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4-ALKYLATED MONOBACTAMS

CHIRAL SYNTHESIS AND ANTIBACTERIAL ACTIVITY

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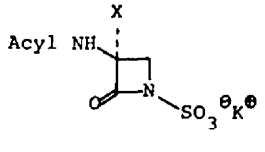
Abstract—The synthesis of 4-alkylated monobactams by a variety of procedures is described. Two complementary procedures have been developed for the chiral synthesis of monobactams: (1) sulfonation of 4-alkyl-3-(protected)amino-2-azetidinones with various complexes of SO_3; and (2) cyclization of β-mesyloxyacyl sulfamates derived from β-alkyl-β-hydroxy-α-amino acids. The most general procedure involves introduction of the alkyl group via a Grignard reaction on 6-APA-derived sulfones **23** or **24** followed by sulfonation. For the specific case of (3*S*,*trans*)-3-amino-4-methylmonobactamic acid (**48**), cyclization of the β-mesyloxyacyl sulfamate **40** derived from (L)-threonine is the preferred route. The introduction of 4-alkyl groups into monobactams results in a decrease in activity against gram-positive bacteria, an increase in activity against gram-negative bacteria, and an increase in β-lactamase stability. Increasing the size of the alkyl group beyond methyl results in diminished intrinsic antibacterial activity. 4β-Alkylmonobactams display better β-lactamase stability than their 4α-counterparts.

INTRODUCTION

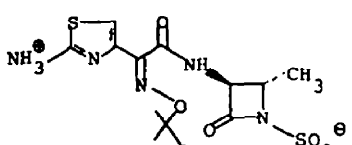
The monobactams are monocyclic β-lactam antibiotics produced by various gram-negative bacteria and characterized by the 2-oxoazetidine-1-sulfonic acid moiety.[1] None of the eight naturally-occurring monobactams[2-6] (general structure **1**) has proved a useful source of material for semi-synthetic modification. This unusual circumstance provided an opportunity for total synthesis to make early and important contributions to monobactam research. The structure and absolute configuration of representative methoxylated and nonmethoxylated naturally-occurring monobactams were proved by total synthesis.[3,4] The mechanism of action of monobactams was explored with the aid of radiolabelled, totally-synthetic derivatives.[7,8] The wide range of structural types made available by total synthesis led to rapid progress in the identification of monobactams with excellent antibacterial activity. One of these, aztreonam (**2**), is currently being studied in the clinic on the basis of its potent and specific activity against gram-negative bacteria and high β-lactamase stability.[9]

The presence of a 4α-Me group on the monobactam nucleus of aztreonam contributed substantially to its biological properties.[10] The synthesis of analogs of **2** with homologous alkyl groups was undertaken for comparison.[11] We have utilized two general strategies for the preparation of monobactams. The first (Path A) relies on the sulfonation of an N-1 unsubstituted azetidinone to introduce the N-sulfonic acid moiety onto a preformed azetidinone[12] ring. The second (Path B) creates the 2-oxoazetidine-1-sulfonic acid moiety by the cyclization of a β-mesyloxyacyl sulfamate.[13]

This paper will detail and evaluate the methods we



1, X = H, OCH_3

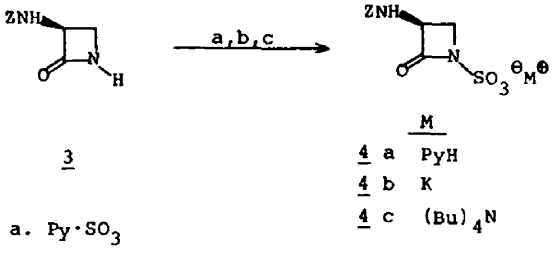


2, aztreonam

have used to prepare nonmethoxylated monobactams and discuss the key relationships between their structures and biologic activities.

Synthesis of monobactams

Three related procedures, illustrated in Scheme 1 with (3*S*)-benzyloxycarbonylamino-2-azetidinone

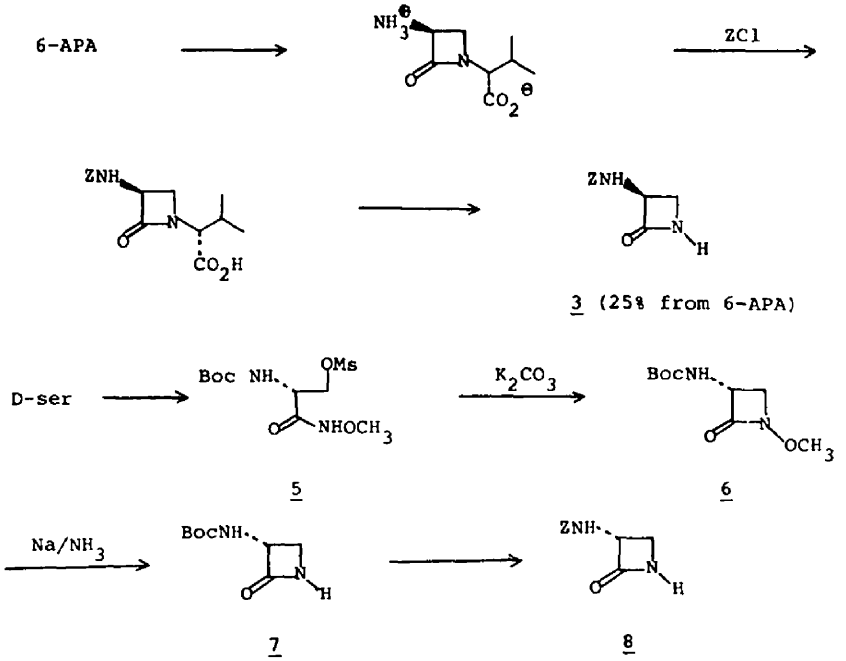


Scheme 1.

(**3**), have been developed for the key N-1 sulfonation required for Path A. The simplest utilizes pyridine–sulfur trioxide (Py–SO_3) complex as the sulfonating agent and results in an essentially quantitative yield of pyridinium salt **4a** after 2 hr in DMF–CH_2Cl_2 (1:1). Various 4-alkylated analogs of **3** react much more slowly under these conditions. The use of excess Py–SO_3, extended reaction times, and elevated temperatures are expedients that we have found useful in such cases. This reaction was independently developed and extensively applied to 3-acylamino-2-azetidinones by the Takeda group.[14] A second procedure relies on chemoselective silylation of azetidinone **3** to give the N-1 silyl derivative which reacts with trimethylsilyl chlorosulfonate at low temperature. The presumed monobactam trimethylsilyl ester is hydrolyzed in buffer solution to afford monobactam potassium salt **4b**. Recently, the Takeda group described, in the patent literature,[15] the Py–SO_3 sulfonation of N-1 t-butyldimethylsilylazetidinones. These procedures are precedented by the acylation of N-1 silylazetidinones.[16] The third and most general procedure we have discovered utilizes the highly reactive sulfonating agent DMF–SO_3 in DMF solution, conveniently prepared by addition of trimethylsilyl chlorosulfonate to DMF,[17] followed by removal *in vacuo* of trimethylsilyl chloride. This reagent sulfonates a range of 3-azido, 3-acylamino- and 3-alkoxycarbonylamino-2-azetidinones and their 4-alkyl derivatives in moderate to excellent yield and has been utilized in the synthesis of SQ 26,180,[3] a naturally-occurring methoxylated monobactam. A convenient workup for this (or any) sulfonation involves addition to 0.5 M KH_2PO_4, followed by addition of an equivalent of *tetra*-n-bytylammonium ion (usually the bisulfate) and the extraction of the ion-paired product into dichloromethane.

With reliable procedures for sulfonation in hand, the synthesis of monobactams was reduced to the preparation of N-1 unsubstituted azetidinones carrying functionality at C-3 compatible with sulfonation conditions and readily convertable to the required (3*S*)-amino group. For the synthesis of naturally-occurring and other 4-unsubstituted monobactams we have utilized 6-APA as a chiral synthon[12] and converted it to (3*S*)-benzyloxycarbonylamino-2-azetidinone (**3**) by a route (Scheme 2), adapted from the procedures of Moll[18] and Kamiya,[19] applicable to kilogram scale.

(3*R*)-Acylaminomonobactams, enantiomeric with the naturally-occurring nonmethoxylated monobactam[4] and those derived from 6-APA, were prepared from D-serine utilizing acyl-hydroxamate methodology[20] modified from the original work of Miller.[21] Conversion of D-serine to mesylate **5** fol-



Scheme 2.

lowed by cyclization (K_2CO_3/acetone) gave N-methoxyazetidinone **6**. Reduction of **6** with Na/NH_3 gave N-1 unsubstituted azetidinone **7**, which was converted to **8** by protecting group interchange.

