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An Efficient Total Synthesis of a New and Highly Active Analog of Lactacystin

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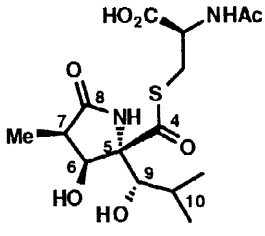
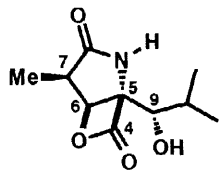
Abstract: *An expeditious total synthesis of the stable and potent proteasome inhibitor* ***3*** *is described.*

Lactacystin (**1**), a microbial product, first isolated by Ōmura *et al.*,[1,2] was originally obtained from a screening effort based on a nerve growth factor-like capacity to induce neurite sprouting in a mouse neuroblastoma cell line. It was later found to inhibit irreversibly the proteolytic activity of the 20 S proteasome,[3,4] a cylindrical complex of 28 protein subunits which is responsible for the hydrolytic fragmentation of ubiquitinated proteins. The thiol ester function of lactacystin is sufficiently reactive to allow spontaneous conversion to the β-lactone **2**[5] which similarly deactivates the 20 S proteasome, but at a much faster rate.[4,5] The cause of inactivation of the 20 S proteasome appears to be the acylation of the *N*-terminal threonine subunit, a key participant in proteolytic catalysis,[3,4] to form inactive proteasome, a result confirmed by X-ray crystallographic studies of the lactacystin inactivated 20 S proteasome at 2.4 Å resolution.[6,7] Because the proteasome machinery is involved in the degradation of many proteins, including misfolded and denatured molecules,[8] and proteins involved in cell cycle progression[9] and regulation of gene transcription,[10] lactacystin has become a valuable tool for the study of protein biochemistry and cell biology.[11] Lactacystin prepared by the route of the first total synthesis[5,12,13] has been used in a large number of biological laboratories. The structural and biological uniqueness of **1** has stimulated the development of a number of other syntheses.[14-16]

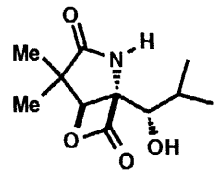
Lactacystin exemplifies dramatically the ability of a small molecule (molecular weight 376) to shut down the functioning of a very large poly-macromolecular machine and to exert this inhibition with great selectivity on the 20 S proteasome in the presence of countless other proteins as potential targets. Most of the structural features of **1** are critical to its activity. First, the C(4) carboxylic function and the hydroxyl at C(6) must be *cis*, as expected for the essentiality of β-lactone (**2**) function for proteasome inactivation.[3,4a] The configuration of the hydroxyl at C(9) and the presence of the isopropyl substituent at C(9) are also very important for activity.[17] For example, when the C(5) substituent is CH_2OH the rate of inactivation of the 20 S proteasome is reduced at least 300 fold.[17] Removal of the methyl substituent at C(7) strongly reduces bioactivity relative to lactacystin,[17] and also leads to chemical instability resulting from facile elimination of the hydroxyl at C(6) to form an α,β-



Lactacystin (**1**)



2



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unsaturated γ-lactam. On the other hand, replacement of the methyl substituent at C(7) by ethyl, *n*-propyl, or *iso*-propyl led to a 2 to 3 fold *increase* in activity.[17] Because an alkyl substituent larger than methyl at C(7) does not cause loss of bioactivity, we decided to develop a synthesis of the 7,7-dimethyl analog (**3**) of β-lactone **2** and to test its ability to inhibit the 20 S proteasome. This paper describes the interesting results of this study.

