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Synthesis and absolute stereochemical reassignment of mukanadin F: A study of isomerization of bromopyrrole alkaloids with implications on marine natural product isolation.

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Abstract

Synthesis of both enantiomers of mukanadin F (**1**) was achieved using a seven step synthesis. Comparison of the optical rotation data of synthetic samples to that reported for the isolated natural product determine that the absolute configuration of the natural product is 9*S* and not the reported 9*R*. Further studies established that the reported low magnitude of optical rotation in the isolated sample is due to compounds of this type undergoing isomerization and racemization under benign laboratory conditions. Additionally the synthetic methods developed were applied to synthesize mukanadins B (**6**) and D (**7**).

Bromopyrrole alkaloids are a diverse family of marine natural products that have been identified as secondary metabolites of marine sponges.[1] Since the mid-1970's, there have been roughly 140 compounds classified as bromopyrrole alkaloids with structures ranging from simple 'linear' to more complicated polycyclic derivatives. These natural products have been attributed with a wide range of biological activities and as such have been of great interest to both natural product and synthetic chemists.

A number of C-9 substituted 'linear' bromopyrrole alkaloids (**Figure 1**) have been isolated from sponges of the orders Agelasida and Axinellida. It is interesting to note that these alkaloids were mostly reported as either racemic or scalemic mixtures.[2–6] Previous studies investigating the racemization of compounds of this type were attempted using debromomukanadin A (**3**) but were inconclusive.[5]

Mukanadin F (**1**) is unique amongst this set in that it was isolated as a single enantiomer with 9*R* configuration established using Mosher's esters.[2] Due to the unique stereochemical nature of mukanadin F (**1**), preparation of both enantiomers was undertaken firstly to confirm its absolute stereochemistry and secondly to give insight into why it alone, in this compound class, has been isolated non-racemically.

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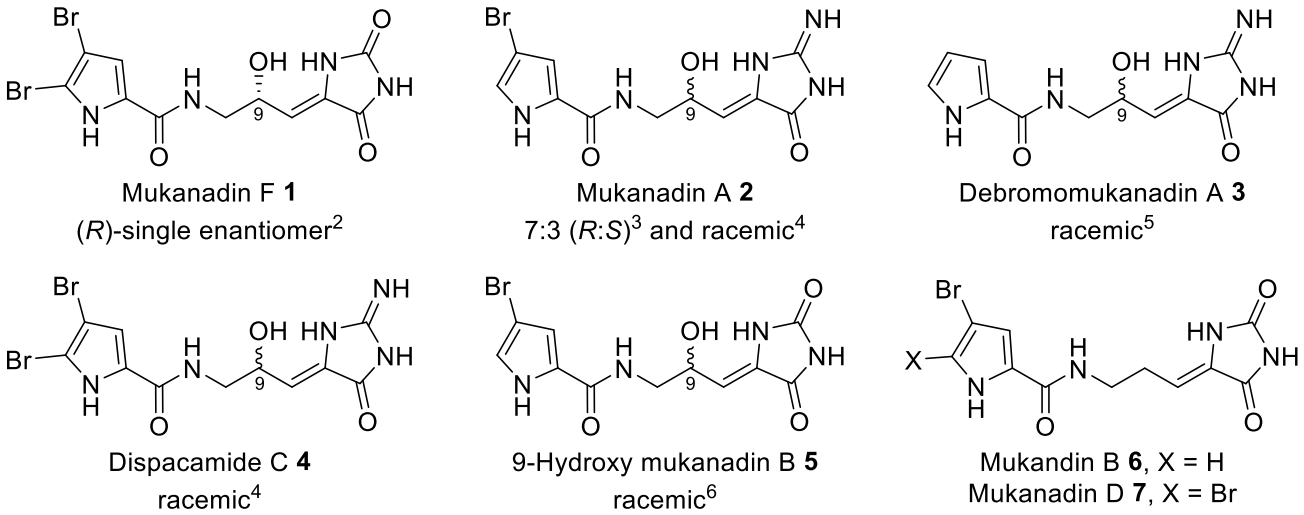
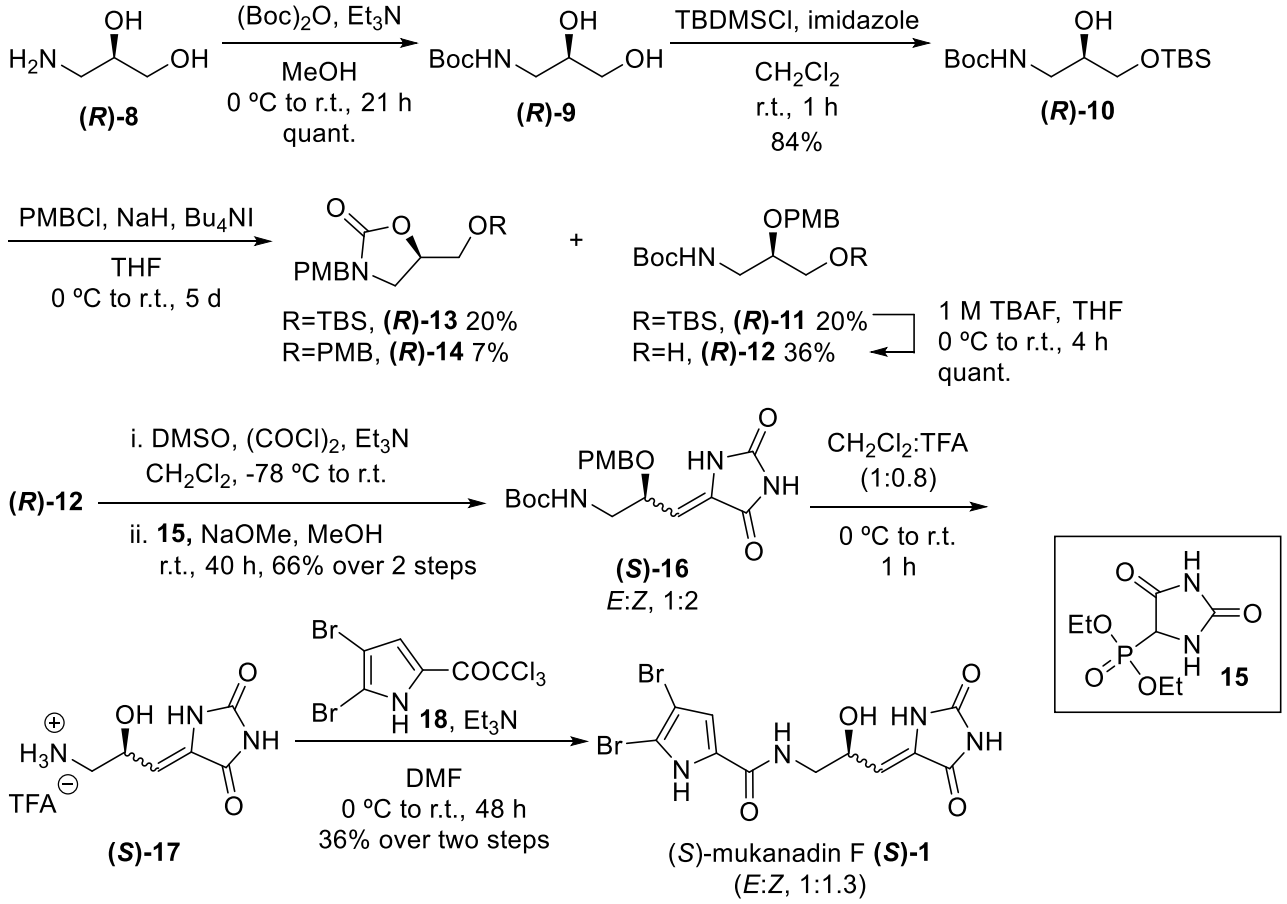
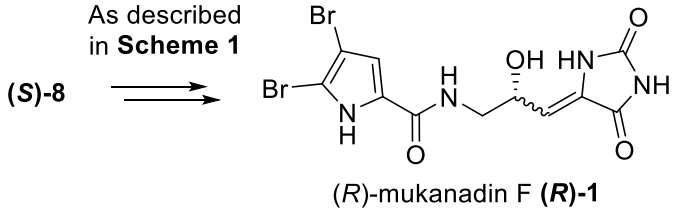


Figure 1. Selected examples of marine bromopyrrole alkaloids.

Both enantiomers of mukanadin F, **(*S*)-1** and **(*R*)-1**, were successfully prepared following the synthesis depicted in **Scheme 1**. The synthesis of (*S*)-mukanadin F **(*S*)-1** began with Boc protection of (*R*)-amino diol **(*R*)-8** to give carbamate **(*R*)-9**, followed by selective primary alcohol protection giving TBDMS ether **(*R*)-10**.[7,8] PMB protection of the remaining alcohol in **(*R*)-10** gave the desired tri-protected **(*R*)-11** as well as, unexpectedly, the deprotected primary alcohol **(*R*)-12** and side products, oxazolidinones **13** and **14**. TBDMS-ether **(*R*)-11** was converted into further alcohol **(*R*)-12**, which underwent Swern oxidation, to give an unstable aldehyde, followed by a Horner-Wadsworth-Emmons reaction with hydantoin phosphonate **15** to give hydantoin **(*S*)-16** as a mixture of *E*/*Z* isomers (*E*/*Z*, 1:2). Next, simultaneous Boc and PMB deprotection was accomplished under acidic conditions to give amine salt **(*S*)-17**, which was used immediately in the subsequent pyrrole coupling reaction with dibromopyrrole **18**,[9] giving (*S*)-mukanadin F **(*S*)-1** as a mixture of *E*/*Z* isomers (*E*/*Z*, 1:1.3). (*R*)-Mukanadin F **(*R*)-1** was synthesized, as a 1:2 mixture of *E*/*Z* isomers, following the same procedure described for **(*S*)-1**, starting from (*S*)-amino diol **(*S*)-8** (**Scheme 2**).



Scheme 1. Synthesis of (*S*)-mukanadin F **(*S*)-1**.



Scheme 2. Synthesis of (*R*)-mukanadin F **(*R*)-1**.

