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(2,3)-α-Methylenepenams: Synthesis and *In Vitro* Activity[1]

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Abstract—A series of α-methylene penicillins was synthesized and SAR were studied. The α-isomers were found to be chemically reactive and biologically active in contrast to the β-isomers. In addition, the α-isomers have broader spectrum of *in vitro* activity than the corresponding penicillins. Generally, the α-isomers are more active against gram-negative bacteria than the corresponding penicillins, but slightly weaker in potency towards gram-positive organisms.

Introduction

A comparison of the various properties of penicillin G and the (2,3)-β-methylenepenam (**2**), led to the conclusion that the "open" conformation of penicillins (**1a**) is the biologically active form,[2] a conclusion also arrived at independently by Cohen[3] (Figure 1). As a corollary, it was predicted that 6-acylamino derivatives of (2,3)-α-methylenepenam (**3**) would possess good antibacterial properties.

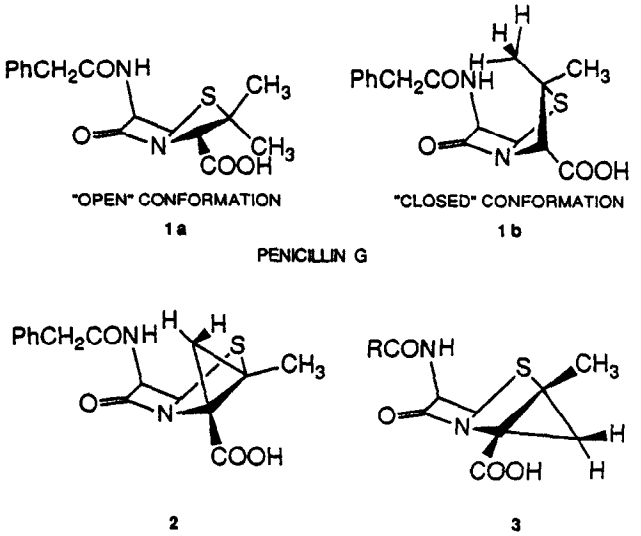
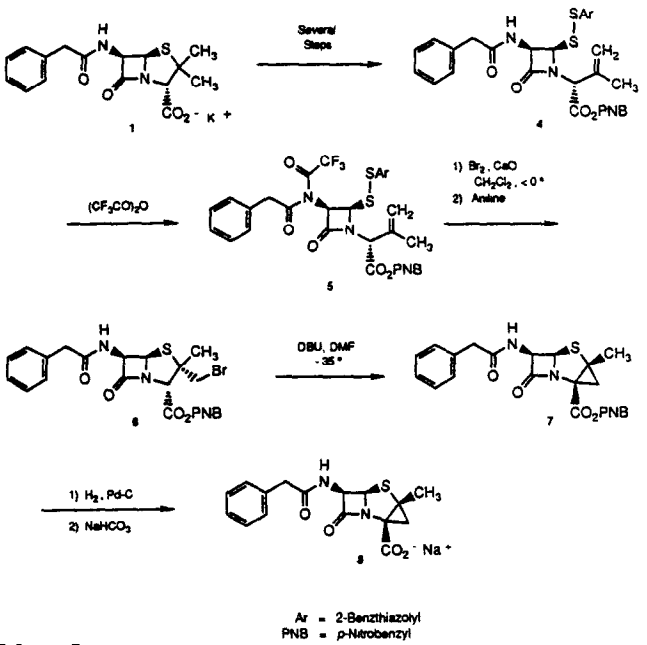


Figure 1.

An interesting feature of **3** is the position of the carboxy substituent on the thiazolidine ring. The carbon bearing it has the *R*-configuration, which, to the best of our knowledge, is the opposite from all known biologically active penicillins. An examination of the Dreiding models reveals that the actual disposition in space of the carboxyl group vis-a-vis the β-lactam carbonyl is quite similar to the spatial arrangement of these groups in cephalosporins. Thus, it was expected that members of this new class of β-lactams would have improved activity against gram-negative bacteria, since many cephalosporins are excellent gram-negative agents.

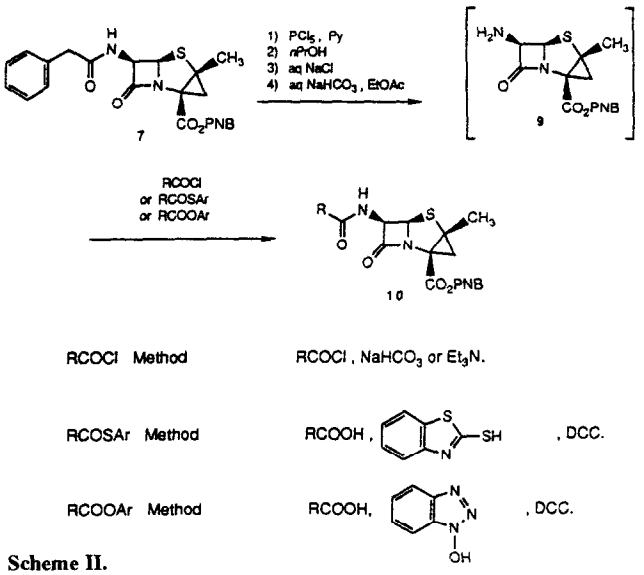
α-Methylenepenam **8** was prepared as outlined in Scheme I. Details of the synthesis were described previously.[4] Since this compound was found to have interesting biological activity, a number of (2,3)-α-methylenepenams with a variety of acylamino side chains were prepared and their antimicrobial activities were evaluated.



Scheme I.

Chemistry

The synthesis of (2,3)-α-methylenepenam analogs is outlined in Scheme II starting from **7**. Due to the reactivity of this class of molecules, numerous conditions were explored before achieving acceptable conversion of **7** to **9**. The optimum conditions in our experience for the deacylation were found to be the reaction of **7** with PCl_5 (1.1 eq.) and dry pyridine (1.9 eq.) in chloroform, followed by treatment with *n*-propanol and then saturated aqueous sodium chloride solution. Compound **9**, without further



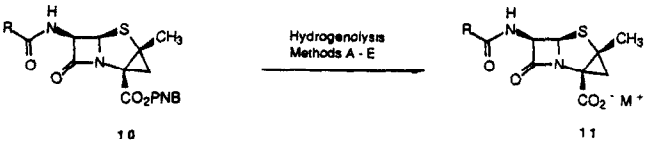
RCOCl Method RCOCl, $NaHCO_3$ or Et_3N.

RCOSAr Method RCOOH, , DCC.

RCOOAr Method RCOOH, , DCC.

