The structure and partial synthesis of imbricatine, a benzyltetrahydroisoquinoline alkaloid from the starfish *Dermasterias imbricata*

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This paper is dedicated to Professor Ross Stewart on the occasion of his 65th birthday

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The structure of imbricatine (**1**), a cytotoxic metabolite of the starfish *Dermasterias imbricata*, has been determined by spectroscopic analysis and chemical degradation. Synthesis of model compounds **7** and **18** provided evidence for the absolute configuration and constitution of the benzyltetrahydroisoquinoline substructure of imbricatine.

Key words: imbricatine, asteroid, alkaloid.

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Faisant appel à des analyses spectroscopiques et à une dégradation chimique, on a déterminé la structure de l'imbricatine (**1**), un métabolite cytotoxique de l'étoile de mer *Dermasterias imbricata*. La synthèse des composés modèles **7** et **18** fournit une preuve de la configuration absolue et de la constitution de la sous-structure benzyltétrahydroisoquinoléine de l'imbricatine.

Mots clés : imbricatine, astéroïde, alcaloïde.

[Traduit par la revue]

Many marine invertebrates exhibit escape responses to chemicals released by their predators. One of the best known examples is the detachment and swimming behavior of the sea anemones *Stomphia coccinea* and *S. didemon* in reaction to contact with certain species of marine asteroids (1, 2). Sea anemones are sessile animals that normally respond to tactile stimulation by contracting and covering their oral disc with a portion of their body column. However, *S. coccinea* and *S. didemon* respond to contact with the starfish *Dermasterias imbricata* in a "striking and extraordinary manner: the anemones release their basal discs from the substratum, and then propel themselves through the water by means of a series of whiplike motions" (2). A number of years ago it was shown that a single metabolite of *D. imbricata* was responsible for eliciting the "swimming response" in *S. coccinea* and *S. didemon* (1–4). Subsequently, we reported the constitution and partial stereochemistry of imbricatine (**1**), the *D. imbricata* metabolite responsible for eliciting the unusual anemone "swimming" behavior (5–7). We now wish to report additional details of the structure elucidation of imbricatine (**1**), including the determination of its absolute configuration and confirmation of the nature of the benzyltetrahydroisoquinoline substructure by synthesis.

Specimens of *Dermasterias imbricata* were collected by hand using SCUBA (−5 to −20 m) in Howe Sound and Barkley Sound, British Columbia. Freshly collected animals were immediately immersed in methanol and allowed to soak at room temperature for 3 days. The methanol was decanted and filtered through Celite to give a crude extract that was capable of eliciting the swimming response in specimens of *S. coccinea* maintained for bioassay purposes in a running seawater aquarium (4, 6, 7). Concentration of the crude methanol extracts *in vacuo* gave an aqueous slurry that was diluted with three equivalent volumes of water and then passed over XAD-4 resin. Elution of the retained substances from the XAD-4 resin with hot methanol gave an active eluate that was further fractionated on a Biogel-P2 column (eluent: H_2O/HOAc 99:1). All of the fractions from the Biogel-P2 chromatography that showed a uv λ_{max} at both 283 and 292 nm and elicited anemone swimming were pooled and further purified by Sephadex LH-20 chromatography (eluent: MeOH/H_2O 8:2) to give pure imbricatine (**1**) (6–7 mg/starfish) as a water soluble white solid. As little as 50 nanograms (50 μL of a 2×10^{-6} M solution) of pure imbricatine (**1**) was capable of eliciting the swimming response in 100% of the bioassay specimens of *S. coccinea* (6). No other swimming elicitors were found in the *D. imbricata* extracts. Imbricatine also displays significant activity in the L1210 (ED_{50} < 1 μg/mL) and P388 (T/C 139 at 0.5 mg/kg) cytotoxicity assays.

