NEW CEPHALOSPORINS WITH 7-ACYL GROUPS DERIVED FROM β-KETOACIDS

I. 7-(β-KETOACYLAMINO)CEPHALOSPORINS

MITSUO NUMATA, MASAYOSHI YAMAOKA, ISAO MINAMIDA, MASAAKI KURITANI and YOSHIO IMASHIRO

Central Research Division, Takeda Chemical Industries, Ltd., Juso, Osaka 532, Japan

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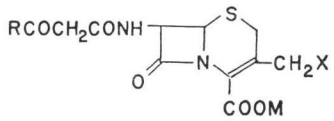
The synthesis and antimicrobial profile of a series of 7-(β-ketoacylamino)cephalosporins (**1**) bearing an acetoxymethyl or a heterocyclicthiomethyl group at the 3-position are described. Of this series, 3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-7-(3-oxobutyrylamino)ceph-3-em-4-carboxylic acid (**1 l**) showed moderate antibacterial activities in *in vitro* and *in vivo* tests.

Antimicrobial properties of cephalosporins seem to be largely dependent on the nature of acyl groups in the side chain at the 7-position.[1] In this connection, we have paid particular attention to the fact that most cephalosporins[2] with potential therapeutic value and currently in clinical use are characterized by the presence of active hydrogen(s) far more acidic than a normal hydrocarbonhydrogen, on the α-carbon of the acyl group. Thus, cephalothin, cephaloridine and cefoxitin involve the thienylacetyl moiety which possesses two hydrogens activated by the adjacent carbonyl and thienyl groups. Likewise, cefazolin, cephacetrile, cephapirin, cefazaflur and CS-1170 bear active α-hydrogens on the methylene between the carbonyl and the aromatic tetrazolyl, the nitrile or the sulfur residues in their acyl groups. Other cephalosporins like cephaloglycin, its analogues and cefamandole all fall into this category. Cefuroxime which lacks the α-hydrogen in the acyl side chain is an exception.

In view of the fact that β-ketoacids[3] are characterized by active hydrogen(s) between the carbonyl and the carboxyl and that no systematic investigations have yet been made to synthesize cephalosporins with β-ketoacyl side chains, we started the synthesis of cephalosporins of general formula **1** and evaluation of the antimicrobial activities of these compounds.

Fig. 1.



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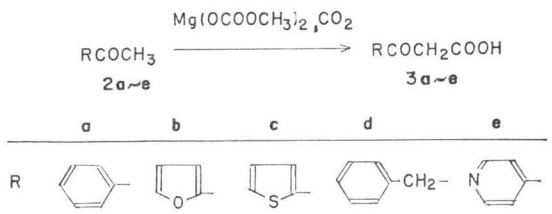
Chemistry

The β-ketoacids (**3**) used in this study were prepared by carboxylation of methyl ketones (**2**) with magnesium methyl carbonate[4] as shown in Scheme 1.

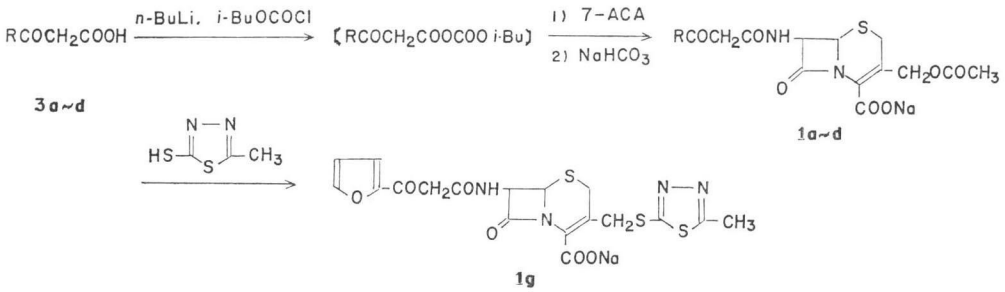
Acylation of 7-aminocephalosporanic acid (7-ACA) with **3** by conventional methods,[5] for example, the mixed anhydride method using isobutyl chlorocarbonate and triethylamine or the activated ester method using 2, 4-dinitrophenol and dicyclohexylcarbodiimide (DCC), failed owing to instant decarboxylation of **3**. Finally the desired 7-(β-ketoacylamino)cephalosporanic acids (**1a**～**d**) were obtained when *n*-butyllithium was used in place of triethylamine in the mixed anhydride method.