Scheme II.

purification, was converted to various derivatives by reaction with activated acyl derivatives. These included acid chlorides (RCOCl) and esters of 2-mercaptobenzothiazole (RCOSAr) or 1-hydroxybenzotriazole (RCOOAr) prepared *in situ* with dicyclohexylcarbodiimide and the corresponding acid. The compounds prepared are listed in Table 1. Deprotection of the *p*-nitrobenzyl ester (PNB) was accomplished by hydrogenolysis with 10% palladium on carbon (Scheme III and Table 2).



Method A) i. 10% Pd-C, H_2 in THF
ii. aq. $NaHCO_3$

Method B) 10% Pd-C, H_2 in EtOAc - 0.1M $NaHCO_3$ (aq.)

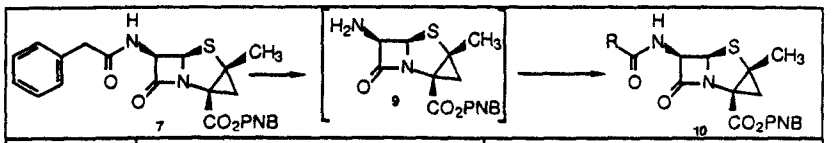
Method C) i. 10% Pd-C, H_2 in CH_3OH-THF (1:1, V/V)
ii. $NaHCO_3$ in H_2O

Method D) i. 10% Pd-C, H_2 in DMF
ii. CH_3OH, aq. $NaHCO_3$

Method E) i. 10% Pd-C, H_2 in CH_3OH
ii. aq. $NaHCO_3$, EtOAc

Scheme III.

Table 1.



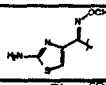
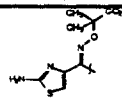
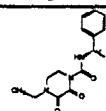
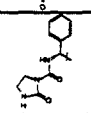
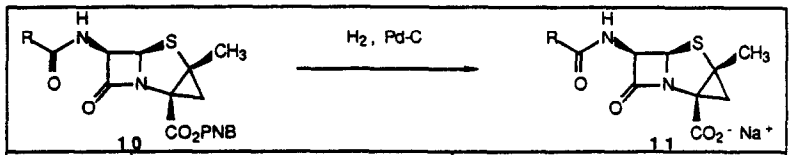
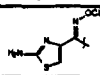
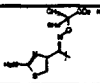
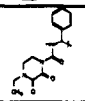
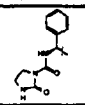
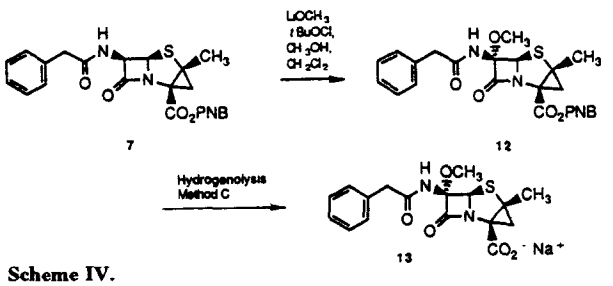
Compound	R	Coupling Method
10a		RCOCl
10b		RCOCl
10c		RCOCl
10d		RCOCl
10e		RCOCl
10f		RCOCl
10g		RCOSAr
10h		RCOSAr
10i		RCOSAr
10j		RCOSAr
10k		RCOSAr
10l		RCOOAr
10m		RCOOAr

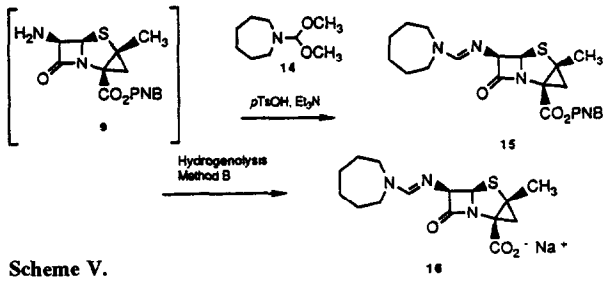
Table 2.



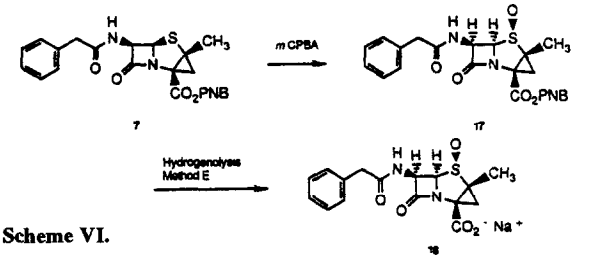
Compound	R	Hydrogenolysis Method
11a		A
11b		A
11c		B
11d		A
11e		A
11g		B
11h		D
11i		C
11j		A
11k		A
11l		B
11m		A



Scheme IV.



Scheme V.



Scheme VI.

Three other derivatives of (2,3)-α-methylenepenams, namely **13**, **16** and **18** were also prepared by the procedures outlined in Schemes IV–VI respectively.

Microbiology

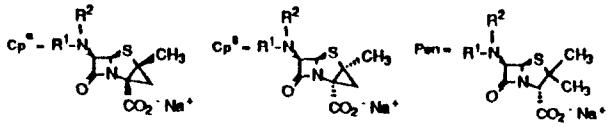
In vitro antimicrobial activities (MICs) were determined in antibiotic medium No. 1 (Seed Agar, BBL) using an agar-diffusion well method.[5] A comparison of *in vitro* antimicrobial activities of selected (2,3)-α-methylene-penams with the corresponding penicillins and cephalosporins is summarized in Tables 3 and 4. An indication of β-lactamase susceptibility was obtained by incorporating a partially purified enzyme preparation into the agar and comparing MICs for *M. luteus* ATCC 9341 with MICs measured in the absence of β-lactamase (Table 5). In general the (2,3)-α-methylenepenam analogs were inactive *in vitro* against *Pseudomonas cepacia, Pseudomonas maltophilia, Entrobacter cloacae,* molds, and yeasts.

The penicillin binding protein (PBP) binding assay was carried out with Triton X-100-solubilized membranes from sonicated bacteria as described by Spratt.[6] PBP binding was measured as inhibition of [^{14}C]-penicillin G binding. Results are summarized in Tables 6 and 7.

