Total synthesis of polyoximic acid

Stephen Hanessian and Jian-min Fu

Abstract: The structure and stereochemistry of polyoximic acid, a degradation product of polyoxins, was originally designated as *trans*-3-ethylidene-L-azetidine-2-carboxylic acid. However, total synthesis revealed that the correct structure was in fact *cis*-3-ethylidene-L-azetidine-2-carboxylic acid, which was confirmed by X-ray crystallography. The synthesis of the *trans*-isomer was also done and its identity was confirmed by X-ray analysis as well. The key step for constructing the four-membered ring was a rhodium catalyzed carbenoid insertion into the N—H bond of a β-amino acid derivative. The stereoselectivity of the *exo*-double bond was controlled by conducting a Horner-Emmons-Wadsworth or a Wittig reaction to generate the *trans*- and *cis*-isomers, respectively. Weinreb's amide was used as a latent methyl group for the separation of *trans* and *cis* mixtures. The double bond stereochemistry of polyoximic acid in the parent polyoxin was also confirmed to be *cis* by extensive 2D NMR studies.

Key words: diazoinsertion, azetidine, olefination.

Résumé : La structure et la stéréochimie originalement attribuées à l'acide polyoximique, un produit de dégradation des polyoxines, étaient celles de l'acide *trans*-3-éthylidène-L-azétidine-2-carboxylique. Une synthèse totale a toutefois permis de démontrer qu'il s'agit plutôt de son isomère, l'acide *cis*-3-éthylidène-L-azétidine-2-carboxylique, ce qui a été confirmé par diffraction des rayons X. On a aussi réalisé la synthèse de l'isomère *trans* et son identité a aussi été confirmée par diffraction des rayons X. L'étape clé dans la construction du cycle à quatre chaînons est une insertion de carbénoïde dans la liaison N—H d'un dérivé d'acide β-aminé, catalysée par le rhodium. La stéréosélectivité de la double liaison *exo* est contrôlée en procédant à une réaction d'Horner-Emmons-Wadsworth ou d'une réaction de Wittig qui conduisent respectivement aux isomères *trans* et *cis*. L'amide de Weinreb a été utilisé comme groupe méthyle latent pour la séparation des mélanges *trans* et *cis*. En se basant sur des études approfondies de RMN 2D, on a aussi confirmé que la stéréochimie de la double liaison de l'acide polyoximique de la polyoxine de base est aussi *cis*.

Mots clés : diazoinsertion, azétidine, oléfination.

[Traduit par la Rédaction]

Introduction

In the 1960's, Isono and co-workers (1*a*, *c*–*j*) first isolated and characterized a series of nucleoside antibiotics designated as the polyoxins from culture broths of *Streptomyces cacacoi var asoensis*. Since then, new members were added to this family that constitute the fifteen known polyoxins A–O (2). These compounds are potent inhibitors of chitin synthetase and could be used as fungicides for treating plant diseases (3) and other applications (for other biological uses of polyoxins, see ref. 4). Consequently a great deal of effort has been devoted to the structural elucidation (5), biosynthesis (6), structural–activity relationships (7), and total synthesis (8) of this small family of antibiotics. The structures and configurations of the polyoxins, represented by polyoxin A and its degradation product polyoximic acid (**1**), were proposed as depicted in Fig. 1.

The structural elucidation was based on the analysis of the degradation products (5). For example, treatment of polyoxin A (Scheme 1) with acid or base led to the generation of 5-hydroxymethyl uracil (**3**), polyoxin C (**4**), polyoxamic acid (**5**), and polyoximic acid (**1**). The latter is a unique amino acid that is a component of polyoxins A, F, H, and K. Total syntheses of polyoxin C (9), polyoxins B and D (10), polyoxins J and L (11), and polyoxamic acid (12) have been reported. Only two reports in the literature address the total synthesis of polyoximic acid (13, 14). Baumann and Duthaler (13) described an approach using a Claisen rearrangement or cycloaddtion reaction for the synthesis of key intermediates. Emmer (14) reported a total synthesis of racemic *trans*-polyoximic acid using a rhodium catalyzed intramolecular carbenoid insertion reaction.

Initially, racemic polyoximic acid (*rac*-**1**) was obtained from polyoxin A by either basic or acidic hydrolysis (Scheme 2) (5). Upon ozonolysis, the characterizable product **6** was produced, thus establishing the atomic composition of the molecule. The stereochemistry of the exocyclic double bond was established as *trans* based on an NOE study using a 60 MHz NMR instrument. Additionally, hydrogenolysis of the parent polyoxin A produced the saturated intermediate

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Dedicated to Professor Victor Snieckus: a friend, scholar, and chemist extraordinaire.

S. Hanessian.[1] Department of Chemistry, Université de Montréal, Montréal, QC H3C 3J7 Canada.
J.-m. Fu. Pharmacia Corp. 7255-209-706, Structural, Analytical and Medicinal Chemistry, 301 Henrietta St., Kalamazoo, MI 49007, U.S.A.

[1]Corresponding author (telephone: (514) 343-6738; fax: (514) 343-5728; e-mail: stephen.hanessian@umontreal.ca).

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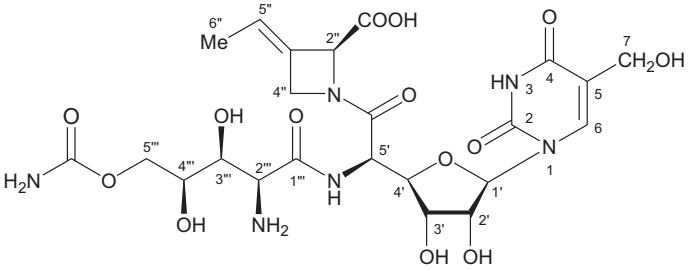
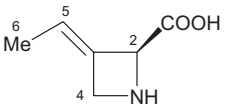
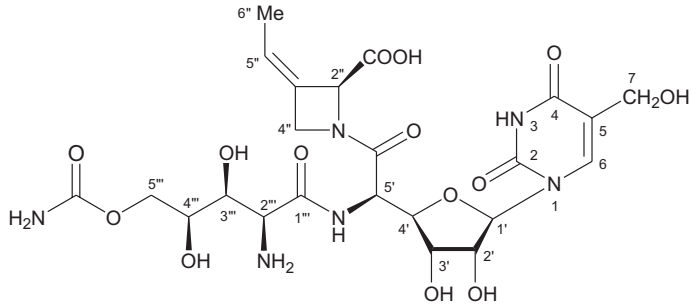
Fig 1.



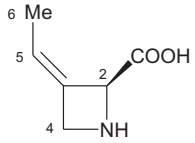
Polyoxin A (original structure)



1. *trans*-Polyoximic acid

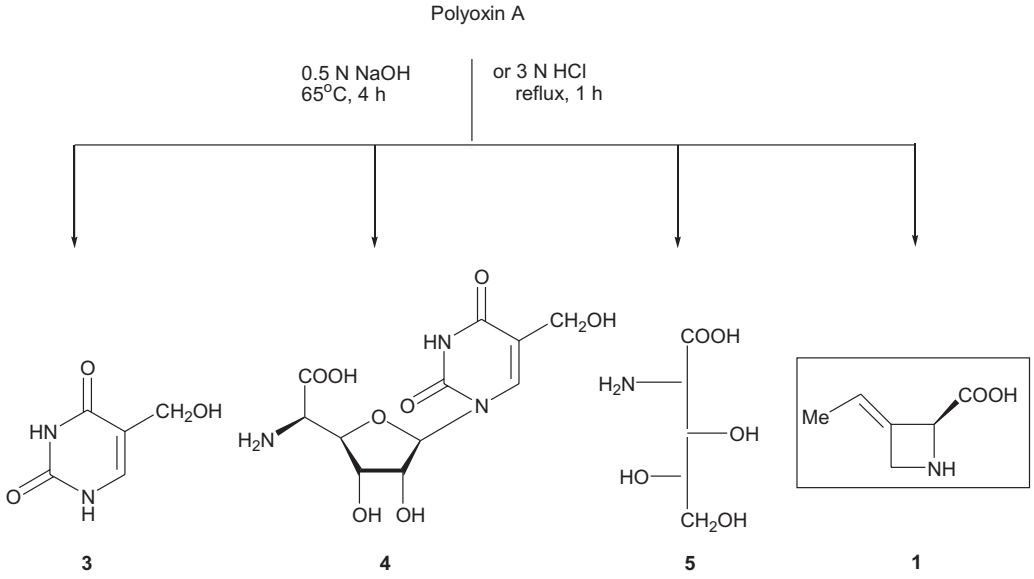


Polyoxin A (revised structure)



2. *cis*-Polyoximic acid

Scheme 1.



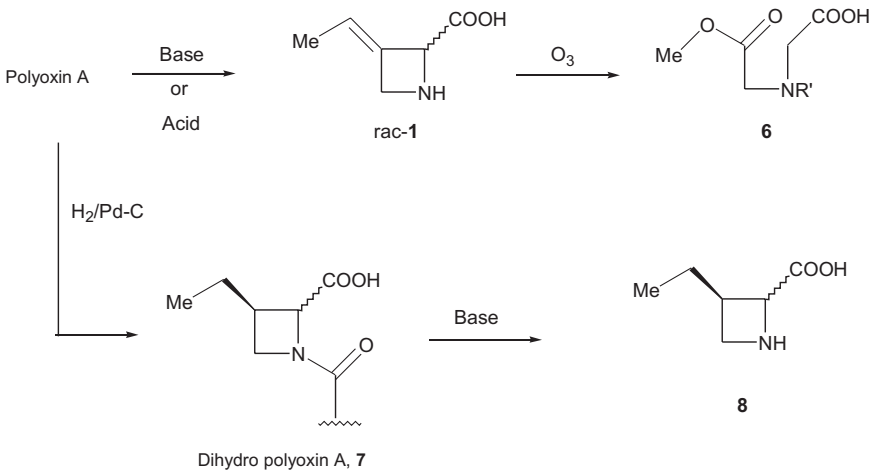
dihydropolyoxin A **7**, and subsequent basic hydrolysis generated the dihydro polyoximic acid **8**, which was converted to its *N*-dithiocarbethoxy derivative whose positive Cotton effect was compared to that of the known analogous derivative prepared from L-azetidine-2-carboxylic acid. Thus, the absolute configuration of the amino acid was established as (*S*) (5).

In the course of our studies (15*a*) directed at the total synthesis of polyoximic acid, we discovered that the structure was originally assigned incorrectly as the *trans*-isomer (15*b*). Here we wish to report in detail the total synthesis of this four-membered amino acid as the *cis*-isomer, as well as a synthesis of the *trans*-isomer.

