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N′-(α-Aminoacyl)- and *N′*-α-(*N*$^{\alpha}$-Alkylamino)acyl Derivatives of Vancomycin and Eremomycin

I. Synthesis of *N′*-(α-aminoacyl)- and *N′*-α-(*N*$^{\alpha}$-Alkylamino)acyl Derivatives of Vancomycin and Eremomycin by Selective Aminoacylation of the amino Sugar of the Disaccharide Branch

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Abstract The acylation of unprotected vancomycin or eremomycin with activated esters of *N*$^{\alpha}$-protected amino acids or *N*$^{\alpha}$-alkyl-*N*$^{\alpha}$-Fmoc-amino acids is directed selectively to the amino group of the disaccharide branch (*N′*) and after Fmoc-group removal leads to the corresponding *N′*-α-aminoacyl derivatives. A series of *N′*-α-aminoacyl and *N′*-α-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin and vancomycin containing hydrophobic moieties has been synthesized. The structures of all derivatives were confirmed by Electrospray Ionization mass-spectral (ESI MS) analysis, and by chemical degradation methods. Position of the introduced *N′*-α-aminoacyl- and *N*-(*N*$^{\alpha}$-alkylamino)acyl groups were determined after Edman degradation and acidic hydrolysis. The structures of the synthesized starting reagents (*N*$^{\alpha}$-alkylamino acids or *N*$^{\alpha}$-alkyl-*N*$^{\alpha}$-Fmocamino acids) were confirmed by NMR-spectra data. In general, *N′*-(*N*-alkylglycyl)-derivatives were more active than the corresponding *N′*-α-(*N*$^{\alpha}$-alkylamino)acylated derivatives containing other amino acids (L-Lys, L-Met, L-Orn, L- and D-Ala, L- and D-Phe and benzyl-*O*-L-Tyr).

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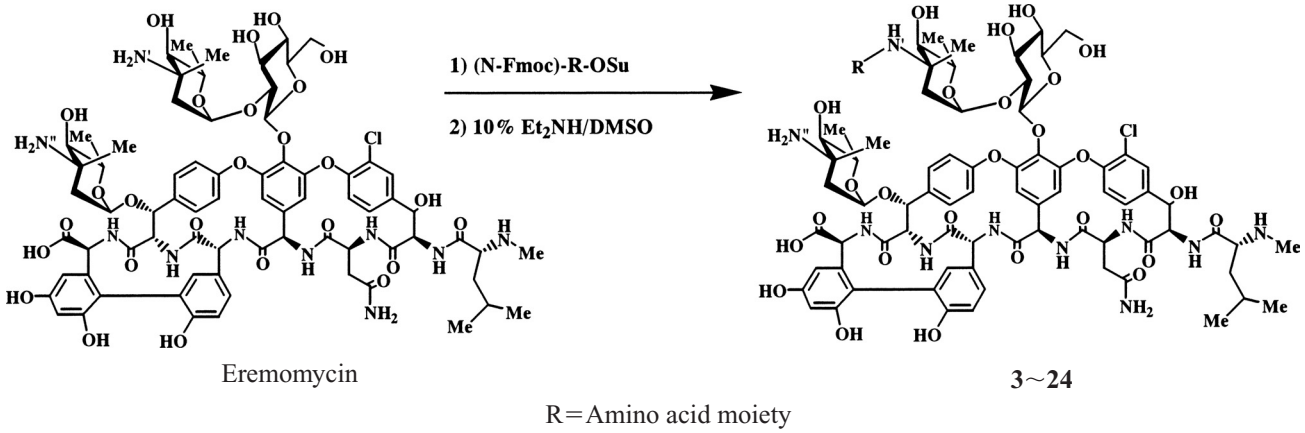
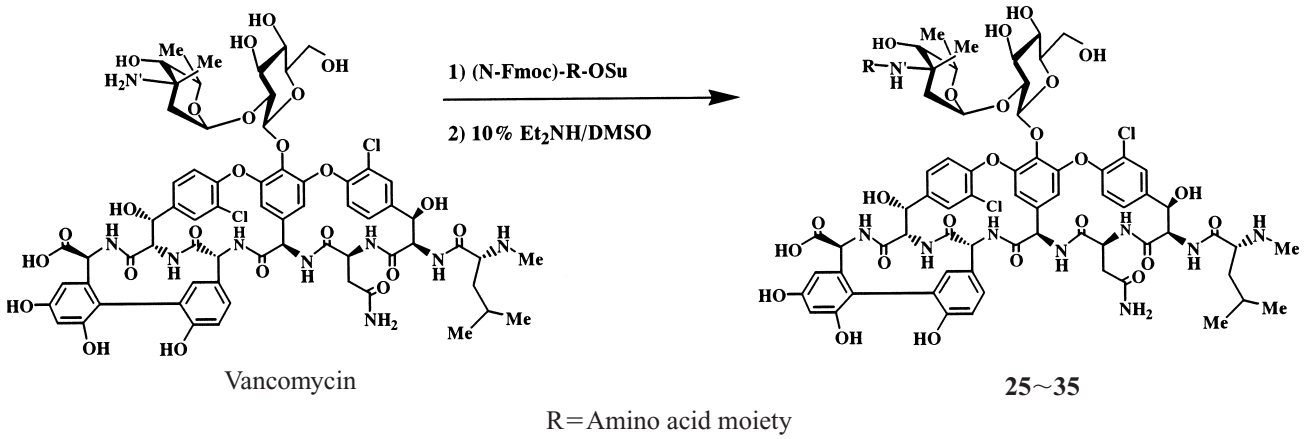
Introduction

The introduction of a hydrophobic substituent into a glycopeptide molecule is a way to obtain derivatives active against glycopeptide-resistant enterococci [1～3]. The introduction of a substituent into a glycopeptide molecule is complicated by low selectivity of reactions caused by the presence of several functional groups. In addition, complicated purification of the synthesized compounds is often necessary [4]. Earlier we demonstrated that the selectivity of acylation of vancomycin group antibiotics depends on the nature of the different acylating agents (Ac-, n-$C_8H_{17}CO$– [5, 6] or Boc-, Cbz- [7] and Fmoc-containing reagents [2]) and of pH. Under alkaline conditions the predominant isomer is a product of *N*-terminal acylation, a mixture of *N*- and *N′*-acylated products being observed with increasing pH. However, we have found that acylation of the unprotected vancomycin or eremomycin with activated esters of *N*-acyl-(*N*-9-fluorenylmethoxycarbonyl or *N*-1-adamantyloxycarbonyl) α-amino acids is directed selectively to the *N′* position of the amino group of the disaccharide branch. We used this reaction for the introduction of α-amino acid moieties containing hydrophobic groups into glycopeptides.



