Semi-synthesis of Polymyxin B (2-10) and Colistin (2-10) Analogs Employing the Trichloroethoxycarbonyl (Troc) Group for Side Chain Protection of α,γ-Diaminobutyric Acid Residues[1)]

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Improved strategies for the chemical conversion of natural polymyxin B and colistin to their *N*-terminal analogs are reported. First, the protection of the side chains of five L-α,γ-diaminobutyric acid (Dab) residues in natural polymyxin B and colistin was achieved with trichloroethoxycarbonyl (Troc), then the resulting pentakis(N^{γ}-Troc)-polymyxin B and pentakis(N^{γ}-Troc)-colistin were treated with trifluoroacetic acid (TFA) : methanesulfonic acid (MSA) : dimethylformamide (DMF) : H_2O (10 : 30 : 55 : 5) at 40 °C in order to remove N^{α}-alkanoyl-Dab(Troc)-OH selectively. The new key compounds, tetrakis(N^{γ}-Troc)-polymyxin B (2-10) and tetrakis(N^{γ}-Troc)-colistin (2-10), were obtained in 19% and 15% yields, respectively, which is higher than previous reports using trifluoroacetyl (Tfa) for tetrakis(N^{γ}-Tfa)-polymyxin B (2-10) and tetrakis(N^{γ}-Tfa)-colistin (2-10), respectively.[2)] Acylation of tetrakis(N^{γ}-Troc)-polymyxin B (2-10) and tetrakis(N^{γ}-Troc)-colistin (2-10) with various hydrophobic acids bearing aliphatic or aromatic ring structures, followed by the deprotection of Troc by Zn in AcOH, produced polymyxin B (2-10) and colistin (2-10) analogs which were used for structure–activity relationship studies. It was found that cyclohexylbutanoyl-, 4-biphenylacetyl-, and 1-adamantaneacetyl-polymyxin B (2-10) showed potent antimicrobial activity equal to that of polymyxin B against three Gram-negative bacterial strains. The lipopolysaccharide (LPS) binding activity of cyclohexylbutanoyl-, 4-biphenylacetyl-, and cyclododecanecarbonyl-polymyxin B (2-10) increased greatly in comparison with that of polymyxin B (2-10). The various N^{α}-acylated polymyxin B (2-10) analogs showed slightly higher antimicrobial and LPS binding activities than the corresponding N^{α}-acylated colistin (2-10) analogs.

Key words polymyxin B (2-10) analog; tetrakis(N^{γ}-Troc)-colistin nonapeptide; semi-synthesis; methanesulfonic acid; trichloroethoxycarbonyl group; antimicrobial activity

Polymyxin B and colistin, produced by *Bacillus polymyxa*[3,4)] and *Bacillus colistinus*,[5)] are cationic cyclic decapeptide antibiotics active against Gram-negative bacteria that bind to the lipid A portion of lipopolysaccharides (LPS).[6)] Both peptides contain six L-α,γ-diaminobutyric acid (Dab) residues. The N^{γ}-amino function of Dab at position 4 (Dab[4]) is acylated by *C*-terminal Thr[10] to form a 23-member lactam ring (Fig. 1). The peptides have identical amino acid sequences except for position 6, which is occupied by D-Phe in polymyxin B and by D-Leu in colistin.[7,8)] The N^{α}-amino function of Dab[1] in polymyxin B_1, the main component of polymyxin B, is acylated by (*S*)-6-methyloctanoic acid, and most of the other minor components are replaced by various fatty acids.[7)] Colistin A (polymyxin E_1) and colistin B (polymyxin E_2) are the main components of colistin. Colistin A is acylated by (*S*)-6-methyloctanoic acid at the *N*-terminus, and colistin B is acylated by 6-methylheptanoic acid.[9—11)]

Therapeutic applications of polymyxin B and colistin have been limited to topical use because of their toxicity to humans.[12)] Most of the toxic activity of these peptide antibiotics

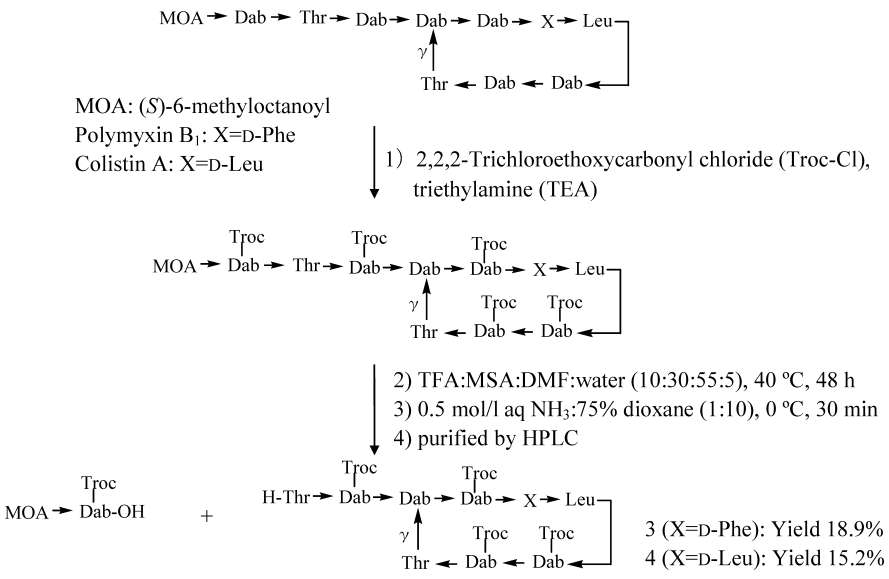

Fig. 1. Synthesis of Tetrakis(N^{γ}-Troc)-polymyxin B (2-10) (**3**) or Tetrakis(N^{γ}-Troc)-colistin (2-10) (**4**) from Polymyxin B_1 or Colistin A

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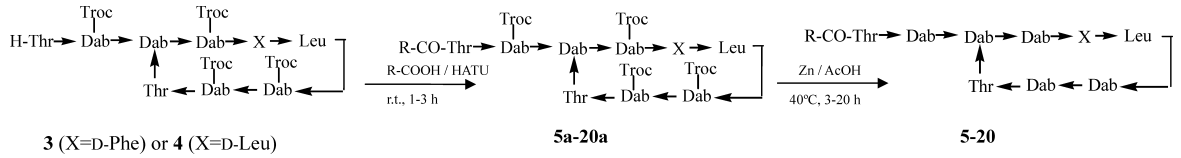
are due to the *N*-terminal fatty acyl-Dab[1]. Polymyxin B (2-10) and colistin (2-10) are cyclic nonapeptides obtained respectively from polymyxin B and colistin by enzymic removal of fatty acyl-Dab-OH with ficin[13] or papain.[14] The toxicity of the nonapeptides is known to be markedly low in comparisons with the parent compounds, polymyxin B and colistin; however, both nonapeptides are very weak bactericidals despite the fact that they retain considerable binding activity to LPS.[15] Therefore, the development of nonapeptide analogs with increased antimicrobial activity and/or higher LPS binding activity is of interest.