Purification of the *E*/*Z* isomers of **1** using flash chromatography proved troublesome with samples containing only the suspected *E*-isomer being found to contain trace amounts of the *Z*-isomer upon NMR analysis, suggesting instability of the *E*-isomer (see below). As a result, only samples of (*S*,*E*)-, (*S*,*Z*)- and (*R*,*Z*)-mukanadin F were able to be obtained in pure form. The olefin geometry of (*S*,*E*)- and (*S*,*Z*)-mukanadin F was confirmed by analysis of $^{3}J_{C\text{-}H}$ values (obtained via proton coupled ^{13}C NMR acquisition). In the case of (*S*,*E*)-mukanadin F, the H-10 - C-12 coupling constant was determined to be 9.9 Hz, while for (*S*,*Z*)-mukanadin F it was 5.6 Hz (**Figure 2**); these values fall within the appropriate ranges for *E*- and *Z*- isomers respectively.[5] Comparison of NMR data of synthetic *Z* samples of mukanadin F (**1**) matched those reported for the natural product, confirming the assigned alkene geometry.[2] Comparing the $[\alpha]_D$ values, neither synthetic *R,Z*-**1** (+22.2 (*c* = 0.3, MeOH)) nor *S,Z*-**1** (-26.7 (*c* = 0.3, MeOH)) had rotations that matched the reported value for the natural product

(-3.9 (*c* = 0.3, MeOH)),[2] and both were notably higher in magnitude. The negative rotation value for synthetic *S,Z*-**1** suggests the natural product, albeit with its lower negative value, has 9*S* stereochemistry, which is opposite to that proposed for the isolated natural sample.

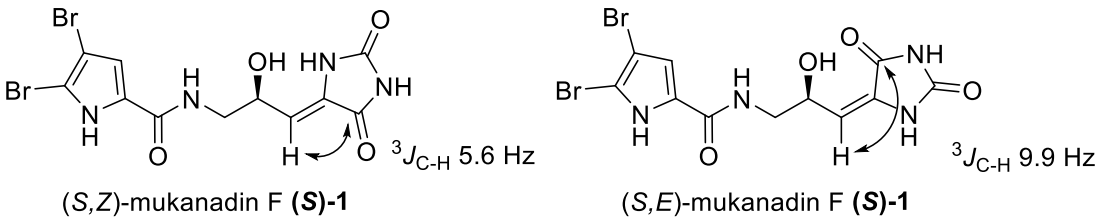
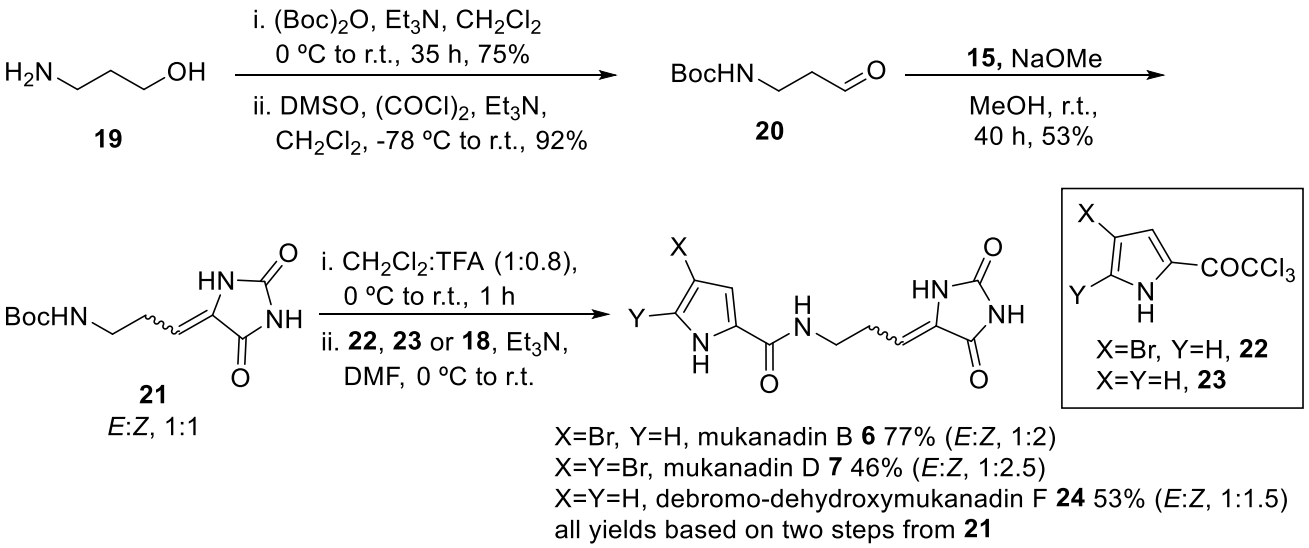


Figure 2. Long range coupling constants determining *E*- and *Z*-mukanadin F **(*S*)-1**.

The noted difficultly in purifying samples of *E*-**1**, combined with inconsistencies in optical rotation data between synthetic and natural samples, suggested that isomerization and racemization are facile amongst this compound class. To determine the extent to which either/both of these processes occur during natural product isolation studies, we initially examined the propensity of simpler, achiral bromopyrrole alkaloids containing this ene-hydantoin to undergo *E*/*Z* isomerization, noting that all have been isolated as *Z*-isomers.

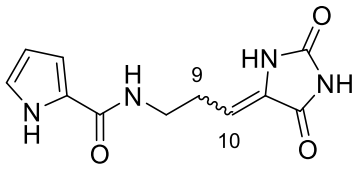
Using similar methodology to that used to prepare **1**, the synthesis of **24**, debromo-dehydroxy-mukanadin F, commenced with Boc protection of amino alcohol **19** followed by Swern oxidation giving aldehyde **20** (**Scheme 3**). Aldehyde **20** then underwent a Horner-Wadsworth-Emmons reaction with phosphonate **15** to give hydantoin **21** as a 1:1 mixture of *E* and *Z* isomers. The 1:1 *E*:*Z* mixture of **21** then underwent Boc deprotection, followed by immediate coupling with pyrrole **23** to give **24** as a mixture of *E*/*Z* isomers (1:1.5, *E*:*Z*). Using this same method we prepared mukanadin B (**6**) and mukanadin D (**7**) using pyrroles **22** or **18**, respectively.

All were obtained as mixtures of isomers, with *Z* always being the major isomer. It was noted that the isomeric ratio became enriched in *Z* after chromatographic purification. After separation the assignment of *E* and *Z* geometry was again confirmed by performing proton coupled ^{13}C NMR experiments. Mukanadin D (**7**) has been synthesized previously but as a mixture of isomers, whilst mukanadin B (**6**) has not been previously prepared. These syntheses along with successful separation of their respective *Z*-isomers, enables confirmation of the geometry of the *Z*-alkene originally assigned to the natural products.[3,10,11]



Scheme 3. Synthesis of mukanadin B (**6**).

Isomerization studies were carried out on the simplest prepared mukanadin, **24**. A chromatographically obtained, *E*-enriched sample, of **24** was dissolved in deuterated methanol and stored in a NMR tube exposed to sunlight. Time course analysis showed that the proportion of *Z*-isomer increased over time, highlighting facile olefin isomerization under these conditions (**Figure 3**) which are commonly encountered in the extraction and purification of marine natural products. Interestingly, when a similar time course experiment was conducted on a sample of *Z*-**24**, no *Z* to *E* isomerization was observed (data not shown). Calculations using Gaussian software show that *Z*-**24** has a lower Gibbs free energy compared to the *E*-**24** (Δ 27.29 kJ mol^{-1}), and hence, is the more stable isomer (see Supplementary for details).



Debromo-dehydroxymukanadin F **24**

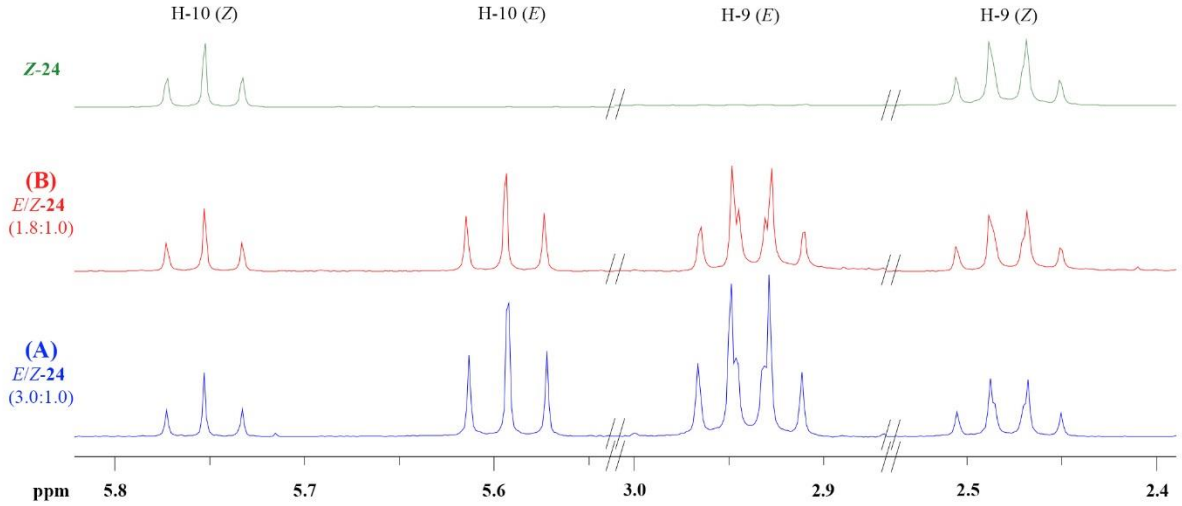


Figure 3. Isomerization of **24**. ^{1}H NMR spectra excerpts of *E*- and *Z*-**24** highlighting changes in H-9 and H-10 ratios over time. (A) T = 0 days or (B) T = 53 days after initial purification.

Having observed the isomerization of *E*-**24** to *Z*-**24** in methanol, similar isomerization studies were performed on mukanadin F (**1**), to establish if this process also occurs for 9-substituted bromopyrrole alkaloids. (*S*,*E*)-Mukanadin F was dissolved in deuterated methanol and exposed to a range of conditions, described in **Table 1**. As observed for **24**, (*S*,*E*)-mukanadin F underwent *E*/*Z* isomerization in methanol and sunlight as well as with the other three conditions (acidic, basic, exclusion of light) trialed, with all the samples becoming enriched with *Z*-isomer over time. It was observed that the rate of isomerization was greatest in acidic conditions. Again, no *Z* to *E* isomerization was observed for (*S*,*Z*)-mukanadin F.

