Total Synthesis of Petrosin, Petrosin A, and Petrosin B

Robert W. Scott, James Epperson, and Clayton H. Heathcock*

Department of Chemistry, University of California, Berkeley, California 94720

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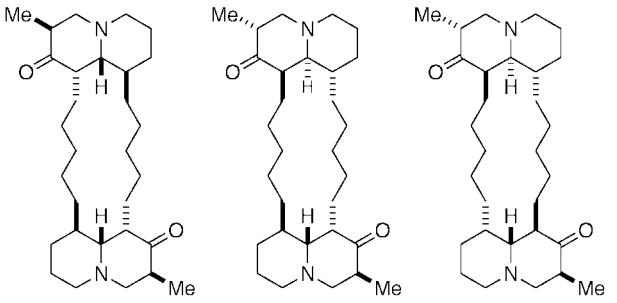
The petrosins are a family of marine alkaloids that includes the chiral, racemic isomer petrosin (**1**), the meso isomer petrosin A (**2**), and the chiral, scalemic isomer petrosin B (**3**). Monte Carlo molecular mechanics calculations indicated that petrosin (**1**) is the most stable isomer of the group, suggesting that it might be synthesized by a route that utilizes thermodynamic control for establishing the relative configurations of the eight stereocenters. The model synthesis summarized in Scheme 1 showed that intramolecular Mannich condensation is a viable route to the quinolizidone subunit of the petrosins and that this synthesis gives isomer **5**, having the relative configuration found in petrosin and petrosin A, as the kinetic product. Equilibration studies with this isomer afforded an approximate equimolar mixture of **5** and diastereomer **6**, having the relative configuration found in one of the two quinolizidone units of petrosin B. On the basis of this model study, a "stereo-uncontrolled" synthesis of petrosin was carried out, as summarized in Schemes 3−5. The key step of this synthesis is a "double-barrelled" intramolecular Mannich condensation of a diamino keto dialdehyde. This transformation provides crystalline petrosin in 23% yield, along with about 37% of a mixture of petrosin diastereomers. Although simple acid- and Lewis acid-mediated equilibrations of this mixture of diastereomers were not successful, the derived mixture of bis-butylimines undergoes equilibration upon treatment with protic acid to give a mixture that is greatly enriched in petrosin, relative to the other isomers. Crystallization of this equilibration mixture provided another 10% of petrosin, bringing the overall yield of petrosin to 33%. In the course of the equilibration studies, pure samples of petrosin A (**2**), petrosin B (**3**), and petrosin B′ (**7**) were isolated and characterized.

Between 1982 and 1988, Braekman and co-workers reported on the isolation and structural determination of three ichthyotoxic bis-quinolizidone alkaloids from the sponge *Petrosia seriata*, collected from the waters off of Papua New Guinea.[1,2] The three diastereomers are petrosin (**1**), petrosin A (**2**), and petrosin B (**3**). These natural products present a fascinating stereochemical and biosynthetic puzzle. Although it contains eight stereocenters, petrosin (**1**) is racemic as isolated. The relative configuration of petrosin was firmly established by X-ray crystallography. Petrosin A (**2**) is also devoid of optical activity, and the presence of only 15 peaks in the ^{13}C NMR spectrum for this isomer confirmed a symmetrical system. A 2-D NMR study of petrosin and petrosin A established that the quinolizidone units in these compounds have the same relative configuration. This led to the meso structure **2**, in which the two halves of the molecule are enantiomeric. Petrosin B (**3**), on the other hand, is optically active ($[\alpha]_{579} = -12$). In this isomer, one of the quinolizidone units has the same relative configuration as that found in petrosin and petrosin A, but the other quinolizidone has a different relative configuration. The structure was determined by 2-D NMR analysis and comparison to the previously obtained data for **1** and **2**.

Petrosin and petrosin A have also been isolated from the sponge *Xestospongia* sp.[3] In this work, the racemic nature of petrosin and the meso structure of petrosin A were confirmed by reduction of each isomer to the diequatorial diol, formation of the diester with a chiral carboxylic acid, and examination of the NMR spectra of these products. These experiments provided unambiguous proof that petrosin is racemic, rather than simply having a very low optical rotation.



Since it is uncommon for natural products to be biosynthesized in racemic form, we wondered if petrosin and petrosin A might be products of some post-biosynthetic equilibration. We evaluated this possibility by carrying out a series of molecular mechanics calculations. Minimizations were first carried out for the three chair−chair conformations of each of the eight diastereomeric forms of trimethylquinolizidone **4**.[4] Two low-energy structures of approximately equal energy, **5** and **6**, were

(1) (a) Braekman, J. C.; Daloze, D.; Macedo de Abreu, P.; Piccini-Leopardi, C.; Germain, G.; Van Meerssche, M. *Tetrahedron Lett.* **1982**, *23*, 4277. (b) Braekman, J. C.; Daloze, D.; Defay, N.; Zimmerman, D. *Bull. Soc. Chim. Belg.* **1984**, *93*, 941. (c) Braekman, J. C.; Daloze, D.; Cimino, G.; Trivellone, E. *Bull Soc. Chim Belg.* **1988**, *97*, 519.

(2) For an excellent recent review of this structurally unique class of marine natural products, see: Matzanke, N.; Gregg, R. J.; Weinreb, S. M. *Org. Prep. Proc. Int.* **1998**, 1−51.

(3) Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 4149.

(4) Calculated molecular mechanics energies of these 24 structures are included in the Supporting Information.

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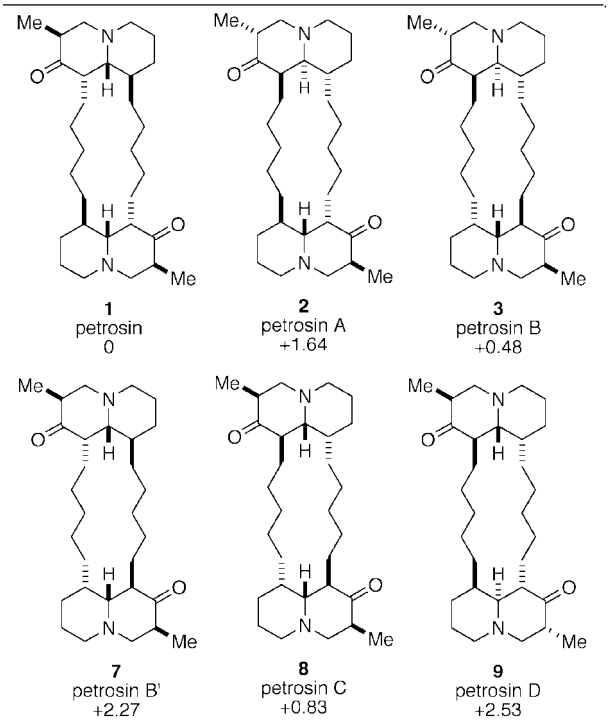
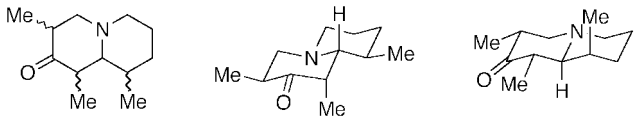
Table 1. Structures and Calculated Relative Energies (kcal mol^{-1}) of Petrosin Diastereomers



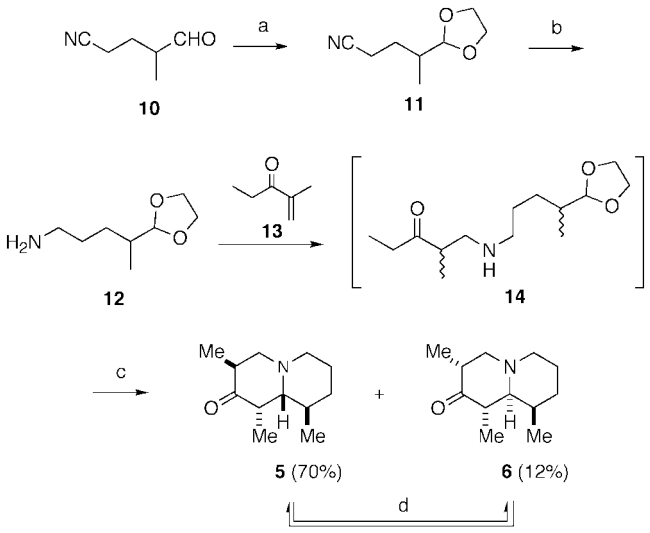
found. Isomer **5** represents the relative configuration present in petrosin, petrosin A, and one-half of petrosin B, whereas isomer **6** contains the unique configuration present in the novel half of petrosin B.



A series of Monte Carlo global minimizations were then carried out using various combinations of the two enantiomeric forms of **5** and **6**, randomizing the cyclohexadecane conformations. In this way, low-energy conformations of six diastereomeric forms of petrosin were generated. These six diastereomers correspond to the three known natural products, petrosin (**1**), petrosin A (**2**), petrosin B (**3**), and three unknown diastereomers, petrosin B′ (**7**),[5] petrosin C (**8**), and petrosin D (**9**). The calculated low-energy conformations of each of these diastereomers is indicated along with their structures in Table 1.

The computed low-energy conformation of petrosin is identical to the X-ray structure[1] and lower in energy than the low-energy conformations of petrosins A, B, B′, C, and D. These calculations suggested the interesting possibility of a synthesis based on thermodynamic control of relative configuration at the eight stereocenters. To this end, we carried out the simple model synthesis depicted in Scheme 1. Cyanoethylation of propanal, by way of the piperidine enamine, provided cyano aldehyde **10**, which was converted into acetal **11** by treatment with ethylene glycol. Reduction of the cyano group with lithium aluminum hydride provided amine **12**, which was added

Scheme 1[a]



[a] Key: (a) $(CH_2OH)_2$, *p*-TsOH; (b) $LiAlH_4$, THF; (c) 5% HOAc, 100 °C; (d) BF_3, CH_2Cl_2.

to enone **13**[6] to obtain **14** as a diastereomeric mixture. Treatment of **14** with aqueous acetic acid provided bicyclic amino ketone **5** in 70% yield, accompanied by about 12% of amino ketone **6** and smaller amounts of several other unidentified isomers. The major isomer was isolated as a crystalline picrate and characterized by single-crystal X-ray analysis. The minor isomer was identified as **6** by ^{1}H NMR analysis. Treatment of a 5.4:1 mixture of **5** and **6** with boron trifluoride in methylene chloride gave an approximate equimolar mixture of the two diastereomers.