The molecular formula of imbricatine, $C_{24}H_{26}N_4O_7S$, was determined from the mass spectra of the parent compound (FABMS (M^+ + H), *m/z*: 515 Da) and the dimethylpentacetyl derivative **2** (FABMS, *m/z*: M^+ + H) 753; HREIMS (M^+ − OAcbenzyl), *m/z*: 603.1756, $C_{27}H_{31}N_4O_{10}S$ (ΔM −0.5 mmu)) prepared from **1** by esterification (MeOH/HCl) and acetylation (Ac_2O/pyridine).

A *N*-methyl-5-thiohistidine fragment could be identified in **1** by comparing ^1H and ^{13}C nmr resonances in the spectrum of

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TABLE 1. ^{1}H nuclear magnetic resonance data. Chemical shifts are in ppm from internal TMS

Proton	**1**[a]	**5**[b]	**6a**[a]	**9**[c]	**18**[c]
1	4.67,dd(8.4,4.5)		4.57,dd(8,4)	4.73,dd(8,7)	5.06,dd(8,7)
3	3.76,dd(12.5,5)		3.12,dd(12,4)	4.49,t(10)	4.48,dd(9,11)
4_{ax}	2.71,dd(12.5,16)		2.71,dd(12,16)	3.21,m	3.24,m
4_{eq}	4.03,dd(16,5)		2.90,dd(16,4)	3.21,m	3.24,m
5	—		6.08,d(2)	7.10,s	6.94,d(2)
7	6.42,s		6.22,d(2)		6.88,d(2)
8				6.67,s	
12	3.22,dd(13.9,4.5)		3.20,dd(14,4)	3.28,dd(14,8)	3.32,dd(14,8)
12	2.85,dd(13.9,8.4)		2.80,dd(14,8)	2.88,dd(14,7)	2.88,dd(14,7)
14	7.04,d(8)		7.01,d(8)	7.15,d(8)	7.04,d(8)
15	6.71,d(8)		6.70,d(8)	6.84,d(8)	7.29,d(8)
2′	7.73,s:8.73,s[b]	8.95,s			
6′	3.30,dd(15,9): 3.34,d(8:2H)[b]	3.44,d(7.5:2H)			
6′	3.02,dd(15,5)	—			
7′	3.60,dd(5,9): 4.31,t(8)[b]	4.29,t(7.5)			
NMe	3.66,s:3.87,s[b]	3.94,s			
CO_2Me				3.84,s	3.85,s
OAc				2.28,s	2.29,s
OAc				2.25,s	2.28,s
OAc					2.20,s
NAc				1.87,s	1.80,s

[a]Recorded in Me_2SO-d_6.
[b]Recorded in D_2O.
[c]Recorded in $CDCl_3$.