This effect of *n*-butyllithium is explicable on the basis of a complex formed with **3**, *i. e.* $RCOCH_2COOLi$, the formation of which would be reasonably explained by PEARSON's Hard and Soft Acids and Bases principle.[6] Thus, $RCOCH_2COO^-$, being a hard base, forms a stable complex with Li^+, which is a hard acid. However, the complex of $RCOCH_2COO^-$ with Et_3NH^+ or protonated DCC is not as stable, because these cations are less hard than Li^+.

Scheme 1.

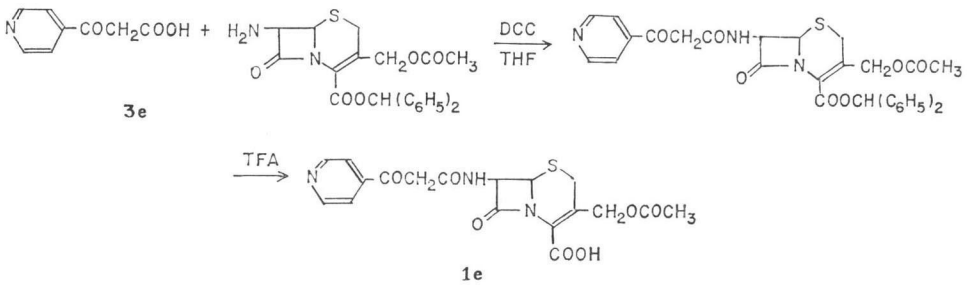


Scheme 2.



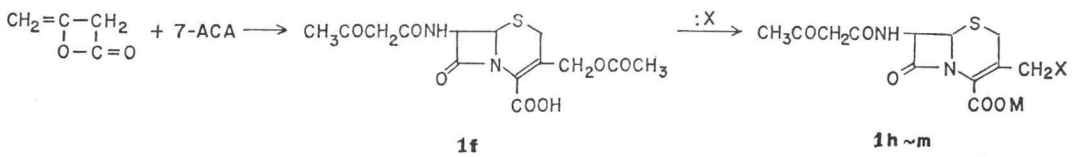
3-Oxo-3-(4-pyridyl)propionic acid (**3e**) was decarboxylated on treatment with *n*-butyllithium, presumably due to the pyridine ring of the side acting as an intramolecular catalyst for decarboxylation. 7-[3-Oxo-3-(4-pyridyl)propionylamino]cephalosporanic acid (**1e**) was, therefore, prepared by the coupling of **3e** to the benzhydryl ester of 7-ACA[7] using DCC followed by the hydrolysis of the ester (Scheme 3).

Scheme 3.



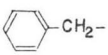
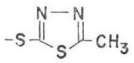
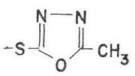
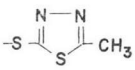
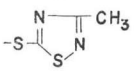
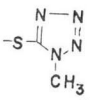
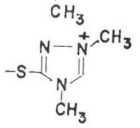
7-(3-Oxobutyrylamino)cephalosporanic acid (**1f**) was prepared by the acylation of 7-ACA with diketene (Scheme 4).

Scheme 4.



Compounds **1b** and **1f** with an acetoxymethyl side chain at the 3-position were subjected to further modification by the nucleophilic displacement[8] of the acetoxy group with five-membered heterocyclic thiols to afford **1g** (Scheme 2) and **1h**~**m** (Scheme 4), respectively.

Table 1. 7-(β-Ketoacylamino)cephalosporins (**1**)