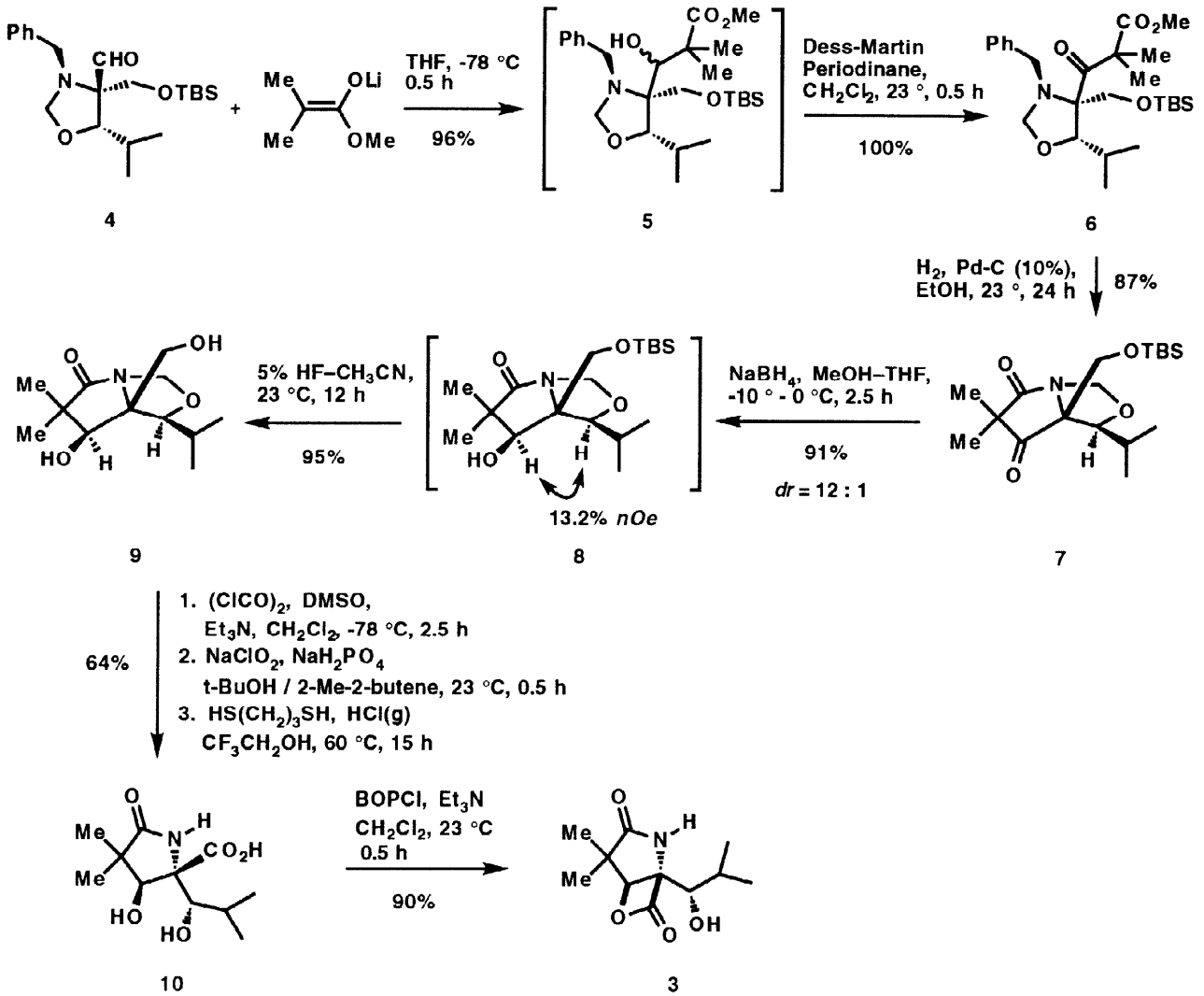
The synthesis of **3**, which is outlined in Scheme 1, commenced with the readily available amino aldehyde **4**.[12,13] Addition of aldehyde **4** to the lithium enolate of methyl isobutyrate at -78 °C led to a rapid and clean aldol reaction to produce a mixture of two diastereomeric β-hydroxy esters **5** (ratio 1.2 : 1) which was directly oxidized by the Dess-Martin periodinane reagent to give the β-keto ester **6** in 96% overall yield from **4**. Hydrogenation of **6** in ethanol with Pd–C and H_2 at 1 atm effected debenzylation and concurrent γ-lactam closure to provide the keto lactam **7** (87% yield).[18] Reduction of **7** with $NaBH_4$ at -10 °C in MeOH–THF proceeded position selectively and with 12 : 1 diastereoselectivity to form the required β-hydroxy γ-lactam **8**, the stereochemistry of which was established by ^1H NMR studies (see positive NOE shown in Scheme 1). The crude γ-lactam **8** was directly desilylated to afford after silica gel column chromatography the diol **9** in 86% overall yield from **7**. The primary alcohol subunit of **9** was oxidized via the corresponding aldehyde to the carboxylic acid, and the methylene protecting group was removed to afford the pure γ-lactam dihydroxy acid **10** in 64% overall yield for all three steps.[19] Treatment of the solid dihydroxy acid **10** [mp 270 °C (dec.)] with bis(2-oxo-3-oxazolidinyl) phosphonic dichloride (BOPCl, Aldrich Co.) and Et_3N in CH_2Cl_2 at 23 °C resulted in rapid and selective formation of the β-lactone (**3**) (mp 184-186 °C)[20] which was isolated by column chromatography on silica gel in 90% yield.

