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Structure activity relationship studies on the antimicrobial activity of novel edeine A and D analogues

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Abstract: Edeines are pentapeptide amide antibiotics composed of four nonprotein amino acids, glycine, and polyamine. They exhibit antimicrobial and immunosuppressive activities and are universal inhibitors of translation. Moreover, it was proven that the free ionizable carboxy group in the (2*R*, 6*S*, 7*R*)-2,6-diamino-7-hydroxyazelaic acid moiety is not essential for biological activity of these compounds. In this paper we describe the synthesis of four novel edeine A and D analogues in which the above-mentioned acid residue was replaced with the (3*R*, 4*S*)- or (3*S*, 4*S*)-4,5-diamino-3-hydroxypentanoic acid moiety. In one compound we also introduced into molecule the 3-*N*,*N*-dimethyl derivative of (*S*)-2,3-diaminopropanoic acid to prevent the transpeptidation process, which results in the loss of biological activity of α-isomers of edeines. All peptides were synthesized applying the active ester and azide methods and on the basis of the coupling of suitable *N*-terminal tripeptides with proper *C*-terminal dipeptide amides. The activities of the newly obtained edeine analogues against selected strains of bacteria and fungi are also presented. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: edeine antibiotics; edeine analogues; peptide synthesis; antimicrobial activity

INTRODUCTION

The *Bacillus brevis* Vm4 strain has been shown to produce a mixture of closely related basic peptide antibiotics named edeines [1–3]. These compounds are pentapeptide amides composed of glycine, polyamine (Sper or Gsper), and four nonprotein amino acids: (*S*)-βTyr or (*S*)-βphe, (*S*)-Ise, (*S*)-A_2pr, and (2*R*, 6*S*, 7*R*)-A_2ha [4–6]. (2*R*, 6*S*, 7*R*)-A_2ha is a rare amino acid detected only in edeines and the method of its synthesis has not been elaborated yet. The major components of the antibiotic complex formed during biosynthesis were edeines A, B, D, and F, but each compound existed as two isomers – the active isomer α and the inactive β (the linkage between the (*S*)-Ise residue and the (*S*)-A_2pr moiety is by α- or β-amino group of (*S*)-A_2pr, respectively) [4–6]. Chemical structures of the α-isomers of edeines are shown in Figure 1.

Edeines exhibit a broad spectrum of antimicrobial activity including gram-positive and gram-negative bacteria, yeasts, mould [7], and *Mycoplasmas* [8], but their high animal toxicity does not allow them to be applied in chemotherapy as antimicrobial agents. These peptides have the ability to eliminate plasmids determining antibiotic resistance from bacterial cells [9], and they demonstrate considerable immunosuppressive effects in mice [10–12]. Edeine antibiotics inhibit specifically and reversibly the biosynthesis of DNA [13–15] and possess the ability to differentiate prokaryotic and eukaryotic microorganisms based on differences in functioning and structure of the DNA replicating systems [16]. Edeines are also universal inhibitors of protein synthesis [17,18]. In 2001, the crystal structure of the complex of the *Thermus thermophilus* small ribosomal subunit with edeine was determined, showing that the binding of this antibiotic might lead to an undesirable stabilization of the subunit conformation and in this way contribute to its inhibitory activity [19]. Edeine antibiotics, as inhibitors of protein synthesis in prokaryotic and eukaryotic systems, have been found to be a useful tool for studying various aspects of translation.

The active α-isomers of edeines are unstable in alkaline aqueous solutions because they undergo intramolecular isomerization – the migration of the acyl residue from the α-amino group of (*S*)-A_2pr to its β-amino group up to the balance state [4].

Currently there is no biotechnological source of edeines. Taking into consideration the unique biological properties of these peptides (the immunological activity and the capacity for universal inhibition of translation) and difficulties in chemical synthesis of natural antibiotics, we have undertaken the synthesis of

Abbreviations: A_2ha, 2,6-diamino-7-hydroxyazelaic acid; A_2ho, 4,8-diamino-3-hydroxyoctanoic acid; A_2hp, 4,5-diamino-3-hydroxypentanoic acid; A_2pr, 2,3-diaminopropanoic acid; Gsper, guanylspermidine; HOSu, *N*-hydroxysuccinimide; Ise, isoserine; A_2pr(Me_2), 2-amino-3-(*N*,*N*-dimethylamino)propanoic acid; βPhe, β-phenyl-β-alanine; rt, room temperature; Sper, spermidine; βTyr, β-tyrosine.

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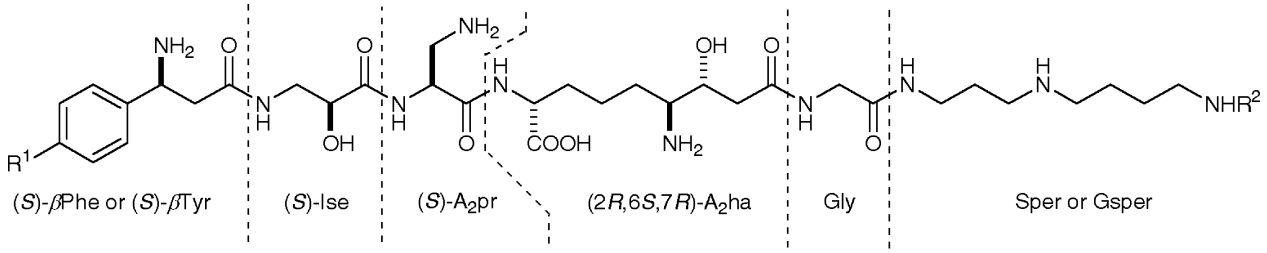

EDEINE	R^1	R^2
A	OH	H
B	OH	$C(NH)NH_2$
D	H	H
F	H	$C(NH)NH_2$

Figure 1 α-Isomers of edeines A, B, D, and F – chemical structures.

edeine analogues with simplified structures (Figure 2). In our previous studies we demonstrated that the presence of the free ionizable carboxy group in the (2*R*, 6*S*, 7*R*)-A_2ha moiety is not necessary for the biological activity of edeines [20,21]. Continuing our research program we have synthesized following edeine antibiotic analogues: (i) edeine D analogues **28a** and **28b**, in which the (2*R*, 6*S*, 7*R*)-A_2ha residue was replaced with the (3*R*, 4*S*)- or (3*S*, 4*S*)-A_2hp moiety, respectively; (ii) edeine A analogue **29**, with (3*R*, 4*S*)-A_2hp instead of (2*R*, 6*S*, 7*R*)-A_2ha; and (iii) edeine D analogue **30**, in which the (3*R*, 4*S*)-A_2hp moiety was substituted for the (2*R*, 6*S*, 7*R*)-A_2ha residue and (*S*)-A_2pr was replaced with (*S*)-A_2pr(Me_2) to prevent intramolecular isomerization. The absolute configuration of the β-hydroxy-γ-amino moiety is unchanged in peptides **28a**, **29**, and **30**, which contain the (3*R*, 4*S*)-A_2hp residue. Compound **28b** has got the inverse configuration of the carbon atom bonded with hydroxy group in comparison with original antibiotics.