Compound	R	X	M	Yield[a] %	Formula[c]	IR(β-lactam) KBr, cm^{-1}
a		$OCOCH_3$	Na	69	$C_{19}H_{17}N_2O_7SNa \cdot H_2O$	1770
b		$OCOCH_3$	Na	17	$C_{17}H_{15}N_2O_8SNa \cdot 1.5H_2O$	1770
c		$OCOCH_3$	Na	28	$C_{17}H_{15}N_2O_7S_2Na \cdot H_2O$	1770
d		$OCOCH_3$	Na	20	$C_{20}H_{19}N_2O_7SNa \cdot 1.75H_2O$	1765
e		$OCOCH_3$	H	[b]	$C_{18}H_{17}N_3O_7S \cdot 1.5H_2O$[d]	1770
f	CH_3	$OCOCH_3$	H	70	$C_{14}H_{16}N_2O_7S$	1780
g			Na	23	$C_{18}H_{15}N_4O_6S_3Na \cdot 1.5H_2O$	1775
h	CH_3		Na	40	$C_{14}H_{13}N_4O_5S_3Na \cdot 0.5H_2O$[e]	1770
i	CH_3		H	48	$C_{15}H_{15}N_4O_6S_2Na \cdot H_2O$[f]	1780
j	CH_3		H	64	$C_{15}H_{16}N_4O_5S_2$[g]	1775
k	CH_3		H	46	$C_{15}H_{16}N_4O_5S_3$[h]	1780
l	CH_3		H	46	$C_{14}H_{16}N_6O_5S_2$[i]	1780
m	CH_3		—	26	$C_{16}H_{19}N_5O_5S_2 \cdot 1.5H_2O$[j]	1765

a: Yields of **1a**~**f** are calculated from 7-ACA, that of **1g** from **1b**, those of **1h**~**m** from **1f**.

b: See experimental.

c: Unless otherwise indicated, analytical results for C, H and N for all compounds were within 0.4% of the theoretical values.

d: H, calcd., 4.52; found, 3.92.

e: N, calcd., 12.58; found, 11.59.

f: Sodium salt was analyzed.

g: N, calcd., 13.08; found, 12.30.

h: C, calcd., 42.04; found, 42.54. N, calcd., 13.08; found, 10.95. Single spot on tlc at Rf=0.36 (carrier, Merck silica gel plate 60F254; solvent, AcOEt - AcOH - H_2O (8: 1: 1)).

i: N, calcd., 20.28; found, 17.63. Single spot on tlc at Rf=0.17 (conditions are same as above).

j: N, calcd., 15.48; found, 14.07. Single spot on tlc at Rf=0.15 (carrier, same as above; solvent, AcOEt-AcOH - H_2O (3: 1: 1)).

The cephalosporins thus prepared were characterized and tested as free acids or as their sodium salts (Table 1).

Antimicrobial Activity

The minimum inhibitory concentrations (MIC's) against Gram-positive and Gram-negative bacteria were measured by the two-fold serial agar dilution method. The results are shown in Table 2.

Of the 3-acetoxymethyl analogues **1a**~**f**, the two compounds **1b** and **1f** showed good activity against Gram-negative bacteria. Replacement of the acetoxy group of **1b** and **1f** with heterocyclic thiols resulted in analogues **1g** and **1h**~**l**, respectively, with improved activity against Gram-positive and Gram-negative bacteria. In the series of cephalosporins with a 3-oxobutyrylamino at the 7-position and varied substituents at the 3-methylene position, the 3-(1-methyltetrazole)thiomethyl analogue (**1l**) was the most active. This finding agrees well with the observations[9] previously reported on other cephalosporins. The compound **1l** was as active as cephalothin (CET) against both Gram-positive and Gram-negative organisms.

Compounds **1g**, **1j** and **1l** were chosen for comparative *in vivo* evaluation with CET. Table 3 shows their ED_{50} values in mice infected with *Escherichia coli* O-111. Among the compounds tested, the most protective compound, **1l**, was 6.5 times as potent as CET.

Table 2. *In vitro* activity of 7-(β-ketoacylamino)cephalosporins (**1**)

Compound	MIC (mcg/ml)[a]			
	S. aureus 209P	*S. aureus* 1840	*E. coli* NIHJ JC-2	*K. pneumoniae* DT
a	>100	>100	>100	>100
b	<0.78	1.56	50	25
c	100	100	100	>100
d	<0.78	1.56	>100	>100
e	1.56	6.25	>100	>100
f	3.13	3.13	50	25
g	<0.78	<0.78	25	25
h	<0.78	1.56	12.5	6.25
i	1.56	3.13	25	12.5
j	<0.78	1.56	25	12.5
k	<0.78	1.56	50	25
l	<0.78	<0.78	6.25	3.13
m	3.13	6.25	>100	>100
CET	<0.78	<0.78	6.25	3.13

a: The MIC's were determined by the two-fold serial dilution method on Tripticase soy agar (BBL).