The kinetics of inhibition of chymotrypsin-like peptidase activity of purified 20 S proteasome from bovine brain were measured as previously described[3] for the β-lactone **2** corresponding to lactacystin and for the β-lactone analog **3**. The values of k_{assoc} ($M^{-1}s^{-1}$) for inactivation were 3060 for β-lactone **2** and 2300 for β-lactone **3**. Thus the new analog **3** is nearly (*ca.* 3/4) as active as the lactacystin derived **2**. This is a noteworthy result since **3** possesses advantages over **2** as a reagent for research use. Specifically, it can be made in better yield and more readily than **2** and it is also more stable, especially against β-elimination.

The following experimental sections provide the details for the aldol reaction/oxidation sequence to form the β-keto ester **6** and the borohydride reduction/desilylation sequence which converts **7** to **9**.

Preparation of β-Keto Ester 6. To a stirred solution of LDA (20 mmol) in THF (80 mL) at -78 °C was added dropwise freshly distilled methyl isobutyrate (2.30 mL, 20 mmol) over 20 min. The resulting mixture was stirred for 1.5 h at -78 °C. A solution of aldehyde **4**[12,13] (7.40 g, 19.6 mmol) in THF (20 mL) was added dropwise to the lithium enolate solution over 15 min at -78 °C. After stirring for 0.5 h at that temperature, the reaction mixture was treated with of sat. aq. NH_4Cl at -78 °C and subjected to extractive workup with ether to give the crude aldol product **5** (9.10 g, 96%, 1 : 1.2 mixture of diastereomers by ^1H-NMR analysis) as a colorless oil. Without further purification, the aldol product was taken up in CH_2Cl_2 (100 mL) and treated with the Dess-Martin periodinane reagent (10.0 g, 23.7 mmol, 1.25 eq) portionwise at 23 °C. After stirring for 0.5 h, the reaction mixture was cooled to 0 °C and treated with a mixture of 20% *w/w* aq. $Na_2S_2O_3$–sat. aq. $NaHCO_3$ (1 : 1, 50 mL). The resulting mixture was stirred vigorously for 0.5 h at 23 °C and extracted with ether. The resulting crude product was purified by flash chromatography on silica gel to afford β-keto ester **6** (8.99g, 96%, 2 steps) as a colorless oil. $[\alpha]_D^{23}$ +60.2° (c = 0.39, EtOAc); FTIR (film) ν_{max}: 2954, 1720, 1709, 1464, 1256, 1092, 840 cm^{-1}; ^1H-NMR (500 MHz, $CDCl_3$) δ 0.06 & 0.08 (each s, 3 H, CH_3Si), 0.89 (s, 9 H, *t*-BuSi), 1.01 & 1.06 (each d, 3 H, *J* = 6.8 Hz, $(CH_3)_2CH$), 1.42 & 1.50 (each s, 3 H, CH_3), 2.08 (sept, 1 H, *J* = 6.8 Hz, $CH(CH_3)_2$), 3.60 (s, 3 H, CH_3O), 3.74 (d, 1 H, *J* = 6.8 Hz, CH-O), 3.94 (d, 1 H, *J* = 13.7 Hz, CH_2Ph), 4.09 (d, 1 H, *J* = 10.6 Hz, CH_2OSi), 4.18 & 4.31 (each d, 1 H, *J* = 4.8 Hz, NCH_2O), 4.28 (d, 1 H, *J* = 10.6 Hz, CH_2OSi), 4.65 (d, 1 H, *J* = 13.7 Hz, CH_2Ph), 7.23 - 7.42 (m, 5 H, ArH) *ppm*; ^{13}C-NMR (125 MHz, $CDCl_3$) δ -5.8; -5.5; 18.3; 20.2; 21.5; 22.1; 25.3; 25.9; 28.6; 51.8; 54.1; 54.3; 63.4; 79.5; 85.2; 89.3; 126.9; 128.3;