Herein, we report the synthesis and antimicrobial activity of novel edeine A and D analogues **28a**, **28b**, **29**, and **30**. All peptides have been tested to check their immunological properties as well. The results of these studies will be published in a specialist journal in a short time.

MATERIALS AND METHODS

All melting points were determined in a capillary tube and are uncorrected. Optical rotations were measured on an AUTOPOL II (Rudolph Research Flanders, New Jersey) polarimeter. ^{1}H NMR spectra were recorded on a Varian Unity Plus 500 MHz spectrometer using TMS as an internal standard. Chemical shifts are given in ppm. MS spectra were recorded on a Trio-3 (VG, Great Britain) spectrometer. TLC was performed on aluminum sheets precoated with silica gel 60 from Merck. Compounds were detected with ninhydrin or cerium reagents. Column chromatography was carried out on silica gel 60 (0.063–0.200 mm) from Merck. Compositions of all solvent systems are given by volume. Diethyl ether was dried and stored over Na. All other solvents and chemicals were of reagent grade.

(*S*)-Boc-Ise-(*S*)-A_2pr(Z)-OMe (**1**) was synthesized following the literature procedure [22]. Nonprotein amino acids and polyamine were obtained according to the literature methods followed by introduction or removal of protecting groups by standard procedures: (*S*)-*N*-benzyloxycarbonyl-βPhe (**3**) [23], (*S*)-*tert*-butoxycarbonyl-Ise (**6**) and (*S*)-Ise (**16**) [24], (*S*)-N^3-benzyloxycarbonyl-N^2-*tert*-butoxycarbonyl-A_2pr (**8**) [25], (*S*)-*O*-benzyl-*N*-*tert*-butoxycarbonyl-βTyr (**12**) [26], (*S*)-A_2pr(Me_2) (**19**) [27], (3*R*, 4*S*)- and (3*S*, 4*S*)-N^4-benzyloxycarbonyl-N^5-*tert*-butoxycarbonyl-A_2hp (**21a**) and (**21b**) [28], and N^4,N^8-dibenzyloxycarbonyl-N^1-glycylspermidine hydrochloride (**22**) [29].

General Procedure for Boc Deprotection

tert-Butoxycarbonyl group protected compound was dissolved in TFA and allowed to stand for 1 h at rt. Then the reaction mixture was evaporated to dryness. The residue was washed with anhydrous diethyl ether several times and dried to afford the corresponding TFA salt.

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A_2pr(Z)-OMe (4)

Compound **2** was obtained from dipeptide **1** according to the general procedure for Boc deprotection. Yield 96%; a hygroscopic solid; TLC R_f 0.58 *n*-butanol : acetic acid : water (4 : 1 : 1).

DPPA (0.81 ml, 3.74 mmol) and TEA (0.99 ml, 7.14 mmol) were added to a stirred solution of TFA salt **2** (1.54 g, 3.40 mmol) and *N*-protected amino acid **3** (1.02 g, 3.40 mmol) in DMF (20 ml). After 1 h stirring at 0 °C and 12 h at rt the mixture was diluted with ethyl acetate and the organic layer was washed with 1 M $KHSO_4$, 1 M $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated. The residue was crystallized from ethyl acetate to give **4** (1.37 g, 65%). Melting point 174 °C (dec.) {176–177 °C [22]}; TLC R_f 0.52 ethyl acetate : methanol (20 : 1); $[\alpha]^{20}_D = -15.9$ (*c* 3.3, methanol : DMF (2 : 1)) {$[\alpha]^{20}_D = -19.4$ (*c* 3.3, methanol : DMF (2 : 1)) [22]}; ^{1}H NMR (500 MHz, DMSO-d_6)

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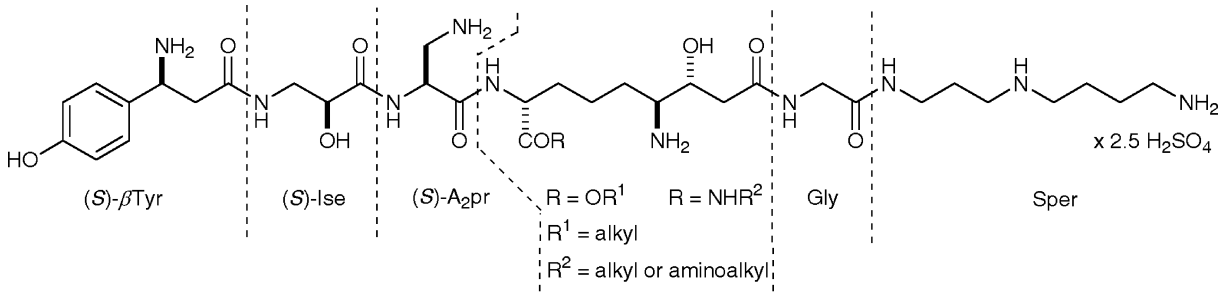
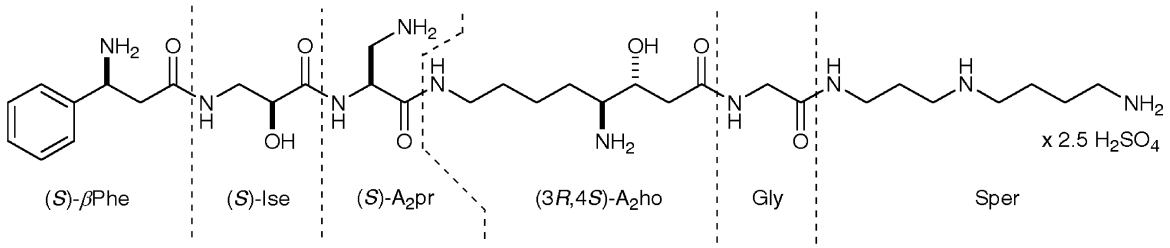
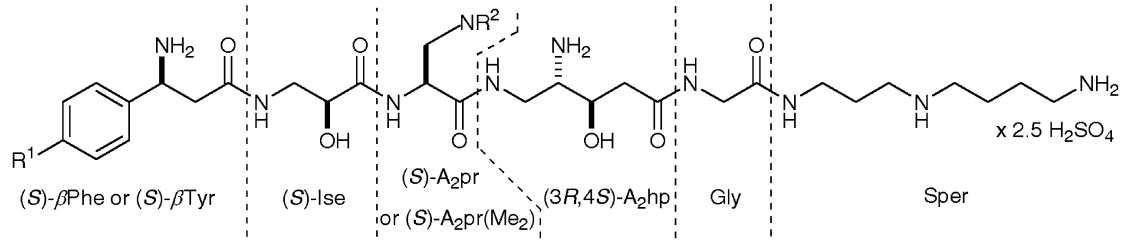


