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PREPARATION OF N^α -ACETYL- N^ϵ -3-(METHYLPYRIDINIUM)LYSINE (MP-LYSINE) AS A HAPTEN OF ANTIBODY

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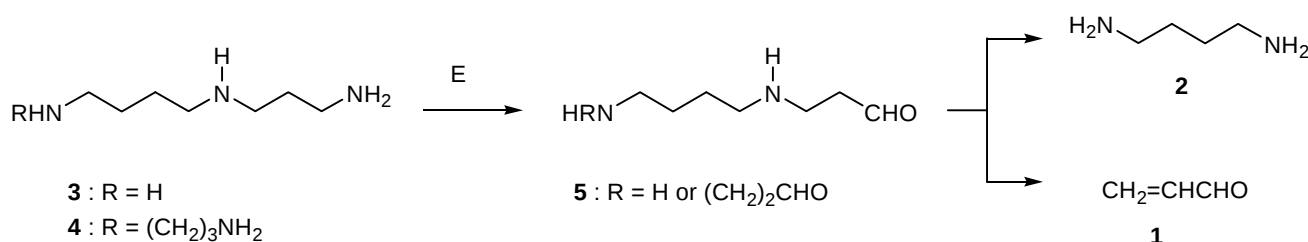
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Dedicated to professor Barry Trost on the occasion of his 65th birthday.

Abstract – The title compound was prepared from α -*tert*-butyl N^α -*tert*-butoxycarbonyl-L-glutamate (Boc-Glu-OBu-*t*) by *N*-alkylation of 3-methylpyridine after one carbon-elongation through six steps.

INTRODUCTION

Acrolein (**1**) is considered to inhibit cell proliferation in living organisms and is, *in vivo*, generated together with putrescine (**2**) by retro-Michael reaction of intermediate aminoaldehydes (**5**) enzymatically derived from spermidine (**3**) and spermine (**4**) as shown in Scheme 1. Thus, acrolein (**1**) could be regarded as a molecule marker of these polyamines; however, it is very difficult to directly estimate the acrolein level in plasma because of its high reactivity with protein.



Scheme 1. Expected production of acrolein (**1**) and putrescine (**2**) from spermidine (**3**) and spermine (**4**)

In 2003, Furuhashi *et al.*¹ reported the production of *N*^α-acetyl-*N*^ε-(3-formyl-3,4-dehydropyridino)lysine (FDP-lysine) and *N*^α-acetyl-*N*^ε-(3-methylpyridinium)lysine (MP-lysine)² (**6**) by treatment of β-chain insulin with acrolein (**1**) followed by hydrolysis. MP-lysine (**6**) is a minor product *in vitro* reaction but has more antigenic activity than FDP-lysine. There is no highly efficient method to synthesize MP-lysine. We had reported the preparation of *N*-hydroxyhomoarginine from commercial available α-*t*-butyl *N*^α-*tert*-butoxycarbonyl-L-glutamate (Boc-Glu-OBu-*t*) (**7**) in the course of nitric oxide (NO) chemistry.³ In this paper we present the synthesis of MP-lysine (**6**) from Boc-Glu-OBu-*t* (**7**) by application of the reported method with slight modification as basic reactions (see, Chart 1).

