SYNTHESIS AND ANTIFUNGAL PROPERTIES OF DIASTEREOMERS AND ANALOGS OF ANTIBIOTIC Sch 37137

RYSZARD ANDRUSZKIEWICZ

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, 80-952 Gdańsk, Poland

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The synthesis of several diastereomeric derivatives of antibiotic Sch 37137 and its structural isomer has been performed and their antifungal activity has been examined.

Antibiotic Sch 37137 (**1a**), *i.e.* N^2-L-alanyl-N^3-[(*trans*)-epoxysuccinamoyl]-L-2,3-diaminopropanoic acid, a new antifungal peptide was recently isolated from the culture filtrate of a *Micromonospora* sp. by the Schering group[1]. A similar peptide produced by *Serratia plymutica*, CB-25-I related to Sch 37137 has also been isolated and characterized[2]. Both compounds exhibited a weak inhibitory activity against a variety of strains of *Candida* in the range that depends on the medium used in the experiments. The biological studies of **1a** have also demonstrated the lack of activity against a broad range of Gram-positive and Gram-negative bacteria. This peptide is closely related to antibiotic A 19009[3,4] isolated from a *Streptomyces* sp. The only difference between compound Sch 37137 and A 19009 is the replacement of an epoxide by a double bond. Since our earlier report on antibiotic A '19009[5] showed its relatively good anticandidal activity, we decided to synthesize several derivatives of antibiotic Sch 37137 and its structural isomer (Fig. 1) and to test them against selected antifungal strains using modified YNB medium.

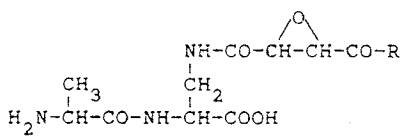
Chemistry

The preparation of products **1a**~**1c** and **2a**~**2c** proceeded as shown in Scheme 1. Taking into account the fact that the original structure elucidation of Sch 37137 performed by ^{1}H NMR revealed only the *trans* configuration of the 2,3-epoxysuccinic acid without determination of its absolute configuration at C-2 and C-3 of the epoxide ring, racemic acid was used throughout the synthesis. Subsequently a recent publication[6] described the total synthesis of Sch 37137 as well as its 2*S*,3*S*-isomer and established its absolute stereochemistry as 2*R*,3*R*. Racemic (*trans*)-2,3-epoxysuccinic acid prepared according to the method of PAYNE[7], was converted into the monoethylester[8] which was activated with *N*-hydroxysuccinimide and *N*,*N*'-dicyclohexylcarbodiimide. The active ester **1** was combined with N^2-(*tert*)-butoxycarbonyl-L-2,3-diaminopropanoic acid[9] to give compound **2** (a mixture of diastereomers) which was either deprotected using trifluoroacetic acid to obtain **3** or was activated again with *N*-hydroxysuccinimide and *N*,*N*'-dicyclohexylcarbodiimide to yield active ester **4**. Product **3** was acylated with Boc-Ala-OSu[10] to give the peptide **5**. Treatment of **5** (the ethyl ester) with ammonia[11] gave the mixture of protected diastereomers of Sch 37137 **6**. Alkaline hydrolysis of **5** furnished the product **7**. Activated product **4** in turn was used for acylation of alanine to afford the peptide **8** which was converted into products **9** and **10**, respectively (ammonolysis followed by alkaline hydrolysis). Final deprotection of the Boc groups using trifluoracetic acid from the peptides **5**, **6**, **7**, **8**, **9** and **10** gave the products **1a**~**1c** and **2a**~**2c**.

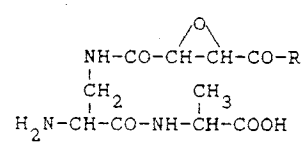
Biological Activity

The antifungal activity of the synthesized products **1a**~**1c** and **2a**~**2c** against selected fungal strains is summarized in Table 1. Of the peptides obtained only the mixtures of diastereomers of Sch 37137 (**1a**) and its isomer (**2a**) showed good activity, the latter being slightly less active than the former. Both products contained the *trans*-2,3-epoxysuccinamide moiety. Activity was measured in YNB medium containing sodium glutamate[12] and was

Fig. 1. Structures of the synthesized compounds.