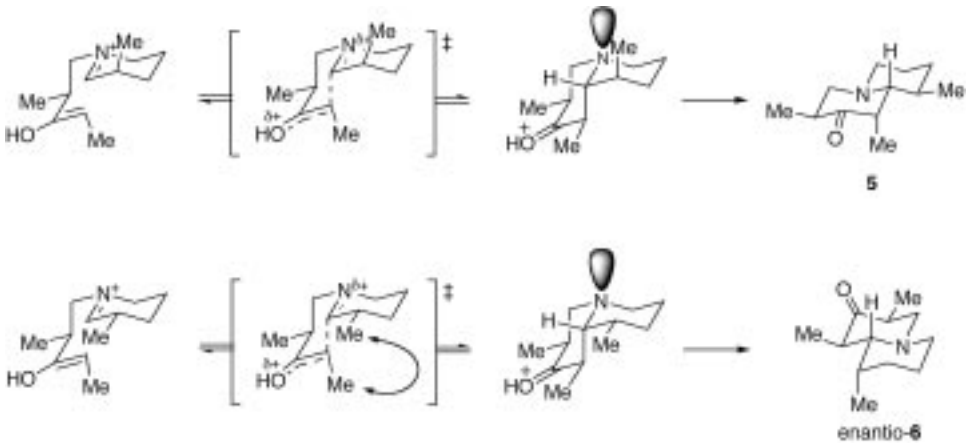
The equilibration experiments with **5** and **6** are in excellent agreement with the molecular mechanics calculations, which showed these two isomers to be of approximately equal energy and at least 1.5 kcal/mol more stable than any other diastereomeric trimethylquinolizidone. It is also notable that the kinetic product of the intramolecular Mannich reaction, isomer **5**, has the relative configuration found in both quinolizidone subunits of the two most abundant petrosin stereoisomers, petrosin and petrosin A. This kinetic preference is understandable if one considers the transition states for the C−C bond-forming step in the two Mannich reactions (Scheme 2). In the transition state leading to isomer **5**, the enol double bond attacks the immonium ion trans to the ring methyl group, leading initially to a "*cis*-decalinic" quinolidine, which isomerizes to **5** by inversion of the tertiary amine and epimerization of one of the methyl stereocenters adjacent to the carbonyl group. In the isomeric transition state leading to **6**, the enol must attack the immonium ion cis to the ring methyl group, resulting in the indicated nonbonded interaction. The experimental result of the model synthesis summarized in Scheme 1 is consistent with a small difference of about 0.6 kcal/mol in the energies of these two transition states (ratio of **5**:**6** = 6:1). Control experiments showed that **5** and **6** do not equilibrate under the conditions of the Mannich reaction in which they are formed.

At this point, it became clear that it should be possible to synthesize petrosin by a route involving a double-

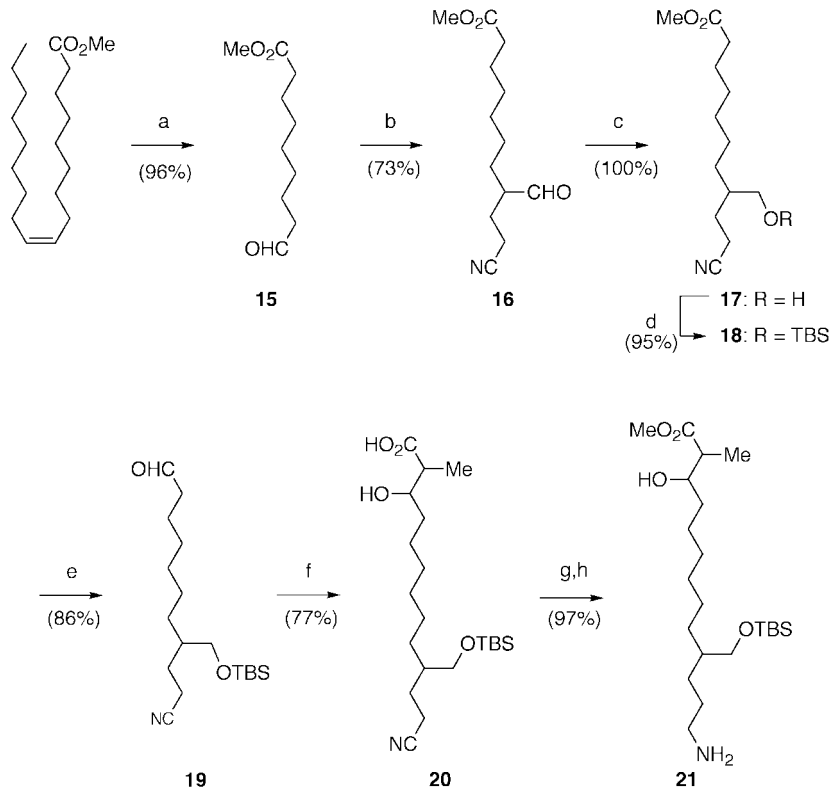
(5) Prior work did not actually distinguish between structures **3** and **7** for petrosin B.

(6) Gore, W. P.; Pearce, G. T.; Silverstein, R. M. *J. Org. Chem.* **1975**, *40*, 170.

Scheme 2



Scheme 3[a]



[a] Key: (a) (i) O_3; (ii) Zn, HOAc; (b) (i) pyrrolidine, K_2CO_3, (ii) CH_2=CHCN, (iii) H_2O; (c) $NaBH_4$; (d) TBS-Cl; (e) DIBAL, −95 °C; (f) CH_3CH=CO_2Li_2; (g) CH_2N_2; (h) H_2, PtO_2, EtOAc, HOAc.

barrelled intramolecular Mannich reaction, without paying any particular attention to stereocontrol.[7,8] The Mannich reactions leading to the quinolizidone subunits should give mainly "type **5**" relative stereochemistry for kinetic reasons. Thus, of the six petrosin isomers depicted in Table 1, a synthesis based on intramolecular Mannich reactions should yield mainly petrosin and petrosin A, smaller amounts of petrosins B and B′, and relatively minor amounts of petrosins C and D. If we were then able to achieve equilibration of this kinetic mixture (for example, by some acid-mediated method involving retro-Mannich reactions), we might be able to enrich the petrosin−petrosin A mixture significantly in favor of petrosin. With this object in mind, we set about a "stereo-uncontrolled" synthesis of petrosin.

Our synthesis began with the natural fatty-acid derivative methyl oleate, which was ozonized to obtain methyl azelaldehyde (**15**) (Scheme 3). The aldehyde was cyanoethylated by way of its pyrrolidine enamine to obtain **16**.[9] The aldehyde was reduced to obtain alcohol **17**, which was protected as the *tert*-butyldimethylsilyl ether **18**. This material was reduced to cyano aldehyde **19** with DIBAL-H at −95 °C. Compound **19** was consistently obtained by this method with yields in the mid-

(7) For a preliminary account of this work, see: Scott, R. W.; Epperson, J.; Heathcock, C. H. *J. Am. Chem. Soc.* **1994**, *116*, 8853.

(8) Hoye and co-workers have reported a thermodynamically controlled synthesis of the biosynthetically related compound xestospongin A: Hoye, T. R.; North, J. T.; Yao, L. J. *J. Am. Chem. Soc.* **1994**, *116*, 2617. Hoye, T. R.; Ye, Z.; Yao, L. J.; North, J. T. *J. Am. Chem. Soc.* **1996**, *118*, 12074.

(9) Stork, G. A.; Brizzolara, H.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207.

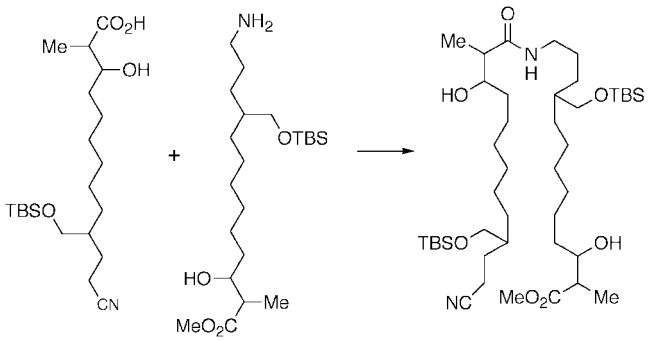
Table 2. Coupling of Acid 20 with Amine 21

entry	catalyst in reduction of **20**	coupling reagents[a]	solvent	yield of **22**, %
1	Raney Ni	DPPA	CH_2Cl_2	20−33
2	Raney Ni	CDI	CH_2Cl_2	21
3	Raney Ni	EDC, HOBT	DMF	40−48
4	Raney Ni	DCC, PFP[b]	CH_2Cl_2	49
5	Raney Ni	DCC, HOBT	THF	46−48
6	PtO_2, HOAc	DCC, HOBT	THF	70

[a] DPPA = diphenyl phosphorazidate; CDI = carbonyldiimidazole; EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; DCC = dicyclohexylcarbodiimide; HOBT = *N*-hydroxybenzotriazole; PFP = pentafluorophenol. [b] In this experiment, the pentafluorophenyl ester was preformed and the amine was then added.

80% range, with the balance of the material being mainly the cyano alcohol resulting from further reduction of the aldehyde. Maintaining a low temperature in this reaction is crucial, and even careful reaction at −78 °C proceeded with 10−15% more overreduction to the alcohol. Treatment of aldehyde **19** with the dianion of propionic acid gave acid **20** as a 1:1 mixture of syn and anti diastereomers.[10] At this point, the material was partitioned, as this precursor is used for both halves of the molecule. One portion is treated with diazomethane to produce the methyl ester, followed by reduction of the nitrile to amine **21**. This provides us with two differentially functionalized pieces to proceed with the sequential amide bond formations, as one segment has a free carboxylic acid and a latent amine (nitrile), whereas the other contains a protected carboxylic acid (ester) and free amine.

Reduction of the methyl ester of **20** was initially carried out over Ra−Ni catalyst under basic conditions, which produced the amino ester **21** in high yield. However, the primary amine was difficult to purify; therefore, **21** was used crude from the hydrogenation reaction in the amide coupling reaction with acid **20**. Under several different amide formation conditions, amide **22** was obtained in no greater than 50% yield (Table 2, entries 1−5). Additionally, it was discovered that acid **20** couples cleanly with a model pure amine and that amine **21** suffered the same low yields when attempts to couple it to a pure model acid were tried. Although the amine from the Ra−Ni reduction was clean by NMR, it was known that it contained some amount of nickel, as the viscous oil was a slightly green color and the recovery yield always several percent over quantitative. It is believed that this nickel complexed to the amine somehow inhibits the amide formation reaction, leading to the lower than expected yields of product. However, this realization presented a new problem, as the other common way to deal with the amine reduction is to perform the hydrogenation with typical catalysts (Pd or Pt) in the presence of an acid source to protonate the amine as formed, thereby limiting its ability to poison the catalyst. Since our substrate contains an acid-labile TBS protecting group; not surprisingly, most hydrogenations in the presence of acid led to the removal of this group. Eventually through trial and error, it was discovered that by carrying out the reduction in EtOAc with approximately 8−9 equiv of acetic acid over a large amount of PtO_2 catalyst (∼30 mol %), the nitrile could be reduced under 3 atm of H_2 to the amine hydroacetate salt in very high yield with only trace removal of the silyl group. The large amount of catalyst was required to speed the reaction enough that it was complete before significant desilylation occurs.