Table 3. Comparison of *in vitro* activity** of selected (2,3)-α-methylenepenams with penicillins

Organisms		Compound: R¹ = PhCH₂CO—			R¹ = 2-thienylacetyl		R¹ = 3-pyridylthioacetyl		R¹ = 2,6-dimethoxybenzoyl		R¹ = 2-phenyl-5-oxo-4-imidazolidinylcarbonyl	
		Pen. Penicillin G	Cpα 8	Cpβ 2	Pen	Cpα 111	Pen	Cpα 11c	Pen Methicillin	Cpα 11e	Pen Azlocillin	Cpα 11k
Pseudomonas aeruginosa	ATCC 8709	250	>1000	>500	250	>500	125	>125	>125	>125	12.5	100
Pseudomonas aeruginosa[a]		1.95	15.6	>500	3.9	31.3	0.49	7.8	7.8	31.3	0.39	12.5
Escherichia coli	ATCC 27856	125	15.6	>500	125	15.6	31.3	3.9	>125	125	100	>100
Klebsiella pneumoniae	ATCC 27858	250	15.6	>500	500	15.6	125	3 9	>125	>125	>100	>100
Serratia marcescens	ATCC 27857	>1000	>1000	>500	>500	>500	>125	>125	>125	>125	50	>100
Serratia sp	ATCC 93	>1000	1000	>500	>500	>500	>125	>125	>125	>125	25	>100
Streptococcus faecium	ATCC 8043	1.95	31.5	62.5	7.8	250	3.9	15.6	>125	>125	6 25	25
Staphylococcus aureus	ATCC 6538p	0.03	0.23	15.6	0.06	0.49	0.03	0.12	1.95	15.6	0.78	0.78
Staphylococcus aureus[b]		125	1.9	500	7.8	1.95	3.9	1.95	3.9	62.6	12.5	6.25
Staphylococcus aureus[c]		500	62.5	>1000	>500	250	>125	31.3			>100	100
Micrococcus luteus	ATCC 9341	0.03	0.45	62.5	0.06	0.98	0 03	0.24	0.24	0.98	0.195	3.13
Bacillus subtilis	NRRL 558	0.03	0.12	7.8	0.06	0.24	0.03	0.03	0.49	125	0.78	0.78

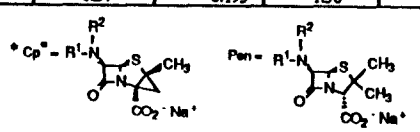
Organisms		Compound: R¹ = 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl		R¹ = 2-hydroxy-2-phenylacetyl		R¹ = 2-carboxy-2-phenylacetyl		R¹,R² = 1-azacycloheptenyl	
		Pen	Cpα 11g	Pen	Cpα 111	Pen Carbenicillin	Cpα 11b	Pen Amdinocillin	Cpα 16
Pseudomonas aeruginosa	ATCC 8709	100	100	>125	>125	25	>100	250	>500
Pseudomonas aeruginosa[a]		1.56	3.13	1.95	31.3	0.78	12.5	15.6	>500
Escherichia coli	ATCC 27856	50	25	31.3	7.8	100	25	0.12	500
Klebsiella pneumoniae	ATCC 27858	100	25	>125	7.8	>100	50	0 12	125
Serratia marcescens	ATCC 27857	50	12.5	>125	62.5	100	50	0 49	>500
Serratia sp	ATCC 93	12.5	6.25	31.3	15.6	12.5	25	0.24	>500
Streptococcus faecium	ATCC 8043	25	>500	3.9	>125	>100	>100	>500	>500
Staphylococcus aureus	ATCC 6538p	0.78	1.56	0.06	0.24	0.78	12.5	31.3	15.6
Staphylococcus aureus[b]		2.56	3.13	3.9	1.95	12.5	100	125	125
Staphylococcus aureus[c]		100	100	>125	125	>100	>100	>500	>500
Micrococcus luteus	ATCC 9341	0.78	0.78	0.03	0.49	3.13	50	31.3	62.5
Bacillus subtilis	NRRL 558	1.56	25	0.12	0.24	3.13	25	15.6	31.3



R^2 = H, unless indicated otherwise.
** MIC (μg/mL) Agar Diffusion Well Method.
[a] β-Lactam sensitive mutant.
[b] Inducible β-lactamase.
[c] Methicillin resistant and β-lactamase producer.

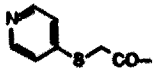
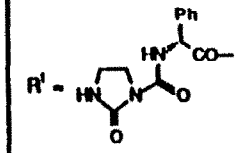
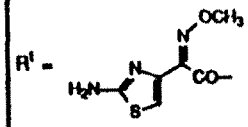
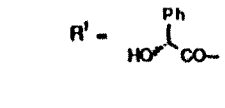
Table 4. Comparison of *in vitro* activity** of (2,3)-α-methylenepenams with penicillins and cephalosporins

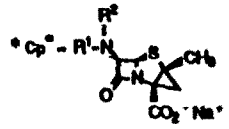
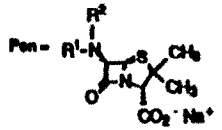
Organisms		Compound*: R = 2-hydroxy-2-phenylacetyl			R = NCCH₂CO—		
		Pen	Cpα 111	Cefamandole	Pen	Cpα 11m	Cephacetrile
Pseudomonas aeruginosa	ATCC 8709	>125	>125	>125	>100	>100	>100
Pseudomonas aeruginosa[a]		1.95	31.3	15.6	3.13	50	3.13
Escherichia coli	ATCC 27856	31.3	7.8	1 95	50	6.25	25
Klebsiella pneumoniae	ATCC 27858	>125	7.8	7.8	>100	12.5	25
Serratia marcescens	ATCC 27857	>125	62.5	>125	>100	100	>100
Serratia sp	ATCC 93	31.3	15.6	15.6	>100	100	>100
Streptococcus faecium	ATCC 8043	3 9	>125	62.5	6 25	6.25	12 5
Staphylococcus aureus	ATCC 6538p	0.06	0.24	0.12	0.195	1.56	0 39
Staphylococcus aureus[b]		3.9	1.95	0.98	6.25	6.25	1.56
Staphylococcus aureus[c]		>125	125	62.5	>100	100	12.5
Micrococcus luteus	ATCC 9341	0.03	0.49	0.24	0.78	12.5	6.25
Bacillus subtilis	NRRL 558	0.12	0.24	0.24	0.195	1.56	0.78



R^2 = H, unless indicated otherwise.
** MIC (μg/mL) Agar Diffusion Well Method.
[a] β-Lactam sensitive mutant.
[b] Inducible β-lactamase.
[c] Methicillin resistant and β-lactamase producer.

