) both 2'-epimers are active; (2) the activity is not much affected by the replacement of phenylalanine by glycine ester linkages; (3) both adenine and thymine derivatives exhibit the inhibitory activity. Furthermore, (4) the compounds do not show acceptor-substrate activity in the peptidyl transferase assay.

The conformation of the molecule seems to display the most important role in the activity of the compounds. In addition to the role of the aliphatic chain and its substitution, the forces involved might consist in the mutual interaction of the heterocyclic base and aminoacyl residue stabilizing the conformation. Such interaction would be strongest with adenine and phenylalanine residues.

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