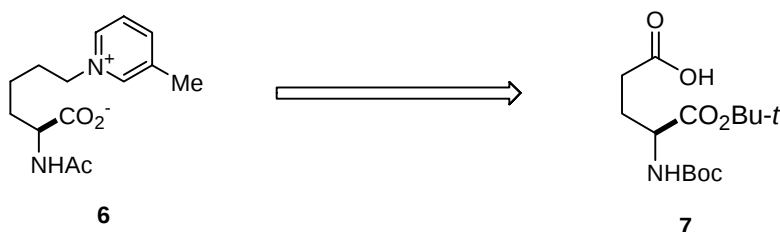


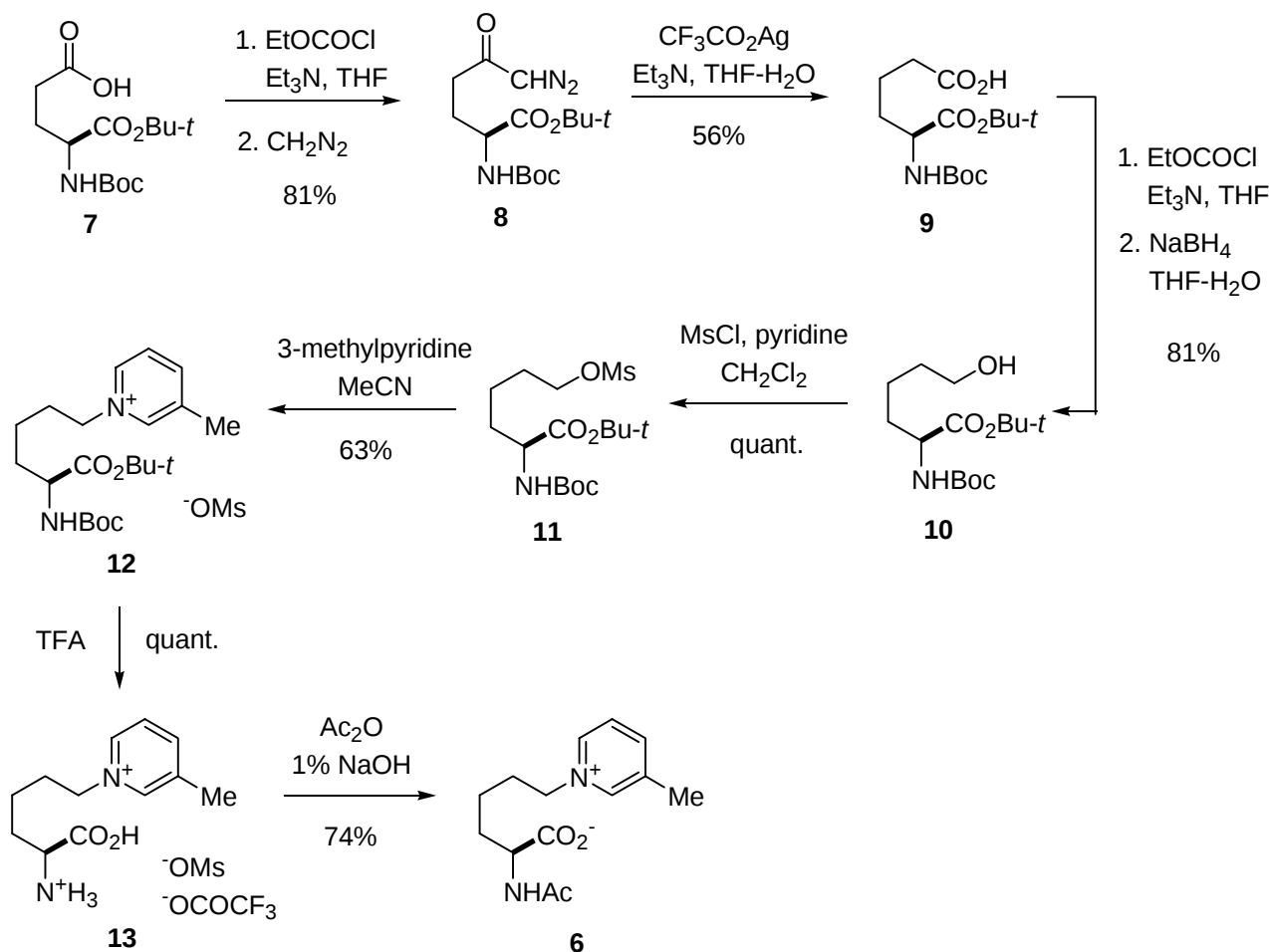
Chart 1. Retrosynthesis of MP-lysine (**6**) from Boc-Glu-OBu-*t* (**7**)

RESULT AND DISCUSSION

Treatment of Boc-Glu-OBu-*t* (**7**) with diazomethane after conversion to a mixed anhydride by reaction with ethoxycarbonyl chloride (EtO₂CCl) in tetrahydrofuran (THF) in the presence of triethylamine (TEA) gave diazo ketone (**8**)⁴ in 81% yield and the subsequent Wolff rearrangement of **8** under protection of light afforded a carbon-elongated carboxylic acid (**9**)⁵ in 56% yield. According to our method³ the acid (**9**) was reduced with sodium borohydride (NaBH₄) in 10% aqueous THF at room temperature for 2 h after conversion to a mixed anhydride under the same conditions (EtO₂CCl/Et₃N/THF) used in the reaction from **7** to **8** afford the corresponding alcohol (**10**)⁶ in 81% yield even with recovery of a small amount of the starting **9** (8%). The alcohol (**10**) was quantitatively converted the methanesulfonate (**11**) by conventional method.

