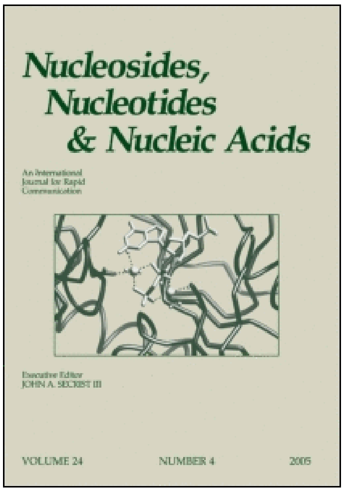
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Peptidinol Adenylates

Annett Kreimeyer[a,b]; Philippe Marliere[a]
[a] Unité de Biochimie Cellulaire, Paris, France [b] Faculté de Pharmacie, Université de Louis Pasteur, Illkirch, France

To cite this Article Kreimeyer, Annett and Marliere, Philippe(1998) 'Synthesis of Peptidinol Adenylates', Nucleosides, Nucleotides and Nucleic Acids, 17: 12, 2339 — 2350

To link to this Article: DOI: 10.1080/07328319808004322

URL: http://dx.doi.org/10.1080/07328319808004322

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES & NUCLEOTIDES, 17(12), 2339-2350 (1998)

Synthesis of Peptidinol Adenylates

Annett Kreimeyer[§], **Philippe Marlière***

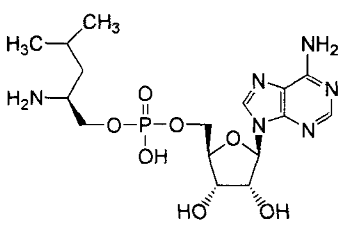
Unité de Biochimie Cellulaire , URA CNRS 1129
Institut Pasteur, 28 rue du Dr. Roux, 75015 Paris, France

Abstract: Peptidinol adenylates were assembled by condensing the carboxyl group of N-blocked peptides with the alkylamino group of leucinol 5' adenosine phosphodiester. The latter was prepared as protected precursor from adenosine and leucinol by phosphite chemistry. The title compounds, which mimic aminoacyl adenylates, are designed to penetrate microorganisms *via* peptide permeases and to interfere with genetic translation.

The chemical diversity of polypeptides synthesized through genetic translation on ribosomes originates from modifications of amino acids before and after esterification of tRNAs.[1] So far, no modification of aminoacyl adenylates, the mixed anhydride compounds that are transiently formed from amino acids and ATP by aminoacyl-tRNA synthetases before reacting with tRNAs has been reported. We undertook to explore the metabolic diversification of polypeptide biosynthesis by delivering synthetic analogues of aminoacyl adenylates within microbial cells. A long term prospect of this study is to develop activating tags different from adenylate, so that any acyl group attached to such a tag would be transferred to a tRNA by a cognate acyl-RNA synthetase specific for that tag, and then incorporated in proteins. Several difficulties are anticipated in reprogramming genetic translation according to this scheme, the first of which being the permeation of fragile entities such as aminoacyl nucleotide anhydrides. As a first step, we opted to study the intracellular delivery of stable analogues of anhydrides, i.e. aminoalcohol adenylates.[2] These compounds were

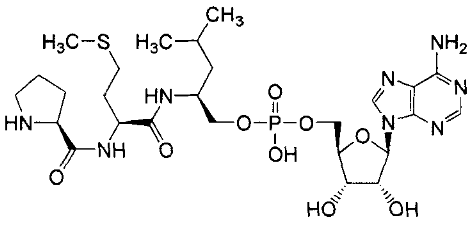
---

[§] present address: Université de Louis Pasteur, Faculté de Pharmacie, 74, route du Rhin, 67401 Illkirch, France described as very potent inhibitors of purified aminoacyl-tRNA synthetases, with dissociation constants in the micromolar range.[2] In spite of this potency, they are innocuous to living cells which they fail to penetrate. A logical approach to overcome this impermeability is to extend aminoalcohol adenylates into peptidinol adenylates so as to permit entry of such adducts *via* oligopeptide permeases in microorganisms, followed by the release of aminoalcohol adenylates by intrabacterial peptidases. Both permeases and peptidases indeed display a broad activity toward diverse substrates.[3] The validity of such a strategy employing peptides as metabolite carriers has already been demonstrated.[3-6] Here we report the preparation of two peptide adducts of leucinol adenylate, by direct C-terminal linkage and through an ε-lysine spacer.