The conversion of L-threonine (**9**) to 4α-methyl azetidinone **12**, via N-hydroxyazetidinone **15**, was accomplished as outlined by Miller.[21] We have modified the $TiCl_3$ reduction of **15** by inclusion of NH_4OAc buffer as suggested by Miller (this precludes the use of a pH-stat and is particularly useful for small scale work). We have used the modified hydroxamate methodology[20] to prepare 4β-methyl azetidinone **13** from L-*allo*-threonine (**10**) as well as to prepare **12** from L-threonine on a multi-kilo scale.[20]

The favorable effect of 4-monomethylation on biological activity[10] suggested the synthesis of the 4,4-dimethyl analog. Uncertain that the cyclization of **16** (Scheme 3) would be feasible, we chose 4,4-dimethyl-2-azetidinone **17**, readily available in racemic form by [2 + 2]-cycloaddition,[23] as starting material (Scheme 4). After conversion to the t-butyldiphenylsilyl derivative **18**, a 3-azido group was introduced with tosylazide.[24] Deprotection with fluoride, followed by sulfonation with DMF–SO_3 gave the required (±)-3-azido-4,4-dimethylmonobactamate **21** in overall 16% yield. The poor yield of *racemic* product obtained in this sequence deterred us from applying it widely.

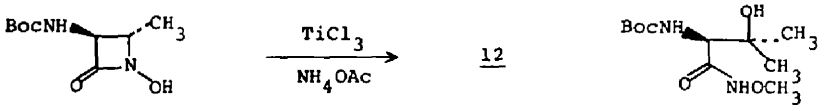
For comparison to 4-monomethylated analogs we have prepared homologous monoalkylated derivatives by two procedures. Racemic 4α-ethylazetidinone **14** (Scheme 3) was prepared from (±)-*threo*-2-amino-3-hydroxypentanoic acid by acyl hydroxamate methodology.[20] Sulfonation of **14** was accomplished with freshly prepared Py–SO_3 in pyridine at 90°. Even with the highly stereoselective syntheses of the requisite β-alkyl-β-hydroxy-α-amino acids,[25] this route is lengthy and, without an added resolution step, produces racemic products. This led us to consider 6-APA (**22**) as a chiral synthon for 4-alkylated monobactams. When we began this work few conversion of penicillins to 4-alkylated azetidinones had been described; within the last two years several strategies have been utilized.[26]

	R	
17	H	19
18	$Si(Ph_2)_2C(CH_3)_3$	20
	$SO_3^{\ominus}(Bu)_4N^{\oplus}$	21

Scheme 4.

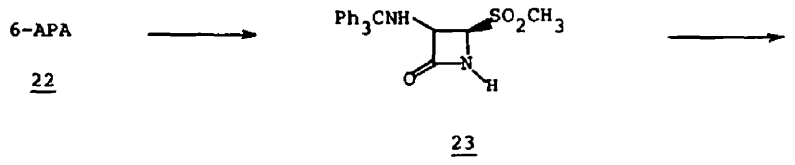
We chose the methyl sulfone **23**, simply prepared from 6-APA,[27] as starting material with the expectation that predominant (or exclusive) substitution *trans* to the large (3S)-tritylamino moiety would occur. Initial experiments with methyl magnesium halides suggested that deprotonation of the methyl sulfone group was a facile and competing process. This suggested the use of less basic acetylenic Grignard reagents. Reaction of **23** with one equivalent of methyl magnesium bromide (to deprotonate the azetidinone NH and conserve trimethylsilyl acetylene), followed by the Grignard reagent prepared from trimethylsilyl acetylene and methyl magnesium bromide, gave an easily separable mixture of 4β- (22%) and 4α- (30%) trimethylsilylethynyl azetidinones **25** and **26**. An excess of the Grignard reagent prepared from methyl acetylene gave a similar mixture from which 4β- (22%) and 4α- (32%) (1-propynyl)azetidinones **27** and **28** were obtained. Vinyl magnesium bromide, intermediate in basicity

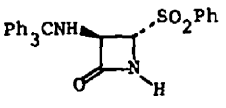
	R_1	R_2	
9 (L-threonine)	H	CH_3	12
10 (L-allothreonine)	CH_3	H	13
11 (±-2-amino-3-hydroxypentanoic acid)	H	CH_2CH_3	14



15 16

Scheme 3.





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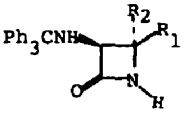


No.	R_1	R_2
25	TMSC≡C	H
26	H	TMSC≡C
27	$CH_3C{\equiv}C$	H
28	H	$CH_3C{\equiv}C$
29	$CH_2{=}CH$	H
30	H	$CH_2{=}CH$
31	HC≡C	H
32	H	HC≡C
33	CH_3CH_2	H
34	$CH_3(CH_2)_2$	H
35	CH_3	H
36	H	CH_3

Scheme 5.

between methyl and ethynyl analogs, led to poor results.

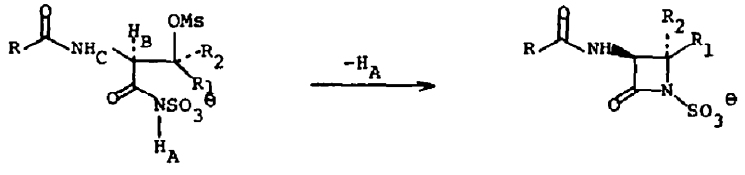
This problem could be overcome by the conversion of **23** to phenylsulfone **24**, obtained as a mixture predominating in the 4α-isomer. 4β- and 4α-Vinyl azetidinones **29** and **30** were separated from the mixture resulting from reaction of **24** with vinyl magnesium bromide. Reaction of phenyl sulfone **24** with methyl magnesium bromide gave a 1:3 mixture of 4β- and 4α-methylazetidinones **35** and **36**, respectively. This latter result suggests that deprotonation of the methylsulfone moiety of **23** is indeed responsible for its poor reactivity. While this work was in progress a Sankyo group[28] reported similar observations.

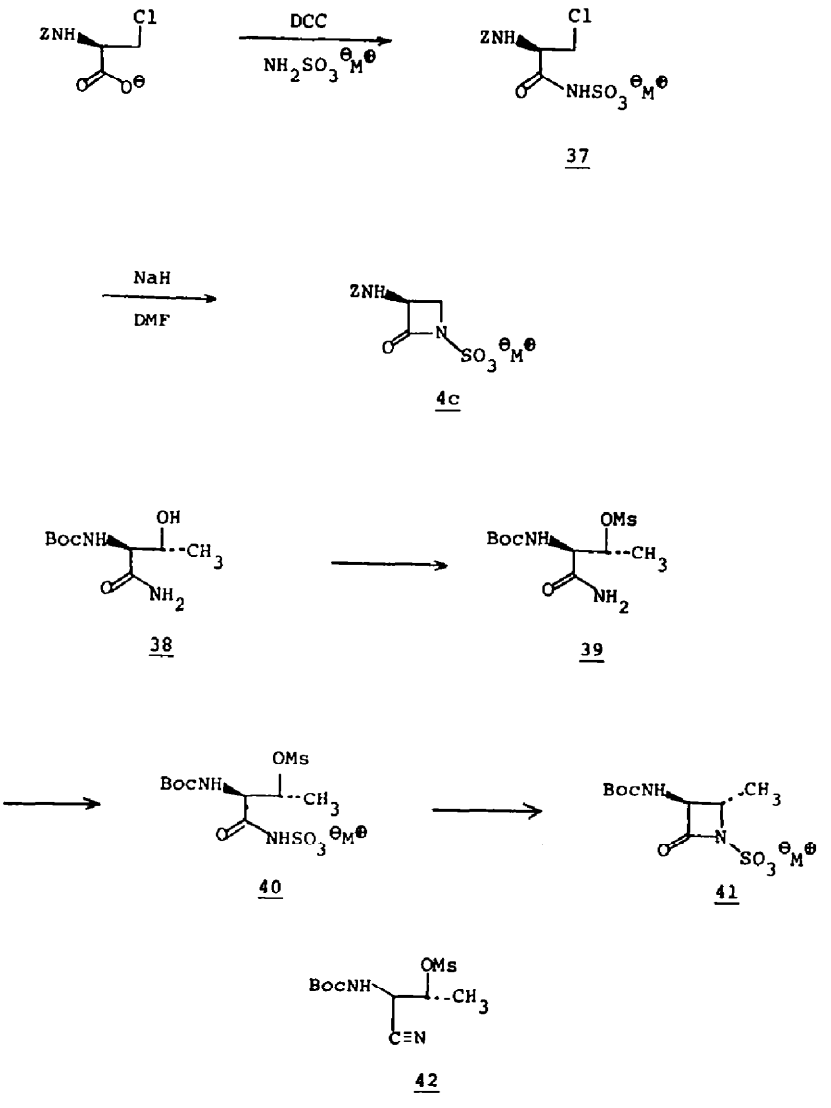
Azetidinones **25** and **26** could be smoothly converted to the parent acetylenes **31** (95%) and **32** (82%) with $(Bu)_4NF$ in CH_2Cl_2. Partial hydrogenation of **31** gave 4β-vinyl derivative **29** (identical to material prepared via vinyl magnesium bromide); and, as expected, complete hydrogenation afforded 4β-ethylazetidinone **33**. Similarly, **27** was converted to 4β-propylazetidinone **34**.