Table 5. Comparison of β-lactamase stability of selected (2,3)-α-methylenepenams with penicillins and cephalosporin

Compound[a] / Organisms & Enzyme	R^1 = $PhCH_2CO-$		R^1 = 		R^1 = 		R^1 = 		R^1 = 			
	Pen Penicillin G	Cp^a 8	Pen	Cp^a 11c	Pen Azlocillin	Cp^a 11k	Pen	Cp^a 11g	Pen	Cp^a 11l	Cefamandole	
	MIC (μg/mL) [Relative Activity**]											
M. luteus ATCC 9341	0.03	0.24	0.03	0.49	0.195	0.78	0.78	0.78	0.03	0.49	0.24	
M. luteus ATCC 9341 + Enterobacter cephalosporinase (Type 1a)	7.8 [256]	125 [512]	0.98 [32]	15.6 [32]	0.39 [2]	12.5 [16]	0.78 [1]	1.56 [2]	0.12 [4]	7.8 [16]	0.49 [2]	
M. luteus ATCC 9341 + Proteus cephalosporinase (Type 1c)	7.8 [256]	>1000 [>4096]	7.8 [256]	>125 [>256]	5.13 [16]	>100 [>128]	6.25 [8]	5.13 [4]	0.24 [8]	>125 [>256]	1.95 [8]	
M. luteus ATCC 9341 + TEM-1	31.3 [1024]	62.5 [256]	15.6 [512]	62.5 [128]	12.5 [64]	50 [64]	50 [64]	1.56 [2]	31.3 [1024]	31.3 [64]	0.24 [1]	
M. luteus ATCC 9341 + Staph. penicillinase	>1000 [>32768]	15.6 [64]	>125 [>4096]	31.3 [64]	>100 [>512]	25 [32]	1.56 [2]	1.56 [2]	>125 [>4096]	31.3 [64]	0.98 [4]	

[a] Cp =  Pen = 

R^2 = H, unless indicated otherwise.

** Relative activity = $\frac{\text{MIC } \textit{M. luteus} \text{ ATCC 9341 + β-lactamase}}{\text{MIC } \textit{M. luteus} \text{ ATCC 9341.}}$

Table 6. Binding of penams and (2,3)-methylenepenams to *E. coli* DC0 PBPs

Compound	Concn (μg/ml) for 90% inhibition of [^{14}C]penicillin G binding					
	PBP 1a (90 kDa)	PBP 1b (90 kDa)	PBP 2 (66 kDa)	PBP 3 (60 kDa)	PBP 4 (49 kDa)	PBP 5/6 (40 kDa)
Penicillin G	10	10	2	2	10	100
8	100	100	100	2	100	>100
2	>100	>100	>100	100	>100	>100
Pen. analogue of **13**	>100	>100	100	100	>100	100
13	>100	>100	>100	10	30	30
Pen. analogue of **11l**	10	10	2	0.5	0.5	100
11l	ND[a]	>100	>100	0.5	100	>100
Pen. analogue of **11d**	2	10	30	0.5	100	>100
11d	100	100	>100	10	>100	>100
Penicillin V	0.5	2	2	0.5	0.1	100
11a	10	100	>100	2	>100	>100
Azlocillin	10	10	2	0.1	0.5	30
11k	2	2	2	0.1	10	>100
Pen. analogue of **11f**	30	100	10	10	100	>100
11f	100	>100	30	30	100	>100
Amdinocillin	>100	>100	0.1	>100	>100	>100
16	100	>100	>100	>100	>100	>100

[a] ND = Not detected.

Table 7. Binding of benzylpenicillin and two (2,3)-methylenepenam analogs to *S. aureus* ATCC 25923 PBPs

Compound	Concn (μg/ml) for 90% inhibition of [^{14}C]penicillin G binding			
	PBP1 (87 kDa)	PBP2 (80 kDa)	PBP3 (75 kDa)	PBP4 (41 kDa)
Penicillin G	0.1	0.1	0.1	>100
8	0.1	0.1	>100	>100
2	0.1	>100	100	>100

Results and Discussion

Penicillin G exhibits excellent antibacterial efficacy against gram-positive bacteria but is less effective against gram-negative strains. The (2,3)-α-methylenepenam analog **8**, which contains the same phenylacetylamino side chain, has good gram-positive activity and also has activity against gram-negative strains. The β-isomer, **2**, however, is the least effective antibacterial, having weak activity against gram-positive strain and no measurable gram-negative activity. These results are in agreement with PBP binding assays in which penicillin G and α-methylenepenam bind effectively to the essential PBPs in both *E. coli* (Table 6) and *S. aureus* (Table 7), while the β-isomer is inactive. The α-methylenepenam is a rigid analog of the "open" conformation available to penams, while the β-isomer is an analog of the "closed" conformation. Our results strongly support the hypothesis of Keith *et al.*[2] that the active conformation of penicillin is the "open" one.

The *in vitro* activities of selected (2,3)-α-methylenepenams are shown in Tables 3 and 4. These compounds have a broader spectrum of activity than the corresponding penicillins. In general, they are more active against gram-negative bacteria, but slightly weaker in potency toward gram-positive organisms. Their activities against gram-negative rods were found to be associated with binding to the essential PBP 3, with inhibitory concentrations comparable to those of the corresponding penams (Table 6). The better activity of (2,3)-α-methylenepenams, as compared to the penicillin series against these organisms, is probably due to a combination of intrinsic potency and better permeability characteristics, as demonstrated in a recent study.[7]

In terms of antibacterial activity, the spectra of α-methylenepenams are closer to their cephalosporin analogs than those of the penicillins (Table 4). The lack of activity of the amidino-(2,3)-α-methylenepenam is similar to the characteristics of the amidino-cephalosporins.[8] In addition, the relative susceptibility of α-methylenepenams to cephalosporinase and penicillinase, as indicated in Table 5, confirms that these derivatives act more like cephalosporins than penicillins. For example, while penicillins were apparently completely inactivated by staphylococcal penicillinase, the activities of the corresponding α-methylenepenams were still detectable. In contrast, α-methylenepenams were extensively inactivated by Type 1c cephalosporinase from *P. vulgaris*, while the corresponding penicillins were much less sensitive.

The β-lactamase results provide a striking illustration of the influence of the position in space of the carboxyl group. Compared to the corresponding penicillins, the α-methylenepenams, in which the spatial orientation of the carboxyl group is similar to that in cephalosporins, were

better substrates for gram-negative chromosomal cephalosporinase, and much poorer substrates for staphylococcal penicillinases.

α-Methylenepenams are highly strained molecules and might be expected to be very reactive toward hydrolysis. Our studies indicate that α-methylenepenam **8** is readily hydrolyzed by pH 7.4 phosphate buffer, at 37 °C, with a half-life of 5.9 h, while penicillin G and β-methylenepenam **2** are relatively stable with half-lives of 98 h and 420 h respectively. Although, the *in vitro* activities of α-methylenepenams are excellent against gram-positive bacteria and moderate against gram-negative strains, their instability to hydrolysis probably reduces their effectiveness as antibacterials.

Summary

(2,3)-α-Methylenepenams, in contrast to the β-methylene isomers, were found to be biologically active. The results confirm that the "open" conformation of penicillins is the biologically active form. The relative position in space of the carboxylic acid was found to profoundly influence the nature of the biological properties. In (2,3)-α-methylenepenam, the absolute configuration at C2 is opposite to that in the naturally occurring penicillins, placing the carboxyl group in a position similar to that of cephalosporins. Consistent with this, the (2,3)-α-methylenepenams exhibited selective activity to PBP 3 of *E. coli* and were more susceptible to cephalosporinase than penicillinase.