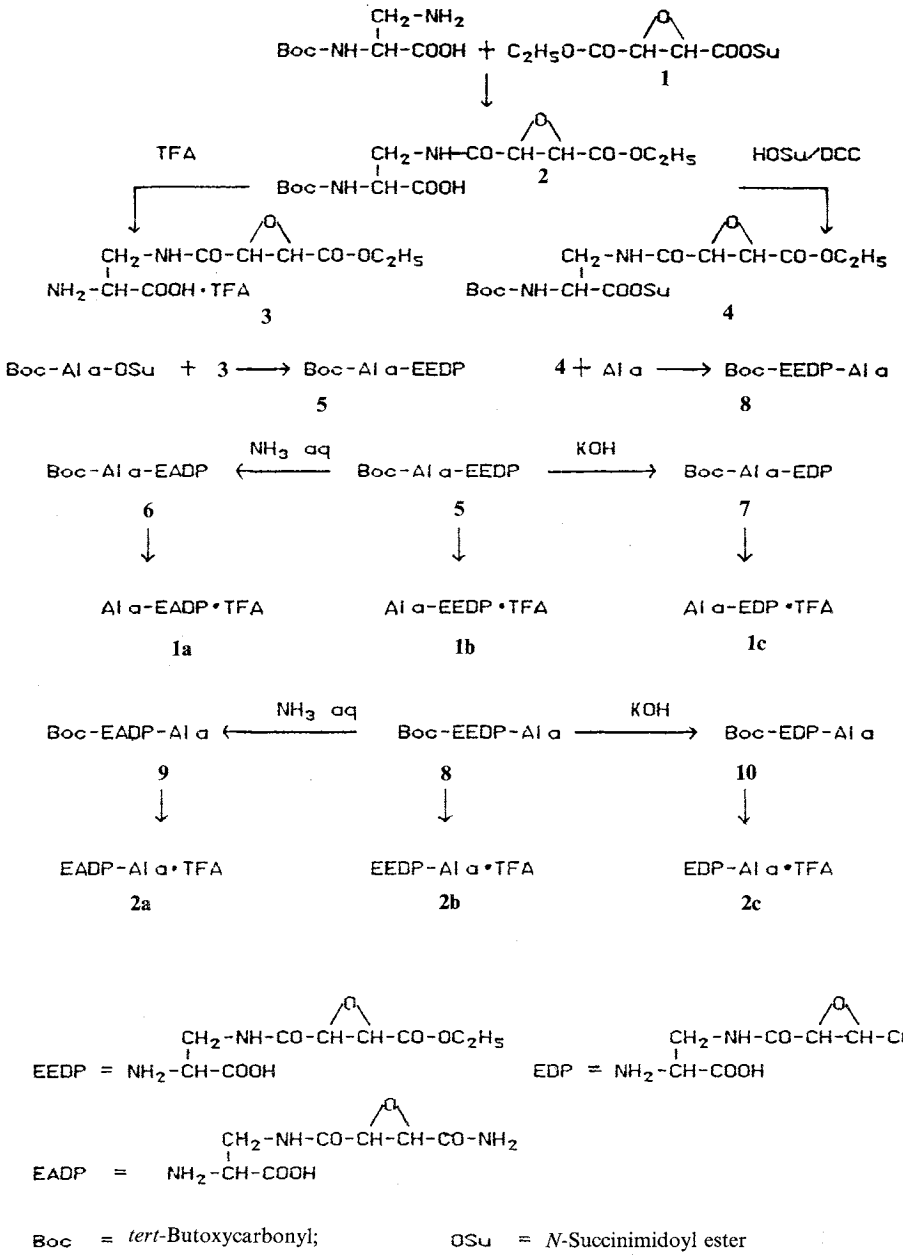


1a R=NH_2 (Ala-EADP)
1b R=OC_2H_5 (Ala-EEDP)
1c R=OH (Ala-EDP)



2a R=NH_2 (EADP-Ala)
2b R=OC_2H_5 (EEDP-Ala)
2c R=OH (EDP-Ala)

Scheme 1. Synthesis of compounds **1a**~**1c** and **2a**~**2c**.