Previously, we reported a strategy for chemically converting natural polymyxin B and colistin to their *N*-terminal analogs.[2] The side chains of Dab residues in the natural peptides were first protected by trifluoroacetyl (Tfa) groups; the key step of the conversion strategy was the treatment of the N^{γ}-protected natural peptides with aqueous methanesulfonic acid (MSA)[16—20] in order to cleave N^{α}-alkanoyl-Dab(Tfa)-OH. Tetrakis(N^{γ}-Tfa)-polymyxin B (2-10) and tetrakis(N^{γ}-Tfa)-colistin (2-10), which have N^{α}-free and N^{γ}-protected amino functions, were useful starting materials for the semi-synthesis of their *N*-terminal derivatives. However, the yields of tetrakis(N^{γ}-Tfa)-polymyxin B (2-10) and tetrakis(N^{γ}-Tfa)-colistin (2-10) were only 8—11%. In this study, we investigated the trichloroethoxycarbonyl (Troc) group as an alternative acid-resistant N^{γ}-protecting group for the Dab residues of polymyxin B and colistin, and sixteen N^{α}-acylated nonapeptide analogs (**5**—**20**) were synthesized by an improved semi-synthetic route in order to develop nonapeptide analogs with potent antimicrobial and LPS binding activities.

Results and Discussion

Semi-synthesis As reported previously,[2] the preparation of N^{α}-free and N^{γ}-protected nonapeptides from N^{γ}-protected cyclic decapeptides was the key step for the semi-synthesis of *N*-terminal nonapeptide analogs of polymyxin B and colistin. In the present study, Troc was examined as an acid-resistant protecting group for the Dab side chains of polymyxin B and colistin. Pentakis(N^{γ}-Troc)-polymyxin B (**1**) and pentakis(N^{γ}-Troc)-colistin (**2**) were prepared almost quantitatively by treating commercially available polymyxin B or colistin with Troc-Cl and triethylamine (TEA) in 90% dimethylformamide (DMF). To avoid derivatizing Thr side chains at positions 2 and 10 with Troc, aqueous DMF was used as the solvent. The resulting pentakis(N^{γ}-Troc)-polymyxin B (**1**) and pentakis(N^{γ}-Troc)-colistin (**2**) were dissolved in trifluoroacetic acid (TFA) : MSA : DMF : H_2O (10 : 30 : 55 : 5), and the solution was allowed to react at 40 °C for 48 h to remove fatty acyl-Dab(Troc)-OH. After cleavage, the product was treated with diluted ammonia to reverse the N–O migration products, as reported previously.[2] Following purification by RP-HPLC, the new key compounds, tetrakis(N^{γ}-Troc)-polymyxin B (2-10) (**3**) and tetrakis(N^{γ}-Troc)-colistin (2-10) (**4**), were obtained in 18.9% and 15.2% yield, respectively (Fig. 1). Higher yields of these key compounds compared to that of their Tfa derivatives may be attributable not only to changing the protecting group from Tfa to Troc, but also to changes in the reaction conditions, since using a very low content of H_2O in the MSA–TFA–DMF mixture should result in higher selectivity during the hydrolysis reaction as reported in the selective chemical removal of acyl amino acids from the *N*-terminally acylated peptides such as pGlu-peptides and Myr-Gly-peptides.[2,16—19] The removal of fatty acyl-Dab(Troc)-OH from **1** and **2** by the cleavage of Dab(Troc)-Thr bond at the positions 1–2 was considered to be facilitated through N–O migration,[2] however, the cleavage of another bond at positions 9–10 seemed to be unavoidable, resulting in the still unsatisfactory yields of **3** and **4**.

The *N*-terminal acylation of tetrakis(N^{γ}-Troc)-polymyxin B (2-10) (**3**) and tetrakis(N^{γ}-Troc)-colistin (2-10) (**4**) with fatty acids was achieved using HATU as the coupling reagent. Eight fatty acids bearing aliphatic or aromatic ring



Peptides	X	R-COOH	Structures of R-CO-
5	D-Phe	1-adamantaneacetic acid	Ada-Ac-
13	D-Leu		
6	D-Phe	2-anthracenecarboxylic acid	2-Anthracene-CO-
14	D-Leu		
7	D-Phe	4-biphenylacetic acid	4-Biphenyl-Ac-
15	D-Leu		
8	D-Phe	cyclohexanebutyric acid	cHex-Bu-
16	D-Leu		
9	D-Phe	1-pyrenebutyric acid	1-Pyrene-Bu-
17	D-Leu		
10	D-Phe	*trans*-4-propylcyclohexane-carboxylic acid	Propyl-cHex-CO-
18	D-Leu		
11	D-Phe	cyclododecanecarboxylic acid	Cyclododecane-CO-
19	D-Leu		
12	D-Phe	cyclopentylphenylacetic acid	cPenPh-Ac-
20	D-Leu		

Fig. 2. Synthesis of Polymyxin B (2-10) and Colistin (2-10) Derivatives

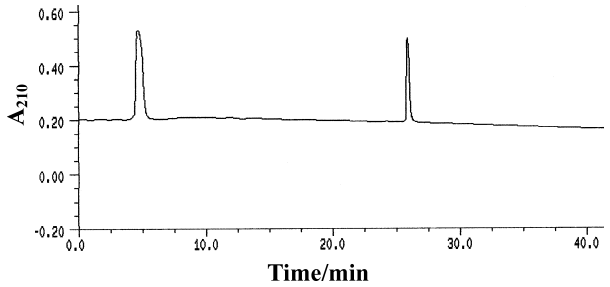


Fig. 3. HPLC Profile of Cyclohexylbutanoyl-polymyxin B (2-10) (**8**) Prepared by Chemical Conversion

