SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 9-(2-AMIDOETHENYLTHIO)-9-DEOXY DERIVATIVES OF CLAVULANIC ACID

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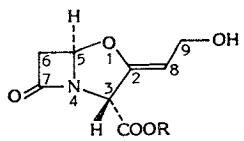
The reaction of activated derivatives of clavulanic acid with substituted amidoethenyl thiolates to give 9-(2-amidoethenylthio)-9-deoxy derivatives is described; the antibacterial/synergistic and β-lactamase inhibitory activities of the thioethers and their corresponding sulfoxides and sulfones are compared.

Clavulanic acid (**1**) is a naturally occurring β-lactamase inhibitor which acts as a synergist for penicillins and cephalosporins, increasing their activity against β-lactamase producing strains of bacteria such as *Escherichia coli*, *Klebsiella aerogenes* and *Staphylococcus aureus*[1]. Since its discovery there has been much interest in preparing analogues and derivatives[2] of this microbial metabolite in the hope of finding new compounds with enhanced biological activity. As part of a program designed to prepare new derivatives of **1** we have prepared a number of substituted amidoethenylthio derivatives and their corresponding sulfoxides and sulfones.

Chemistry

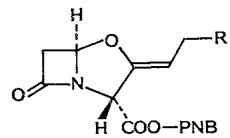
The syntheses of 9-(2-amidoethenylthio)-9-deoxy derivatives of clavulanic acid (**1**) are outlined in Scheme 1 and the compounds prepared are summarised in Table 1. Reaction of activated derivatives of clavulanic acid, such as the chloride (**3**)[3] and the dichloroacetate (**4**)[4], with the appropriate thiolates (**13a**~**13f**) afforded thioethers (**5a**~**5f**) as mixtures of *Z* and *E* isomers, which were separated by column chromatography. Oxidation of the thioethers with 1.1 equivalents of *m*-chloroperoxybenzoic acid gave the sulfoxides (**6a** and **6b**) whilst 2.2 equivalents of *m*-chloroperoxybenzoic acid provided the sulfones (**7a**, **7b**, **7d** and **7f**).

The *p*-nitrobenzyl protecting group of the thioethers (**5a**~**5f**) was removed by iron-ammonium chloride dissolving metal reduction. This method failed in the case of the sulfoxides (**6a** and **6b**) and the removal of the protecting group was accomplished by hydrogenolysis over palladium on charcoal in tetrahydrofuran solution. The sulfones (**7a**, **7b**, **7d** and **7f**) were also deprotected using iron-am-



1 R = H

2 R = PNB

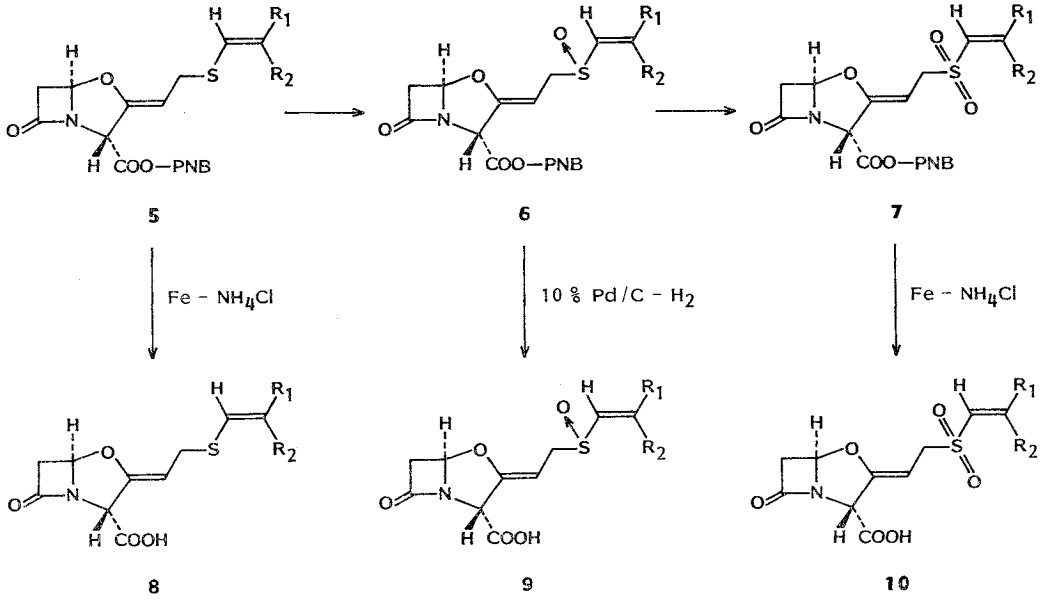


3 R = Cl

4 R = $OCOCHCl_2$

PNB=*p*-Nitrobenzyl

Scheme 1. Preparation of 9-(2-amidoethenylthio)-9-deoxy clavulanic acid derivatives.