Sulfonation of these trityl-protected azetidinones with $Py{-}SO_3$ in hot pyridine, followed by ion-pair workup, gave crude tetrabutylammonium salts. These were directly converted to the corresponding zwitterionic 3-aminomonobactamates with formic acid (see Scheme 7).

A conceptually different and more efficient synthesis of monobactams would result from cyclization of β-mesyloxyacyl sulfamates derived from α-amino-β-hydroxy acids. For this route to be successful, the acidity of $-CONH_ASO_3^-$ and the *intramolecular* nucleophilicity of dianion $-CONSO_3^-$ must favor this mode of reaction over elimination (H_B removal) or oxazolidine formation (H_C removal).

Initial experiments with β-chloroalanine derivative **37**, prepared by DCC-mediated coupling of sulfamic acid and (*Z*)-β-chloroalanine, demonstrated the fea-





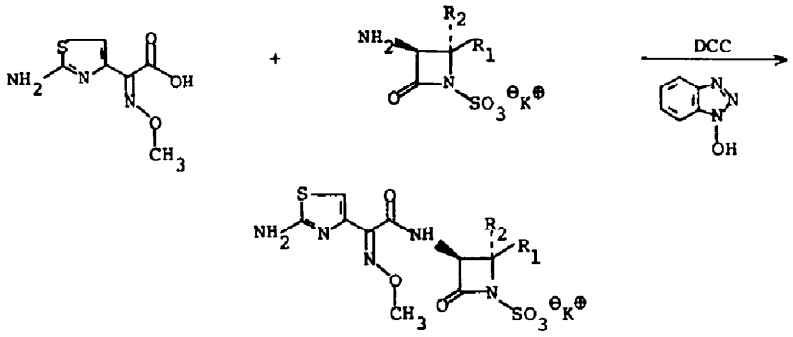
Scheme 6 ($M^{\oplus} = (Bu)_4N^{\oplus}$).

sibiltiy of this approach. Cyclization of **37** (NaH/DMF) gave monobactam **4c** which was identical to material prepared either from L-serine via acyl hydroxamate methodology[20] or from 6-APA. Attempts to apply this procedure to L-threonine were initially frustrated by our inability to couple (Boc)-threonine with sulfamic acid.

Unable to generate the requisite acyl sulfamate moiety by coupling, we turned to an alternate construction: sulfonation of a suitably protected derivative of threonine amide **38**. Because sulfonation of the OH group was expected to be a competing process, we utilized a mesylate function as both the protecting group during sulfonation and the leaving group in the subsequent cyclization.[20] Although inert to Py–SO_3, threonine amide derivative **39** was converted to acyl sulfamate **40** in high yield by 2-picoline–SO_3. Significant and variable amounts of nitrile **42** were encountered with the more reactive sulfonating agent 2,6-lutidine–SO_3. The separate preparation and isolation of hygroscopic 2-picoline–SO_3 could be avoided by its generation *in situ* from 2-picoline and chlorosulfonic acid; the rate of sulfonation using this mixture, which contains one equivalent of 2-picoline hydrochloride, was much slower and required more forcing conditions. Refluxing **39** with such a solution for 16–20 hr afforded **40** in high yield, however.

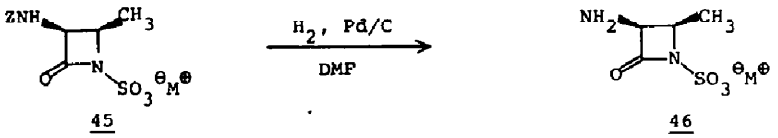
Much effort was expended to find reproducible conditions for cyclization of **40**. It was eventually found that aqueous bicarbonate effected a simple, economical, and reproducible cyclization to give **41**, conveniently isolated in high yield after ion-pair extraction. This procedure works equally well with L-serine and L-*allo*-threonine and is compatible with both urethane and amide functionality at C-3.[13]

For this investigation, access to both 4α- and 4β-alkylmonobactams in chiral form was important. Thus, the reaction of sulfones **23** and **24** with Grignard reagents, which afforded easily separable mixtures of both azetidinones, proved to be ideal. As

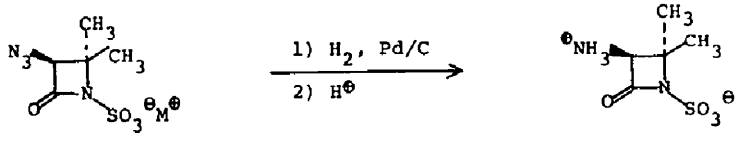


No.	R_1	R_2
50a	H	H
50b	H	H(3R)
50c	CH_3	H(±)
50d	H	CH_3
50e	CH_3	CH_3
50f	CH_3CH_2	H

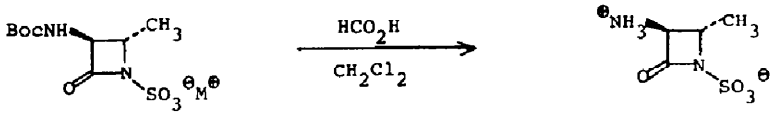
No.	R_1	R_2
50g	H	CH_3CH_2 (±)
50h	$CH_3(CH_2)_2$	H
50i	CH_2=CH	H
50j	H	CH_2=CH_2
50k	HC≡C	H
50l	H	HC≡C



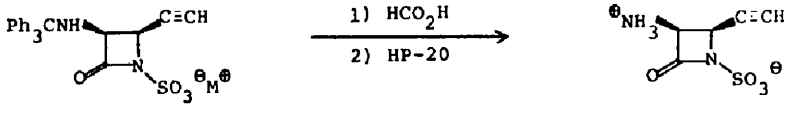
45 46



21 47



41 48



31 49

Scheme 7.

a general method for the preparation of chiral 4-alkylazetidinones, however, procedures that lead to higher or complete stereoselectivity would be preferred. The recent description of complete *trans* stereoselectivity in the reaction of alkyl and allyl cuprates with 4-acetoxy-3-phenoxyacetylamino-2-azetidinone is one such procedure.[30]

The efficient utilization of acyl hydroxamate routes to 4-alkylazetidinones requires chiral β-hydroxy-α-amino acids, as does the direct acyl sulfamate route to monobactams. For the specific case of (3*S*, *trans*)-3-amino-4-methylmonobactamic acid **48** (the precursor of aztreonam) the acyl sulfamate route represents a most efficient approach in view of the availability of L-threonine.

Structure activity relationships

This discussion will be limited to monobactams bearing the (*Z*)-α-methoxyimino-2-aminothiazol-4-yl-acetyl side chain. These compounds were prepared by DCC-N-hydroxybenzotriazole-mediated coupling of acid **43** with the appropriate 3-aminomonobactamate **44**, available by the deprotection protocols illustrated in Scheme 7 by single examples.

Hydrogenolysis of benzyloxycarbonyl-protected **45** in DMF gave a solution of the corresponding amine **46** that was directly acylated. Reduction of azide **21** in methanol, followed by acidification, gave zwitterionic monobactam **47** (95%). Removal of t-butyloxycarbonyl groups was conveniently done with 98% formic acid; simple filtration after dilution with CH_2Cl_2 provided moderate to excellent yields of zwitterions as illustrated for **48** (the key intermediate for aztreonam). The same protocol was utilized with trityl-protected azetidinones. Especially for 4β-substituted analogs, isolation of the more soluble zwitterionic products was accomplished by HP-20 chromatography (illustrated in the Experimental by the conversion of **31**–**49**).

Monobactam **50b** of (3*R*)-absolute configuration, enantiomeric with the antibacterially-active **50a** and the naturally-occurring nonmethoxylated monobactam,[4] was devoid of antibacterial activity and was not a substrate for β-lactamase. Incorporation of a 4β- or 4α-Me group, to give **50c** and **50d**, respectively, was attended by dramatic results. Activity against gram-positive bacteria (represented in Table 1 by *Staphylococcus aureus* SC 2399) was decreased substantially, while activity against gram-negative bacteria (represented in Table 1 by *Eschericia coli* SC8294) increased just as remarkably. A third effect of 4-methylation is revealed by comparison of the MIC at low (10^4 CFU) and high (10^6 CFU) inoculuum levels for *Eschericia coli* SC 10,404 and *Klebsiella aerogenes* SC 10,436. These organisms carry the plasmid-mediated R-TEM β-lactamase and chromosomally-mediated K1 β-lactamase, respectively. The 4-unsubstituted parent **50a** is hydrolyzed readily by these lactamases as seen by the increase in MIC on going from the low to high inoculuum level. Incorporation of a 4-Me group stabilizes the resulting monobactam to such hydrolysis; 4β-Me derivative **50c** is more active (and thus, β-lactamase stable) than its 4α-Me counterpart **50d**, especially against *Klebsiella aerogenes*. Interestingly, the 4,4-dimethyl analog **50e** was much less active than its parent **50a**.

The 4-Et derivatives **50f** and **50g** were prepared to further examine these effects. It was found that their activity against both gram-positive and gram-negative bacteria was decreased slightly, although significantly. The β-lactamase stability of these Et derivatives was further increased, especially with regard to the K1 β-lactamase (as evidenced by activity against *Klebsiella aerogenes*). Homologation to give the 4β-propyl derivative **50h** resulted in a further decrease in intrinsic activity.