Column, Tosoh TSK-GEL ODS-120T (4.6×250 mm); linear 40 min gradient elution from 19.0 to 57.0% CH_3CN in 0.1% TFA; flow, 1 ml/min; detection, 210 nm.

structures were examined in this study (Fig. 2). The resulting N^{α}-fatty acylated tetrakis(N^{γ}-Troc)-polymyxin B (2-10) (**5a**—**12a**) and N^{α}-fatty acylated tetrakis(N^{γ}-Troc)-colistin (2-10) (**13a**—**20a**) were treated with Zn in AcOH to remove the Troc groups. The products were purified first by RP-HPLC and then by gel-filtration, using 5 mmol/l hydrochloric acid as the eluent, to yield fatty acyl-nonapeptides as their tetrahydrochlorides. The structures of the synthetic polymyxin B (2-10) analogs (**5**—**12**) and colistin (2-10) analogs (**13**—**20**), shown in Fig. 2, were confirmed by FAB-MS. The purity of these synthetic peptides was demonstrated by analytical HPLC as representatively shown in Fig. 3 for analog **8** (the purity 97%), as well as HP-TLC. Thus, starting from the new key compounds **3** and **4** highly pure fatty acyl-nonapeptides were obtained using a single coupling reaction, followed by deprotection. Recently, a strategy for the synthesis of tetra-*tert*-butyloxycarbonyl (Boc)-protected polymyxin B nonapeptide was reported by O'Dowd *et al.*[21] employing non-protected polymyxin B (2-10) as the starting material, and the selective tetra-Boc-protection of the Dab side chain amino groups to produce N^{α}-free tetrakis(N^{γ}-Boc)-polymyxin (2-10) was described.

Concerning the bactericidal activity of synthetic polymyxin peptides, there were the discrepancies among published reports. Octanoyl-colistin (2-10) synthesized in our previous study showed the potent activity against *Escherichia coli* (*E. coli*) with the MIC value of 1 nmol/l,[2] however, the same compound reported by Chihara was 10—20 times less active, which was prepared by the reaction of octanoyl chloride directly with colistin nonapeptide bearing five free amino functions.[22] This product was contaminated with 20% of N^{γ}-octanoyl-colistin nonapeptide(s), which were estimated by quantitative analysis of N^{α}-DNP-Thr and N^{γ}-DNP-Dab in the dinitrophenylated octanoyl-colistin nonapeptide preparation.[22] The activity of our synthetic myristoyl-colistin (2-10) also differed from that reported 30 years ago.[23] Thus, we demonstrated in the previous paper[2] that the *N*-terminal structure–activity relationship of polymyxin B and colistin should be re-examined using highly pure synthetic peptides as these descrepancies are believed to be due to differences in the purities of the synthetic peptides. In the present study, the chemical conversion route described above was applied to search for potent bioactive acyl-nonapeptide analogs bearing aliphatic or aromatic ring structure in the acyl group (Fig. 2).

Antimicrobial Activity of Synthetic Peptides Introduction of fatty acids bearing aliphatic or aromatic ring structures to the nonapeptides polymyxin B (2-10) and colistin (2-10) greatly increased their bactericidal activity. Three polymyxin B (2-10) analogs, acylated with 1-adamantaneacetyl- (**5**), 4-biphenylacetyl- (**7**), and cyclohexylbutanoyl- (**8**), showed potent antimicrobial activities equal to that of polymyxin B. 2-Anthracenecarbonyl- (**6**), 1-pyrenebutanoyl- (**9**), and cyclododecanecarbonyl- (**11**) analogs exhibited slightly reduced antimicrobial activity, equal to that of octanoyl-polymyxin B (2-10)[24] and octanoyl-colistin (2-10),[2] which were recently reported to have MIC values of 1.0—4.0 nmol/ml against *E. coli* and *Salmonella* Typhimurium (*S.* Typhimurium), respectively. *trans*-4-Propylcyclohexanecarbonyl-polymyxin B (2-10) (**10**) and 2-cyclopentylphenylacetyl-polymyxin B (2-10) (**12**) showed reduced activities, with MIC values of 4 and 8 nmol/ml, respectively (Table 1), similar to that reported by Tsubery for Fmoc-polymyxin B (2-10), which has a MIC value of 8 μg/ml.[25] It seems likely that the antimicrobial activities of polymyxin B (2-10) analogs bearing various fatty acyl groups are slightly higher against *E. coli* and *S.* Typhimurium than those of the corresponding colistin (2-10) analogs. The activities of various colistin (2-10) analogs varied in an approximately similar manner as the corresponding polymyxin B (2-10) analogs. These results suggest that, for antimicrobial activity, D-Phe at position 6 is superior to D-Leu in this family of peptides.

It is well known that the nonapeptides polymyxin B (2-10) and colistin (2-10) have almost no antimicrobial activity.[13,14,24] However, introduction of octanoyl to the N^{α}-amino function of nonapeptides greatly increases their activity.[2,24] In this study it was found that cyclohexylbutanoyl-polymyxin B (2-10) (**8**) showed two-fold higher antimicrobial activity against *E. coli* compared with polymyxin B, and its activity against *S.* Typhimurium and *Pseudomonas aeruginosa* (*P. aeruginosa*) was equal to that of polymyxin B. Analog **8** is the most potent synthetic polymyxin B nonapeptide analog reported to date. The cyclohexyl ring structure located at the terminus (the 4-position of butanoyl) in **8** seems to cause favorable interactions with the bacterial cell membrane. However, a cyclohexyl ring located at the carbonyl of the acyl group seems to be unfavorable, since *trans*-4-propylcyclohexylcarbonyl-polymyxin B (2-10) (**10**) shows low activity. In this regard, biphenylacetyl-analogs (**7**, **15**) show high activity, while 2-cyclopentylphenylacetyl-analogs (**12**, **20**) with a branched bulky structure close to the *N*-terminus of Thr^2 show low activity. The sensitivity of *E. coli* and *S.* Typhimurium to polymyxin B (2-10) and colistin (2-10) analogs varied according to the *N*-terminal fatty acyl group on the nonapeptide, but the sensitivity of *P. aeruginosa* did not. Previously we reported that acetyl-polymyxin B (2-10) and acetyl-colistin (2-10) retained the potent bactericidal activity only against *P. aeruginosa*, but not against *E. coli* and *S.* Typhimurium.[2] Taking into account the previous results, it is possible to say that the peptide portions of polymyxin B and colistin contributes greater to destroy the cell membrane of *P. aeruginosa* than fatty acyl portion, and it seems likely that the constitution of the *P. aeruginosa* cell membrane differs from that of other Gram-negative bacteria.