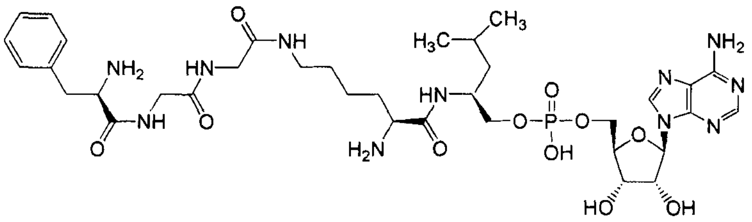
Leucinol-AMP

1



Pro—Met—Leucinol-AMP

2



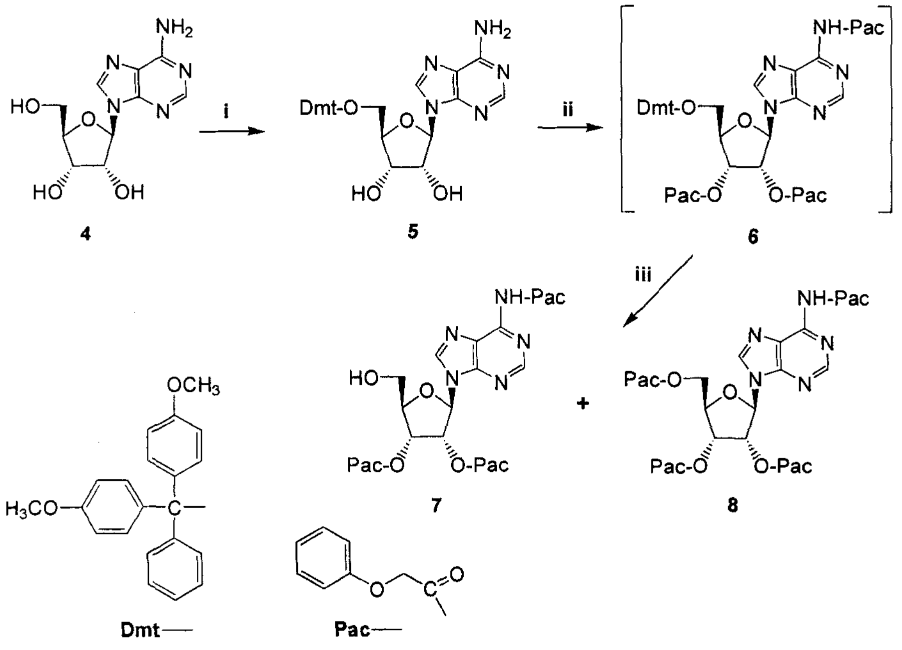
Phe— Gly—Gly— Nε-Lys— Leucinol-AMP

3

The synthetic route to peptidyl leucinol adenylate derivatives (**2**, **3**) proceeds through condensation of adenosine and leucinol as building unit *via* a phosphorylation reagent, which is then linked to two different peptides, Pro-Met and Phe-Gly-Gly-Lys. A published synthesis of several aminoalcohol adenosine 5′-monophosphate derivatives required long reaction times especially when performed on a large scale.[7] Therefore, an alternative to the construction of the phosphodiester linkage between adenosine and the aminoalcohol leucinol was elaborated, using phosphorylation methods developed for nucleic acid chemistry.

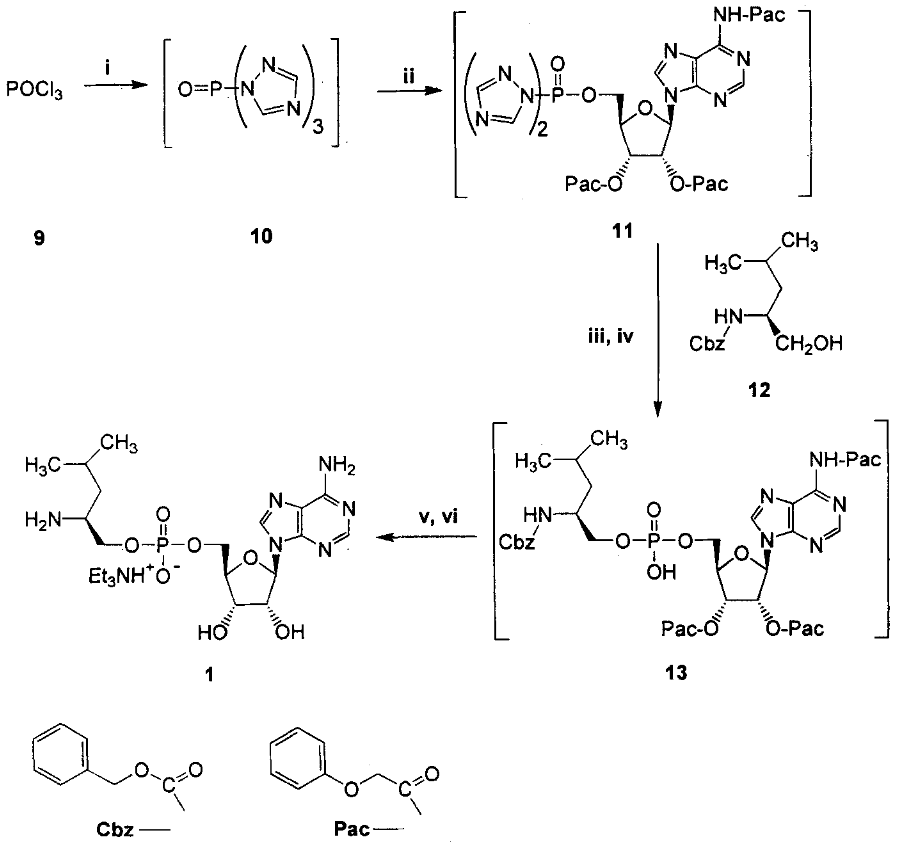
The main problem associated with the preparation of compound **1** is the combination of protecting groups. Indeed, it is known that drastic basic conditions used for the deblocking of common N-acyl protecting groups of adenosine result in the cleavage of the nucleotide-peptide phosphodiester bond.[8,9] However, no decomposition was observed in weak alkaline solution.[10] Recently, the phenoxyacetyl (Pac) group has been introduced into oligonucleotide synthesis for the protection of the amino function of adenine.[11] It can be removed by methanolic ammonia solution in less than one minute.[12] These findings led us to examine the use of the Pac group to protect the exocyclic amino function of the base as well as the hydroxyls at positions 2′ and 3′ of the sugar moiety of adenosine. Since the presence of a free amino group in the vicinity of the phosphodiester bond is reported to reduce the stability of this linkage in alkaline solutions[8], the benzyloxycarbonyl (Cbz) group was introduced to protect the amino function of leucinol (**12**). The three-step synthetic pathway to the protected derivative of adenosine (**4**) is outlined in Scheme 1. It consisted in the tritylation of the 5' hydroxy function of adenosine followed by the acylation of its 2′ hydroxy, 3′ hydroxy and amino groups with phenoxyacetic anhydride and removal of the dimethoxytrityl (Dmt) group to give the triphenoxyacetyladenosine derivative **7** in 48 % yield together with compound **8** in 4 % yield, resulting from a partial loss of the trityl group during the acylation step.