significantly improved (approximately one order of magnitude) relative to that determined in MA medium (a glucrose-salt medium containing glutamate and biotin) (data not shown). The activities of the ethyl ester derivative (**1b** and **2b**) were markedly reduced. Unsubstituted carboxyl group in products **1c** and **2c** resulted in almost complete loss of activity. Moreover, all the products were inactive (MIC > 150 μg/ml; data not presented) against dermatophytes (*Epidermophyton floccosum*, *Trichophyton rubrum* and *Scopulariopsis brevicaulis*) when tested in the modified YNB medium. From these results, it can be concluded that the structural element essential for antifungal activity of the examined compounds is the amide group. It is likely that this group is needed to ensure that the products **1a** and **2a** may reach the target site inside the cells thus inhibiting fungal growth.

Table 1. Antifungal activity of the obtained products.

No.	Prod.	MIC[a], μg/ml				
		S. cerevisiae ATCC 9763	*C. albicans* ATCC 26278	*C. albicans* 4477	*C. crusei*	*Torula candida*
1a	Ala-EADP	2.0	1.2	1.5	2.5	2.5
2a	EADP-Ala	3.75	1.8	2.0	3.0	3.0
1b	Ala-EEDP	25	50	60	75	75
2b	EEDP-Ala	25	65	70	80	80
1c	Ala-EDP	100	100	>100	>100	>100
2c	EDP-Ala	150	150	>150	>150	>150
	AMB	0.25	0.25	0.375	N.T.	N.T.

[a] Measured in YNB medium (Yeast nitrogen base—Difco, containing 200 μg/ml of sodium glutamate), 10^5 cfu/ml, 30°C, 24 hours AMB—amphotericin B.

Experimental

Melting points were determined with a heated microscope (HMK Dresden, Germany). ^{1}H NMR spectra were recorded at 60 MHz on a Varian 360 spectrometer. Optical rotations were measured in a Polamat (Carl Zeiss, Jena) polarimeter. Microbiological assays were carried out as described by MILEWSKI *et al.*[12].

N-Succinimidoyl Ethyl DL-(*trans*)-Epoxysuccinate **1**

To a solution of ethyl hydrogen DL-(*trans*)-epoxysuccinate (2.4 g; 15 mmol) and *N*-hydroxysuccinimide (1.72 g; 15 mmol) in THF (20 ml), *N*,*N*'-dicyclohexylcarbodiimide (3.09 g; 15 mmol) in THF (10 ml) was added dropwise at 0~5°C for 1 hour. After 20 hours the urea was filtered off and the filtrate evaporated to dryness leaving a crystalline residue which was crystallized from THF-ethyl ether to give 3.2 g (83%) of **1**. MP 122~123°C; ^{1}H NMR (DMSO-d_6) δ 1.3 (3H, t, J=7 Hz), 2.9 (4H, s), 3.85 (1H, d, J=2 Hz), 4.0 (1H, d, J=2 Hz), 4.3 (2H, q, J=7 Hz).

Anal Calcd for $C_{10}H_{11}NO_7$: C 46.69, H 4.31, N 5.44.
Found: C 46.61, H 4.45, N 5.62.

N^2-(*tert*)-Butoxycarbonyl-N^3-(DL-(*trans*)-4-ethoxy-epoxysuccinoyl)-L-2,3-diaminopropanoic Acid **2**

N^2-(*tert*)-Butoxycarbonyl-L-2,3-diaminopropanoic acid (2.04 g; 10 mmol) and $NaHCO_3$ (0.84 g; 10 mmol) were dissolved in a water-methanol solution (1 : 1, v/v; 20 ml). The *N*-succinimidoyl ethyl DL-(*trans*)-epoxysuccinate (2.57 g; 10 mmol) was added with stirring at 0~5°C and was kept at room temperature overnight. The solvents were removed *in vacuo*. The residue was dissolved in 10 ml of water, acidified to pH 2 (saturated $KHSO_4$) and extracted with ethyl acetate (3 × 50 ml). The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to give 2.51 g (72%) of **2**, which was crystallized from a mixture of ethyl acetate-hexane. MP 54~56°C; $[\alpha]_{578}^{25}$ −10.1° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J=7 Hz), 1.25 (9H, s), 3.5~3.7 (2H, m), 3.6 (2H, m), 4.1 (1H, m), 4.3 (2H, q, J=7 Hz), 6.6 (1H, m), 7.2 (1H, m).