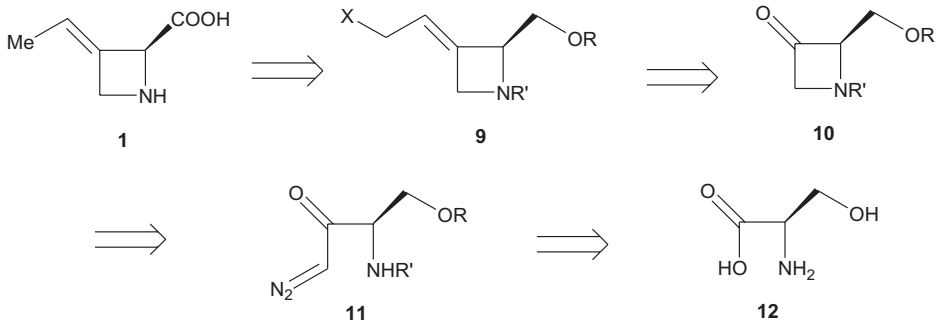
Results and discussion

We envisioned that the total synthesis of polyoximic acid could be accomplished from D-serine, as illustrated by the disconnections shown in Scheme 3. The carboxylic acid could be derived from a latent functional group like an alcohol **9**, and the *exo*-double bond would originate from a Wittig reaction on the azetidinone **10**. The four-membered ring of **10**

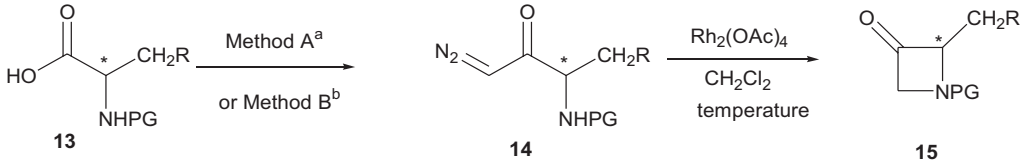
Scheme 2.



Scheme 3.



Scheme 4.



would be constructed by an intramolecular rhodium catalyzed carbene insertion into the N—H bond of the diazoketone, which in turn could be generated from the amino acid D-serine (**12**). This approach to the synthesis does not require us to build any new stereogenic centers on an sp^3 carbon, and relies on the general design principles embodied in the "Chiron" approach (16).

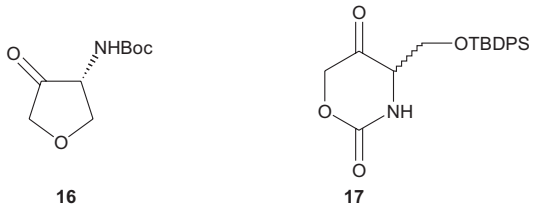
Thus, the formation of azetidinone **10**, from the rhodium catalyzed intramolecular carbenoid insertion reaction, would be the pivotal step of the total synthesis. Although rhodium catalyzed carbene insertions to C—H and O—H bonds have been extensively studied (17), to our knowledge, there was no precedence in the literature of using rhodium catalysis to promote an intramolecular carbene insertion into an N—H bond to form a four-membered ring from enantiopure α-amino acids (18). Hence, a methodological study was carried out first on simple amino acid templates such as phenylalanine and alanine (Scheme 4, Table 1) to explore the appropriate conditions. The diazoketones **14a–f** were prepared from different amino acids by reacting with diazomethane via either a mixed anhydride (method A) or an acyl chloride (method B). Subjection of the phenylalanine diazoketone **14a** to catalysis by rhodium(III) at different temperatures (rt, –20, –40, –78°C) revealed that –40°C was the optimal temperature giving a 70% yield of the expected 3-azetidinone. This temperature (–40°C) appeared to work well for the alanine system (Table 1, entries 6 and 7) with Cbz and Ts as the amino protecting groups. In contrast, when the amino protecting group was changed to Boc, the reaction led to the formation of unidentified materials (Table 1, entries 5 and 9) and no product could be isolated. Normally, carbene insertion reactions are carried out at rt or higher (17).

These low temperature conditions were then applied to the synthesis of polyoximic acid, starting with D-serine (**12**). In sharp contrast to the phenylalanine and alanine systems, the Boc group emerged as a better amino protecting group than Cbz (Scheme 4, Table 1, entries 10 and 11). In addition, the TBDPS (19) hydroxyl protecting group was found better than TBDMS (20). From the rhodium catalyzed reaction of diazoketone **14h**, two side products **16** and **17** were isolated in addition to the desired product **15h**. Compound **16** was formed by insertion of the carbene into the oxygen—silicon

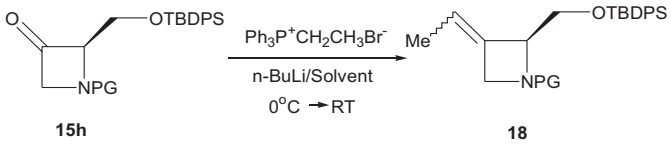
Table 1.

Entry	R	PG	Configuration	Method[a]	Product **14** (yield, %)	Temp. (°C)	Product **15** (yield, %)
1	Ph	Cbz	*R*	A	**14a** (74)	rt	**15a** (0)
2	Ph	Cbz	*R*	A		–20 → rt	**15a** (61)
3	Ph	Cbz	*R*	A		–40 → rt	**15a** (70)
4	Ph	Cbz	*R*	A		–78 → rt	**15a** (46)
5	Ph	Boc	*R*	A	**14b** (71)	–40 → rt	**15b** (0)
6	H	Cbz	*R*	A	**14c** (69)	–40 → rt	**15c** (71)
7	H	Cbz	*S*	A	**14d** (75)	–40 → rt	**15d** (68)
8	H	Ts	*R*	B	**14e** (77)	–40 → rt	**15e** (75)
9	H	Boc	*rac*	A	**14f** (95)	–78 → rt	**15f** (0)
10	OTBDMS	Cbz	*R*	A	**14g** (35)	–40 → rt	**15g** (19)
11	OTBDPS	Boc	*R*	A	**14h** (97)	–40 → rt	**15h** (50)
12	OTBDPS	Boc	*R*	A		–78 → rt	**15h** (57)
13	OTBDPS	Boc	*R*	A		–95 → rt	**15h** (67)

[a]Method A: *t*-BuOCOCl, *N*-methylmorpholine, $CH_2N_2 \cdot Et_2O$, CH_2Cl_2, –10 to 0°C ; Method B: (*i*) PCl_5, Et_2O, (*ii*) $CH_2N_2 \cdot Et_2O$, 0°C.



Scheme 5.



bond, and **17** was derived from the ylide, which was formed by reaction of the carbene with the Boc carbonyl. Since the four-membered ring product **15h** was the kinetic product, lower temperature was presumed to be beneficial. When the reaction was carried out at –78°C, the yield increased to 57% (Table 1, entry 12). A further decrease of the reaction temperature to –95°C furnished **15h** in 67% yield. A temperature lower than –95°C was not pursued since the reaction mixture starts to freeze at –95°C in CH_2Cl_2. The highest yield (75%) was observed in the case of the N-Ts analog **15e**. However, the synthesis was continued with the N-Boc derivative **15h** for ease of deprotection.

Having secured a method to construct the four-membered ring skeleton, the next stage of the synthesis was to introduce the ethylidene group by conducting the Wittig reaction. Under normal Wittig conditions (Scheme 5 and Table 2, entry 1), a 1:2 mixture of *E*:*Z* olefins **18** was produced in favor of the *Z* isomer, which were not separable by normal chromatography methods. The stereoselectivity of the double bond formation was not improved by solvent variations (toluene, HMPA, Table 2, entries 2 and 3), or by using salt free conditions (entry 4). In addition, Schlosser's conditions (21) (entry 5) reversed the stereoselectivity in favor of the *E* isomer (*E*:*Z*, 2:1).

Table 2.

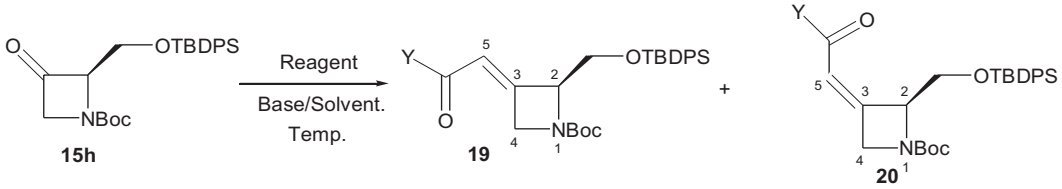
Entry	Solvent	Yield (%)	*E*:*Z*
1	THF	54	33:67
2	Toluene	50	37:63
3	HMPA	19	37:63
4	THF[a]	55	37:63
5	THF[b]	24	61:39

[a]Salt free conditions: NaHMDS, 0°C.
[b]Wittig–Schlosser conditions (ref. 21).

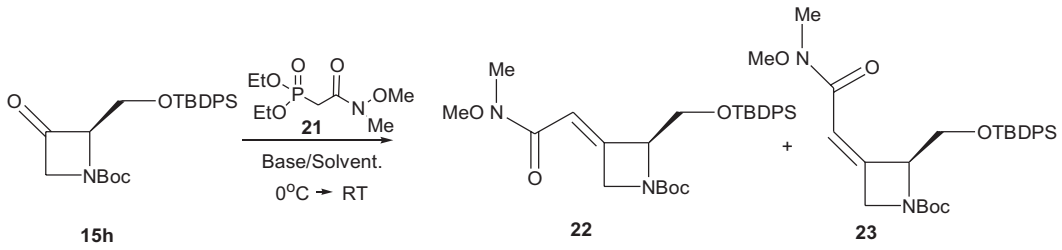
To better control the stereochemistry of the double bond, we changed the Wittig ylide from an unstabilized one to a stabilized one with either an aldehyde or ester (Scheme 6), since stabilized ylides normally favor *E*-isomer formation (22). Moreover, the aldehyde or ester could be considered as a latent methyl group as required in polyoximic acid. The results (Table 3, entries 1–6) showed that the stereoselectivity was not improved, and the *E*:*Z* mixture of olefins was still inseparable for both the aldehyde and the ester cases. Alternatively, an Horner-Emmons-Wadsworth reaction was considered. Indeed, *E*-isomer formation was favored, and the ratio was improved as expected (Table 3, entries 7–10). We then turned our attention to the cyano group (Table 3, entries 11–17), and found that the *E*:*Z* mixture of cyano olefins could be separated by iterative preparative TLC. It appeared that the phosphine oxide was the best choice with respect to obtaining a higher *E*:*Z* ratio (Table 3, entries 16 and 17). With both isomers in hand, we could determine the double bond geometry by an NOE study (Fig. 2). Although the enhancement was not significant, the *E*- and *Z*-isomers were differentiated by comparing the corresponding enhancements of the two isomers. Retrospectively, this evidence was used to confirm the stereochemical outcome of the Wittig and Horner-Emmons-Wadsworth reactions for the inseparable *E*- and *Z*-product mixtures (Schemes 5 and 6) by comparing their ^{1}H NMR spectra.