Since the Et derivative represented a reasonable compromise between intrinsic activity and β-lactamase stability, the analogous two-carbon ethenyl (**50i** and **50j**) and ethynyl (**50k** and **50l**) analogs were examined. The intrinsic activity of all of these two-carbon substituted compounds is similar, although a small increase in activity against gram-positive bacteria is noted for the 4β-ethynyl derivative **50j**. β-Lactamase stability appears to decrease in the order ethyl > ethenyl > ethynyl, suggesting that the major effect is steric in nature.

In summary, both intrinsic activity against gram-negative bacteria and β-lactamase stability are increased by 4-alkylation, while activity against gram-positive bacteria is decreased. These considerations, in combination with the synthetic factors discussed above, led to the selection of a 4α-methyl-monobactam (aztreonam, **2**) for development.

EXPERIMENTAL

All reagents and solvents were used directly as purchased unless otherwise noted. With the exception of aqueous reactions, all reactions were conducted under N_2 or argon. M.ps were obtained on a Thomas Hoover capillary apparatus and are uncorrected. Optical rotations were determined using a Perkin–Elmer 141 polarimeter. ^{1}H NMR spectra were recorded on either Varian T-60 or XL-100 instruments. Values are reported in δ units relative to TMS. ^{13}C NMR spectra were recorded on a Joel FX60-Q instrument. IR spectra were obtained on a Perkin–Elmer 257 spectrometer except for samples run in KBr where a Perkin–Elmer 621 spectrometer was used. Analytical data were obtained by the Squibb Analytical Department.

(3S) - (*Benzyloxycarbonyl*)*amino* - 2 - *azetidinone* (**3**). A slurry of 6-APA (12.98 g, 60 mmol) in 140 ml water containing 5.18 g $NaHCO_3$ (stirred for *ca* 10 min without complete soln) was added in one portion to a well-stirred (mechanical stirrer) suspension of Raney Ni (W-2, washed with water to pH < 8.0, 260 ml of slurry ≃ 130 g) in a 70° oil bath. After 15 min the slurry was cooled, filtered, and the filtrate treated with 5.18 g $NaHCO_3$ and a soln of 11.94 g (70 mmol) benzyl chloroformate in 12 ml acetone. After 30 min, the soln was acidified to pH = 2.5 and extracted with CH_2Cl_2. The organic layer was dried, evaporated and triturated with ether–hexane to give a total of 6.83 g of (3*S*) - (benzyloxycarbonyl)amino - α - isopropyl - 2 - oxoazetidine - 1-acetic acid.

A soln of 6.83 g (2.13 mmol) of this acid in 213 ml acetonitrile (Burdick–Jackson) was treated with 1.95 g (1.07 mmol) cupric acetate [$Cu(OAc)_2 \cdot H_2O$ ground in a mortar] and 9.5 g (2.13 mmol) of $Pb(OAc)_4$ (dried *in vacuo* to remove AcOH). The slurry was immersed in a 65° oil bath and stirred with a stream of N_2 bubbling *through* the slurry. After TLC shows the disappearance of starting material (only traces remaining after 2–3/4 hr), the slurry was filtered and the solids washed with EtOAc. The combined filtrate and washings were evaporated *in vacuo* and the residue taken up in 100 ml each of EtOAc and water and adjusted to pH

Table I.

No.	Molecular Formula	Calcd. C	H	N	S	K	Found C	H	N	S	K	Homogeneity Index (%) TLE[a]	TLC[b]
50a	$C_{10}H_{12}N_5O_6SK$	26.66	2.99	17.27	15.82	9.64	27.02	2.77	17.35	15.79	9.66	99.1	99.8
50b	$C_{10}H_{12}N_5O_6SK \cdot H_2O$	26.66	2.94	17.27	15.82	9.64	26.72	2.75	17.07	15.89	9.65	100	99.9
50c	$C_{11}H_{14}N_5O_6SK \cdot 1/2\ H_2O$	29.26	3.19	17.05			29.30	3.31	16.66				96.7
50d	$C_{11}H_{14}N_5O_6SK$	29.92	3.01	17.45	15.97	9.74	30.32	3.49	15.82	13.95	10.45	99.9	99.7
50e	$C_{11}H_{16}N_5O_6SK \cdot 2\ H_2O$	29.26	3.99	15.52	14.19		29.47	3.48	14.98	13.35			81.3
50f	$C_{12}H_{16}N_5O_6SK \cdot 1/2\ H_2O$	31.14	3.56	16.51	15.11		31.37	3.09	16.69	15.02		99.7	99.4
50g	$C_{12}H_{16}N_5O_6SK\ H_2O$	30.47	3.72	16.16	14.83	9.02	30.16	3.25	15.21	13.17	8.51	84	
50h	$C_{13}H_{18}N_5O_6SK \cdot 2\ H_2O$	30.96	4.33	15.04	13.78	8.40	30.93	3.69	15.06	13.78	8.85	93.3	93
50i	$C_{12}H_{14}N_5O_6SK$	31.95	2.93	16.94	15.51		31.59	2.90	16.71	15.23		99.6	99.6
50j	$C_{12}H_{14}N_5O_6SK$	31.95	2.93	16.94	15.51		32.24	2.91	17.17	15.43		99.6	99.6
50k	$C_{12}H_{12}N_5O_6SK \cdot H_2O$	30.75	3.79	16.30	14.92		30.95	2.43	16.44	14.84		95.6	99.6
50l	$C_{12}H_{12}N_5O_6SK \cdot H_2O$	30.75	2.79	16.30	14.92		31.01	2.43	16.34	14.67		98.6	97.5

[a]By densitometry at 300 nm after thin layer electrophoresis (20 volts/cm for 1 hour on cellulose) at pH 4.0 (pyridine/acetate buffer.

[b]By densitometry at 300 nm after thin layer chromatography [silica gel, *n*-butanol-acetic acid-water (3:1:1)].

Table 2.

No.	R_1	R_2	*S. aureus* SC 2399	*E. coli* SC 8294	*E. Coli* SC 10,404		*K. aerogenes* SC 10,436	
50a	H	H	6.3[a]	0.8[a]	6.3[b]	>100[c]	>100[b]	>100[c]
50b	H	H	>100	>100	N.T.[d]	N.T.	N.T.	N.T.
50c	CH_3	H	50	0.1	0.2	0.1	12.5	>100
50d	H	CH_3	100	0.1	0.4	1.6	>100	>100
50e	CH_3	CH_3	>100	25	N.T.	N.T.	N.T.	N.T.
50f	CH_3CH_2	H	>100	0.4	0.1	0.4	0.2	0.8
50g	H	CH_3CH_2	>100	0.4	0.2	0.8	12.5	>100
50h	$CH_3(CH_2)_2$	H	>100	1.6	0.8	1.6	0.8	1.6
50i	CH_2=CH	H	100	0.2	0.1	0.2	6.3	>100
50j	H	CH_2=CH	>100	0.8	0.2	0.8	100	>100
50k	CH≡C	H	25	0.2	0.4	0.2	>100	>100
50l	H	CH≡C	>100	0.8	3.1	12.5	>100	>100

[a]MIC at an inocula of $5x10^4$ CFU. [b]MIC at an inocula of 10^4CFU. [c]MIC at an inocula of 10^6 CFU.

[d]N.T. = not tested.

7. The EtOAc layer was separated, dried and evaporated to give 6.25 g crude (3*S*) - benzyloxycarbonylamino - 1 - [α - (acetoxy)isobutyl] - 2 - azetidinone as a mixture of diastereomers.

A soln of 3.12 g (0.93 mmol) of the above acetate in 70 ml MeOH and 7 ml H_2O was cooled to −15° and 1.33 g K_2CO_3 and 349 mg $NaBH_4$ was added. The reaction was stirred at 0° and monitored by TLC (SiO_2, 9:1 EtOAc–MeOH). After the reaction was complete (*ca* 2 hr), the mixture was neutralized to pH 7 with 2N HCl and concentrated *in vacuo*. The concentrate was adjusted to pH 5.8, saturated with salt and extracted with EtOAc (3 times). The organic layer was dried and evaporated *in vacuo*. The residue was combined with material from a similar experiment and triturated with ether to give 3.30 g of TLC pure **3** (25% from 6-APA): m.p. (EtOAc) 163–164°; $[\alpha]_D$ −17.8° (*c* 0.72, CH_3OH); ^1H NMR (CD_3CN) $\delta = 3.24$ (1H; dd, J = 5 Hz), 3.52 (1H; appt, J = 5 Hz), 4.78 (1H; m), 5.08 (2H; s) and 7.30 (5H; s) ppm. (Found: C, 59.91; H, 5.36; N, 12.62. Calc for $C_{11}N_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72%.)