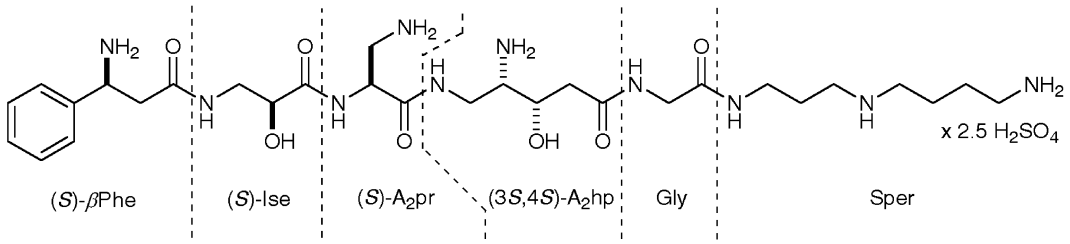
ESTERS AND AMIDES OF EDEINE A - ACTIVE [20]



DECARBOXY ANALOGUE OF EDEINE D - ACTIVE [21]



R^1	R^2	
H	H_2	**28a**
OH	H_2	**29**
H	Me_2	**30**



28b

NOVEL EDEINE A AND D ANALOGUES

Figure 2 Analogues of edeine A and D.

δ 2.50–2.60 (m, 2H, 2H-α–βPhe), 3.05 (m, 1H, H-β), 3.36–3.45 (m, 3H, 3H-β), 3.60 (s, 3H, OCH_3), 3.92 (m, 1H, H-α), 4.39 (m, 1H, H-α), 4.93–5.04 (m, 5H, H-β–βPhe, $2CH_2C_6H_5$), 5.89 (d, 1H, J = 4.4 Hz, OH), 7.18–7.38 (m, 15H, $3C_6H_5$), 7.47 (m, 1H, NH), 7.82–7.87 (m, 2H, 2NH), 8.03 (d, 1H, J = 7.3 Hz, NH).

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A_2pr(Z) (5)

Tripeptide **4** (0.558 g, 0.90 mmol), dissolved in a mixture of methanol (10 ml) and DMF (5 ml), was treated with 1 M NaOH (0.90 ml, 0.90 mmol) and the reaction mixture was stirred for 7 days at rt Within this time the additional portions of 1 M

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NaOH were added under TLC control to complete the reaction. Then the resulting mixture was diluted with water, acidified with 1 M $KHSO_4$ to pH 2, and extracted with ethyl acetate. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent and crystallization of a crude product with ethyl acetate yielded **5** (0.239 g, 44%). Melting point 153–156 °C; TLC R_f 0.42 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -12.0$ (*c* 1, methanol); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.50–2.60 (m, 2H, 2H-α–βPhe), 3.05 (m, 1H, H-β), 3.35–3.48 (m, 3H, 3H-β), 3.90 (m, 1H, H-α), 4.33 (m, 1H, H-α), 4.92–5.06 (m, 5H, H-β–βPhe, 2CH_2C$_6$H$_5$), 5.86 (d, 1H, $J = 4.9$ Hz, OH), 7.18–7.38 (m, 15H, 3C$_6$H$_5$), 7.41 (t, 1H, $J = 5.6$ Hz, NH), 7.80–7.87 (m, 2H, 2NH), 7.89 (d, 1H, $J = 7.8$ Hz, NH), 12.89 (s, 1H, COOH); FAB-MS 608 $[M + H]^+$.

(*S*)-Boc-Ise-(*S*)-A$_2$pr(Z) (10)

Compound **9** was synthesized from amino acid derivative **8** following the general procedure for Boc deprotection. Yield 85%; a hygroscopic solid; TLC R_f 0.35 ethyl acetate : methanol : water (5 : 1 : 0.75).

N-Protected amino acid **6** (0.360 g, 1.750 mmol), HOSu (0.200 g, 1.750 mmol), and DCC (0.360 g, 1.750 mmol) were dissolved in DMF (5 ml) and stirred for 30 min at 0 °C and then for 5 h at rt. Then the precipitated DCU was filtered off and a solution of active ester **7** [TLC R_f 0.50 ethyl acetate : *n*-hexane (5 : 2)] was added dropwise at 0 °C to a stirred suspension of TFA salt **9** (0.710 g, 2.015 mmol) in DMF (10 ml) in the presence of TEA (0.585 ml, 4.210 mmol). Stirring was continued for 30 min at 0 °C and 12 h at rt. Then the reaction mixture was worked up as described for compound **5**. The crude product was purified by column chromatography on silica gel with chloroform : methanol : water (30 : 8 : 1) solvent system to afford pure **10** (0.500 g, 67%) as an oil. TLC R_f 0.40 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -4.0$ (*c* 1, methanol); ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C) δ 1.39 (s, 9H, C(CH_3)$_3$), 3.05 (m, 1H, H-β), 3.31 (m, 1H, H-β), 3.34–3.39 (m, 2H, 2H-β), 3.92 (m, 1H, H-α), 4.07 (m, 1H, H-α), 5.03 (s, 2H, CH_2C$_6$H$_5$), 6.27 (brs, 1H, NH), 6.92 (brs, 1H, NH), 7.27–7.38 (m, 5H, C$_6$H$_5$), 7.76 (d, 1H, $J = 6.3$ Hz, NH–A$_2$pr).

(*S*)-Boc-βTyr(Bzl)-(*S*)-Ise-(*S*)-A$_2$pr(Z) (14)

Compound **11** was obtained from dipeptide **10** according to the general procedure for Boc deprotection. Yield 97%; an amorphous powder; TLC R_f 0.16 ethyl acetate : methanol : water (5 : 1 : 0.75).

Protected amino acid **12** (0.339 g, 0.913 mmol), HOSu (0.105 g, 0.913 mmol), and DCC (0.188 g, 0.913 mmol) were dissolved in DMF (3 ml) and stirred for 30 min at 0 °C and 12 h at rt. Then the precipitated DCU was filtered off and a solution of active ester **13** [TLC R_f 0.78 ethyl acetate : *n*-hexane (5 : 2)] was dropped at 0 °C into a stirred solution of TFA salt **11** (0.461 g, 1.050 mmol) in DMF (4 ml) containing TEA (0.307 ml, 2.205 mmol). The reaction mixture was worked up according to the procedure described for preparation of dipeptide **10**. Crystallization of a crude tripeptide from ethyl acetate resulted in pure **14** (0.542 g, 87%). Melting point 139–141 °C; TLC R_f 0.55 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -27.4$ (*c* 1, methanol); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (s, 9H, C(CH_3)$_3$), 2.40–2.50 (m, 2H, 2H-α–βTyr), 3.03 (m, 1H, H-β), 3.35–3.50 (m, 3H, 3H-β), 3.91 (m, 1H, H-α), 4.35 (m, 1H, H-α), 4.85 (m, 1H, H-β–βTyr), 5.00 (s, 2H, CH_2C$_6$H$_5$), 5.05 (s, 2H, CH_2C$_6$H$_5$), 5.85 (s, 1H, OH), 6.90–7.45 (m, 16H, aromatic H, 2NH), 7.78 (m, 1H, NH), 7.89 (d, 1H, $J = 8.3$ Hz, NH), 12.88 (s, 1H, COOH); FAB-MS 680 $[M + H]^+$.