Treatment of **11** with an excess amount of 3-methylpyridine (9.5 M eq.) in acetonitrile (MeCN) at 50 °C for 27 h afforded a desired pyridine-substituted product (**12**) in 63% yield with the recovery of the starting **11** in 24% yield. Use of limited amounts of 3-methylpyridine (1.2 M) at the same temperature for 18 h **12** was produced; however, unreacted **11** was mainly recovered (54%). On the other hand the formation of side products was observed under more harsh conditions (70 °C, 26 h). Concomitant deprotection of both the Boc and the *t*-Bu groups in **12** was successfully achieved by treatment with trifluoroacetic acid (TFA) without solvent for 6.5 h to give deacetyl MP-lysine (**13**) in quantitative yield. Either the use of

dichloromethane (DCM) as solvent in the above reaction or the use of saturated hydrogen chloride in dioxane as an acid source resulted in no reaction or incomplete deprotection, in which the *t*-Bu ester function partially remained. Acetylation of the deacetyl MP-lysine (**13**) with acetic anhydride in 1% sodium hydroxide (NaOH) aq. at room temperature for 2 h under sonication afforded a desired MP-lysine (**6**) in 74% yield.



Scheme 2. Preparation of MP-lysine (**6**) from Boc-Glu-OBu-*t* (**7**)

CONCLUSION

In conclusion Mp-lysine (**6**) was synthesized from Boc-Glu-OBu (**7**) through 7 steps in overall 18% yield. Its affinity ability with aclorein-bounded antibody is under investigation in our laboratory.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a micro melting point hot-stage instrument (Yanagimoto) and are uncorrected. IR spectra were recorded on a JASCO FT/IR-300E spectrophotometer. Specific rotation, $[\alpha]_D$, was recorded on a JASCO DIP-140 spectrophotometer. ¹H-

and ^{13}C -NMR spectra were recorded with JEOL JNM ECP 400 and GSX-500 α spectrometers in CDCl_3 unless otherwise stated. FABMS and HRFABMS spectra were recorded on a JEOL JMX-HX 110A spectrometer with a direct inlet system. For column chromatography silica gel 60 or 60N (spherical 70-230 mesh, Kanto) was used.

(S)-tert-Butyl 2-tert-Butoxycarbonylamino-6-diazo-5-oxohexanoate (8). A mixture of Boc-Glu-OBu-*t* (**7**) (1.187 g, 3.91 mmol), EtO_2CCl (0.52 mL, 5.44 mmol), and TEA (1.20 mL, 8.61 mmol) in THF (14 mL) was stirred at $-15\text{ }^\circ\text{C}$ for 50 min under argon. To the mixture was added an ethereal solution of diazomethane, which was prepared by reaction using *N*-methyl-*N*-nitrosourea (4.48 g, 47 mmol), 40% KOH aq (28 mL), and ether (85 mL), at $0\text{ }^\circ\text{C}$ and the whole was stirred at the same temperature for 4.5 h and then at rt for 1.5 h. After evaporation of the solvent, residue was dissolved in ether (180 mL). The ethereal solution was washed with sat NaHCO_3 aq (30 mL), sat NH_4Cl aq (30 mL), and brine (30 mL), dried (Na_2SO_4), and evaporated. Purification of the residue by column chromatography (SiO_2 60, n-hexane : EtOAc = 2 : 1) afforded a yellow oil (1.04 g, 81%). IR (neat) ν_{max} 3352, 2106, 1712, 1639 cm^{-1} ; ^1H -NMR δ : 1.44 and 1.46 (each 9H, s, *t*-Bu), 1.92 (1H, dddd, $J = 16.9$, 8.6, 8.6, 6.0 Hz, 3- CH_2), 2.15 (1H, m, 3- CH_2), 2.28-2.49 (2H, m, 4- CH_2), 4.17 (1H, m, 2-CH), 5.14 (1H, br d, $J = 7.5$ Hz, NH), 5.31 (1H, br s, 2-CH); ^{13}C -NMR δ : 27.9 (*t*-Bu-Me), 28.2 (*t*-Bu-Me), 36.6 (2- and 3- CH_2), 53.5 (4- CH_2), 54.6 (1-CH), 79.7 (*t*-Bu-C), 82.2 (*t*-Bu-C), 155.4 (CONH), 171.3 (COOR), 193.8 (COCHN $_2$); FABMS m/z : 328 (MH^+); $[\alpha]_{\text{D}}^{24} +17.2^\circ$ ($c = 1.0$, CHCl_3).