LPS Binding Activity The lipopolysaccharide (LPS, derived from *E. coli*) binding activities of the synthetic analogs were examined according to the method reported previously[24] (Figs. 4, 5). The activities of 2-anthracenecarbonyl-

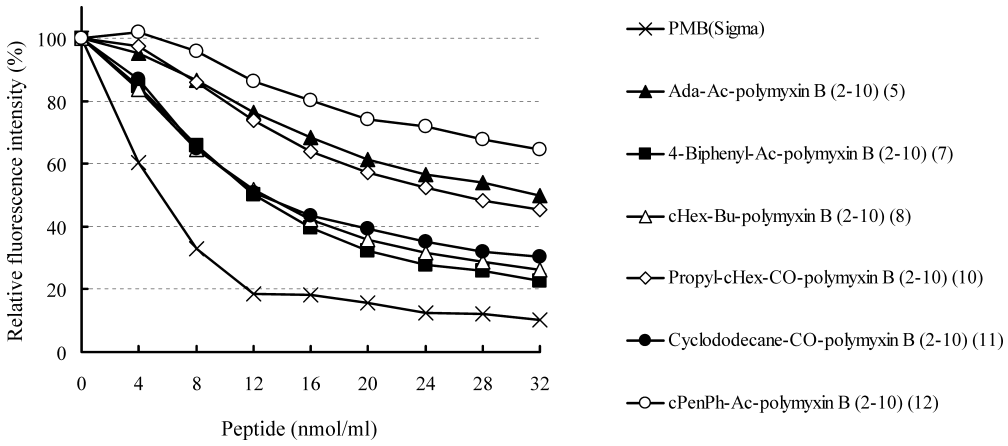


Fig. 4. Displacement Assay of Synthetic Polymyxin Derivatives

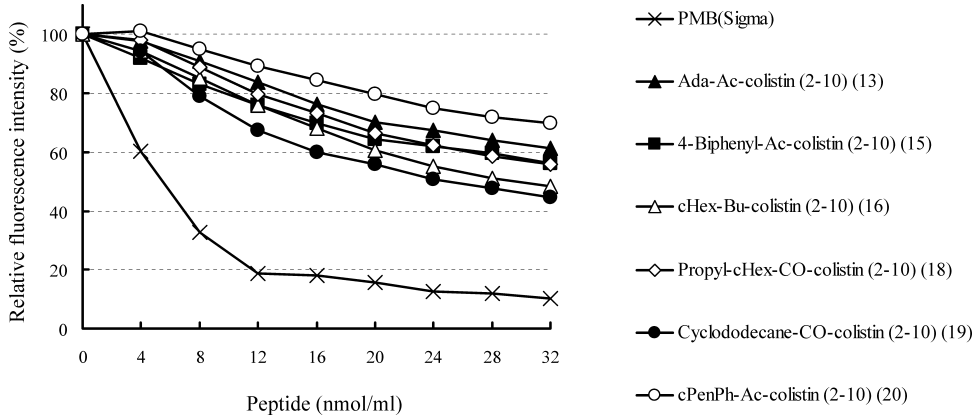


Fig. 5. Displacement Assay of Synthetic Colistin Derivatives

Table 1. Antimicrobial Activity of Synthetic Polymyxin Analogs

Peptide	MIC (nmol/ml)		
	Escherichia coli	*Salmonella* Typhimurium	*Pseudomonas aeruginosa*
Polymyxin B (Sigma)	1.0	0.5	1.0
Ada-Ac-polymyxin B (2-10) (**5**)	1.0	0.5	1.0
2-Anthracene-CO-polymyxin B (2-10) (**6**)	4.0	2.0	2.0
4-Biphenyl-Ac-polymyxin B (2-10) (**7**)	0.5	1.0	1.0
cHex-Bu-polymyxin B (2-10) (**8**)	0.5	0.5	1.0
1-Pyrene-Bu-polymyxin B (2-10) (**9**)	2.0	2.0	2.0
Propyl-cHex-CO-polymyxin B (2-10) (**10**)	4.0	4.0	2.0
Cyclododecane-CO-polymyxin B (2-10) (**11**)	2.0	2.0	2.0
cPenPh-Ac-polymyxin B (2-10) (**12**)	8.0	8.0	2.0
Ada-Ac-colistin (2-10) (**13**)	2.0	1.0	1.0
2-Anthracene-CO-colistin (2-10) (**14**)	4.0	2.0	2.0
4-Biphenyl-Ac-colistin (2-10) (**15**)	1.0	2.0	1.0
cHex-Bu-colistin (2-10) (**16**)	2.0	1.0	1.0
1-Pyrene-Bu-colistin (2-10) (**17**)	2.0	2.0	1.0
Propyl-cHex-CO-colistin (2-10) (**18**)	8.0	8.0	2.0
Cyclododecane-CO-colistin (2-10) (**19**)	4.0	2.0	2.0
cPenPh-Ac-colistin (2-10) (**20**)	16.0	16.0	2.0

analogs (**6**, **14**) and 1-pyrenebutanoyl-analogs (**9**, **17**) were not examined because these compounds disturbed fluorescence measurements of the dansyl group. Although none of the synthetic nonapeptides examined showed LPS binding activity comperable to polymyxin B, 4-biphenylacetyl-polymyxin B (2-10) (**7**), cyclohexylbutanoyl-polymyxin B (2-10) (**8**), and cyclododecanecarbonyl-polymyxin B (2-10) (**11**) showed LPS binding activities close to that observed for octanoyl-polymyxin B (2-10).[24] 1-Adamantaneacetyl-polymyxin B (2-10) (**5**) and propylcyclohexanecarbonyl-polymyxin B (2-10) (**10**) showed low LPS binding activity. Analog **5**, as well as **7** and **8**, were potent bactericidals, com-

perable to polymyxin B (Table 1). Thus, the antimicrobial and LPS binding activities of these compounds are not parallel. These results suggest that 1-adamantaneacetyl-polymyxin B (2-10) (**5**) is a potent bactericidal agent that exhibits moderate activity for neutralizing the endotoxin. Previously we reported that myristoyl-polymyxin B (2-10) and myristoyl-colistin (2-10) show potent LPS binding activity but have lower antimicrobial activity than polymyxin B, with a MIC value of 4.0 nmol/ml against *E. coli*.[2] Thus, myristoyl-polymyxin B (2-10) may have potent neutralizing activity against endotoxin while retaining moderate antimicrobial activity. 2-Cyclopentylphenylacetyl-polymyxin B (2-10) (**12**) and cyclopentylphenylacetyl-colistin (2-10) (**20**), which have a bulky structure close to the *N*-terminus of Thr[2], showed very low LPS binding activity and 8—16 fold lower antimicrobial activity. Colistin (2-10) analogs less effectively displaced dansylated polymyxin B_3 bound to LPS than polymyxin B (2-10) analogs, suggesting that D-Phe at position 6 is superior to D-Leu for LPS binding activity in this family of peptides.