(*S*)-Z-βPhe-(*S*)-Ise (17)

A solution of active ester **15** {TLC R_f 0.71 ethyl acetate : *n*-hexane (5 : 2)}, obtained from *N*-protected amino acid **3** (0.631 g, 2.11 mmol), HOSu (0.243 g, 2.11 mmol), and DCC (0.435 g, 2.11 mmol) in DMF (4 ml) following the procedure described for compound **13**, was dropped at 0 °C into a stirred solution of amino acid **16** (0.244 g, 2.32 mmol) in a mixture of DMF (1 ml) and water (1.5 ml) in the presence of TEA (0.352 ml, 2.53 mmol). Small volumes of DMF or water were added to prevent precipitation of reagents. Then the reaction mixture was worked up as described for preparation of dipeptide **10**. The crude product was purified by crystallization from ethyl acetate to give pure **17** (0.560 g, 69%). Melting point 184–187 °C [182–183 °C [22]]; TLC R_f 0.35 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -17.2$ (*c* 1, methanol : DMF (1 : 1)); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.50–2.58 (m, 2H, 2H-α–βPhe), 3.08 (ddd, 1H, $J_1 = 5.8$ Hz, $J_2 = 7.1$ Hz, $J_3 = 13.7$ Hz, H-β_1–Ise), 3.38 (ddd, 1H, $J_1 = 4.4$ Hz, $J_2 = 5.8$ Hz, $J_3 = 13.7$ Hz, H-β_2–Ise), 3.93 (dd, 1H, $J_1 = 4.4$ Hz, $J_2 = 7.1$ Hz, H-α–Ise), 4.94–5.02 (m, 3H, H-β–βPhe, CH_2C$_6$H$_5$), 5.39 (s, 1H, OH), 7.18–7.38 (m, 10H, 2C$_6$H$_5$), 7.82 (d, 1H, $J = 8,5$ Hz, NH–βPhe), 7.89 (t, 1H, $J = 5.8$ Hz, NH–Ise), 12.52 (brs, 1H, COOH).

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A$_2$pr(Me$_2$) (20)

A solution of active ester **18** {TLC R_f 0.80 ethyl acetate : methanol : water (5 : 1 : 0.75)}, prepared from dipeptide **17** (0.658 g, 1.705 mmol), HOSu (0.196 g, 1.705 mmol), and DCC (0.351 g, 1.705 mmol) in DMF (5 ml) according to the procedure used for preparation of compound **13**, was added dropwise at 0 °C to a stirred solution of amino acid **19** (0.225 g, 1.705 mmol) in a mixture of DMF (1.5 ml) and water (1.5 ml) containing TEA (0.260 ml, 1.876 mmol). Small volumes of DMF or water were added to prevent precipitation of the reagents. The reaction mixture was stirred for 30 min at 0 °C and 12 h at rt. Then the solvents were evaporated under reduced pressure at 45 °C. Tripeptide **20** was isolated by column chromatography on silica gel with chloroform : methanol : water (30 : 8 : 1) as an eluent (0.371 g, 44%). Melting point 120–124 °C; TLC R_f 0.23 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -11.2$ (*c* 1, methanol); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.52–2.60 (m, 2H-α–βPhe), 2.64 (s, 6H, N(CH_3)$_2$–A$_2$pr(Me$_2$)), 2.89 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 11.7$ Hz, H-β_1–A$_2$pr(Me$_2$)), 3.04–3.11 (m, 2H, H-β–Ise, H-β_2–A$_2$pr(Me$_2$)), 3.37 (m, 1H, H-β–Ise), 3.88 (dd, 2H, $J_1 = 3,9$ Hz, $J_2 = 6.8$ Hz, H-α–Ise), 4.08 (ddd, 1H, $J_1 = 5.8$ Hz, $J_2 = 5.9$ Hz, $J_3 = 9.3$ Hz, H-α–A$_2$pr(Me$_2$)), 4.94–5.01 (m, 3H, H-β–βPhe, CH_2C$_6$H$_5$), 5.97 (s, 1H, OH), 7.19–7.38 (m, 10H, 2C$_6$H$_5$), 7.78 (d, 1H, $J = 5.8$ Hz, NH–A$_2$pr(Me$_2$)), 7.85 (d, 1H, $J = 8,3$ Hz, NH–βPhe), 7.94 (t, 1H, $J = 5,6$ Hz, NH–Ise); FAB-MS 502 $[M + H]^+$.

(3*R*,4*S*)-*N*^{4}-Z-*N*^{5}-Boc-A$_2$hp-Gly-*N*^{8}-Z-Sper(Z) (23a)

DPPA (0.215 ml, 0.999 mmol) and TEA (0.266 ml, 1.907 mmol) were added to a stirred solution of hydrochloride **22** (0.460 g,

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0.908 mmol) and *N*-protected amino acid **21a** (0.347 g, 0.908 mmol) in DMF (8 ml), and the reaction mixture was stirred for 1 h at 0 °C and then for 48 h at rt. Within this time the additional quantities of DPPA and TEA were added under TLC control to complete the reaction. Then the reaction mixture was worked up as described for compound **4** and a crude product was purified by column chromatography on silica gel with chloroform : methanol (gradually changed from 30 : 1 to 15 : 1) solvent system to give pure **23a** (0.453 g, 60%) as an oil. TLC R_f 0.76 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = +1.6$ (*c* 1.24, methanol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9H, $C(CH_3)_3$), 1.43–1.60 (m, 4H, $ZNCH_2CH_2CH_2CH_2NHZ$), 1.66–1.73 (m, 2H, $NHCH_2CH_2CH_2NZ$), 2.45 (m, 1H, CH_2CO–A_2hp), 2.57 (m, 1H, CH_2CO–A_2hp), 3.10–3.55 (m, 10H, $5NCH_2$–A_2hp, Sper), 3.63–3.74 (m, 2H, CH_2–Gly), 3.97 (m, 1H, C*H*NHZ–A_2hp), 4.14 (m, 1H, C*H*OH–A_2hp), 4.85 (brs, 1H, NH), 5.05–5.23 (m, 7H, $3CH_2C_6H_5$, NH), 5.89 (brs, 1H, NH), 6.75 (brs, 1H, NH), 7.22–7.40 (m, 16H, $3C_6H_5$, NH); FAB-MS 837 $[M + H]^+$.