Scheme 1 Scheme for synthesis of *N′*-(*N*-acyl)glycyl-, *N′*-(*α*-aminoacyl)- and *N′*-*α*-(N$^{\alpha}$-alkylamino)acyl derivatives of eremomycin (**3**~**24**) (R see Table 1).



Scheme 2 Scheme for synthesis of *N′*-(*α*-aminoacyl)- and *N′*-*α*-(N$^{\alpha}$-alkylamino)-acyl derivatives of vancomycin (**25**~**35**) (R see Table 1).

Results and Discussion

N′-Fmoc-glycyl, *N′*-Adoc-glycylderivatives of eremomycin, and a series of *N′*-(*α*-aminoacyl) and *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin or vancomycin were synthesized by the treatment of eremomycin or vancomycin with the corresponding activated *N*-protected *N*-oxysuccinimide esters (–OSu) of different amino acids or N$^{\alpha}$-alkyl-*α*-amino acids. Deprotection of the corresponding *N′*-(*N*-Fmoc)derivatives gave the final *N′*-(*α*-aminoacyl) or *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of vancomycin or eremomycin. Some of these compounds were transformed into the corresponding carboxamides by the method described earlier [8]. The antibacterial activities of new derivatives of eremomycin and vancomycin were investigated [9, 10].

N′-(*N*-Fmoc)glycyl or *N′*-(*N*-Adoc)glycylderivatives of eremomycin (**1**, **2**), and *N′*-(*N*-alkylglycyl) derivatives of eremomycin (**3**~**18**) (Scheme 1; Table 1) or vancomycin (**25**~**31**) (Scheme 2; Table 1) were prepared in 30~60% yield by the treatment of unprotected eremomycin or vancomycin with equivalent amounts of the corresponding *N*-protected glycine or *N*-alkyl-glycine *N*-oxysuccinimide esters followed by deprotection with 10% diethylamine in DMSO. *N′*-(*α*-aminoacyl) and *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin (**19**~**24**) or vancomycin (**32**~**35**) containing other *α*-amino acids were prepared in 10~50% yield by a similar method starting with the antibiotic and the corresponding OSu-esters of *N*-Fmoc *α*-amino or *N*-Fmoc-*N*$^{\alpha}$-alkylamino acids followed by deprotection with 10% diethylamine in DMSO (Scheme 1, 2; Table 2). The purification of these compounds was performed analogously to the purification of **28**; the yields

Table 1 The structures of the eremomycin and vancomycin derivatives

Compound	R	Molecular formula	Calculated: Exact mass (Molecular mass)	ESI-MS Found (M)
Eremomycin derivatives				
1	FmocNHCH$_2$CO	$C_{90}H_{102}N_{11}O_{29}Cl$	1835.65 (1837.3)	1835.7*
2	AdocNHCH$_2$CO	$C_{86}H_{106}N_{11}O_{29}Cl$	1791.7 (1793.3)	1791.7**
3	*p*-(*p*-ClPh)PhCHNHCH$_2$CO	$C_{88}H_{101}N_{11}O_{27}Cl_2$	1813.6 (1815.7)	1814.6**
4	*p*-BuPhCH$_2$NHCH$_2$CO	$C_{86}H_{106}N_{11}O_{27}Cl$	1759.7 (1761.3)	1759.9**
5	C_9H_{19}CONHCH$_2$CO	$C_{85}H_{110}N_{11}O_{28}Cl$	1767.7 (1769.3)	1768.2**
6	*p*-Bu$_2$NPhCH$_2$NHCH$_2$CO	$C_{90}H_{115}N_{12}O_{27}Cl$	1830.8 (1832.4)	1830.8*
7	*p*-PhCH=CHPhCH$_2$NHCH$_2$CO	$C_{90}H_{104}N_{11}O_{27}Cl$	1805.7 (1807.3)	1806.2**
8	*p*-BuOPhCH$_2$NHCH$_2$CO	$C_{86}H_{106}N_{11}O_{28}Cl$	1775.7 (1777.3)	1775.7*
9	$(C_{10}H_{21})_2$NCH$_2$CO	$C_{95}H_{132}N_{11}O_{27}Cl$	1893.9 (1895.6)	1894.2*
10	*p*-octylOPhCH$_2$NHCH$_2$CO	$C_{90}H_{114}N_{11}O_{28}Cl$	1831.75 (1833.4)	1832.5**
11	*p*-PhCH$_2$OPhCH$_2$NHCH$_2$CO	$C_{89}H_{104}N_{11}O_{28}Cl$	1809.7 (1811.3)	1810.6*
12	5-PhCH$_2$OIndol-3-yl-CH$_2$NHCH$_2$CO	$C_{91}H_{105}N_{12}O_{28}Cl$	1848.7 (1850.3)	1851.0**
13	1-PhCH$_2$Indol-3-yl-CH$_2$NHCH$_2$CO	$C_{91}H_{105}N_{12}O_{27}Cl$	1832.7 (1834.35)	1833.7*
14	Phenanthren-9-yl-CH$_2$NHCH$_2$CO	$C_{90}H_{102}N_{11}O_{27}Cl$	1803.7 (1805.3)	1804.1**
15	Fluorenyl-2-CH$_2$NHCH$_2$CO	$C_{89}H_{102}N_{11}O_{27}Cl$	1791.7 (1793.3)	1791.8**
16	*p*-F-PhCH$_2$NHCH$_2$CO	$C_{82}H_{97}N_{11}O_{27}ClF$	1721.6 (1723.2)	1721.6*
17	*p*-CF$_3$-PhCH$_2$NHCH$_2$CO	$C_{83}H_{97}N_{11}O_{27}ClF_3$	1771.6 (1773.2)	1772.1**
18	Quinolinyl-2-CH$_2$NHCH$_2$CO	$C_{85}H_{99}N_{12}O_{27}Cl$	1754.6 (1756.2)	1755.6**
19	PhCH$_2$CH(NH$_2$)CO (D-isomer)	$C_{82}H_{96}N_{11}O_{27}Cl$	1703.6 (1705.2)	1703.8**
20	PhCH$_2$CH(NH$_2$)CO (L-isomer)	$C_{82}H_{96}N_{11}O_{27}Cl$	1703.6 (1705.2)	1704.3***
21	PhCH$_2$OPhCH$_2$CH(NH$_2$)CO (L-isomer)	$C_{89}H_{104}N_{11}O_{28}Cl$	1809.7 (1811.3)	1810.9**
22	NH$_2$(CH$_2$)CH(NH$_2$)CO (L-isomer)	$C_{79}H_{101}N_{12}O_{27}Cl$	1684.7 (1686.2)	1686.2**
23	*p*-BuPhCH$_2$NH$^{\alpha}$-CH[(CH$_2$)$_3$NH$_2^{\delta}$]CO (L-isomer)	$C_{89}H_{113}N_{12}O_{27}Cl$	1816.75 (1818.4)	1818.5*, 1816.6**
24	*p*-octylOPhCH$_2$NHCH(CH$_3$)CO (L-isomer)	$C_{91}H_{116}N_{11}O_{28}Cl$	1845.8 (1847.4)	1845.9**
Vancomycin derivatives				
25	*p*-(*p*-ClPh)PhCH$_2$NHCH$_2$CO	$C_{81}H_{87}N_{10}O_{25}Cl_3$	1704.5 (1707.0)	1706.5**
26	*p*-BuPhCH$_2$NHCH$_2$CO	$C_{79}H_{92}N_{10}O_{25}Cl_2$	1650.56 (1652.26)	1652.3**
27	*p*-BuOPhCH$_2$NHCH$_2$CO	$C_{79}H_{92}N_{10}O_{26}Cl_2$	1666.6 (1668.6)	1668.5*
28	*p*-OctylOPhCH$_2$NHCH$_2$CO	$C_{80}H_{100}N_{10}O_{26}Cl_2$	1722.6 (1724.6)	1722.7
29	*p*-F-PhCH$_2$NHCH$_2$CO	$C_{75}H_{83}N_{10}O_{25}Cl_2F$	1612.5 (1614.4)	1612.6**
30	*p*-CF$_3$PhCH$_2$NHCH$_2$CO	$C_{76}H_{83}N_{10}O_{25}Cl_2F_3$	1662.5 (1664.45)	1664.3**
31	Quinolinyl-2-CH$_2$NHCH$_2$CO	$C_{78}H_{85}N_{11}O_{25}Cl_2$	1645.5 (1647.5)	1647.2**
32	*p*-OctylOPhCH$_2$NH$^{\alpha}$-CH[(CH$_2$)$_3$NH$_2^{\delta}$]CO (L-isomer)	$C_{86}H_{107}N_{11}O_{26}Cl_2$	1779.7 (1781.7)	1781.6**
33	*p*-Octyl-OPhCH$_2$NHCH(CH$_3$)CO (L-isomer)	$C_{84}N_{102}N_{10}O_{26}Cl_2$	1736.6 (1738.7)	1738.6**
34	*p*-OctylO-PhCH$_2$NHCH(CH$_3$)-CO (D-isomer)	$C_{84}H_{102}N_{10}O_{26}Cl_2$	1736.6 (1738.7)	1736.6**
35	*p*-OctylOPhCH$_2$NHCH(CH$_2$CH$_2$SCH$_3$)CO (L-isomer)	$C_{96}H_{106}N_{10}O_{26}Cl_2S$	1796.6 (1798.8)	1798.6**