Table 3. *In vivo* activity of 7-(β-ketoacylamino)cephalosporins (**1**) and CET

Organism	Route	ED_{50} (mg/kg)*			
		1g	**1j**	**1l**	CET
E. coli O-111	sc	56.3	6.22	5.97	38.8

* The ED_{50} values are expressed as the dose of compound which afforded protection to 50% of the mice (male mice; Slc-ICR strain) challenged intraperitoneally with 10^5 CFU/animal of the test organism. A single dose (5 mice per one dose) was administered subcutaneously immediately after challenge.

Experimental

Infrared spectra were measured in KBr disks using a Hitachi EPI-S_2 infrared spectrophotometer. NMR spectra were determined on a Varian HA-100 spectrometer, using tetramethylsilane as a standard. All melting points are uncorrected.

β-Ketoacids (**3**)

General procedure: To a mixture of an appropriate methyl ketone (0.025~0.030 mol) and 3~4 molar equivalent of magnesium methyl carbonate in dry DMF (80~100 ml) was introduced carbon dioxide gas at 110~120°C for 2~3 hours. Crushed ice was added to the reaction mixture which was cooled in an ice-water bath. The resulting agar-like material was acidified to pH 0.5~2.0 with 2N HCl and extracted with Et_2O-AcOEt (**3e** was obtained as a precipitate by adjusting pH to 2.5~3.0 with 2N NaOH). The extract was dried and evaporated to yield crystalline solid, which was collected and washed with petroleum ether to give **3a~d**. Yield and mp (dec.) of each compound are as follows.

3a: 76%, 102~103°C (lit.[10] 99.5~100°C); **3b**: 51%, 65~67°C; **3c**: 61%, 77~79°C; **3d**: 71%, 69~71°C (lit.[11] 72°C); **3e**: 93%, 130°C.

Sodium 7-[3-(2-furyl)-3-oxopropionylamino]cephalosporanate (**1b**)

To a stirred solution of 3-(2-furyl)-3-oxopropionic acid (2.7 g) in tetrahydrofuran (THF) (20 ml) was added 20% *n*-BuLi (8 ml, *n*-hexane solution) under N_2 stream. Isobutyl chlorocarbonate (2.4 g) was added dropwise at -40 ± 5°C thereto, and the solution was kept stirring for 20 minutes. To a stirred solution of 7-ACA (3.95 g) and Et_3N (3.0 g) in CH_2Cl_2 (50 ml) at −40°C was added the above solution in one portion. The mixture was stirred for 30 minutes at −40°C, for 30 minutes at −30~−20°C and for 3 hours at 0°C, and then evaporated. The residue was mixed with 10% NaCl and washed with AcOEt. The aqueous layer was separated, acidified to about pH 2 with 50% H_3PO_4 and extracted with AcOEt. The extract was dried over $MgSO_4$ and evaporated. Trituration of the residue with Et_2O gave powder of **1b** free acid (3.4 g, 52%). A solution of this powder (0.41 g) in 10% aq. $NaHCO_3$ (10 ml) was chromatographed on Amberlite XAD-2 column (Rohm & Haas Co., 100~200 mesh, 3.4×30 cm) with water as eluent. The desired fractions were lyophilized to yield **1b** (0.151 g, 17%). NMR (D_2O) δ 2.16 (s, $OCOCH_3$), 3.41 & 3.71 (ABq, J=18 Hz, C_2–H_2), 4.76 & 4.95 (ABq, J=13 Hz, C_3–CH_2), 5.17 (d, J=5 Hz, C_6–H), 5.73 (d, J=5 Hz, C_7–H), 6.77, 7.59 & 7.91 (m, furan–H).

1a, c, d were prepared by the same method. NMR data of these compounds are as follows.

1a: (D_2O) δ 2.14 (s, $OCOCH_3$), 3.35 & 3.65 (ABq, J=18 Hz, C_2–H_2), 4.74 & 4.94 (ABq, J=12 Hz, C_3–CH_2), 5.12 (d, J=5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H), 7.4~8.1 (m, Ph).