(3*S*,4*S*)-N^4-Z-N^5-Boc-A_2hp-Gly-N^8-Z-Sper(Z) (23b)

Compound **23b** was prepared from hydrochloride **22** and *N*-protected amino acid **21b** following the procedure used for preparation of dipeptide amide **23a**. Yield 39%; an oil; TLC R_f 0.80 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -9.1$ (*c* 1.24, methanol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 1.45–1.60 (m, 4H, $ZNCH_2CH_2CH_2CH_2NHZ$), 1.63–1.72 (m, 2H, $NHCH_2CH_2CH_2NZ$), 2.33 (m, 1H, CH_2CO–A_2hp), 2.47 (m, 1H, CH_2CO–A_2hp), 3.05–3.35 (m, 10H, $5NCH_2$–A_2hp, Sper), 3.62–3.72 (m, 2H, CH_2–Gly), 3.86 (m, 1H, C*H*NHZ–A_2hp), 4.19 (m, 1H, C*H*OH–A_2hp), 4.90 (brs, 1H, NH), 5.04–5.15 (m, 6H, $3CH_2C_6H_5$), 5.27 (brs, 1H, NH), 5.42 (d, 1H, $J = 8.8$ Hz, CHN*H*Z–A_2hp), 6.40 (brs, 1H, NH), 7.18 (brs, 1H, NH), 7.27–7.40 (m, 15H, $3C_6H_5$).

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A_2pr(Z)-(3*R*,4*S*)-N^4-Z-A_2hp-Gly-N^8-Z-Sper(Z) (25a)

Compound **24a** was prepared from dipeptide amide **23a** following the general procedure for Boc deprotection. Yield 98%; an oil; TLC R_f 0.46 ethyl acetate : methanol : water (5 : 1 : 0.75).

To a stirred solution of TFA salt **24a** (0.270 g, 0.318 mmol) and tripeptide **5** (0.193 g, 0.318 mmol) in DMF (4 ml) DPPA (0.075 ml, 0.349 mmol) and TEA (0.094 ml, 0.673 mmol) were added at 0 °C. Then the reaction mixture was worked up as described for compound **23a** to yield a crude product that was crystallized from acetic acid–ethyl acetate solvent mixture to afford **25a** (0.204 g, 48%). Melting point 186 °C (dec.); TLC R_f 0.67 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -14.0$ (*c* 1, acetic acid); FAB-MS 1325 $[M + H]^+$.

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A_2pr(Z)-(3*S*,4*S*)-N^4-Z-A_2hp-Gly-N^8-Z-Sper(Z) (25b)

Compound **24b** was obtained from dipeptide amide **23b** according to the general procedure for Boc deprotection. Yield 98%; an oil; TLC R_f 0.49 ethyl acetate : methanol : water (5 : 1 : 0.75).

Peptide **25b** was synthesized from TFA salt **24b** and tripeptide **5** following the procedure described for the preparation of compound **25a** except that a crude product was crystallized from ethyl acetate. Yield 42%; m.p. 146–153 °C; TLC R_f 0.67 ethyl acetate : methanol : water (5 : 1 : 0.75); $[\alpha]^{20}_D = -32.5$ (*c* 1, acetic acid); FAB-MS 1326 $[M + H]^+$.

(*S*)-Boc-βTyr(Bzl)-(*S*)-Ise-(*S*)-A_2pr(Z)-(3*R*,4*S*)-N^4-Z-A_2hp-Gly-N^8-Z-Sper(Z) (26)

Peptide **26** was obtained from TFA salt **24a** and tripeptide **14** according to the procedure used for the preparation of compound **25a** except that a crude product was crystallized from ethyl acetate. Yield 74%; m.p. 181–183 °C; TLC R_f 0.69 chloroform : methanol : water (30 : 8 : 1); $[\alpha]^{20}_D = -22.0$ (*c* 1, acetic acid); FAB-MS 1396 $[M + H]^+$.

(*S*)-Z-βPhe-(*S*)-Ise-(*S*)-A_2pr(Me_2)-(3*R*,4*S*)-N^4-Z-A_2hp-Gly-N^8-Z-Sper(Z) (27)

Peptide **27** was synthesized from TFA salt **24a** and tripeptide **20** following the azide method, as described for compound **25a** except that after completion of the reaction DMF was evaporated under reduced pressure at 45 °C. Then compound **27** was isolated by column chromatography on silica gel with chloroform : methanol : 25% NH_4OH (at the beginning 28 : 4 : 0.1, then 14 : 2 : 0.1) solvent system. Yield 39%; an oil; TLC R_f 0.43 chloroform : methanol : water (30 : 8 : 1); $[\alpha]^{20}_D = -9.2$ (*c* 1, acetic acid); FAB-MS 1218 $[M + H]^+$.

General Procedure for Preparation of Pentapeptide Amide Sulphates

The protected pentapeptide amide was dissolved in acetic acid (**25a**, **25b**, and **27**) or in formic acid (**26**) and 10% Pd/C catalyst (0.05 g/0.1 mmol) was added. The reaction mixture was vigorously stirred under an H_2 atmosphere for 18 h at rt. Then the catalyst was removed by filtration and the solvent was evaporated. The residue was treated with 0.1 M H_2SO_4 (2.5 ml/0.1 mmol) and concentrated to a small volume (1 ml/0.15 mmol). The solution was dropped with stirring into a chilled methanol : 99.8% ethanol (3 : 1) (25 ml/0.1 mmol) solvent mixture followed by the addition of diethyl ether (several ml). The resulting suspension was stored overnight at −10 °C. Then the precipitate was centrifuged, washed with acetone : *n*-hexane (1 : 1) and *n*-hexane, and dried to afford corresponding edeine analogue sulphate (**28a**, **28b**, **29**, and **30**).

(*S*)-βPhe-(*S*)-Ise-(*S*)-A_2pr-(3*R*,4*S*)-A_2hp-Gly-Sper x 2.5 H_2SO_4 (28a)

Yield 82%; m.p. 185 °C (dec.); TLC R_f 0.26 isopropyl alcohol : 25% NH_4OH : water (6 : 4 : 3); ^{1}H NMR (500 MHz, D_2O) δ 1.57–1.69 (m, 4H, $NHCH_2CH_2CH_2CH_2NH_2$), 1.72–1.81 (m, 2H, $NHCH_2CH_2CH_2NH$), 2.44–2.58 (m, 2H, CH_2CO), [2.84–3.00 (m, 7H), 3.12–3.53 (m, 9H), CH_2CO, $7NCH_2$–Ise, A_2pr, A_2hp, Sper], 3.77 (s, 2H, CH_2–Gly), [4.11 (m, 1H), 4.23 (m, 1H), 4.58–4.69 (m, 3H), C*H*NHZ–A_2hp, C*H*OH–A_2hp, H-β–βPhe, H-α–Ise, H-α–A_2pr], 7.29–7.40 (m, 5H, C_6H_5).