20 **21** **22**

The optimized procedure for preparation of amine **21** resulted in a good yield of amide **22** when coupling was carried out with dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole.[11] Our first attempts to achieve macrocyclization were carried out directly on the amino acid with Mukiayama's reagent.[12] These reactions led to several products, none of which could be identified as the desired macrolactam. In our next approach, compound **22** was treated with hydrogen over Adams' catalyst in ethanolic hydrochloric acid. In this way, the cyano group was reduced to a primary amine, which was trapped as the hydrochloride, and both silyl protecting groups were removed. After neutralization with $NaHCO_3$, the amine was protected as the BOC derivative to give **23** (Scheme 4). This compound possesses suitable functionality for a stepwise activation/macrolactamization procedure, without the necessity of protecting the hydroxy groups. The successful macrolactamization was accomplished by a four-step procedure that was optimized on the basis of several literature precedents.[13] The methyl ester of **23** was saponified and the resulting carboxylic acid converted to the pentafluorophenyl ester by treatment with dicyclohexylcarbodiimide and pentafluorophenol. Deprotection of the BOC group with standard TFA/CH_2Cl_2 conditions followed by concentration of the crude reaction mixture did lead to successful deprotection; however, as a side reaction, varying amounts of trifluoroacetates were formed on the free hydroxyls (as determined by ^1H and ^{19}F NMR and FAB-MS). To avoid this undesired reaction, the crude active ester was treated with anhydrous HCl in dioxane to remove the BOC group, and simultaneously protect the amine as the hydrochloride salt. A solution of this hydrochloride salt in dioxane−THF was slowly added with a syringe pump to a hot 5:1 dioxane−pyridine solution, providing the desired 28-membered macrolactam **24** in 78% overall yield for the three steps.

The amide linkages were reduced with lithium aluminum hydride to the diamine tetraol. Attempted oxidation

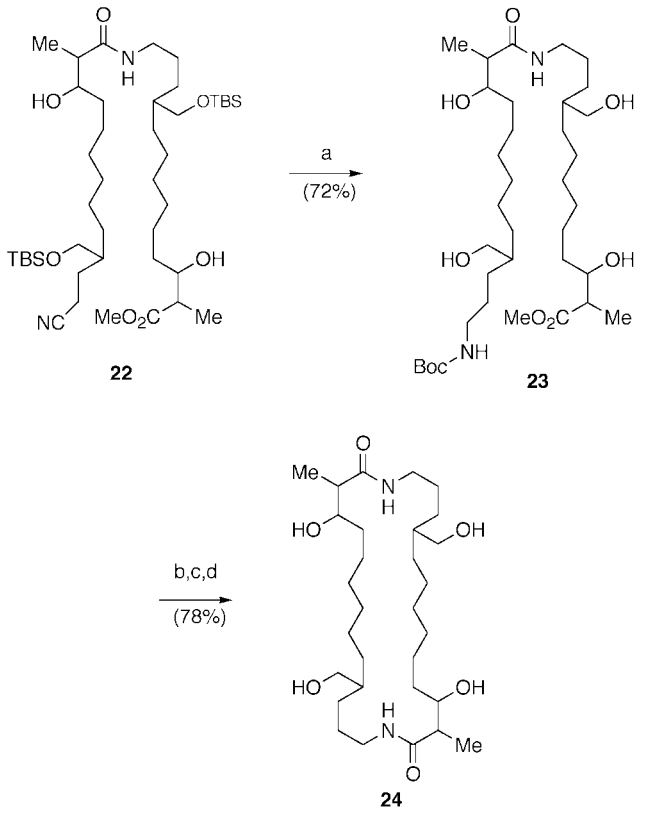
(10) Moersch, G. W.; Burkett, A. R. *J. Org. Chem.* **1971**, *36*, 1149.

(11) Windridge, G. C.; Jorgensen, E. C. *J. Am. Chem. Soc.* **1971**, *93*, 6318.

(12) Bald, E.; Saigo, K.; Mukaiyama, T. *Chem. Lett.* **1975**, 1163.

(13) (a) Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063. (b) Schmidt, U.; Meyer, R.; Leitenberger, V.; Griesser, H.; Lieberknecht, A. *Synthesis* **1982**, 1025. (c) Schmidt, U.; Leitenberger, V.; Griesser, H.; Schmidt, J.; Meyer, R. *Synthesis* **1992**, 1248. (d) Schmidt, U.; Griesser, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 280. (e) Schmidt, U.; Kroner, M.; Griesser, H. *Synthesis* **1991**, 294. (f) Schmidt, U.; Utz, R.; Lieberknecht, A.; Griesser, H.; Potzolli, B.; Wagner, K.; Fischer, P. *Synthesis* **1987**, 236.

Scheme 4[a]



[a] Key: (a) (i) 0.23 M HCl, EtOH, H_2, PtO_2; (ii) $(Boc)_2O$, dioxane, H_2O; (b) 1 M NaOH, MeOH, THF; (c) DCC, C_6F_5OH; (d) (i) 6 N HCl, dioxane, (ii) syringe pump into dioxane/pyridine.

of the diamine−tetraol with $(COCl)_2$/DMSO, SO_3•pyridine, CrO_3•pyridine, $AgCO_3$/Celite, $Na_2Cr_2O_7$/DMSO, or Dess−Martin periodinane led to unidentifiable products in all cases. These reactions were monitored by TLC and ^1H NMR using authentic natural material as a reference sample and it can be concluded that no more than trace amounts of petrosin isomers were formed under these conditions. As a result, the crude diamine obtained from the $LiAlH_4$ reduction was protected as the bis-BOC derivative, which seemed to eliminate the competing reactions in the oxidation as well as provide a product that could be purified after the $LiAlH_4$ reduction. The hydroxy groups of the di-BOC-protected compound were oxidized with Dess−Martin periodinane,[14] which accomplishes the tetraoxidation quickly (<30 min) and efficiently. The BOC groups were removed with HCl in aqueous ethanol, and the intramolecular Mannich condensation was achieved by treatment of the crude product with 0.2 M acetic acid in relfuxing ethanol.

The cyclization product, obtained in approximately 60% for the three-step procedure, was a mixture of petrosin and several of its stereoisomers. Crystalline petrosin, isolated from the crude product in 23% yield, was found to be identical by ^1H NMR, ^{13}C NMR, TLC, and melting point with an authentic sample provided by Professor Kitagawa.[2] The mixture of isomers can be analyzed by ^{13}C NMR, using the resonance for the methine carbon attached to nitrogen at about 70−72 ppm. This region of the ^{13}C NMR spectrum is relatively uncluttered, as the

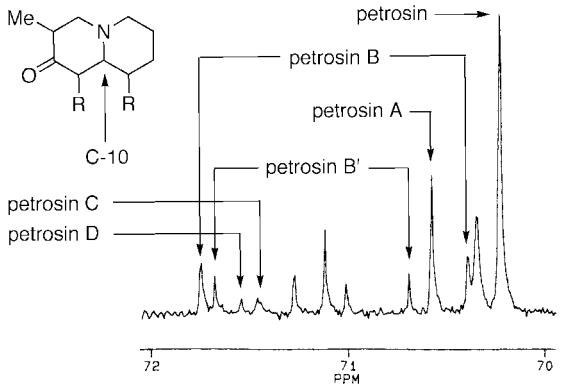


Figure 1. Portion of the ^{13}C NMR spectrum of a typical cyclization mixture (Scheme 5).

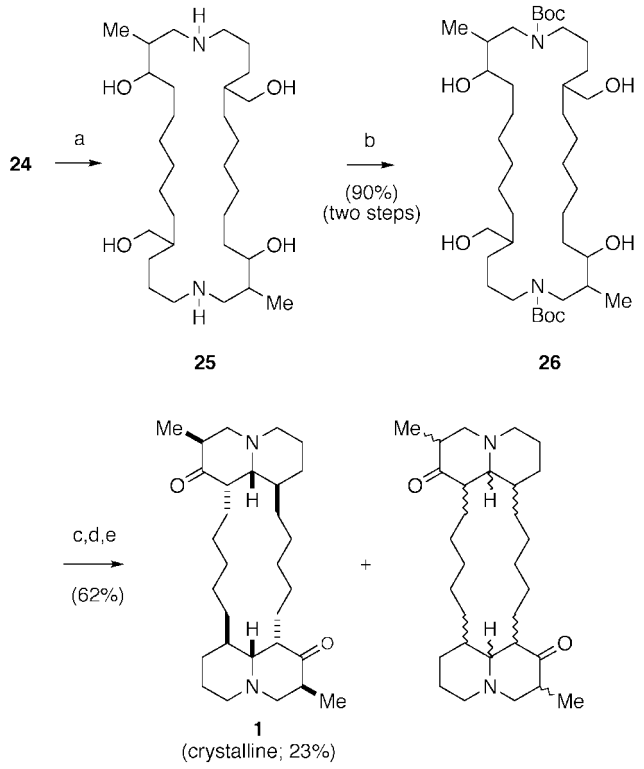
resonance for the C10 methine is well-removed from other resonances in petrosin and its isomers. Furthermore, this carbon is believed to be in a similar environment for all the isomers, attached to a nitrogen and flanked by one equatorial and one axial side-chain substituent, so the NOE effects and relaxation delays of this carbon should be similar enough in all the isomers of interest that ^{13}C NMR line heights can be used as an excellent measure of isomer ratios. An example of the 70−72 ppm region of the ^{13}C NMR spectrum for a typical cyclization mixture, prior to crystallization of the petrosin fraction, is shown in Figure 1. At the outset, we had the published chemical shifts for the C-10 methine resonances in the three isomeric natural products, petrosin (**1**), petrosin A (**2**), and petrosin B (**3**). In the course of this study, we were able to isolate pure samples of each of these isomers, in addition to petrosin B′ (**7**) (vide infra). Evaluation of the ^{13}C NMR peak heights in Figure 1 gives a kinetic isomer ratio of 48:22:17:13 for these four isomers. In addition, we subsequently acquired synthetic samples of petrosins C and D (**8** and **9**),[15] permitting us to identify small amounts of these isomers in the kinetic cyclization mixture.