Experimental Section

Infrared spectra (IR) were recorded on a Digilab FTS 14 spectrometer. Mass spectra (MS) were obtained on a VG7070E-HF mass spectrometer running in the positive ion fast atom bombardment mode using thioglycerol as the solvent. Proton nuclear magnetic resonance spectra (NMR) were recorded on a varian XL-400 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane, and coupling constants (*J*) are expressed in hertz (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet).

Silica gel 60 (230–400 mesh), and plates precoated with silica gel 60 F-254 (both from E. Merck) were used for column and thin layer chromatography (TLC), respectively. Reverse phase high performance liquid chromatography were performed with a Waters analytical instrument using a Whatman Partisil M9 10/25 ODS-2 column. Standard penicillins and cephalosporins used for comparisons were either obtained from commercial sources or prepared by literature methods.

Preparation of ***9***

To a solution of **7** (94 mg, 0.2 mmol) and pyridine (dry, 32 μL, 0.38mmol) in dry chloroform (2 mL) was added PCl_5 (46 mg, 0.22 mmol) at -4 to -2 °C. The mixture was stirred at this temperature for 1 h. About 30 min after the addition of PCl_5, a white precipitate formed. *n*-Propanol dried over molecular sieve (4Å) (0.3 mL) and then chloroform (1 mL) were added at -4 to -2 °C. After the reaction mixture was stirred for about 45 min at the same temperature, a clear solution was obtained. If precipitate still remained after 45 min, additional PCl_5 (2 mg) was added and stirring was continued for 15 min until the reaction became clear. The addition was repeated if the precipitate did not dissolve. Usually, 2 to 4 mg PCl_5 was required. The reaction was further stirred for an additional 15 min after the clear solution was obtained. Subsequently, saturated aqueous sodium chloride solution (0.5 mL) was added at 0 °C and the reaction stirred for 15 min. Ethyl acetate (6 mL), saturated aqueous sodium chloride solution (3 mL) and saturated aqueous sodium bicarbonate solution (0.5 mL) were then added successively. The two layers were separated, and the organic phase was washed with saturated aqueous sodium chloride solution (2 x 3 mL), dried over magnesium sulfate and filtered. The filtrate was used as is for subsequent acylation reactions. In some experiments dichloromethane was used instead of ethyl acetate during the extractive workup. This material has R_f 0.35 with silica gel TLC using ethyl acetate as solvent.

Preparation of ***10*** *by RCOCl method*

The acid chloride (1 eq.) in dry chloroform was added at 0 °C to the mixture of crude **9** (prepared immediately before use) in chloroform and saturated aqueous sodium bicarbonate. This mixture was stirred under argon atmosphere at 0 °C for 2 h. The two phases were separated and the aqueous phase extracted with ethyl acetate twice. The organic layers were combined, washed with saturated aqueous sodium chloride solution, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was then chromatographed on silica gel to give **10**.

Compound **10a**: 23% yield from **7**; NMR ($CDCl_3$) δ 1.65 (d, 1, *J* = 8 Hz), 1.81 (s, 3), 1.84 (d, 1, *J* = 8 Hz), 4.55 (AB q, 2, *J* = 13 Hz), 4.82 (d, 1, *J* = 4 Hz), 5.36 (AB q, 2, *J* = 14 Hz), 5.84 (dd, 1, *J* = 9, 4 Hz), 6.94 (d, 1, *J* = 8Hz), 7.06 (t, 1, *J* = 8 Hz), 7.35 (m, 3), 7.65 (d, 2, *J* = 9 Hz), 8.27 (d, 2, *J* = 9Hz).

Compound **10b**: 24% yield from **7** as a 1:1 mixture of two diastereomers; IR ($CHCl_3$) 1800, 1732, 1682, 1525, 1350 cm^{-1}; MS 647 (M^+ + H), 669 (M^+ + Na); NMR ($CDCl_3$) δ 1.61, 1.62 (2d, 1, *J* = 8 Hz), 1.76, 1.80 (2s, 3), 1.81 (m, 1), 4.64 (d, 1, *J* = 12 Hz), 4.75, (dd, 1, *J* = 12, 4 Hz), 5.30 (m, 4), 5.74 (m, 1), 7.27 (m, 2), 8.19 (2d, 2, *J* = 8 Hz), 8.25 (2d, 2, *J* = 8 Hz).

Compound **10c**: 25% yield from **7**; NMR ($CDCl_3$) δ 1.56 (d, 1, *J* = 8 Hz), 1.70 (s, 3), 1.73 (d, 1, *J* = 8 Hz), 2.78 (d, 1, *J* = 19 Hz), 3.64 (d, 1, *J* = 19 Hz), 4.68 (d, 1, *J* = 5 Hz), 5.25 (d, 1, *J* = 18 Hz), 5.36 (d, 1, *J* = 18 Hz), 5.66 (dd, 1, *J* = 12 and 5 Hz), 7.12 (d, 2, *J* = 9 Hz), 7.40 (d, 1, *J* = 12 Hz), 7.61 (d, 2, *J* = 11 Hz), 8.24 (d, 2, *J* = 11 Hz), 8.48 (d, 2, *J* = 9 Hz).

Compound **10d**: 25% yield from **7**; IR ($CHCl_3$) 3420, 1801, 1740, 1526, 1402, 1352 cm^{-1}; NMR ($CDCl_3$) δ

1.65 (d, 1, J = 8 Hz), 1.81 (s, 3), 1.83 (d, 1, J = 8 Hz), 4.80 (d, 1, J = 7 Hz), 5.32 (d, 1, J = 16 Hz), 5.40 (d, 1, J = 16 Hz), 5.74 (dd, 1, J = 10, 7 Hz), 6.25 (s, 1), 6.88 (d, 1, J = 10 Hz), 7.42 (m, 1), 7.64 (d, 2, J = 10 Hz), 8.22 (s, 1), 8.26 (d, 1, J = 10 Hz).

Compound **10e**: 40% yield from **7**; NMR ($CDCl_3$) δ 1.64 (d, 1, J = 5 Hz), 1.79 (s, 3), 1.86 (d, 1, J = 5 Hz), 3.86 (s, 6), 4.91 (d, 1, J = 3 Hz), 5.31 (d, 1, J = 8 Hz), 5.41 (d, 1, J = 3 Hz), 6.02 (dd, 1, J = 8, 3 Hz), 6.55 (d, 2, J = 5 Hz), 7.29 (t, 1, J = 5 Hz), 7.63 (d, 2, J = 5 Hz), 8.06 (d, 1, J = 8 Hz), 8.24 (d, 2, J = 5 Hz).