After separation of the cyano olefins **19** and **20**, the *E*-isomer was subjected to reduction to obtain the corresponding aldehyde. Unfortunately, using DIBAL-H (1–5 equiv.) in

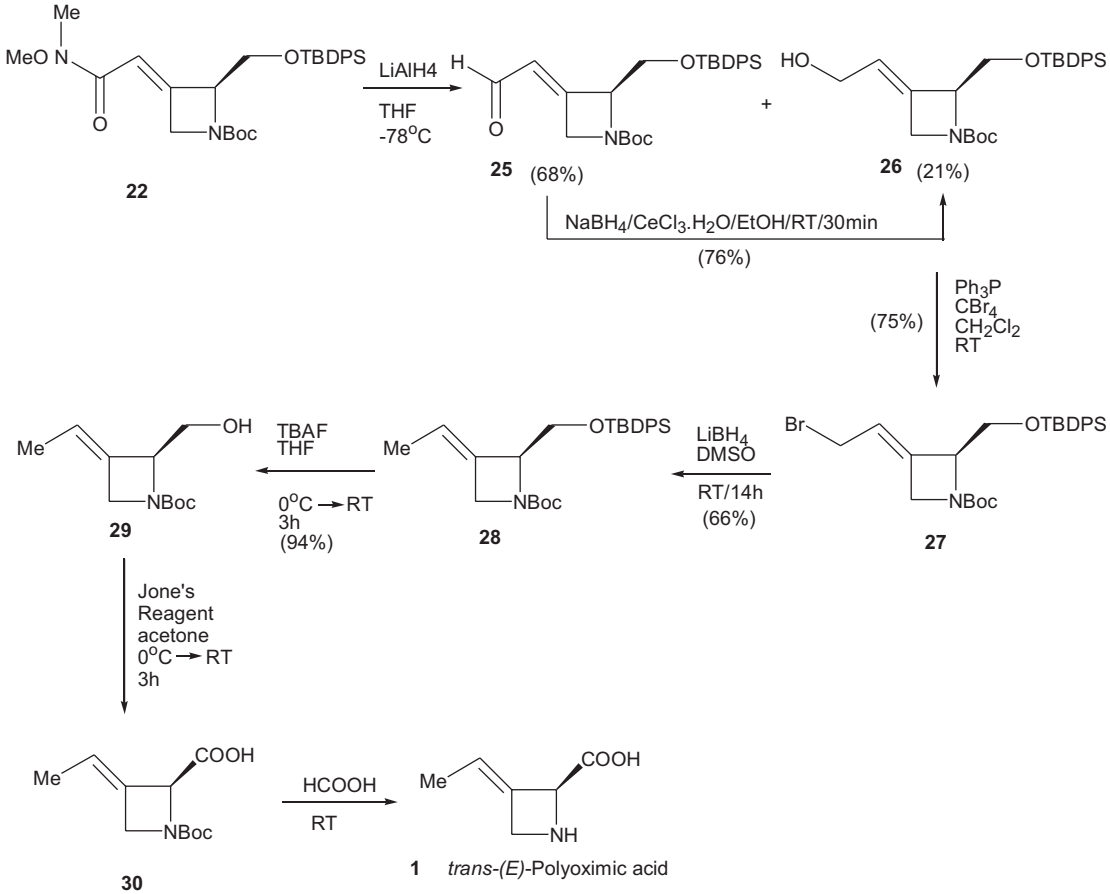
Scheme 6.



Scheme 7.



Scheme 8.



Et_2O or toluene at 0, –40, or –78°C gave at best 30% yield of the expected aldehyde. Either products of decomposition or starting material was recovered from the reduction. Therefore this route using the cyano group as the latent methyl group in the natural product was not pursued further.

Nevertheless, we discovered that the phosphonate amide by Nahm and Weinreb (23) provided a solution for this problem since it could be reduced more easily than the cyano group. Different conditions for the Horner-Emmons-Wadsworth reaction (Scheme 7) of compound **15h** with reagent **21** were explored. A mixture of *E*- (**22**) and *Z*- (**23**) isomers was obtained and separated by column chromatography. Sodium hydride in THF (Table 4, entry 5) was found to give the best result with a 94:6 ratio in favor of the *E*-iso-

Table 3.

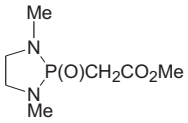
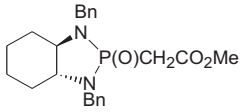
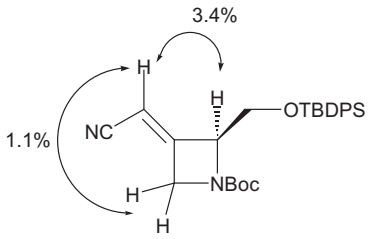
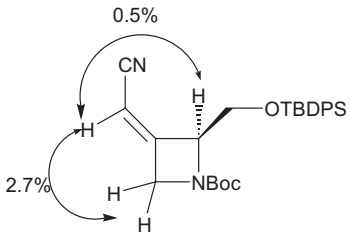
Entry	Reagent	Base	Solvent	Temp. (°C)	Y	Yield (%)	*E*:*Z*
1	$Ph_3P{=}CHCO_2Et$	—	CH_3CN	Reflux	EtO	77	26:74
2	$Ph_3P{=}CHCO_2Et$	—	MeOH	0 → rt	EtO	78	17:83
3	$Ph_3P{=}CHCO_2Et$	—	MeOH	–78 → rt	EtO	84	14:86
4	$Ph_3P{=}CHCO_2Et$	—	DMF	0 → rt	EtO	93	25:75
5	$Ph_3P{=}CHCO_2Et$	—	CF_3CH_2OH	0 → rt	EtO	84	61:39
6	$Ph_3P{=}CHCHO$	—	CH_3CN	Reflux	H	35	50:50
7	$(i\text{-}PrO)_2P(O)CH_2CO_2Et$	*n*-BuLi	DME	rt	EtO	82	80:20
8	$(EtO)_2P(O)CH_2CO_2\text{-}t\text{-}Bu$	*n*-BuLi	DME	rt	*t*-BuO	52	87:13
9		NaH	THF	0 → rt	MeO	71	80:20
10		NaH	THF	0 → rt	MeO	32	91:9
11	$(EtO)_2P(O)CH_2CN$	*n*-BuLi	DME	rt	CN	78	73:27
12	$(EtO)_2P(O)CH_2CN$	NaH	DME	rt	CN	61	83:17
13	$(EtO)_2P(O)CH_2CN$	KH	DME	rt	CN	77	79:21
14	$Ph_3P{=}CHCN$	KH	CH_3CN	Reflux	CN	78	55:45
15	$Ph_3P{=}CHCN$	KH	PhH	Reflux	CN	78	64:36
16	$Ph_2P(O)CH_2CN$	KH	DME	rt	CN	53	89:11
17	$Ph_2P(O)CH_2CN$	KH	THF	rt	CN	51	77:23

Fig 2.



19 *trans, (E-)*



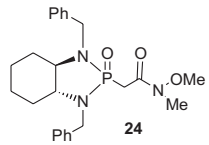
20 *cis, (Z-)*

mer. The chiral non-racemic phosphonamide reagent **24**, developed in our laboratories (24), was also attempted and gave an *E*:*Z* ratio of 88:12.

Our efforts toward the synthesis of the *trans*-(*E*)-isomer of polyoximic acid is shown in Scheme 8. Reduction of the amide **22** with LAH gave a mixture of the α,β-unsaturated aldehyde **25** and the alcohol **26**. The aldehyde was reduced under

Table 4.

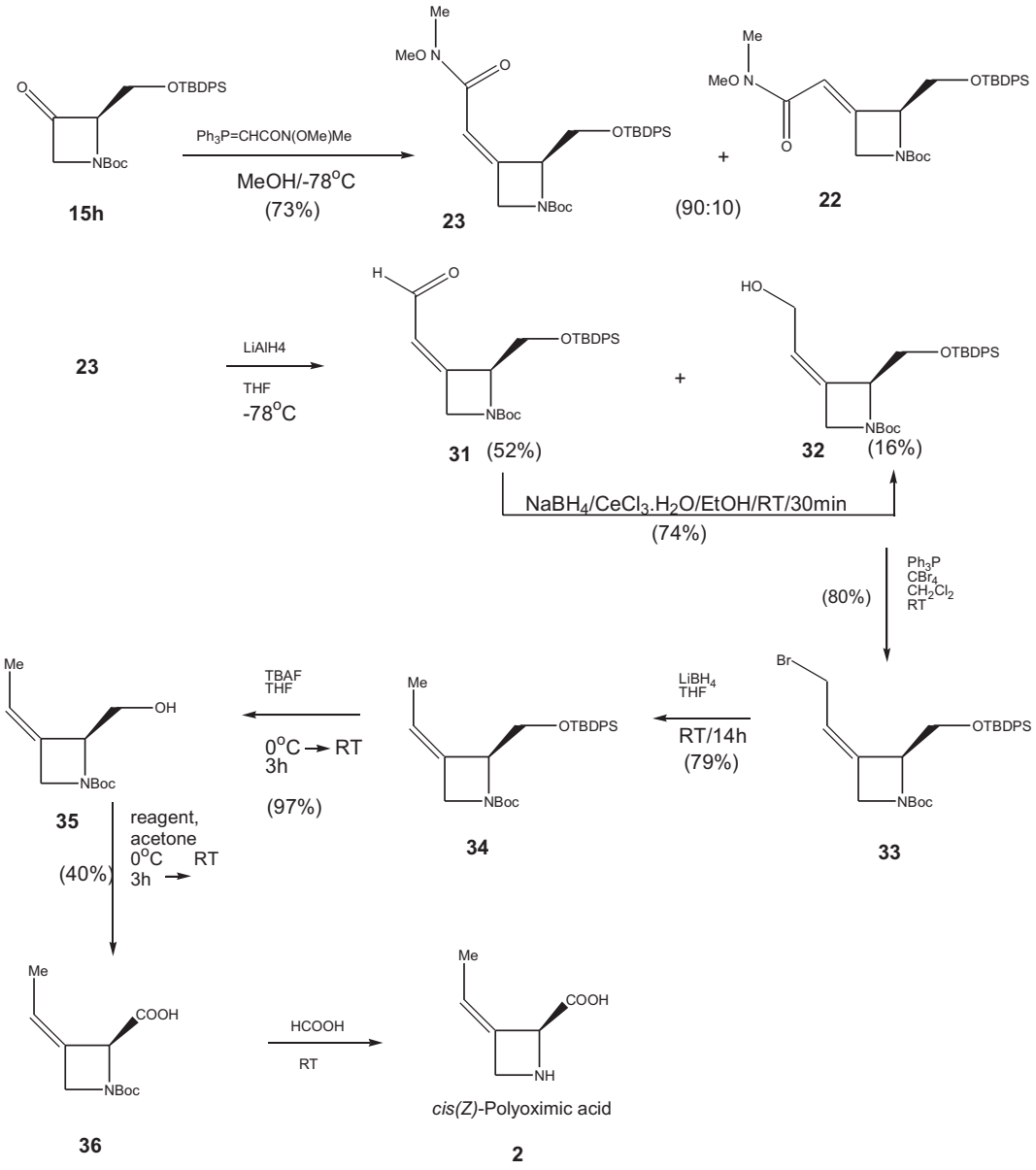
Entry	Base	Solvent	Yield (%)	**22**:**23**
1	*n*-BuLi	DME	65	88:12
2	NaH	DME	61	87:13
3	KH	DME	66	85:15
4	*n*-BuLi	THF	56	86:14
5	NaH	THF	7	94:6
6[a]	NaH	THF	83	88:12

[a] 

24

Luche conditions (25) to the alcohol **26** in good yield. The latter was converted to the bromide **27**, which was further reduced to the methyl compound **28**. Removal of the TBDPS group of **28** and attempts to oxidize the alcohol group in **29** to the carboxylic acid **30** proved problematic. The corresponding aldehyde, obtained from Swern or Dess-Martin oxidations was unstable. Eventually, the classic Jones oxidation emerged as the best method to yield **30** in 40% yield. Finally, the *N*-Boc protecting group was removed by stirring compound **30** with formic acid to produce the *trans*-(*E*)-polyoximic acid **1**, as a crystalline solid, whose structure was definitively confirmed by X-ray analysis. After completing the synthesis of *trans*-(*E*) **1**, and being unaware of the incorrect stereochemistry of the natural product, we compared the ^{1}H NMR spectrum of the synthetic sample with an

Scheme 9.



authentic one kindly provided by Professor Isono. The spectra were in fact different, and we had synthesized the incorrect isomer.

Faced with this dilemma, we were fortunate that the sample of racemic polyoximic acid received from Professor Isuno was in excellent physical state and amenable to X-ray analysis. The result shown in Fig. 3 confirms unambiguously that the geometry of the exocyclic double bond of the natural product is indeed *cis*-(*Z*), contrary to the original assignment (5).