In a first attempt to combine **7** and **12** *via* a phosphodiester linkage, we tried to perform the phosphoramidite phosphite triester approach using 2-cyanoethyl-



SCHEME 1. **i**: dimethoxytrityl chloride, pyridine/DMF (1/1), 0 °C, 12 h; **ii**: phenoxyacetic anhydride, pyridine, 0 °C, 4.5 h; **iii**: 2 % benzenesulphonic acid in dichloromethane/methanol (7/3), 5 °C, 20 min.

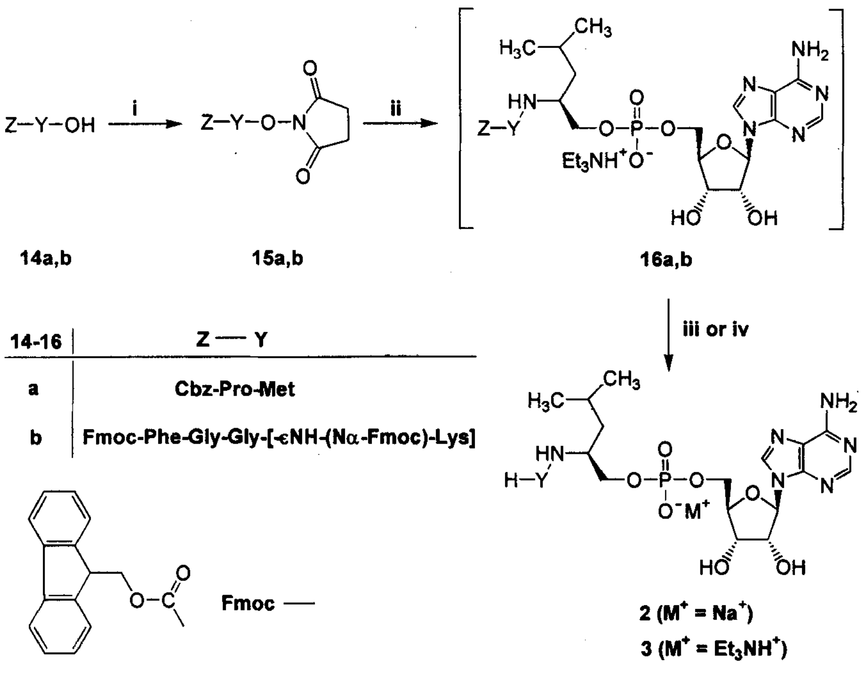
N,N,N′,N′-tetraisopropylphosphordiamidite as the reagent. However, we found that although the phosphorylation of the nucleoside **7** proceeded quantitatively, no reaction occurred after addition of the alcohol **12**. Substituting acetonitrile with dichloromethane had no effect. Inversion of the reaction sequence by treating first the leucinol derivative **12** with 2-cyanoethyl-N,N,N′,N′-tetraisopropylphosphordiamidite gave no product. Finally, the compound **1** was synthesized using phosphoryl tris-triazole (**9**) as reagent for the coupling step between compounds **7** and **12** (Scheme 2). The reaction was performed as a one-pot procedure and led to complete phosphorylation of adenosine within 10 minutes. Treatment of **11** with **12** and addition of water afforded **13**. Phosphorylated by-products were most probably due to impurities in the initially prepared phosphoryl tris-triazole reagent (**9**).[13] Removal of the Pac groups followed by cleavage of the Cbz group afforded crude compound **1**, which was further purified by reverse-phase silica gel chromatography and HPLC, giving the pure product in 29 % yield.



SCHEME 2: **i**: triazole, triethylamine, dioxane, 60 min, RT; **ii**: **7**, 10 min, RT; **iii**: **12**, 90 min, RT; **iv**: water, 12 h, RT. **v**: 9 N aqueous ammonia, 30 min, 5 °C; **vi**: H_2, Pd/C, methanol containing 0.06 % acetic acid, 12 h.

The coupling step of the peptides **14a** and **14b** with leucinol adenylate is depicted in Scheme 3. The tetrapeptide **14b** was prepared by condensation of the N-hydroxysuccinimide ester derivative of Fmoc-Phe-Gly-Gly with Nα-Fmoc-Lys. The Cbz group was used to protect the amino function of the dipeptide **14a**. Both peptides were converted to their N-hydroxysuccinimide esters (**15a, b**) and the successful coupling to the peptidinol adenylates (**16a, b**) was achieved following a standard N-hydroxysuccinimide coupling protocol. The removal of the Cbz group in **16a** was accomplished by catalytic hydrogenation. Complete deblocking of **16b** was effected with 1 % sodium methoxide. Both peptidinol adenylates (**2, 3**) were purified by reverse-phase silica gel chromatography, providing **2** in 53 % yield. **3** was further purified by HPLC giving the desired conjugate in 5 % yield.