With access to reasonable amounts of petrosin and the isomeric mixture, we commenced a series of equilibration experiments, using ^{13}C NMR spectroscopy as the principal analytical tool. Several attempts to equilibrate the mixture of petrosin isomers remaining after crystallization of most of the petrosin led only to recovered mixtures that appeared by NMR to be largely unchanged. The most definitive of this series of experiments were carried out with BF_3•Et_2O under the same or more forcing conditions that successfully equilibrated quinolizidones **5** and **6**. However, these experiments gave unchanged starting material or resulted in general decomposition under the more forcing conditions. To eliminate the possibility of reagent or concentration differences, we carried out careful comparison experiments with quinolizidones **5** and **6**, and their equilibration was shown to be quite reproducible. Since the steric environment in the petrosin skeleton seems modeled by **5** and **6**, we attribute the difficulty of the equilibration to the dimeric nature of the petrosin skeleton. As shown in Scheme 6, to equilibrate all stereocenters within one quinolizidone unit, it is only necessary to enolize the ketone and do retro-Mannich and Mannich reactions. However, to fully

(14) (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(15) Brown, R. C. D.; Norman, T.; Heathcock, C. H *J. Org. Chem.* **1998**, *63*, 0000.

Scheme 5[a]



[a] Key: $LiAlH_4$; (b) Boc_2O; (c) Dess−Martin; (d) 1 M HCl; (e) 0.2 M HOAc.

equilibrate one quinolizidone system relative to the other, one must deprotonate the intermediate immonium ion to the corresponding enamine. That is, trimethylquinolizidone **5** can be interconverted with its diastereomer **6** by undergoing a retro-Mannich reaction to immonium ion **28**, which can then close on the other face to give **29**. After epimerization of the C3-methyl stereocenter, diastereomer **6** is obtained. In this transformation, the C7 stereocenter is unchanged. On the other hand, to interconvert petrosin (**1**) with its diastereomer petrosin A (**2**), it is necessary that the intermediate immonium ions **32** or **34** be deprotonated to enamine **33**, since the overall transformation requires epimerization of one of the C7 stereocenters. We think that the low probability of the deprotonation step is one factor that makes it difficult to equilibrate the petrosins under the same conditions that suffice to equilibrate **5** and **6**.

Of course, there is another major difference between **5** and **1**, in that compound **5** has only one nitrogen, whereas petrosin and its isomers have two. In order for the retro-Mannich reaction to occur, it is necessary that the carbonyl oxygen be coordinated with the acid while the nitrogen is not. Because of the large difference in basicity of an sp^3 nitrogen and an sp^2 oxygen, this is a low-probability event even with **5**. If we envision even one of the nitrogens being associated with the Lewis acid, it could greatly decrease the basicity of the oxygen lone pair, even on the other end of the macrocycle, as the molecule itself would already carry a positive charge. The combination of these events may prevent the retro-Mannich reaction for the dimeric petrosin case.

We thought that we might ameloriate the difficulties by carrying out the equilibrations on the bis-imine derivatives, rather than on the ketones. To this end, the mixture of petrosin isomers was dissolved in butylamine and heated at 85−90 °C for 1−2 days in the presence of molecular sieves (Scheme 7). This treatment did convert the mixture into a mixture of bis-imines, as determined by IR spectroscopy of the crude reaction mixture. The crude bis-imine was then subjected to various acidic conditions in order to effect a retro-Mannich equilibration pathway.[16] Several different conditions were screened, with the ratios of the known petrosin isomers compared by line height of the C-10 resonance in the ^{13}C NMR spectrum. The results of some of these experiments are presented in Table 3, and the partial ^{13}C NMR spectrum of a typical run is shown in Figure 2.

Scheme 6

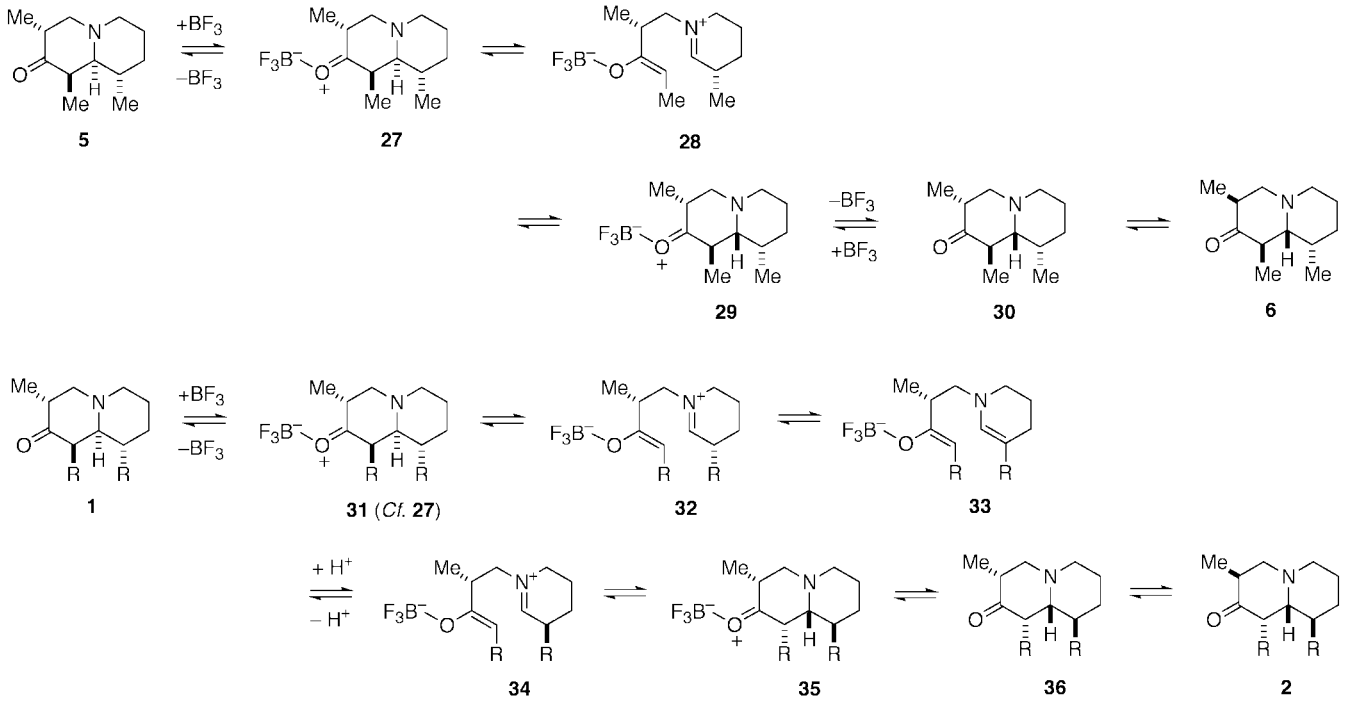
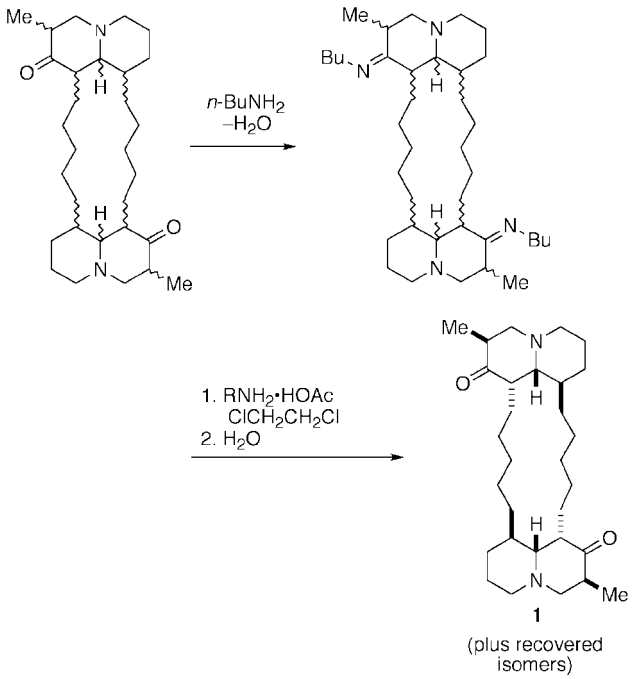


Table 3. Equilibration of Petrosin Bis-Imines (Scheme 7)

entry	acid	solvent	time (h)	*T* (°C)	initial ratio[a] **1**:**2**:**3**:**7**	final ratio[a] **1**:**2**:**3**:**7**
1	$PrNH_3$ OAc	dichloroethane	108	90	36:39:18:6	54:25:13:8
2	Et_3N, Et_3N•HCl	dichloroethane	25	90	19:52:21:8	36:40:15:9
3	$PrNH_3$ OAc	dichloroethane	157	90	22:38:26:14	50:21:18:11
4	Et_3N, Et_3N•HCl	DMF	67	95	18:46:24:13	54:32:10:5
5	pyrrolidine•HOAc	pyrrolidine	60	95	17:45:26:13	46:29:15:10
6	$PrNH_3$ OAc	dichloroethane	50	90	100:0:0:0	90:0:10:0
7	Et_3N, Et_3N•HCl	dichloroethane	72	95	100:0:0:0	84:3:13:0

[a] Ratios are given for petrosin (**1**), petrosin A (**2**), petrosin B (**3**), and petrosin B′ (**7**), normalized to a total of 100%. Small amounts of petrosins C and D (**8** and **9**) and other unidentified compounds are also present.

Scheme 7



The data in Table 3 clearly show that equilibration of the bis-imine derivative leads to mixtures that are enriched in petrosin, relative to the starting sample. However, even with this more favorable system, we are far from equilibrium, as shown by entries 6 and 7 in Table 2, which are two runs starting with pure, crystalline petrosin. The pure petrosin equilibration experiments are important for several reasons. First, they represent the "reverse" of the equilibrium proposed in the isomer mixture experiments and provide further evidence that these isomers are interconverted. This is important as it eliminates the concern that the uncharacterized "isomers" in our mixture are really some other species that we have misidentified and that produce petrosin by some other pathway under the "equilibration" conditions. Second, entries 6 and 7 show that the first isomer formed by equilibration is petrosin B. As has been previously discussed, and is illustrated in Scheme 6, it is easier to interconvert the quinolizidone centers relative to one another than to equilibrate C7 relative to C7′. Therefore, the observation that petrosin gave rise only to petrosin B in the experiment shown in entry 6 is consistent with these two isomers having the same relative configuration at C7 and C7′ (Scheme 8). That is, to convert petrosin (**1**) to structure **3**, it is necessary to equilibrate three stereocenters (marked with X in Scheme 8); the retro-Mannich reaction must occur, but it is not necessary that the intermediate immonium ion be deprotonated to the corresponding enamine. To convert **1** (or **3**) to petrosin A (**2**), equilibration of the position marked Y must also occur.

It is interesting to note that the bis-imine equilibration provides clear enrichment in petrosin in each experiment. However, the ratio of petrosin B to petrosin B′ is not changed very much, even though the transformation that converts petrosin A to petrosin would also convert petrosin B′ to petrosin B. However, it should be remembered that these ratios are obtained by simple measurement of ^{13}C NMR peak heights and are not very accurate for the minor isomers.