* $[M+H]^+$ Molecular mass (M) calculated from the corresponding peaks (*m*/*z*) for $[M+H]^+$ ion in ESI mass-spectra.

** Molecular mass (M) calculated from the corresponding peaks (*m*/*z*) for $[M+2H]^{2+}$ ion in ESI mass-spectra.

*** Molecular mass (M) calculated from the peaks (*m*/*z*) corresponding $[M+Na]^+$ ion in MALDI (TOF) mass-spectra.

were 30～50% for **28** and **19**～**24** and about 10% for **21** and **23**.

The starting *N*-Fmoc-α-amino or *N*-Fmoc-N^{α}-alkyl-α-amino acid OSu-esters of Gly, Lys, Tyr, Phe, OSu ester of N^{α}, N^{ε}-Di-Fmoc-L-Lys and OSu-esters of N^{α}, N^{δ}-Di-Fmoc-(N^{α}-alkyl)-L-Orn were synthesized as shown on Scheme 3 and Scheme 4. The structures of the synthesized starting reagents (N^{α}-alkylamino acids or N^{α}-alkyl-N^{α}-Fmoc-amino acids) were confirmed by ^{1}H-NMR-spectra data.

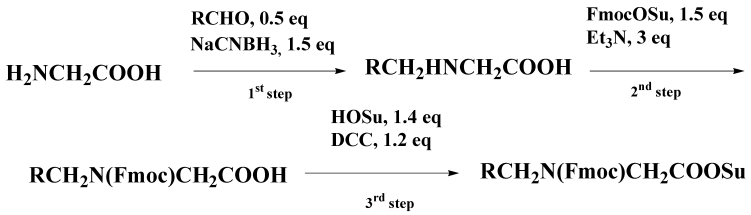
The doubly modified derivatives-carboxamides of *N*′-(α-aminoacyl)- and *N*′-α-(N^{α}-alkylamino)acyl derivatives of eremomycin (**36**～**41**) or vancomycin (**42**～**44**) (Fig. 1;

Table 2 The structures of the carboxamides of eremomycin and vancomycin derivatives

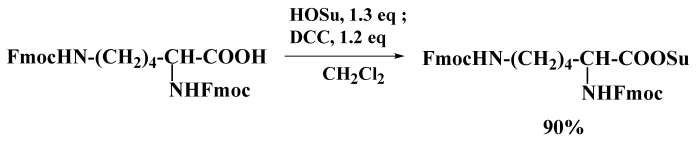
Compound	R^1	R^2	Molecular formula	Calculated: Exact mass (Molecular mass)	ESI MS Found (M)
Eremomycin derivatives					
36	*p*-$BuPhCH_2NHCH_2CO$	$NHCH_3$	$C_{87}H_{109}N_{12}O_{26}Cl$	1772.7 (1774.3)	1772.5**
37	*p*-$BuPhCH_2NHCH_2CO$	NHAdam-2	$C_{96}H_{121}N_{12}O_{26}Cl$	1892.8 (1894.5)	1893.6*
38	*p*-$BuPhCH_2NHCH_2CO$	$NHCH(CH_3)$Adam-1	$C_{98}H_{125}N_{12}O_{26}Cl$	1920.85 (1922.6)	1921.1*
39	*p*-$OctylOPhCH_2NHCH_2CO$	NHAdam-2	$C_{100}H_{129}N_{12}O_{27}Cl$	1964.9 (1966.6)	1967.0**
40	*p*-$OctylOPhCH_2NHCH_2CO$	$NHCH(CH_3)$Adam-1	$C_{102}H_{133}N_{12}O_{27}Cl$	1992.9 (1994.7)	1995.0**
41	*p*-(*p*-ClPh)$PhCH_2NHCH_2CO$	$NHCH_2$Ph-F-*p*	$C_{95}H_{107}N_{12}O_{26}Cl_2F$	1920.7 (1922.9)	1923.8**
Vancomycin derivatives					
42	*p*-$BuPhCH_2NHCH_2CO$	$NHCH_2$Ph-F-*p*	$C_{86}H_{98}N_{11}O_{24}Cl_2F$	1757.6 (1759.7)	1758.8**
43	*p*-$OctylOPhCH_2NHCH_2CO$	$NHCH_2$Ph-F-*p*	$C_{90}H_{106}N_{11}O_{25}Cl_2F$	1829.7 (1831.8)	1832.8**
44	*p*-$OctylOPhCH_2NHCH_2CO$	$NH(CH_2)N(CH_3)_2$	$C_{88}H_{112}N_{12}O_{25}Cl_2$	1806.7 (1808.8)	1807.9**

* $[M+H]^+$ Molecular mass (M) calculated from the corresponding peaks (*m*/*z*) for $[M+H]^+$ ion in ESI mass-spectra.