Sulfonation procedures

(Py–SO_3): (3S) - (*Benzyloxycarbonyl*)*amino* - 2 - *oxo* - *azetidine* - 1 - *sulfonic acid, pyridinium salt* (**4a**). A soln of **3** (440 mg) in 2 ml each CH_2Cl_2 (dried with Al_2O_3–I basic) and DMF (dried with 4A sieves) was stirred for 2 hr at room temp under N_2 with 350 mg Py–SO_3. The bulk of the solvent was then removed *in vacuo* and the residue triturated with EtOAc to give 758 mg solid (*ca* 100%). TLC and electrophoresis indicate this material is pyridinium salt **4a** with a small amount of the β-lactam hydrolysis product of the starting material: ^1H-NMR (CD_3OD) $\delta = 3.64$ (1H; dd, J = 5, 3 Hz), 3.93 (1H; appt, J = 5 Hz), 4.87 (1H; m), 5.10 (2H; s), 7.26 (5H; s) and 8.0–9.1 ppm (*ca* 7H; m's).

A soln of 600 mg of **4a** in 2 ml water was mixed with 15 ml of 0.5 M pH 5.5 KH_2PO_4 buffer. The slurry was cooled to 0°, filtered, washed with cold buffer, cold 50% EtOH, EtOH and ether to give 370 mg of K-salt **4b**.

A soln of 280 mg of **4b** in 10 ml H_2O was applied to a 100-ml HP-20 column. The column was eluted with 200 ml water and then water–acetone (9:1). Fractions (50 ml) were collected; evaporation of fraction 7 gave a solid. Trituration with acetone, filtration and drying *in vacuo* gave 164 mg of **4b**: m.p. 193–196°; ^1H NMR (D_2O) $\delta = 3.70$ (1H; dd, J = 5, 3 Hz), 3.89 (1H; appt, J = 5 Hz), 4.75 (1H; m), 5.10 (2H; s) and 7.33 ppm (5H; s). (Found: C, 38.19; H, 3.24; N, 8.15; S, 9.12; K, 11.53. Calc for $C_{11}H_{11}N_2O_6SK \cdot 0.5\,H_2O$: C, 38.02; H, 3.48; N, 8.06; S, 9.23; K, 11.25%.)

(TMSCl/$TMSOSO_2Cl$). A slurry of 1.1 g (5.0 mmol) of **3** in 11 ml CCl_4 at 0° was treated with 0.7 ml (5.0 mmol) Et_3N and then 0.63 ml (5.0 mmol) of TMSCl in 2 ml CCl_4. After 1 hr at 0° and 1 hr at room temp, 2 ml hexane was added and the slurry stirred 10 min and filtered. The filtrate was evaporated *in vacuo* to give 1.29 g of (3*S*) - (benzyloxycarbonyl) - amino - 1 - trimethylsilyl - 2 - azetidinone (87%): ^1H NMR ($CDCl_3$) $\delta = 0.23$ (9H; s), 3.22 (1H; dd, J = 5, 3 Hz), 3.41 (1H; appt, J = 5 Hz), 4.80 (1H; m), 5.06 (2H; s) and 7.26 (5H; s).

A soln of 1.29 g (4.3 mmol) of this material in 22 ml CH_2Cl_2 at 0° was stirred for 30 min with 0.657 ml (4.3 mmol) trimethylsilyl chlorosulfonate (added over 3 min in 3 ml CH_2Cl_2). The soln was then added dropwise to 30 ml cold 0.5M KH_2PO_4 and the pH was maintained between 4–4.8 by simultaneous addition of 3% KOHaq. After 20 min the aqueous layer was separated and evaporated to dryness. The residue was triturated with 50 ml MeOH, the solid filtered and discarded, and the filtrate evaporated *in vacuo*. This residue was dissolved in 25 ml of 0.02M KH_2PO_4 and applied to a 75-ml HP-20 column. Elution with water (200 ml) and acetone–water (200 ml, 1:9; 200 ml, 1:3) gave 620 mg (33% from **3**) of K-salt **4b** after evaporation of fractions (50 ml) 9–11.

(DMF/SO_3). A slurry of 220 mg (1.0 mmol) of **3** in 3 ml CH_2Cl_2 was cooled to 0° and 1.5 ml 1M DMF–SO_3 in DMF added. After 20 min the soln was poured into 50 ml 0.5M KH_2PO_4 (pH 4.5). The layers were separated and 340 mg (1.0 mmol) $(Bu)_4NHSO_4$ added to the aqueous layer. Extraction with CH_2Cl_2 (3 times) gave 500 mg (92%) of **4c** after solvent removal and trituration with Et_2O: m.p. 114–116°. (Found: C, 59.24; H, 8.70; N, 7.63; S, 5.89. Calc for $C_{27}H_{17}O_6N_5S \cdot 0.2\,H_2O$: C, 59.40; H, 8.76; N, 7.76; S, 5.92%.)

(3R) - (*Benzyloxycarbonyl*)*amino* - 2 - *azetidinone* (**8**). A slurry of 1.0 g (5.37 mmol) of **7** (prepared in 70.5% yield as described for the (3*S*)-enantiomer)[20] in 3 ml each of CH_2Cl_2 and anisole was cooled to −15° and 25 ml cold trifluoroacetic acid added. After 1 hr the soln was evaporated *in vacuo* (benzene added 3 times and evaporated) and residual anisole removed by trituration with hexane. The resulting salt was dissolved at 0° in 20 ml acetone containing 1.2 ml benzyl chloroformate and stirred for 1 hr keeping the pH at 7.0 by addition of 5% $NaHCO_3$aq. The resulting slurry was diluted with 20 ml sat NaClaq and extracted with EtOAc (4 × 100 ml) to give a white solid. Filtration through a 50-ml silica gel column with $CHCl_3$–EtOAc (3:1) gave 428 mg of TLC homogeneous **8**. Crystallization from EtOAc–hexane gave 330 mg of **8**: m.p. 163–164°; $[\alpha]_D + 18.8°$ (*c* 0.925, MeOH). (Found: C, 59.62; H, 5.32; N, 12.62. Calc for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72%.)

(3S,*trans*) - 3 - *t* - (*Butyloxycarbonyl*)*amino* - 4 - *methyl* - 2 - *azetidinone* (**12**) *via* $TiCl_3/NH_4OAc$ *reduction of* **15**. N_2 was bubbled through a soln of 2.05 g (9.5 mmol) of **15** in 60 ml MeOH and 40 ml 4.5 M NH_4OAcaq for 5 min and then 20 ml of 1.5 M aqueous $TiCl_3$ added. After 135 min the green soln was diluted with an equal volume of 8% NaClaq and extracted with EtOAc (3 × 100 ml). Evaporation and trituration with ether gave 1.65 g of **12**: m.p. (ether) 133.5–134.5° (lit.[20] 135–137°). (Found: C, 54.22 H, 8.43; N, 13.88. Calc for $C_9H_6N_2O_3$: C, 53.98; H, 8.06; N, 13.99%.)

(±) - 1 - *t* - *Butyldiphenylsilyl* - 4,4 - *dimethyl* - 2 - *azetidinone* (**18**). A soln of 12.87 g (130 mmol) of **17**[23] in 20 ml DMF was added (10 min) to a cold (0°) soln of 40.5 ml (136 mmol) t-butyldiphenyl chlorosilane and 22 ml (156 mmol) Et_3N in 112 ml DMF. After 18 hr the soln was poured into 400 ml ice–water and extracted with ether–EtOAc (2:1, 3 × 150 ml). The combined extract was washed with 0.5 M KH_2PO_4 (4 × 100 ml), sat $NaHCO_3$aq (150 ml), water (2 × 150 ml), dried and concentrated *in vacuo* to yield 33.03 g of **18** as a white solid: NMR ($CDCl_3$): $\delta = 1.03$ (6H; s), 1.20 (9H, s), 2.83 (2H; brs), 7.1–7.83 (10H; m) ppm.

(±) - 3 - *Azido* - 1 - *t* - *butyldiphenylsilyl* - 4,4 - *dimethyl* - 2 - *azetidinone* (**20**). A soln of 4.25 ml (6.8 mmol) of 1.6 M (in hexane) n-BuLi and 11 ml dry THF was prepared at −50° under argon. A soln of 0.083 g triphenylmethane in 1 ml THF was added. The resulting pink soln was cooled to −60°, and 1.0 ml (7.5 mmol) diisopropylamine was added dropwise by syringe. This was stirred for 15 min and then cooled to −78°. A soln of 2.3 g (6.8 mol) of **18** in 8 ml THF was added slowly by syringe. The resulting yellow soln was stirred for 20 min at −78°, during which time heavy precipitation occurred and uniform stirring became difficult. A soln of 1.33 g (6.8 mmol) *p*-toluenesulfonyl azide in 5 ml THF was added dropwise. The resulting mixture was allowed to stir at −78° for 20 min, and 2 ml (16 mmol) trimethylsilyl chloride was added dropwise. The mixture was warmed to ambient temp and stirred for 1 hr. Then the mixture was cooled to 0° and poured into 150 ml of EtOAc at 0°. Enough 0.5 M KH_2PO_4 buffer was added to make both the aqueous and organic layers clear. The two layers were separated and the organic layer was washed with 0.5 M KH_2PO_4 (3 × 150 ml), 8% NaClaq (1 × 150 ml) sat NaClaq (3 × 150 ml) and dried (Na_2SO_4). The soln was concentrated *in vacuo* to 2.83 g of a pale yellow oil, which upon trituration with hexane yielded 1.67 g of the title compound as a white solid (65%): ^1H NMR ($CDCl_3$): $\delta = 0.96$ (3H; s), 1.03 (3H; s), 1.20 (9H; s), 4.30 (1H; s), 7.23–7.77 (10H; m) ppm.