With this important stereochemical revision, we turned our attention to the synthesis of the natural *cis*-(*Z*)-polyoximic acid **2**. The ready availability of the 3-azetidinone **15h** (Scheme 6), urged us to develop methodology directed at enriching the desired *Z*-isomer. Our studies on olefin formation had shown that the Wittig reaction favors the *Z*-product particularly in a protic solvent (Table 3, compare with Table 4). Therefore the Weinreb ester of the Wittig reagent became a logical choice as a (*Z*)-olefinating reagent in methanol (Scheme 9). Under these conditions, the *E*:*Z*-mixture of olefins was obtained in 73% yield and in a ratio of 10:90. In acetonitrile or THF, the ratios were 64:36 and 61:39, respectively, as determined by ^{1}H NMR spectroscopy. With a satisfactory method to obtain the (*Z*)-isomer **23**, the synthesis was carried out in a manner similar to that of the (*E*)-isomer, affording crystalline *cis*-(*Z*)-polyoximic acid **2** (Scheme 9).

Comparison of the spectroscopic and physical data of the synthetic material **2** with that of the natural product, confirmed the proposed structure, and the geometry of the double bond. The spectra in CD_3OD for the *cis*-(*Z*)- and *trans*-(*E*)-synthetic products, as well as that of the racemic natural product are shown in Fig. 4. It is interesting that the vinylic proton and the methyl protons in the *trans*-isomer **1** are shifted downfield and upfield, respectively, compared to the signals of the *cis*-isomer **2**. This pattern was also found in

Scheme 10.

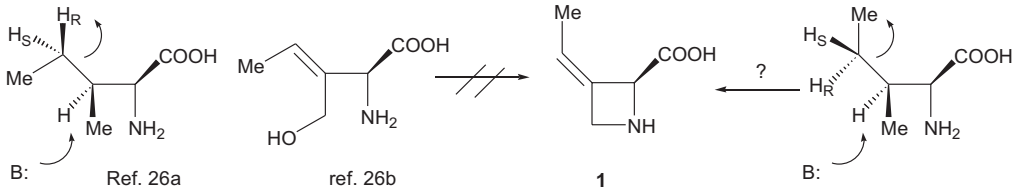
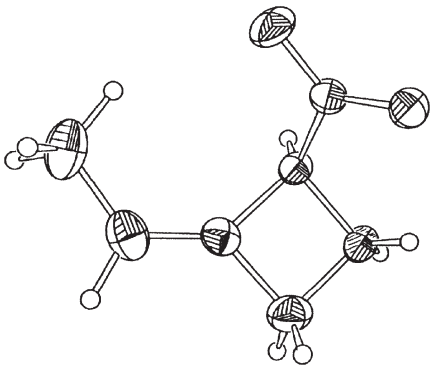


Fig. 3. Ortep drawing and structure of racemic *cis*-polyoximic acid.



D_2O and DMSO-d_6. The amino acid **2** was partially racemized in methanol containing a drop of Et_3N (~50% within 20 h at rt).

The geometry of the exocyclic double in the 3-azetidinone **15h**, could be controlled by conducting either the Horner-Emmons-Wadworth reaction or the Wittig reaction to generate a (*E*)- or (*Z*)-olefin preponderantly. Apparently, the Horner-Emmons-Wadsworth reaction gives the thermodynamic product as a major isomer at 0°C, and the Wittig reaction gives the kinetic product. To what extent, the nature of the Weinreb amide influences the respective transition states compared to normal esters is a matter of speculation (Tables 3 and 4). Dipolar and stereoelectronic effects could be playing important roles in both reactions. In addition, the nature of the solvent and cation is important in the phosphonate case, while the protic medium may promote a faster collapse of the more congested (*Z*)-betaine via the corresponding oxaphosphetane.

Since polyoximic acid was the degradation product of polyoxin, we had to be assured that no isomerization of the double bond had occurrred during the hydrolysis process or subsequent isolation. The 1D ^1H NMR spectrum of polyoxin A provided by Professor Isono is shown in Fig. 5, where the 6″-methyl, 5″-vinylic, and 2″-α-carboxyamide protons are clearly distinguishable. These correspond in pattern and in chemical shift differences to the *cis*- rather than the *trans*-polyoximic acid moiety.

Proton connectivities by the J-correlated spectroscopy (COSY) technique located the "intact" polyoximic acid moiety. Next, we carried out a 2D NOE correlated spectroscopy experiment (NOESY) to secure the *cis* orientation of the ethylenic group vis-à-vis the carboxamide group. Positive enhancements were observed between the ethylene methyl group and the α-carboxamide hydrogen, which is consistent with a *cis*-type geometry. These were independently corroborated by NOE studies on the *cis*- and *trans*-polyoximic acids in D_2O and DMSO-d_6.

With the establishment of the *cis* stereochemistry for polyoximic acid, the proposal of an elimination of the pro-*R* hydrogen at C-4 of L-isoleucine (26*a*), and the possible intermediacy of a hypothetical precursor, L-2-amino-3-hydroxymethyl-3-pentenoic acid (26*b*), must be reassessed (Scheme 10). If the double bond is indeed introduced by an enzymatic process, it most likely involves a synperiplanar elimination.

Finally, it is of interest to point out that the structures of polyoximic acids **1** and **2**, and related structures resulting from the methodology developed in this work represent constrained analogs of amino acids (27) such as L-isoleucine, L-vinyl glycine (28), and their congeners. As such, they are interesting structures for exploitation as β-turn mimetics (for selected reviews, see ref. 29) in a variety of peptide-like motifs.

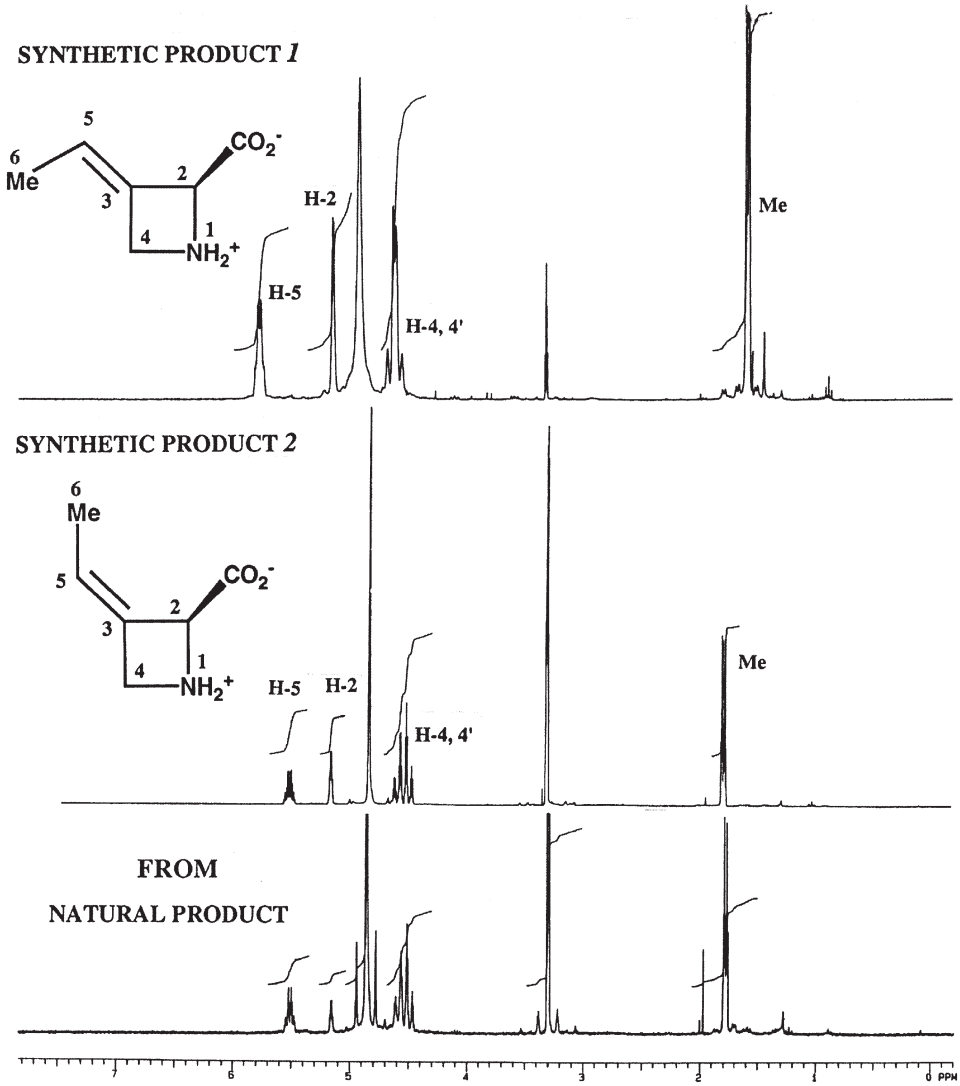
In summary, after more than 30 years since the isolation and structure determination of polyoxin (5), the diminutive yet intriguing amino acid polyoximic acid was synthesized in enantiopure form, and the geometry of the double bond was reassigned as *cis* rather than *trans* through NMR spectroscopy and X-ray crystallography.

Experimental

General procedure

Flash chromatography was carried out using 230–400 mesh silica gel. Mixtures of ethyl acetate–hexanes were used as the eluents unless otherwise specified. Analytical thin layer chromatography (TLC) was performed on glass plates coated with 0.02 mm layer of silica gel 60 F-254. Melting points were uncorrected. IR spectra were recorded as films. ^1H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, and the chemical shifts are reported in parts per million on the δ scale with $CDCl_3$ as reference unless otherwise specified. MS and HRMS spectra were recorded using electron ionization (EI) or by the FAB technique. Elemental analyses were performed by Guelph Laboratories, Guelph, Ontario. Optical rotations were measured in $CHCl_3$ at 23°C. All reactions were carried out under nitrogen or argon atmosphere unless otherwise specified. The –78°C temperature is approximate as achieved by a dry ice – acetone bath. The –95°C temperature is approximate as achieved by a liquid nitrogen – methanol bath. The term "normal workup" means the addition of saturated aqueous NH_4Cl solution to the reaction mixture, followed extraction with ethyl acetate, drying over Na_2SO_4, filtration, and evaporation of the filtrate in vacuo to afford the crude product.

Fig. 4. 300 MHz ^{1}H NMR (CD_3OD) spectra of synthetic *trans* **1**, synthetic *cis*-**2** obtained by acid hydrolysis (ref. 5).



General procedure for the preparation of diazoketones (method A)

To a solution of the protected amino acid (1.00 mmol) in dichloromethane at –10°C was added simultaneously isobutyl chloroformate (1.07 mmol) and 4-methylmorpholine (1.07 mmol). After stirring for 45 min at –10°C, a solution of freshly prepared diazomethane in ether (18.75 mL, 6.68 mmol) was added, and stirring continued for 7 h at rt, followed by concentration in vacuo to dryness. The residue was subjected to column chromatography to afford the desired product.