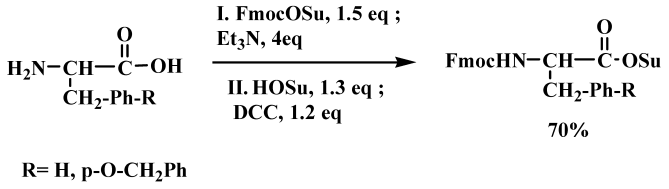
** Calculated from *m*/*z* peaks from the corresponding peaks (*m*/*z*) for $[M+2H]^{2+}$ ion in ESI mass-spectra.



a) N-Alkyl-N-Fmoc-Gly-OSu [for N-alkyl-Gly derivatives (**3** ~ **18**)]

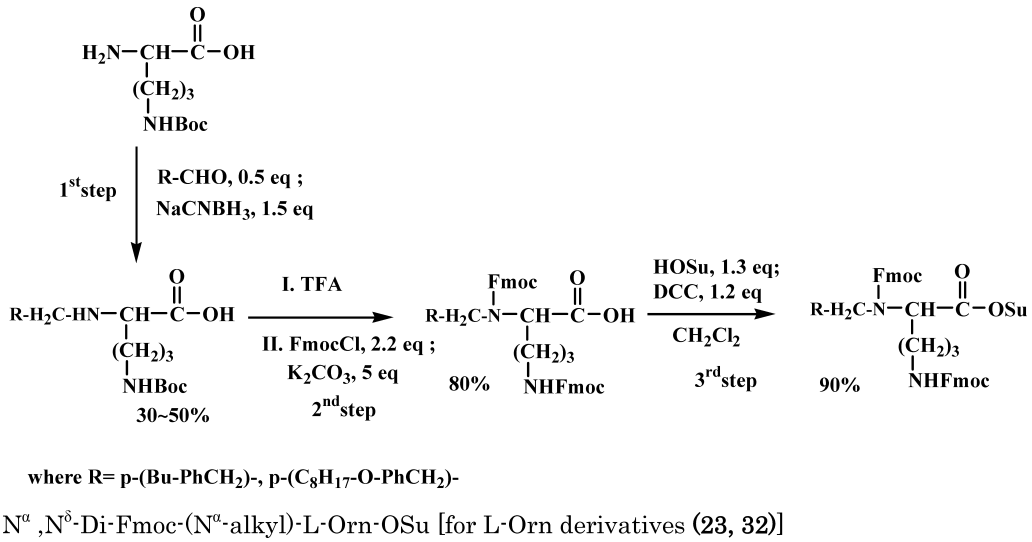


b) N^{α}, N^{ε}- Di-Fmoc-L-Lys-OSu [for L-Lys derivative (**22**)]



c) N^{α}-Fmoc-Phe- or N^{α}-Fmoc-(O-$PhCH_2$)-Tyr-OSu [for L-Tyr, L- or D-Phe derivatives (**19~21**)]

Scheme 3 Syntheses of the starting activated OSu esters of *N*-substituted glycine, L-lysine and L-phenyl and L-tyrosine derivatives.



Scheme 4 Synthesis of the starting *N*$^{\alpha}$,*N*$^{\delta}$-Di-Fmoc-(*N*$^{\alpha}$-alkyl)-L-Orn-OSu (**23**, **32**)

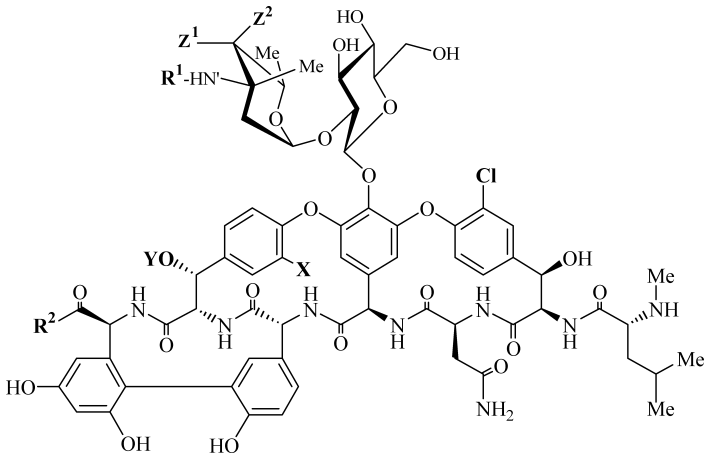


Fig. 1 The structures of carboxamides of *N*′-(*α*-aminoacyl)- or *N*′-*α*-(*N*$^{\alpha}$-alkylamino)-acyl derivatives of eremomycin (R^1=H, R^2=OH; X=H; Y=*α*-eremosaminyl; Z^1=H; Z^2=OH) (**36**~**41**) and Vancomycin (R^1=H; R^2=OH; X=Cl; Y=H; Z^1=OH; Z^2=H) (**42**~**44**) (R^1 and R^2 see in Table 2).

Table 2) were obtained by the interaction of the corresponding compounds (**3**, **4**, **10**) or (**26**, **28**) with different amines in the presence of the condensing reagents HBPyU or PyBOP by the method described earlier [8].

The purification of the eremomycin and vancomycin derivatives was performed using column chromatography on silanized silica gel. The progress of the reactions, the components of the column eluates and the purity of the final compounds were checked by TLC and HPLC in three systems.