Table 1. Isomerization of (*S*,*E*)-mukanadin F **(*S*)-1**.

		E/*Z* ratio		
		Day 0	Day 7	Day 39
(*S*,*E*)-mukanadin F **(*S*)-1**	Without sunlight[a]	10.0:1.0	3.1:1.0	3.0:1.0
	With sunlight[a]	10.0:1.0	3.0:1.0	2.4:1.0
	TFA[a,b,c]	10.0:1.0	0.9:1.0	0.7:1.0
	Et_3N[a,b]	10.0:1.0	2.5:1.0	2.4:1.0

a Samples were approx. 4.4 mM in deuterated methanol. *b* one drop of each reagent was added to a 2.5 mL sample. *c* ^{1}H NMR analysis also indicated the formation of a unidentifiable new species (1%) as well as isomerization.

Based on the isomerization findings, further experiments were performed to study the effects of the four conditions on the optical rotation of the prepared samples (**Figure 4**). Four samples each of (*S*,*Z*)- and (*S*,*E*)-mukanadin F (**1**) (4.4 mM) were prepared in deuterated methanol, and exposed to the same four conditions; sunlight, protected from light, presence of TFA, and presence of Et_3N (**Figure 4**). Under all conditions (*S*,*Z*)-mukanadin F was found to racemize over time to give a scalemic mixture, with sunlight having the greatest effect on the rate of racemization. This change in the optical rotation can only be due to the racemization at C-9, as no *Z* to *E* isomerization was observed for *Z*-isomers. Similarly, (*S*,*E*)-mukanadin F also racemizes to a scalemic mixture over time, with acidic conditions having the greatest effect on the rate of racemization. In this case, the change in optical rotation can be attributed to both the racemization at C-9 as well as *E*/*Z* isomerization. The added factor of *E*/*Z* isomerization accounts for the observed change in sign for the optical rotation value. Overall, both *E* and *Z* isomers were found to racemize, with optical rotation values in all cases approaching 0.

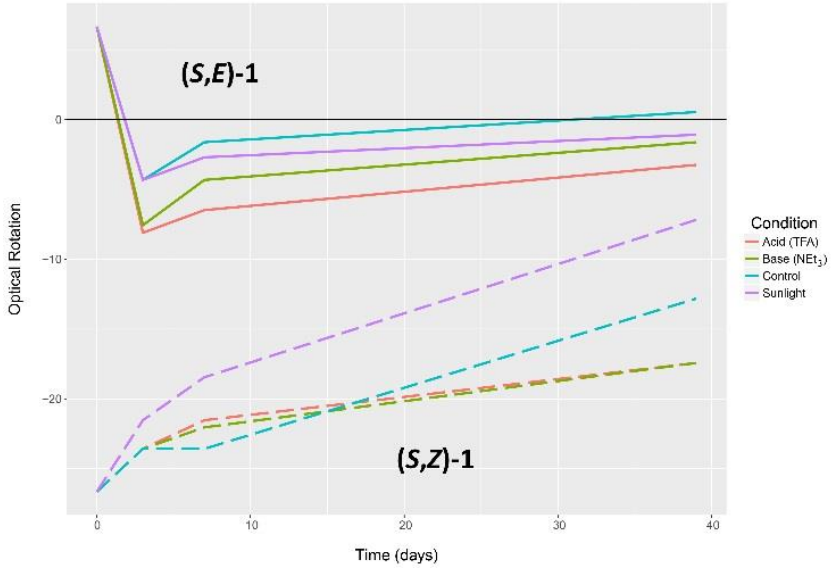


Figure 4. Racemization studies of (*S*,*Z*)- and (*S*,*E*)-mukanadin F **(*S*)-1**.

Proposed mechanisms of isomerization and racemization were formulated to further understand the role methanol could be having on the results we had observed (**Figure 5**). Isomerization of the alkene in (*S*,*E*)-mukanadin F (**1**) may occur through the addition of a nucleophile, such as methanol, to C-10, giving an enol which could then undergo C-10,11 bond rotation, ultimately allowing the formation of (*S*,*Z*)-mukanadin F (**1**). Racemization of (*S*,*Z*)-mukanadin F (**1**) could proceed through the removal of the acidic proton at C-9. This would lead to the formation of a planar enolate, which upon protonation would racemize the chiral center at C-9. There is evidence of this second mechanism observed in the proton NMR where it was observed that relative integral of H-9 to H-10 decreased over time when left in deuterated methanol solvent, implying that the proton was being replaced by deuterium.

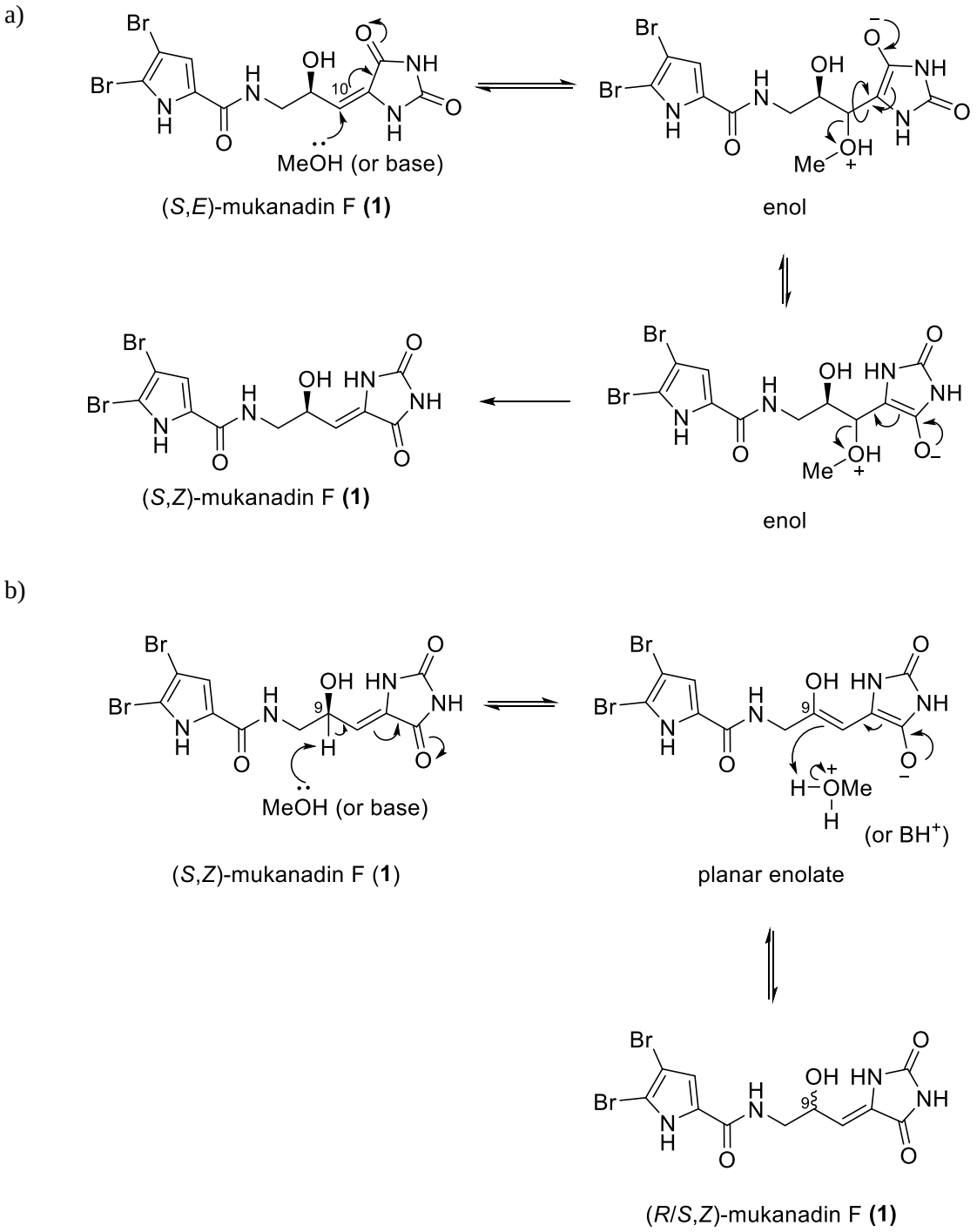


Figure 5. a) Proposed isomerization mechanism of (*S*,*E*)-mukanadin F (**1**) in methanol. b) Proposed racemization mechanism of (*S*,*Z*)-mukanadin F (**1**) in methanol.

The results from the isomerization and racemization studies can be used to explain the differences observed in optical rotation values of isolated natural product to that of synthetic samples of mukanadin F (**1**). The isomerization and racemization experiments performed on synthetic **1** suggest that the isolated natural product was characterized as a scalemic mixture enriched in the 9*S* enantiomer.[12] These results establish the ability of C-9 to undergo racemization under normal laboratory conditions. The potential reactivity at C-9 has recently been observed with the isolation of 9-*O*-ethyl-mukanadin F, the

authors suggesting that this compound could in fact be an artefact arising from extraction and isolation procedures utilizing ethanol.[13] Similarly, 9-*O*-methyl-debromomukanadin F was recently isolated, using extraction methods utilizing methanol, however, the authors do not speculate as to whether the new compound could in fact be an artefact.[14] It should be noted that we did not observe any 9-*O*-methyl products during our isomerization studies.

The occurrence of artefacts and modifications to natural products as a result of isolation and/or purification processes have been well documented.[15] Little has been investigated within this group of alkaloids, with structures reported without mention of possible effects from these processes. These findings have implications on the isolation and characterization of C-9 functionalized ene-hydantoin/imidazole marine natural products. The results presented herein suggest that not only isomerization but also racemization occurs under conditions regularly used during isolation and purification procedures, and as such, caution needs to be exercised when assigning the C-9 configuration of marine natural products containing this α,β-unsaturated system. In the racemization studies, extrapolation of the trends see the samples racemizing to give scalemic mixtures with optical rotation values approaching 0. While many of the chiral marine natural products shown in **Figure 1** have been isolated and reported as racemic mixtures, the isolation of mukanadin A (**2**) as both a scalemic[3] and racemic[4] mixture leads to the possibility that this class of compounds do exist in nature as pure enantiomers, which upon exposure to isolation and/or purification procedures undergo C-9 racemization. Therefore, care should be taken when assigning the configuration of these types of marine natural products.

