A54145, A NEW LIPOPEPTIDE ANTIBIOTIC COMPLEX: MICROBIAL AND CHEMICAL MODIFICATION†

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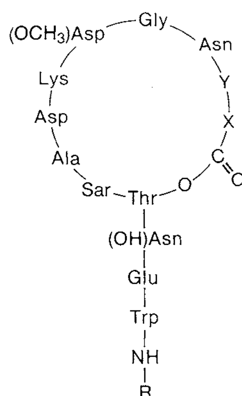
A54145 is a complex of acidic lipopeptide antibiotics produced by *Streptomyces fradiae* NRRL 18158, NRRL 18159, and NRRL 18160. Each antibiotic factor consists of a peptide core bearing an *N*-terminal acyl substituent. *N*-Lys-*tert*-BOC-protected A54145 complex was deacylated by *Actinoplanes utahensis*; three protected core peptides were isolated. A54145 antibiotic analogs were synthesized by acylation of the tryptophan *N*-terminus with 2,4,5-trichlorophenyl active esters, followed by deblocking with trifluoroacetic acid.

Previous communications in this series have described the taxonomy and fermentation of culture A54145[1] (*Streptomyces fradiae* NRRL 18158, NRRL 18159, and NRRL 18160) and the isolation, characterization[2], and structure elucidation[3] of eight acidic lipopeptide factors (A, A_1, B, B_1, C, D, E, and F, see Fig. 1) produced by the culture. Like the members of the A21978C[4] antibiotic complex, each

Fig. 1. Structures of A54145 complex antibiotics.



Compound No.	Factor	X	Y	R
1	A	Ile	Glu	8-Methylnonanoyl (iC_{10})
2	A_1	Ile	Glu	*n*-Decanoyl (nC_{10})
3	B	Ile	3-MethylGlu	*n*-Decanoyl (nC_{10})
4	B_1	Ile	3-MethylGlu	8-Methylnonanoyl (iC_{10})
5	C	Val	3-MethylGlu	8-Methyldecanoyl (aC_{11})
6	D	Ile	Glu	8-Methyldecanoyl (aC_{11})
7	E	Ile	3-MethylGlu	8-Methyldecanoyl (aC_{11})
8	F	Val	Glu	8-Methylnonanoyl (iC_{10})

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factor consists of a peptide core or nucleus which is acylated at the *N*-terminal tryptophan residue. However, unlike A21978C, structural variations occur in the nuclei as well as the side chains. In the A54145 antibiotics four peptide cores, (A54145A, B, C, and F nuclei) differ by methyl substitution on the *C*-terminal (Val/Ile) and penultimate (Glu/3-MeGlu) residues. Naturally-occurring *N*-acyl substituents include 8-methylnonanoyl (factors A, B_1, and F), *n*-decanoyl (factors A_1 and B), and 8-methyldecanoyl (factors C, D, and E). The microbial deacylation of A54145 factors to provide individual A54145 core peptides has been reported in a previous communication[3]. This paper describes the production of semi-synthetic *N*-acyl A54145 analogs by means of the microbial deacylation of appropriately protected A54145 complex.

Modification of A54145 Acyl Substituents

A limited number of A54145 antibiotic analogs were made to complete a simple structure activity

Fig. 2. Preparation of semi-synthetic A54145 factors and analogs.