1c: (D_2O) δ 2.16 (s, $OCOCH_3$), 3.36 & 3.67 (ABq, J=18 Hz, C_2–H_2), 4.75 & 4.94 (ABq, J=12 Hz, C_3–CH_2), 5.14 (d, J=5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H), 7.28 (t, J=4 Hz, thiophene-H), 7.98 (d, J=4 Hz, thiophene-H).

1d: (D_2O) δ 2.14 (s, $OCOCH_3$), 3.35 & 3.65 (ABq, J=18 Hz, C_2–H_2), 3.98 (s, $PhC\underline{H}_2$), 4.76 & 4.95 (ABq, J=13 Hz, C_3–CH_2), 5.12 (d, J=5 Hz, C_6–H), 5.66 (d, J=5 Hz, C_7–H), 7.2~7.5 (m, Ph).

7-[3-Oxo-3-(4-pyridyl)propionylamino]cephalosporanic acid (**1e**)

To a solution of 3-oxo-3-(4-pyridyl)propionic acid (0.106 g) and benzhydryl 7-aminocephalosporanate[7] (0.438 g) in THF (10 ml) was added DCC (2.66 g) and the mixture was stirred for 5 hours at room temperature. The precipitated crystals were removed by filtration and the filtrate was evaporated. Trituration of the residue with *n*-hexane gave benzhydryl 7-[3-oxo-3-(4-pyridyl)propionylamino]cephalosporanate (0.445 g) as powder. NMR ($CDCl_3$) δ 1.97 (s, $OCOCH_3$), 3.27 & 3.54 (ABq, J=18 Hz, C_2–H_2), 3.94 (s, enol OH), 4.73 & 5.03 (ABq, J=13 Hz, C_3–CH_2), 5.72 (s, enol olefin), 6.90 (s, $C\underline{H}Ph_2$), 7.14~7.80 (m, aromatic-H), 8.5~8.8 (m, CONH).

To a solution of anisole (0.25 ml) and CF_3COOH (0.75 ml) was added the benzhydryl ester (0.292 g) obtained above with stirring at −10°C. The mixture was gradually warmed to room temperature. After stirring for 20 minutes, Et_2O was added to the mixture and the supernatant was removed by decantation. The residue was washed twice with Et_2O. A solution of this crude product and $NaHCO_3$ (0.1 g) in water (3 ml) was acidified to about pH 2 and extracted with AcOEt. The extract was dried over $MgSO_4$ and evaporated. Trituration of the residue with Et_2O yielded **1e** (0.098 g). NMR (DMSO-d_6) δ 1.98 (s, $OCOCH_3$), 3.25~3.75 (m, C_2–H_2), 4.02 (s, enol OH), 4.65 & 4.97 (ABq, J=14 Hz,

C_3–CH_2), 5.11 (d, J=5 Hz, C_6–H), 5.6~5.9 (m, C_7–H), 6.06 (s, enol olefin), 7.6~8.9 (m, pyridine-H & CONH).

7-(3-Oxobutyrylamino)cephalosporanic acid (**1f**)

To a solution of 7-ACA (2.72 g) and Et_3N (2.02 g) in CH_2Cl_2 (80 ml), was added a solution of diketene (1.0 g) in CH_2Cl_2 (10 ml) with stirring under ice-cooling. After stirring for 3 hours, the mixture was concentrated *in vacuo*. Water and AcOEt were added to the residue and the mixture was shaken. The aqueous layer was separated, acidified to pH 2 with 50% H_3PO_4 and extracted twice with AcOEt. The combined extracts were washed with saturated NaCl and dried over $MgSO_4$. After evaporation of the solvent, the residue was cooled to −40°C. Crystals separated were collected and washed with cold (−40°C) AcOEt, to yield **1f** (1.8 g), mp 148~150°C (dec.). NMR (DMSO-d_6) δ 2.02 (s, $OCOCH_3$), 2.14 (s, CH_3CO), 3.41 (s, $COCH_2CO$), 3.41 & 3.65 (ABq, J=18 Hz, C_2–H_2), 4.68 & 4.99 (ABq, J=14 Hz, C_3–CH_2), 5.08 (d, J=5 Hz, C_6–H), 5.66 (dd, J=5 & 8 Hz, C_7–H), 8.98 (d, J=8 Hz, CONH).