(S)-tert-Butyl 2-tert-Butoxycarbonylamino-6-hydroxycarbonyl-1-hexanoate (9). To a solution of diazo ketone (**8**) (0.953 g, 2.91 mmol) in THF (7.8 mL) and H_2O (0.8 mL) was added a solution of silver trifluoroacetate (72 mg, 0.32 mmol) in TEA (1.13 mL, 8.11 mmol) at $-10\text{ }^\circ\text{C}$ (inner temperature) for 3 h under protection of light. After evaporation of the solvent, residue was repeatedly collected with sat NaHCO_3 aq (15 mL x 5). After the non-acidic substances were removed from the NaHCO_3 solution by extraction with ether (25 mL), the residual aqueous solution was adjusted to be pH 2-3 by addition of 18% HCl aq and extracted with EtOAc. The organic solution was dried (Na_2SO_4) and evaporated to give a colorless solid (0.530 g, 56%), mp $74\text{--}76\text{ }^\circ\text{C}$ after trituration with hexane; IR (ATR) ν_{max} 3244, 1709 cm^{-1} ; ^1H -NMR δ : 1.44 and 1.47 (each 9H, s, *t*-Bu), 1.68-1.83 (4H, m, 3- and 4- CH_2), 2.36 and 2.44 (each 1H, ddd, $J = 15.5$, 7.1, 7.1 Hz, 5- CH_2), 4.19 (1H, br s, 2-CH), 5.10 (1H, d, $J = 8.2$ Hz, NH, exchangeable); ^{13}C -NMR δ : 20.3 (4- CH_2), 27.9 (*t*-Bu-Me), 28.3 (*t*-Bu-Me), 32.1 (2- or 3- CH_2), 33.3 (2- or 3- CH_2), 53.6 (5-CH), 79.8 (*t*-Bu-C), 82.1 (*t*-Bu-C), 155.4 (CONH), 171.7 (COOR), 178.4 (COOH); FABMS m/z : 318 (MH^+); $[\alpha]_{\text{D}}^{25} +6.5^\circ$ ($c = 1.0$, CHCl_3).

(S)-tert-Butyl 2-N-(tert-Butoxycarbonyl)amino-6-hydroxyhexanoate (10). A mixture of acid (**9**) (0.237 g, 0.75 mmol), chloro ethyl carbonate (0.11 mL, 1.14 mmol), and TEA (0.31 mL, 2.22 mmol) in THF (3.7 mL) was stirred at -15 °C for 70 min under argon. To the mixture was added a solution of sodium borohydride (129 mg, 3.07 mmol) in 80% THF aq (3.7 mL) and the whole was stirred at rt for 2 h. After evaporation of the solvent residue was diluted with H₂O (3 mL) and adjusted to be pH ca. 2 by addition of 5% HCl aq and repeatedly extracted with EtOAc (total 80 mL). The organic solution was washed with 10% NaOH aq (8 mL) and brine (8 mL), dried (MgSO₄), and evaporated. The residue was purified by column chromatography [SiO₂ 60 (neutral), n-hexane : EtOAc = 3 : 2] to give a colorless oil (0.183 g, 81%); IR (ATR) $\nu_{\max}$ 3367, 1700 cm⁻¹; ¹H-NMR δ : 1.44 and 1.46 (each 9H, s, *t*-Bu), 1.44-1.46 (2H, m, 4-CH₂), 1.53-1.81 (4H, m, 3- and 5-CH₂), 2.64 (1H, br s, OH, exchangeable), 3.62 (2H, t, *J* = 6.2 Hz, 6-CH₂), 4.16 (1H, dd, *J* = 13.0, 8.1 Hz, 2-CH), 5.21 (1H, d, *J* = 8.1 Hz, NH); ¹³C-NMR δ : 21.3 (4-CH₂), 27.8 (*t*-Bu-Me), 28.2 (*t*-Bu-Me), 32.0 (2- or 3-CH₂), 32.5 (2- or 3-CH₂), 53.7 (5-CH), 62.0 (6-C), 79.5 (*t*-Bu-C), 81.6 (*t*-Bu-C), 155.4 (CONH), 172.0 (COOR); FABMS *m/z*: 304 (MH⁺); [α]_D²⁵ +6.7° (*c* = 1.0, CHCl₃).