In light of the equilibration of the petrosin skeleton isomers to produce mainly petrosin, we thought we might improve the synthesis by adding an equilibration step after crystallization of petrosin from the kinetic cyclization mixture (Scheme 5). Though the equilibration experiments described always proceed with some decomposition, even one cycle does significantly improve the yield of petrosin. Specifically, the final sequence produces a 62% yield of isomers, approximately 1/3 of which is pure petrosin obtained by crystallization. Formation of the bis-imine of the isomer mixture remaining after petrosin removal followed by equilibration with propylammonium acetate in 1,2-dichloroethane leads, after aqueous workup, to an 80% recovery of material, which is now a 1:2 mixture of petrosin/other petrosin isomers. In this mixture, petrosin is the major single isomer present. Crystallization of petrosin from this mixture provides an additional 10% of this isomer. This equilibration process raises the total yield of crystalline petrosin for the final sequence to 33%, while the combined yield of petrosin isomers (mostly petrosin A and petrosin B) is reduced to 20%.

In the course of studying the equilibration of petrosin and its isomers, we were able to obtain reasonably pure samples of the three known isomers, petrosin (**1**), petrosin A (**2**), and petrosin B (**3**), as well as the previously unknown isomer petrosin B′ (**7**). Petrosin is the easiest isomer to obtain in a pure state, as it crystallizes directly. The other isomers were isolated by repeated chromatographies of mixtures of the isomers or of the diols produced by $NaBH_4$. The detailed separation scheme is given in the Supporting Information. The ^{13}C NMR spectrum of petrosin B′ is quite similar to that of the known petrosin B, as is true with petrosin and petrosin A, which also differ only in the absolute stereochemical relationship of the two quinolizidone units. In particular, the spectra of petrosin and petrosin A, which are both symmetrical, are much simpler than those of petrosin B and petrosin B′, which have two diastereomeric quinolizidone units.

(16) A control experiment where the bis-imine was formed and subsequently hydrolyzed showed that negligible equilibration occurs during this step.

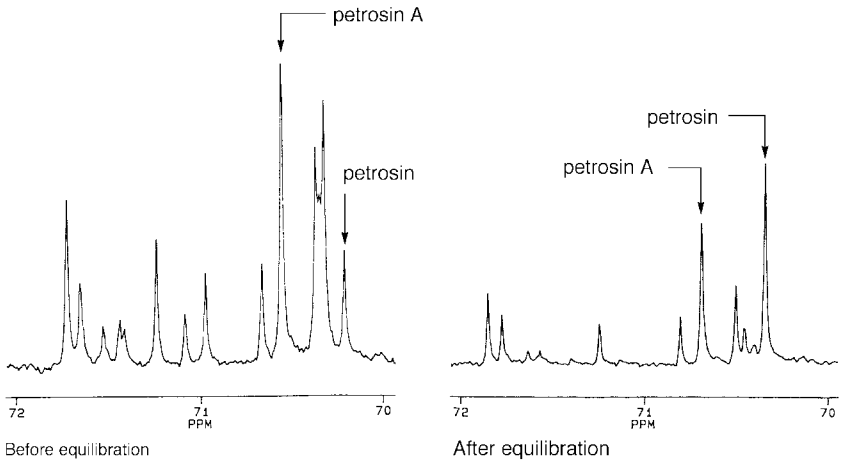


Figure 2. Portion of the ^{13}C NMR spectrum of a typical equilibration mixture (Scheme 7).

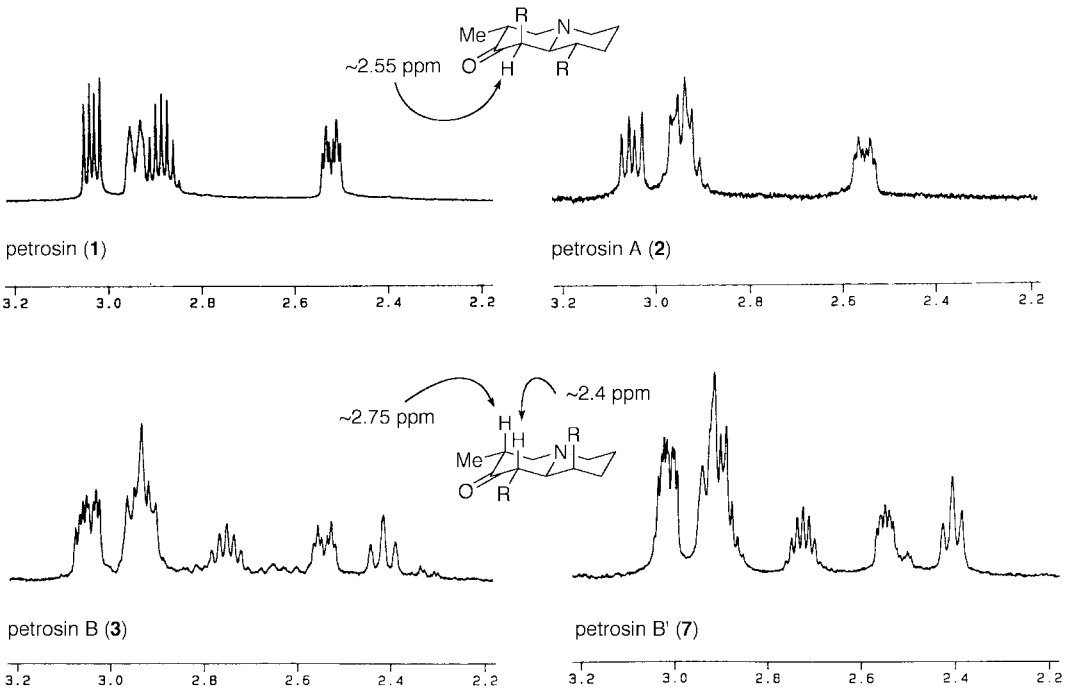


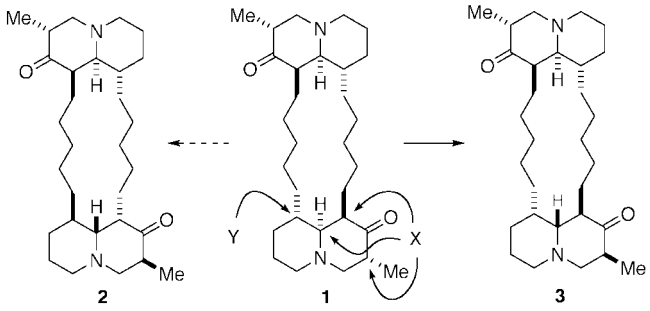
Figure 3. Portions of the ^1H NMR spectra of petrosin (**1**), petrosin A (**2**), petrosin (B) (**3**), and petrosin B′ (**7**).

The ^1H NMR spectrum of petrosin B and petrosin B′ both show a characteristic "triplet" structure at about 2.4 ppm, due to the indicated axial proton, which has large coupling constants with the axial angular hydrogen and one of the hydrogens of the linking pentamethylene chain. These key regions of the spectra are shown in Figure 3.

In summary, we have worked out a relatively straightforward synthesis of petrosin that totally ignores the issue of stereochemistry in its design. The final "double Mannich cyclization" provides crystalline petrosin (**1**) in more than 30% yield. In addition, we have isolated the other two diastereomeric natural products, petrosin A (**2**) and petrosin B (**3**), in pure form. Finally, we have isolated and characterized the previously unknown diastereomer, petrosin B′ (**7**), and obtained evidence that permits us to assign its relative configuration. In the following paper in this issue,[15] we describe the synthesis and characterization of two other petrosin diastereomers, petrosin C (**8**) and petrosin D (**9**).

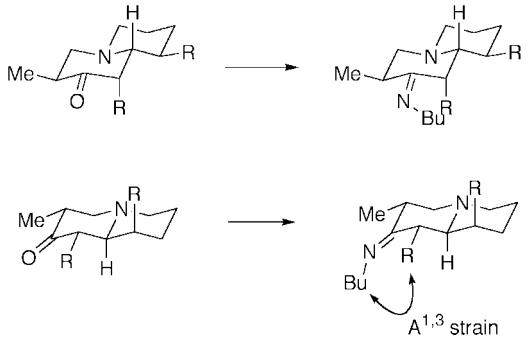
At the onset of the project, we thought it was possible, perhaps probable, that the petrosin isomers would interconvert quickly under mild acid catalysis, thereby producing a mixture of isomers, the composition of which is governed by thermodynamic stability of the various isomers. However, this theory seems unlikely if one

Scheme 8[a]



[a] For clarity and simplicity of presentation, the enantiomer depicted for petrosin has opposite handedness to that depicted in the remainder of the paper.

Scheme 9



considers the drastically differing ratios of the isomers that were found in the isolations by Braekman (ca. 7:1 ratio of petrosin/petrosin A) and Kitagawa (ca. 1:1 mixture of petrosin/petrosin A). In addition, whereas Braekman found a small amount of petrosin B in his isolation, the Kitagawa isolation yielded none of this isomer. One possible explanation for this discrepancy may be the labor of isolation of the natural products from the naturally occurring mixtures. The repeated and painstaking separations necessary to obtain pure compounds undoubtedly lead to isolation ratios that do not reflect the naturally occurring ratios but rather reflect the ability to obtain pure fractions chromatographically or by crystallization. However, it must be remembered that petrosin A and petrosin B are practically inseparable, and it is therefore unlikely that a significant quantity of petrosin B existed in the *Xestospongia* sp. isolation of Kitagawa because it would have coeluted with petrosin A. Furthermore, since we have been unable to find simple acidic conditions that will bring about equilibration of the petrosin isomers, it is highly unlikely that petrosin is racemic because of equilibration during isolation and laboratory processing.

However, the successful bis-imine equilibrations may provide a clue to the mystery of why petrosin is racemic whereas petrosin B is optically active. It may be that the petrosins are all biosynthesized in optically active form but that there is an in vivo analogue of our bis-imine equilibration that permits the retro-Mannich sequence to operate, thereby equilibrating petrosin with its meso diastereomer, petrosin A. The crucial point would then be that, because of $A^{1,3}$ strain, the imine can only easily form if the quinolizidone has the petrosin-type relative configuration such as that found in compound **5** (Scheme 9). Thus, the bis-imines of petrosin and petrosin A could equilibrate with one another and since petrosin A is a meso compound, the isolated petrosin would be racemic. On the other hand, petrosin B could only equilibrate with its optically active diastereomer petrosin B′. Extension of this theory to the currently unknown diastereomers petrosin C and petrosin D suggests that these isomers would not be able to equilibrate with one another. Therefore, if petrosin C is eventually found as a natural product, it should be optically active.