In summary, a preparation of peptidinol adenylates was elaborated, which includes a new approach of aminoalcohol adenylate synthesis. Even though yields were not optimized, the synthetic route that we have elaborated provides a straightforward access to the adduction of aminoalcohol adenylates that should fit combinatorial synthetic procedures. The effect of the peptidinol adenylates described here toward microorganisms will be the subject of a separate report.



14-16	Z — Y
a	Cbz-Pro-Met
b	Fmoc-Phe-Gly-Gly-[ε-NH-(Nα-Fmoc)-Lys]

SCHEME 3. **i**: N-hydroxysuccinimide, DCC, DMF, 4 °C, 12 h; **ii**: **1**, $NaHCO_3$, dioxane/H_2O (1/1), RT, 30 min - 3h; **iii**: H_2, Pd/C, methanol containing 0.06 % acetic acid, RT, 48 h; **iv**: 1 % sodium methoxide, 0 °C, 15 min, Dowex AG50WX8 H^+ form, 5 °C.

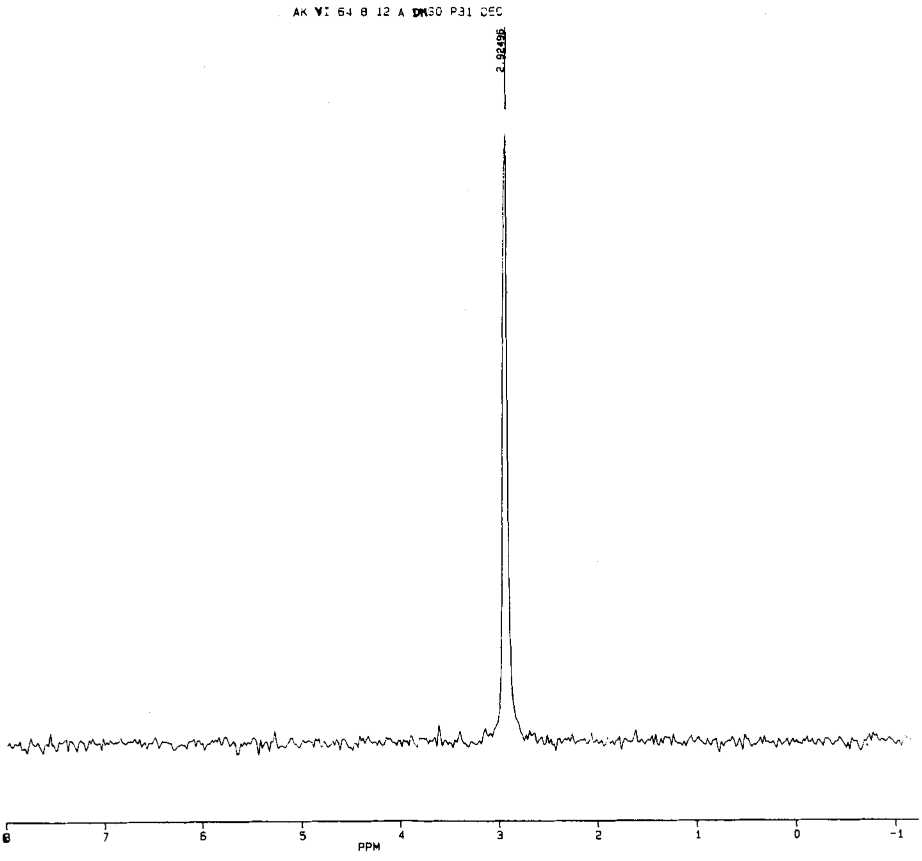


Figure 1. ^{31}P-NMR of Leucinol adenylate (1).

EXPERIMENTAL

Standard benchtop conditions were used for working under anhydrous conditions. Solvents were dried and distilled prior to use: dioxane from sodium / benzophenone and dichloromethane from phosphorus pentoxide. Dry acetonitrile was purchased from Merck. TLC were performed on silica gel 60 F_{254} precoated plates. The spots were visualized with UV light, anisaldehyde-sulphuric acid for the nucleoside moiety, ninhydrin for free aliphatic amino groups and a modified Ditmer and Lester[14] reagent for phosphate revelation. Silica gel 7734 (70 - 230 mesh) from Merck was used for column chromatography. Chromatography was performed on a Chromatotron Model 8924 (Harrison Research) using silica gel 60 PF_{254} containing gypsum from Merck. Reverse-phase column chromatography was conducted on LiChroprep RP-18 silica gel from Merck using a 0 - 35 % water-acetonitrile gradient. Purification by HPLC was carried out on a Perkin Elmer system using a 5C18 column. The elution was effected with the gradient of acetonitrile (solvent A) in aqueous 10 mM triethylammonium acetate solution (solvent B) at pH = 7.0 at a flow rate of 5.5 mL/min. The gradients and retention times are indicated below. ^{1}H-NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz. Chemical shifts were reported in ppm (δ) relative to tetramethylsilane as external standard in DMSO-d_6, D_2O and $CDCl_3$. ^{31}P-NMR spectra were taken at 121 MHz using 85 % phosphoric acid as external standard. *m*-Nitrobenzyl alcohol was used as the matrix for mass spectra determinated by fast atom bombardment (FAB).