Compound **10f**: 39% yield from **7**; IR ($CHCl_3$) 3320, 1791, 1733, 1705, 1523, 1348 cm^{-1}; MS 460 (M^+ + H); NMR ($CDCl_3$) δ 1.66 (d, 1, J = 8 Hz), 1.83 (s, 3), 1.93 (d, 1, J = 8 Hz), 4.86 (d, 1, J = 6 Hz), 5.30–5.50 (m, 4), 5.67 (dd, 1, J = 8, 6 Hz), 7.69 (d, 2, J = 8 Hz), 8.25 (d, 2, J = 8 Hz), 9.02 (s, 1), 9.41 (d, 1, J = 8 Hz).

*Preparation of **10** by RCOSAr method*

Dicyclohexylcarbodiimide (1.1 eq.) was added in one portion to a solution of carboxylic acid (1 eq.) and 2-mercaptobenzothiazole in ethyl acetate (5 mL per mmol) with magnetic stirring at room temperature. A white precipitate was formed. After 1 h, a dried solution of **9** (1 eq.) in chloroform was added to this suspension. This mixture was stirred at room temperature for an additional 2 h. Insoluble material was filtered off, and the filtrate was concentrated and chromatographed on silica gel to give the desired product.

Compound **10g**: 32% yield from **7**; NMR ($CDCl_3$) δ 1.64 (d, 1, J = 8 Hz), 1.80 (s, 3), 1.83 (d, 1, J = 8 Hz), 4.08 (s, 3), 4.87 (d, 1, J = 6 Hz), 5.15 (br s, 2), 5.35 (AB q, 2), 5.89 (dd, 1, J = 10, 6 Hz), 6.91 (d, 1, J = 10 Hz), 6.94 (s, 1), 7.63 (d, 2, J = 9 Hz), 8.24 (d, 2, J = 9 Hz).

Compound **10h**: NMR (Me_2SO-d_6) δ 1.52 (s, 3), 1.54 (s, 3), 1.62 (m, 2), 1.70 (s, 3), 5.12 (d, 1, J = 4 Hz), 5.35 (AB q, 2), 5.41 (s, 2), 5.73 (dd, 1, J = 8, 4 Hz), 6.72 (s, 1), 7.32 (s, 2), 7.66 (d, 2, J = 9 Hz), 7.74 (d, 2, J = 9 Hz), 8.14 (d, 2, J = 9 Hz), 8.27 (d, 2, J = 9 Hz), 9.50 (d, 1, J = 8 Hz).

Compound **10i**: 31% yield from **7**; IR (KBr) 1775, 1667, 1520 cm^{-1}; NMR ($CDCl_3$) δ 1.62 (d, 1, J = 8 Hz), 1.76 (s, 3), 1.81 (d, 1, J = 8 Hz), 3.85 (s, 2), 4.77 (d, 1, J = 5 Hz), 5.34 (AB q, 2, J = 5 Hz), 5.75 (dd, 1, J = 12, 5 Hz), 6.76 (d, 1, J = 12 Hz), 7.01 (m, 2), 7.31 (m, 1), 7.63 (d, 2, J = 11 Hz), 8.27 (d, 2, J = 11 Hz).

Compound **10j**: 22% yield from **7**; NMR ($CDCl_3$) δ 1.18 (t, 3, J = 7 Hz), 1.54 (d, 1), 1.59 (s, 3), 1.70 (d, 1), 3.52 (m, 2), 3.52 (q, 2, J = 7 Hz), 4.02 (m, 2), 4.64 (d, 1, J = 4 Hz), 5.28 (AB q, 2), 5.38 (d, 1, J = 7.5 Hz), 5.70 (dd, 1, J = 8.5, 4 Hz), 6.38 (d, 1, J = 8.5 Hz), 7.38 (s, 5), 7.59 (d, 2, J = 8.5 Hz), 8.22 (d, 2, J = 8.5 Hz), 9.94 (d, 1, J = 7.5 Hz).

Compound **10k**: 11% yield from **7**; IR ($CHCl_3$) 3595, 3430, 1722, 1062, 1048 cm^{-1}; MS 703 (M^+ + H), 595 (M^+ + H); NMR ($CDCl_3$) δ 1.59 (d, 1, J = 8 Hz), 1.74 (s, 3), 1.78 (d, 1, J = 8 Hz), 3.50 (m, 2), 3.96 (m, 2), 4.72 (d, 1, J = 6 Hz), 4.85 (s, 1), 5.32 (AB q, 2), 5.38 (d, 1, J = 7 Hz), 5.72 (dd, 1, J = 10, 7 Hz), 6.51 (d, 1, J = 10 Hz), 7.38 (s, 5), 7.62 (d, 2, J = 8 Hz), 8.24 (d, 2, J = 8 Hz), 9.05 (d, 1, J = 8 Hz).

*Preparation of **10** by RCOOAr method*

Dicyclohexylcarbodiimide (1.1 eq.) was added in one portion to the suspension of carboxylic acid and 1-hydroxybenzotriazole hydrate (1.1 eq.) in dichloromethane (5 mL per mmol) with magnetic stirring in an ice–water bath. After 90 min, a dried solution of **9** (1 eq.) in dichloromethane was added to this suspension. The mixture was stirred with cooling in an ice–water bath for 2 h followed by stirring at room temperature for 1 h. The reaction mixture was chromatographed on silica gel to give the desired product.

Compound **10l**: 19% yield from **7**; IR (KBr) 3315, 1800, 1728, 1659, 1522, 1345 cm^{-1}; NMR ($CDCl_3$) δ 1.63 (d, 1, J = 8 Hz), 1.78 (s, 3), 1.80 (d, 1, J = 8 Hz), 3.04 (d, 1, J = 5 Hz), 4.78 (d, 1, J = 5 Hz), 5.12 (d, 1, J = 5 Hz), 5.31 (d, 1, J = 14 Hz), 5.39 (d, 1, J = 14 Hz), 5.73 (dd, 1, J = 12, 5 Hz), 7.04 (d, 1, J = 12 Hz), 7.40 (m, 5), 7.63 (d, 2, J = 9 Hz), 8.75 (d, 2, J = 9 Hz).

Compound **10m**: 42% yield from **7**; IR ($CHCl_3$) 3410, 1801, 1743, 1711, 1528, 1351 cm^{-1}; NMR ($CDCl_3$) δ 1.68 (d, 1, J = 8 Hz), 1.84 (s, 3), 1.85 (d, 1, J = 8 Hz), 3.48 (m, 2), 4.83 (d, 1, J = 6 Hz), 5.38 (AB q, 2), 5.75 (dd, 1, J = 10, 6 Hz), 6.67 (d, 1, J = 10 Hz), 7.55 (d, 2, J = 8 Hz), 8.37 (d, 2, J = 8 Hz).

*Hydrogenolysis of **10***

Using the hydrogenolysis methods as indicated in Table 2, the following compounds were prepared.