Experimental Section

General Methods. All manipulations involving air-sensitive reagents were carried out under house nitrogen, using flame-dried glassware. Flash chromatography was carried out using Merck 60 230−400 mesh silica gel as the stationary phase. Thin-layer chromatography was performed with Merck silica gel 60 F-254 glass plates (0.25 mm). Diethyl ether and tetrahydrofuran (THF) were distilled under nitrogen from sodium/benzophenone immediately prior to use. Methylene chloride (CH_2Cl_2), pyrrolidine, diisopropylamine, and triethylamine were distilled under nitrogen from calcium hydride prior to use. Reagents were used as received from commercial suppliers unless otherwise noted. The concentration of commercially available *n*-butyllithium in hexanes was periodically checked by titration of diphenylacetic acid. Unless otherwise specified, extracts were dried with anhydrous magnesium sulfate and concentrated under reduced pressure with a rotary evaporator. Unless otherwise indicated, IR spectra were of thin films on NaCl plates and NMR spectra measured in $CDCl_3$. The NMR chemical shifts are reported in ppm of the δ scale using $CHCl_3$ as an internal reference and the coupling constants reported in Hz.

4-Formylvaleronitrile Dioxolane (11). A solution of 4-formylvaleronitrile (4.64 g, 41.8 mmol), ethylene glycol (10.0 g, 161 mmol), *p*-toluenesulfonic acid (0.4 g, 2.1 mmol), and benzene (100 mL) was refluxed for 16 h with azeotropic removal of water. The solution was allowed to cool to room temperature, washed with saturated solutions of NaCl, $NaHCO_3$, and NaCl again, dried, and concentrated. The resulting oil was purified by distillation from a Kugelrohr apparatus to yield 5.57 g (86%) of **11** (bp 75−77 °C, 2.5 Torr) as a clear oil. IR: 2260 cm^{-1}. ^1H NMR: δ 0.98 (d, 3, J = 6.8), 1.52−1.56 (m, 1), 1.83−1.89 (m, 2), 2.15−2.47 (m, 2), 3.83−3.87 (m, 2), 3.91−3.94 (m, 2), 4.66 (d, 1, J = 4.1). ^{13}C NMR: δ 14.12, 15.17, 27.11, 35.89, 64.88, 65.02, 106.85, 119.80. Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 62.0; H, 8.19; N, 9.07.

4-Formylpentylamine Dioxolane (12). Lithium aluminum hydride (1.94 g, 51.1 mmol) was suspended in THF (80 mL), and a solution of **11** (6.47, 41.7 mmol) in THF (5 mL) was added slowly from a dropping funnel. The reaction mixture was stirred for 0.5 h at room temperature and then quenched by addition of 2.0 mL of H_2O, 2.0 mL of 15% aqueous NaOH, and 6.0 mL of H_2O. The solution was filtered, washed with saturated NaCl solution, and concentrated. The resulting oil was purified by distillation from a Kugelrohr apparatus to yield 5.270 g (80%) of **12** (bp 118−120 °C, 25 Torr) as a clear oil. IR: 3380, 3300 cm^{-1}. ^1H NMR: δ 0.95 (d, 3, J = 6.8), 1.15−1.22 (m, 3), 1.42−1.44 (m, 1), 1.54−1.58 (m, 2), 1.69−1.72 (m, 1), 2.67−2.70 (m, 2), 3.83−3.87 (m, 2), 3.93−3.97 (m, 2), 5.32 (d, 1, J = 4.5). ^{13}C NMR: δ 28.66, 31.43, 36.77, 42.50, 64.96, 107.56, 110.47. Anal. Calcd for $C_8H_{17}NO_2$: C, 60.35; H, 10.76. Found: C, 60.59; H, 10.52.

Quinolizidones 5 and 6. Amine **12** (439 mg, 2.76 mmol) was dissolved in MeOH (3.0 mL) and cooled to 0 °C. Enone **13** (275 mg, 2.80 mmol) dissolved in MeOH (1.0 mL) was slowly added from an addition funnel. The solution was allowed to warm to room temperature, stirred for 4 h, and concentrated and the residue taken up in 5% AcOH and heated to 100 °C for 4 h. The cooled reaction mixture was quenched with a 5% solution of $NaHCO_3$ and extracted into CH_2Cl_2. The solution was dried and concentrated to afford 510 mg (95%) of a brown oil, which was a mixture of isomers (mainly **5** and **6** in a ratio of approximately 5.4:1, identified by ^1H NMR and GC/MS). Isomer **5** was isolated in approximately 70% yield by MPLC. IR: 2805, 2760, 1720 cm^{-1}. ^1H NMR: δ 0.82 (d, 3, J = 6.5), 0.96 (d, 3, J = 6.5), 1.05 (m, 1), 1.17 (d, 3, J = 7.2), 1.85 (m, 4), 1.87 (m, 1), 1.95 (m, 2), 2.58 (m, 1), 2.93 (m, 2), 3.02 (m, 1). ^{13}C NMR: δ 11.2, 12.0, 17.6, 25.2, 31.8, 32.6, 40.2, 45.9, 55.9, 64.2, 71.3, 215.0. Anal. Calcd for $C_{12}H_{21}NO$: C, 73.80; H, 10.84; N, 7.17. Found: C, 74.02; H, 10.86; N, 7.02.

Methyl Azelaldehyde (15). A solution methyl oleate (24.68 g, 0.0832 mol) in 500 mL of a 2:1 mixture of MeOH/CH_2Cl_2 was cooled to −78 °C. Ozone was bubbled through the solution until a persistent purple color appeared. The solution was purged with N_2 while being warmed to room temperature until all the color had dissipated. The ozonide was decomposed by adding glacial acetic acid (35 mL) in one portion, followed by powdered Zn (13 g, 0.20 mol) in small portions to control heat evolution. After all the Zn was added, the mixture was allowed to stir 30 min and then filtered through Celite to remove unreacted Zn, and H_2O (150 mL) was added to the

mixture to prevent acetal formation. The solution was concentrated to about one-half initial volume in vacuo and added to saturated $NaHCO_3$ (200 mL). The mixture was extracted with CH_2Cl_2 (3 × 250 mL). The organic phases were dried and concentrated. The crude product was distilled to produce nonanal (bp 41 °C, 0.6 Torr) and 14.82 g (96%) of **15** (bp 93 °C, 0.6 Torr) as a colorless oil. IR: 2930, 2860, 2720, 1720, 1740, 1200, 1170 cm^{-1}. ^1H NMR (400 MHz): δ 1.31 (br s, 6), 1.61 (br s, 4), 2.30 (t, 2, J = 7.5), 2.41 (dt, 2, J = 1.7, 7.3), 3.66 (s, 3), 9.75 (t, 1, J = 1.7). ^{13}C NMR (125 MHz): δ 21.91, 24.76, 28.82, 28.87, 28.90, 33.94, 43.77, 51.36, 174.11, 202.62. Anal. Calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.50; H, 9.78.

Methyl 10-Cyano-8-(hydroxymethyl)decanoate (17). To a solution of pyrrolidine (0.3799 g, 5.342 mmol) and anhydrous K_2CO_3 (0.455 g) in ether (2 mL) at 0 °C was added dropwise from an addition funnel aldehyde **15** (0.9936 g, 5.335 mmol) in ether (2 mL). After addition was complete, the ice bath was removed and the slurry stirred 5 h. The solution was filtered through a fine glass frit to remove K_2CO_3 and rinsed with 5 mL of ether. The crude enamine was concentrated and added with CH_3CN (5 mL) to a reaction tube containing acrylonitrile (0.3402 g, 6.411 mmol) in CH_3CN (5 mL). The tube was sealed and heated at approximately 80 °C for 20 h. After the mixture was cooled to room temperature, H_2O was added (5 mL) and heating resumed for 1 h. The mixture was poured into 5% aqueous HCl (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried and concentrated. The product was purified by flash chromatography (ethyl acetate/hexane 20/80 → 30/70) to obtain 0.93 g (73%) of aldehyde **16** as a slightly yellow, semipure oil. IR: 2240, 1740, 1720 cm^{-1}. ^1H NMR (500 MHz): δ 1.24 (br s, 6), 1.38−1.45 (m, 1), 1.49−1.54 (m, 2), 1.59−1.69 (m, 2), 1.89−1.96 (m, 1), 2.20 (t, 2, J = 7.5), 2.24−2.42 (m, 3), 3.56 (s, 3), 9.55 (d, 1, J = 1.5). ^{13}C NMR (125 MHz): δ 15.02, 23.83, 24.63, 26.40, 28.39, 28.68, 29.09, 33.82, 50.08, 51.32, 118.98, 173.93, 202.83.

To a solution of **16** (0.5935 g, 2.480 mmol) in MeOH (10 mL) at 0 °C was slowly added $NaBH_4$ (0.0482 g, 1.274 mmol). After 5 min, the solution was poured onto 5% HCl (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried and concentrated, and the crude oil was purified by Kugelrohr distillation to provide 0.5971 g (100%) of alcohol **17** as a colorless oil. IR: 3500, 2260, 1740 cm^{-1}. ^1H NMR (500 MHz): δ 1.27 (br s, 8), 1.54−1.59 (m, 3), 1.61−1.68 (m, 1), 1.70−1.77 (m, 1), 2.10 (br s, 1), 2.26 (t, 2, J = 7.5), 2.37−2.41 (m, 2), 3.47 (dd, 1, J = 6.0, 10.9), 3.58 (dd, 1, J = 4.4, 10.9), 3.62 (s, 3). ^{13}C NMR (125 MHz): δ 14.90, 24.66, 26.41, 27.11, 28.80, 29.27, 30.32, 33.86, 39.26, 51.36, 64.27, 120.00, 174.25. Anal. Calcd for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.44; H, 9.75; N, 5.98.

Methyl 10-Cyano-8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]decanoate (18). To a solution of alcohol **17** (11.02 g, 0.0457 mol) in DMF (20 mL) was added imidazole (7.83 g, 0.115 mol) followed by *tert*-butyldimethylsilyl chloride (8.26 g, 0.055 mol). The solution was stirred overnight at room temperature and then poured onto 1 N HCl (200 mL) and extracted with ether (2 × 225 mL). The combined organic phases were then washed with 100-mL portions of 1 N HCl, H_2O, and saturated NaCl solution. The organic phase was dried, concentrated, and purified by Kugelrohr distillation (bp 225 °C at 0.3 Torr) to provide 15.43 g (95%) of the protected alcohol **18**. IR: 2240, 1740 cm^{-1}. ^1H NMR (500 MHz): δ 0.02 (s, 6), 0.86 (s, 9), 1.27 (br s, 8), 1.55−1.76 (m, 5), 2.28 (t, 2, J = 7.5), 2.38 (t, 2, J = 7.5), 3.43 (dd, 1, J = 6.0, 10.2), 3.55 (dd, 1, J = 4.2, 10.2), 3.64 (s, 3). ^{13}C NMR (125 MHz): δ −5.63, −5.58, 14.99, 18.12, 24.79, 25.78, 26.58, 27.60, 28.94, 29.40, 30.50, 33.95, 39.31, 51.34, 64.75, 120.06, 174.10. Anal. Calcd for $C_{19}H_{37}NO_3Si$: C, 64.17; H, 10.49; N, 3.94. Found: C, 63.99; H, 10.42; N, 3.99.