Scheme 1



128.4; 139.7; 173.8; 212.0 *ppm*; HRMS (FAB, 3-NBA + NaI) *m/z* calcd for $C_{26}H_{44}NO_5Si$, 478.2989; found for $[M+H]^+$, 478.2991.

Preparation of β-Hydroxy γ-Lactam 9. To a stirred solution of β-keto γ-lactam **7** (4.60 g, 12.96 mmol) in THF (100 mL) at -10 °C was added portionwise sodium borohydride (1.22 g, 32.4 mmol, 2.5 eq) over 15 min. The resulting slurry mixture was treated dropwise with methanol (4.0 mL) over 30 min. After stirring for 2.5 h at -10 ° ~ 0 °C, the reaction mixture was treated with water and extracted with ether to give the crude alcohol (4.60 g, 100%, α-OH / β-OH = 1 : 12 by ^{1}H-NMR analysis) as a colorless oil. A pure sample of the major isomer was obtained by flash chromatography on silica gel. $[\alpha]_D^{23}$ -2.0° (c = 0.25, EtOAc); FTIR (film) ν_{max}: 3409, 2936, 2954, 1684, 1104 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.08 & 0.09 (each s, 3 H, CH_3Si), 0.89 (s, 9 H, *t*-BuSi), 1.01 & 1.02 (each d, 3 H, *J* = 6.8 Hz, $(CH_3)_2CH$), 1.22 (br s, 6 H, 7-*gem*-dimethyl), 1.66 (m, 1 H, $CH(CH_3)_2$), 2.86 (d, 1 H, *J* = 10.4 Hz, CH-O), 3.48 (d, 1 H, *J* = 11.5 Hz, CH_2OSi), 3.72 (d, 1 H, *J* = 10.9 Hz, CHOH), 3.93 (d, 1 H, *J* = 11.5 Hz, CH_2OSi), 4.00 (d, 1 H, *J* = 10.9 Hz, HOCH), 4.58 & 5.16 (each d, 1 H, *J* = 4.8 Hz, NCH_2O) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ -5.6; -5.3; 18.3; 18.5; 20.2; 20.7; 25.9; 27.9; 49.3; 60.3; 71.0; 75.6; 80.4; 91.2; 181.3 *ppm*; HRMS (FAB, 3-NBA + NaI) *m/z* calcd for $C_{18}H_{35}NO_4SiNa$, 380.2233; found for $[M+Na]^+$, 380.2225.