Experimental

General Experimental Procedures. Oven-dried glassware and freshly distilled solvents were used when carrying out reactions, with reaction vessels purged with an inert gas such as nitrogen unless stated otherwise. Chemical reagents were not subjected to quality analysis and were used as purchased. A MicroTOF-Q mass spectrometer was used to perform mass spectrometry analysis on compounds (low- and high-resolution), using methods of electrospray ionization (ESI) or chemical ionization (CI). Nuclear magnetic resonance (NMR) spectroscopic analysis was performed on Bruker 300, 400 or 500 MHz spectrometers. ^{1}H NMR analysis is presented as position (δ), relative integration, multiplicity, coupling constant (*J*, Hz), followed by the assignment of the atom. ^{13}C NMR analysis is presented as position (δ) followed by the assignment of the atom. The position (δ) of signals in the NMR are reported relative to the signal of either chloroform (δ 7.26 for ^{1}H and δ 77.00 for ^{13}C), dimethyl sulfoxide (δ 2.50 for ^{1}H and δ 39.52 for ^{13}C), or methanol (δ 3.31 for ^{1}H and δ 49.00 for ^{13}C) where stated. Assignments of atoms were performed using 2D NMR techniques of HSQC, COSY and HMBC. All reported theoretical energy calculations were performed using the Gaussian 09 package. The level of theory employed for the theoretical calculation was RB3LYP level and DGDZVP level of theory. Due to the use of methanol in the calculations, SCRF was used in the DFT calculation in order to model the reaction under solvation conditions. Furthermore, an implicit solvent model, PCM was used. The Gibbs free energies were reported in hartrees and converted to kJ mol^{-1} using the conversion 1 hartree = 2625.50 kJ mol^{-1}.

***tert*-Butyl (*R*)-(2,3-dihydroxypropyl)carbamate (*R*)-9.** To a stirred solution of (*R*)-3-amino-1,2-diol **(*R*)-8** (2.5 g, 27 mmol) in dry methanol (75 mL) at 0 ºC was added first triethylamine (3.80 mL, 41 mmol) and then Boc_2O (9.46 mL, 41 mmol) in portions. The reaction mixture was allowed warm to room temperature and stirred for 21 h before the solvent was removed *in vacuo* to give the crude product which was purified by gradient flash chromatography (firstly with 1:4 *n*-hexanes:ethyl acetate then ethyl acetate) to afford the *title compound* **(*R*)-9** (5.38 g, quant.) as a clear colorless oil which solidifies to a

white crystalline solid after being frozen. m.p. 51-53 ºC. (lit. m.p. 53-55 ºC).[7] R_f = 0.30 (ethyl acetate). $[\alpha]_D$ -8.9 (*c* = 1.46, $CHCl_3$). (lit. $[\alpha]_D$ -8.3 (*c* = 1.4, $CHCl_3$)).[8] δ_H (300 MHz, $CDCl_3$) 1.43 (9H, s, CMe_3), 2.90 (2H, br s, 2 x OH), 3.23-3.28 (2H, m, H-1), 3.57 (2H, br d, *J* = 3.9 Hz, H-3), 3.72 (1H, br p, *J* = 4.8 Hz, H-2), 4.95 (1H, br s, NH). These values were in agreement with literature values.[16] ***tert*-Butyl (S)-(2,3-dihydroxypropyl)carbamate (*S*)-9** was prepared using the same procedure as above, starting with **(*S*)-8**, giving the *title compound* as a white solid. m.p. 49-51 ºC. (lit. m.p. 51-52 ºC).[7] $[\alpha]_D$ +10.8 (*c* = 0.5, $CHCl_3$). (lit. $[\alpha]_D$ +6.7 (*c* = 0.5, $CHCl_3$)).[7] All spectroscopic data was identical to that of the previously prepared **(*R*)-9**.

***tert*-Butyl (*R*)-(3-((*tert*-butyldimethylsilyl)oxy)-2-hydroxypropyl)carbamate (*R*)-10.** To a stirred solution of diol **(*R*)-9** (5.28 g, 28 mmol) in dry CH_2Cl_2 (130 mL) at room temperature was added imidazole (5.64 g, 83 mmol) and then TBSCl (4.58 g, 30 mmol) in one portion. The reaction mixture was stirred for 1 h before being quenched with sat. aq. ammonium chloride (30 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were then dried ($MgSO_4$) before the solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (3:1 *n*-hexanes:ethyl acetate) to afford the *title compound* **(*R*)-10** (7.08 g, 84%) as a clear colorless oil. R_f = 0.60 (1:1 *n*-hexanes:ethyl acetate). $[\alpha]_D$ +8.2 (*c* = 0.916, $CHCl_3$). (lit. $[\alpha]_D$ +9.5 (*c* = 0.9, $CHCl_3$)).[8] δ_H (300 MHz, $CDCl_3$) 0.06 (6H, s, $SiMe_2$), 0.89 (9H, s, $SiCMe_3$), 1.43 (9H, s, $OCMe_3$), 2.80 (1H, br s, OH), 3.11 (1H, ddd, *J* = 13.8, 6.3, 4.8 Hz, 1-H_B), 3.31-3.35 (1H, br m, 1-H_A), 3.52 (1H, dd, *J* = 9.9, 6.3 Hz, 3-H_B), 3.63 (1H, dd, *J* = 9.9, 4.8 Hz, 3-H_A), 3.68-3.77 (1H, m, H-2), 4.94 (1H, br s, NH). These values were in agreement with literature values.[8] ***tert*-Butyl (S)-(3-((*tert*-butyldimethylsilyl)oxy)-2-hydroxypropyl)carbamate (*S*)-10** was prepared using the same procedure as above, starting with **(*S*)-9**, giving the *title compound* as a clear colorless oil. $[\alpha]_D$ -7.9 (*c* = 0.92, $CHCl_3$). All spectroscopic data was identical to that of the previously prepared **(*R*)-10**.

***tert*-Butyl (*R*)-(3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*R*)-11, (*R*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-(4-methoxybenzyl)oxazolidin-2-one (*R*)-13, (*R*)-3-(4-methoxybenzyl)-5-(((4-methoxybenzyl)oxy)methyl)oxazolidin-2-one (*R*)-14** and ***tert*-butyl (*R*)-(3-hydroxy-2-((4-methoxybenzyl)oxy)propyl)carbamate (*R*)-12**. To a stirred mixture of alcohol (*R*)-10 (1.0 g, 3.3 mmol), sodium hydride (60% dispersion in oil w/w, 0.14 g, 3.4 mmol) and tetrabutylammonium iodide (0.12 g, 0.33 mmol) in dry THF (10 mL) at 0 ºC was added PMBCl (0.49 mL, 3.6 mmol) slowly. The reaction mixture was stirred for 1 h at 0 ºC before being allowed to warm to room temperature and stirred for a further 5 d before being quenched with sat. aq. ammonium chloride (5 mL) followed by extraction with ether (3 x 5 mL). The combined organic extracts were then washed with sat. aq. sodium thiosulphate (10 mL) and dried ($MgSO_4$) before the solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (gradient elution first with *n*-hexanes then 9:1 *n*-hexanes:ethyl acetate) to afford the *title compound* **(*R*)-11** (0.28 g, 20%) as a yellow oil. R_f = 0.67 (3:1 *n*-hexanes:ethyl acetate). $[\alpha]_D$ +18.6 (*c* = 4.78, $CHCl_3$). (* denotes minor rotamer) δ_H (400 MHz, $CDCl_3$) -0.02 and -0.01 (6H, s, $SiMe_2$), 0.01 (6H, s, $SiMe_2$*), 0.81 (9H, s, $SiCMe_3$*), 0.83 (9H, s, $SiCMe_3$), 1.35 (9H, s, $OCMe_3$), 1.36 (9H, s, $OCMe_3$*), 3.06-3.34 (4H, m, H-1 and H-3*), 3.45 (1H, p, *J* = 5.2 Hz, H-2), 3.53-3.64 (4H, m, H-3 and H-1*), 3.69 (3H, s, OCH_3), 3.81-3.84 (1H, m, H-2*), 4.36 (2H, s, CH_2Ar*), 4.44 (1H, d, *J* = 11.4 Hz, CH_BAr), 4.46 (1H, d, *J* = 11.4 Hz, CH_AAr), 4.78-4.81 (1H, m, NH*), 4.89-4.92 (1H, m, NH), 6.77-6.80 (4H, m, Ar-H and Ar-H*), 7.15 -7.19 (4H, m, Ar-H and Ar-H*). δ_C (75 MHz, $CDCl_3$) -5.7 and -5.7 ($Si(CH_3)_2$ and $Si(CH_3)_2$*), 17.9 (Si*C*$(CH_3)_3$*), 18.0 (Si*C*$(CH_3)_3$), 25.6 (SiC(*C*$H_3)_3$*), 25.7 (SiC(*C*$H_3)_3$), 28.2 (OC(*C*$H_3)_3$), 41.4 (C-1), 54.9 (OCH_3), 63.7 (C-3 and C-1*), 70.1 (C-2*), 71.4 (*C*H_2Ar), 72.1 (C-3*), 72.8 (*C*H_2Ar*), 77.5 (C-2), 78.6 (O*C*$(CH_3)_3$), 113.5 (Ar-CH*), 113.6 (Ar-CH), 129.0 (Ar-CH*), 129.1 (Ar-CH), 130.0 (Ar-C*), 130.3 (Ar-C), 155.7 (C=O*), 155.8 (C=O), 159.0 (*C*OCH_3*), 159.0 (*C*OCH_3). ν_{max}(ATR)/cm^{-1} 3364, 2930, 2857, 1715, 1513, 1464, 1365, 1247, 1171, 834. *m/z* (ESI^+): 448 (MNa^+, 100%), 392 (12%); HRMS (ESI^+) found