Sodium 7-[3-(2-furyl)-3-oxopropionylamino]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]ceph-3-em-4-carboxylate (**1g**)

Reaction of the free acid of **1b** (0.445 g) with 5-methyl-1, 3, 4-thiadiazole-2-thiol (0.224 g) by the same procedure described for **1l**, gave 0.112 g (23%) of **1g**. NMR (D_2O) δ 2.76 (s, CH_3), 3.43 & 3.81 (ABq, J=18 Hz, C_2–H_2), 4.02 & 4.51 (ABq, J=14 Hz, C_3–CH_2), 5.12 (d, J=5 Hz, C_6–H), 5.70 (d. J=5 Hz, C_7–H), 6.74, 7.57 & 7.88 (m, furan-H).

3-[[(1-Methyl-1H-tetrazol-5-yl)thio]methyl]-7-(3-oxobutyrylamino)ceph-3-em-4-carboxylic acid (**1l**)

The mixture of **1f** (0.35 g), $NaHCO_3$ (0.084 g), 1-methyl-1H-tetrazole-5-thiol (0.122 g) and phosphate buffer solution (pH 6.4, 20 ml) was stirred for 16 hours at 60~70°C. After cooling, the reaction mixture was washed with AcOEt and acidified to pH 2 with 1N HCl and saturated with NaCl. The solution was extracted with AcOEt five times. The combined extracts were washed with saturated NaCl and dried over $MgSO_4$. After evaporation of the solvent the residue was triturated with Et_2O to yield **1l** (0.19 g), mp 65~70°C (dec.). NMR (DMSO-d_6) δ 2.14 (s, CH_3CO), 3.41 (s, $COCH_2CO$), 3.57 & 3.79 (ABq, J=18 Hz, C_2–H_2), 3.92 (s, tetrazole-CH_3), 4.20 & 4.37 (ABq, J=14 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.65 (dd, J=5 & 8 Hz, C_7–H), 9.02 (d, J=8 Hz, CONH).

1i (free acid), **j** and **k** were obtained by the same method. **1h**, **i** and **m** were obtained from the reaction mixture by the column chromatography on Amberlite XAD-2 (Rohm & Haas Co.) with water as eluent and subsequent lyophilization. NMR data of these compounds are as follows.

1h: (D_2O) δ 2.38 (s, CH_3CO), 3.52 & 3.88 (ABq, J=18 Hz, C_2–H_2), 4.15 & 4.60 (ABq, J=14 Hz, C_3–CH_2), 5.17 (d, J=5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H), 9.48 (s, thiadiazole-H).

1i: (DMSO-d_6) δ 2.12 (s, CH_3CO), 2.43 (s, oxadiazole-CH_3), 3.39 (s, $COCH_2CO$), 3.51 & 3.77 (ABq, J=18 Hz, C_2–H_2), 4.11 & 4.35 (ABq, J=14 Hz, C_3–CH_2), 5.05 (d, J=4 Hz, C_6–H), 5.65 (dd, J=4 & 8 Hz, C_7–H), 8.99 (d, J=8 Hz, CONH).

1j: (DMSO-d_6) δ 2.10 (s, CH_3CO), 2.62 (s, thiadiazole-CH_3), 3.37 (s, $COCH_2CO$), 3.50 & 3.74 (ABq, J=18 Hz, C_2–H_2), 4.15 & 4.46 (ABq, J=13 Hz, C_3–CH_2), 5.02 (d, J=5 Hz, C_6–H), 5.62 (dd, J=5 & 8 Hz, C_7–H), 8.95 (d, J=8 Hz, CONH).

1k: (DMSO-d_6) δ 2.11 (s, CH_3CO), 2.49 (s, thiadiazole-CH_3), 3.38 (s, $COCH_2CO$), 3.49 & 3.76 (ABq, J=18 Hz, C_2–H_2), 4.21 & 4.57 (ABq, J=13 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.64 (dd, J=5 & 8 Hz, C_7–H), 8.98 (d, J=8 Hz, CONH).

1m: (D_2O) δ 2.39 (s, CH_3CO), 3.58 & 3.91 (ABq, J=18 Hz, C_2–H_2), 3.94 & 4.15 (each s, 2×CH_3), 5.19 (d, J=5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H).

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