Without further purification, the above alcohol was taken up in CH_3CN (40 mL) and treated with 48% HF (2.1 mL). After stirring for 12 h at 23 °C, the reaction mixture was directly placed on a pad of silica gel packed with ethyl acetate. The pad was eluted with 10% MeOH / EtOAc (200 mL) and the eluent was concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography on silica gel to give the desired γ-lactam **9** (2.72 g, 85%, 2 steps) as a colorless oil. $[\alpha]_D^{23}$ +11.7° (c = 0.31, EtOAc); FTIR (film) ν_{max}: 3386, 2966,

1683, 1406 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.01 (t, 6 H, *J* = 6.7 Hz, $(CH_3)_2CH$), 1.20 & 1.22 (each s, 3 H, CH_3), 1.71 (m, 1 H, $CH(CH_3)_2$), 2.89 (d, 1 H, *J* = 10.4 Hz, CH-O), 3.47 (br s, 1 H, OH), 3.72 (dd, 1 H, *J* = 4.3; 11.9 Hz, CH_2OH), 3.77 (d, 1 H, *J* = 10.2 Hz, CHOH), 3.96 (dd, 1 H, *J* = 7.0; 11.9 Hz, CH_2OH), 3.97 (d, 1H, *J* = 10.2 Hz, CHOH), 4.62 & 5.10 (each d, 1 H, *J* = 4.9 Hz, OCH_2N) *ppm*; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 18.2; 20.3; 20.6; 25.8; 27.8; 49.6; 58.9; 71.3; 75.4; 80.2; 91.3; 181.6 *ppm*; HRMS (FAB, 3-NBA + NaI) *m/z* calcd for $C_{12}H_{21}NO_4Na$, 266.1368; found for $[M+Na]^+$, 266.1376.