The structures of all derivatives were confirmed by electrospray ionization mass-spectral (ESI MS) analysis (Tables 1 and 2), and by chemical degradation methods as was described earlier [8, 11, 12]. The presence of the unsubstituted *N*-Me-D-leucine, that is the *N*-terminal amino acid in the eremomycin or vancomycin derivatives was confirmed by Edman degradation: TLC demonstrated the presence of 5-isopropyl-10-methyl-3-phenyl-2-thiohydantoin by the comparison with an authentic sample, which was obtained in the parallel experiment by Edman degradation of eremomycin or vancomycin. Mild hydrolysis of eremomycin or vancomycin in 1 N HCl at 100°C for 10 minutes leads to the splitting off eremosamine or respectively vancosamine from the disaccharide branch. The *N*′-aminoacyl derivatives obtained do not produce unsubstituted eremosamine or vancosamine but do produce

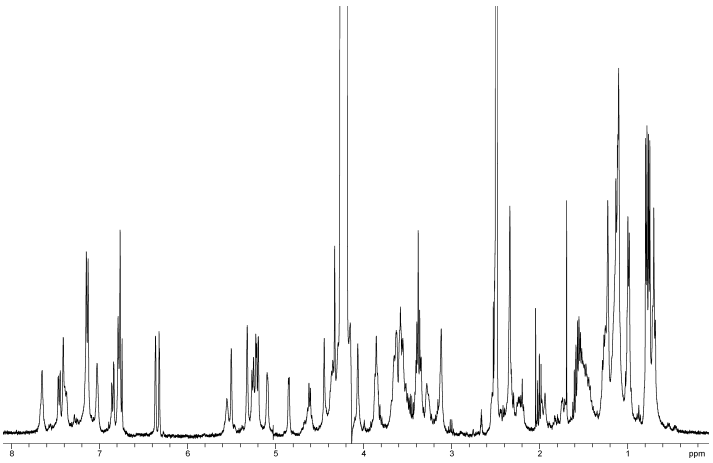


Fig. 2A ^{1}H NMR spectrum of compound **28** in D_2O+DMSO-d_6 (2 : 1), T=60°C.

V1 and V2: the signals of remaining protons of respective D-solvents (HOD: δ 4.22, $CD_3S(O)CD_2H$: δ 2.49); * and **: the signals of the traces of respective H-solvents in the sample (acetone: δ 2.05 and acetic acid anion: δ 1.69).

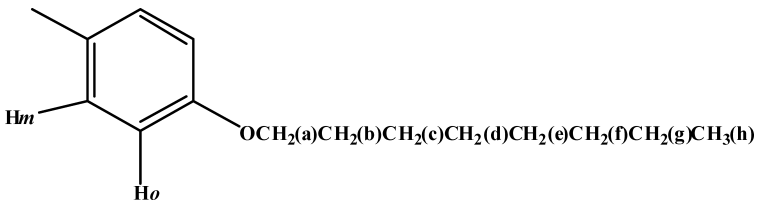


Fig. 2B The introduced moiety of the compound **28**.

The ^{1}H NMR signals of *p*-octyl-*O*-phenyl-residue, δ (ppm): H*m*: 7.14 d, ^{3}J=8.2 Hz; H*o*: 6.78 d; CH_2(a): 3.86t, ^{3}J=6.3 Hz; CH_2(b): 1.54 m; CH_2(c): 1.24 m; CH_2(d): 1.13 m; CH_2(e), CH_2(f), CH_2(g): 1.10 m; CH_3(h): 0.70 t, ^{3}J=6.7 Hz.

des-(eremosaminyl) eremomycin [5, 7, 11] or des-(vancosaminyl)vancomycin under these conditions [7, 12]. After drastic hydrolysis (conc. HCl, room temperature, 4 hours), when all sugars of the glycopeptide antibiotics are split off, the formation of unmodified eremomycin or vancomycin aglycons were observed. The presence of unmodified eremosamine formed after splitting off the monosaccharide branch from eremomycin derivatives [8] was observed as well. The presence of the unsubstituted eremosamine, vancosamine, des-(eremosaminyl)eremomycin and des-(vancosaminyl)vancomycin was demonstrated by paper and TLC chromatography by the comparison with authentic compounds [11]. The ^{1}H NMR spectrum of **28** is presented in Fig. 2A. The assignments were confirmed with double resonance and 2D COSY spectra. The introduced *p*-octyl-*O*-phenyl-residue of **28** is presented in Fig. 2B.

Conclusion

The acylation of unprotected vancomycin or eremomycin with activated esters of *N*-Fmoc-α-amino acids or *N*-alkyl-N^{α}-Fmoc-amino acids is a selective reaction that allows the preparation of highly active hydrophobic antibacterial compounds capable of overcoming resistance to vancomycin [9, 10].

Experimental

Material and Methods

Eremomycin sulfate was produced at the pilot plant of the Gause Institute of New Antibiotics, Moscow. All reagents and solvents were purchased from Aldrich, Fluka and Merck. *p*-(*p*-Chlorophenyl)benzaldehyde was synthesized at Advanced Medicine East, Inc. The progress of the reactions, column eluates and all final samples were

analyzed by TLC using Merck Silica Gel 60 F_{254} plates in systems containing EtOAc - PrOH - 25%-NH_4OH, 3 : 2 : 2, or 1 : 1 : 1. In addition, purity of the final compounds was demonstrated by HPLC in the conditions described earlier [8]. HPLC analyses were performed on a Shimadzu HPLC instrument of the LC 10 series. Analytical reverse phase HPLC was carried out on a Diasorb C16 column (particle size 7 μm) at an injection volume of 10 μl and a wave length 280 nm. The sample concentration was 0.05～0.2 mg/ml. Three systems were used to assess the purity or identity of the compounds obtained: System A comprised 0.2 M $HCOONH_4$ at pH 7.6 and acetonitrile, the proportion of acetonitrile varied from 10 to 80% for 40 minutes with flow rate 1.0 ml/minute. System B comprised 0.1 M $NH_4H_2PO_4$ at pH 3.75 and acetonitrile, the proportion of acetonitrile varied from 5 to 40% for 25 minutes and from 40 to 45% for 15 minutes with flow rate 1.0 ml/minute. System C was similar to B system, but linear gradient of elution varied from 5 to 40% for 17 minutes and from 40 to 45% for 23 minutes with the same flow rate.

Reaction products were purified by reversed-phase column chromatography on Merck Silanized Silica Gel (0.063～0.2 mm). Mass spectra were determined by electrospray ionization (ESI) on a Finnigan SSQ7000 single quadrupole mass spectrometer; and by MALDI TOF MS on Kompact MALDI III mass spectrometer (Kratos, UK). All ^1H NMR measurements were obtained with Varian VXR-400 instrument and Varian Unity Plus-400 instrument operated at 400 MHz. The structures of compound **28** and the synthesized starting reagents (N^{α}-alkylamino acids or N^{α}-alkyl-N^{α}-Fmoc-amino acids) were confirmed by NMR-spectral data.