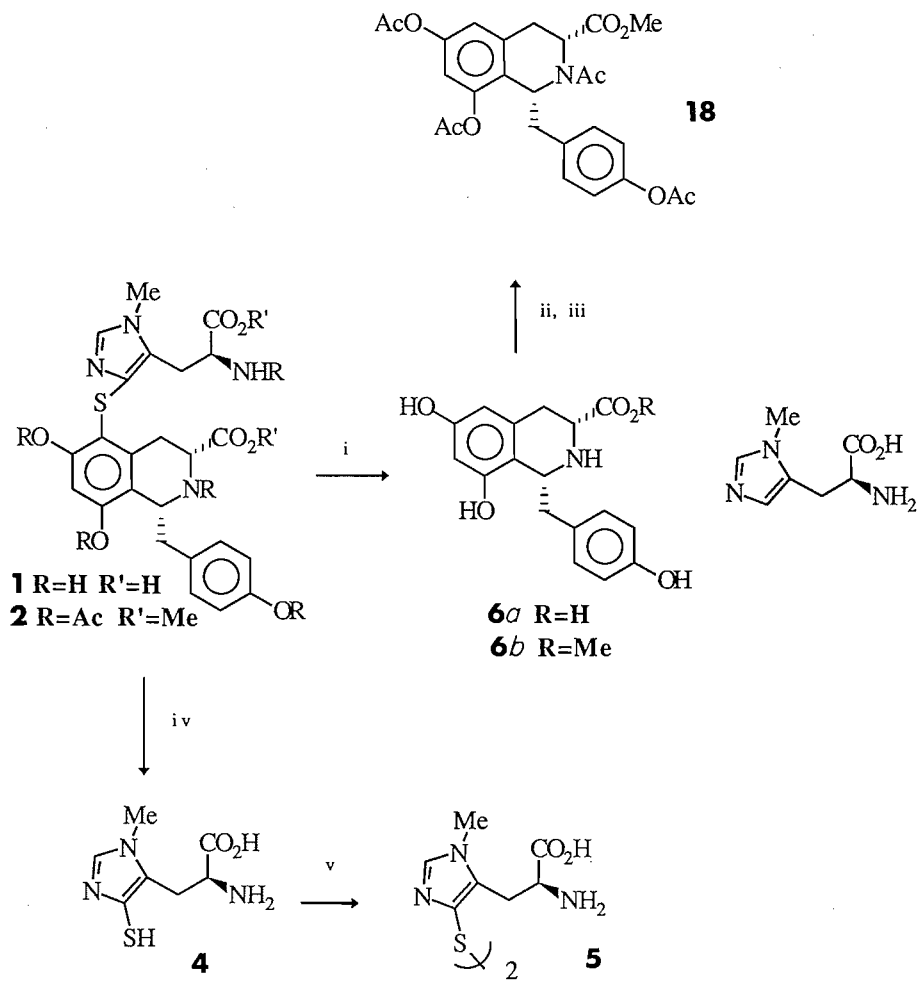
imbricatine to the values reported for the symmetrical disulfide **3** first isolated from echinoderm eggs (8, 9) (Tables 1 and 2). Reduction of imbricatine (**1**) with Raney nickel (Scheme 1) liberated *N*-methyl histidine, which was shown by tlc analysis to be identical to 3-methylhistidine and different from 1-methylhistidine. This result indicated that the position of *N*-methylation in the histidine residue of imbricatine (3-methyl) was different from that reported for the disulfide from echinoderm eggs (1-methyl). It was subsequently shown by an unambiguous synthesis of disulfide **5** that the structure originally assigned to the disulfide from echinoderm eggs (i.e., **3**) was incorrect and needed to be revised as **5** (9). Treatment of imbricatine with red phosphorus in refluxing HI (Scheme 1) liberated the histidine thiol **4**, which was oxidized by iodine in aqueous HCl to the disulfide **5** (10). The spectral data collected on the disulfide **5** obtained from imbricatine were in complete agreement with the literature values (8, 9) and its specific rotation ($[\alpha]_D$ +67° (lit. (9) $[\alpha]_D$ +77°) indicated that the histidine residues had the L configuration.

The second product obtained from the Raney nickel reduction of imbricatine was the benzyltetrahydroisoquinoline fragment **6a**. Compound **6a** gave a parent ion ($M^+ + H$) in the CIMS at *m/z* 316 Da and fragment ions at 270 ($M^+ - CO_2H$), 242 ($M^+ - NH{=}CHCO_2H$ via a retro Diels–Alder), 208 ($M^+ -$ HO-benzyl) and 164 ($M^+ -$ (HO-benzyl + CO_2)) Da typical of benzyltetrahydroisoquinolines with a carboxylic acid substituent at C3 (11). The EIHRMS spectrum of **6a** failed to show a parent ion; however, an intense fragment ion at *m/z* 208.1611 Da ($C_{10}H_{10}NO_4$, ΔM +0.1 mmu), which was assigned to a $M^+ -$ (HO-benzyl) fragment ion, was consistent with a molecular formula of $C_{17}H_{17}NO_5$ for the intact molecule. Only 15 resonances were observed in the ^{13}C nmr spectrum of **6a** (Table 2), demonstrating that the molecule contained some element of symmetry. The ^{1}H nmr resonances at δ 6.70 (d, J = 8 Hz, 2H) and 7.01 (d, J = 8 Hz, 2H) (Table 1) and ^{13}C nmr resonances at δ 126.9 (C), 130.2 (2 × CH), 115.0 (2 × CH), and 156.0 (C) (Table 2) were assigned to the phenyl ring in the benzyl residue of **6a**. The ^{1}H and ^{13}C chemical shifts of these resonances were in close agreement with those that we recorded for tyrosine, suggesting that the phenyl ring in **6a** contained only a *para*