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References and Notes

1. Ōmura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113-116.
2. Ōmura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117-118.
3. Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726-731.
4. (a) Fenteany, G.; Standaert, R. F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3358-3362. (b) See also Dick, L. R.; Cruikshank, A. A.; Grenier, L.; Melandri, F. D.; Nunes, S. L.; Stein, R. L. *J. Biol. Chem.* **1996**, *271*, 7273-7276.
5. Corey, E. J.; Reichard, G. A.; Kania, R. *Tetrahedron Lett.* **1993**, *34*, 6977-6980.
6. Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463-471.
7. See also, Bogyo, M.; McMaster, J. S.; Gaczynska, M.; Tortorella, D.; Goldberg, A. L.; Ploegh, H. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6629-6634.
8. Rock, K. L.; Gramm, C.; Rothstein, L.; Clark, K.; Stein, R.; Dick, L.; Hwang, D.; Goldberg, A. L. *Cell* **1994**, *78*, 761-771.
9. King, R. W.; Deshaies, R. J.; Peters, J.-M.; Kirschner, M. W. *Science* **1996**, *274*, 1652-1659.
10. Pahl, H. L.; Baeuerle, P. A. *Curr. Opin. Cell Biol.* **1996**, *8*, 340-347.
11. Fenteany, G.; Schreiber, S. L. *J. Biol. Chem.* **1998**, *273*, 8545-8548.
12. Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677-10678.
13. Corey, E. J.; Li. W.; Reichard, G. A. *J. Am. Chem. Soc.* **1998**, *120*, 2330-2336.
14. (a) Sunazuka, T.; Nagamitsu, T.; Matsuzaki, K.; Tanaka, H.; Ōmura, S.; Smith, III, A. B. *J. Am. Chem. Soc.* **1993**, *115*, 5302. (b) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Ōmura, S.; Sprengeler, P. A.; Smith, III, A. B. *J. Am. Chem. Soc.* **1996**, *118*, 3584-3590.
15. Uno, H.; Baldwin, J. E.; Russell, A. T. *J. Am. Chem. Soc.* **1994**, *116*, 2139-2140.
16. (a) Chida, N.; Takeoka, J.; Tsutsumi, N.; Ogawa, S. *J. Chem. Soc., Chem. Commun.* **1995**, 793-794. (b) Chida, N.; Takeoka, J.; Ando, K.; Tsutsumi, N.; Ogawa, S. *Tetrahedron* **1997**, *53*, 16287-16298.
17. (a) Corey, E. J.; Li, W.; Nagamitsu, T. *Angew. Chem., Int. Ed. Eng.* **1998**, *37*, 1676-1679. (b) Corey, E. J.; Li, W.-D. Z.; Nagamitsu, T.; Fenteany, G. *Tetrahedron* in press (Barton memorial issue 1998).
18. Spectral data for γ-lactam **7**. $[\alpha]_D^{23}$ +41.0° (c = 0.41, EtOAc); FTIR (film) ν_{max}: 2958, 2935, 1717, 1113, 838 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.002 & 0.01 (each s, 3 H, CH_3Si), 0.83 (s, 9 H, *t*-BuSi), 0.98 (d, 6 H, *J* = 6.6 Hz, $(CH_3)_2CH$), 1.19 & 1.27 (each s, 3 H, CH_3), 1.68 (m, 1 H, $CH(CH_3)_2$), 3.03 (d, 1 H, *J* = 10.1 Hz, CH-O), 3.68 (d, 1 H, *J* = 10.4 Hz, CH_2OSi), 4.05 (d, 1 H, *J* = 10.4 Hz, CH_2OSi), 4.67 & 5.38 (d, 1 H, *J* = 4.8 Hz, NCH_2O) *ppm*; ^{13}C-NMR (125 MHz, $CDCl_3$) δ -5.6; -5.5; 18.4; 18.5; 20.9; 21.8; 25.9; 28.1; 49.8; 59.7; 75.5; 77.7; 84.6; 179.3; 210.4 *ppm*; HRMS (FAB, 3-NBA + NaI) *m/z* calcd for $C_{18}H_{33}NO_4SiNa$, 378.2077; found for $[M+Na]^+$, 378.2075.
19. Spectral data for dihydroxy acid **10**. $[\alpha]_D^{23}$ -9.1° (c = 0.11, MeOH); FTIR (film) ν_{max}: 3416, 1709, 1649 cm^{-1}; ^{1}H-NMR (300 MHz, Py-d_5) δ 1.31 & 1.37 (each d, 3 H, *J* = 6.8 Hz, $(CH_3)_2CH$), 1.57 & 1.70 (each s, 3 H, CH_3), 2.46 (sept, 1 H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.61 (d, 1 H, *J* = 5.5 Hz, CHCHOH), 5.40 (s, 1 H, CHOH), 8.44 (br s, COOH), 8.93 (s, 1 H, NH) *ppm*; ^{13}C-NMR (125 MHz, Py-d_5) δ 19.2; 20.5; 21.9; 25.5; 32.4; 44.2; 72.8; 76.8; 79.1; 176.6; 182.2 *ppm*; LRMS (FAB, 3-NBA + NaI) *m/z* $[M+H]^+$ 246 (100%).
20. Spectral data for β-lactone **3**. $[\alpha]_D^{23}$ -99° (c = 0.10, EtOAc); FTIR (film) ν_{max}: 3547, 3188, 1824, 1715, 1644 cm^{-1}; ^{1}H-NMR (500 MHz, Py-d_5) δ 1.21 & 1.22 (each d, 3 H, *J* = 6.6 Hz, $(CH_3)_2CH$), 1.40 & 1.42 (each s, 3 H, CH_3), 2.16 (sept, 1 H, *J* = 6.6 Hz, $CH(CH_3)_2$), 4.11 (t, 1 H, *J* = 7.2 Hz, CHOH), 5.20 (s, 1 H, CH-OCO), 7.64 (d, 1 H, *J* = 7.6 Hz, HOCH), 10.40 (s, 1 H, NH) *ppm*; ^{13}C-NMR (125 MHz, Py-d_5) δ 18.0; 19.1; 20.5; 23.9; 30.9; 42.8; 72.6; 80.2; 82.0; 171.8; 180.8 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $C_{11}H_{21}N_2O_4$, 245.1501; found for $[M+NH_4]^+$, 245.1503.