General Synthetic Procedure for the *N′*-(α-Aminoacyl)- and *N′*-α-(N^{α}-Alkylamino)acyl Derivatives of Eremomycin (1～24) or Vancomycin (25～35)

N′-(*N*-Fmoc)-Gly or *N′*-(*N*-Adoc)Gly-eremomycin (**1**, **2**) were prepared by the treatment of eremomycin with Fmoc-GlyOSu or Adoc-GlyOSu. *N′*-(α-aminoacyl)- and *N′*-α-(N^{α}-alkylamino)acyl eremomycin (**3**～**24**) or vancomycin (**25**～**35**) derivatives were prepared in 30～60% yields by the treatment of the eremomycin or vancomycin (bases) with OSu esters of *N*-Fmoc-α-amino acids or *N*-Fmoc-α-(N^{α}-alkylamino acids (alkyl is a hydrophobic radical from the appropriate aldehyde) followed by deprotection with 10% diethylamine in DMSO.

Purification of eremomycin and vancomycin derivatives was performed using column chromatography on silanized silica gel. The progress of the reactions, the components of the column eluates and the purity of the final compounds were checked by TLC in the systems: EtOAc - *n*-PrOH - 25% NH_4OH 1 : 1 : 1 or 3 : 2 : 2 and *n*-BuOH - AcOH - H_2O 5 : 1 : 1. Additionally, the purity of the derivatives for *in vivo* study was controlled by HPLC.

Detailed typical procedure of the synthesis and purification of the compounds is presented in the method of synthesis of *N′*-(*p*-octylOPhCH_2Gly)vancomycin (**28**).

The starting *N*-alkyl-*N*-Fmoc-derivatives of glycine or other amino acids and their Osu esters were synthesized according to typical procedures.

***N′*-(*p*-OctylOPhCH_2NHCH_2CO)vancomycin (28)**

To a stirred solution of 1800 mg (1.25 mmol) of vancomycin (base) in 30 ml of DMSO : H_2O (4 : 1) were added 0.16 ml (1.25 mmol) of Et_3N and 1165 mg (1.9 mmol) of *p*-octylOPhCH_2N(Fmoc)-CH_2COOSu. The reaction mixture was stirred at room temperature for 5 hours, and then 3 ml of Et_2NH was added. The reaction mixture was stirred at room temperature for 1 hour, and than added to 200 ml of acetone. The solid precipitate was filtered off, washed with acetone and dried *in vacuo*. The solid was dissolved in H_2O : THF (1 : 1) and evaporated *in vacuo* with a small amount of silanized silica gel. The residue was applied to a chromatographic column of silanized silica gel (3×120 cm) preequilibrated with H_2O. The column was eluted firstly with H_2O (1000 ml) at a rate 10 ml/hour, while collecting 5 ml fractions. The fractions containing vancomycin were collected. The column was then eluted with 0.02 M CH_3COOH (1000 ml) at a rate 10 ml/hour, while collecting 5 ml fractions, and then with 15% MeOH in 0.02 M CH_3COOH (500 ml) at the same rate. Fractions containing the product of the reaction were collected. An additional amount of the desired product was obtained after elution with 30% MeOH in 0.02 M CH_3COOH (1000 ml) at the same rate, as suitable fractions containing the product of the reaction were collected. All the fractions containing pure *N′*-(*p*-octylOPhCH_2NHCH_2CO)vancomycin were combined and concentrated *in vacuo* to a small volume (～10 ml). Then 30 ml of acetone was added and this mixture was added to 250 ml of Et_2O to precipitate the product. The solid precipitated was filtered off, washed with Et_2O and dried *in vacuo* to give 904 mg (42%) of pure *N′*-(*p*-octylOPhCH_2NHCH_2CO)vancomycin (**28**).

Carboxamides of *N′*-(α-Aminoacyl) and *N′*-α-(N^{α}-Alkylamino)acyl Derivatives of Eremomycin (36～41) or Vancomycin (42～44) (Fig. 1)

Carboxamides **36**～44 were synthesized starting from the corresponding *N′*-(α-aminoacyl)- and *N′*-α-(N^{α}-alkylamino)acyl derivatives of eremomycin (**3**, **4**, **10**) or vancomycin (**26**, **28**) and the respective methyl-, adamantyl-2-, 1-(adamantyl-1)ethyl-, *p*-fluorobenzyl- and

N,*N*-dimethyl-3-aminopropyl- amines in the presence of the coupling reagents HBPyU [*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate] or PyBOP [(benzotriazol-1-yloxy)-tris-(pyrrolidino)phosphonium-hexafluorophosphate] by the method described earlier [7].

Typical Procedure for $RCH_2N(Fmoc)CH_2COOSu$ (OSu-Esters of N^{α}-Fmoc-derivatives of N^{α}-Alkyl-Gly-OH) (Scheme 3a)

1st step: RCH_2NHCH_2COOH (Reductive Alkylation of NH_2-group of Gly)

To a stirred solution of glycine (2 mmol) in THF : H_2O (1 : 1) at room temperature were added portion-wise a solution of 1 mmol of an appropriate aldehyde in THF and 1.5 mmol of $NaCNBH_3$. The reaction mixture was stirred for 4 hours, and then water was added. The resulting mixture was evaporated *in vacuo* to remove THF and was extracted three times with petroleum. The aqueous fraction was evaporated *in vacuo* with silica gel to dryness and applied to a chromatographic column of silica gel preequilibrated with $CHCl_3$. The column was eluted with $CHCl_3$: MeOH : 25% NH_4OH (60 : 20 : 1) system at a rate 10 ml/hour, collecting 5 ml fractions. Suitable fractions were combined and evaporated *in vacuo* to dryness. The yields are 30～50%.

^{1}H NMR-spectra data (δ ppm, solvent, room temperature) for starting reagents:

p-(*p*-ClPh)$PhCH_2NHCH_2COOH$ (CD_3OD) 8.00, 7.90, 7.80 and 7.55 (8H, 4d, 2 Ph), 4.17 (2H, s, Ph-$\underline{CH_2}$-NH), 3.40 (2H, s, NH-$\underline{CH_2}$-COOH);

p-$BuPhCH_2NHCH_2COOH$ ($CDCl_3$) 7.38 and 7.05 (4H, 2d, Ph), 4.15 (2H, m, Ph-$\underline{CH_2}$-NH), 3.32 (2H, s, NH-$\underline{CH_2}$-COOH), 2.55 (2H, m, Ph-$\underline{CH_2}$), 1.60～1.20 (4H, 2m, 2C-CH_2-C), 0.92 (3H, t, CH_3);

