HETEROCYCLES, Vol. 67, No. 1, 2006, pp. 361 - 367. © The Japan Institute of Heterocyclic Chemistry
Received, 28th July, 2005, Accepted, 24th August, 2005, Published online, 26th August, 2005. COM-05-S(T)50

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

Kohsaku Matsumoto,[1] Takuya Kumamoto,[1] Tsutomu Ishikawa,[1,*] Erika Ishii,[2] Hideyuki Tomitori,[2] and Kazuei Igarashi[2]

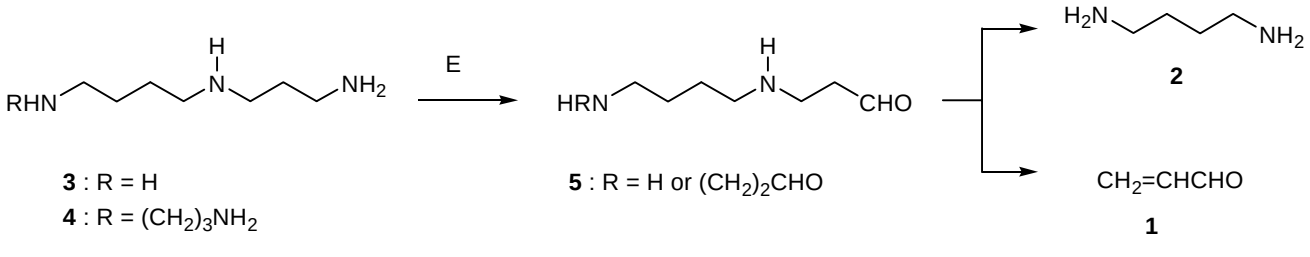
[1]Department of medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan
[2]Department of Clinical Biochemistry, Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan
E-mail address: benti@p.chiba-u.ac.jp

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.

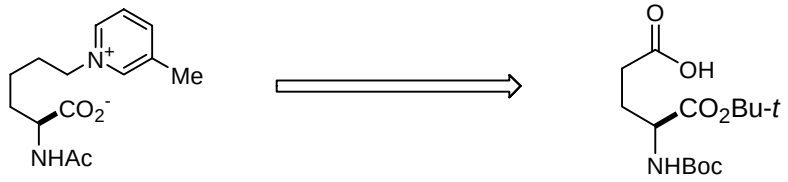
INTRODUCTION

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 puterscine (**2**) from spermidine (**3**) and spermine (**4**)

In 2003, Furuhata *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).



6 **7**

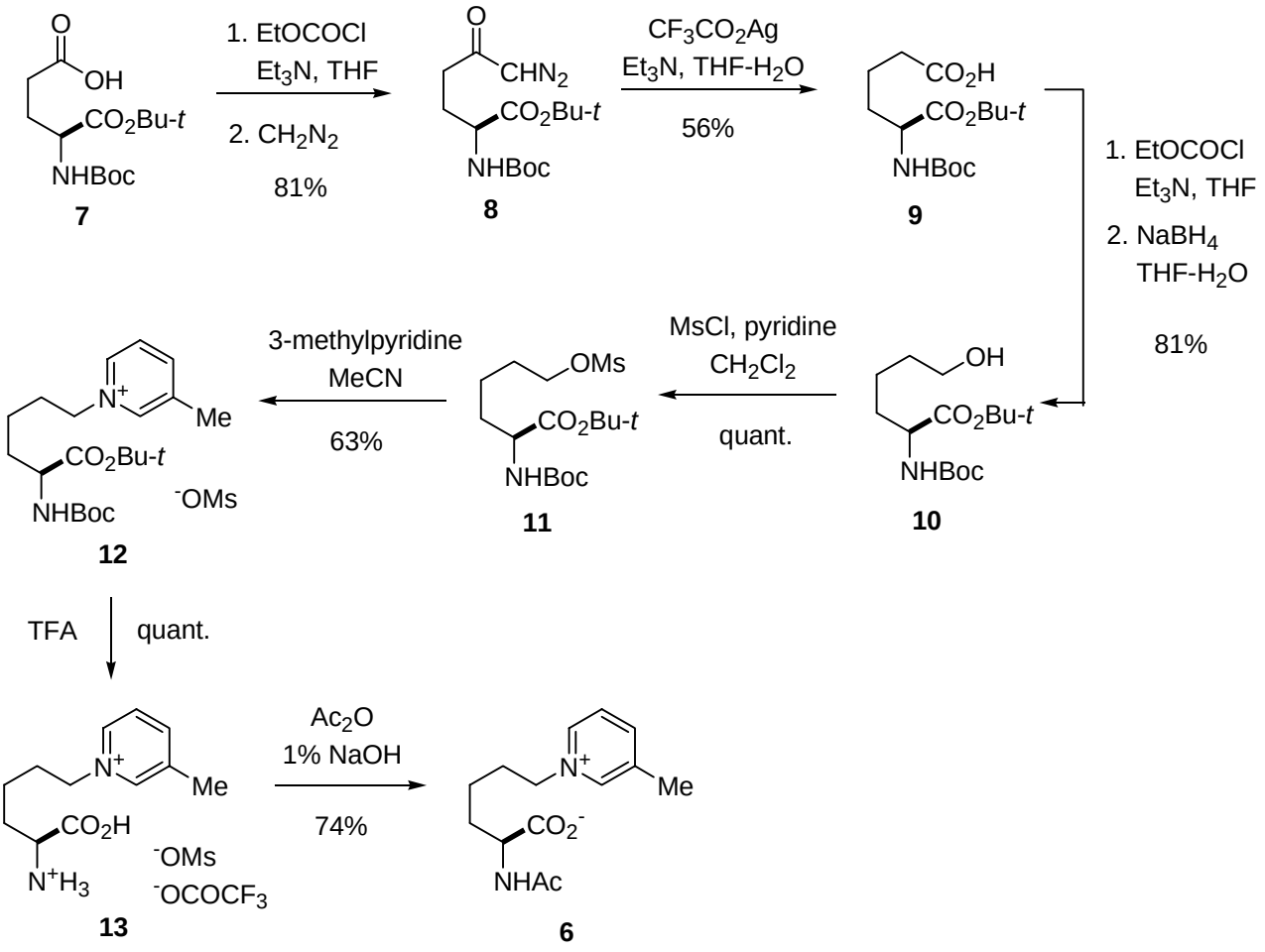
Chart 1. Retrosynthesis of MP-lysine (**6**) from Boc-Glu-OBu-*t* (**7**)