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(*S*)-β-Phe-(*S*)-Ise-(*S*)-A_2pr-(3*S*,4*S*)-A_2hp-Gly-Sper x 2.5 H_2SO_4 (28b)

Yield 90%; m.p. 190 °C (dec.); TLC R_f 0.25 isopropyl alcohol : 25% NH_4OH : water (6 : 4 : 3); ^{1}H NMR for acetate (500 MHz, D_2O) δ 1.56–1.67 (m, 4H, $NHCH_2CH_2CH_2CH_2NH_2$), 1.71–1.79 (m, 2H, $NHCH_2CH_2CH_2NH$), 1.82 (s, 15H, $5CH_3COOH$), 2.44 (m, 1H, CH_2CO), 2.56 (m, 1H, CH_2CO), [2.82–2.97 (m, 7H), 3.02–3.46 (m, 9H), CH_2CO, $7NCH_2$–Ise, A_2pr, A_2hp, Sper], 3.77 (s, 2H, CH_2–Gly), [3.99–4.08 (m, 2H), 4.56–4.67 (m, 3H), CHNHZ–A_2hp, CHOH–A_2hp, H-β–βPhe, H-α–Ise, H-α–A_2pr], 7.28–7.39 (m, 5H, C_6H_5).

(*S*)-βTyr-(*S*)-Ise-(*S*)-A_2pr-(3*R*,4*S*)-A_2hp-Gly-Sper x 2.5 H_2SO_4 (29)

Yield 92%; m.p. 187 °C (dec.); TLC R_f 0.40 isopropyl alcohol : 25% NH_4OH : water (6 : 4 : 3); ^{1}H NMR for formate (500 MHz, D_2O) δ 1.55–1.67 (m, 4H, $NHCH_2CH_2CH_2CH_2NH_2$), 1.70–1.80 (m, 2H, $NHCH_2CH_2CH_2NH$), 2.42–2.53 (m, 2H, CH_2CO), [2.78–2.96 (m, 8H), 3.08–3.20 (m, 3H), 3.25 (m, 1H), 3.30–3.47 (m, 4H), CH_2CO, $7NCH_2$–Ise, A_2pr, A_2hp, Sper], 3.77 (s, 2H, CH_2–Gly), [4.06 (m, 1H), 4.22 (m, 1H), 4.50–4.67 (m, 3H), CHNHZ–A_2hp, CHOH–A_2hp, H-β–βTyr, H-α–Ise, H-α–A_2pr], 6.80 (d, 2H, $J = 7.8$ Hz, aromatic H), 7.19 (d, 2H, $J = 7.8$ Hz, aromatic H), 8.25 (s, 5H, 5HCOOH).

(*S*)-β-Phe-(*S*)-Ise-(*S*)-A_2pr(Me$_2$)-(3*R*,4*S*)-A_2hp-Gly-Sper x 2.5 H_2SO_4 (30)

Yield 83%; m.p. 177 °C (dec.); TLC R_f 0.37 isopropyl alcohol : 25% NH_4OH : water (6 : 4 : 3); ^{1}H NMR for acetate (500 MHz, D_2O) δ 1.56–1.67 (m, 4H, $NHCH_2CH_2CH_2CH_2NH_2$), 1.71–1.79 (m, 2H, $NHCH_2CH_2CH_2NH$), 1.82 (s, 15H, $5CH_3COOH$), 2.42–2.52 (m, 2H, CH_2CO), 2.79 (s, 6H, $N(CH_3)_2$–A_2pr(Me$_2$)), [2.83–3.00 (m, 8H), 3.13–3.48 (m, 7H), 3.53 (m, 1H), CH_2CO, $7NCH_2$–Ise, A_2pr(Me$_2$), A_2hp, Sper], 3.77 (s, 2H, CH_2–Gly), [4.06 (m, 1H), 4.22 (m, 1H), 4.58–4.69 (m, 2H), 4.77 (m, 1H), CHNHZ–A_2hp, CHOH–A_2hp, H-β–βPhe, H-α–Ise, H-α–A_2pr(Me$_2$)], 7.28–7.40 (m, 5H, C_6H_5).

Antimicrobial Activity Assay

All microorganisms (listed in Table 1) were from the Polish Collection of Microorganisms (Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, Wrocław (or Wroclaw), Poland).

The minimum Inhibitory concentration (MIC) was determined using a microbroth dilution method with either the Mueller–Hinton (MH) broth or the Sabouraud Dextrose broth (Becton Dickinson, Le Pont de Claix, France) and an initial inoculum of 10^5–10^6 CFU/ml (for bacteria) and 10^2–10^3 CFU/ml (for fungi). Polypropylene 96-well plates (Nunc GmbH & Co. KG, Germany) were incubated in air for 18 h at 37 °C (for bacteria), 72 h at 25 °C (for *Candida albicans*), and 5 days at 25 °C (for *Aspergillus niger*). The MIC was taken as the lowest drug concentration at which a noticeable growth was inhibited. Experiments were performed in triplicate.

Table 1 Antimicrobial Activity of Edeine A and D Analogues, Edeine B (EB), and Chloramphenicol (CHL)

Organism	MIC (μg/ml)/compound					
	28a	**28b**	**29**	**30**	EB	CHL
Gram-positive bacteria						
Bacillus subtilis ATCC 9372	256	512	512	512	4	2
Staphylococcus aureus ATCC 6538	>512	>512	>512	>512	64	16
Gram-negative bacteria						
Escherichia coli ATCC 8739	>512	>512	>512	>512	64	16
Proteus vulgaris NCTC 4635	>512	>512	>512	>512	64	2
Serratia marcescens ATCC 274	>512	>512	>512	>512	32	8
Fungi						
Candida albicans ATCC 10231	512	512	512	512	8	—
Aspergillus niger ATCC 16404	512	512	512	512	64	—

RESULTS AND DISCUSSION

Synthesis

The new edeine A and D analogues **28a**, **28b**, **29**, and **30** have been obtained according to the general idea of coupling suitable *N*-terminal tripeptides **5**, **14**, and **20** with proper *C*-terminal dipeptide amides **23a** and **23b** based on our previous synthetic experience connected with edeines and their analogues [21,22,30–32].