In conclusion, the synthetic strategy for converting natural polymyxin B and colistin to their *N*-terminal analogs was improved twice in the present study with regard to the yields of the key intermediates, tetrakis(Troc)-polymyxin B (2-10) and tetrakis(Troc)-colistin (2-10). Acylation of these key intermediates with various fatty acids, followed by deprotection with Zn–AcOH to remove the Troc protecting group, provided various nonapeptide analogs in good yields. The present study demonstrates that fatty acylated polymyxin B (2-10) analogs bearing aliphatic or aromatic ring structures are synthetic antibiotics whose characteristics depend on the specific fatty acyl group in the compound. Among sixteen synthetic analogs, cyclohexylbutanoyl-polymyxin B (2-10) (**8**) was found to be as potent a bactericidal agent as polymyxin B against *E. coli*, *S.* Typhimurium and *P. aeruginosa*, but its binding activity was slightly lower than polymyxin B or myristoyl-polymyxin B (2-10). 1-Adamantaneacetyl-polymyxin B (2-10) (**5**) showed lower LPS binding activity than polymyxin B, but the analog **5** was a potent bactericidal.

Experimental

General Fast-atom bombardment mass spectra (FAB-MS) were obtained on a JMS-DX300 mass spectrometer (JEOL Ltd., Japan). The optical rotations of the peptides were measured using a DIP-370 digital polarimeter (JASCO Co., Ltd., Japan). HPLC was performed on a system comprised of a PX-8010 gradient controller (Tosoh, Japan), two CCPD pumps (Tosoh), a Rheodyne 7125 injector (Rheodyne Inc., U.S.A.) or an AS-8020 automatic sample injector (Tosoh), a UV 8000 detector (Tosoh), and an 805 data station (Waters). HP-TLC was performed on precoated silica-gel plates (Kieselgel 60, E. Merck, Germany). Rf^1: BuOH : AcOH : AcOEt : H_2O (1 : 1 : 1 : 1), Rf^2: BuOH : pyridine : AcOH : H_2O (30 : 20 : 6 : 24).

Pentakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (1) Polymyxin B sulfate (Wako, Japan; 868 mg, 0.60 mmol, calculated as Polymyxin B_1 sulfate) was suspended in 95% DMF (80 ml) containing TEA (834 μl, 6.0 mmol), and Troc-Cl (822 mg, 6.0 mmol) was added while the mixture was cooled with ice. The mixture was allowed to react for 24 h at room temperature while the pH was maintained at 7.8 with TEA. The product was solidified by addition of cold H_2O (320 ml), collected by filtration, dried, and then reprecipitated from MeOH–AcOEt–ether–petr. ether. Yield: 1019 mg (81.7%, calculated as pentakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B_1). The crude product was used for the next reaction without further purification. A small amount of material from the main peak of this product was isolated by RP-HPLC on a YMC Pack D-ODS-5ST column (150×20 mm). The purified product was lyophilized and characterized. $[\alpha]_D^{27}$ −27.2° (c=0.5, DMF), FAB-MS m/z: 2081.3 (Calcd for $C_{71}H_{104}Cl_{15}N_{16}O_{23}$: 2081.47). HP-TLC: Rf^1 0.89, Rf^2 0.87.

Pentakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2) Colistin sulfate (Wako, 847.89 mg, 0.60 mmol, calculated as Colistin A sulfate) was suspended in 95% DMF (80 ml) containing TEA (834 μl, 6.0 mmol), and Troc-Cl (822 mg, 6.0 mmol) was added while the mixture was cooled with ice. The mixture was allowed to react for 24 h at room temperature while the pH was maintained at 7.8 with TEA. The product was solidified by addition of cold H_2O (320 ml), collected by filtration, dried and then reprecipitated from MeOH–AcOEt–ether–petr. ether. Yield: 1025.91 mg (83.5%, calculated as pentakis(N^{γ}-trichloroethoxycarbonyl)-colistin A). The crude product was used for the next reaction without further purification. Small amounts of material from the two main peaks of this product were isolated by RP-HPLC on a CAPCELL PAK UG80 column (20×250 mm). Purified products were lyophilized and characterized. Pentakis(N^{γ}-trichloroethoxycarbonyl)-colistin A: $[\alpha]_D^{27}$ −28.3° (c=0.5, DMF), FAB-MS m/z: 2047.3 (Calcd for $C_{68}H_{106}Cl_{15}N_{16}O_{23}$: 2047.46). HP-TLC: Rf^1 0.90, Rf^2 0.88. Pentakis(N^{γ}-trichloroethoxycarbonyl)-colistin B: $[\alpha]_D^{27}$ −28.3° (c=0.5, DMF), FAB-MS m/z: 2033.3 (Calcd for $C_{67}H_{104}Cl_{15}N_{16}O_{23}$: 2033.43). HP-TLC: Rf^1 0.90, Rf^2 0.88.

Tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) Hydrochloride (3) Compound **1** (93.58 mg, 45 μmol) was dissolved in trifluoroacetic acid (TFA) (3 ml) cooled in ice, then a mixture of methanesulfonic acid (MSA) : dimethylformamide (DMF) : water (30 : 55 : 5) (27 ml) was added and the reaction mix was stirred at 40 °C for 48 h. The mixture was added directly to a Wakogel 50C18 column (5×6 cm), which was washed with H_2O (400 ml) to remove MSA. Reaction products were eluted with 75% aqueous dioxane (350 ml). The eluates were combined, cooled in an ice bath, and a dilute ammonia solution (0.5 mol/l, 35 ml) was then added. The mixture was stirred for 30 min while cooled with ice, then the solvent was removed under vacuum. The residue was re-dissolved in 90% aqueous dioxane (50 ml), and then lyophilized. The obtained powder was dissolved in 90% dioxane, and then purified by RP-HPLC on a YMC Pack D-ODS-5ST column (150×20 mm) using a linear gradient (30 min) from 45.6 to 56.05% CH_3CN in 0.1% TFA at a flow rate of 5 ml/min. The eluate was evaporated and lyophilized, then the obtained product was re-lyophilized from 75% dioxane (50 ml) containing 1 mol/l HCl (0.1 ml). The yield of **3** was 14.49 mg (18.9%). $[\alpha]_D^{27}$ −49.7° (c=0.2, 50% AcOH), FAB-MS m/z: 1665.2 (Calcd for $C_{55}H_{79}Cl_{12}N_{14}O_{19}$: 1665.73). HP-TLC: Rf^1 0.71, Rf^2 0.78.

Tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) Hydrochloride (4) Pentakis(N^{γ}-trichloroethoxycarbonyl)-colistin (**2**) (92.05 mg, 45 μmol) was dissolved in trifluoroacetic acid (TFA) (3 ml) cooled with ice, a solution of methanesulfonic acid (MSA) : dimethylformamide (DMF) : water (30 : 55 : 5) (27 ml) was added, then the mixture was stirred at 40 °C for 48 h. The product was isolated in the same manner as described for **3**. Yield: 11.37 mg (15.2%). $[\alpha]_D^{27}$ −34.3° (c=0.2, 50% AcOH), FAB-MS m/z: 1631.2 (Calcd for $C_{52}H_{81}Cl_{12}N_{14}O_{19}$: 1631.71). HP-TLC: Rf^1 0.70, Rf^2 0.81.

1-Adamantaneacetyl-polymyxin B (2-10) Tetrahydrochloride (5) To a solution of **3** (25.5 mg, 15 μmol) and *N*-methylmorpholine (NMM) (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 1-adamantaneacetic acid (11.66 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 3 h and its pH was maintained at 7.8 with NMM. The mixture was applied to a Toyopearl HW-40 column (100×2 cm) and eluted with DMF : water (9 : 1). The eluate fractions containing the main product were combined and evaporated *in vacuo*. The residue was lyophilized from dioxane (3 ml) to give 1-adamantaneacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**5a**). Yield: 30.39 mg (84.2%). $[\alpha]_D^{27}$ −25.2° (c=0.5, DMF), FAB-MS m/z: 1841.3 (Calcd for $C_{67}H_{95}Cl_{12}N_{14}O_{20}$: 1841.99). HP-TLC: Rf^1 0.88, Rf^2 0.85.

Compound **5a** (27.6 mg, 15 μmol) was treated with Zn (6 mmol, 392.28 mg)–AcOH (5 ml) at 40 °C overnight. After filtration, the product was purified by RP-HPLC on a YMC Pack D-ODS-5ST column (20×150 mm) using linear gradient elution (30 min) from 25.65 to 30.4% CH_3CN in 0.1% TFA at a flow rate of 5 ml/min. The eluate fractions containing the main peak product were collected, evaporated and lyophilized. The product was applied to a Toyopearl HW-40 column (100×2 cm) and eluted with 25% CH_3CN in 5 mM HCl. The collected eluate fractions containing the main product were combined, evaporated and lyophilized. Purified **5** was re-lyophilized from 75% dioxane. Yield (**5**): 10.46 mg (54.3%). $[\alpha]_D^{27}$ −62.8° (c=0.5, 12% AcOH), FAB-MS m/z: 1139 (Calcd for $C_{55}H_{91}N_{14}O_{12}$: 1139). HP-TLC: Rf^1 0.38, Rf^2 0.44.

2-Anthracenecarbonyl-polymyxin B (2-10) Tetrahydrochloride (6) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 2-anthracenecarboxylic acid (13.33 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added. The mixture was

stirred at room temperature for 3 h and treated in the same manner as described for **5a** to give 2-anthracenecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**6a**). Yield: 27.01 mg (96.5%). $[\alpha]_D^{27}$ −11.2° (c=0.5, DMF), FAB-MS m/z: 1869.2 (Calcd for $C_{70}H_{87}Cl_{12}N_{14}O_{20}$: 1869.96). HP-TLC: Rf^1 0.88, Rf^2 0.85.

Compound **6a** (18.68 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 18 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**6**): 3.72 mg (28.4%). $[\alpha]_D^{27}$ −42.5° (c=0.5, 12% AcOH), FAB-MS m/z: 1167 (Calcd for $C_{58}H_{83}N_{14}O_{12}$: 1167). HP-TLC: Rf^1 0.37, Rf^2 0.43.

4-Biphenylacetyl-polymyxin B (2-10) Tetrahydrochloride (7) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 4-biphenylacetic acid (12.73 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the reaction mix was cooled with ice. The mixture was stirred at room temperature for 5 h and treated in the same manner as described for **5a** to give 4-biphenylacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**7a**). Yield: 27.85 mg (100.0%). $[\alpha]_D^{27}$ −21.2° (c=0.5, DMF), FAB-MS m/z: 1859.3 (Calcd for $C_{69}H_{89}Cl_{12}N_{14}O_{20}$: 1859.96). HP-TLC: Rf^1 0.88, Rf^2 0.86.

Compound **7a** (18.58 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 3 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**7**): 6.74 mg (51.8%). $[\alpha]_D^{27}$ −61.6° (c=0.5, 12% AcOH), FAB-MS m/z: 1157 (Calcd for $C_{57}H_{85}N_{14}O_{12}$: 1157). HP-TLC: Rf^1 0.37, Rf^2 0.42.

Cyclohexylbutanoyl-polymyxin B (2-10) Tetrahydrochloride (8) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclohexylbutyric acid (10.22 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the reaction mix was cooled with ice. The mixture was stirred at room temperature for 2 h and treated in the same manner as described for **5a** to give cyclohexylbutanoyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**8a**). Yield: 23.10 mg (84.8%). $[\alpha]_D^{27}$ −25.2° (c=0.5, DMF), FAB-MS m/z: 1817.3 (Calcd for $C_{65}H_{95}Cl_{12}N_{14}O_{20}$: 1817.97). HP-TLC: Rf^1 0.88, Rf^2 0.86.

Compound **8a** (18.16 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 3 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**8**): 6.94 mg (55.1%). $[\alpha]_D^{27}$ −67.3° (c=0.5, 12% AcOH), FAB-MS m/z: 1115 (Calcd for $C_{53}H_{91}N_{14}O_{12}$: 1115). HP-TLC: Rf^1 0.37, Rf^2 0.43.