(MNa^+): 448.2476, $C_{22}H_{39}NNaO_5Si$ requires 448.2490. In a separate fraction **5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-(4-methoxybenzyl)oxazolidin-2-one (*R*)-13** (0.23 g, 20%) was also isolated, as a clear colourless oil. R_f = 0.17 (3:1 *n*-hexanes:ethyl acetate). $[\alpha]_D$ -28.6 (*c* = 0.41, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 0.04 (6H, s, $SiMe_2$), 0.84 (9H, s, CMe_3), 3.29-3.40 (2H, m, H-4), 3.66 (1H, dd, *J* = 11.0, 3.6 Hz, 6-H_B), 3.74 (1H, dd, *J* = 11.0, 4.8 Hz, 6-H_A), 3.79 (3H, s, OCH_3), 4.35 (2H, d, *J* = 4.0 Hz, H-7), 4.45-4.50 (1H, m, H-5), 6.86 (2H, d, *J* = 8.6 Hz, Ar-H), 7.20 (2H, d, *J* = 8.6 Hz, Ar-H). δ_C (100 MHz, $CDCl_3$) -5.5 and -5.5 ($Si(CH_3)_2$), 18.2 ($C(CH_3)_3$), 25.7 ($C(CH_3)_3$), 45.3 (C-4), 47.6 (C-7), 55.2 (OCH_3), 63.4 (C-6), 72.9 (C-5), 114.1 (Ar-CH), 127.8 (Ar-C), 129.5 (Ar-CH), 157.9 (C-2), 159.3 ($COCH_3$). ν_{max}(ATR)/cm^{-1} 2929, 1744, 1514, 1440, 1361, 1245, 834. *m/z* (ESI^+): 374 (MNa^+, 100%), 352 (MH^+, 10%); HRMS (ESI^+) Found (MNa^+): 374.1794, $C_{18}H_{29}NNaO_4Si$ requires 374.1758. Found (MH^+): 352.1961, $C_{18}H_{30}NO_4Si$ requires 352.1939. In a separate fraction **3-(4-methoxybenzyl)-5-(((4-methoxybenzyl)oxy)methyl)oxazolidin-2-one (*R*)-14** (78 mg, 7%) was also isolated, as a yellow solid. m.p. 74-76 ºC. R_f = 0.03 (3:1 *n*-hexanes:ethyl acetate). $[\alpha]_D$ -39.0 (*c* = 0.20, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 3.25 (1H, dd, *J* = 8.6, 6.0 Hz, 4-H_B), 3.40 (1H, t, *J* = 8.6 Hz, 4-H_A), 3.51-3.59 (2H, m, H-6), 3.78 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.29-4.39 (2H, m, H-7), 4.43-4.51 (2H, m, O-CH_2Ar), 4.55-4.61 (1H, m, H-5), 6.80-6.88 (4H, m, ArH), 7.15-7.21 (4H, m, ArH). δ_C (100 MHz, $CDCl_3$) 45.8 (C-4), 47.5 (C-7), 70.0 (C-6), 71.8 (C-5), 73.2 (OCH_2Ar), 113.8 and 114.0 (Ar-CH), 127.6 (ArC), 129.3 and 129.3 (Ar-CH), 129.5 (ArC), 157.7 (C-2), 159.2 and 159.3 ($COCH_3$). ν_{max}(ATR)/cm^{-1} 2960, 1728, 1513, 1448, 1347, 1250, 810. *m/z* (ESI^+): 380 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 380.1474, $C_{20}H_{23}NNaO_5$ requires 380.1468. In a separate fraction alcohol **(*R*)-12** (0.36 g, 36%) was also isolated.

***tert*-Butyl (*S*)-(3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*S*)-11** was prepared using the same procedure as above, starting with **(*S*)-10**, giving the *title compound* as a clear colorless oil. $[\alpha]_D$ -14.4 (*c* = 4.8, $CHCl_3$). All spectroscopic data was identical to that of the previously prepared **(*R*)-11**.

***tert*-Butyl (*R*)-(3-hydroxy-2-((4-methoxybenzyl)oxy)propyl)carbamate (*R*)-12.** To a stirred solution of silyl **(*R*)-11** (0.41 g, 0.96 mmol) in THF (10 mL) at 0 ºC was added a solution of TBAF (0.61 g, 1.9 mmol) in THF (1M). The reaction mixture was allowed to warm to room temperature and stirred for 4 h before being washed with sat. aq. ammonium chloride (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic extracts were then washed with brine (10 mL) and dried ($MgSO_4$) before the solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (gradient 3:1 *n*-hexanes:ethyl acetate then 1:1 *n*-hexanes:ethyl acetate) to afford the *title compound* **(*R*)-12** (0.31 g, quant.) as a colorless oil. $[\alpha]_D$ -25.0 (*c* = 0.58, $CHCl_3$). R_f = 0.06 (3:1 *n*-hexanes:ethyl acetate). (* denotes minor rotamer) δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, CMe_3), 3.0-3.15 (1H, m, OH), 3.22-3.42 (2H, m, H-1), 3.37-3.48 (2H, m, H-1*), 3.50-3.55 (1H, m, H-2), 3.57-3.61 (3H, m, H-3 and H-2*), 3.79 (3H, s, OCH_3), 4.46 (2H, s, CH_2Ar*), 4.51 (2H, s, CH_2Ar), 5.00-5.06 (1H, m, NH and NH*), 6.86-6.88 (2H, m, Ar-H), 7.23-7.27 (2H, m, Ar-H). δ_C (100 MHz, $CDCl_3$) 28.2 ($C(CH_3)_3$), 40.2 (C-1), 55.2 (OCH_3), 61.0 (C-3), 71.3 (CH_2Ar), 71.5 (C-1*), 73.0 (CH_2Ar*), 77.6 (C-2), 79.4 and 79.6 ($C(CH_3)_3$ and $C(CH_3)_3$*), 113.76 and 113.82 (Ar-CH and Ar-CH*), 129.4 (Ar-CH and Ar-CH*), 129.8 and 130.0 (Ar-C and Ar-C*), 156.6 and 157.0 (C=O and C=O*), 159.2 and 159.3 ($COCH_3$ and $COCH_3$*). ν_{max}(ATR)/cm^{-1} 3357, 2932, 2874, 1689, 1513, 1455, 1366, 1246, 1170, 1033, 821. *m/z* (ESI^+): 334 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 334.1617, $C_{16}H_{25}NNaO_5$ requires 334.1625. ***tert*-Butyl (*S*)-(3-hydroxy-2-((4-methoxybenzyl)oxy)propyl)carbamate (*S*)-12** was prepared using the same procedure as above, starting with **(*S*)-11**, giving the *title compound* as a clear colorless oil. $[\alpha]_D$ +20.6 (*c* = 0.50, $CHCl_3$). All spectroscopic data was identical to that of the previously prepared **(*R*)-12**.

***tert*-Butyl (*R*)-(2-((4-methoxybenzyl)oxy)-3-oxopropyl)carbamate.** To a stirred solution of oxalyl chloride (0.45 mL, 5.3 mmol) in CH_2Cl_2 (13 mL) at -78 ºC under nitrogen was added DMSO (0.56 mL, 7.9 mmol) dropwise. After 15 min of stirring, alcohol **(*R*)-12** (0.82 g, 2.6 mmol) in CH_2Cl_2 (11 mL) was added to the reaction mixture which was maintained at -78 ºC and stirred for a further 30 min. Triethylamine (2.2 mL, 16 mmol) was then added and the reaction stirred for a further 30 min at -78 ºC followed by a further 30 min at 0 ºC. The reaction was then quenched with sat. aq. ammonium chloride (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were then washed with water (20 mL), sat. aq. sodium bicarbonate (20 mL), brine (20 mL) and dried ($MgSO_4$) before the solvent was removed *in vacuo* to give the *title compound* which was used in the next reaction without further purification. ***tert*-Butyl (*S*)-(2-((4-methoxybenzyl)oxy)-3-oxopropyl)carbamate** was prepared using the same procedure as above, starting with **(*S*)-12**, giving the *title compound* which was used in the next reaction without further purification.