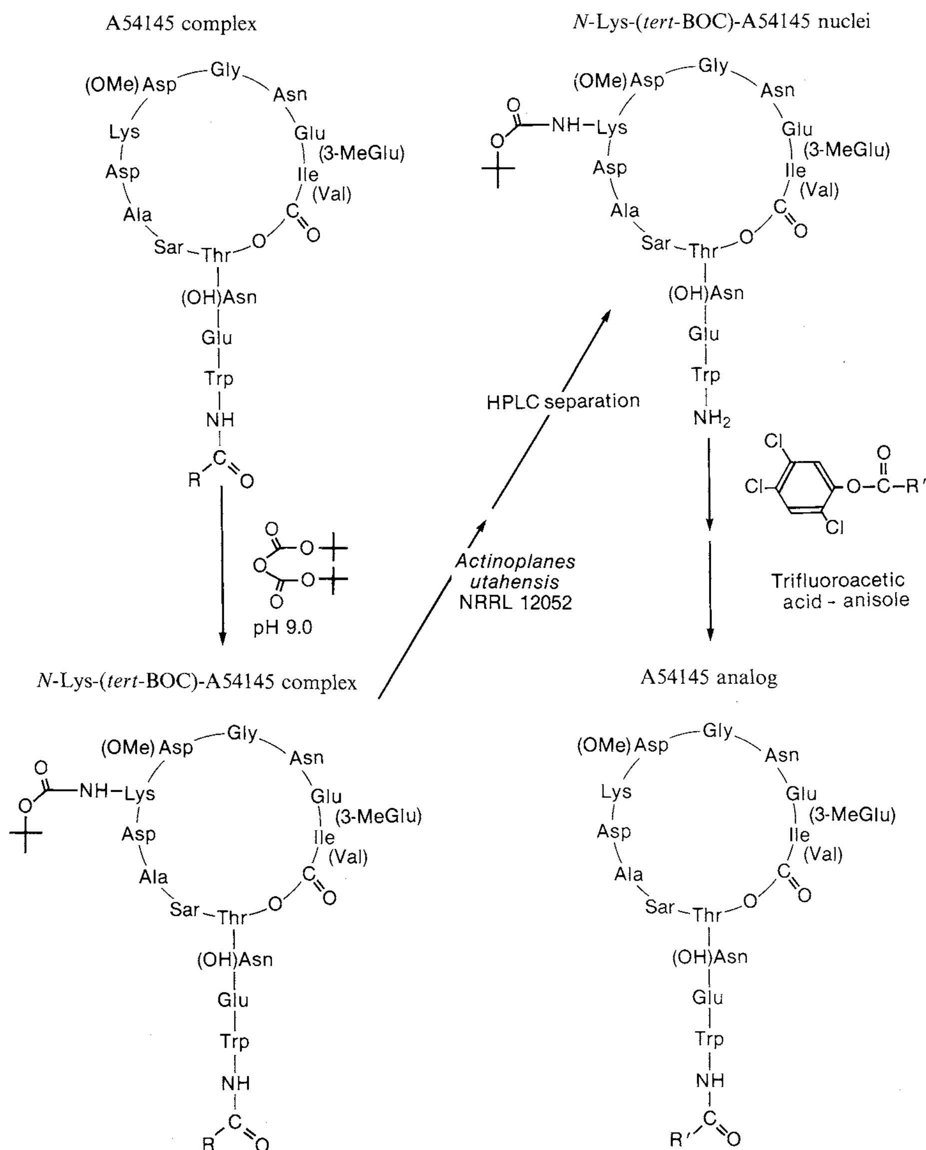


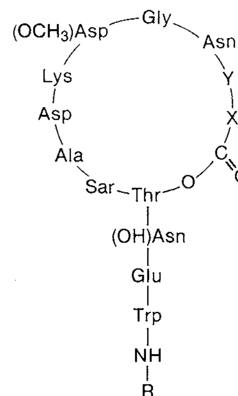
Table 1. Physical characteristics of A54145 derivatives.

Compound No.	FAB-MS (m/z) (M+H)	UV λ_{max}^{EtOH} nm (ε)	Rt (minutes)[a]
9	1,588	220 (16,300), 282 (2,490), 290 (2,200)	2.3
10	1,630	220 (39,124), 279 (5,459)	6.9
2	1,644	220 (35,870), 280 (5,419)	11.3
1	1,644	218 (30,000), 278 (4,100), 287 (3,900)	10.4
11	1,658	220 (34,413), 280 (4,752)	19.1
12	1,672	221 (32,400), 281 (4,600), 289 (3,880)	32.3
13	1,700	219 (32,200), 281 (4,940), 289 (4,260)	40.2
3	1,658	221 (29,100), 282 (4,390), 290 (3,710)	12.8
14	1,630	221 (17,600), 280 (2,460), 290 (2,110)	8.9

[a] HPLC (system C).

relationship and confirm the structures of several naturally-occurring factors. *Actinoplanes utahensis* has been used previously to effect the deacylation of *N*-Orn-*tert*-BOC-protected A21978C lipopeptide complex without hydrolyzing the protecting group[5,6] in a route to A21978C analogs. The isolation of A54145 complex lipopeptide antibiotics was complicated by the presence of four possible peptide cores, rather than one, as in the case of A21978. In order to minimize the number of purifications required, we adopted the strategy of deacylating protected A54145 complex rather than deacylating pure protected A54145 factors. Since our interests required *N*-Trp acylated A54145 nuclei, not *N*-Lys, *N*-Trp diacylated products, we employed the mono-*tert*-BOC protecting group to prevent acylation at the lysine residue during the later reacylation step. The resulting mixture of *N*-Lys-*tert*-BOC protected A54145 nuclei was purified to give the protected A54145A, B, and F nuclei. The *N*-Lys-*tert*-BOC-A54145C nucleus was not isolated in practical quantities, due to the low quantities of A54145C in the starting material.