PNB=*p*-Nitrobenzyl

monium chloride dissolving metal reduction. The products were isolated as their lithium or sodium salts by neutralising an aqueous solution of the free acids (**8a**~**8f**, **9a**, **9b**, **10a**, **10b**, **10d** and **10f**) with 0.1 M aqueous lithium carbonate or 0.1 M sodium bicarbonate and freeze-drying the aqueous solution and were sufficiently pure for biological testing.

Thiolate (**13a**) was prepared by cleavage of 2,3-dihydro-4*H*-(1,4-thiazin-3-one) (**14**) using sodium in liquid ammonia[5]. A more general and convenient route to the thiolates (**13a**~**13f**) is outlined in Scheme 2[6]. Reaction of bromoacetaldehyde diethyl acetal with ethanethiol and sodium ethoxide in ethanol gave 1,1-diethoxy-2-ethylthioethane (**11**), which on acid catalysed (*p*-toluene sulfonic acid) reaction with the appropriate amide gave the unsaturated sulfides (**12a**~**12f**) as mixtures of *Z* and *E* isomers (compounds **12c** and **12d** could be separated by column chromatography at this stage). Cleavage of the sulfides with sodium in liquid ammonia gave the sodium thiolates (**13a**~**13f**).

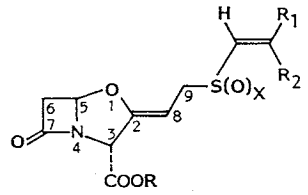
The numbering follows that used in penicillins, shown in **1**.

Biology

The biological activities of these derivatives are given in Table 2. All derivatives showed poor inhibitory activity against the cephalosporinase of *Enterobacter cloacae*, but all were potent inhibitors of TEM-1 and staphylococcal β-lactamases, with activity similar to that of clavulanic acid.

All were equally effective synergists of amoxycillin *in vitro* against *S. aureus*, but differences could be seen against a TEM-1 producing *E. coli* presumably reflecting different rates of penetration into the bacterial periplasm. The four sulfones (**10a**, **10b**, **10d** and **10f**) were 2- to 4-fold more active than their corresponding sulfides (**8a**, **8b**, **8d** and **8f**) with the two sulfoxides giving a somewhat mixed spectrum of activity; the *E* isomer (**9b**) was as active as the sulfone analogue (**10b**) while the *Z* isomer (**9a**) was much less active.

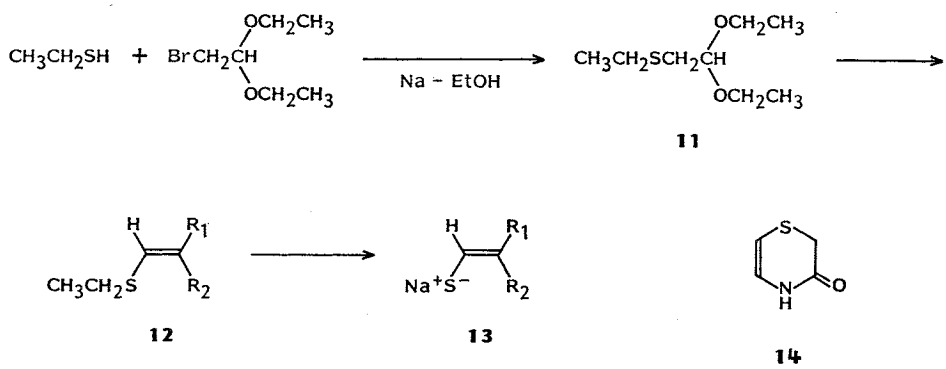
Table 1. 9-(2-Amidoethenylthio)-9-deoxy derivatives of clavulanic acid.