*(3*R*)-1-Diazo-3-(*N*-benzyloxycarbonylamino)-4-phenyl-2-butanone (**14a**)*

According to method A, the title compound was prepared in 74% yield: mp 82–83°C (hexanes–CH_2Cl_2); $[\alpha]_D$ –13 (*c* 1.2, $CHCl_3$). FAB-MS (NBA) *m/z* (rel intensity): 324 ($[M + 1]^+$, 3), 307 (13), 289 (16), 154 (100), 136 (91), 106 (58). HRMS calcd. for $C_{18}H_{18}N_3O_3$ + H: 324.1349; found: 324.1329. IR (KBr) υ_{max} (cm^{-1}): 3335, 3082, 2118, 1700, 1626. ^{1}H NMR δ: 3.04 (d, *J* = 6.6 Hz, 2H), 4.46–4.55 (m, 1H), 5.08 (s, 2H), 5.20 (s, 1H), 5.38–5.40 (m, 1H), 7.15–7.38 (m, 10H). ^{13}C NMR δ: 54.6, 58.7, 67.0, 127.0, 128.0, 128.1, 128.4, 128.5, 128.6, 129.1, 129.2, 135.8, 136.0, 155.6, 192.6.

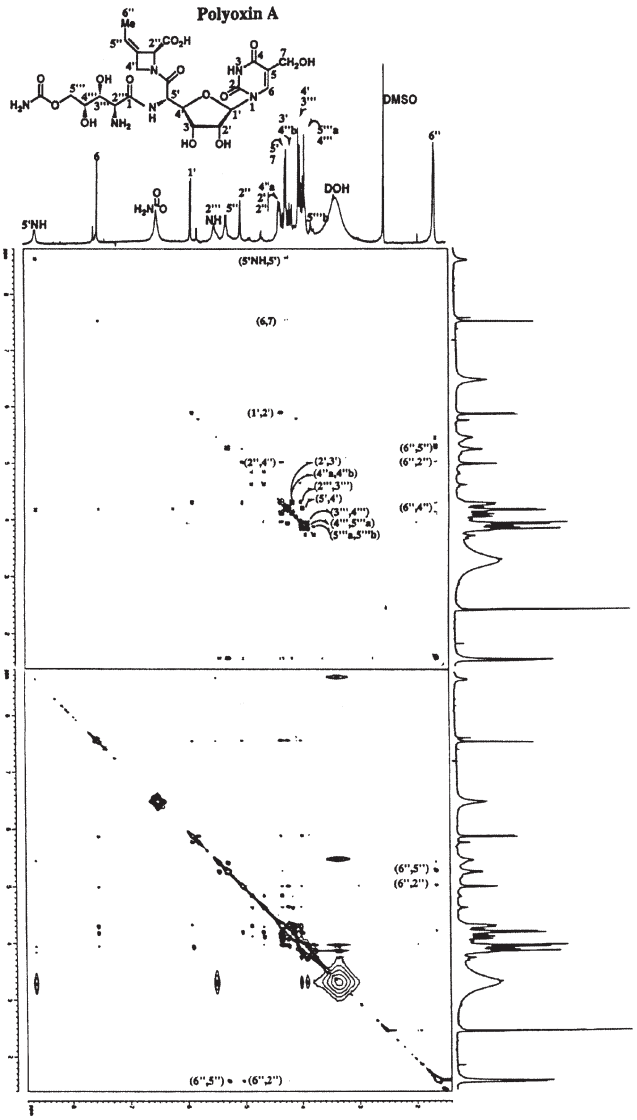
*(3*R*)-1-Diazo-3-(*N*-tert-Butyloxycarbonylamino)-4-phenyl-2-butanone (**14b**)*

According to method A, the title compound was prepared in 71% yield: mp 87–89°C (hexanes–CH_2Cl_2); $[\alpha]_D$ –8.1 (*c* 1.02, $CHCl_3$). FAB-MS (NBA) *m/z* (rel intensity): 290 ($[M + 1]^+$, 18), 206 (100), 164 (31), 154 (70), 136 (52), 120 (84). HRMS calcd. for $C_{15}H_{20}N_3O_3$: 290.1506; found: 290.1472. IR (KBr) υ_{max} (cm^{-1}): 3340, 2102, 1690, 1640. ^{1}H NMR δ: 1.39 (s, 9H), 2.98–3.09 (m, 2H), 4.35–4.48 (br s, 1H), 5.14 (d, *J* = 7.7 Hz, 1H), 5.23 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 28.1, 38.4, 54.2, 58.3, 79.9, 126.8, 128.5, 129.2, 136.2, 155.0, 193.2.

*(3*R*)-1-Diazo-3-(*N*-benzyloxycarbonylamino)-2-butanone (**14c**)*

According to method A, the title compound was prepared in 69% yield: mp 92–94°C (hexanes–CH_2Cl_2); $[\alpha]_D$ +4.1 (*c* 1.0, $CHCl_3$). FAB-MS (NBA) *m/z* (rel intensity): 268 ($[M + 1]^+$, 16), 240 (38), 197 (62), 154 (100), 139 (20). HRMS calcd. for $C_{12}H_{14}N_3O_3$ + H: 248.1036; found: 248.1037. IR ($CHCl_3$) υ_{max} (cm^{-1}): 3326, 2124, 1730, 1650. ^{1}H NMR δ:

Fig. 5. 2D COSY NMR spectrum of natural polyoxin A in DMSO-d_6 at 400 MHz (top); 2D NOESY NMR spectrum in DMSO-d_6 at 400 MHz (bottom).



1.35 (d, *J* = 7.1 Hz, 3H), 4.20–4.37 (m, 1H), 5.11 (s, 2H), 5.40 (s, 1H), 5.47 (br, 1H), 7.31–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 18.5, 53.4, 53.5, 66.9, 128.0, 128.1, 128.4, 136.0, 155.5, 193.6.

rac-*1-Diazo-3-(*N-tert-*butyloxycarbonylamino)-2-butanone (**14f**)*

According to method A, the title compound was prepared in 95% yield: mp 87–89°C (hexanes–CH_2Cl_2). FAB-MS (NBA) *m*/*z* (rel intensity): 214 ([M + 1]$^+$, 35), 158 (50), 154 (80), 144 (26), 136 (71), 130 (100). ^{1}H NMR δ: 1.31 (d, *J* = 7.1, 3H), 1.43 (s, 9H), 4.14–4.29 (br s, 1H), 5.09–5.21 (br s, 1H), 5.44 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 18.3, 28.2, 53.0, 53.2, 79.9, 155.0, 194.3.

*(3*R*)-1-Diazo-3-(*N-tert-*butyloxycarbonyl)amino-4-*tert-*butyl-diphenyl-silyloxy-2-butanone (**14h**)*

According to method A, the title compound was prepared in 97% yield: mp 89–90°C (hexanes). [α]$_D$ –0.67 (*c* 1.05, $CHCl_3$). FAB-MS (NEA) *m*/*z* (rel intensity): 468 ([M + H]$^+$, 49), 410 (16), 199 (64), 135 (100). HRMS calcd. for $C_{25}H_{34}N_3O_4Si$ (M + H): 468.2320; found: 468.2266. IR ($CHCl_3$) υ$_{max}$ (cm^{-1}): 2103, 1711, 1641. ^{1}H NMR δ: 1.06 (s, 9H), 1.46 (s, 9H), 3.79–3.84 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.96–4.01 (dd, *J* = 10.2, 3.9 Hz, 1H), 4.20–4.38 (br s, 1H), 5.35–5.38 (d, *J* = 7.8 Hz, 1H), 5.49 (s, 1H), 7.40–7.45 (m, 6H), 7.61–7.64 (m, 4H). ^{13}C NMR δ: 19.1, 26.4, 28.2, 54.1, 59.0, 64.0, 80.0, 127.7, 129.8, 132.6, 135.4, 155.2, 192.8.

(3*R*)-(+)-1-Diazo-3-(*N*-4-toluenesulphonylamino)-2-butanone (14e) (method B)

To a solution of *N*-4-toluenesulphonyl-D-alanine (5.73 g, 23.6 mmol) in ether (55.0 mL) was added pentachlorophosphorus (10.1 g, 48.3 mmol). After vigorous stirring at rt for 1 h, the reaction mixture was filtered. To the filtrate was added hexane (200 mL). The generated solid was filtered and collected to give 5.00 g (82%) of the chloride compound. The chloride was dissolved in ether (50.0 mL). To this solution was added diazomethane-etherate solution (140 mL) at 0°C. After 1 h, the reaction mixture was filtered through a pad of silica gel. The filtrate was concentrated in vacuo to dryness. The residue was subjected to column chromatography (EtOAc–hexane, 1:2) to afford 3.94 g (77%) of the desired product: oil. [α]$_D$ +116.2 (*c* 2.36, $CHCl_3$). FAB-MS *m*/*z* (rel intensity): 268 ([M + 1]$^+$, 16), 240 (35), 197 (61), 154 (100). HRMS calcd. for $C_{11}H_{14}N_3O_3S$: 268.0757; found: 268.0788. IR (neat) υ$_{max}$ (cm^{-1}): 3260, 2105, 1735, 1641, 1366. ^{1}H NMR δ: 1.24 (d, *J* = 7.1 Hz, 3H), 2.40 (s, 3H), 3.76–3.89 (m, 1H), 5.45 (s, 1H), 5.51 (d, *J* = 7.5 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.69–7.73 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 19.4, 21.4, 53.9, 55.2, 127.0, 129.7, 136.6, 143.7, 192.7.

General procedure for the preparation of azetidinone 15 (method C)

To a solution of the diazo compound **14** (1.00 mmol) in dichloromethane (10.0 mL) at the reaction temperature indicated was added rhodium acetate dimer (0.009 g, 0.020 mmol). The resulting reaction mixture was stirred that reaction temperature for 1 h and allowed to warm to rt for 14 h. The solvent was removed and the residue was subjected to column chromatography.

*(2*R*)-1-Benzyloxycarbonyl-2-benzyl-3-azetidinone (**15a**)*

The reaction was carried out at –40°C to give 70% of product **15a**: mp 85–87°C (hexanes–CH_2Cl_2). [α]$_D$ –124.6 (*c* 1.0, $CHCl_3$). EI-MS *m*/*z* (rel intensity): 295 (M$^+$, 3), 267 (18), 204 (38), 132 (30), 88 (100). HRMS calcd. for $C_{18}H_{17}NO_3$: 295.1209; found: 295.1238. IR (neat) υ$_{max}$ (cm^{-1}): 1819, 1691. ^{1}H NMR δ: 3.14 (dd, *J* = 14.3, 4.0 Hz, 1H), 3.24 (dd, *J* = 14.3, 6.3 Hz, 1H), 4.08 (dd, *J* = 16.5, 4.4 Hz, 1H), 4.59 (d, *J* = 16.5 Hz, 1H), 5.20 (s, 2H), 5.21–5.25 (m, 1H), 7.12–7.15 (m, 2H), 7.23–7.29 (m, 2H), 7.35–7.41 (m, 6H). ^{13}C NMR ($CDCl_3$) δ: 35.4, 67.3, 69.2, 83.5, 126.9, 128.1, 128.2, 128.4, 128.5, 129.7, 134.9, 136.0, 155.8, 198.7.

*(2*R*)-1-Benzyloxycarbonyl-2-methyl-3-azetidinone (**15c**)*

The reaction was carried out at –40°C to give 71% of product **15c**: mp 80–81°C (hexane–CH_2Cl_2). [α]$_D$ –39.7

(*c* 0.99, $CHCl_3$). EI-MS *m*/*z* (rel intensity): 219 (M^+, 1), 191 (4), 91 (100). HRMS calcd. for $C_{12}H_{13}NO_3$: 219.0896; found: 219.0935. IR ($CHCl_3$) υ_{max} (cm^{-1}): 1816, 1694. ^1H NMR δ: 1.49 (d, *J* = 7.0 Hz, 3H), 4.67 (dd, *J* = 16.5, 4.2 Hz, 1H), 4.97–5.07 (m, 1H), 5.17 (s, 2H), 7.27–7.39 (m, 2H).