Fig. 3. Structures of semi-synthetic A54145 factors and analogs.



Compound No.	Fatty acyl derivatives		
	X	Y	R
9	Ile	Glu	*n*-Hexanoyl
10	Ile	Glu	*n*-Nonanoyl
2	Ile	Glu	*n*-Decanoyl
1	Ile	Glu	8-Methylnonanoyl
11	Ile	Glu	*n*-Undecanoyl
12	Ile	Glu	*n*-Dodecanoyl
13	Ile	Glu	*n*-Tetradecanoyl
3	Ile	3-MethylGlu	*n*-Decanoyl
14	Val	Glu	*n*-Decanoyl

The acylation of the protected A54145 nuclei was accomplished with the use of 2,4,5-trichlorophenyl active esters which were prepared from the appropriate fatty acids or acid chlorides. The deprotection of *N*-Lys-*tert*-BOC-*N*-Trp-alkanoyl A54145 nuclei was readily accomplished by hydrolysis with trifluoroacetic acid. The route to A54145 antibiotic analogs is summarized in Fig. 2. Physical characteristics of the analogs prepared are listed in Table 1. The microbiological evaluation of A54145 complex antibiotics and analogs, including the effects of side chain and nucleus variation is described separately[7]. An alternative means of producing A54145 antibiotic analogs, as well as controlling factor ratios, *via* side chain precursing is described in a separate communication[8].

Experimental

General Methods

UV spectra were run on a Cary model 118 or model 219 spectrophotometer. IR spectra were recorded on a Nicolet MX-1 FT-IR spectrometer. FAB mass spectra were run on a Varian-MAT 731, VG Analytical ZAB 3, or VG Analytical ZAB 2SE mass spectrometer.

Analytical HPLC of *N*-Lys-*tert*-BOC-A54145 Complex

N-Lys-*tert*-BOC-A54145 complex was analyzed by HPLC on HPLC system A: A Waters NOVA-PAK C_{18} RadialPak cartridge (8 mm × 10 cm) using CH_3CN - 0.5% aq $NH_4H_2PO_4$ (20 : 80) at a flow rate of 2.0 ml/minute, with UV detection at 223 nm or on HPLC system B: A DuPont Zorbax ODS column (4.6 mm × 25 cm, 5 μm particle size) using a mobile phase of CH_3CN - MeOH - 0.04 M aq NH_4OAc (25 : 12.5 : 62.5) at a flow rate of 2 ml/minute with UV detection at 289 and 223 nm.

Analytical HPLC of *N*-Trp-alkanoyl Derivatives of A54145 Nuclei

N-Trp-alkanoyl derivatives of A54145 nuclei were analyzed by HPLC system C: A DuPont Zorbax 150-C_8 column (4.6 mm × 25 cm), with UV detection at 223 nm and flow rate of 2 ml/minute. The solvent program consisted of isocratic elution with CH_3CN - 0.2% aq triethylamine (adjusted to pH 3.0 with H_3PO_4) (35 : 65) for 25 minutes followed by a 10-minute linear gradient to a final isocratic hold at a ratio of 45 : 55.

A. utahensis Growth

A. utahensis first vegetative medium consisted of sucrose 2.0%, soybean flour 1%, K_2HPO_4 0.12%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.025%, and SAG 471 defoamer (Union Carbide) 0.03% in deionized water. The production medium was the same as previously described. Stock *A. utahensis* liquid nitrogen suspension[6] was used to inoculate vegetative medium (800 ml), which was then incubated at 30°C on a shaker for 72 hours. Second stage medium (950 liters) in a 1,400-liter vessel was then inoculated and stirred at 155 rpm at 30°C with air flow of 793 liters/minute for 72 hours. Production medium (810 liters in a 1,400-liter vessel) was inoculated from the second stage fermentation, then fermented at 30°C with an air flow of 283 liters/minute and stir rate of 130 rpm.