TABLE 2. ^{13}C nuclear magnetic resonance assignments

Carbon	**1**[a]	**6**[a]	**5**[b]
1	52.9	53.0	
3	54.9	55.0	
4	27.2	30.0	
5	110.0	106	
6	159.7	157.0	
7	104.0	100.9	
8	155.8	154.7	
9	112.7	110.8	
10	137.7	136.0	
11	169.7	169.2	
12	37.3	37.8	
13	126.5	126.9	
14	130.5	130.2	
15	115.3	115.0	
16	156.4	156.0	
2	137.8		141.1
4′	126.8		130.0
5′	131.1		134.8
6′	25.1		25.5
7′	52.3		54.5
8′	170.5		173.3
9′	32.3		34.0

[a]Recorded in Me_2SO-d_6.
[b]Recorded in D_2O.



Reagents and reaction conditions: (i) Raney nickel, reflux, 1 h; (ii) HCl, MeOH, reflux, 2.5 h; (iii) Ac_2O, pyridine, r.t., 24 h; (iv) red phosphorus, aqueous HI (57%), N_2, reflux, 48 h; (v) 0.1 N HCl, I_2, r.t., 26 h.

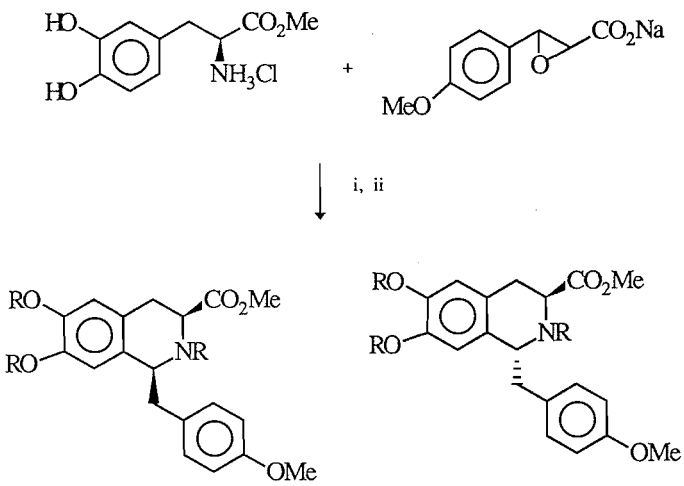
SCHEME 1.

hydroxyl substituent, thereby accounting for the symmetry required by the ^{13}C nmr data. Additional deshielded ^1H resonances at δ 6.08 (d, J = 2 Hz) and 6.22 (d, J = 2 Hz) were assigned to protons on the aromatic ring of the tetrahydroisoquinoline moiety. The magnitude of the scalar coupling between these protons (2 Hz) showed that they were *meta* to each other and their chemical shifts suggested that the other substituents on the ring were a pair of *meta* hydroxyls. The remaining resonances in the ^1H nmr spectrum of **6***a* were assigned to the two CH-CH_2 fragments (δ (4.57, dd, J = 8, 4 Hz, H1), 3.12 (dd, J = 14, 4 Hz, H12), 2.80 (dd, J = 14, 8 Hz, H12′), 3.20 (dd, J = 12, 4 Hz, H3), 2.90 (dd, J = 16, 4 Hz, H4), 2.71 (dd, J = 12, 16 Hz, H4′)) in the reduced ring and the benzyl substituent of the isoquinoline nucleus.