Anal Calcd for $C_{14}H_{22}N_2O_8$: C 48.54, H 6.40, N 8.09.
Found: C 48.30, H 6.15, N 8.02.

N^3-(DL-(*trans*)-4-Ethoxy-epoxysuccinoyl)-L-2,3-diaminopropanoic Acid Trifluoroacetate **3**.

N^2-*tert*-Butoxycarbonyl-N^3-(DL-(*trans*)-4-ethoxy-epoxysuccinoyl)-L-2,3-diaminopropanoic acid (1.75 g; 5 mmol) was dissolved in a cold trifluoroacetic acid (15 ml) and stirred for 3 hours at room temperature. Excess of TFA was removed under reduced pressure and the oily residue was triturated with dry ethyl ether, dried *in vacuo* over KOH to yield 1.72 g (95%) of **3** as its trifluoroacetate salt. MP 112~114°C; $[\alpha]_{578}^{25}$ −18.2° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.2 (3H, t, J=7 Hz), 3.5~3.6 (2H, m), 3.7 (2H, m), 4.0 (2H, q), 4.1 (1H, m), 6.4 (1H, m), 7.8~8.3 (4H, m).

Anal Calcd for $C_9H_{14}N_2O_6CF_3COOH$:
C 36.67, H 4.19, N 7.77.
Found: C 36.30, H 4.05, N 7.42.

N-Succinimidoyl N^2-(*tert*)-Butoxycarbonyl-N^3-(DL-(*trans*)-4-ethoxy-epoxysuccinoyl)-L-2,3-diaminopropanoate **4**

N^2-(*tert*)-Butoxycarbonyl-N^3-(DL-(*trans*)-4-ethoxy-epoxysuccinoyl-L-2,3-diaminopropanoic acid (1.04 g; 3 mmol) and *N*-hydroxysuccinimide (0.34 g; 3 mmol) were dissolved in ethyl acetate (20 ml) cooled to 0°C and *N*,*N*'-dicyclohexyl-

carbodiimide (0.6 g; 3 mmol) dissolved in ethyl acetate (5 ml) was added. The mixture was stirred overnight, the urea was filtered off, the filtrate evaporated to dryness, leaving an oily residue which was crystallized upon addition of hexane to yield 1.22 g (92%) of **4**. MP 111~114°C; $[\alpha]_{578}^{25}$ −25.4° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.1 (9H, s), 1.2 (3H, t, J=7 Hz), 2.4 (4H, s), 3.5~3.6 (2H, m), 3.7 (2H, m), 4.2 (1H, m), 4.3 (1H, m), 6.5 (1H, m), 7.2 (1H, m).

Anal Calcd for $C_{18}H_{25}N_3O_{10}$:
C 48.76, H 5.68, N 9.47.
Found: C 48.49, H 5.41, N 9.19.

N^2-[*N*-(*tert*)-Butoxycarbonyl-L-alanyl]-N^3-(DL-(*trans*)-4-ethoxy-epoxysuccinoyl-L-2,3-diaminopropanoic Acid **5**

To a cooled (0°C) solution of N^3-[DL-(*trans*)-4-ethoxy-epoxysuccinoyl] L-2,3-diaminopropanoic acid trifluoroacetate (1.84 g, 4 mmol) and $NaHCO_3$ (0.69 g, 8 mmol) in a mixture of water-methanol (1:1, v/v; 10 ml) Boc-Ala-OSu (1.14 g, 4 mmol) in MeOH (10 ml) was added immediately with stirring. The reaction mixture was stirred for 1 hour in a bath, then at room temperature overnight. After usual work up and evaporation of the solvents 1.48 g (82%) of **5** was obtained as a foam. MP 73~76°C; $[\alpha]_{578}^{25}$ −12.1° (*c* 1, MeOH).

Anal Calcd for $C_{17}H_{27}N_3O_9$:
C 48.91, H 6.52, N 10.06.
Found: C 48.71, H 6.45, N 9.92.