Tripeptide **5** was synthesized starting from its *C*-terminal amino acid residue following the modified literature procedure [22] (Figure 3). Compound **1** was prepared by the method mentioned, but removal of its Boc group was carried out with TFA and the resulting salt **2** was coupled with *N*-protected amino acid **3** in the presence of TEA instead of Boc deprotection with formic acid and subsequent neutralization of formic salt

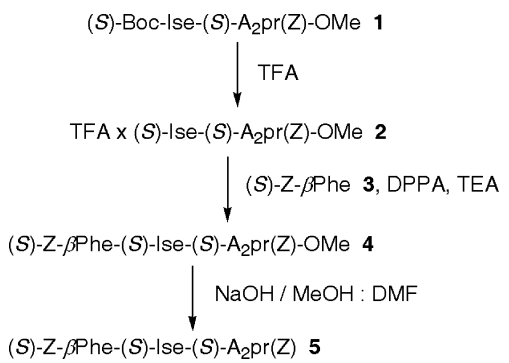

Figure 3 Synthesis of protected *N*-terminal tripeptide of two edeine D analogues **28a** and **28b**.

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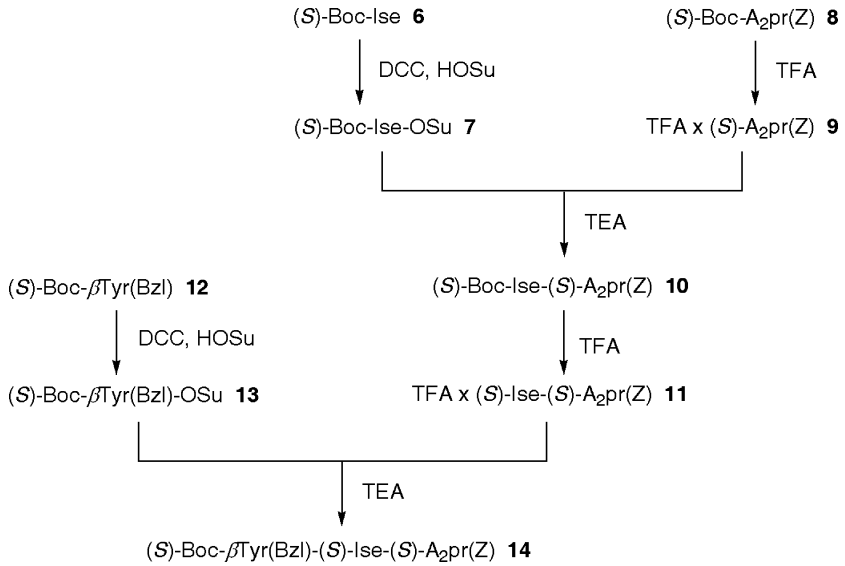

Figure 4 Synthetic route to protected *N*-terminal tripeptide of edeine A analogue **29**.

by ion-exchanged chromatography, which led to a free base in a poor yield because of extensive by-product formation. The peptide bond between **2** and **3** was formed by means of DPPA [33]. The hydrolysis of methyl ester in protected peptide **4** had to be performed in the mixture of methanol and DMF (because of the low solubility of compound **4** in methanol) with the excess of NaOH and was accompanied by partial racemization of the (*S*)-A_2pr residue (Andruszkiewicz R, unpublished data). A formed in minority as a side-product diastereoisomer was successfully separated by crystallization from ethyl acetate. For this reason, methods that needed protection of the (*S*)-A_2pr carboxy group with methyl or ethyl esters were not applied in the next syntheses.

Thus, protected *N*-terminal tripeptide **14** of edeine A analogue was obtained from its *C*-terminus following the HOSu method [34], as shown in Figure 4. It should be emphasized that the active ester **7** was generated *in situ* from the (*S*)-Ise derivative **6** without protection of its hydroxy group in good purity, and then it was reacted with TFA salt **9** in the presence of TEA to provide dipeptide **10** in satisfactory yield. The reaction was performed in DMF. In the same manner, compound **14** was prepared from protected amino acid **12** and corresponding to peptide **10** TFA salt **11**.

Similar to the preparation of peptide **14**, compound **20** was synthesized according to the active ester method [34], but the synthesis was carried out from its *N*-terminal amino acid residue (Figure 5). Dipeptides composed of *N*-protected (*S*)-Ise and (*S*)-A_2pr(Me_2) or its methyl ester derivative are well soluble in water. This feature makes it difficult to separate them from the reaction mixture. HOSu esters **15** and **18** were formed *in situ* and used in the next step without purification. Tripeptide **20** was isolated from the reaction mixture by column chromatography on silica gel, which was preceded with the evaporation of solvents under

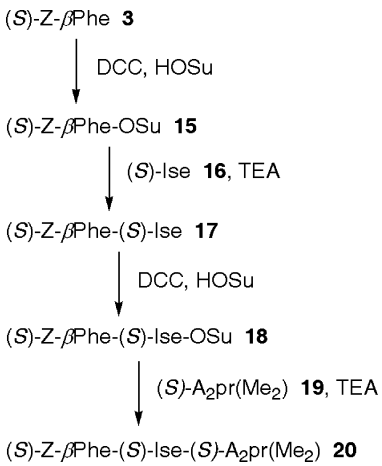

Figure 5 Synthesis of protected *N*-terminal tripeptide of edeine D analogue **30**.

reduced pressure. The presence of dimethyl amino group and free carboxy group in one molecule prevented us from the application of extraction. The *N*-protected aromatic amino acid residue contained in compound **20** decreases the strong adsorption of this peptide on silica gel, and that is a favorable condition.

Diastereoisomeric dipeptide amides **23a** and **23b** were obtained following the azide method [33] (Figure 6). The formation of a by-product was detected during the condensation of amino acid derivative **21a** and **21b** with hydrochloride **22**. This process happened more extensively in case of the synthesis of compound **23b**, which has got the β-hydroxy-γ-amino moiety absolute configuration as (3S, 4S). The reaction was controlled by TLC. It was necessary to use a large excess (up to 50%) of DPPA in order to complete the peptide bond formation. Crude products were purified by column chromatography on silica gel to give

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pure peptides **23a** and **23b** in yield of 60% and 39%, respectively.

The formation of peptide bonds between tripeptides **5**, **14**, **20** and dipeptide amides **23a** and **23b** leading to protected edeine analogues **25a**, **25b**, **26**, and **27** (Figure 7) was performed by means of DPPA [33]. In each case a considerable excess of carboxyl group activating agent had to be added so as to complete the reaction, just like during the synthesis of compounds **23a** and **23b**. The aim of a next synthetic step was to remove Z and Boc protecting groups. Catalytic hydrogenation of *N*-protected peptides **25a**, **25b**, and **27** was carried out in acetic acid, whereas compound **26** was hydrogenized in formic acid for the simultaneous cleavage of the Boc group. After that, all novel edeine analogues were converted into the corresponding sulphates **28a**, **28b**, **29**, and **30** on the basis of the results of our previous studies [21,30]. The Sulphates of edeine antibiotics and their analogues are not hygroscopic in contrast to other salts (e.g. chlorides and bromides). They can be stored in a dry place for a long time.