***tert*-Butyl (*S*,*E*)-(3-(2,5-dioxoimidazolidin-4-ylidene)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*S*,*E*)-16 and *tert*-butyl (*S*,*Z*)-(3-(2,5-dioxoimidazolidin-4-ylidene)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*S*,*Z*)-16**. To a solution of sodium methoxide (prepared from sodium (73 mg, 3.2 mmol) in dry methanol (7 mL)) was added hydantoin phosphonate 15 (0.75 g, 3.2 mmol) and the reaction placed under an atmosphere of nitrogen. After 5 min a solution of *tert*-butyl (*R*)-(2-((4-methoxybenzyl)oxy)-3-oxopropyl)carbamate (0.82 g, 2.6 mmol) in dry methanol (3 mL) was added and the reaction stirred for 40 h before being quenched with sat. aq. ammonium chloride (5 mL). The solvent was removed *in vacuo* and the remaining aqueous mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extracts were washed with sat. aq. sodium bicarbonate (10 mL), brine (10 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (2:1 ethyl acetate:*n*-hexanes) afford the *title compounds* **(*S*,*Z*)-16** and **(*S*,*E*)-16** (0.68 g, 66% over two steps) as a 2:1 mixture of *Z* to *E* isomers, as a cream foam. Further purification to obtain an analytical sample of each isomer afforded **(*S*,*Z*)-16** as a yellow gum (* denotes minor rotamer). R_f = 0.56 (3:1 ethyl acetate:*n*-hexanes). δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, CMe_3), 3.15-3.43 (3H, m, H-1 and H-2*), 3.81 (3H, s, OCH_3), 4.05 (2H, d, *J* = 6.4 Hz, H-1*), 4.31 (1H, br s, H-2), 4.38-4.58 (4H, m, CH_2Ar and CH_2Ar*), 4.95 (1H, br s, 1-NH*), 5.30 (1H, t, *J* = 6.6 Hz, 1-NH), 5.76 (1H, d, *J* = 5.1 Hz, H-3), 6.89 (2H, dd, *J* = 8.6, 2.7 Hz, Ar-H), 7.24 (2H, dd, *J* = 8.6, 2.0 Hz, Ar-H), 8.19 (1H, br s, NH), 8.26-8.36 (3H, m, 2 x NH* and NH). δ_C (100 MHz, $CDCl_3$) 28.3 ($C(CH_3)_3$), 37.2 (C-1*), 44.0 (C-1), 50.9 (C-2*), 55.3 (OCH_3), 71.7 ($ArCH_2$), 73.3 ($ArCH_2$*), 75.5 (C-2), 79.7 and 80.2 ($C(CH_3)_3$ and $C(CH_3)_3$*), 110.6 (C-3), 114.0, 114.1 and 114.2 (Ar-C), 125.8 and 126.4 (C-4' and C-4'*), 128.7, 129.6 and 129.8 (Ar-C), 130.9 (Ar-C), 152.4 and 153.2 (C-5' and C-5'*), 156.3 (C=O), 159.4 and 159.7 ($COCH_3$ and $COCH_3$*), 162.9 and 163.5 (C-2' and C-2'*). ν_{max}(ATR)/cm^{-1} 3213, 2976, 1725, 1678, 1512, 1458, 1366, 1162, 1031, 763. *m/z* (ESI^+): 414 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 414.1620, $C_{19}H_{25}N_3NaO_6$ requires 414.1636. In a separate fraction **(*S*,*E*)-16** was also isolated, as a yellow semisolid. R_f = 0.44 (3:1 ethyl acetate:*n*-hexanes). δ_H (500 MHz, CD_3OD) 1.42 (9H, s, CMe_3), 3.20-3.23 (2H, m, H-1), 3.77 (3H, s, OCH_3), 4.46-4.53 (2H, m, CH_2Ar), 5.20-5.26 (1H, m, H-2), 5.31 (1H, d, *J* = 9.6 Hz, H-3), 6.85-6.92 (2H, m, Ar-H), 7.24-7.31 (2H, m Ar-H). δ_C (100 MHz, $CDCl_3$) 28.4 ($C(CH_3)_3$), 44.6 (C-1), 55.3 (OCH_3), 71.0 ($ArCH_2$), 71.8 (C-2), 89.1 ($C(CH_3)_3$), 113.8 and 114.1 (Ar-CH), 117.1 (C-3), 127.8 (C-4'), 129.4 and 129.5 (Ar-CH), 130.1 (Ar-C), 156.2 (C=O), 159.1 ($COCH_3$), 159.3 (C-2'), 163.1 (C-5'). ν_{max}(ATR)/cm^{-1} 3212, 2980, 2930, 1725, 1677, 1514, 1457, 1367, 1159, 1031, 762. *m/z* (ESI^+): 414 (MNa^+, 70%), 390 (33%), 358 (100%), 314 (26%); HRMS (ESI^+) found (MNa^+): 414.1627, $C_{19}H_{25}N_3NaO_6$ requires 414.1636. ***tert*-Butyl (*R*,*E*)-(3-(2,5-dioxoimidazolidin-4-ylidene)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*R*,*E*)-16** and ***tert*-butyl (*R*,*Z*)-(3-(2,5-dioxoimidazolidin-4-ylidene)-2-((4-methoxybenzyl)oxy)propyl)carbamate (*R*,*Z*)-16** was prepared using the same

procedure as above, starting with *tert*-butyl (*S*)-(2-((4-methoxybenzyl)oxy)-3-oxopropyl)carbamate, giving the *title compounds* as a 1.0:1.5 mixture of *Z* to *E* isomers, as a yellow foam. All spectroscopic data was identical to that of the previously prepared **(*S*,*E*)-16** and **(*S*,*Z*)-16**.

(*S*,*E*)- and (*S*,*Z*)-5-(3-Amino-2-hydroxypropylidene)imidazolidine-2,4-dione (*S*,*E*)-17 and (*S*,*Z*)-17. To a solution of carbamate **(*S*)-16** (0.42 g, 1.1 mmol) in CH_2Cl_2 (1.0 mL) at 0 ºC was added TFA (0.8 mL) slowly. The reaction mixture was allowed to warm to room temperature and stirred for 1 h before the solvent was removed *in vacuo* to give the crude product which was used immediately in the next reaction without further purification. **(*R*,*E*)- and (*R*,*Z*)-5-(3-Amino-2-hydroxypropylidene)imidazolidine-2,4-dione (*R*,*E*)-17 and (*R*,*Z*)-17** was prepared using the same procedure as above, starting with a mixture of **(*R*,*E*)-** and **(*R*,*Z*)-16**, giving the *title compounds* which were used in the next reaction without further purification.

1-(1*H*-Pyrrol-2-yl)-2,2,2-trichloroethanone 23. To a stirring solution of trichloroacetyl chloride (16 mL, 0.146 moles) in dry ether (26 mL), under an atmosphere of nitrogen, was added dropwise a solution of pyrrole (10 mL, 0.143 mol) in dry ether (78 mL) over 2 h. After 18 h, a solution of potassium carbonate (13 g, 0.1 mol) in water (40 mL) was added carefully. The organic layer was separated, dried ($MgSO_4$), filtered and then stirred with activated charcoal for 10 min. The organic layer was then filtered again and the solvent was removed *in vacuo* to give the crude product which was recrystallized from *n*-hexanes to afford the *title compound* **23** (25 g, 82%) as a purpley-white metallic solid. m.p. 71-72 ºC. (lit. m.p. 73-75 ºC).[17] δ_H (300 MHz, $CDCl_3$) 6.37-6.40 (1H, m, H-4), 7.15-7.17 (1H, m, H-5), 7.37-7.39 (1H, m, H-3), 9.46 (1H, br s, NH). The NMR values were in agreement with literature values.[17]

1-(4,5-Dibromo-1*H*-pyrrol-2-yl)-2,2,2-trichloroethanone 18. To a stirring solution of pyrrole **23** (1 g, 4.71 mmol) in acetic acid (5 mL), under an atmosphere of nitrogen, was added a solution of bromine (0.49 mL, 9.4 mmol) in acetic acid (4.5 mL) at such a rate as to maintain the reaction temperature at 18 ºC. After the addition the reaction was heated at 60 ºC for 2 h before being cooled to room temperature. The solvent was removed *in vacuo* to afford the *title compound* **18** (1.75 g, 100%) as a dark brown solid. m.p. 135-136 ºC. (lit. m.p. 136-139 ºC).[9] δ_H (400 MHz, $CDCl_3$) 7.34 (1H, d, J = 2.4 Hz, H-3), 9.81 (1H, br s, NH). The spectroscopic data was in agreement with literature values.[9]

General Procedure A: To a solution of amine salt (1 equiv.) and pyrrole (1 equiv.) in dry DMF at 0 ºC, under an atmosphere of nitrogen, was added triethylamine (2 equiv.) dropwise. The mixture was allowed to warm to room temperature and stirred until completion indicated by TLC. The solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography, using LiChroprep NH_2 silica, to afford the desired product.

(*S*,*E*)- and (*S*,*Z*)-Mukanadin F (1).

The reaction was carried out following General Procedure A using salt **(S)-17** (0.31 g, 1.1 mmol), bromopyrrole **18** (0.40 g, 1.1 mmol) and triethylamine (0.30 mL, 2.2 mmol) in DMF (15 mL) stirring for 48 h before the solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (9:1 CH_2Cl_2:CH_3OH) using LiChroprep NH_2 to afford the *title compound* **(*S*,*Z*)-1** (94 mg, 21% over 2 steps) as a white solid, m.p. 151-153 ºC. R_f = 0.26 (9:1 CH_2Cl_2:CH_3OH). $[\alpha]_D$ -26.7 (*c* = 0.3, CH_3OH). δ_H (500 MHz, $(CD_3)_2SO$) 3.24-3.40 (2H, m, H-8), 4.41-4.46 (1H, m, H-9), 5.33 (1H, d, J = 5.0 Hz, OH), 5.45 (1H, d, J = 8.0 Hz, H-10), 6.94 (1H, s, H-3), 8.06 (1H, t, J = 6.0 Hz, NH-7), 10.03 (1H, s, NH-13), 11.03 (1H, br s, NH-15), 12.69 (1H, br s, NH-1). δ_C (125 MHz, $(CD_3)_2SO$) 44.4 (C-8), 65.6 (C-9), 97.8 (C-3), 104.6 (C-2), 111.6 (C-10), 112.9 (C-4), 128.0 (C-5), 130.4 (C-11), 154.7 (C-14), 159.0 (C-6), 164.6 (C-12). ν_{max}(ATR)/cm^{-1} 3184, 2923, 1716, 1561, 1387, 1326, 1067, 779, 751, 650. *m/z* (ESI$^+$): 447 ($^{81,81}Br_2MNa^+$, 53%), 445 ($^{79,81}Br_2MNa^+$, 100%), 443 ($^{79,79}Br_2MNa^+$, 48%), 312 (50%); HRMS (ESI$^+$) found ($^{81,81}Br_2MNa^+$): 446.8902, $C_{11}H_{10}{}^{81,81}Br_2N_4NaO_4$ requires 446.8922. Found ($^{79,81}Br_2MNa^+$): 444.8939, $C_{11}H_{10}{}^{79,81}Br_2N_4NaO_4$ requires 444.8941. Found ($^{79,79}Br_2MNa^+$):