Compound No.	R	R_1	R_2	X
5a	PNB	H	$NHCOCH_3$	0
5b	PNB	$NHCOCH_3$	H	0
5c	PNB	H	NHCHO	0
5d	PNB	NHCHO	H	0
5e	PNB	H	$NHCOCH_2CH_3$	0
5f	PNB	$NHCOCH_2CH_3$	H	0
6a	PNB	H	$NHCOCH_3$	1
6b	PNB	$NHCOCH_3$	H	1
7a	PNB	H	$NHCOCH_3$	2
7b	PNB	$NHCOCH_3$	H	2
7d	PNB	NHCHO	H	2
7f	PNB	$NHCOCH_2CH_3$	H	2
8a	H	H	$NHCOCH_3$	0
8b	H	$NHCOCH_3$	H	0
8c	H	H	NHCHO	0
8d	H	NHCHO	H	0
8e	H	H	$NHCOCH_2CH_3$	0
8f	H	$NHCOCH_2CH_3$	H	0
9a	H	H	$NHCOCH_3$	1
9b	H	$NHCOCH_3$	H	1
10a	H	H	$NHCOCH_3$	2
10b	H	$NHCOCH_3$	H	2
10d	H	NHCHO	H	2
10f	H	$NHCOCH_2CH_3$	H	2

PNB: *p*-Nitrobenzyl.

Scheme 2. Preparation of amidoethenyl thiolates.



For **12** and **13**

a	R_1=H	R_2=$NHCOCH_3$	**d**	R_1=NHCHO	R_2=H
b	R_1=$NHCOCH_3$	R_2=H	**e**	R_1=H	R_2=$NHCOCH_2CH_3$
c	R_1=H	R_2=NHCHO	**f**	R_1=$NHCOCH_2CH_3$	R_2=H

Table 2. Synergistic and β-lactamase inhibitory activity.

Compound	I_{50} (μg/ml)			Amoxycillin MICs (μg/ml) in presence of 1 μg/ml inhibitor		*In vivo* relative potency[a]
	Staphylococcus aureus	TEM-1	*Enterobacter cloacae*	*S. aureus* Russell	*Escherichia coli* TEM-1	
8a	0.02	0.01	42	NT	16	0.18
8b	0.14	0.07	50	0.16	16	2.78
8c	0.03	0.05	47	0.31	16	0.23
8d	0.03	0.06	23	0.31	8	1.14
8e	0.03	0.05	20	0.62	512	<0.13
8f	0.03	0.02	45	0.62	16	0.13
9a	0.09	0.09	20	1.25	512	0.25
9b	0.05	0.03	6	0.62	4	0.88
10a	0.01	0.05	18	NT	8	2.70
10b	0.03	0.01	50	0.31	4	4.00
10d	0.05	0.06	39	0.31	4	1.60
10f	0.02	0.03	37	0.62	4	1.25
Clavulanic acid	0.03	0.03	30	0.31	32	1.00
Amoxycillin alone				125.00	>512	<0.13

[a] (CD_{50} Amox + clavulanic acid)/(CD_{50} Amox + compound) against a TEM-1 producing *E. coli* in mice.
NT: Not tested.

In vivo, the differences in potency were much clearer. The sulfones were once again more potent than the corresponding sulfoxides or sulfides, and the *E* isomers (**8b**, **8d**, **8f**, **9b** and **10b**) were more active than the corresponding *Z* isomers (**8a**, **8c**, **8e**, **9a** and **10a**). In two cases (**8a** vs. **8b** and **9a** vs. **9b**), the relative activities of the *E* and *Z* isomers *in vitro* were not reflected *in vivo*, perhaps due to differing distribution characteristics or metabolic stability of the isomer pairs. Optimal activity was seen with the *E*-acetamido derivative (**10b**).

Experimental

β-Lactamase inhibition studies were carried out on isolated enzyme preparations by spectrophotometric monitoring of hydrolysis of a standard (ampicillin) in the presence and absence of a β-lactamase inhibitor[7].

MIC determinations were carried out in Microtiter plates by serial dilution of amoxycillin (Amox) in broth, followed by addition of inhibitor (1 μg/ml) and organism (approx 2×10^6 cfu/ml), as described previously[1].

The 50%-curative dose (CD_{50}) determinations were performed in mice. The organism (*E. coli* E96) was suspended in 3% hog gastric mucin+1% carboxymethyl cellulose at $100\times LD_{50}$, and 0.5 ml of suspension was injected ip into groups of five mice. Compounds were administered subcutaneously at 2 mg/kg with varying doses of amoxycillin at 1 and 5 hours post infection, and survivors were recorded over a 4-day period. The CD_{50} of amoxycillin in the presence of 2 mg/kg inhibitor was calculated by log probit analysis, and relative potencies were defined as (CD_{50} Amox+2 mg/kg clavulanic acid)/(CD_{50} Amox+2 mg/kg derivative). This test had a high level of reproducibility with the amoxycillin/clavulanic acid CD_{50} of 30 ± 13 mg/kg.