(S)-tert-Butyl 2-tert-Butoxycarbonylamino-6-methanesulfonyloxyhexanoate (11). A mixture of alcohol (**10**) (0.084 g, 0.28 mmol), methanesulfonyl chloride (0.06 mL, 0.78 mmol), and pyridine (0.07 mL, 0.87 mmol) in DCM (0.6 mL) was stirred at rt for 2 h under argon. After evaporation of the solvent residue was extracted with EtOAc (20 mL x 2). The organic solution was washed with sat CuSO₄ aq (7 mL), sat NaHCO₃ aq (7 mL), and brine (7 mL), dried (K₂CO₃), and evaporated. The residue was purified by column chromatography (SiO₂ 60, benzene : EtOAc = 2 : 1) to give colorless oil (0.111 g, quant.); IR (neat) $\nu_{\max}$ 3394, 1704, 1639 cm⁻¹; ¹H-NMR δ : 1.45 and 1.47 (each 9H, s, *t*-Bu), 1.62-1.69 (2H, m, 4-CH₂), 1.73-1.85 (4H, m, 3- and 5-CH₂), 3.01 (3H, s, SCH₃), 4.18 (1H, dd, *J* = 13.2, 7.1 Hz, 2-CH), 4.23 (2H, t, *J* = 6.4 Hz, 6-CH₂), 5.05 (1H, d, *J* = 8.2 Hz, NH); ¹³C-NMR δ : 21.2 (4-CH₂), 28.0 (*t*-Bu-Me), 28.3 (*t*-Bu-Me), 28.6 (2- or 3-CH₂), 32.4 (2- or 3-CH₂), 37.3 (SCH₃), 53.5 (5-CH₂), 69.6 (6-CH), 79.7 (*t*-Bu-C), 82.0 (*t*-Bu-C), 155.4 (CONH), 171.7 (COOR); HRFABMS *m/z*: 382.1872, calcd for C₁₆H₃₂NO₇S: 382.1899; [α]_D²⁵ +7.9° (*c* = 1.1, CHCl₃).

N-Boc-MP-lysine tert-Butyl Ester Methanesulfate (12). A mixture of methanesulfonate (**11**) (0.029 g, 0.075 mmol) and 3-methylpyridine (0.08 mL, 0.71 mmol) in MeCN (0.55 mL) was stirred at 50 °C for 27 h under argon. After evaporation of the solvent, residue was purified by column chromatography (SiO₂ 60, benzene : EtOAc = 2 : 1 followed by CHCl₃ : MeOH = 2 : 1) to give a colorless oil (0.023 g, 63%); IR (neat) $\nu_{\max}$ 3436, 1708 cm⁻¹; ¹H-NMR δ : 1.43 and 1.44 (each 9H, s, *t*-Bu), 1.63-1.72 and 1.77-1.85 (each 1H, m, 4-CH₂), 2.03-2.12 (2H, m, 3- or 5-CH₂), 2.63 (3H, s, ArCH₃), 2.76 (3H, s, SCH₃), 4.18 (1H, m,

2-CH), 4.23 (2H, t, $J = 6.4$ Hz, 6-CH₂), 5.44 (1H, d, $J = 8.0$ Hz, NH, exchangeable), 7.97 (1H, br t, $J = ca.$ 7.1 Hz, ArH), 8.20 (1H, d, $J = 7.9$ Hz, ArH), 9.00-9.03 (2H, br, ArH); ¹³C-NMR δ : 18.6 (ArCH₃), 22.0 (4-CH₂), 28.0 (*t*-Bu-Me), 28.3 (*t*-Bu-Me), 30.9 (3- or 5-CH₂), 32.0 (3- or 5-CH₂), 39.5 (SCH₃), 53.4 (2-CH₂), 69.6 (6-CH), 79.7 (*t*-Bu-C), 82.1 (*t*-Bu-C), 127.7 (Ar), 139.8 (Ar), 142.4 (Ar), 144.7 (Ar), 145.3 (Ar), 155.7 (CONH), 171.7 (COOR); HRFABMS m/z : 379.2597, calcd for C₂₁H₃₅N₂O₄: 379.2597; $[\alpha]_D^{23} +5.7^\circ$ ($c = 1.1$, CHCl₃).