N^2-[*N*-(*tert*)-Butoxycarbonyl-L-alanyl]-N^3-(DL-(*trans*)-epoxysuccinamoyl)-L-2,3-diaminopropanoic Acid **6**

A sample of **5** (0.417 g, 1 mmol) was stirred with 20 ml of cold ammonia (29% ammonia) using a strong magnetic stirrer for 2 hours. Ammonia was evaporated, the crude residue was dissolved in a small amount of water (5 ml) and passed through a short column of Dowex 50W×8 (H^+). The product was eluted with water-methanol (2:1, 50 ml), evaporated to dryness leaving an oily residue which was crystallized upon addition of ethyl ether. Yield 0.3 g (77%). MP 104~106°C; $[\alpha]_{578}^{25}$ −16.8° (*c* 1, MeOH).

Anal Calcd for $C_{15}H_{24}N_4O_8 \cdot H_2O$:
C 44.32, H 6.44, N 13.78.
Found: C 44.04, H 6.21, N 13.48.

N^2-[*N*-(*tert*)-Butoxycarbonyl-L-alanyl]-N^3-(DL-(*trans*)-epoxysuccinoyl)-L-2,3-diaminopropanoic acid **7**

To a ethanolic solution (5 ml) of dipeptide **5** (0.417 g, 1 mmol), 1 N KOH in ethanol (2.1 ml, 2.1 mmol) was added under ice cooling and stirred for 2 hours at the same temperature. Ethanol was evaporated off, the residue was dissolved in 5 ml of water and passed through a small column of Dowex 50W×8 (H^+), which was washed with water-methanol (1:1, 50 ml). Fractions containing **7** were collected, evaporated to dryness and crystallized from ethyl ether to give **7**, 0.28 g (72%). MP 138~140°C; $[\alpha]_{578}^{25}$ −15.1° (*c* 1, MeOH).

Anal Calcd for $C_{15}H_{23}N_3O_9$:
C 46.26, H 5.95, N 10.79.
Found: C 45.98, H 5.75, N 10.52.

N^2-(*tert*)-Butoxycarbonyl-N^3-DL-(*trans*)-4-ethoxy-epoxysuccinoyl)-L-2,3-diaminopropanoyl-L-alanine **8**

Starting from the active ester **4** (2.02 g, 5 mmol), L-alanine (0.45 g, 5 mmol) and $NaHCO_3$ (0.42 g, 5 mmol), peptide **8** was obtained by the procedure used to prepare **5**. Yield 1.46 g (71%). MP 71~74°C; $[\alpha]_{578}^{25}$ +5.3° (*c* 1, MeOH).

Anal Calcd for $C_{17}H_{27}N_3O_9$:
C 48.91, H 6.52, N 10.06.
Found: C 48.68, H 6.30, N 10.14.

N^2-(*tert*)-Butoxycarbonyl-N^3-DL-(*trans*)-epoxy-succinamoyl-L-2,3-diaminopropanoyl-L-alanine **9**

Compound **9** was obtained analogously as compound **6**. Yield 0.35 g (90%). MP 118~120°C; $[\alpha]_{578}^{25}$ −10.4° (*c* 1, MeOH).

Anal Calcd for $C_{15}H_{24}N_4O_8 \cdot H_2O$:
C 44.32, H 6.44, N 13.78.
Found: C 43.95, H 6.16, N 13.44.

N^2-(*tert*)-Butoxycarbonyl-N^3-DL-(*trans*)-epoxy-succinoyl-L-2,3-diaminopropanoyl-L-alanine **10**

According for the procedure used for the preparation of **7**, compound **10** was obtained. Yield 0.28 g (72%). MP 138~140°C; $[\alpha]_{578}^{25}$ −15.1° (*c* 1, MeOH)

Anal Calcd for $C_{15}H_{23}N_3O_9$:
C 46.26, H 5.95, N 10.79.
Found: C 46.05, H 5.84, N 10.66.

N^2-L-Alanyl-N^3-DL-(*trans*)-epoxysuccinamoyl-L-2,3-diaminopropanoic Acid Trifluoroacetate **1a**

Deprotection of **9** according to the procedure used for deprotection of **3** yielded **1a** in 95%. MP

136~139°C; $[\alpha]_{578}^{25}$ +2.1° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.5 (3H, d, J=7.0 Hz), 3.5~3.6 (2H, m), 3.7 (2H, s), 4.1 (1H, q, J=7.0 Hz), 4.4 (1H, m).