UV spectra were recorded on a Pye-Unicam SP 8000 or a Perkin-Elmer 554 spectrophotometer. Unless otherwise stated IR spectra were recorded for solutions in $CHCl_3$ on a Perkin-Elmer 197 or 457 machine. ^1H NMR spectra were recorded at 60 MHz on a Varian EM 360 and at 90 MHz on a Perkin-Elmer R32 instrument for solutions in $CDCl_3$ with TMS as internal standard unless otherwise stated. The purity of all compounds was tested by TLC on Merck precoated Silica gel 60 F_{254} plates.

Preparative chromatography was carried out on columns of Merck Silica gel 60 (230~400 mesh ASTM) with eluants as stated using the slightly increased pressure provided by a Medcalf Hy-flo pump. MP's were determined with a Kofler hot-stage apparatus and are uncorrected. Compounds **2**[4], **3**[3], **4**[4], **13a** and **14**[5] were prepared by published procedures and the syntheses of the amidoethenyl thiolates (**13a**~**13f**) may be found in the patent literature[6]. The following general synthetic procedures illustrate the methods used to prepare 9-(2-amidoethenylthio)-9-deoxy derivatives of clavulanic acid and further examples are described in ref 6.

General Procedure for the Preparation of Thioethers (**5a**~**5f**)

The preparation of thioethers (**5a** and **5b**) was typical of the method used.

A 1:1 mixture of sodium *Z*-2-acetamidovinylthiolate and sodium *E*-2-acetamidovinylthiolate (**13a** and **13b**) (4.15 g, 0.03 mol) was added to a stirred, ice-cooled solution of *p*-nitrobenzyl 9-chloro-9-deoxyclavulanate (**3**) (6.78 g, 0.02 mol) in dry DMF (200 ml) and stirring continued for 45 minutes. EtOAc (400 ml) was added and the mixture washed with water (3×300 ml), dried over magnesium sulfate and evaporated. Chromatography of the residue using EtOAc - petrol (bp 60~80°C) as eluent gave as the less polar product (1.33 g, 16%) the *Z* isomer (**5a**) as a yellow foam: $[\alpha]_D^{20}$ −19.2° (*c* 0.8, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3430, 1808, 1760, 1692, 1627; UV λ_{max}^{dioxan} nm (ε) 266.5 (17,800); ^1H NMR (90 MHz, $CDCl_3$) δ 2.07 (3H, s, $COCH_3$), 3.05 (1H, d, *J*=17 Hz, 6β-CH), 3.32 (2H, d, *J*=8 Hz, 9-CH_2), 3.51 (1H, dd, *J*=17 and 2.5 Hz, 6α-CH), 4.72 (1H, dt, *J*=8 and 1 Hz, 8-CH), 5.0~5.4 (4H, m, 3-CH, SCH= and CH_2Ar), 5.68 (1H, d, *J*=2.5 Hz, 5-CH), 7.04 (1H, dd, *J*=11.5 and 8 Hz, NHC*H*=), 7.4~7.8 (3H, m, NH and 2Ar-H), 8.21 (2H, d, 2Ar-H). The more polar product was the *E* isomer (**5b**) (1.78 g, 21%) as a yellow foam: $[\alpha]_D^{20}$ −19.2° (*c* 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3460, 1807, 1758, 1695, 1623; UV λ_{max}^{EtOH} nm (ε) 260 (sh, 13,800), 269 (17,400); ^1H NMR (90 MHz, $CDCl_3$) δ 1.97 (3H, s, $COCH_3$), 3.07 (1H, d, *J*=17 Hz, 6β-CH), 3.27 (2H, d, *J*=8 Hz, 9-CH_2), 3.49 (1H, dd, *J*=17 and 2.5 Hz, 6α-CH), 4.71 (1H, t, *J*=8 Hz, 8-CH), 5.11 (1H, br s, 3-CH), 5.29 (2H, s, CH_2Ar), 5.5~5.8 (2H, m, 5-CH and SCH=), 6.97 (1H, dd, *J*=14 and 10 Hz, =C*H*NH), 7.53 and 8.31 (4H, 2d, Ar-H), 8.57 (1H, d, *J*=10 Hz, NH); MS *m/z* 433.0949 (M^+, calcd for $C_{19}H_{19}N_3O_7S$ 433.0958).