Preparation of *N*-Lys-*tert*-BOC-A54145 Complex

In a typical preparation crude A54145 complex (25 g) was dissolved in water (200 ml) and adjusted to pH 9.0 with 5 N NaOH. Di-*tert*-butyldicarbonate (7 ml) was added with stirring over a 30-minute period at ambient temperature while the pH was maintained at 8.5 ~ 9.0 by addition of NaOH. The reaction was judged to be complete by HPLC analysis after a total of 80 minutes. The solution was lyophilized, then dissolved in H_2O (400 ml) and extracted with dichloromethane (3 × 400 ml). The aqueous phase was concentrated under reduced pressure, then lyophilized to give *N*-Lys-*tert*-BOC-A54145 complex (weight not determined).

Preparation of *N*-Lys-*tert*-BOC-A54145A and F Nuclei

N-Lys-*tert*-BOC-A54145 complex prepared as above from A54145 complex (50 g, dissolved in water) was added to 15 liters of production fermentation in a 68-liter fermenter 43 hours after inoculation. The fermentation was maintained at 30°C until HPLC analysis indicated that the deacylation was completed.

The mycelial methanol extract (4 liters) was concentrated, lyophilized, reconstituted in H_2O (0.5 liter), and combined with the filtered broth and mycelial H_2O wash. The combined solution was adjusted to pH 4.7 with HCl and stirred with Diaion HP-20 macroreticular resin (2.4 liters). After 2 hours the mixture was poured into a column (5 × 150 cm) and washed with dilute HOAc (pH 3.5, 14.5 liters). The column was eluted with an CH_3CN - H_2O step gradient (5 : 95, 4 liters; 1 : 9, 4 liters; 15 : 85, 2 liters and 3 : 7, 8 liters). Upon concentration and lyophilization the 3 : 7 eluate yielded purified *N*-Lys-*tert*-BOC-A54145 nucleus complex (23.0 g). Preparative reverse phase high performance liquid chromatography (RPHPLC) of the protected nucleus complex (23.0 g) on a Chromatospac 100 column (4 liters) packed with octadecylsilanized Quantum LP-1 silica gel (LP-1/C_{18}), eluting with pyridine - HOAc - H_2O - CH_3CN - MeOH (1 : 1 : 73 : 15 : 10)

at a flow rate of 60 ml/minute yielded *N*-Lys-*tert*-BOC-A54145F nucleus (1.8 g) followed by *N*-Lys-*tert*-BOC-A54145A nucleus (2.6 g). *N*-Lys-*tert*-BOC-A54145F nucleus has the following properties: HRFAB-MS (*m/z*) Calcd for $C_{66}H_{98}N_{17}O_{28}$: 1,576.67666 (M+H), Found: 1,576.67723 (M+H); UV λ_{max}^{EtOH} nm (ε) 218 (sh, 35,500), 281 (5,590), 289 (4,890), IR (KBr) cm^{-1} 3339, 1668, 1539, HPLC Rt 4.2 minutes in system A (see above). *N*-Lys-*tert*-BOC-A54145A nucleus has the following properties: HRFAB-MS (*m/z*) Calcd for $C_{67}H_{100}N_{17}O_{28}$ (M+H): 1,590.69231, Found: 1,590.69229 (M+H); UV λ_{max}^{EtOH} nm (ε) 218 (36,600), 282 (4,400), 289 (3,690), IR (KBr) cm^{-1} 3338, 1669, 1536, HPLC Rt 6.6 minutes in system A (see above).

Preparation of *N*-Lys-*tert*-BOC-A54145B Nucleus

N-Lys-*tert*-BOC-A54145 complex (120 g) was deacylated by 10 liters of *A. utahensis* production broth as described above to give *N*-Lys-*tert*-BOC-A54145 nucleus complex (79.65 g). A portion (21.1 g) of *N*-Lys-*tert*-BOC-A54145B nucleus-enriched material was obtained by Diaion HP-20 chromatography and preparative RPHPLC on a Chromatospac column as described in the preceding example, using a mobile phase consisting of pyridine - HOAc - H_2O - CH_3CN (1 : 1 : 83 : 15). Purified *N*-Lys-*tert*-BOC-A54145B nucleus (0.38 g) was obtained by RPHPLC on columns (2~2.2 × 30 cm) of DuPont Zorbax ODS (12 μm) using pyridine - HOAc - H_2O - CH_3CN (1 : 1 : 49 : 49) followed by rechromatography using pyridine - HOAc - H_2O - CH_3CN (1 : 1 : 78 : 20). *N*-Lys-*tert*-BOC-A54145B nucleus has the following properties: HRFAB-MS (*m/z*) Calcd for $C_{68}H_{102}N_{17}O_{28}$ (M+H) 1,604.70796, Found: 1,604.70644 (M+H); UV λ_{max}^{EtOH} nm (ε) 218 (34,900), 281 (4,530), 289 (3,790), IR (KBr) cm^{-1} 3340, 1668, 1538, HPLC Rt 8.6 minutes in system A (see above).