$C_9H_{19}CONHCH_2COOH$ (CD_3OD) 4.10 (2H, t, $\underline{CH_2}$-CONH), 3.49 (2H, s, NH-$\underline{CH_2}$-COOH), 1.8～1.45 (14H, m, C-$(CH_2)_7$-C), 0.98 (3H, t, CH_3);

p-$Bu_2NPhCH_2NHCH_2COOH$ ($CDCl_3$) 7.35 and 6.98 (4H, 2d, Ph), 4.22 (2H, dd, Ph-$\underline{CH_2}$-N), 4.15 (2H, s, NH-$\underline{CH_2}$-COOH), 3.56 (4H, m, $\underline{CH_2}$-N(Ph)-$\underline{CH_2}$), 1.70～1.22 (8H, m, 2C-$(CH_2)_2$-C), 0.90 (6H, 2t, 2 CH_3);

p-PhCH=CH$PhCH_2NHCH_2COOH$ (CD_3OD) 7.43～7.06 (9H, m, 2Ph), 6.65 (2H, m, HC=CH), 3.95 (2H, s, Ph-$\underline{CH_2}$-NH), 3.45 (2H, s, NH-$\underline{CH_2}$-COOH);

p-$BuOPhCH_2NHCH_2COOH$ (CD_3OD, t=40°C) 7.42 and 6.99 (4H, 2d, Ph), 4.17 (2H, s, Ph-$\underline{CH_2}$-NH), 4.02 (2H, t, α-$\underline{CH_2}$ of Bu group), 3.49 (2H, s, NH-$\underline{CH_2}$-COOH), 1.79 (2H, m, β-$\underline{CH_2}$ of Bu group), 1.54 (2H, m, γ-$\underline{CH_2}$ of Bu group), 1.03 (3H, t, $\underline{CH_3}$);

$(C_{10}H_{21})_2NCH_2COOH$ ($CDCl_3$) 4.18 (2H, s, NH-$\underline{CH_2}$-COOH), 3.80 (4H, m, $\underline{CH_2}$-N-$\underline{CH_2}$), 1.65～1.15 (32H, m, 2C-$(CH_2)_8$-C), 0.92 (6H, 2t, $2CH_3$);

p-$PhCH_2OPhCH_2NHCH_2COOH$ ($CDCl_3$) 7.30～7.02 (9H, m, Ph), 4.98 (2H, s, Ph-$\underline{CH_2}$-O), 4.21 (2H, s, Ph-$\underline{CH_2}$-N), 3.46 (2H, s, NH-$\underline{CH_2}$-COOH).

Phenanthren-9-yl-CH_2-$NHCH_2COOH$ (DMSO-d_6 - TFA) 9.00～7.65 (9H, m, Ar), 4.81 (2H, s, Ar-$\underline{CH_2}$-NH), 4.14 (2H, s, NH-$\underline{CH_2}$-COOH);

Fluorenyl-2-CH_2NHCH_2COOH (DMSO-d_6 - TFA+D_2SO_4) 8.00～7.30 (7H, m, Ar), 4.26 (2H, s, Ar-$\underline{CH_2}$-NH), 3.96 (2H, s, NH-$\underline{CH_2}$-COOH);

p-F-$PhCH_2NHCH_2COOH$ (CD_3OD) 7.55 and 7.28 (4H, 2m, Ph), 4.21 (2H, s, Ph-$\underline{CH_2}$-NH), 3.51 (2H, s, NH-$\underline{CH_2}$-COOH);

Quinolinyl-2-CH_2NHCH_2COOH (CD_3OD) 8.20～7.60 (6H, m, Ar protons of quinolinyl group), 4.25 (2H, s, Ar-$\underline{CH_2}$-NH), 3.60 (2H, s, NH-$\underline{CH_2}$-COOH);

p-Octyl-$OPhCH_2NHCH(CH_3)COOH$ (L-isomer) (DMSO-d_6: CF_3COOD) 7.40 and 7.00 (4H, 2d, Ph), 4.10 (2H, m, $\underline{CH_2}$-O-Ph), 3.99～3.91 (3H, m, $\underline{CH_2}$-NH and $\underline{CH}$-CH_3), 1.8～1.2 (15H, 4m, 6C-CH_2-C and CH_3 of Ala), 0.90 (3H, t, CH_3 of octyl group);

p-Octyl-$OPhCH_2{}^{\alpha}NHCH[(CH_2)_3{}^{\delta}NH_2]COOH$ (L-isomer) (CD_3OD) 7.42 and 6.98 (4H, 2d, Ph) 4.15 (2H, dd, Ph-$\underline{CH_2}$-NHα), 3.98 (2H, t, CH_2-O), 3.72 (1H, 2d, NH^{α}-$\underline{CH}$-COOH), 2.96 (2H, t, $\underline{CH_2}$-$NH_2{}^{\alpha}$), 1.75 and 1.50～1.25 (16H, 3m, 8C-CH_2-C of Orn and octyl group), 0.90 (3H, t, CH_3);

p-Octyl-$OPhCH_2$-NH-$CH(CH_2CH_2SCH_3)COOH$ (L-isomer) ($CDCl_3$ - MeOD - CF_3COOD) 7.18 and 7.73 (4H, 2d, Ph), 3.97 (2H, dd, Ph-$\underline{CH_2}$-NH), 3.78 (3H, m, CH_2-O and NH-$\underline{CH}$-COOH), 2.45 (2H, m, CH-$\underline{CH_2}$-C of Met), 2.05 (2H, m, CH_2-S), 1.90 (3H, s, S-CH_3), 1.60 and 1.30～1.05 (12H, 3m, 6C-CH_2-C of octyl group);

2nd step: $RCH_2N(Fmoc)CH_2COOH$ (N^{α}-Fmoc-derivatives of N^{α}-alkyl-Gly-OH)

To a stirred solution of RCH_2NHCH_2COOH (1 mmol) in THF : H_2O mixture (1 : 1) at room temperature 3 mmol of triethylamine and a solution of 1.5 mmol of FmocOSu in THF were added portionwise. The reaction mixture was stirred for 4 hours, and then water was added. The resulting mixture was evaporated *in vacuo* to remove THF and was extracted with petroleum (3 times) and then the aqueous fraction was evaporated *in vacuo* with silica gel to dryness and applied to a chromatographic column with silica gel preequilibrated with $CHCl_3$. The column was eluted with $CHCl_3$ - MeOH - 25% NH_4OH (5 : 1 : 0.05) system at a rate 10 ml/hour, while collecting 5 ml fractions. The suitable fractions were combined and evaporated *in vacuo* to dryness. The yields are 50～80%.