(±) - 3 - *Azido* - 4,4 - *dimethyl* - 2 - *azetidinone* (**19**). A total of 3.25 ml (78 mmol) of 48% HF was added over 6.5 hr to a

soln of 1.52 g (4 mmol) of **20** in 25 ml MeCN. The soln was cooled to 0°, neutralized with satd $NaHCO_3$aq and extracted with EtOAc (120 ml) to give 1.34 g of impure **19** after drying and concentration of the organic layer. Chromatography on a 27-g silica gel column eluting with hexane–EtOAc (2:1) gave 0.358 g (64%) of **19** as an off-white solid: ^{1}H NMR ($CDCl_3$): $\delta = 1.40$ (3H; s), 1.47 (3H; s), 4.25 (1H; d, J = 2 Hz), 6.0–6.6 (1H; b) ppm.

(±) - 3 - *Azido* - 4,4 - *dimethyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid, tetrabutylammonium salt* (**21**). A soln of 0.10 g (7 mmol) of **19** in 2.8 ml 0.5 M DMF–SO_3 in DMF was prepared at 0°, stirred for 45 min at room temp and poured into 0.5 M KH_2PO_4. Work-up as described above gave 0.31 g yellow oil (*ca* 50%, by ^{1}H NMR a 1:1 mixture with DMF): ^{1}H NMR ($CDCl_3$): $\delta = 0.73$–1.90 (34H; m), 2.87 (3H; s; DMF), 2.93 (3H; s; DMF), 3.03–3.53 (8H; m), 4.07 (1H; s), 7.95 (1H; bs; DMF) ppm.

(3R,*trans*) - 4 - *Phenylsulfonyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**24**). A mixture of 30 g of **23**,[27] 40 g sodium benzenesulfinate, 25 g of tetra-n-butylammonium bromide, 400 ml 1,2-dichloroethane, and 100 ml water were refluxed under N_2 for 30 min. The dichloroethane was removed *in vacuo* and the residue was extracted with 700 ml EtOAc. The extract was washed with sat $NaHCO_3$aq, then water, then sat NaClaq. The extract was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a 50 × 280 mm silica gel column eluted with 1000 ml 1:4 EtOAc:hexane, then 1000 ml 1:1 EtOAc:hexane. (3*R*,*trans* - 4 - phenylsulfonyl - 3 - triphenylmethylamino - 2 - azetidinone (25.3 g) was obtained predominantly as the *trans* isomer. **24**: NMR ($CDCl_3$): $\delta = 2.85$ (1H; d, J = 8 Hz), 4.15 (1H; d, J = 8 Hz), 4.55 (1H; d, J = 2 Hz), 6.43 (1H; bs), 7.1–7.7 (20H; m).

(3S,cis)- *and* (3S,trans) - 4 - *Trimethylsilylethynyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**25** and **26**). A soln of bromo(trimethylsilylethynyl)magnesium was prepared in a flame-dried 50-ml flask under N_2 by the addition of 5.05 ml of 3.06 M (15.5 mmol) MeMgBr in Et_2O to a soln of 2.20 ml (15.5 mmol) trimethylsilylethyne in 20 ml dry THF. The mixture was stirred for 140 min at 20°. Meanwhile, 5.77 g (14.2 mmol) of **23** was added to 60 ml dry THF in a 250-ml flask maintained under positive N_2 pressure. The soln was cooled in a dry ice/i-PrOH bath and 4.65 ml of 3.06 M (14.2 mmol) MeMgBr in Et_2O was added dropwise with rapid stirring. After another 5 min, the bromo(trimethylsilylethynyl)magnesium soln prepared above was added via a Teflon tube with positive N_2 pressure; another 7 ml dry THF was used to rinse the flask. The cold bath was removed and the mixture was allowed to warm slowly. After 45 min, a soln of 3.5 g $KHSO_4$ in 20 ml water was added to the mixture. Most of the THF was removed on the rotary evaporator. The residue was extracted with Et_2O (3 × 50 ml). The combined organic layer was washed with sat NaClaq, dried over Na_2SO_4 and evaporated *in vacuo* to give a yellow foam. The residue was chromatographed on a 50 × 330-mm silica gel column eluted sequentially with CH_2Cl_2 (2 l.), 1% Et_2O in CH_2Cl_2 (1 l.), 2% Et_2O in CH_2Cl_2 (2 l.) and 10% Et_2O in CH_2Cl_2 (1.5 l.) [fraction 1 = 1 l., fractions 2 and 3 = 500 ml, fractions 4–20 = 250 ml]. The fractions were analyzed by TLC [SiO_2; $Et_2O/CHCl_3$, 1:4). Pure *cis* isomer **25** (1.30 g, 22%) was contained in fractions 2–8, pure *trans* isomer **26** (1.80 g, 30%) in fractions 12–19. Fractions 9–11 contained 1.19 g (20%) of a mixture of **25** and **26**. *cis* Isomer **25**: ^{1}H NMR ($CDCl_3$): $\delta = 0.13$ (9H; s), 3.32 (1H; d, J = 10 Hz), 3.67 (1H; d, J = 5 Hz), 4.35 (1H; dd, J = 10, 5 Hz), 6.48 (1H; s), 7.1–7.7 (15H, m). *trans* Isomer **26**: ^{1}H NMR ($CDCl_3$): $\delta = 0.28$ (9H; s), 3.10 (1H; bd, J = 10 Hz), 3.58 (1H; d, J = 2 Hz), 4.42 (1H; bd, J = 10 Hz), 6.78 (1H; s), 7.1–7.7 (15H, m).

(3S,cis)- *and* (3S,trans) - 3 - *Triphenylmethylamino* - 4 - (1 - *propynyl*) - 2 - *azetidinone* (**27** and **28**). Utilizing the procedure described above, 12.4 g (30.5 mmol) of **23**, 21.2 ml (71 mmol) MeMgBr and 14 ml (excess) propyne gave 10.4 g crude product. Chromatography of 8.4 g on a Waters Prep 500 [silica gel, CH_2Cl_2 (1 l.) followed by CH_2Cl_2–Et_2O (19:1, 4 l.)] gave a total of 2.0 g (22.2%) early-eluting *cis*-isomer **27** and 2.9 g (32.2%) later-eluting *trans*-isomer **28**. *cis*-Isomer **27**: ^{1}H NMR ($CDCl_3$) $\delta = 1.81$ (3H; d, J = 2.5 Hz), 3.75 (1H; d, J = 6 Hz), 4.1 (1H; d, J = 6 Hz), 7–7.5 (15 H; m) ppm. *trans*-Isomer **28**: ^{1}H NMR ($CDCl_3$) $\delta = 1.81$ (3H; d, J = 2.5 Hz), 3.8 (1H; d, J = 2.6 Hz), 4.1 (1H; d, 2.6 Hz), 7–7.5 (15H; m) ppm.

(3S,cis)- *and* (3S,trans) - 4 - *Ethenyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**29** *and* **30**). Dry THF (60 ml) was added to 6.32 g (13.5 mmol) **24** in a dry 250-ml flask maintained under positive N_2 pressure and equipped with a magnetic stirring bar and septum cap. Vinyl magnesium bromide (43.4 ml of a 0.934 M soln in THF, 40.5 mmol) was added dropwise to the mixture cooled in a dry ice/acetone bath. When the addition was complete, the dry ice bath was replaced with a −15° ice/acetone bath. After 30 min, the mixture was poured into 50 ml rapidly stirred 10% NH_4Claq. The THF was removed *in vacuo*. Additional NH_4Cl was added to dissolve the solids, then the mixture was extracted with Et_2O. The organic extract was washed with sat NaClaq, dried over Na_2SO_4 and evaporated. The residue was chromatographed on a 50 × 280-mm silica gel column eluted with 15% Et_2O/CH_2Cl_2. Pure fractions [TLC: silica gel, Et_2O–$CHCl_3$ (1:4)] were combined to afford 1.0 g (21%) *cis*-isomer **29** and 1.9 g (40%) *trans*-isomer **30**. A mixed fraction, 0.69 g (14%) was also obtained. *cis*-Isomer **29**: ^{1}H NMR ($CDCl_3$): $\delta = 2.53$ (1H; d, J = 10 Hz), 3.65 (1H; t, J = 5 Hz); 4.2–5.3 (4H; complex), 5.87 (1H; bs), 7.0–7.6 (15H, m) ppm. *trans*-Isomer **30**: NMR ($CDCl_3$): $\delta = 2.67$ (1H; bs), 3.35 (1H; bs), 3.70 (1H; bs), 4.73 (3H; bs), 5.97 (1H; bs), 7.0–7.6 (15H; m) ppm.