1-Pyrenebutanoyl-polymyxin B (2-10) Tetrahydrochloride (9) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 1-pyrenebutyric acid (17.3 mg, 60 μmol) and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 2 h and treated in the same manner as described for **5a** to give 1-pyrenebutanoyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**9a**). Yield: 27.36 mg (94.3%). $[\alpha]_D^{27}$ −22.4° (c=0.5, DMF), FAB-MS m/z: 1935.3 (Calcd for $C_{75}H_{93}Cl_{12}N_{14}O_{20}$: 1936.06). HP-TLC: Rf^1 0.88, Rf^2 0.86.

Compound **9a** (19.34 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 20 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**9**): 6.87 mg (49.9%). $[\alpha]_D^{27}$ −59.2° (c=0.4, 12% AcOH), FAB-MS m/z: 1233 (Calcd for $C_{63}H_{89}N_{14}O_{12}$: 1233). HP-TLC: Rf^1 0.37, Rf^2 0.43.

***trans*-4-Propylcyclohexanecarbonyl-polymyxin B (2-10) Tetrahydrochloride (10)** To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of *trans*-4-Propylcyclohexanecarboxylic acid (17.3 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the reaction mix was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give *trans*-4-propylcyclohexanecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**10a**). Yield: 23.03 mg (84.54%). $[\alpha]_D^{27}$ −23.7° (c=0.2, DMF), FAB-MS m/z: 1817.3 (Calcd for $C_{65}H_{95}Cl_{12}N_{14}O_{20}$: 1817.97). HP-TLC: Rf^1 0.88, Rf^2 0.86.

Compound **10a** (18.16 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 5 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**10**): 8.41 mg (66.8%). $[\alpha]_D^{27}$ −61.5° (c=0.5, 12% AcOH), FAB-MS m/z: 1115 (Calcd for $C_{53}H_{91}N_{14}O_{12}$: 1115). HP-TLC: Rf^1 0.37, Rf^2 0.44.

Cyclododecanecarbonyl-polymyxin B (2-10) Tetrahydrochloride (11) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclododecanecarboxylic acid (12.74 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give cyclododecanecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**11a**). Yield: 23.30 mg (83.60%). $[\alpha]_D^{27}$ −27.0° (c=0.4, DMF), FAB-MS m/z: 1859.4 (Calcd for $C_{68}H_{101}Cl_{12}N_{14}O_{20}$: 1860.05). HP-TLC: Rf^1 0.88, Rf^2 0.87.

Compound **11a** (18.58 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C overnight. After filtration, the product was purified in the same manner as described for **5**. Yield (**11**): 6.84 mg (52.53%). $[\alpha]_D^{27}$ −66.8° (c=0.5, 12% AcOH), FAB-MS m/z: 1157 (Calcd for $C_{56}H_{97}N_{14}O_{12}$: 1157). HP-TLC: Rf^1 0.38, Rf^2 0.46.

Cyclopentylphenylacetyl-polymyxin B (2-10) Tetrahydrochloride (12) To a solution of **3** (25.5 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclopentylphenylacetic acid (12.26 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give cyclopentylphenylacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-polymyxin B (2-10) (**12a**). Yield: 23.45 mg (84.5%). $[\alpha]_D^{27}$ −27.6° (c=0.5, DMF), FAB-MS m/z: 1851.3 (Calcd for $C_{98}H_{93}Cl_{12}N_{14}O_{20}$: 1851.98). HP-TLC: Rf^1 0.88, Rf^2 0.86.

Compound **12a** (18.50 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml) at 40 °C for 5 h. After filtration, the product was purified in the same manner as described for **5**. Yield (**12**): 5.61 mg (43.35%). $[\alpha]_D^{27}$ −51.7° (c=0.4, 12% AcOH), FAB-MS m/z: 1149 (Calcd for $C_{56}H_{89}N_{14}O_{12}$: 1149). HP-TLC: Rf^1 0.38, Rf^2 0.46.

1-Adamantaneacetyl-colistin (2-10) Tetrahydrochloride (13) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 1-adamantaneacetic acid (11.66 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give 1-adamantaneacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**13a**). Yield: 22.83 mg (84.3%). $[\alpha]_D^{27}$ −28.4° (c=0.5, DMF), FAB-MS m/z: 1807.3 (Calcd for $C_{64}H_{97}Cl_{12}N_{14}O_{20}$: 1807.97). HP-TLC: Rf^1 0.88, Rf^2 0.87.

Compound **13a** (18.06 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**13**): 7.44 mg (59.52%). $[\alpha]_D^{27}$ −54.9° (c=0.5, 12% AcOH), FAB-MS m/z: 1105 (Calcd for $C_{52}H_{93}N_{14}O_{12}$: 1105). HP-TLC: Rf^1 0.37, Rf^2 0.47.

2-Anthracenecarbonyl-colistin (2-10) Tetrahydrochloride (14) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 2-anthracenecarboxylic acid (13.33 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give 2-anthracenecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**14a**). Yield: 27.78 mg (100.8%). $[\alpha]_D^{27}$ −9.6° (c=0.5, DMF), FAB-MS m/z: 1835.3 (Calcd for $C_{67}H_{89}Cl_{12}N_{14}O_{20}$: 1835.94). HP-TLC: Rf^1 0.89, Rf^2 0.87.

Compound **14a** (18.34 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**14**): 4.26 mg (33.33%). $[\alpha]_D^{27}$ −31.2° (c=0.5, 12% AcOH), FAB-MS m/z: 1133 (Calcd for $C_{55}H_{85}N_{14}O_{12}$: 1133). HP-TLC: Rf^1 0.37, Rf^2 0.45.

4-Biphenylacetyl-colistin (2-10) Tetrahydrochloride (15) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 4-biphenylacetic acid (12.73 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 2 h and treated in the same manner as described for **5a** to give 4-biphenylacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**15a**). Yield: 23.67 mg (86.5%). $[\alpha]_D^{27}$ −22.3° (c=0.2, DMF), FAB-MS m/z: 1825.3 (Calcd for $C_{66}H_{91}Cl_{12}N_{14}O_{20}$: 1825.95). HP-TLC: Rf^1 0.89, Rf^2 0.88.