^{1}H-NMR-spectra data (δ ppm) for starting reagents:

p-OctylOPhCH$_2$ NH(Fmoc)CH$_2$COOH ($CDCl_3$) 7.77～6.80 (12H, Ph and Fmoc groups), 4.55 (2H, dd, Ph-CH$_2$-NH), 4.40 and 4.45 (2H, 2s, CH$_2$ of Fmoc group), 4.26 (1H, m, CH-CH$_2$), 3.96 (2H, m, Ph-CH$_2$-O), 3.36 and 3.50 (2H, 2s, NH-CH$_2$-COOH), 1.78 and 1.50～1.22 (12H, 3m, 6C-CH$_2$-C), 0.99 (3H, t, CH$_3$);

5-PhCH$_2$O-Indol-3-yl-CH$_2$NH(Fmoc)CH$_2$COOH (CD_3OD-$CDCl_3$) 7.75～6.95 (18H, m, Ar protons of Ph, Ind and Fmoc groups), 5.32 and 5.24 (2H, 2s, Ph-CH$_2$-O), 4.73 and 4.48 (2H, 2s, Ind-CH$_2$-O), 4.60 and 4.43 (2H, 2d, CH$_2$ of Fmoc group), 4.26, 4.22 (1H, 2t, CH-CH$_2$), 3.83 (2H, d, NH-CH$_2$-COOH);

(1-PhCH$_2$Indol-3-yl)-CH$_2$NH(Fmoc)CH$_2$COOH (CD_3OD) 7.80～6.85 (17H, m, Ar protons of Ph, Ind and Fmoc groups), 4.97 and 4.85 (2H, 2s, Ph-CH$_2$-O), 4.72 (2H, s, Ind-CH$_2$-O), 4.45 and 4.40 (2H, 2d, CH$_2$ of Fmoc group), 4.31, 4.23 (1H, 2t, CH-CH$_2$), 3.85 and 3.77 (2H, 2s, NHCH$_2$-COOH);

p-CF$_3$-PhCH$_2$NH(Fmoc)CH$_2$COOH ($CDCl_3$) 7.80～7.05 (12H, m, Ar protons of Ph and Fmoc groups), 4.58 (m), 4.50 (d) and 4.30 (s) (4H, Ph-CH$_2$ and CH$_2$ of Fmoc group), 4.22 (1H, m, CH-CH$_2$), 3.92 and 3.80 (2H, 2s, NH-CH$_2$-COOH);

Fmoc-NH$^{\alpha}$(CH$_2$)$_4$CH(NH$^{\alpha}$Fmoc)COOH (L-isomer) (Py-d5) 7.78～7.33 (16H, m, Ar protons of 2 Fmoc groups), 5.04～4.82 (4H, m, 2CH$_2$ of 2 Fmoc groups), 4.66 and 4.59 (2H, 2t, 2 CH-CH$_2$ of 2 Fmoc groups), 3.85 (2 H, m, CH$_2$-NH$^{\delta}$), 3.59 (1H, m, NH-CH$_2$-COOH), 2.46～1.84 (6H, m, 3C-CH$_2$-C of Lys).

p-BuPhCH$_2$NH$^{\alpha}$(Fmoc)CH[(CH$_2$)$_3$NHδ(Fmoc)]COOH (L-isomer) ($CDCl_3$) 7.70～6.75 (20H, m, Ar protons of Ph and 2 Fmoc groups), 4.55 (4H, m, O-CH$_2$ of 2 Fmoc groups), 4.40 (2H, m, 2CH of 2 Fmoc groups), 4.25～4.05 (4H, m, Ph-CH$_2$-NH$^{\alpha}$ and CH$_2$-NH$_2$), 3.10 (1H, s, NH$^{\alpha}$-CH-COOH), 2.50 (2H, m, Ph-CH$_2$), 1.70～1.15 (8H, 3m, 4C-CH$_2$-C of Orn and Bu group), 0.89 (3H, t, CH$_3$);

3rd step: RCH$_2$N(Fmoc)CH$_2$COOSu (OSu-Esters of N^{α}-Fmoc-derivatives of N^{α}-alkyl-Gly-OH)

To a stirred solution of RCH$_2$N(Fmoc)CH$_2$COOH (1 mmol) in CH_2Cl_2 at 0～5°C were added 1.3 mmol of HOSu followed by a solution of 1.2 mmol of DCC in THF added drop-wise. The reaction mixture was stirred for 4 hours, and then the precipitate of dicyclohexylurea was filtered off. The filtrate was concentrated *in vacuo* to a small volume, and precipitated dicyclohexylurea was filtered off again. The filtrate was evaporated *in vacuo* to dryness to give the desired activated ester.

p-Octyl-OPhCH$_2$N(Fmoc)CH$_2$COOSu was prepared by the typical procedure for RCH$_2$N(Fmoc)CH$_2$COOSu (1st～3rd steps) in 20% yield starting from glycin and poctylOPhCHO.

N^{α},N^{ε}-Di-Fmoc-L-Lys-OSu was prepared by the typical procedure for RCH$_2$N(Fmoc)CH$_2$COOSu (3rd step) in 90% yield starting from N^{α}, N^{ε}-Di-Fmoc-L-lysine and HOSu, 1.3 eq. and DCC, 1.2 eq., in CH_2Cl_2 (Scheme 3b).

N^{α}-Fmoc-Phe or N^{α}-Fmoc-(O-PhCH$_2$)-Tyr-OSu were prepared by the typical procedure as for RCH$_2$N-(Fmoc)CH$_2$COOSu (2nd and 3rd steps) in 70% total yield starting from L- or D-phenylalanine and *O*-benzyl-L-tyrosine and the following reagents: Fmoc-OSu, 1.5 eq., Et_3N, 4 eq. (in the 2nd step) and HOSu, 1.3 eq., DCC, 1.2 eq. (in the 3rd step) (Scheme 3c).

N^{α},N^{δ}-Di-Fmoc-(N^{α}-alkyl)-L-Orn-OSu was prepared by the typical procedure for RCH$_2$N(Fmoc)CH$_2$COOSu starting from N^{δ}-Boc-L-ornitine and the following reagents: *p*-Bu-PhCHO or *p*-octyl-*O*-PhCHO, 0.5 eq. and $NaCNBH_3$ 1.5 eq. (the 1st step); Fmoc-Cl, 2.2 eq. (instead of FmocOSu), K_2CO_3 (instead of Et_3N), 5 eq. after elimination of Boc-group with TFA (the 2nd step), and HOSu, 1.3 eq., DCC, 1.2 eq. (the 3rd step). The yield in the last step was 90% (Scheme 4).

N^{α}-Fmoc-(N^{α}-*p*-octyl-*O*-PhCH$_2$)-Ala-OSu (L- or D-isomer) and N^{α}-Fmoc-(N^{α}-*p*-octyl-OPhCH$_2$)-L-Met-OSu were prepared by the typical procedure for RCH$_2$N-(Fmoc)CH$_2$COOSu (1st～3rd steps) in 20% and 10% total yields respectively starting from L- or D-Ala or L-Met and poctyl-OPhCHO.

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