It should be emphasized that solubility of described peptides, which contain the protected aromatic amino acid residue (except compounds **20** and **27**), in many organic solvents (e.g. methanol, chloroform, ethyl acetate, and acetone) is rather low. It makes their isolation difficult and limits the methods of their purification.

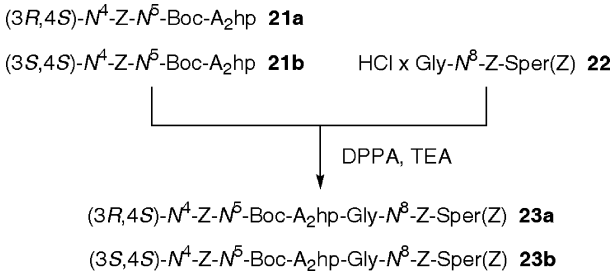

Figure 6 Synthetic route to protected *C*-terminal dipeptide amides of edeine A and D analogues **28a**, **28b**, **29**, and **30**.

Antimicrobial Activity

Data presented in Table 1 indicate that the new edeine A and D analogues **28a**, **28b**, **29**, and **30** exhibit very weak antimicrobial activity. Moreover, it can be

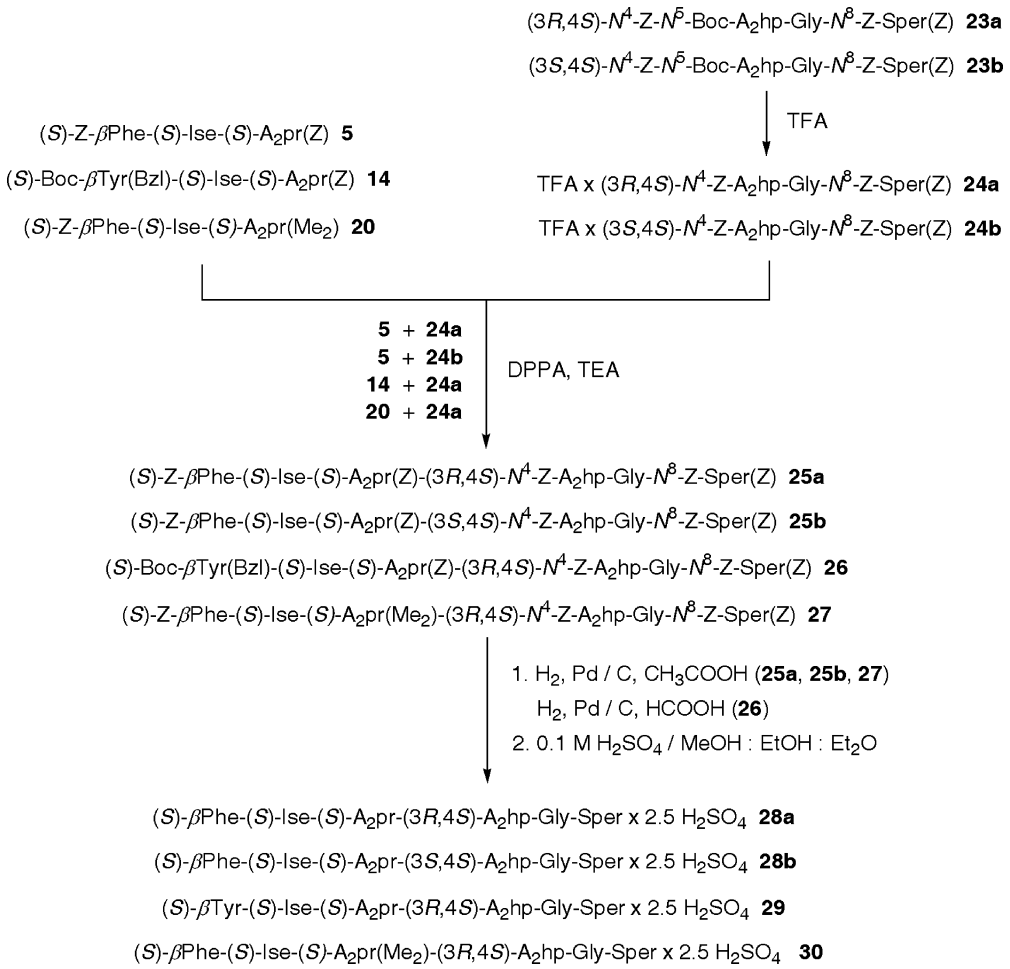

Figure 7 Synthesis of pentapeptide amides **28a**, **28b**, **29**, and **30**.

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stated that these compounds are inactive against gram-negative bacteria.

These results enabled us to establish several structure activity relationships. The most important one is that the replacement of (2*R*, 6*S*, 7*R*)-A_2ha with A_2hp in edeine A and D leads to peptides that essentially have no antibiotic activity. There are no important differences in the MIC values between peptides **28a** and **28b**. Therefore the absolute configuration of the β-hydroxy-γ-amino moiety is not essential for this type of biological activity of compounds deriving from edeine D and having replaced the (2*R*, 6*S*, 7*R*)-A_2ha residue with the (3*R*, 4*S*)- or (3*S*, 4*S*)-A_2hp moiety. The introduction of (*S*)-A_2pr(Me_2) instead of (*S*)-A_2pr into edeine D analogue containing the (3*R*, 4*S*)-A_2hp residue also does not influence the level of antimicrobial activity significantly (compounds **28a** and **30**).

CONCLUSIONS

Our studies confirm that the synthesis of edeines and their analogues by coupling of their *N*-terminal tripeptides with *C*-terminal dipeptide amides reported in the literature [21,22,30–32] is a suitable method for the preparation of these compounds, because such a procedure reduces the number of synthetic steps after introduction into peptide chain the (2*R*, 6*S*, 7*R*)-A_2ha analogue, the synthesis of which is the most difficult and time-consuming among the edeine amino acids. Additionally, new synthetic experience has been gained in the presented work. *N*-terminal tripeptides of natural and modified edeine antibiotics could be obtained in good yield and purity following the HOSu ester method [34] without protection of the hydroxy group in the (*S*)-Ise moiety. Peptides that derive from the *N*-terminal fragments of edeine antibiotics and contain at their *C*-terminus (*S*)-A_2pr in the protected form of methyl ester are prone to racemization of this amino acid residue during the removal of the protecting group by alkaline hydrolysis.

It has been demonstrated that edeine analogues that include in their molecules (3*R*, 4*S*)- or (3*S*, 4*S*)-A_2hp instead of (2*R*, 6*S*, 7*R*)-A_2ha (compounds **28a**, **28b**, **29**, and **30**) are very weak antimicrobial agents. However, some of these peptides exhibit interesting immunological properties (data not presented here) and in these cases a lack of antimicrobial activity might be recommended.

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