442.8963, $C_{11}H_{10}{}^{79,79}Br_2N_4NaO_4$ requires 442.8961. The ^1H and ^{13}C NMR data was in agreement with literature values.[2] In a separate fraction *title compound* **(*S*,*E*)-1** (71 mg, 16 % over 2 steps) was also isolated, as a white solid, m.p. 163-164 ºC. R_f = 0.17 (9:1 CH_2Cl_2-CH_3OH). $[\alpha]_D$ +6.7 (*c* = 0.3, CH_3OH). δ_H (500 MHz, $(CD_3)_2SO$) 3.16-3.40 (2H, m, H-8), 5.15-5.26 (2H, m, H-9 and OH), 5.29 (1H, d, *J* = 9.0 Hz, H-10), 6.92 (1H, s, H-4), 8.05 (1H, t, *J* = 5.0 Hz, NH-7), 10.01 (1H, s, NH-13), 11.01 (1H, br s, NH-15), 12.62 (1H, br s, NH-1). δ_C (125 MHz, $(CD_3)_2SO$) 44.7 (C-8), 63.6 (C-9), 97.7 (C-3), 104.3 (C-2), 112.8 (C-4), 117.2 (C-10), 128.2 (C-5), 129.7 (C-11), 154.0 (C-14), 158.9 (C-6), 164.3 (C-12). ν_{max}(ATR)/cm^{-1} 3147, 2925, 1718, 1564, 1389, 1328, 1059, 748, 650. *m/z* (ESI$^+$): 447 ($^{81,81}Br_2MNa^+$, 52%), 445 ($^{79,81}Br_2MNa^+$, 100%), 443 ($^{79,79}Br_2MNa^+$, 51%), 413 (39%), 367 (22%); HRMS (ESI$^+$) found ($^{81,81}Br_2MNa^+$): 446.8911, $C_{11}H_{10}{}^{81,81}Br_2N_4NaO_4$ requires 446.8922. Found ($^{79,81}Br_2MNa^+$): 444.8940, $C_{11}H_{10}{}^{79,81}Br_2N_4NaO_4$ requires 444.8941. Found ($^{79,79}Br_2MNa^+$): 442.8958, $C_{11}H_{10}{}^{79,79}Br_2N_4NaO_4$ requires 442.8961. **(*R*,*E*)-** and **(*R*,*Z*)-Mukanadin F (1)** was prepared using the same procedure as above, starting with a mixture of **(*R*,*E*)-** and **(*R*,*Z*)-16**, giving the *title compounds* as a 0.5:1.0 mixture of *E* to *Z* isomers. Purification by flash chromatography (9:1 CH_2Cl_2:CH_3OH) using LiChroprep NH_2 afforded **(*R*,*Z*)-Mukanadin F (1)** only, as a cream solid, m.p. 152-154 ºC. $[\alpha]_D$ +22.2 (*c* = 0.30, CH_3OH). All spectroscopic data was identical to that of the previously prepared **(*S*,*Z*)-1**.

***tert*-Butyl 3-hydroxypropylcarbamate.** To a stirring solution of 3-amino-propan-1-ol (2.0 g, 26.6 mmol) in dry CH_2Cl_2 (50 mL), under an atmosphere of nitrogen, was added di-*tert*-butyl dicarbonate (9.65 g, 39.9 mmol). Triethylamine (5.56 mL, 39.9 mmol) was added and the mixture was stirred for 35 h before being quenched with sat. aq. ammonium chloride (20 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were washed with brine (30 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the crude product which was purified by flash chromatography (gradient elution firstly with 4:1 *n*-hexanes:ethyl acetate and then with 1:1 *n*-hexanes:ethyl acetate) to afford the *title compound* (3.48 g, 75%) as a pale yellow oil. R_f = 0.26 (1:1 *n*-hexanes:ethyl acetate). δ_H (300 MHz, $CDCl_3$) 1.45 (9H, s, $C(CH_3)_3$), 1.67 (2H, p, *J* = 6.0 Hz, H-2), 3.01 (1H, br s, OH), 3.28 (2H, t, *J* = 6.0 Hz, H-1), 3.66 (2H, t, *J* = 6.0 Hz, H-3), 4.82 (1H, br s, NH). These values were in agreement with literature values.[18]

***tert*-Butyl 3-oxopropylcarbamate 20.** To a stirred solution of DMSO (1.2 mL, 17.1 mmol) in dry CH_2Cl_2 (54 mL), under an atmosphere of nitrogen, at -78 ºC was added oxalyl chloride (0.73 mL, 8.6 mmol) and the mixture was stirred for 15 min before a solution of *tert*-butyl 3-hydroxypropylcarbamate (1 g, 5.7 mmol) in dry CH_2Cl_2 (5 mL) was added drop wise. After stirring for a further 1 h at -78 ºC, triethylamine (4 mL, 28.5 mmol) was added and the reaction was allowed to warm to room temperature. After stirring for a further 30 min, the reaction was quenched with 10% aqueous HCl (10 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with sat. aq. sodium bicarbonate (20 mL), brine (20 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford the *title compound* **20** (0.91 g, 92%) as a pale yellow oil. δ_H (300 MHz, $CDCl_3$) 1.43 (9H, s, $C(CH_3)_3$), 2.70 (2H, t, *J* = 6.0 Hz, H-2), 3.42 (2H, t, *J* = 6.0 Hz, H-1), 4.92 (1H, br s, NH), 9.81 (1H, s, H-3). The ^1H NMR values were in agreement with literature values.[19,20]

(*E*/*Z*)-*tert*-Butyl 3-(2,5-dioxoimidazolidin-4-ylidene)propylcarbamate 21. To a solution of sodium ethoxide (prepared from sodium (0.13 g, 5.5 mmol) in dry ethanol (13 mL)) was added hydantoin phosphonate **15** (1.3 g, 5.5 mmol) and the reaction placed under an atmosphere of nitrogen. After 5 min a solution of aldehyde **20** (0.8 g, 4.6 mmol) in dry ethanol (2 mL) was added and the reaction stirred for a further 90 min before being quenched with 10% aq. HCl (5 mL). The solvent was removed *in vacuo* and the remaining aqueous mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extracts were washed with sat. aq. sodium bicarbonate (10 mL), brine (10 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo* to afford the *title compound* **21** (0.62 g, 53%) as a pale yellow foam in a 1:1 mixture of *E*:*Z* isomers. δ_H (300 MHz, CD_3OD, *E*/*Z*-

isomer ~ 0.85:1) 1.47 (18H, s, $C(CH_3)_3$, *E* and *Z*), 2.41 (2H, dt, *J* = 9.0, 6.0 Hz, H-2, *Z*), 2.86 (2H, dt, *J* = 9.0, 6.0 Hz, H-2, *E*), 3.17-3.25 (4H, m, H-1, *E* and *Z*), 5.59 (1H, t, *J* = 9.0 Hz, H-3, *E*), 5.75 (1H, t, *J* = 9.0 Hz, H-3, *Z*). δ_C (75 MHz, CD_3OD) 27.8 and 28.6 (C-2), 28.8 ($C(CH_3)_3$), 40.3 and 41.0 (C-1), 80.0 and 80.2 ($C(CH_3)_3$), 111.4 and 116.8 (C-3), 131.7 and 132.9 (*C*=CH), 156.3 and 156.9, 158.5, 166.3 (C=O). ν_{max}(ATR)/cm^{-1} 3349, 1764, 1725, 1683, 1168. *m/z* (ESI^+): 278 (MNa^+, 100%), 222 (31%), 200 (25%), 178 (12%); HRMS (ESI^+) found (MNa^+): 278.1101, $C_{11}H_{17}N_3NaO_4$ requires 278.1111.

3-(2,5-Dioxoimidazolidin-4-ylidene)propan-1-amine trifluoroacetate salt. To a stirred solution of carbamate **21** (0.1 g, 0.39 mmol) in dry CH_2Cl_2 (0.35 mL) at 0 ºC was added TFA (0.28 mL) slowly. The reaction was placed under an atmosphere of nitrogen and allowed to warm to room temperature. After 2 h the solvent was removed *in vacuo* to afford the *title compound* (0.11 g, quantitative) as a dark orange which was used in the next step without further purification.

1-(4-Bromo-1*H*-pyrrol-2-yl)-2,2,2-trichloroethanone 22. To a solution of pyrrole **23** (2.13 g, 10 mmol) in chloroform (10 mL) at 0 ºC, was added bromine (1.71 g, 11 mmol) dropwise. The mixture was allowed to warm to room temperature and stirred for 10 min before being poured onto water and the organic layer extracted with chloroform (3 x 10 mL). The combined organic extracts were washed with sat. aq. sodium bicarbonate (20 mL), water (20 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the crude product which was recrystallized from *n*-hexanes to afford the *title compound* **22** (2.0 g, 69%) as a black solid. m.p. 133-134 ºC. (lit. m.p. 134-136 ºC).[21] δ_H (400 MHz, $CDCl_3$) 7.14-7.16 (1H, m, H-5), 7.35-7.36 (1H, m, H-3), 9.48 (1H, br s, NH). The spectroscopic data was in agreement with literature values.[22,23]

(*E*)- and (*Z*)-Mukanadin B[24] **(6)**. The reaction was carried out following General Procedure A using 3-(2,5-dioxoimidazolidin-4-ylidene)propan-1-amine trifluoroacetate salt (53 mg, 0.20 mmol), pyrrole **22** (58 mg, 0.20 mmol) and triethylamine (54.0 µL, 0.40 mmol) in DMF (2 mL) stirring for 2.5 h to give the crude product which was purified by flash chromatography (gradient elution firstly with 9:1 CH_2Cl_2:CH_3OH and then with 9:1 CH_3OH:NH_3) to afford the *title compounds* **(*Z*)-6** and **(*E*)-6** (0.049 g, 77%) as a 1:2 mixture of *E* to *Z* isomers. Further purification by flash chromatography (9:1 CH_2Cl_2:CH_3OH) using LiChroprep NH_2 to obtain pure samples of each isomer afforded *(Z)-mukanadin B* **(*Z*)-6** (5.4 mg, 8%) as a white solid, m.p. 131-133 ºC. R_f = 0.44 (9:1 CH_2Cl_2:CH_3OH). δ_H (400 MHz, CD_3OD) 2.46 (2H, dt, *J* = 7.6, 7.2 Hz, H-9), 3.43 (2H, t, *J* = 7.2 Hz, H-8), 5.73 (1H, t, *J* = 7.6 Hz, H-10), 6.75 (1H, d, *J* = 1.6 Hz, H-4), 6.91 (1H, d, *J* = 1.6 Hz, H-2). δ_C (100 MHz, CD_3OD) 28.2 (C-9), 39.2 (C-8), 97.5 (C-3), 110.9 (C-10), 113.2 (C-4), 122.9 (C-2), 127.4 (C-5), 133.0 (C-11), 156.9 (C-14), 162.8 (C-6), 166.4 (C-12). ν_{max}(ATR)/cm^{-1} 3237, 2926, 1724, 1687, 1625, 1567, 1389, 1327, 1129, 674, 581. *m/z* (ESI^+): 381 (100%), 353 (16%), 351 ($^{81}BrMNa^+$, 9%), 349 ($^{79}BrMNa^+$, 10%), 301 (12%), 227 (18%), 159 (12%); HRMS (ESI^+) found (MNa^+): 350.9882, $C_{11}H_{11}{}^{81}BrN_4NaO_3$ requires 350.9887. Found (MNa^+): 348.9898, $C_{11}H_{11}{}^{79}BrN_4NaO_3$ requires 348.9907. In a separate fraction *(E)-mukanadin B* **(*E*)-6** (1.7 mg, 3%) was also isolated as a white film. R_f = 0.41 (9:1 CH_2Cl_2:CH_3OH). δ_H (400 MHz, CD_3OD) 2.93 (2H, dt, *J* = 8.0, 6.8 Hz, H-9), 3.44 (2H, t, *J* = 6.8 Hz, H-8), 5.58 (1H, t, *J* = 8.0 Hz, H-10), 6.74 (1H, d, *J* = 1.6 Hz, H-4), 6.90 (1H, d, *J* = 1.6 Hz, H-2). δ_C (100 MHz, CD_3OD) 27.3 (C-9), 40.0 (C-8), 97.4 (C-3), 113.1 (C-4), 116.3 (C-10), 122.7 (C-2), 127.6 (C-5), 131.9 (C-11), 156.3 (C-14), 166.3 (C-6), 170.9 (C-12). ν_{max}(ATR)/cm^{-1} 3421, 3204, 2920, 1720, 1678, 1618, 1564, 1387, 1325, 1133, 769, 655. *m/z* (ESI^+): 351 ($^{81}BrMNa^+$, 100%), 349 ($^{79}BrMNa^+$, 99%); HRMS (ESI^+) found (MNa^+): 350.9910, $C_{11}H_{11}{}^{81}BrN_4NaO_3$ requires 350.9887. Found (MNa^+): 348.9917, $C_{11}H_{11}{}^{79}BrN_4NaO_3$ requires 348.9907.