RESULT AND DISCCUSSION

Treatment of Boc-Glu-OBu-*t* (**7**) with diazomethane after conversion to a mixed anhydride by reaction with ethoxycarbonyl chloride (EtO_2CCl) 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 method[3] the acid (**9**) was reduced with sodium borohydride ($NaBH_4$) in 10% aqueous THF at room temperature for 2 h after conversion to a mixed anhydride under the same conditions (EtO_2CCl/Et_3N/THF) used in the reaction from **7** to **8** afford the corresponding alcohol (**10**)[6] in 81% yield even with recovery of a small of 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 ℃ 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 ℃, 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-^{t}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. ^{1}H-

and ^{13}C-NMR spectra were recorded with JEOL JNM ECP 400 and GSX-500α 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 ºC for 50 min under argon. To the mixture was added an etheral 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 ºC 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}; ^{1}H- 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^+); $[\alpha]_D^{24}$ +17.2º (*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 ºC (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 74-76 ºC after trituration with hexane; IR (ATR) ν_{max} 3244, 1709 cm^{-1}; ^{1}H-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^+); $[\alpha]_D^{25}$ +6.5º (*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 ℃ for 70 min under argon. To the mixture was added a solution of sodium borohydride (129 mg, 3.07 mmol) in 80% THF aq (3.7mL) and the whole was stirred at rt for 2 h. After evaporation of the solvent residue was diluted with H_2O (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 ($MgSO_4$), and evaporated. The residue was purified by column chromatography [SiO_2 60 (neutral), n-hexane : EtOAc = 3 : 2] to give a colorless oil (0.183 g, 81%); IR (ATR) ν_{max} 3367, 1700 cm^{-1}; ^{1}H-NMR δ: 1.44 and 1.46 (each 9H, s, *t*–Bu), 1.44-1,46 (2H, m, 4-CH_2), 1.53-1.81 (4H, m, 3- and 5-CH_2), 2.64 (1H, br s, OH, exchangeable), 3.62 (2H, t, *J* = 6.2 Hz, 6-CH_2), 4.16 (1H, dd, *J* = 13.0, 8.1 Hz, 2-CH), 5.21 (1H, d, *J* = 8.1 Hz, NH); ^{13}C-NMR δ: 21.3 (4-CH_2), 27.8 (*t*–Bu-Me), 28.2 (*t*–Bu-Me), 32.0 (2- or 3-CH_2), 32.5 (2- or 3-CH_2), 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^+); $[\alpha]_D^{25}$ +6.7º (*c* = 1.0, $CHCl_3$).

(*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 $CuSO_4$ aq (7 mL), sat $NaHCO_3$ aq (7 mL), and brine (7 mL), dried (K_2CO_3), and evaporated. The residue was purified by column chromatography (SiO_2 60, benzene : EtOAc = 2 : 1) to give colorless oil (0.111 g, quant.); IR (neat) ν_{max} 3394, 1704, 1639 cm^{-1}; ^{1}H-NMR δ: 1.45 and 1.47 (each 9H, s, *t*–Bu), 1.62-1.69 (2H, m, 4-CH_2), 1.73-1.85 (4H, m, 3- and 5-CH_2), 3.01 (3H, s, SCH_3), 4.18 (1H, dd, *J* = 13.2, 7.1 Hz, 2-CH), 4.23 (2H, t, *J* = 6.4 Hz, 6-CH_2), 5.05 (1H, d, *J* = 8.2 Hz, NH); ^{13}C-NMR δ: 21.2 (4-CH_2), 28.0 (*t*–Bu-Me), 28.3 (*t*–Bu-Me), 28.6 (2- or 3-CH_2), 32.4 (2- or 3-CH_2), 37.3 (SCH_3), 53.5 (5-CH_2), 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 $C_{16}H_{32}NO_7S$: 382.1899; $[\alpha]_D^{25}$ +7.9º (*c* = 1.1, $CHCl_3$).