(3S,cis) - 4 - *Ethenyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**29**) *via partial hydrogenation of* **31**. A slurry of 50 mg Lindlar catalyst in 5 ml benzene was stirred in an atmosphere of H_2 until uptake ceased (3 hr). A soln of 100 mg (0.28 mmol) of **31** in 2.5 ml benzene was added and the slurry stirred until uptake of H_2 (5.7 ml, 0.254 mmole) ceased. Filtration and evaporation gave **29** identical to material prepared above.

(3S,trans) - 4 - *Methyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**36**). Phenylsulfone **24** (2.00 g, 4.27 mmol) was dissolved in 30 ml dry THF under N_2. The flask was cooled in a dry ice/acetone bath and MeMgBr (3.06 M in Et_2O, 4.2 ml, 12.8 mmol) was added dropwise via syringe. After 5 min, the cold bath was replaced with a −15° ice/acetone bath. After 30 min, the mixture was poured into sat NH_4Claq. The mixture was extracted three times with Et_2O; the combined extract was washed once with brine, dried over Na_2SO_4, filtered and evaporated to give 1.2 g of an oily residue. NMR showed two high field Me doublets at $\delta = 0.37$ and 0.57 ppm in the ratio of *ca* 3:1, corresponding to the *trans* and *cis* isomers, respectively. The crude product was chromatographed on a 50 × 240 mm silica gel column eluted with CH_2Cl_2–Et_2O (9:1). Fractions containing pure *trans* isomer were combined to give 610 mg (42%) of **36**: ^{1}H NMR ($CDCl_3$): $\delta = 0.37$ (3H; d, J = 6 Hz), 2.68 (1H; bs), 3.00 (1H; qd, J = 6, 2 Hz), 3.60 (1H; d, J = 2 Hz), 6.03 (1H; bs), 7.2–7.4 (15H; m) ppm.

Preparation of authentic **36** *from* **12**. Azetidinone **12** (3.00 g, 15 mmol), 9 ml CH_2Cl_2, 6 ml anisole and 90 ml pre-cooled trifluoroacetic acid were mixed and stirred at 0°. After 90 min, the volatiles were removed *in vacuo*. CH_2Cl_2 (30 ml) and 4.5 g (16 mmol) triphenylmethyl chloride were added. Et_3N was added slowly until the mixture was basic to pH paper. After 45 min, the mixture was washed twice with water, once with brine and dried over Na_2SO_4. The residue after evaporation was chromatographed on a 25 × 310-mm silica gel column eluted with 550 ml CH_2Cl_2, CH_2Cl_2–Et_2O (19:1, 500 ml) and CH_2Cl_2–Et_2O (9:1, 500 ml). Fractions containing pure **36** (TLC: silica gel, Et_2O) were combined, evaporated and dried *in vacuo* to afford 3.01 g (59%) of **36** as a white solid. This material was identical to that obtained from the Grignard reaction.

(3S,trans) - 4 - *Ethynyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**32**). To a soln of 2.97 g (3*S*,*trans*)-**26** in 30 ml CH_2Cl_2 was added 330 mg tetra-n-butylammonium fluoride (containing 20–25% water). After 20 min, the solvent was removed *in vacuo* and the residue was taken up in EtOAc and water. The organic layer was separated, washed once with water and once with sat NaClaq, dried over Na_2SO_4 and filtered. The solvent was removed to give a yellow oil which was stirred with 60 ml pentane for 15 min to give a light yellow powder, isolated by filtration and dried *in vacuo* to give 2.35 g (95%) of the *trans*-acetylene **32**: ^1H NMR ($CDCl_3$): $\delta = 2.08$ (1H; d, J = 2 Hz), 2.88 (1H; bd, J = 10 Hz), 3.27 (1H; bs), 4.22 (1H; bd, J = 10 Hz), 6.53 (1H; bs), 7.0–7.6 (15H; m) ppm.

(3S,cis) - 4 - *Ethyl* - 3 - *triphenylmethylamino* - 2 - *azetidinone* (**35**). A soln of 1.3 g crude **31** in 70 ml EtOAc–MeOH (1:1) was stirred in an atmosphere of H_2 with 250 mg 5% Pd/$BaSO_4$ for 3 hr. After filtration of the catalyst, evaporation *in vacuo* gave 1.1 g of **33**: ^1H NMR ($CDCl_3$): $\delta = 1.2$ (3H; t, J = 6.5 Hz), 3.56 (1H; t, J = 2.6 Hz), 3.8–4.4 (3H; complex), 7.76 (15H, m) ppm.

Benzyloxycarbonyl - L - *β* - *chloroalanine amide* N - *sulfonate*, *tetrabutylammonium salt* (**37**). A soln of 1.29 g (5 mmol) (*Z*)-*β*-chloroalanine, 1.70 g (5 mmol) tetrabutylammonium sulfamate and 1.04 g (5.05 mmol) DCC in 20 ml of CH_2Cl_2 was stirred for 150 min, concentrated and filtered. The filtrate was evaporated to give 3.02 g crude product. Chromatography of 2.6 g on 270 g-silica gel column gave 1.06 g (37%) of **37** in fractions (100 ml) 57–64 [eluent: 5 l. CH_2Cl_2–CH_3OH (99:1), then CH_2Cl_2–CH_3OH (97:3)]: ^1H NMR ($CDCl_3$) $\delta = 4.0$ (2H; m), 4.9 (1H; m), 5.1 (2H; s) and 7.4 (5H; s) ppm.

(3S) - (*Benzyloxycarbonyl*)*amino* - 2 - *oxoazetidine* - 1 - *sulfonic acid*, *tetrabutylammonium salt* (**4c**) *from* **37**. A soln of 0.58 g (1 mmol) of **37** in 25 ml DMF was stirred with an excess of NaH overnight. The mixture was diluted with 100 ml of 0.5 M pH 4.5 KH_2PO_4 buffer and extracted with CH_2Cl_2. Solvent removal gave a residue that was chromatographed on silica gel [eluent CH_2Cl_2–MeOH (99:1)–(97:3)] to give 0.14 g of **4c** contaminated with the corresponding elimination product. TLC and ^1H NMR comparison clearly demonstrated the formation of **4c**.

t-*Butyloxycarbonyl*-L-*threonine amide* (**38**). Under an atmosphere of N_2, a flask containing 500 ml MeOH was cooled to −5° (ice/brine) and 130 ml (excess) $SOCl_2$ was added at such a rate as to maintain the temp between 0–10°. After recooling to −5°, 59.5 g (0.5 mmol) L-threonine was added and the mixture was allowed to reach room temp and stir for 16 hr. The mixture was concentrated and evacuated at 10^{-1} torr for 2 hr to yield a colorless viscous oil. This material was dissolved in 2.5 l. MeOH and cooled to −5° (ice/brine). The soln was saturated with ammonia gas and the cooling bath removed. The sealed flask was allowed to stand for 3 days by which time TLC analysis indicated only a trace of starting ester. After concentration to *ca* 1/3 volume, 250 ml 3N KOH was added and the mixture concentrated to a viscous soln. This was diluted with 500 ml water and 120 g (1.1 equiv) of 97% Boc_2O was added as a soln in a minimal amount of t-BuOH. The resulting mixture was stirred overnight at room temp and saturated with NaCl. The reaction was then extracted with several protions of EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated to a solid which was recrystallized from 200 ml EtOAc. After cooling and collection of the crystalline material the mother liquor was concentrated and diluted with hexane to afford additional product. The combined yield was 77.5 g (71% based on the starting threonine) of **38**: m.p. 121–122°; $[\alpha]_D + 12.4°$ (*c* 5, CH_3OH); ^1H NMR (CD_3COCD_3) $\delta = 1.12$ (3H; d, J = 6.5 Hz), 1.4 (9H; s), 3.93–4.50 (2H; m) pp. (Found: C, 49.34; H, 8.31; N, 12.85. Calc for $C_9H_{18}N_2O_4$: C, 49.53; H, 8.31; N, 12.84%.)

t-*Butyloxycarbonyl*-L-*threonine amide* (O-*mesylate*) (**39**). After dissolving 21.8 g (100 mmol) of **38** in 500 ml CH_2Cl_2 the soln was cooled to −5° (ice–brine) and 16.8 ml (120 mmol) Et_3N was added followed by the slow addition (*ca* 15 min) of 8.5 ml (110 mmol) methanesulfonyl chloride. After the addition was complete, the reaction was stirred for 2 hr and slowly reached 20° whereupon the mixture was poured into a separatory funnel and washed with water (2 × 125 ml) and 200 ml brine–1N HCl (1:1). The CH_2Cl_2 soln was then dried over $MgSO_4$ and concentrated. The solid residue was dissolved in about 100 ml EtOAc with *slight* warming, followed by the addition of about 100 ml hexane. After 30 min, the ppt was filtered off and dried *in vacuo* to yield 24.4 g (82%) of **39**: m.p. 129–131°; $[\alpha]_D + 18.9°$ (*c* 10, MeOH); ^1H (CD_3COCD_3) $\delta = 1.46$ (9H; s), 3.03 (3H; s, 4.33 (1H; dd, J = 12, 5 Hz) and 5.10 (1H; m) ppm; ^{13}C NMR ($CDCl_3$) 170 ppm (amide carbonyl). (Found: C, 40.61; H, 6.76; N, 9.44; S, 10.62. Calc for $C_{10}H_{20}N_2O_6S$: C, 40.54; H, 6.76; N, 9.46; S, 10.81%.)