5′-O-Dimethoxytrityladenosine (5)

About 7.0 g of compound **5** was synthesized in 62 % yield according to the procedure of Hakimelahi et al.[15] After work-up the crude product was recrystallized from xylene. Rf (CH_2Cl_2/MeOH, 9/1) = 0.48. ^{1}H-NMR (DMSO-d_6) δ 3.19 (2H, d, H5′,H5′′), 3.71 (6H, s, 2 x OCH_3), 4.04 (1H, dd, H4′), 4.3 (1H, dd, H3′), 4.69 (1H, dd, H2′), 5.21 (1H, d, 2′-OH), 5.54 (1H, d, 3′-OH), 5.91 (1H, d, H-1′), 6.81 (4H, m, NH_2 and arom. of Dmt), 7.27 (11H, m, arom. of Dmt), 8.08 (1H, s, H-2), 8.25 (1H, s, H-8). MS (FAB positive, $C_{31}H_{31}N_5O_6$) m/z 570.1 $[M+H]^+$.

5′-O-Dimethoxytrityl-2′-O,3′-O,6-N-triphenoxyacetyladenosine (6)

Compound **5** (4.56 g, 8 mmol) was dried by coevaporation with pyridine and then dissolved in pyridine (50 mL). To the cooled solution was added phenoxyacetic anhydride[17] (15.3 g, 60 mmol) and the mixture was left stirring at 5 °C. TLC (CH_2Cl_2/MeOH, 95/5) showed that the reaction was completed after about 4.5 h. Excess of anhydride was destroyed by addition of water (9 mL). After an additional 30 min the reaction mixture was poured in dichloromethane (150 mL). The solution was washed with 5 % $NaHCO_3$, then water and dried over sodium sulphate. After evaporation of the solvent, the residual gum was dissolved in few dichloromethane. Petroleum ether was added and the mixture was shaken vigorously. The precipitated product was filtered off. The crude compound was used without further purification for the next step. Chromatotron chromatography on silica gel using a 0-1.5 % gradient of methanol in dichloromethane provided an analytical example. Rf (CH_2Cl_2/MeOH, 95/5) = 0.77. ^{1}H-NMR ($CDCl_3$) δ 3.58 (2H, ddd, H5′, H5′′), 3.86 (6H, s, 2 x OCH_3), 4.42 (1H, dd, H4′), 4.58 (2H, d, CH_2 Pac), 4.70 (2H, s, CH_2 Pac), 4.92 (2H, s, CH_2 Pac), 5.90 (1H, m, H3′), 6.29 (1H, d, H1′), 6.38 (1H, t, H2′), 7.17 (28H, m, arom. of Dmt and Pac), 8.15 (1H, s, H2), 8.77 (1H, s, H8), 9.44 (1H, s, NH). MS (FAB positive, $C_{55}H_{49}N_5O_{12}$) m/z 972.4 $[M+H]^+$.

2′-O,3′-O,6-N-Triphenoxyacetyladenosine (7)

The fully protected adenosine derivative **6** was detritylated using a cold 2 % solution of benzenesulphonic acid in a dichloromethane/methanol mixture (7/3) for 20 min. Aqueous 10 % Na_2CO_3 was added until the solution became colourless. The organic layer was washed with water and dried (Na_2SO_4). Removal of the solvent afforded a white foam, which was dissolved in dichloromethane and precipitated with petroleum ether as described for compound **6**. Chromatography on silica gel with a 10-30 % ethyl acetate/dichloromethane gradient afforded 2.57 g of compound **7** (48 % yield) as well as 257.2 mg of 2′-O,3′-O,5′-O,6-N-tetraphenoxyacetyladenosine (**8**) as a by-product (4 % yield).

7: Rf (CH_2Cl_2/ethyl acetate, 7/3) = 0.28. ^{1}H-NMR ($CDCl_3$) δ 3.73 (2H, m, H5′, H5″), 4.21 (1H, d, H4′), 4.30 (2H, d, CH_2 Pac at the NH group), 4.53 (2H, s, CH_2 Pac), 4.71 (2H, s, CH_2 Pac), 5.63 (2H, m, H1′, 5′-OH), 6.02 (1H, dd, H2′), 7.02 (15H, phenyl of Pac), 7.64 (1H, s, H2), 8.62 (1H, s, H8), 9.52 (1H, s, NH). MS (FAB positive, $C_{34}H_{31}N_5O_{10}$) m/z 669.8 $[M+H]^+$.

8: Rf (CH_2Cl_2/ethyl acetate, 7/3) = 0.58. ^{1}H-NMR ($CDCl_3$) δ 4.41 (2H, m, H5′, H5″), 4.49 (2H, d, CH_2 Pac at the NH group), 4.57 (1H, m, H4′), 4.59 (2H, d, CH_2 Pac), 4.63 (2H, s, CH_2, Pac). 4,79 (2H, s, CH_2 Pac), 5.72 (1H, t, H3′), 6.08 (2H, m. H2′, H1′), 7.08 (20H, m, phenyl 4 x Pac), 8.07 (1H, s, H2), 8.72 (1H, s, H8), 9.57 (1H, s, NH).