(*E*)- and (*Z*)-Mukanadin D[10,11] **(7)**. The reaction was carried out following General Procedure A using 3-(2,5-dioxoimidazolidin-4-ylidene)propan-1-amine trifluoroacetate salt (47 mg, 0.30 mmol), pyrrole **18** (112 mg, 0.30 mmol) and triethylamine (84.0 µL, 0.60 mmol) in DMF (2 mL) stirring for 22 h to give the crude product which was purified by flash chromatography (gradient elution CH_2Cl_2 then

9:1 CH_2Cl_2:CH_3OH) to afford the *title compounds* **(*Z*)-7** and **(*E*)-7** (0.057 g, 46%) as a 1:2.5 mixture of *E* to *Z* isomers. δ_H (300 MHz, CD_3OD, *E*/*Z*-isomer ~ 1:2.5) 2.48 (2H, dt, *J* = 7.8, 6.9 Hz, H-9, *Z*), 2.93 (2H, dt, *J* = 8.4, 6.9 Hz, H-9, *E*), 3.45 (2 x 2H, t, *J* = 6.9 Hz, H-8, *E* and *Z*), 5.60 (1H, t, *J* = 8.4 Hz, H-10, *E*), 5.74 (1H, t, *J* = 7.8 Hz, H-10, *Z*), 6.81 (1H, d, *J* = 3.0 Hz, H-4, *E*), 6.83 (1H, d, *J* = 5.4 Hz, H-4, *Z*). δ_C (75 MHz, CD_3OD) 27.3 and 28.2 (C-9), 39.3 and 40.0 (C-8), 100.0 and 100.1 (C-3), 106.0 and 106.2 (C-2), 111.1 and 116.5 (C-10), 114.3 and 114.4 (C-4), 128.7 (C-5), 131.8 and 133.0 (C-11), 156.3 and 156.9 (C-14), 161.7 and 161.9 (C-6), 166.3 (C-12). Further purification by flash chromatography (9:1 CH_2Cl_2:CH_3OH) using LiChroprep NH_2 to obtain pure samples of each isomer afforded only *(Z)-mukanadin D* **(*Z*)-7** (9 mg, 7%) as a pale yellow solid, m.p. decomp. at 228 ºC. R_f = 0.51 (9:1 CH_2Cl_2:CH_3OH). δ_H (400 MHz, CD_3OD) 2.45 (2H, dt, *J* = 7.8, 6.9 Hz, H-9), 3.41 (2H, t, *J* = 6.9 Hz, H-8), 5.71 (1H, t, *J* = 7.8 Hz, H-10), 6.78 (1H, s, H-4). δ_C (100 MHz, CD_3OD) 28.1 (C-9), 39.3 (C-8), 99.9 (C-3), 106.3 (C-2), 110.7 (C-10), 114.3 (C-4), 128.8 (C-5), 133.2 (C-11), 157.3 (C-14), 162.0 (C-6), 166.7 (C-12). ν_{max}(ATR)/cm^{-1} 3134, 2920, 1707, 1674, 1629, 1563, 1389, 1320, 1224, 676, 636, 587. *m/z* (ESI^+): 431 ($^{81,81}Br_2MNa^+$. 50%), 429 ($^{79,81}Br_2MNa^+$, 100%), 427 ($^{79,79}Br_2MNa^+$, 50%), 408 (35%), 233 (35%); HRMS (ESI+) found (MNa^+): 430.8982, $C_{11}H_{10}{}^{81,81}Br_2N_4NaO_3$ requires 430.8971. Found (MNa^+): 428.8997, $C_{11}H_{10}{}^{79,81}Br_2N_4NaO_3$ requires 428.8992. Found (MNa^+): 426.9021, $C_{11}H_{10}{}^{79,79}Br_2N_4NaO_3$ requires 426.9012.

(*E*)- and **(*Z*)-Debromo-dehydroxymukanadin F (24).** The reaction was carried out following General Procedure A using 3-(2,5-dioxoimidazolidin-4-ylidene)propan-1-amine trifluoroacetate salt (53 mg, 0.20 mmol), pyrrole **23** (42 mg, 0.20 mmol) and triethylamine (54.0 µL, 0.40 mmol) in DMF (2 mL) stirring for 2.5 h to give the crude product which was purified by flash chromatography (gradient elution CH_2Cl_2 then 9:1 CH_2Cl_2:CH_3OH) to afford the *title compounds* **(*Z*)-24** and **(*E*)-24** (0.036 g, 53%) as a 1:1.5 mixture of *E* to *Z* isomers. δ_H (300 MHz, CD_3OD, *E*/*Z*-isomer ~ 1:1.5) 2.45-2.53 (2H, m, H-9, *Z*), 2.91-2.98 (2H, m, H-9, *E*), 3.46 (2H, t, *J* = 6.9 Hz, H-8, *Z*), 3.47 (2H, t, *J* = 6.6 Hz, H-8, *E*), 5.61 (1H, t, *J* = 8.4 Hz, H-10, *E*), 5.76 (1H, t, *J* = 8.0 Hz, H-10, *Z*), 6.15-6.18 (1H, m, H-3, *E* and *Z*), 6.75-6.78 (1H, m, H-4, *E* and *Z*), 6.90-6.93 (1H, m, H-2, *E* and *Z*). δ_C (75 MHz, CD_3OD) 27.5 and 28.3 (C-9), 39.2 and 39.9 (C-8), 110.2 and 110.2 (C-3), 111.1 and 116.6 (C-10), 111.6 and 111.7 (C-4), 122.9 and 123.0 (C-2), 126.7 and 129.3 (C-5), 131.8 and 133.0 (C-11), 156.9 (C-14), 164.0 (C-6), 166.4 (C-12). Further purification by flash chromatography (9:1 CH_2Cl_2:CH_3OH) using LiChroprep NH_2 to obtain pure samples of each isomer afforded only **(Z)-24** (14 mg, 29%) as a white solid, m.p. 240-242 ºC. R_f = 0.36 (9:1 CH_2Cl_2:CH_3OH). δ_H (400 MHz, CD_3OD) 2.47 (2H, dt, *J* = 8.0, 6.9 Hz, H-9), 3.43 (2H, t, *J* = 6.9 Hz, H-8), 5.75 (1H, t, *J* = 8.0 Hz, H-10), 6.15-6.16 (1H, m, H-3), 6.75-6.77 (1H, m, H-4), 6.90-6.91 (1H, m, H-2). δ_C (100 MHz, CD_3OD) 28.3 (C-9), 39.2 (C-8), 110.2 (C-3), 111.0 (C-10), 111.6 (C-4), 122.9 (C-2), 126.7 (C-5), 133.0 (C-11), 157.0 (C-14), 164.0 (C-6), 166.4 (C-12). ν_{max}(ATR)/cm^{-1} 3288, 2978, 1723, 1688, 1620, 1566, 1408, 1331, 1125, 748. *m/z* (ESI^+): 248 (M^+, 6%), 123 (23%), 94 (100%), 86 (95%); HRMS (ESI^+) found (M^+): 248.09021, $C_{11}H_{12}N_4O_3$ requires 248.09094. The spectroscopic data was in agreement with literature values.[25]

ASSOCIATED CONTENT

Supporting Information. NMR spectra for all novel compounds.

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Notes

The authors declare no competing financial interest.

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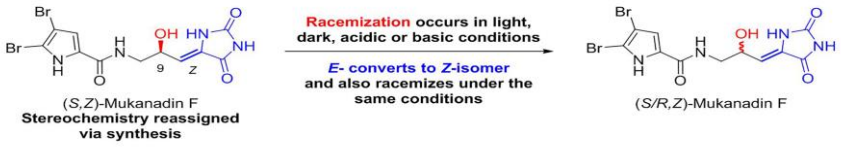

Spectroscopic analysis of synthetic, enantiopure, bromopyrrole alkaloid mukanadin F determined that the reported absolute stereochemistry of the natural product was misassigned. Furthermore, mukanadin F and similar compounds undergo racemization and alkene-isomerization under benign conditions such as sunlight and solvent.

Natural Products

*Michelle van Rensburg, Brent R. Copp and David Barker**

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Synthesis and absolute stereochemical reassignment of mukanadin F: A study of isomerization of bromopyrrole alkaloids with implications on marine natural product isolation.