*(2*R*)-1-*p-*Toluenesulphonyl-2-methyl-3-azetidinone (**15e**)*

According to method C, the reaction was carried out at –40°C to give 75% of product **15e**: mp 76–78°C (hexane–CH_2Cl_2). $[\alpha]_D$ –65.1 (*c* 1.09, $CHCl_3$). EI-MS *m*/*z* (rel intensity): 240 (M^+, 8), 211 (16), 155 (20), 105 (17), 91 (100). HRMS calcd. for $C_{11}H_{14}NO_3S$: 239.0617; found: 239.0605. IR (KBr) υ_{max} (cm^{-1}): 1831, 1599, 1345. ^1H NMR δ: 1.46 (d, *J* = 7.0 Hz, 3H), 2.47 (s, 3H), 4.43–4.60 (m, 2H), 4.76–4.80 (m, 1H), 7.38–7.41 (d, *J* = 8.3 Hz, 2H), 7.78–7.82 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 15.6, 21.5, 69.5, 80.9, 128.3, 130.0, 131.8, 144.9, 196.7.

*(2*R*)-1-*tert-*Butyloxycarbonyl-2-*tert-*butyldiphenylsilyloxymethylene-3-azetidinone (**15h**)*

The reaction was carried out at –95°C to give 67% of product **15h** as a colorless oil. $[\alpha]_D$ –41.9 (*c* 1.50, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 440 ($[M + H]^+$, 4), 384 (10), 326 (57), 306 (63), 198 (78), 197 (77), 135 (100). HRMS calcd. for $C_{25}H_{34}NO_4Si$ (M + H): 440.2258; found: 440.2294. IR (film) υ_{max} (cm^{-1}): 1831, 1705. ^1H NMR δ: 1.03 (s, 9H), 1.46 (s, 9H), 3.90–3.94 (dd, *J* = 11.4, 1.7 Hz, 1H), 4.00–4.20 (br s, 1H,), 4.68–4.70 (m, 2H), 4.88 (br s, 1H), 7.37–7.47 (m, 6H), 7.62–7.73 (m, 4H). ^{13}C NMR δ: 19.1, 26.5, 28.2, 60.3, 70.0, 80.6, 84.0, 127.7, 129.8, 132.6, 135.4, 155.1, 198.9.

(2*R*)-2-(*tert*-Butyloxycarbonylamino)-4-oxacyclopentanone (16)

Compound **16** was isolated as a side product when preparing compound **15h** (12%): oil. IR (neat) υ_{max} (cm^{-1}): 3431, 1770, 1711. ^1H NMR δ: 1.45 (s, 9H), 3.78 (dd, *J* = 10.4, 9.0 Hz, 1H), 3.93 (d, *J* = 17.2 Hz, 1H), 4.09–4.21 (br s, 1H), 4.20 (d, *J* = 17.2 Hz, 1H), 4.64–4.72 (m, 1H), 4.91–5.01 (br s, 1H).

***rac*-2-(*tert*-Butyldiphenylsilyloxymethyl)-3-aza-5-oxa-1,4-cyclohexanedione (17)**

Compound **17** was isolated as a side product when preparing compound **15h** (8%): mp 137–139°C. EI-MS *m*/*z* (rel intensity): 327 ($[M + 1]^+$, 38), 326 (M^+, 57), 296 (62), 282 (46), 248 (48), 199 (100). HRMS calcd. for $C_{17}H_{16}NO_4Si$: 326.0849; found: 326.0872. IR (KBr) υ_{max} (cm^{-1}): 3270, 1736, 1720. ^1H NMR δ: 1.04 (s, 9H), 3.80 (dd, *J* = 10.3, 2.8 Hz, 1H), 3.91–3.94 (br s, 1H), 4.56 (d, *J* = 17.0 Hz, 1H), 4.65 (d, *J* = 17.0 Hz, 1H), 6.49 (s, 1H), 7.37–7.46 (m, 6H), 7.58–7.67 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 19.0, 26.5, 61.1, 64.6, 71.8, 127.9, 130.1, 132.0, 135.5, 154.3, 200.9.

(2*S*,*E*/*Z*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-ethylidene azetidine (18)

To a solution of (ethyl)triphenylphosphonium bromide (1.94 g, 5.23 mmol) in THF (30.0 mL) at rt was added *n*-butyllithium (2.12 mL, 2.19 M in hexanes, 4.65 mmol). The resulting orange red solution was stirred for 30 min and cooled down to 0°C. A solution of compound **15h** (0.511 g, 1.16 mmol) in THF (10.0 mL) was added. After stirring at rt for 14 h, water (30.0 mL) was added, THF was removed in vacuo and the aqueous solution was extracted with EtOAc (3 × 30 mL). The extracts were dried (Na_2SO_4) and filtered. Column chromatography (EtOAc–hexanes, 1:8) afforded 0.281 g (54%) of product **18** as a colorless oil. IR (film) υ_{max} (cm^{-1}): 1705. ^1H NMR δ: 1.05 (s, 9H), 1.40 (s, 9H), 1.58–1.60 (m, 3H), 3.79–3.86 (m, 1H), 3.90–4.10 (br s, 1H), 4.29–4.41 (m, 2H), 4.67 (br s, 1H), 4.80 (br s, 1H), 7.34–7.46 (m, 6H), 7.65–7.74 (m, 4H).

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-cyanocarbonyl methylidene-azetidine (19) and (2*S*,*Z*)-1-*tert*-butoxycarbonyl-2-*tert*-butyldiphenysilyloxymethyl-3-cyanocarbonyl-methylidene-azetidine (20)

To a solution of diethyl cyanomethylphosphonate (62.0 mg, 0.250 mmol) in DME (1.50 mL) at 0°C was added *n*-butyllithium (0.14 mL, 2.30 M in hexanes, 0.32 mmol). The resulting light yellow solution was stirred at 0°C for 20 min and rt for 20 min. The solution of compound **15h** (0.128 g. 0.29 mmol) in DMF (1.50 mL) was added. After stirring at rt for 2 h, the reaction was quenched with saturated NH_4Cl solution (1.00 mL). The aqueous solution was extracted with EtOAc (3 × 5.00 mL) and the combined EtOAc solutions were dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:3) afforded 77.0 mg (57%) of product **19** and 22.0 mg (16%) of product **20**, both as colorless oils. For **19**: $[\alpha]_D$ –24.0 (*c* 1.22, $CHCl_3$). FAB-MS *m*/*z* (rel intensity): 463 ($[M + H]^+$, 47), 407 (66), 349 (46), 329 (52), 198 (52), 154 (70), 137 (71). 106 (59), 57 (100). HRMS calcd. for $C_{27}H_{34}N_2O_3Si$ (M+ H): 463.2419; found: 463.2435. IR (film) υ_{max} (cm^{-1}): 2221, 1710. ^1H NMR δ: 1.07 (s, 9H), 1.42 (s, 9H), 3.85–3.90 (dd, *J* = 10.6, 3.3 Hz, 1H), 3.97–4.08 (br s, 1H), 4.63–4.65 (dd, *J* = 2.9, 2.9 Hz, 2H), 4.84 (m, 1H), 5.38 (m, 1H). 7.37–7.48 (m, 6H), 7.62–7.67 (m, 4H). ^{13}C NMR δ: 19.1, 26.6, 28.1, 56.7, 62.8, 70.5, 80.5, 93.1, 114.3, 127.7, 129.8, 132.5, 135.4, 155.4. 160.7.

For **20**: $[\alpha]_D$ +61.3 (*c* 1–22, $CHCl_3$). FAB-MS *m*/*z* (rel intensity): 463 ($[M + H]^+$, 66), 407 (71), 349 (49), 329 (52), 307 (42), 137 (37), 106 (68), 57 (100). HRMS calcd for $C_{27}H_{34}N_2O_3Si$ (M + H): 463.2419; found: 463.2397. IR (film) υ_{max} (cm^{-1}): 2219, 1706. ^1H NMR δ: 1.06 (s, 9H), 1.42 (s, 9H), 4.08–4.25 (br s, 2H), 4.51–4.58 (ddd, *J* = 15.4, 2.0, 1.9 Hz, 1H), 4.61–4.68 (ddd, *J* = 15.4, 4.2, 2.3 Hz, 1H), 4.95–4.97 (m, 1H), 5.45–5.47 (m, 1H). 7.36–7.47 (m, 6H), 7.63–7.73 (m, 4H). ^{13}C NMR δ: 19.3. 26.7. 28.3. 57.2, 61.2. 72.3, 80.4, 92.9, 114.2, 127.7. 129.8, 132.9, 135.5, 155.2, 160.4.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-(*N*-methoxy-*N*-methylcarbamoyl)methylidene-azetidine (22)

To a suspension of NaH (1.76 g, 60% in oil, 44.0 mmol, washed three times with ether) in THF (500 mL) at 0°C was added the solution of diethyl (*N*-methoxy-*N*-methylcarbamoylmethyl)phosphonate **21** (11.0 g, 46.0 mmol) in THF (50.0 mL). The resulting reaction mixture was stirred at 0°C for 10 min and at rt for 20 min, a solution of compound **15h** (17.9 g, 42.0 mmol) in THF (50.0 mL) was added. After stirring at rt for 14 h, water (30.0 mL) was added, THF was evaporated, and the aqueous solution was extracted with

EtOAc (3 × 30.0 mL). The combined EtOAc solution was dried (Na_2SO_4) and filtered. Column chromatography (CH_2Cl_2–C_6H_6–EtOAc, 4:2:1) afforded 16.0 g (73%) of product **22** as a colorless oil and 1.0 g (4.6%) of product **23** as a colorless solid. For **22**: $[\alpha]_D$ +3.2 (*c* 1.7 1, $CHCl_3$). FAB-MS *m*/*z* (rel intensity): 525 ($[M + H]^+$, 52), 469 (73), 451 (18), 411 (47), 213 (21), 197 (72), 154 (76), 136 (100). HRMS calcd. for $C_{29}H_{40}N_2O_5Si$ (M + H): 525.2786; found: 525.2723. IR (film) υ_{max} (cm^{-1}): 1718, 1644. ^1H NMR δ: 1.06 (s, 9H), 1.40 (s, 9H), 3.23 (s, 3H), 3.65 (s, 3H), 3.93–4.01 (br s, 2H), 4.83–4.91 (m, 3H), 6.52–6.55 (br s, 1H), 7.34–7.46 (m, 6H), 7.65–7.69 (m, 4H). ^1H NMR (C_6D_6) δ: 1.13 (s, 9H), 1.39 (s, 9H), 2.87 (s, 3H), 3.10 (s, 3H), 3.86–4.30 (br s, 2H), 4.72–4.79 (br s, 1H), 5.12–5.27 (m, 2H), 6.60–6.70 (br s, 1H), 7.21–7.26 (m, 6H), 7.73–7.80 (m, 4H). ^{13}C NMR δ: 19.1, 26.6, 28.2, 31.8, 59.3, 61.6, 63.3, 70.2, 79.6, 110.3, 127.6, 129.6, 129.7, 132.9, 135.4, 154.4, 165.9. ^{13}C NMR (C_6D_6) δ: 19.5, 26.9, 28.4, 31.8, 60.2, 61.1, 63.7, 70.7, 79.2, 110.9, 128.1, 128.6, 130.0. 133.6, 136.0, 154.8, 166.0.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-(*N*-methoxy-*N*-methylcarbamoyl)methylidene-azetidine (23)