4-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-11-oxoundecanitrile (19). To a solution of ester **18** (6.00 g, 16.9 mmol) in CH_2Cl_2 (60 mL) at −93 °C (MeOH/liquid N_2) was added DIBALH (18.5 mL, 1.0 M solution in CH_2Cl_2) over 12 min. After being stirred for 8 min, the reaction was quenched by adding 15% NaOH (50 mL). The mixture was warmed to room temperature with stirring over 45 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic phases were dried, concentrated, and purified by flash chromatography (ethyl acetate/hexanes 10/90) to produce 4.37 g (80%) of **19** as a colorless oil. On a larger scale (20.00 g), this procedure produced an 86% yield of purified aldehyde (the reaction time was extended to 2 h). IR: 2260, 1730 cm^{-1}. ^1H NMR (400 MHz): δ 0.02 (s, 6), 0.86 (s, 9), 1.28 (br s, 8), 1.53−1.77 (m, 5), 2.36−2.43 (m, 4), 3.44 (dd, 1, J = 5.8, 10.2), 3.55 (dd, 1, J = 4.2, 10.2), 9.74 (t, 1, J = 1.8). ^{13}C NMR (100 MHz): δ −5.61, −5.56, 15.01, 18.14, 21.97, 25.80, 26.57, 27.60, 28.97, 29.50, 30.50, 39.31, 43.76, 64.75, 120.05, 202.56. Anal. Calcd for $C_{18}H_{35}NO_2Si$: C, 66.40; H, 10.84; N, 4.30. Found: C, 66.64; H, 10.74; N, 4.11.

12-Cyano-10-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-hydroxy-2-methyldodecanoic Acid (20). A solution of LDA was prepared by adding *n*-butyllithium (3.28 mL, 8.59 mmol) to diisopropylamine (0.8697 g, 8.595 mmol) in THF (12.5 mL) at 0 °C. A solution of propionic acid (0.3180 g, 4.293 mmol) in THF (7.5 mL) was added dropwise, and the reaction mixture was allowed to stir at room temperature for 1 h. The enolate solution was cooled to 0 °C, and a solution of aldehyde **19** (1.40 g, 4.30 mmol) in THF (10 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and then poured into 1 N HCl (30 mL). The mixture was concentrated in vacuo to remove most of the THF, and then the aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL). The crude acid was dried, concentrated, and then dissolved in 1 M NaOH (50 mL), which was extracted with ether (2 × 15 mL). The aqueous layer was brought to pH = 1 with 12 M HCl and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried and concentrated to produce 1.33 g (77%) of a 1:1 mixture of diastereomers of **20** as a viscous, slightly yellow oil. IR: 3420, 3120, 2220, 1720 cm^{-1}. ^1H NMR (500 MHz): δ 0.02 (s, 12), 0.87 (s, 18), 1.18 (d, 3, J = 7.1), 1.22 (d, 3, J = 7.2), 1.28 (br s, 20), 1.40−1.77 (m, 10), 2.39 (t, 4, J = 7.5), 2.51−2.57 (m, 2), 3.44 (dd, 2, J = 6.0, 10.2), 3.57 (dd, 2, J = 4.2, 10.2), 3.65−3.69 (m, 1), 3.91−3.95 (m, 1). ^{13}C NMR (100 MHz): δ −5.59, −5.54, 10.36, 14.10, 15.02, 18.15, 25.33, 25.81, 26.71, 27.62, 29.31, 26.69, 30.55, 33.58, 34.47, 39.36, 44.11, 45.13, 64.89, 71.66, 73.15, 120.06, 180.58, 180.70. Anal. Calcd for $C_{21}H_{41}NO_4Si$: C, 63.11; H, 10.34; N, 3.50. Found: C, 63.39; H, 10.40; N, 3.27.

Methyl 12-Cyano-10-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-hydroxy-2-methyldodecanoate (Methyl Ester of 20. To acid **20** (5.42 g, 13.6 mmol) dissolved in ether (50 mL) was added an excess of freshly prepared diazomethane (from the reaction of Diazald and KOH) in ether. The solution was concentrated to provide 5.61 g (100%) of the ester as a 1:1 mixture of diastereomers as a slightly yellow oil. IR: 3500, 2240, 1740 cm^{-1}. ^1H NMR (500 MHz): δ 0.02 (s, 12), 0.87 (s, 18), 1.16 (d, 3, J = 7.2), 1.19 (d, 3, J = 7.2), 1.27 (br s, 20), 1.34−1.76 (m, 10), 2.39 (t, 4, J = 7.5), 2.48−2.56 (m, 4), 3.43 (dd, 2, J = 6.0, 10.2), 3.56 (dd, 2, J = 4.1, 10.2), 3.62−3.65 (m, 1), 3.69 (s, 6), 3.83−3.87 (m, 1). ^{13}C NMR (100 MHz): δ −5.59, −5.54, 10.61, 14.25, 15.03, 18.16, 25.43, 25.82, 25.90, 26.75, 27.64, 29.38, 29.41, 30.57, 33.76, 34.65, 39.37, 44.21, 45.17, 51.63, 51.70, 64.83, 71.66, 73.24, 120.09, 176.37. Anal. Calcd for $C_{22}H_{43}NO_4Si$: C, 63.87; H, 10.48; N, 3.39. Found: C, 64.05; H, 10.58; N, 3.59.

Methyl 13-Amino-10-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-hydroxy-2-methyltridecanoate (21). The methyl ester of **20** (5.17 g, 12.5 mmol) was dissolved in ethyl acetate (200 mL) in a Parr bottle (500 mL capacity). To the solution was added acetic acid (6.61 g, 110 mmol), followed by PtO_2 (1.01 g, 4.30 mmol). The solution was shaken under an H_2 atmosphere (53 psi) for 42 h. The solution was filtered through Celite and then extracted with 1 M NaOH (250 mL). The aqueous layer was extracted with ethyl acetate (2 × 150 mL). The combined organic phases were dried (K_2CO_3) and concentrated to provide 5.06 g (97%) of the crude amine as a colorless oil. IR: 3360, 1740, 1600 cm^{-1}. ^1H NMR (400 MHz): δ 0.01 (s, 12), 0.87 (s, 18), 1.16 (d, 3, J = 7.3), 1.18 (d,

3, $J = 7.3$), 1.20−1.50 (m, 34), 2.47−2.54 (m, 2), 2.65 (br s, 4), 3.43 (dd, 2, $J = 5.7, 10.0$), 3.46 (dd, 2, $J = 5.5, 10.0$), 3.61−3.66 (m, 1), 3.69 (s, 6), 3.83−3.86 (m, 1). ^{13}C NMR (100 MHz): δ −5.42, 10.70, 14.22, 18.26, 25.45, 25.90, 26.70, 28.15, 29.45, 29.91, 30.85, 33.92, 34.66, 40.26, 42.64, 44.33, 45.29, 51.61, 51.69, 65.55, 71.63, 73.17, 176.40. Anal. Calcd for $C_{22}H_{47}NO_4Si$: C, 63.26; H, 11.34; N, 3.35. Found: C, 63.40; H, 11.27; N, 3.57.

Methyl 13-[[12-Cyano-10-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-hydroxy-2-methyl-1-oxododecyl]amino]-10-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-hydroxy-2-methyltridecanoate (22). Acid **20** (2.98 g, 7.46 mmol) and amine **21** (3.11 g, 7.45 mmol) were added to a 50 mL flask with THF (23 mL). To the solution was added hydroxybenzotriazole hydrate (1.20 g, 8.91 mmol), and after dissolution the solution was cooled in an ice−water bath. Dicyclohexylcarbodiimide (1.84 g, 8.90 mmol) was added, and the solution was stirred in the ice bath for 1 h. The cold bath was removed and the solution allowed to warm to room temperature. After 12 h, the solution was filtered to remove the solid dicyclohexylurea. After concentration, the crude product was purified by flash chromatography to provide 4.20 g (70%) of amide **22**. IR: 3350, 2250, 1740, 1645, 1545, 1465 cm^{-1}. ^{1}H NMR (500 MHz):[17] δ 0.01 (s, 6), 0.02 (s, 6), 0.87 (s, 18), 1.13−1.76 (m, 38), 2.17−2.26 (m, 1), 2.40 (t, 2, $J = 7.5$), 2.49−2.55 (m, 1), 3.17−3.25 (m, 2), 3.40−3.49 (m, 4), 3.55−3.58 (m, 2), 3.60−3.67 (m, 1), 3.69 (s, 3), 3.80−3.88 (m, 1), 5.90 (br s, 1). ^{13}C NMR (100 MHz):[17] δ −5.57, −5.52, −5.43, −5.42, 10.69, 11.10, 14.24, 15.06, 15.82, 18.17, 18.26, 25.41, 25.75, 25.83, 25.91, 25.96, 26.66, 26.74, 26.84, 26.88, 27.64, 28.33, 29.39, 29.43, 29.74, 29.76, 29.82, 30.57, 30.83, 33.55, 33.83, 34.67, 35.45, 39.36, 39.61, 39.65, 40.06, 44.28, 44.56, 45.21, 45.90, 51.64, 51.71, 64.92, 65.52, 71.74, 71.91, 73.28, 73.75, 120.13, 175.98, 176.41, 176.53. Anal. Calcd for $C_{43}H_{86}N_2O_7Si_2$: C, 64.61; H, 10.85; N, 3.51. Found: C, 64.76; H, 10.84; N, 3.64.