Method A. Compound **10** (0.1 mmol) was dissolved in dry tetrahydrofuran (10 mL). To this solution was added 10% palladium on charcoal (60 mg). The suspension was stirred under hydrogen at room temperature and atmospheric pressure until hydrogen uptake stopped. Catalyst was removed by filtration through Celite (pre-washed with tetrahydrofuran), and the filter-cake washed with dry tetrahydrofuran (10 mL). The filtrate and washing were combined and added to a solution of sodium bicarbonate (10 mg, 0.12 mmol) in water (20 mL). This mixture was concentrated under reduced pressure to remove tetrahydrofuran. The resulting aqueous solution was washed with dichloromethane, then further concentrated to about 4 mL under reduced pressure. After filtration, this aqueous solution was purified by reverse-phase high performance liquid chromatography using acetonitrile–water mixtures as solvent. After combining the appropriate fractions and evaporation under reduced pressure to remove acetonitrile, lyophilization gave **11** as a white solid.

Method B. Compound **10** was hydrogenated in the mixture of ethyl acetate and 0.1 M aqueous sodium bicarbonate

(1:1, V/V) over 10% palladium on charcoal until the appropriate volume of hydrogen was consumed. The mixture was filtered through Celite, washed with water and ethyl acetate. The layers were separated. The aqueous portion was washed once with ethyl acetate. The organic layer was extracted once with water. Aqueous extracts were combined, concentrated and chromatographed as in Method A to give pure product.

Method C. Identical to Method A except for substituting methanol–tetrahydrofuran (1:1, V/V) as solvent instead of tetrahydrofuran during the hydrogenolysis.

Method D. Identical to Method A except for substituting dimethylformamide as solvent instead of tetrahydrofuran during the hydrogenolysis.

Method E. Identical to Method A except for substituting methanol as solvent instead of tetrahydrofuran during the hydrogenolysis.

Compound **11a**: 43% yield; NMR (D_2O) δ 1.44 (d, 1, J = 8 Hz), 1.64 (s, 3), 1.67 (d, 1, J = 8 Hz), 5.01 (d, 1, J = 4 Hz), 5.52 (d, 1, J = 4 Hz), 7.03 (d, 2, J = 8 Hz), 7.11 (t, 1, J = 8 Hz), 7.41 (t, 2, J = 8 Hz).

Compound **11b**: 63% yield as a 1:1 mixture of two diastereomers; IR (KBr) 3400, 1762, 1665, 1610 cm^{-1}; MS 421 (M^+ + H), 443 (M^+ + Na); NMR (D_2O) δ 1.42 (2d, 1, J = 8 Hz), 1.67, 1.71 (2s, 3), 1.66, 1.70 (2d, 1, J = 8 Hz), 4.59 (d, 1, J = 4 Hz), 4.72 (s, 1), 5.49 and 5.56 (2d, 1, J = 4 Hz), 7.42 (m, 5).

Compound **11c**: 15% yield; IR (KBr) 3420, 1768, 1665, 1618 cm^{-1}; MS 388 (M^+ + H); NMR (D_2O) δ 1.42 (d, 1, J = 8 Hz), 1.63 (s, 3), 1.64 (d, 1, J = 8 Hz), 4.01 (AB q, 2, J = 17 Hz), 5.43 (d, 1, J = 3 Hz), 7.37 (d, 2, J = 5 Hz), 8.04 (d, 2, J = 5 Hz).

Compound **11d**: 26% yield; IR (KBr) 3410, 1768, 1735, 1698, 1612, 1520 cm^{-1}; MS 421 (M^+ + Na), 399 (M^+ + H); NMR (D_2O) δ 1.23 (d, 1, J = 8 Hz), 1.43 (s, 3), 1.52 (d, 1, J = 8 Hz), 4.82 (m, 1), 5.35 (m, 1), 6.11 (s, 1), 7.40 (m, 5), 8.23 (s, 1).

Compound **11e**: 16% yield; IR (KBr) 3410, 1767, 1673, 1618, 1598 cm^{-1}: MS 401 (M^+ + H); NMR (D_2O) δ 1.45 (d, 1, J = 8 Hz), 1.69 (s, 3), 1.75 (d, 1, J = 8 Hz), 3.89 (s, 6), 5.06 (d, 1, J = 3 Hz), 5.68 (d, 1, J = 3 Hz), 6.80 (d, 2, J = 9 Hz), 7.48 (t, 1, J = 9 Hz).

Compound **11f**: 37% yield; IR (KBr) 3400–3200, 1766, 1685, 1605 cm^{-1}; MS 347 (M^+ + H); NMR (D_2O) δ 1.44 (d, 1, J = 8 Hz), 1.70 (s, 3), 1.71 (d, 1, J = 8 Hz), 5.00 (d, 1, J = 4 Hz), 5.52, (d, 1, J = 4 Hz), 5.56 (m, 2), 9.18 (s, 1).

Compound **11g**: 33% yield; IR (KBr) 3400, 1765, 1661, 1615, 1531 cm^{-1}; MS 398 (M^+ + H of free acid); NMR (Me_2SO-d_6) δ 1.19 (d, 1, J = 8 Hz), 1.45 (d, 1, J = 8 Hz), 1.69 (s, 3), 3.84 (s, 3), 4.82 (d, 1, J = 5 Hz), 5.34 (dd, 1, J = 8, 5 Hz), 6.70 (s, 1), 7.19 (br s, 2), 9.41 (br d, J = 8 Hz).

Compound **11h**: NMR (Me_2SO-d_6) δ 1.26 (d, 1, J = 8 Hz), 1.92 (s, 3), 1.93 (d, l, J = 8 Hz), 1.95 (s, 3), 2.09 (s, 3), 4.89 (m, 1), 5.20 (m, 1), 6.72 (s, 1), 7.16 (s, 2).

Compound **11i**: 61% yield; IR (KBr) 3400, 1765, 1668, 1602, 1518 cm^{-1}; NMR (D_2O) δ 1.46 (d, 1, J = 8 Hz), 1.71 (m, 4), 3.95 (s, 2), 5.00 (d, 1, J = 4 Hz), 5.50 (d, 1, J = 4 Hz), 7.09 (m, 2), 7.42 (m, 1).

Compound **11j**: 71% yield; IR (KBr) 3300, 1770, 1718, 1683, 1612, 1515, 1400, 1370, 1190 cm^{-1}; MS 538.1373 (M^+ + H); NMR (D_2O) δ 1.20 (t, 3, J = 7 Hz), 1.38 (d, 1, J = 8 Hz), 1.58 (s, 3), 1.61 (d, 1, J = 8 Hz), 3.53 (m, 2), 3.72 (m, 2), 4.05 (m, 2), 4.89 (d, 1, J = 4 Hz), 5.49 (s, 1), 5.51 (d, 1, J = 4 Hz), 7.50 (s, 5).