A mixture of compound **15h** (0.647g, 1.47 mmol) and *N*-methoxy-*N*-methyl-2-(triphenylphosphoranylidene)acetamide (0.642 g, 1.77 mmol) in MeOH (15.0 mL) was stirred at –78°C for 1 h and at rt for 24 h. After concentration to dryness, column chromatography (CH_2Cl_2–C_6H_6–EtOAc, 4:2:1) afforded 0.515 g (67%) of product **23** and 0.059 g (7.7%) of product **22**. For **23**: mp 119–121°C (CH_2Cl_2–hexanes). $[\alpha]_D$ +15.9 (*c* 0.79, $CHCl_3$). FAB-MS *m*/*z* (rel intensity): 525 ($[M + H]^+$, 44), 469 (27), 451 (16), 411 (40), 212 (54), 197 (66), 154 (82), 136 (100). HRMS calcd. for $C_{29}H_{40}N_2O_5Si$ (M + H): 525.2786; found: 525.2836. IR (film) υ_{max} (cm^{-1}): 1711, 1645. ^1H NMR δ: 1.03 (s, 9H), 1.40 (s, 9H), 3.18 (s, 3H), 3.70 (s, 3H), 4.05–4.15 (br s, 1H), 4.34–4.39 (m, 1H), 4.44–4.53 (m, 1H), 4.64–4.73 (m, 1H), 5.18–5.20 (br s, 1H), 6.34–6.41 (br s, 1H), 7.29–7.41 (m, 6H), 7.59–7.68 (m, 4H). ^1H NMR (C_6D_6) δ: 1.16 (s, 9H), 1.40 (s, 9H), 2.86 (s, 3H), 3.06 (s, 3H), 4.22–4.40 (br s, 2H), 4.66–4.76 (m, 2H), 5.37–5.38 (br s, 1H), 6.17 (br s, 1H), 7.21–7.29 (m, 6H), 7.76–7.84 (m, 4H). ^{13}C NMR δ: 18.9, 26.4, 28.0, 31.6, 56.9, 61.0, 61.3, 73.4, 79.2, 110.7, 126.8, 129.0, 133.1, 133.4, 135.1, 153.2, 165.1. ^{13}C NMR (C_6D_6) δ: 19.6, 27.1, 28.5, 31.8, 57.4, 61.1, 61.8, 74.1, 79.1, 111.6, 128.6, 129.8, 134.0, 134.3, 136.1, 153.8, 165.9. Anal. calcd for $C_{29}H_{40}N_2O_5Si$: C 66.38, H 7.68, N 5.34; found: C 66.38, H 7.62, N 5.25.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-(formyl)methylidene-azetidine (25)

To a solution of **22** (8.70 g, 16.6 mmol) in THF (170 mL) at –78°C was added lithium aluminum hydride (3.15 g, 82.9 mmol). The resulting reaction mixture was stirred at –78°C for 1 h and quenched with H_2O (3.15 mL) at –78°C. After warming to rt, a 15% NaOH solution (3.15 mL) was added and the slurry was stirred for 15 min at rt followed by addition of H_2O (6.30 mL). The reaction mixture was filtered and the filtrate was concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:3) afforded 5.23 g (68%) of product **25** as a colorless oil and 1.65 g (21%) of product **26** as a colorless oil. For **25**: $[\alpha]_D$ –16.0 (*c* 2.01, $CHCl_3$). FAB-MS (NEA) *m*/*z* (rel intensity): 466 ($[M + H]^+$, 5), 427 (9), 352 (12), 197 (74), 154 (64), 135 (100). HRMS calcd. for $C_{27}H_{36}N_3O_4Si$ (M + H): 466.2415; found: 466.2390. IR ($CHCl_3$) υ_{max} (cm^{-1}): 2739, 1703, 1696. ^1H NMR δ: 1.05 (s, 9H), 1.43 (s, 9H), 3.87–3.91 (dd, *J* = 10.6, 2.7 Hz, 1H), 4.00–4.17 (br s, 1H), 4.86–4.88 (m, 2H), 4.93 (br s, 1H), 6.07–6.10 (m, 1H), 7.35–7.47 (m, 6H), 7.61–7.67 (m, 4H), 9.62–9.65 (d, *J* = 6.8 Hz, 1H). ^{13}C NMR δ: 19.4, 26.9, 28.4, 60.0, 62.2, 63.4, 71.6, 79.6, 122.4, 128.6, 130.1, 133.9, 135.9, 155.4, 159.2, 187.8.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenyisilyloxymethyl-3-(hydroxymethyl)methylidene-azetidine (26)

To a solution of **25** (5.11 g, 11.0 mmol) in ethanol (300 mL) at rt was added sodium borohydride (0.415 g, 11.0 mmol) and cerium trichloride heptahydrate (4.10 g, 11.0 mmol) simultaneously. The resulting reaction mixture was stirred at rt for 30 min and quenched with H_2O (300 mL). The aqueous solution was extracted with ethyl acetate (3 × 500 mL) and the combined ethyl acetate solution was dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo to dryness. Column chromatography (CH_2Cl_2–hexanes–EtOAc, 2:1:1) afforded 4.54 g (89%) of product **26** as a colorless oil: $[\alpha]_D$ –13.3 (*c* 1.56, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 468 ($[M + H]^+$, 20), 429 (18), 354 (44), 334 (43), 307 (40), 198 (63), 154 (90), 136 (100). HRMS calcd. for $C_{27}H_{38}NO_4Si$ (M + H): 468.2571; found: 468.2618. IR ($CHCl_3$) υ_{max} (cm^{-1}): 3420, 1700, 1676. ^1H NMR δ: 1.06 (s, 9H), 1.41 (s, 9H), 3.83–3.87 (dd, *J* = 10.4, 3.0 Hz, 1H), 3.95–4.09 (br s, 1H), 4.10–4.12 (d, *J* = 5.7 Hz, 2H), 4.51 (br s, 2H), 4.73 (br s, 1H), 5.61 (m, 1H), 7.35–7.43 (m, 6H), 7.65–7.70 (m, 4H). ^{13}C NMR δ: 19.2, 26.7, 28.3, 55.8, 60.0, 63.5, 70.1, 79.5, 120.8, 127.6, 129.6, 133.3, 133.4, 135.5, 155.8.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-(bromomethyl)methylidene-azetidine (27)

To a solution of **26** (1.98 g, 4.23 mmol) in dichloromethane (85.0 mL) at rt was added triphenylphosphine (1.67 g, 6.35 mmol) and carbon tetrabromide (2.11 g, 6.35 mmol). The resulting reaction mixture was stirred at rt for 14 h and concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:6) afforded 1.67 g (75%) of product **27** as a colorless oil: $[\alpha]_D$ –10.9 (*c* 2.27, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 532 ($[M + H]^+$, 3), 530 ($[M + H]^+$, 1), 418 (14), 416 (12), 199 (55), 197 (52), 135 (100). HRMS calcd. for $C_{27}H_{37}BrNO_3Si$ (M + H): 530.1727; found: 530.1783. IR ($CHCl_3$) υ_{max} (cm^{-1}): 1702. ^1H NMR δ: 1.06 (s, 9H), 1.41 (s, 9H), 3.84–3.88 (m, 3H), 3.90–4.10 (br s, 1H), 4.51 (br s, 2H), 4.76 (br s, 1H), 5.77 (m, 1H), 7.36–7.46 (m, 6H), 7.66–7.73 (m, 4H). ^{13}C NMR δ: 19.1, 26.6, 27.3, 28.2, 54.3, 63.1, 69.9, 79.7, 118.4, 127.6, 129.6, 133.1, 135.5, 138.3, 155.6.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyloxymethyl-3-ethylidene-azetidine (28)

To a solution of **27** (2.85 g, 5.36 mmol) in dimethyl sulphoxide (110 mL) at rt was added lithium borohydride (5.36 mL, 2.0 M solution in THF, 10.7 mmol). The resulting reaction mixture was stirred at rt for 14 h. Water (110 mL) was added followed by extraction with ethyl acetate (3 ×

200 mL). The combined ethyl acetate solution was dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:8) afforded 1.60 g (66%) of product **28** as a colorless oil: $[\alpha]_D$ –10.7 (*c* 1.34, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 452 ($[M + H]^+$, 9), 396 (7), 338 (100), 318 (84), 199 (83), 154 (42), 135 (100). HRMS calcd. for $C_{27}H_{37}NO_3Si$ (M + H): 452.2685; found: 452.2667. IR ($CHCl_3$) υ_{max} (cm^{-1}): 1701. ^1H NMR δ: 1.05 (s, 9H), 1.40 (s, 9H), 1.58–1.60 (m, 3H), 3.79–3.83 (dd, *J* = 10.3, 2.9 Hz, 1H), 3.90–4.10 (br s, 1H), 4.41 (br s, 2H), 4.67 (br s, 1H), 5.39–5.50 (m, 1H), 7.34–7.46 (m, 6H), 7.65–7.72 (m, 4H). ^{13}C NMR δ: 13.5, 19.5, 26.6, 28.3, 55.1, 63.6, 69.9, 79.2, 116.8, 127.5, 129.5, 131.5, 133.4, 135.5, 155.9.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-hydroxymethyl-3-ethylidene-azetidine (29)

To a solution of compound **28** (0.589 g, 1.30 mmol) in THF (40.0 mL) at 0°C was added tetrabutylammonium fluoride (1.96 mL, 1.0 M solution in THF, 1.96 mmol). The resulted reaction mixture was stirred towards rt for 3 h. The reaction mixture was concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:3) afforded 0.261 g (94%) of product **29** as a colorless oil: $[\alpha]_D$ –8.9 (*c* 1.56, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 214 ($[M + H]^+$, 78), 182 (54), 158 (100), 136 (98), 126 (81), 107 (96), 97 (74). HRMS calcd. for $C_{11}H_{20}NO_3$ (M + H): 214.1444; found: 214.1422. IR ($CHCl_3$) υ_{max} (cm^{-1}): 3425, 1701, 1658. ^1H NMR δ: 1.43 (s, 9H), 1.50–1.53 (m, 3H), 3.66–3.78 (m, 2H), 4.31–4.42 (m, 2H), 4.81 (br s, 1H), 5.30–5.40 (m, 1H). ^{13}C NMR δ: 13.3, 28.1, 55.1, 65.6, 71.3, 80.3, 117.7, 128.7, 156.9.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-carboxylic acid 3-ethylidene-2-azetidine (30)

To a solution of **29** (64.6 mg, 0.30 mmol) in acetone (3.00 mL) at 0°C was added chromic acid (1.16 mL, 2.6 M solution, 3.03 mmol). The resulting reaction mixture was stirred towards rt for 3 h. Isopropanol was added to destroy the residual chromic acid followed by the addition of water (5.00 mL). The mixture was extracted with ether (3 × 10.0 mL) and the combined ether solution was dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo to dryness. Column chromatography (EtOAc–hexanes, 1:2, with 1% of AcOH) afforded 26.5 mg (39%) of product **30**: mp 138–140°C (CH_2Cl_2–hexane). $[\alpha]_D$ –63.5 (*c* 1.28, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 228 ($[M + H]^+$, 8), 220 (45), 172 (25), 154 (100), 136 (98), 107 (89). HRMS calcd. for $C_{11}H_{18}NO_4$ (M + H): 228.1236; found: 228.1244. IR ($CHCl_3$) υ_{max} (cm^{-1}): 3700–2500, 1729, 1707, 1687. ^1H NMR δ: 1.45 (s, 9H), 1.57–1.61 (m, 3H), 4.39–4.57 (m, 2H), 5.19 (br s, 1H), 5.78 (br s, 1H). ^{13}C NMR δ: 13.5, 28.2, 55.5, 67.9, 81.7, 120.7, 124.9, 156.6, 171.7. Anal. calcd for $C_{11}H_{17}NO_4$: C 58.14, H 7.54, N 6.16; found: C 57.99, H 7.57, N 6.12.