The ^1H nmr spectrum of imbricatine (**1**), in contrast to that of the Raney nickel reduction product **6***a*, contained only a single resonance (δ 6.42, s) that could be assigned to a proton on the aromatic ring of the tetrahydroisoquinoline portion of the molecule (Table 1). Therefore, the other nonphenolic position of the ring had to be occupied by a thioether linkage to the methylhistidine residue. Both the $H4_e$ and $H3_a$ resonances were strongly deshielded in imbricatine (**1**: δ 4.03, $H4_e$: 3.76, $H3_a$) relative to the resonances assigned to the corresponding protons in the Raney nickel reduction product (**6***a*: δ 2.90, $H4_e$: 3.12, $H3_a$). The observed difference in chemical shifts for these two protons in compounds **1** and **6***a* could best be explained if the thioether linkage in imbricatine was attached to C5 of the isoquinoline nucleus, which in turn placed the two phenolic hydroxyls at C6 and C8. A pair of SINEPT experiments optimized for polarization transfer through a J $^{13}C/^1H$ of 7 Hz supported this assignment. Irradiation of H1 (δ 4.67) in imbricatine (**1**) gave polarization transfer into C8 (δ 155.8) and C10 (137.7), while irradiation of H7 (δ 6.42) gave polarization transfer into C5 (δ 110.0), C6 (159.7), C8 (155.8), and C9 (112.7).

Difference nOe experiments involving irradiation of H1 and H3 in imbricatine (**1**) failed to provide definitive proof for the relative stereochemistries at C1 and C3. Therefore, we synthesized the model compounds **9** and **10**, starting from optically pure L-dopa, in order to obtain reference data for the determination of both the relative and absolute configurations at C1 and C3 in the Raney nickel reduction product **6** and ultimately in imbricatine (**1**) itself.

Scheme 2 outlines the synthetic route used to prepare model compounds **9** and **10**. Earlier workers had found that the *cis* isomer was the major product (3.2:1 *cis*/*trans*) formed in the Pictet–Spengler coupling reaction when the phenylglycidate reactant contained methoxy substituents at both C3 and C4 on the phenyl ring (12). We found an identical 3.2:1 ratio of the *cis* to *trans* products with the 4-methoxyphenylglycidate shown. The *cis* configuration of the C1 and C3 substituents in **9** was



7 R=H
9 R=Ac

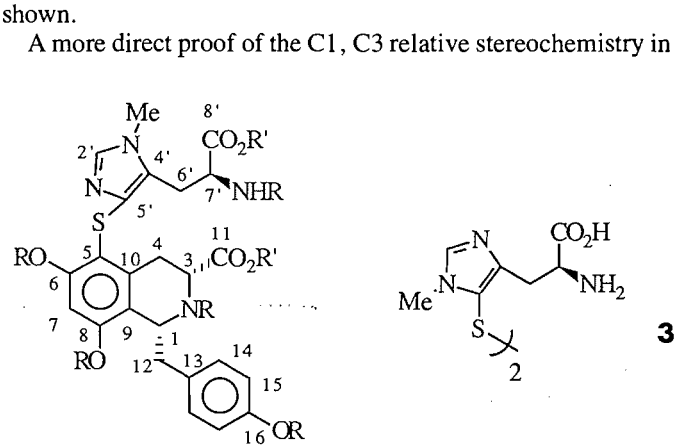
8 R=H
10 R=Ac

Reagents and reaction conditions: (i) H_2O, HOAc, 35°C, 36 h; (ii) Ac_2O, pyridine, r.t., 24 h

SCHEME 2.