Alternative Preparation of Thioether (**5a**)

Sodium *Z*-2-acetamidovinylthiolate (**13a**) (0.35 g, 1.77 mmol as a 1:1 mixture with NaCl) was added to a stirred, ice-cooled solution of **3** (0.63 g, 1.78 mmol) in dry DMF (20 ml) and stirring continued for 30 minutes. EtOAc (100 ml) was added and the mixture washed with water (2×100 ml), dried over anhydrous magnesium sulfate and evaporated. The residue was chromatographed using EtOAc - petrol (bp 60~80°C) as eluent to give, as a pale yellow foam (0.40 g, 52%), a compound identical in all respects to the *Z* isomer (**5a**) described above.

Preparation of the Sulfoxides (**6a** and **6b**)

The ester (**5a**) (0.25 g) was dissolved in dichloromethane (20 ml), ice-cooled with stirring and treated dropwise over 5 minutes with a solution of *m*-chloroperoxybenzoic acid (0.10 g) in dichloromethane (5 ml). After stirring for 30 minutes the solution was washed with 1 M sodium bicarbonate (2×20 ml) and water (20 ml), dried over $MgSO_4$ and evaporated. Chromatography of the residue on silica gel using EtOAc - petrol (bp 60~80°C) as eluent gave the sulfoxide (**6a**) (0.12 g, 46%) as a colourless foam: $[\alpha]_D^{20}$ −12.2° (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3300, 1810, 1758, 1705(s), 1692, 1630; UV λ_{max}^{dioxan} nm (ε) 268 (19,320); ^1H NMR (90 MHz, $CDCl_3$) δ 2.05 (3H, s, $COCH_3$), 3.08 and 3.11 (1H, 2d, *J*=17 Hz, 6β-CH), 3.4~4.0 (3H, m, 6α-CH and 9-CH_2), 4.7~5.1 (2H, m, 8-CH and SOCH=), 5.20 (1H, s, 3-CH), 5.27 (2H, s, CH_2Ar), 5.73 (1H, d, *J*=2.5 Hz, 5-CH), 7.3~7.6 (3H, m, 2Ar-H and =C*H*NH), 8.22 (2H, d, 2Ar-H), 10.30 (1H, d, *J*=11 Hz, NH). An analogous procedure employing the *E* isomer (**5b**) provided the *E* sulfoxide (**6b**) as a colourless foam (69%).

General Procedure for the Preparation of Sulfones (**7a**, **7b**, **7d** and **7f**)

The preparation of the sulfone (**7a**) was typical.

A stirred, ice-cooled solution of the ester (**5a**) (0.60 g) in dichloromethane (50 ml) was treated dropwise over 10 minutes with a solution of *m*-chloroperoxybenzoic acid (0.50 g) in dichloromethane

(10 ml). The mixture was stirred at 0~5°C for 2 hours, washed with 1 M aqueous sodium bicarbonate solution (2×50 ml) and water (50 ml), dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel using EtOAc - petrol (bp 60~80°C) as eluent to give the sulfone (**7a**) as a white microcrystalline solid (0.39 g, 60%): MP 138~140°C; $[\alpha]_D^{20}$ −8.9° (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3380, 1810, 1758, 1722, 1698(s), 1629; UV λ_{max}^{dioxan} nm (ε) 257 (22,580); ^1H NMR (90 MHz, DMSO-d_6) δ 2.09 (3H, s, $COCH_3$), 3.10 (1H, d, *J*=17 Hz, 6β-CH), 3.67 (1H, dd, *J*=17 and 3 Hz, 6α-CH), 3.94 (2H, d, *J*=8 Hz, 9-CH_2), 4.78 (1H, t, *J*=8 Hz, 8-CH), 5.33 (2H, s, CH_2Ar), 5.45 (1H, d, *J*=9.5 Hz, SO_2CH=), 5.54 (1H, s, 3-CH), 5.77 (1H, d, *J*=3 Hz, 5-CH), 7.45 (1H, dd, *J*=12 and 9.5 Hz, CH=NH), 7.64 and 8.23 (4H, 2d, Ar-H), 9.67 (1H, d, *J*=12 Hz, NH).