N-Benzyloxycarbonylleucinol (12)

To a solution of benzyloxycarbonyl chloride (2.86 mL, 20 mmol) in dried diethyl ether (40 mL) was added N-methylimidazole (1.2 mL, 15.1 mmol) at room temperature and the obtained suspension was stirred for 1 h. The precipitate was filtrated and washed twice with diethyl ether to afford the 1-(benzyloxycarbonyl)-3-methylimidazolium salt. Leucinol (1.29 mL, 10 mmol) was dissolved in water (12 mL) and the imidazolium salt was added. After stirring for 2 h the reaction mixture was washed with diethyl ether and dried (Na_2SO_4). The crude product was purified by Chromatotron chromatography eluting with a 0-5 % gradient of methanol in dichloromethane. Thus, 2.06 g of compound **12** (82 % yield) were obtained. Rf (CH_2Cl_2/MeOH, 95/5) = 0.4. ^{1}H-NMR (DMSO-d_6) δ 0.84 (6H, m, 2 x CH_3), 1.25 (2H, m, β-CH_2), 1.57 (1H, m, γ-CH), 3.25 (2H, M, $\underline{CH_2}$-OH), 3.49 (1H, t, J = 5.7 Hz, CH_2-$\underline{OH}$), 4.99 (2H, s, CH_2 phenyl), 6.94 (1H, d, J = 8.8 Hz, NH), 7.29 (5H, m, phenyl). MS (EI, $C_{14}H_{21}NO_3$) m/z 251.3 [M].

Fmoc-Phe-Gly-Gly-(OH)

The amino function of the peptide Phe-Gly-Gly[17] was protected by the 9-fluorenyloxycarbonyl (Fmoc) group, introduced according to the method of

Meienhofer et al.[18] Further purification by Chromatotron chromatography using a 1-5 % gradient of methanol in dichloromethane afforded 481.5 mg of the desired compound (96 % yield). Rf (CH_2Cl_2/MeOH/acetic acid, 85/10/5) = 0.49. ^{1}H-NMR (DMSO-d_6) δ 2.79 (1H, t, 1H of CH_2 Phe), 3.05 (1H, dd, 1H of CH_2 Phe), 3.77 (4H, d, 2 x CH_2 Gly), 4.15 (2H, s, CH_2 Fmoc), 4.28 (2H, m, CH Fmoc, CH Phe), 7.29 (9H, m, arom. of Fmoc and Phe), 7.63 (3H, m, NH and arom. of Fmoc), 7.87 (2H, d, arom. of Fmoc), 8.15 (1H, t, NH), 8.36 (1H, t, NH).

Leucinol adenylate (1)

To an ice-cooled solution of dried triazole (249 mg, 3.6 mmol) in dioxane (6 mL) was added $POCl_3$ (0.11 mL, 1.2 mmol), then a solution of triethylamine (0.502 mL, 3.6 mmol) in dioxane (1 mL). After stirring for 1 h on ice the reaction mixture was directly filtrated under argon into a flask containing dried **7** (536 mg, 0.8 mmol). Compound **7** was consumed as judged by TLC (CH_2Cl_2/MeOH, 95/5) after 10 min. N-benzyloxycarbonylleucinol (**12**) (221 mg, 0.88 mmol) dissolved in 2 mL dioxane, was added and the solution was stirred for 1 h. TLC (EtOH/1 M $CH_3CO_2NH_4$, 5/2) showed that a major product was formed together with some minor ones. Stirring was continued for 30 min without any detectable change. The reaction was treated with water (0.03 mL, 1.6 mmol) and stirred overnight. Filtration of the mixture and evaporation of the organic phase gave crude **13**, which was dissolved in dichloromethane, washed with 5 % $NaHCO_3$ and dried. After evaporation of the solvent the residue was treated by 9 N aqueous ammonia (5 mL) at 5 °C for 30 min. Dowex AG50WX8 (H^+ form) was added to neutralize the reaction mixture. After filtration and rinsing with water the solution was lyophilized. In a second stage, the benzyloxycarbonyl protecting group was removed by catalytic hydrogenation. Thus, a suspension of 10 % palladium on charcoal (100 mg) and the lyophilized product in methanol containing 0.06 % acetic acid was stirred overnight under an atmosphere of hydrogen. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The crude product (**1**) was purified by column chromatography on reverse-phase silica gel eluting with a 0-35 % gradient of acetonitrile in water. Fractions containing compound **1** were repurified by reverse-phase HPLC using a 5-50 % gradient of solvent A in solvent B in 20 min. After lyophilisation, 131.1 mg of compound **1** were obtained (29 % yield). The product gave a positive response with ninhydrin. Rf (EtOH/1 M $CH_3CO_2NH_4$, 5/2) = 0.7. ^{1}H-NMR (DMSO-d_6) δ 0.83 (6H, d, J = 6.5 Hz, 2 x CH_3), 0.96 (3H, t, triethylamine), 1.32 (2H, t, β-CH_2), 1.60 (1H, m, γ-CH), 2.5 (m, triethylamine), 3.16 (2H, d, CH_2-O), 3.63 - 3.97 (3H, m, α-CH_2, H-5′, H-5″), 4.05 (1H, d, H-4′), 4.20 (1H, t, H-3′), 4.62 (1H, t, H-2′), 5.92 (1H, d, H-1′), 7.29 (2H, s, -NH_2 of adenosine), 8.16 (1H, s, H-2), 8.43 (1H, s, H-

8). The 2′ and 3′ hydroxy groups of the ribose moiety and the amino group of leucinol gave no detectable signals. ^{31}P-NMR (121 MHz, DMSO-d_6, proton decoupled): δ 2.92 (s). HPLC: 7.6 min.

Procedure for the preparation of N-hydroxysuccinimide ester of peptides 14a, 14b and Fmoc-Phe-Gly-Gly-OH.