Preparation of 2,4,5-Trichlorophenyl Active Esters

2,4,5-Trichlorophenyl active esters were prepared by the acid chloride or *N,N'*-dicyclohexylcarbodiimide method[5]. The following examples are representative: 1) *n*-Dodecanoyl chloride (2.5 g, 11.4 mmol) and 2,4,5-trichlorophenol (1.75 g, 8.86 mmol) were stirred in 40 ml of anhydrous diethyl ether under nitrogen at room temperature until completely dissolved. Pyridine (2 ml) was added and the diethyl ether was removed under a stream of nitrogen. The precipitate was collected and washed well with diethyl ether. The combined filtrates were concentrated to give the crude product. This material was chromatographed over a column (2.5 × 30 cm) of Woelm silica gel. Elution with toluene gave the desired ester. Combination and concentration of these fractions gave 3.0 g of 2,4,5-trichlorophenyl *n*-dodecanoate. 2) *n*-Undecanoic acid (7.5 g, 40 mmol) was added to a solution of 2,4,5-trichlorophenol (8.6 g, 43.7 mmol) in anhydrous THF (175 ml), with stirring under nitrogen. *N,N'*-Dicyclohexylcarbodiimide (9.0 g, 43.7 mmol) was then added in one portion. The reaction mixture was stirred overnight at room temperature, and then concentrated to approximately 100 ml volume and filtered to remove the dicyclohexylurea (DCU). The filtrate was evaporated to dryness, then dissolved in diethyl ether - hexanes. Additional precipitate was removed by filtration. Upon further concentration of the filtrate a white precipitate was collected and dried to give 5.27 g of 2,4,5-trichlorophenyl *n*-undecanoate.

Preparation of *N*-Lys-*tert*-BOC-*N*-Trp-alkanoyl A54145 Nucleus

The following example is representative of the preparation of *N*-Trp-acylated *N*-Lys-protected A54145 nuclei: 2,4,5-Trichlorophenyl *n*-undecanoate (300 mg) and hydroxybenzotriazole (8 mg) were added to a solution of *N*-Lys-*tert*-BOC-A54145A nucleus (500 mg) in dry DMF (15 ml) under a nitrogen atmosphere. After stirring for 21 hours under nitrogen at room temperature, the reaction mixture was concentrated to approximately one-tenth volume and diethyl ether (30 ml) was added. The resulting precipitate was washed with diethyl ether (2 × 30 ml) and dried under reduced pressure to give *N*-Lys-*tert*-BOC-*N*-Trp-*n*-undecanoyl A54145A nucleus (425 mg).

Preparation of *N*-Trp-alkanoyl A54145 Nucleus

The *N*-Lys-*tert*-BOC-*N*-Trp-alkanoyl A54145 nuclei were deblocked with TFA as illustrated in the following example: A solution of *N*-Lys-*tert*-BOC-*N*-Trp-*n*-undecanoyl A54145A nucleus, TFA (5 ml), and anisole (0.5 ml) was stirred under nitrogen at room temperature for 1 hour. The solution was concentrated under reduced pressure to minimal volume and CH_2Cl_2 - diethyl ether (2 : 1, 30 ml) added. The resulting precipitate was washed with CH_2Cl_2 - diethyl ether (2 : 1, 2 × 30 ml), dried, and redissolved in water (10 ml).

The solution was adjusted to pH 6.2 by the addition of pyridine then lyophilized to give crude product (645.5 mg). This crude product was purified by RPHPLC on a column (2.2 × 30 cm) packed with DuPont Zorbax ODS (12 μm), eluting with pyridine - HOAc - H_2O - CH_3CN - MeOH (1 : 1 : 53 : 35 : 10) at 9 ml/minute, to give *N*-Trp-*n*-undecanoyl A54145A nucleus (241 mg).

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