Methyl 13-[[13-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-10-(hydroxymethyl)-2-methyl-1-oxotridecyl]amino]-3-hydroxy-10-(hydroxymethyl)-2-methyltridecanoate (23). The amide **22** (7.78 g, 9.73 mmol) was added to a Parr bottle (500 mL capacity) with 0.23 M HCl in ethanol solvent (4 mL of concd HCl + 200 mL of absolute ethanol), followed by PtO_2 (0.23 g, 1.0 mmol). The solution was shaken under an H_2 atmosphere (53 psi) for 18 h. The solution was filtered through Celite and concentrated. To the crude oil were added H_2O (20 mL) and dioxane (20 mL), followed by the slow addition of $NaHCO_3$ (10 g). A solution of di-*tert*-butyl dicarbonate (2.56 g, 11.7 mmol) in dioxane (12 mL) was added, and the mixture was allowed to stir for 12 h. The solution was concentrated and added to a separatory funnel with H_2O (50 mL) and CH_2Cl_2 (100 mL). The aqueous layer was extracted further with CH_2Cl_2 (2 × 40 mL), and the combined organic layers were dried and concentrated. The crude oil was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to provide 4.75 g (72%) of **23** as a colorless oil. IR: 3330, 1710, 1685, 1640, 1530, 1450, 1360 cm^{-1}. ^{1}H NMR (500 MHz):[17] δ 1.12−1.56 (m, 49), 2.20−2.60 (m, 4), 2.76−2.90 (m, 1), 3.02−3.35 (m, 4), 3.40−3.67 (m, 5), 3.69 (s, 3), 3.73−3.90 (m, 2), 4.60−4.80 (m, 1), 6.34−6.45 (m, 1). ^{13}C NMR (100 MHz):[17] δ 10.89, 11.30, 13.89, 15.50, 15.54, 25.29, 25.42, 25.67, 25.75, 26.44, 26.62, 27.16, 27.88, 28.29, 29.18, 29.26, 29.59, 29.70, 30.67, 30.79, 33.50, 33.97, 34.34, 35.27, 39.18, 39.33, 39.77, 39.87, 39.95, 40.74, 44.52, 44.68, 45.33, 45.65, 45.84, 50.25, 51.51, 51.57, 64.80, 64.87, 71.73, 71.93, 73.06, 73.48, 73.55, 78.93, 156.16, 176.28, 176.35, 176.61. Anal. Calcd for $C_{36}H_{70}N_2O_9$: C, 64.06; H, 10.45; N, 4.15. Found: C, 63.70; H, 10.09; N, 3.97.

4,18-Dihydroxy-11,25-bis(hydroxymethyl)-3,17-dimethyl-1,15-diazacyclooctacosane-2,16-dione (24). The ester **23** (0.73 g, 1.1 mmol) was added to a 50 mL flask with MeOH (7.5 mL), THF (10 mL), and 1 N NaOH (7.5 mL) and the solution stirred at room temperature for 1.75 h. The solution was reduced to approximately one-half volume under reduced pressure followed by the addition of 1 N HCl (7.5 mL) until pH = 1. The acidic solution was extracted (EtOAc, 1 × 40 mL, 2 × 25 mL), and the combined organic layers were dried and concentrated. The crude acid was added to a 25 mL flask with the aid of THF (8 mL) and cooled in an ice−water bath. Pentafluorophenol (0.59 g, 3.2 mmol) was added in THF (2 mL), followed by the addition of dicyclohexylcarbodiimide (0.26 g, 1.3 mmol), and the solution stirred for 3 h. The cold bath was removed and the solution allowed to stir at room temperature for 10 h. The solution was filtered through a glass wool plug to remove the solid dicyclohexylurea formed and concentrated. To the crude active ester was added dioxane saturated with anhydrous HCl (16 mL) and the solution stirred for 1 h at room temperature. The solution was concentrated to an oil and was taken up into a gastight syringe along with dioxane (5 mL) and THF (5 mL). The flask was rinsed (3 mL of dioxane, 5 mL of THF) and the rinse combined in the syringe. The solution was added via syringe pump over 3.5 h to a 5:1 dioxane/pyridine solution (600 mL) maintained at 85−90 °C. The syringe was rinsed with THF (3 mL) and the rinse added at the same rate. The solution was stirred in the heated bath for an additional 4.5 h and then was cooled and concentrated. The crude oil was purified by flash chromatography (1:9 MeOH/CH_2Cl_2 → 1.5:8.5 MeOH/CH_2Cl_2) to provide 0.46 g (78%) of the macrocycle **24** as a slightly yellow glass. The absence of dimeric products was confirmed by FAB-MS. IR (KBr pellet): 3320, 1640, 1540, 1460 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD):[17] δ 1.16 (d, 6, $J = 6.8$), 1.20−1.57 (m, 34), 2.18−2.26 (m, 1), 2.35−2.43 (m, 1), 2.79−3.62 (m, 10). ^{13}C NMR (100 MHz, CD_3OD):[17] δ 14.98, 15.03, 15.14, 15.20, 26.79, 26.98, 27.70, 27.86, 29.30, 30.70, 31.00, 31.13, 31.21, 32.01, 32.05, 32.13, 32.20, 36.17, 36.25, 40.42, 40.50, 41.46, 41.50, 41.57, 41.62, 46.55, 46.62, 48.87, 65.56, 65.63, 74.09, 74.63, 74.74, 177.91, 177.99. Anal. Calcd for $C_{30}H_{58}N_2O_6$: C, 66.38; H, 10.77; N, 5.15. Found: C, 66.03; H, 10.44; N, 5.04.

Bis(1,1-dimethylethyl) 4,18-Dihydroxy-11,25-bis(hydroxymethyl)-3,17-dimethyl-1,15-diazacyclooctacosane-1,15-dicarboxoate (26). The bis-amide **24** (0.2813 g, 5.183 mmol) was added to a 50 mL flask with the aid of methanol (approximately 10 mL). The solution was reduced in volume with a rotary evaporator until an oil remained, and THF (35 mL) was added. Solid $LiAlH_4$ (0.6260 g, 16.50 mmol) was added in portions slowly. The solution was heated at reflux for 23 h. TLC of the solution showed that the starting material was still present, so additional $LiAlH_4$ (0.2090 g, 5.507 mmol) was added and heating continued for 13 h. To the cooled solution were added H_2O (0.8411 g), 15% aqueous NaOH (0.8670 g), and H_2O (2.5201 g), and the solution was stirred a few minutes until all the solids were white. The solution was dried with Na_2SO_4 and filtered through a glass frit, rinsing several times with small portions of THF. The solution was concentrated to provide 0.2743 g (103%) of crude diamine **25**. The crude amine was added to a 10 mL flask with THF and rotovapped down to an oil. Dioxane (1 mL) and H_2O (0.6 mL) were added followed by di-*tert*-butyl carbonate (0.2717, 1.245 mmol) in dioxane (0.6 mL). Bubbling was evident immediately. The solution was stirred for 45 min and the solvent removed. Purification by flash chromatography (1:9 MeOH/CH_2Cl_2) provided 0.3337 g (90%) of the di-Boc tetraol **26** as a colorless glass. IR: 3420, 1670 cm^{-1}. ^{1}H NMR (500 MHz):[17] δ 0.78−0.82 (m, 3), 0.87−0.92 (m, 3), 1.18−1.90 (m, 54), 2.59−4.05 (m, 18). ^{13}C NMR (100 MHz):[17] δ 9.95, 10.15, 15.18, 25.49, 25.94, 25.99, 26.07, 26.16, 26.20, 26.36, 26.57, 27.40, 27.48, 27.73, 27.81, 28.35, 28.42, 29.17, 29.35, 29.52, 29.77, 30.12, 30.21, 30.54, 30.64, 31.12, 33.38, 34.27, 36.20, 36.53, 37.71, 38.20, 38.29, 39.88, 40.08, 48.37, 49.34, 49.66, 51.57, 52.17, 65.43, 65.65, 68.37, 68.65, 69.02, 74.25, 79.46, 79.56, 79.93, 156.29, 157.22. Anal. Calcd for $C_{40}H_{78}N_2O_8$: C, 67.19; H, 11.00; N, 3.92. Found: C, 66.90; H, 10.66; N, 3.97.

Final Ring Closure. The tetraol **26** (0.2436 g, 0.3407 mmol) was dissolved in CH_2Cl_2 (13 mL). To the stirred

(17) Because of the large number of diastereomers present in compounds **22**−**26**, the spectra were often largely uninterpretable. For these compounds, only the common multiplets are listed for ^{1}H NMR. This is also the reason for the very large number of peaks in the ^{13}C NMR spectra. However, the data as presented are still useful for structure determination.

solution was added solid Dess−Martin periodinane (1.0443 g, 2.462 mmol) and the solution stirred at room temperature for 1 h. The reaction mixture was added to a separatory funnel containing 1 M NaOH (40 mL) and Et_2O (40 mL). After separation of the layers, the aqueous layer was extracted with Et_2O (1 × 40 mL). The combined organic layer was reduced to an oil, and 95% ethanol (40 mL), H_2O (8 mL), and 12 M HCl (4 mL) were added. The solution was refluxed for 3 h. The solution was concentrated to a thin oil and added to a seperatory funnel with 1.25 M NaOH (40 mL) and $CHCl_3$ (40 mL). The layers were separated, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL), and the combined organic layers were concentrated. To the crude mixture were added 95% ethanol (40 mL) and acetic acid (0.5108 g). The solution was heated at reflux for 24 h. After concentration of the solvent, the oil was added to a separatory funnel with 1 M NaOH (30 mL) and CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were dried over K_2CO_3. Filtration and concentration produced the crude oil, which was purified by flash chromatography (9.5:9.5:1 hexane/Et_2O/Et_3N) to produce 0.0988 g (62%) of the petrosin skeleton as a mixture of stereoisomers. The oil was dissolved in Et_2O (1.75 mL) and put in the freezer overnight to induce crystallization. Centrifugation and decantation, followed by washing of the crystals with ether and repeating the process, provided 0.0374 g of pure petrosin (**1**) and 0.0601 g of petrosin isomers. The petrosin was identical to an authentic sample by ^1H NMR, ^{13}C NMR, IR, and TLC. The isomers were identified mainly by ^{13}C NMR, IR, and MS.

Equilibration (Typical Procedure, Entry 3 from Table 3 Provided Below). To one-half of the mixture of petrosin isomers from above (after removal of crystalline petrosin, 0.0305 g, 0.0648 mmol) were added butylamine (1.5 mL) and several 3 Å molecular sieves. The solution was heated in a sealed tube at approximately 95 °C until the ketone peak was no longer clearly observed in the IR (68 h). The crude imine was separated from the molecular sieves, which were rinsed with CH_2Cl_2 (3 × 3 mL), and the solution concentrated. The crude oil was added to a reaction tube with propylammonium acetate (0.0076 g, 0.0638 mmol) and 1,2-dichloroethane (2 mL) and the solution heated at approximately 95 °C for 7 days. To the solution was added H_2O (1 mL) and heating resumed for 1 h. The mixture was added to a separatory funnel with 1 M NaOH (5 mL) and CH_2Cl_2 (10 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 7 mL), and the combined organic layers were dried with K_2CO_3. Concentration produced the crude oil, which was purified by flash chromatography (9.5:9.5:1 hexane/Et_2O/Et_3N) to produce 0.0245 g (80%) of a mixture of isomers enriched in petrosin. To the crude oil was added Et_2O (1 mL) and the solution put in the freezer overnight to induce crystallization. The mixture was centrifuged, and crystals of pure petrosin (0.0080 g) were obtained along with an oily mixture of isomers (0.0164 g).

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Supporting Information Available: Separation scheme for isolation of petrosin, petrosin A, petrosin B, and petrosin B′, table of ^{13}C NMR chemical shifts for the four isomers, and calculated molecular mechanics energies for 24 isomers of compound **4** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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