1 mmol of N-protected peptide[17] and 1.1 mmol of N-hydroxysuccinimide were dissolved in 3 mL dimethylformamide (DMF). To the ice-cooled solution were added 1.5 mmol dicyclohexylcarbodiimide (DCC) and the reaction mixture was stirred overnight at 4 °C. After filtration, the solution was evaporated, and then coevaporated with xylene. The protected activated esters Fmoc-Phe-Gly-Gly-NHS and **15b** were used without purification in the next step. The Cbz protected ester **15a** was recrystallized from 2-propanol giving 290.7 mg (61 % yield). Rf (CH_2Cl_2/ethyl acetate, 6/4) = 0.69. ^{1}H-NMR (DMSO-d_6) δ 1.81 (3H, m, 1H of β-CH_2 Met, γ-CH_2 Pro), 1.98 (m, 1H of β-CH_2 Met, CH_3 Met), 2.05 (s, CH_3 Met), 2.61 (2H, m, β-CH_2 Pro) 2.79 (4H, s, 2 x CH_2 NHS), 3.42 (2H, m, δ-CH_2 Pro), 4.26 (1H, m, α-CH Pro), 4.99 (1H, α-CH Met), 5.05 (2H, s, CH_2 Cbz), 7.31 (5H, m, phenyl of Cbz), 8.71 (1H, t, NH). MS (FAB positive, $C_{22}H_{27}N_3O_7S_1$) m/z 478.0 $[M+H]^+$.

Fmoc-Phe-Gly-Gly-(Fmoc-Lys)-OH (14b)

A solution of Fmoc-Phe-Gly-Gly-NHS (1.35 g, 2.25 mmol) in dioxane (45 mL) was added dropwise to a solution of Fmoc-Lys-OH (743 mg, 1.8 mmol) and $NaHCO_3$ (302 mg, 3.6 mmol) in a dioxane/water mixture (40 mL/40 mL). The reaction mixture was stirred for 2 h, and then dioxane was evaporated. Concentrated hydrochloric acid was added to the cooled aqueous solution until reaching pH = 2. The precipitated product was collected by filtration and recrystallized from aqueous ethanol to give 540 mg of compound **14b** (31 % yield). Rf (CH_2Cl_2/MeOH/acetic acid, 89/10/1) = 0.36. ^{1}H-NMR (DMSO-d_6) δ 1.2 (4H, m, γ-CH_2 Lys, δ-CH_2 Lys), 1.48 (2H, m, β-CH_2 Lys), 2.63 (1H, dd -CH_2 Phe), 2.90 (3H, m, 4H, m, -CH_2 Phe, ε-CH_2 Lys), 3.52 (2H, d, CH_2 Gly), 3.63 (2H, d, CH_2 Gly), 3.72 (3H, m, α-CH Lys, 2 x CH Fmoc), 4.04 (5H, α-CH Phe, 2 x CH_2 Fmoc), 7.13 (13H, m, phenyl of Phe and arom. of 2 x Fmoc), 7.62 (11H, arom. of Fmoc, 3 x NH), 7.89 (1H, t, NH), 8.30 (1H, s, broad, NH). MS (FAB positive, $C_{49}H_{49}N_5O_9$) m/z 874.3 $[M-H+Na]^+$.

Pro-Met-Leucinol-adenylate (2)

To a solution of **1** (56.5 mg, 0.1 mmol) and $NaHCO_3$ (16.8 mg, 0.2 mmol) in a water/dioxane mixture (2 mL/1 mL) was added dropwise **15a** (95.5 mg, 0.2 mmol) dissolved in dioxane (2 mL). The mixture was stirred at room temperature and the reaction was complete in about 30 min. After evaporation of the organic layer in vacuo, the solution was lyophilized. The Cbz protecting group was removed by catalytic hydrogenation as describe for compound **1**. After 12 h stirring under an atmosphere of hydrogen, palladium on charcoal (10 %) was added again and the reaction was continued for 24 h. The solvent was evaporated and the residue was applied to a reverse-phase chromatography eluting with a 0 - 35 % gradient of acetonitrile in water. Lyophilization of the corresponding fractions gave 36.6 mg of compound **2** (53 % yield). Rf (EtOH/1 M $CH_3CO_2NH_4$, 5/2) = 0.49. ^{1}H-NMR (300 MHz, D_2O) δ 0.75 (6H, m, 2 x CH_3 of leucinol), 1.2 (2H, m, β-CH_2 of leucinol), 1.53 (1H, m, γ-CH of leucinol), 1.79 (3H, m, 1H of β-CH_2 Met, γ-CH_2 Pro), 1.95 (s, CH_3 Met), 2.02 (s, CH_3 Met), 2.10 (1H, m, 1H of β-CH_2 Met), 2.37 (2H, m, β-CH_2 Pro), 3.05 (2H, m, CH_2-O of leucinol), 3.42 (3H, m, α-CH of leucinol, γ-CH_2 Met), 3.7 (2H, m, H-5', H-5''), 4.0 (2H, m, δ-CH_2 Pro), 4.08 (1H, s, H-4'), 4.2 (1H, s, H-3'), 4.59 (1H, t, H-2'), 5.03 (2H, m, α-CH Pro, α-CH Met), 5.6 (1H, s, -NH- Pro), 5.94 (1H, d, H-1'), 7.3 (2H, s, NH_2 of adenosine), 7.68 (d, Met-NH-leucinol), 7.83 (d, Met-NH-leucinol), 8.17 (1H, s, H-2), 8.3 (1H, t, Pro-NH-Met), 8.37 (1H, s, H-8). ^{31}P-NMR (121 MHz, D_2O, proton decoupled): δ 0.85 (s).