***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 ℃ for 27 h under argon. After evaporation of the solvent, residue was purified by column chromatography (SiO_2 60, benzene : EtOAc = 2 : 1 followed by $CHCl_3$: MeOH = 2 : 1) to give a colorless oil (0.023 g, 63%); IR (neat) ν_{max} 3436, 1708 cm^{-1}; ^{1}H-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-CH_2), 2.03-2.12 (2H, m, 3- or 5-CH_2), 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, SCH_3), 4.18 (1H, m,

2-CH), 4.23 (2H, t, *J* = 6.4 Hz, 6-CH_2), 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); ^{13}C-NMR δ: 18.6 ($ArCH_3$), 22.0 (4-CH_2), 28.0 (*t*–Bu-Me), 28.3 (*t*–Bu-Me), 30.9 (3- or 5-CH_2), 32.0 (3- or 5-CH_2), 39.5 (SCH_3), 53.4 (2-CH_2), 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 $C_{21}H_{35}N_2O_4$: 379.2597; $[\alpha]_D^{23}$ +5.7º (*c* = 1.1, $CHCl_3$).

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 ºC for 6.5 h under argon and evaporated to give a yellow oil (0.048 g, quant.); IR (ATR) ν_{max} 3409, 1739 cm^{-1}; ^{1}H-NMR δ: 1.35-1.57 (2H, m, 4-CH_2), 1.87-1.99 (2H, m, 3-CH_2), 2.00-2.08 (2H, dt, *J* = 15.0, 7.5 Hz, 5-CH_2), 2.50 (3H, s, $ArCH_3$), 2.77 (3H, s, SCH_3), 3.96 (1H, t, *J* = 6.2 Hz, 2-CH), 4.55 (2H, t, *J* = 7.3 Hz, 6-CH_2), 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); ^{13}C-NMR δ: 20.3 ($ArCH_3$), 23.8 (4-CH_2), 31.8 (3-CH_2), 32.6 (5-CH_2), 41.2 (SCH_3), 55.3 (2-CH_2), 63.7 (6-CH), 130.2 (Ar), 142.8 (Ar), 144.0 (Ar), 146.4 (Ar), 148.9 (Ar), 165.5 ($CF_3CO_2^-$), 171.7 (COOH); HRFABMS *m/z*: 223.1442, calcd for $C_{12}H_{19}N_2O_2$: 223.1447; $[\alpha]_D^{21}$ +71.7º (*c* = 1.1, H_2O).

MP-Lysine (6). A mixture of deacetyl MP-lysine (**13**) (0.012 g, 0.025 mmol), Ac_2O (0.009 mL, 0.095 mmol), and 1% NaOH aq (0.3 mL, 0.1 mmol) was stirred at 25 ºC for 20 min under sonication and co-evaporated with toluene. The residue was purified by column chromatography (SiO_2 60, MeOH) to give a light brown oil (0.005 g, 74%); ^{1}H-NMR[7] (D_2O) δ: 1.23-1.38 (2H, m, 4-CH_2), 1.59-1.69 and 1.73-1.82 (each 1H, m, 3-CH_2), 1.91-1.99 (2H, m, 5-CH_2), 1.94 (3H, s, Ac), 2.49 (3H, s, $ArCH_3$), 4.07 (1H, dd, *J* = 8.6, 4.8 Hz, 2-CH), 4.51 (2H, m, 6-CH_2), 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); ^{13}C-NMR δ: 20.4 ($ArCH_3$), 24.5 (4-CH_2), 24.6 (Ac), 32.7 (5-CH_2), 33.6 (3-CH_2), 57.5 (2-CH_2), 64.1 (6-CH), 130.2 (Ar), 142.7 (Ar), 144.1 (Ar), 146.5 (Ar), 148.8 (Ar), 176.2 (CONH), 181.7 (CO_2^-); HRFABMS *m/z*: 265.1554, calcd for $C_{14}H_{21}N_2O_3$: 265.1552; $[\alpha]_D^{23}$ +12.6º (*c* = 1.0, MeOH).

REFERENCES AND NOTES

1. A. Furuhata, T. Ishii, S. Kumazawa. T. Yamada, T. Nakayama, and K. Uchida, *J. Biol. Chem*., 2003, **278**, 48658.
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.

3. H. Kabasawa, M. Yoshida, and T. Ishikawa, *Arkivoc*, 2003, **viii**, 180.
4. L. Anastasia and M. Arastasia, *J. Chem. Soc., Perkin Trans. 1*, 2001, 2404.
5. N. Sakura, K. Hirose, and T. Hashimoto, *Chem. Pharm. Bull*., 1986, **34**, 3506.
6. F. Yokokawa, H. Sugiyama, T. Shioiri, N. Katagiri, O. Oda, and H. Ogawa, *Tetrahedron*, 2001, **57**, 4759.
7. In literature 1 the ^{1}H-NMR data of MP-lysine (**6**) were wrongly given as follows: ^{1}H-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).