Deacetyl MP-Lysine Methanesulfate Trifluoroacetate (13). A mixture of the pyridinium ester (12) (0.041 g, 0.11 mmol) and TFA (0.9 mL, 11.7 mmol) was stirred at 0 °C for 6.5 h under argon and evaporated to give a yellow oil (0.048 g, quant.); IR (ATR) $\nu_{\max}$ 3409, 1739 cm⁻¹; ¹H-NMR δ : 1.35-1.57 (2H, m, 4-CH₂), 1.87-1.99 (2H, m, 3-CH₂), 2.00-2.08 (2H, dt, $J = 15.0, 7.5$ Hz, 5-CH₂), 2.50 (3H, s, ArCH₃), 2.77 (3H, s, SCH₃), 3.96 (1H, t, $J = 6.2$ Hz, 2-CH), 4.55 (2H, t, $J = 7.3$ Hz, 6-CH₂), 7.99 (1H, t like, $J = 8.0, 6.4$ Hz, ArH), 8.33 (1H, d, $J = 8.0$ Hz, ArH), 8.61 (1H, d, $J = 6.0$ Hz, ArH), 8.66 (1H, s, ArH); ¹³C-NMR δ : 20.3 (ArCH₃), 23.8 (4-CH₂), 31.8 (3-CH₂), 32.6 (5-CH₂), 41.2 (SCH₃), 55.3 (2-CH₂), 63.7 (6-CH), 130.2 (Ar), 142.8 (Ar), 144.0 (Ar), 146.4 (Ar), 148.9 (Ar), 165.5 (CF₃CO₂⁻), 171.7 (COOH); HRFABMS m/z : 223.1442, calcd for C₁₂H₁₉N₂O₂: 223.1447; $[\alpha]_D^{21} +71.7^\circ$ ($c = 1.1$, H₂O).

MP-Lysine (6). A mixture of deacetyl MP-lysine (13) (0.012 g, 0.025 mmol), Ac₂O (0.009 mL, 0.095 mmol), and 1% NaOH aq (0.3 mL, 0.1 mmol) was stirred at 25 °C for 20 min under sonication and co-evaporated with toluene. The residue was purified by column chromatography (SiO₂ 60, MeOH) to give a light brown oil (0.005 g, 74%); ¹H-NMR⁷ (D₂O) δ : 1.23-1.38 (2H, m, 4-CH₂), 1.59-1.69 and 1.73-1.82 (each 1H, m, 3-CH₂), 1.91-1.99 (2H, m, 5-CH₂), 1.94 (3H, s, Ac), 2.49 (3H, s, ArCH₃), 4.07 (1H, dd, $J = 8.6, 4.8$ Hz, 2-CH), 4.51 (2H, m, 6-CH₂), 7.87 (1H, br t, $J = ca.$ 6.0 Hz, ArH), 8.31 (1H, d, $J = 7.7$ Hz, ArH), 8.59 (1H, br s, ArH), 8.64 (1H, s, ArH); ¹³C-NMR δ : 20.4 (ArCH₃), 24.5 (4-CH₂), 24.6 (Ac), 32.7 (5-CH₂), 33.6 (3-CH₂), 57.5 (2-CH₂), 64.1 (6-CH), 130.2 (Ar), 142.7 (Ar), 144.1 (Ar), 146.5 (Ar), 148.8 (Ar), 176.2 (CONH), 181.7 (CO₂⁻); HRFABMS m/z : 265.1554, calcd for C₁₄H₂₁N₂O₃: 265.1552; $[\alpha]_D^{23} +12.6^\circ$ ($c = 1.0$, MeOH).

REFERENCES AND NOTES

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2. The reported nomenclature of compound (6) as *N*^α-acetyl-*N*^ε-3-methylpyridinium lysine is improper and should be revised to be (S)-2-(N-acetylamino)-6-[1-(3-methylpyridinium)]hexanoate. However,

in this paper we use the reported name for convenience of readers.

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6. F. Yokokawa, H. Sugiyama, T. Shioiri, N. Katagiri, O. Oda, and H. Ogawa, *Tetrahedron*, 2001, **57**, 4759.
7. In literature 1 the ^1H -NMR data of MP-lysine (**6**) were wrongly given as follows: ^1H -NMR (D_2O) δ : 1.34 (2H, m), 1.61 (2H, m), 1.79 (1H, m), 1.95 (1H, m), 2.10 (3H, s), 2.90 (2H, t, $J = 8.00$ Hz), 4.23 (1H, m), 7.28 (1H, t, $J = 7.04$ Hz), 8.26 (1H, d, $J = 8.08$ Hz), 8.52 (1H, d, $J = 6.02$ Hz), 8.57 (1H, s).