Phe-Gly-Gly-Leucinol-adenylate (3)

Starting from **1** (56.5 mg, 0.1 mmol) and the activated ester **15b** (190.6 mg, 0.2 mmol) the coupling step proceeded as described for compound **2** within a reaction time of 3 h. To a cooled solution of the lyophilized Fmoc protected compound (**16b**) in methanol was added 1 % sodium methoxide (3 eq/Fmoc-group) and the solution was stirred for 15 min. The mixture was neutralized by addition of dry ion exchange resin (Dowex AG50WX8, H^+ form), filtered, rinsed with methanol. Water was added to the solution and product precipitating was removed by filtration. Evaporation of the organic phase and lyophilisation of the aqueous layer gave the crude compound **3**, which was applied to a reverse-phase column chromatography purification. Fractions containing the product of interest were further purified by HPLC using a 17 - 27 % gradient of solvent A in solvent B. Lyophilization afforded 4.8 mg of compound **3** (5 % yield). Rf (EtOH/ 1 M $CH_3CO_2NH_4$, 5/2) = 0.55. ^{1}H-NMR (300 MHz, D_2O) δ 0.61 (3H, d, CH_3 of leucinol), 0.7 (3H, d, CH_3 of leucinol), 0.75 (2H, m, γ-CH_2 Lys), 1.14 (2H, m, β-CH_2 Lys), 1.28 (5H, m, β-CH_2 of leucinol, triethylamine), 1.47 (1H, m, γ-CH of leucinol), 1.71 (2H, m, δ-CH_2 Lys), 2.97 (2H, d, CH_2-O of leucinol), 3.15 (5H, m, ε-CH_2 Lys, 1H of CH_2 Phe, triethylamine), 3.53 (2H, m, 1H of CH_2 Phe, α-CH of leucinol), 3.69 (1H, t, CH Lys), 3.81 (2H, s, CH_2 Gly), 3.9 (4H, m, CH_2 Gly, H-5', H-5''), 4.08 (2H, s, CH

Phe, H-4′), 4.32 (1H, t, H-3′), 4.51 (1H, m, H-2′), 6.07 (1H, d, H-1′), 7.24 (5H, m, phenyl Phe), 8.21 (1H, s, H-2), 8.45 (1H, s, H-8). ^{31}P-NMR (121 MHz, D_2O, proton decoupled): δ 0.51 (s). MS (FAB positive, $C_{35}H_{54}N_{11}O_{11}P_1$) m/z 836.3 $[M+H]^+$ HPLC: 7.9 min.

REFERENCES

1. Bock, A.; Forchhammer, K.; Heider, J.; Leinfelder, W.; Sawers, G.; Veprek, B.; Zinoni, F. *Molecular Microbiology* **1991**, *5*, 515 - 520.
2. Cassio, D.; Lemoine, F.; Waller, J.-P.; Sandrin, E.; Boissonnas, R. A. *Biochemistry* **1967**, *6*, 827 - 836.
3. Fickel T. E.; Gilvarg, C. *Nature New Biology* **1973**, *241*, 161 - 163.
4. Higgins, C. *Nature* **1987**, *327*, 655 - 656.
5. Kingsbury, W. D.; Boehm, J. C.; Mehta, R. J.; Grappel, S. F. *J. Med. Chem.* **1983**, *26*, 1725 - 1729.
6. Kingsbury, W. D.; Boehm, J. C.; Perry, D.; Gilvarg, C. *Proc. Natl. Acad. Sci.* **1984**, *81*, 4573 - 4576.
7. Sandrin, E.; Boissonnas, R. A. *Helv. Chim. Acta.* **1966**, *49*, 76 - 82.
8. Shabarova, Z. A. in *Progress in Nucleic Acid Research and Molecular Biology*; Academic Press, New York **1970**; pp 145 - 182.
9. Beaucage, S. L.; Radhakrishnan, P. I. *Tetrahedron* **1993**, *49*, 10441 - 10488.
10. Robles, J.; Pedroso, E.; Grandas, A. *Tetrahedron Lett.* **1991**, *32*, 4389 - 4392.
11. Wu, T.; Ogilivie, K. K.; Pon, R. T. *Tetrahedron Lett.* **1988**, *29*, 4249 - 4252.
12. Wu, T.; Ogilvie, K. K.; Pon, R. T. *Nucleic Acid Research* **1989**, *17*, 3501 - 3517.
13. ^{31}P-NMR of freshly prepared phosphoryl tris-triazol (**9**) (121 MHz, $CDCl_3$, proton decoupled) δ -19.43 (s), -5.95 (s, by-product), -21.64 (s, by-product).
14. Ryu, E. K.; MacCoss, M. *J. Lipid Res.* **1979**, *20*, 5610 - 563.
15. Hakimelahi, G. H.; Proba, Z. A., Ogilivie, K. K. *Can. J. Chem.* **1982**, *60*, 1106 - 1113.
16. Phenoxyacetic anhydride was prepared as described by Smalley, R. K.; Suschitzky, H. *J. Chem. Soc.* **1964**, 755 - 758.
17. Phe-Gly-Gly-OH and Cbz-Pro-Met-OH were purchased from Bachem (France).
18. Meienhofer, J.; Waki, M.; Heimer, E. P.; Lambros, T. J.; Makofske, R. C.; Chang, Ch. *Int. J. Peptide Protein Res.* **1979**, *13*, 35 - 42.

Received 12/12/97
Accepted 6/17/98