(2*S*,*E*)-3-Ethylidene-azetidine-2-carboxylic acid (1)

A solution of compound **30** (0.104 g, 0.459 mmol) in formic acid (5.00 mL) was stirred at rt for 5 h. After filtration through a fine sintered glass funnel, the filtrate was concentrated in vacuo on an oil pump to dryness to give 0.058 g (100%) of product **1**: mp 147–149°C (triturated with methanol and isopropanol). $[\alpha]_D$ +17.3 (*c* 2.10, MeOH). CI-MS *m*/*z* (rel intensity): 129 ($[M + H]^+$, 98), 84 (100). HRMS calcd. for $C_6H_{10}NO_2$ (M + H): 128.0794; found: 128.0722. IR (neat) υ_{max} (cm^{-1}): 3700–2000, 1614, 1379, 1291, 759, 690. ^1H NMR (CD_3OD) δ: 1.56–1.58 (m, 3H), 4.50–4.70 (m, 2H), 5.09 (br s, 1H), 5.70–5.83 (m, 1 H). ^1H NMR (D_2O) δ: 1.53–1.56 (m, 3H), 4.60–4.73 (m, 2H), 5.24 (br s, 1H), 5.66–5.77 (m, 1H). ^{13}C NMR (CD_3OD) δ: 13.1, 53.3, 70.7, 121.3, 128.4, 170.6.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyl-oxymethyl-3-(formyl)methylidene-azetidine (31)

Compound **23** (0.505 g, 0.962 mmol) was reduced with lithium aluminum hydride (0.192 g, 95%, 4.81 mmol) at –78°C for 2 h to afford 0.274 g (61%) of **31** and 0.109 g (24%) of **32** both as colorless oils. For **31** : $[\alpha]_D$ +35.9 (*c* 1.79, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 466 ($[M + H]^+$, 31), 427 (17), 352 (12), 198 (72), 154 (76), 135 (100). HRMS calcd. for $C_{27}H_{36}NO_4Si$ (M + H): 466.2415; found: 466.2371. IR ($CHCl_3$) υ_{max} (cm^{-1}): 2739, 1701, 1685. ^1H NMR δ: 1.06 (s, 9H), 1.44 (s, 9H), 3.87–3.91 (d, *J* = 9.3 Hz, 1H), 4.55–4.61 (m, 1H), 4.62–4.69 (m, 2H), 5.20 (br s, 1H), 6.09–6.13 (m, 1H), 7.35–7.47 (m, 6H), 7.61–7.76 (m, 4H), 9.68–9.71 (d, *J* = 7.3 Hz, 1H). ^{13}C NMR δ: 19.1, 26.6, 28.2, 56.9, 62.7, 63.1, 71.7, 80.2, 123.8, 127.6, 129.7, 129.8, 132.6, 155.1, 159.3, 189.0.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyl-oxymethyl-3-(hydroxymethyl)methylidene-azetidine (32)

Compound **31** (1.40 g, 3.01 mmol) was reduced with sodium borohydride (0.125 g, 3.31 mmol) in the presence of cerium trichloride heptahydrate (1.24 g, 3.31 mmol) to afford 1.16 g (82%) of **32** as a colorless oil: $[\alpha]_D$ –0.2 (*c* 1.53, $CHC1_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 468 ($[M + H]^+$, 21), 412 (14), 354 (52), 334 (46), 198 (71), 154 (85), 136 (100). HRMS calcd. for $C_{27}H_{38}NO_4Si$ (M + H): 468.2571, found 468.2598. IR (neat) υ_{max} (cm^{-1}): 3420, 1700, 1676. ^1H NMR δ: 1.08 (s, 9H), 1.34 (s, 9H), 3.89–3.92 (dd, *J* = 10.3, 2.8 Hz, 1H), 3.95–4.21 (br s, 3H), 4.37–4.49 (m, 2H), 4.96 (br s, 1H), 5.63 (m, 1H), 7.36–7.45 (m, 6H), 7.66–7.70 (m, 4H). ^{13}C NMR δ: 19.1, 26.7, 28.2, 56.0. 59.7, 64.0, 70.0, 79.5, 122.3, 127.7, 129.7, 132.8, 133.3, 135.4, 155.6.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyl-oxymethyl-3-(bromomethyl)methylidene-azetidine (33)

Compound **32** (0.315 g, 0.672 mmol) was reacted with triphenylphosphine (0.265 g, 1.01 mmol) and carbon tetrabromide (0.335 g, 1.01 mmol) to afford 0.285 g (80%) of **33** as a colorless oil: $[\alpha]_D$ +60.3 (*c* 1.19, $CHCl_3$). FAB-MS (NBA) *m*/*z* (rel intensity): 532 ($[M + H]^+$, 3), 530 ($[M + H]^+$, 2), 417 (11), 415 (10), 198 (37), 195 (34), 135 (100). HRMS calcd. for $C_{27}H_{37}BrNO_3Si$ (M + H): 530.1727; found: 530.1754. IR (neat) υ_{max} (cm^{-1}): 1701. ^1H NMR δ: 1.09 (s, 9H), 1.39 (s, 9H), 3.81–3.96 (m, 2H), 3.96–4.15 (br s, 2H), 4.39–4.53 (m, 2H), 4.93 (br s, 1H), 5.73 (m, 1H), 7.37–7.47 (m, 6H), 7.69–7.72 (m, 4H). ^{13}C NMR δ: 19.1, 26.7, 27.5, 28.2, 55.7, 63.2, 69.9, 79.6, 119.4, 127.6, 129.6, 132.9, 135.4, 137.4, 155.4.

(2*S*,*E*)-1-*tert*-Butyloxycarbonyl-2-*tert*-butyldiphenylsilyl-oxymethyl-3-ethylidene-azetidine (34)

Compound **33** (0.285 g, 0.537 mmol) was reacted with

lithium borohydride (0.54 mL, 2.0 M solution in THF, 1.08 mmol) to afford 0.181 g (79%) of **34** as a colorless oil: $[\alpha]_D$ +4.4 (*c* 1.38, $CHCl_3$). FAB-MS (NBA) *m/z* (rel intensity): 452 ($[M + H]^+$, 20), 396 (19), 352 (18), 338 (54), 318 (51), 225 (52), 199 (61), 154 (82), 136 (100). HRMS calcd. for $C_{27}H_{38}NO_3Si$ (M + H): 452.2685; found: 452.2598. IR (neat) υ_{max} (cm^{-1}): 1700. ^1H NMR δ: 1.08 (s, 9H), 1.43 (s, 9H), 1.59–1.61 (m, 3H), 3.85–3.89 (dd, *J* = 10.7, 2.1 Hz, 1H), 4.00–4.25 (br s, 1H), 4.30–4.52 (m, 2H), 4.82 (br s, 1H), 5.39–5.50 (m, 1H), 7.35–7.47 (m, 6H), 7.67–7.75 (m, 4H). ^{13}C NMR δ: 13.6, 19.2, 26.8, 28.3, 56.1, 62.5, 70.2, 79.1, 117.3, 127.5, 129.5, 130.8, 133.3, 135.5, 155.6.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-2-hydroxymethyl-3-ethylidene-azetidine (35)

Compound **34** (0.912 g, 2.02 mmol) was reacted with tetrabutylammonium fluoride (3.03 mL, 1.0 M solution in THF, 3.03 mmol) to afford 0.418 g (97%) of **35** as a colorless oil: $[\alpha]_D$ –12.3 (*c* 1.98, $CHCl_3$). FAB-MS (NBA) *m/z* (rel intensity): 214 ($[M + H]^+$, 20), 158 (55), 136 (100), 107 (92). HRMS calcd. for $C_{11}H_{20}NO_3$ (M + H): 214.1444; found: 214.1460. IR (neat) υ_{max} (cm^{-1}): 3423, 1701, 1658. ^1H NMR δ: 1.45 (s, 9H), 1.57–1.61 (m, 3H), 3.78–3.85 (dd, *J* = 11.6, 7.6 Hz, 1H), 3.90–3.95 (dd, *J* = 11.6, 7.6 Hz, 1H) 4.32–4.34 (m, 2H), 4.96 (br s, 1H), 5.31–5.42 (m, 1H). ^{13}C NMR δ: 13.6, 28.2, 55.9, 64.5, 71.3, 80.4, 119.0, 128.1, 154.6.

(2*S*,*Z*)-1-*tert*-Butyloxycarbonyl-3-ethylidene-azetidine-2-carboxylic acid (36)

Compound **35** (0.178 g, 0.840 mmol) was oxidized with chromic acid (3.22 mL, 2.6 M solution, 8.36 mmol) to afford 76.0 mg (40%) of **36**: mp 83–84°C (CH_2Cl_2–hexane). $[\alpha]_D$ –15.3 (*c* 2.10, $CHCl_3$). EI-MS *m/z* (rel intensity): ($[M + H]^+$, 21), 182 (52), 172 (58), 154 (33), 142 (58), 126 (74), 76 (100). HRMS calcd. for $C_{11}H_{18}NO_4$ (M + H): 228.1236; found: 228.1214. IR (neat) υ_{max} (cm^{-1}): 3700–2500, 1731, 1702, 1690. ^1H NMR δ: 1.45 (s, 9H), 1.69–1.72 (m, 3H), 4.36–4.42 (m, 1H), 4.50–4.58 (m, 1H), 5.21 (m, 1H), 5.48–5.59 (m, 1H). ^{13}C NMR δ: 13.5, 28.2, 56.8, 68.0, 81.0, 121.3, 125.1, 155.8, 172.4. Anal. calcd for $C_{11}H_{17}NO_4$: C 58.14, H 7.54, N 6.16; found: C 57.81, H 7.42, N 6.06.

(2*S*,*Z*)-3-Ethylidene-azetidine-2-carboxylic acid (2)

A solution of **36** (0.107 g, 0.472 mmol) in formic acid (5.00 mL) was stirred at rt for 5 h. After filtration through a fine sintered glass funnel, the filtrate was concentrated in vacuo on an oil pump to dryness to afford 0.060 g (100%) of **2**: mp 153–155°C (triturated with methanol and EtOAc). $[\alpha]_D$ +41.3 (*c* 0.55, MeOH). CI-MS *m/z* (rel intensity): 128 ($[M + H]^+$, 20), 84 (43), 82 (100), 68 (48). HRMS calcd. for $C_6H_{10}NO_2$ (M + H): 128.0794; found: 128.0722. IR (neat) υ_{max} (cm^{-1}): 3700–2000, 1640, 1383, 1290, 851, 690. ^1H NMR (CD_3OD) δ: 1.76–1.80 (m, 3H), 4.45–4.63 (m, 2H), 5.14–5.16 (br s, 1H), 5.46–5.56 (m, 1H). ^1H NMR (D_2O) δ: 1.63–1.68 (m, 3H), 4.49–4.68 (m, 2H), 5.29 (m, 1H), 5.50–5.60 (m, 1H). ^{13}C NMR (CD_3OD) δ: 13.6, 54.7, 71.2, 122.8, 127.7, 170.1.

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