Anal Calcd for $C_{10}H_{16}N_4O_6 \cdot CF_3COOH$:
C 35.82, H 4.26, N 13.92.
Found: C 35.50, H 4.08, N 13.66.

N^2-L-Alanyl-N^3-DL-(*trans*)-4-ethoxy-epoxysuccinoyl-L-2,3-diaminopropanoic Acid Trifluoroacetate **1b**

Compound **1b** was obtained from **5** in 92% using the same procedure as for **3**. MP 105~107°C; $[\alpha]_{578}^{25}$ +5.1° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.3 (3H, t, J=6.5 Hz), 1.5 (3H, d, J=7.0 Hz), 3.5~3.6 (2H, m), 3.7 (2H, s), 4.0 (1H, q, J=6.5 Hz), 4.2 (1H, q, J=7.0 Hz), 4.4 (1H, m).

Anal Calcd for $C_{12}H_{19}N_3O_7 \cdot CF_3COOH$:
C 38.39, H 4.67, N 9.74.
Found: C 38.32, H 4.33, N 9.48.

N^2-L-Alanyl-N^3-DL-(*trans*)-epoxysuccinoyl-L-2,3-diaminopropanoic Acid Trifluoroacetate **1c**

Using the same procedure as for compound **3**, **1c** was prepared in 96%. MP 163~166°C; $[\alpha]_{578}^{25}$ +3.2° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.55 (3H, d, J=7.0 Hz), 3.5~3.6 (2H, m), 3.75 (2H, s), 4.15 (1H, q, J=7.0 Hz), 4.35 (1H, m).

Anal Calcd for $C_{10}H_{15}N_3O_7 \cdot CF_3COOH$:
C 35.74, H 3.99, N 10.41.
Found: C 35.51, H 3.66, N 10.22.

N^3-DL-(*trans*)-Epoxysuccinamoyl-L-2,3-diaminopropanoyl-L-alanine Trifluoroacetate **2a**

Compound **2a** was prepared in the same manner as **1a**. Yield 93%. MP 132~134°C; $[\alpha]_{578}^{25}$ −11.8° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.45 (3H, d, J=7.0 Hz), 3.4~3.6 (2H, m), 3.7 (2H, s), 4.0~4.1 (1H, m), 4.3 (1H, m).

Anal Calcd for $C_{10}H_{16}N_4O_6 \cdot CF_3COOH$:
C 35.82, H 4.26, N 13.92.
Found: C 35.50, H. 4.03, N 13.60.

N^3-DL-(*trans*)-4-Ethoxy-epoxysuccinoyl-L-2,3-diaminopropanoyl-L-alanine Trifluoroacetate **2b**

According to the procedure used for **1b**, compound **2b** was obtained in 95%. MP 112~114°C; $[\alpha]_{578}^{25}$ −17.8° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.35 (3H, t, J=6.5 Hz), 1.45 (3H, d, J=7.0 Hz), 3.4~3.55 (2H, m), 3.75 (2H, s), 4.0 (1H, q, J=6.5 Hz), 4.2~4.3 (1H, q, J=7.0 Hz), 4.45 (2H, m).

Anal Calcd for $C_{12}H_{19}N_3O_7 \cdot CF_3COOH$:
C 38.39, H 4.67, N 9.74.
Found: C 38.07, H 4.43, N 9.56.

N^3-DL-(*trans*)-Epoxysuccinoyl-L-2,3-diaminopropanoyl-L-alanine Trifluoroacetate **2c**

Analogously as for preparation of **1c**, compound **2c** was obtained in 95%. MP 157~160°C; $[\alpha]_{578}^{25}$ −13.2° (*c* 1, MeOH); ^{1}H NMR (D_2O) δ 1.45 (3H, d, J=7.0 Hz), 3.4~3.5 (2H, m), 3.75 (2H, s), 4.2 (1H, q, J=7.0 Hz), 4.4 (1H, m).

Anal Calcd for $C_{10}H_{15}N_3O_7 \cdot CF_3COOH$:
C 35.74, H 3.99, N 10.41.
Found: C 35.44, H 3.72, N 10.30.

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