Compound **11k**: 25% yield; IR (KBr) 1765, 1725, 1672, 1615, 1532 cm^{-1}; MS 482 (M^+ + H), 504 (M^+ + Na); NMR (D_2O) δ 1.38 (d, 1, J = 8 Hz), 1.58 (s, 3), 1.60 (d, 1, J = 8 Hz), 3.55 (m, 2), 3.94 (m, 2), 4.90 (d, 1, J = 5 Hz), 5.41 (s, 1), 5.47 (d, 1, J = 5 Hz), 7.48 (s, 5).

Compound **11l**: 37% yield; IR (KBr) 3390, 1765, 1678, 1615, 1520 cm^{-1}; NMR (D_2O) δ 1.44 (d, 1, J = 8 Hz), 1.65 (s, 3), 1.67 (d, 1, J = 8 Hz), 5.00 (d, 1, J = 6 Hz), 5.50 (d, 1, J = 6 Hz), 7.50 (s, 5).

Compound **11m**: 21% yield; IR (KBr) 3400, 2262, 1768, 1680, 1612, 1540 cm^{-1}; MS 304 (M^+ + H); NMR (D_2O) δ 1.11 (d, 1, J = 7 Hz), 1.39 (d, 1, J = 7 Hz), 1.69 (s, 3), 3.77 (AB q, 2), 4.75 (m, 1), 5.25 (m, 1), 9.02 (d, 1, J = 8 Hz).

Preparation of ***12***

To a stirred solution of **7** (93.0 mg, 0.2 mmol) in anhydrous dichloromethane (1 mL) at -30 °C under an argon atmosphere, was simultaneously added *tert*-butyl hypo-chlorite (32.3 mg, 0.27 mmol) and a methanolic lithium methoxide solution (170 μL containing 8 mg of LiOMe, 0.2 mmol). After stirring at -30 °C for 30 min, the mixture was purified by high performance liquid chromatography (Whatman M9 Partisil 10, linear gradient 25%–70% solvent mixture B in solvent mixture A; A = isooctane–dichloromethane, 1:2, V/V; B = ethyl acetate–dichloromethane, 1:2, V/V) to afford recovered starting material **7** (23 mg), a mixture (12 mg) of β-lactam ring opened methyl esters of product and starting material, and the desired methoxylated product **12** (27 mg, 36% based on unrecovered starting material) as a pale yellow, tacky solid: NMR ($CDCl_3$) δ 1.57 (d, 1, J = 8 Hz), 1.60 (s, 3), 1.63 (d, 1, J = 8 Hz), 3.30 (s, 3), 3.56 (s, 2), 4.81 (s, 1), 5.24 (s, 2), 6.39 (br s, 1), 7.21 (m, 5), 7.48 (d, 2, J = 8 Hz), 8.09 (d, 2, J = 8 Hz).

Preparation of ***13***

Compound **12** (25 mg, 0.05 mmol) was hydrogenated by Method C to give, after workup and chromatography, **13** (15.4 mg, 80%) as a white lyophilized powder: m.p. 159–161 °C; IR (KBr) 3650–2800, 1763, 1675, 1618, 1400, 1040 cm^{-1}; MS 407 (M^+ + Na); NMR (D_2O) δ 1.51 (d, 1,

J = 8 Hz), 1.54 (s, 3), 1.57 (d, 1, J = 8 Hz), 3.49 (s, 3), 3.75 (s, 2), 5.03 (s, 1), 7.40 (m, 5).

Preparation of ***15***

To a solution of crude **9** (prepared from 186.8 mg of **7**, 0.4 mmol) in dichloromethane was added a solution of *p*-toluenesulfonic acid monohydrate (57.0 mg, 0.3 mmol), and triethylamine (42 μL, 0.3 mmol) in dichloromethane (2.0 mL). To the resulting mixture was added **14** (89% pure, 520 mg, 2.67 mmol) at 0 °C. The reaction mixture was stirred at 0 °C overnight under an argon atmosphere. The solvent was removed at 10 °C/10 mm mercury pressure. The crude residue was extracted twice with ethyl acetate and backwashed three times with water, once with saturated aqueous solution of sodium bicarbonate, twice with water. The ethyl acetate extracts were combined, dried over anhydrous sodium sulfate, then filtered. The filtrate was concentrated to dryness under reduced pressure to afford crude **15** as a dark oil.

Preparation of ***16***

Crude **15** was hydrogenated by Method B to give, after workup and chromatography, pure **16** (16.5 mg, 12% overall yield from **7**) as a while lyophilized solid: NMR (D_2O) δ 1.51 (d, 1, J = 8 Hz), 1.63 (m, 4), 1.67 (s, 3), 1.70 (d, 1, J = 8 Hz), 1.86 (m, 4), 3.56 (m, 2), 3.73 (m, 2), 5.05 (d, 1, J = 5 Hz), 5.33 (d, 1, J = 5 Hz), 8.05 (s, 1).

Preparation of ***17***

The cooled (ice–water bath) solution of **7** (50.0 mg, 0.106 mmol) in dichloromethane (3.0 mL) was treated with *meta*chloroperbenzoic acid (22.7 mg 0.131 mmol). The reaction was stirred for 80 min then treated with two additional portions of per acid (4.2 and 4.8 mg respectively) 15 min apart. After 10 min additional stirring, the solution was washed three times with saturated aqueous sodium bicarbonate solution (1 mL each), and three times with saturated aqueous sodium sulfite solution (1 mL each). The dichloromethane solution was dried (magnesium sulfate), filtered, and concentrated under reduced pressure. This residue was chromatographed on silica gel to give pure **17** (44.4 mg, 85%): NMR ($CDCl_3$) δ 1.48 (d, 1, J = 8 Hz), 1.67 (d, 1, J = 8 Hz), 1.97 (s, 3), 3.58 (s, 2), 4.37 (d, 1, J = 5 Hz), 5.36 (d, 1, J = 18 Hz), 5.43 (d, 1, J = 18 Hz), 6.18 (dd, 1, J = 12, 5 Hz), 7.30 (m, 5), 7.65 (d, 2, J = 10 Hz), 8.26 (d, 2, J = 10 Hz).

Preparation of ***18***

Compound **17** (43.9 mg, 0.09 mmol) was hydrogenated by Method E to give, after workup and chromatography **18** (11.7 mg, 34%) as a white lyophilized solid: IR (KBr) 3450, 1788, 1685, 1618 cm^{-1}; MS 371 (M^+ + H); NMR (D_2O) δ 1.56 (d, 1, J = 8 Hz), 1.67 (d, 1, J = 8 Hz), 1.82 (s, 3), 3.73 (s, 2), 4.80 (d, 1, J = 3 Hz), 5.96 (d, 1, J = 3 Hz), 7.40 (m, 5).

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