Compound **15a** (18.24 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**15**): 9.17 mg (72.3%). $[\alpha]_D^{27}$ −51.5° (c=0.5, 12% AcOH), FAB-MS m/z: 1123 (Calcd for $C_{54}H_{87}N_{14}O_{12}$: 1123). HP-TLC: Rf^1 0.37, Rf^2 0.44.

Cyclohexylbutanoyl-colistin (2-10) Tetrahydrochloride (16) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclohexylbutyric acid (10.22 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give cyclohexylbutanoyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**16a**). Yield: 25.32 mg (94.7%). $[\alpha]_D^{27}$ −23.3° (c=0.2, DMF), FAB-MS m/z: 1783.3

(Calcd for $C_{62}H_{97}Cl_{12}N_{14}O_{20}$: 1783.95). HP-TLC: Rf^1 0.88, Rf^2 0.88.

Compound **16a** (17.82 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**16**): 9.30 mg (75.9%). $[\alpha]_D^{27}$ −48.3° (c=0.5, 12% AcOH), FAB-MS m/z: 1081 (Calcd for $C_{50}H_{93}N_{14}O_{12}$: 1081). HP-TLC: Rf^1 0.37, Rf^2 0.45.

1-Pyrenebutanoyl-colistin (2-10) Tetrahydrochloride (17) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of 1-pyrenebutyric acid (17.3 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give 1-pyrenebutanoyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**17a**). Yield: 26.09 mg (91.5%). $[\alpha]_D^{27}$ −23.2° (c=0.5, DMF), FAB-MS m/z: 1901.3 (Calcd for $C_{72}H_{95}Cl_{12}N_{14}O_{20}$: 1902.04). HP-TLC: Rf^1 0.88, Rf^2 0.87.

Compound **17a** (20.9 mg, 11 μmol) was treated with Zn (4.4 mmol, 284.67 mg)–AcOH (2.75 ml) at 40 °C for 20 h, and the product was isolated in the same manner as described for **5**. Yield (**17**): 5.82 mg (39.4%). $[\alpha]_D^{27}$ −50.0° (c=0.5, 12% AcOH), FAB-MS m/z: 1199 (Calcd for $C_{60}H_{91}N_{14}O_{12}$: 1199). HP-TLC: Rf^1 0.37, Rf^2 0.44.

***trans*-4-Propylcyclohexanecarbonyl-colistin (2-10) Tetrahydrochloride (18)** To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of *trans*-4-propylcyclohexanecarboxylic acid (17.3 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give *trans*-4-propylcyclohexanecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**18a**). Yield: 21.6 mg (81.0%). $[\alpha]_D^{27}$ −24.8° (c=0.5, DMF), FAB-MS m/z: 1783.3 (Calcd for $C_{62}H_{97}Cl_{12}N_{14}O_{20}$: 1787.95). HP-TLC: Rf^1 0.89, Rf^2 0.88.

Compound **18a** (17.82 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**18**): 9.70 mg (79.1%). $[\alpha]_D^{27}$ −48.3° (c=0.5, 12% AcOH), FAB-MS m/z: 1081 (Calcd for $C_{50}H_{93}N_{14}O_{12}$: 1081). HP-TLC: Rf^1 0.37, Rf^2 0.46.

Cyclododecanecarbonyl-colistin (2-10) Tetrahydrochloride (19) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclododecanecarboxylic acid (12.74 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 1 h and treated in the same manner as described for **5a** to give cyclododecanecarbonyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**19a**). Yield: 23.0 mg (84.0%). $[\alpha]_D^{27}$ −25.5° (c=0.5, DMF), FAB-MS m/z: 1825.4 (Calcd for $C_{65}H_{103}Cl_{12}N_{14}O_{20}$: 1826.03). HP-TLC: Rf^1 0.87, Rf^2 0.87.

Compound **19a** (18.24 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**19**): 9.55 mg (75.3%). $[\alpha]_D^{27}$ −53.1° (c=0.5, 12% AcOH), FAB-MS m/z: 1123 (Calcd for $C_{53}H_{99}N_{14}O_{12}$: 1123). HP-TLC: Rf^1 0.38, Rf^2 0.47.

Cyclopentylphenylacetyl-colistin (2-10) Tetrahydrochloride (20) To the solution of **4** (25.0 mg, 15 μmol) and NMM (3.06 μl, 30 μmol) in DMF (600 μl), a mixture of cyclopentylphenylacetic acid (12.26 mg, 60 μmol) and HATU (22.8 mg, 60 μmol) in DMF (300 μl) was added while the mixture was cooled with ice. The mixture was stirred at room temperature for 4 h and treated in the same manner as described for **5a** to give cyclopentylphenylacetyl-tetrakis(N^{γ}-trichloroethoxycarbonyl)-colistin (2-10) (**20a**). Yield: 24.1 mg (88.4%). $[\alpha]_D^{27}$ −19.2° (c=0.5, DMF), FAB-MS m/z: 1817.3 (Calcd for $C_{65}H_{95}Cl_{12}N_{14}O_{20}$: 1817.97). HP-TLC: Rf^1 0.88, Rf^2 0.87.

Compound **20a** (18.16 mg, 10 μmol) was treated with Zn (4 mmol, 261.52 mg)–AcOH (2.5 ml), and the product was isolated in the same manner as described for **5**. Yield (**20**): 5.1 mg (40.48%). $[\alpha]_D^{27}$ −38.0° (c=0.2, 12% AcOH), FAB-MS m/z: 1115 (Calcd for $C_{53}H_{91}N_{14}O_{12}$: 1115). HP-TLC: Rf^1 0.38, Rf^2 0.46.

Antimicrobial Activities of Synthetic Polymyxin B and Colistin Analogs The antimicrobial activities of the synthetic peptides were estimated using the standard micro plate dilution method reported previously.[24]

LPS Binding Activity LPS binding activity was examined according to the method reported by Moore.[26] A solution of [Dab(Dansyl-Gly)^1]-polymyxin B_3 (4 nmol) and 30 μg of LPS (*E. coli*, serotype005:B5, Sigma Chemical Co.) in 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.2, 1 ml) was incubated in a quartz cuvette at 30 °C for 1 h, then a peptide solution (1 μm/ml) (4 μl each) was added in the same manner as reported previously.[24] The fluorescence spectra were measured using a fluorescence spectrophotometer F-4500 (Hitachi Instrument Co., Tokyo, Japan) at an excitation wavelength of 330 nm and an emission wavelength of 490 nm.

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References and Notes

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