t-*Butyloxycarbonyl*-L-*threonine amide* (O-*mesylate*) N-*sulfonate*, *tetrabutylammonium salt* (**40**). A soln of 2-picoline (17.8 ml, 180 mmol) in 90 ml of CH_2Cl_2 was cooled to −5° (ice–brine) and chlorosulfonic acid 5.97 ml (90 mmol) was added at such a rate as to maintain the internal temp below 5°. The resulting pale yellow soln was stirred for 15 min and 8.9 g (30 mmol) of **39** was added as a soln in 30 ml CH_2Cl_2 with an additional 10 ml used as a rinse. The resulting soln was then refluxed for 16–20 hr and poured into 500 ml of pH 4.5 phosphate buffer (0.5 M) and further diluted with 120 ml CH_2Cl_2. The separated organic layer was then washed once with 100 ml buffer soln and the combined aqueous phases were treated with 10.2 g (30 mmol) tetrabutylammonium hydrogensulfate and extracted with CH_2Cl_2 (1 × 300 ml and 2 × 150 ml). After drying the combined organic extracts over Na_2SO_4, the soln was concentrated to yield 17.2 g (93%) of a white foam. Analysis of the crude product by TLC (SiO_2; 4:1 EtOAc–MeOH) indicated this material was quite pure **40**: ^{13}C NMR ($CDCl_3$) $\delta = 166.6$ ppm (acylsulfamate carbonyl).

(3S,trans) - 3 - *t* - (*Butyloxycarbonyl*)*amino* - 4 - *methyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid*, *tetrabutylammonium salt* (**41**). A soln of 7.8 g (12.5 mmol) of **40** in 160 ml 1,2-dichloroethane was refluxed with 5.52 g (40 mmol) $KHCO_3$ in 20 ml water for 30 min. The mixture was cooled, CH_2Cl_2 added and the layers separated. The organic phase was dried and evaporated to give 6.3 g (99%) of **41**, very pure by TLC and NMR analysis. Recrystallization of a similar sample from EtOAc gave **41**: m.p. 144–146°; $[\alpha]_D - 14.6$ (*c* 2, CH_3OH); ^{13}C NMR ($CDCl_3$) $\delta = 13.27$, 17.7, 19.2, 23.4, 27.8, 58.0, 58.6, 62.3, 79.7, 154.4 and 162.9 ppm. (Found: C, 57.40; H, 9.93; N, 7.96; S, 5.90. Calc for $C_{25}H_{51}N_3O_6S$: C, 57.55; H, 9.85; N, 8.05; S, 6.15%.)

(3S) - trans - 3 - *Amino* - 4 - *methyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid* (**48**). The crude **41** from the 12.5 mmol run described above was dissolved in 30 ml 98% formic acid and stirred at room temp for 5 hr. The slurry was diluted with CH_2Cl_2 and filtered to give 1.53 g (56% from mesylate **39**) of **48**. A similar sample prepared in the same way had m.p. 223–225°; $[\alpha]_D^2 - 41.5°$ (*c* 1, H_2O); ^1H NMR (D_2O) $\delta = 1.58$ (3H; d, J = 7 Hz), 4.21 (1H, dq, J = 6.5, 3 Hz); ^{13}C NMR (D_2O) $\delta = 17.5$, 57.5, 60.1 and 161.3 ppm. (Found: C, 26.69; H, 4.42; N, 15.85; S, 17.71. Calc for $C_4H_8N_2O_4S$: C, 26.67; H, 4.44; N, 15.55; S, 17.77%.)

(±) - cis - 3 - *Amino* - 4 - *methyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid*, *tetrabutylammonium salt* (**46**). A soln of 81 mg (0.15 mmol) of **45** in 4 ml DMF was stirred in an atmosphere of H_2 with 40 mg 10% Pd/C for 2 hr. The catalyst was filtered off and washed with 1 ml DMF. The combined filtrate and washings was used directly for acylation as described below.

(±) - 3 - *Amino* - 4,4 - *dimethyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid* (**47**). A soln of 0.155 g (3.36 mmol) crude **19** in 0.6 ml MeOH was stirred over 10% Pd/C for 20 min in an atmosphere of H_2. The catalyst was removed by filtration and the filtrate treated with 0.123 ml (3.3 mmol) 97% HCO_2H. After standing for 1 hr at 5°, the white solid was filtered off to yield 66.4 mg (95%) of **47**: m.p. 200–202° (dec):

^{1}H NMR (D_2O): δ = 1.64 (3H; s), 1.68 (3H; s) and 4.42 (1H; s) ppm. (Found: C, 28.79; H, 5.70; N, 13.28; S, 15.44. Calc for $C_5H_{10}N_2O_4S{\cdot}H_2O$: C, 28.49; H, 5.68; N, 13.17; S, 15.06%.)

(3S,trans) - 3 - *Amino* - 4 - *methyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid* (**48**) *via* **36**. *trans*-Azetidinone **36** (600 mg, 1.75 mmol) and 840 mg (5.26 mmol) Py–SO_3 were heated under N_2 in 6 ml pyridine for 30 min. The mixture was poured into a rapidly stirred mixture of 16 g K_2HPO_4, 50 ml water and 50 ml EtOAc. The aqueous layer was separated, acidified to pH 3 with 3N HCl and extracted three times with EtOAc. The combined organic layer was dried over Na_2SO_4 and evaporated. The residue was taken up in 10 ml CH_2Cl_2 and 10 ml 98% HCO_2H. After 15 min, the mixture was evaporated and the residue was triturated with CH_2Cl_2 and MeOH. The solid was isolated by filtration and dried under vacuum to give 230 mg (73%) of **48**, identical to material prepared via the sulfamate route described above.

(3S,cis) - 3 - *Amino* - 4 - *ethynyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid* (**49**). *cis*-Acetylene **31** (504 mg, 1.43 mmol) and Py–SO_3 (700 mg, 4.4 mmol) were weighed into a 25-ml flask. The flask was flushed with N_2 and 5 ml dry pyridine was added. The mixture was heated at 80–85° for 3 hr, then poured into a stirred mixture of 15 g KH_2PO_4, 100 ml water and 50 ml EtOAc. The pH was adjusted to 3.0 with H_3PO_4. Tetra-n-butylammonium bromide (450 mg, 1.40 mmol) and 100 ml CH_2Cl_2 were added. The water layer was separated and extracted once with CH_2Cl_2. The combined organic extract was washed once with water, dried over Na_2SO_4 and filtered. The solvents were removed *in vacuo* to afford a dark foam. CH_2Cl_2 (10 ml) and 98% HCO_2H (6 ml) were added. After 20 min, the solvents were evaporated; 20 ml CH_2Cl_2 and 20 ml water were added to the residue. After 10 min, the mixture was filtered and the water layer was separated. The water layer was concentrated *in vacuo* to 2 ml and chromatographed on a 150-ml HP-20 column eluted with water. Fractions (30 ml) were checked by TLC (silica gel, 3:1:1 n-BuOH:HOAc:H_2O). Fractions 4 and 5 were combined and evaporated to give 120 mg of **49** (44%) as a clear glass: NMR (D_2O): δ = 3.08 (1H; m), 4.70 (1H; d, J = 2.6 Hz) and 4.9 (1H; d, J = 2.6 Hz) ppm.

(±) - cis - [(2 - *Amino* - 4 - *thiazolyl*)(*methoxyimino*)*acetyl*] - *amino*] - 4 - *methyl* - 2 - *oxoazetidine* - 1 - *sulfonic acid, potassium salt* (**50c**). The soln of **46** obtained above was stirred overnight at room temp under N_2 with 31 mg (0.15 mmol) of **43**, 27 mg (0.15 mmol) of N-hydroxybenzotriazole·H_2O, and 31.5 mg (0.15 mmol) of DCC, filtered and the filtrate evaporated *in vacuo*. The residue was triturated with 3 ml acetone and centrifuged. The soln was decanted and treated with 51 mg (0.15 mmol) solid potassium perfluorobutanesulfonate. Dilution with 5 ml ether gave crude **50c** which dissolved in a small amount water and chromatographed on a 40-ml HP-20 column. Elution with water gave 23 mg (37.3%) of **50c** in fractions (20 ml each) 3–5 after evaporation and trituration with ether: ^{1}H NMR (D_2O) δ = 1.46 (3H; d, J = 7 Hz), 3.97 (3H, s), 4.46 (1H; m), 5.36 (d, J = 5 Hz) and 6.97 (s) ppm. (Found: C, 29.30; H, 3.31; N, 16.66. Calc for $C_{10}H_{12}N_5O_6S_2K{\cdot}0.5\ H_2O$: C, 29.26; H, 3.19; N, 17.05%.)

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