Anal Calcd for $C_{19}H_{19}N_3O_9S$: C 49.0, H 4.1, N 9.0, S 6.9.
Found: C 49.0, H 4.4, N 9.0, S 6.9.

Similarly, thio ethers (**5b**, **5d** and **5f**) were converted into their corresponding sulfones (**7b**, **7d** and **7f**).

General Procedure for De-esterification of Thioethers (**5a~5f**) and Sulfones (**7a**, **7b**, **7d** and **7f**) with Iron-ammonium Chloride

The de-esterification of **5a** was typical.

A solution of the ester (**5a**) (0.143 g, 0.33 mmol) was dissolved in THF (14 ml) with stirring, ice-cooled and treated with 1 M aqueous ammonium chloride solution (4 ml) and iron powder (0.5 g). After 20 minutes a further 0.3 ml of 1 M aqueous ammonium chloride solution and 0.5 g of iron powder were added and stirring continued for 45 minutes. EtOAc (20 ml) was added and H_2S bubbled through the mixture for 10 minutes with ice-cooling. The mixture was filtered through Celite and the residue washed with water (20 ml). The aqueous layer of the filtrate (including washings) was saturated with sodium chloride, acidified with 1 N HCl to pH 2.5 and separated from the organic layer. The aqueous layer was further extracted with EtOAc (2×20 ml) and the combined EtOAc extracts dried over $MgSO_4$. After filtration the EtOAc was extracted with pH 7 phosphate buffer (3×20 ml). The combined aqueous extracts were saturated with sodium chloride, acidified to pH 2.5 with 1 N HCl and extracted with EtOAc (3×20 ml). The EtOAc extracts were dried over $MgSO_4$, evaporated, and the residue quickly taken up in THF (10 ml) and water (10 ml). This solution was brought to pH 7 by addition of 0.1 M lithium carbonate solution, washed with ether (10 ml) and freeze-dried to give the lithium salt (**8a**) (0.054 g, 54%) as a pale yellow solid: $[\alpha]_D^{20}$ +49.5° (*c* 0.7, H_2O); IR ν_{max} (KBr) cm^{-1} 1780, 1670(s), 1620; UV $\lambda_{max}^{H_2O}$ nm (ε) 224 (10,210), 268 (9,010); ^1H NMR (90 MHz, D_2O) δ 2.10 (3H, s, $COCH_3$), 3.06 (1H, d, *J*=17.5 Hz, 6β-CH), 3.2~3.7 (3H, m, 6α-CH and 9-CH_2), 4.5~5.1 (2H, m, 3-CH and 8-CH), 5.50 (1H, d, *J*=8 Hz, SCH=), 5.68 (1H, d, *J*=2.5 Hz, 5-CH), 6.86 (1H, d, *J*=8 Hz, =CHNH). Thioethers (**5b~5f**) and sulfones (**7a**, **7b**, **7d** and **7f**) were deprotected using analogous procedures.

De-esterification of Sulfoxides (**6a** and **6b**)

A solution of the ester (**6a**) (0.138 g) in THF (50 ml) was added to a prehydrogenated suspension of 10% palladium on charcoal (0.20 g) in THF (50 ml). Hydrogenation at 1 atmosphere was continued for 2 hours, the suspension filtered through Celite and the filtrate evaporated to 5 ml. Water (20 ml) was added followed by a solution of 0.1 M lithium carbonate (1.53 ml). The solution was washed with ether (3×20 ml), adjusted to pH 7 with 0.5 N HCl and freeze-dried to give the lithium salt of **9a** as a yellow solid (0.07 g, 71%): $[\alpha]_D^{20}$ +36.8° (*c* 0.8, DMSO); IR ν_{max} (KBr) cm^{-1} 1783, 1685, 1620; UV $\lambda_{max}^{H_2O}$ nm (ε) 236 (10,400); ^1H NMR (90 MHz, D_2O) δ 2.06 (3H, s, $COCH_3$), 3.04 (1H, d, *J*=17.5 Hz, 6β-CH), 3.52 (1H, dd, *J*=17.5 and 2.5 Hz, 6α-CH), 3.75 (2H, br d, *J*=8 Hz, 9-CH_2), 5.55 (1H, d, *J*=8 Hz, SOCH=), 5.69 (1H, br s, 5-CH), 7.34 (1H, d, *J*=8 Hz, =CHNH), 8-CH and 3-CH obscured by DOH. An analogous procedure using the *E* isomer (**6b**) gave the *E* sulfoxide lithium salt **9b**.

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