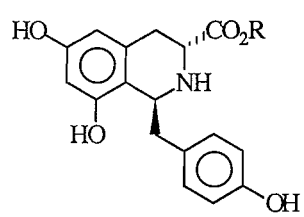
confirmed by a difference nOe experiment involving irradiation of H1 in compound **7** that induced nOe's in H3 and one of the H12 protons. Comparison of the ^1H nmr spectra of the model compounds **9** ($(CDCl_3)$ δ: 1.87 (s, CH_3CON), 2.88 (dd, J = 14, 8 Hz, H12), 3.21 (m, $H4_a$ and $H4_e$), 3.28 (dd, J = 14, 6 Hz, H12′), 4.49 (t, J = 10 Hz, $H3_a$), 4.73 (dd, J = 8, 6 Hz, $H1_a$)) and **10** (see Experimental) to that of derivative **18** ($(CDCl_3)$ δ: 1.80 (s, CH_3CON), 2.88 (dd, J = 14, 8 Hz, H12), 3.24 (m, $H4_a$ and $H4_e$), 3.30 (dd, J = 14, 6 Hz, H12′), 4.48 (t, J = 10 Hz, $H3_a$), 5.04 (dd, J = 8, 6 Hz, $H1_a$)) confirmed the benzyltetrahydroisoquinoline nature of the reduction product **6***a* and demonstrated that the C1 and C3 substituents in **6***a* were *cis* as shown.

A more direct proof of the C1, C3 relative stereochemistry in **6***a* was obtained from a difference nOe experiment carried out on its methyl ester **6***b*. Irradiation of the resonance assigned to H1 (δ 4.45: acetone-d_6 plus four drops of benzene-d_6) in **6***b* induced nOe's in the resonances assigned to H3 (δ 3.42) and H12 (δ 3.48) consistent with the *cis* relative stereochemistry shown. The *trans* reduction product **20***a* was produced as a minor side product in the Raney nickel reduction reaction (Scheme 1) if the catalyst was added in large excess and the reaction was allowed to reflux for at least 3 h. The circular dichroism (CD) spectrum of **20***b* (Fig. 1, see discussion below) indicated that it was epimeric with **6***b* at C1.



1 R=H R′=H
2 R=Ac R′=Me

3



20*a* R=H
20*b* R=Me

The CD spectra of model compounds **7** and **8** prepared from L-dopa, as indicated in Scheme 2, and the CD spectra of the methyl esters **6***b* and **20***b* of the *cis* and *trans* Raney nickel reduction products **6***a* and **20***a* are shown in Fig. 1. The Cotton effects observed for the *cis* (1*S*,3*S*) model compound **7** and the *cis* Raney nickel reduction product methyl ester **6***b* in the long wavelength region (290–298 nm), the middle range wavelength region (234–238 nm), and in the short wavelength region (200–220 nm) are each of opposite signs for the two compounds. Since the model compound **7** has the 1*S*,3*S* absolute configuration, the CD results demonstrate that the *cis* Raney nickel reduction product **6***a* and its methyl ester **6***b* have the 1*R*,3*R* absolute configuration. Similarly, the CD curve of the *trans* Raney nickel reduction product methyl ester **20***b* corresponds to the mirror image of the spectrum for the *trans* model compound **8** (Fig. 1) and, therefore, **20***b* must have the 1*S*,3*R* absolute configuration. Imbricatine has the same absolute configurations at C1 and C3 as the *cis* Raney nickel reduction product **6***a* and its methyl ester **6***b*; therefore, the absolute configuration of imbricatine (**1**) is 1*R*,3*R*,7′*S* as shown.

Imbricatine (**1**) is apparently the first example of a benzylisoquinoline alkaloid to be isolated from a nonplant source and it appears to be the first member of this class of alkaloids to have the C6/C8 hydroxylation pattern and a thiohistidine substituent. The biogenetic origin of the hydroxyl substitutents at C6/C8 is of considerable interest since all the plant alkaloids in this family are derived from L-dopa, which gives rise to C6/C7 hydroxylation (13). Then D amino acid configuration at C3 and the C6/C8 hydroxylation pattern indicate that the isoquinoline fragment of imbricatine (**1**) is probably not derived from L-dopa but rather from some other precursor such as D-3-(3,5-dihydroxyphenyl)-alanine, an amino acid that has not been previously described. We have prepared 3-(3,5-dihydroxyphenyl)alanine methyl ester **13** as the racemate and used it as a starting point in a biogenetic-type synthesis of **18**, a derivative of the benzyltetrahydroisoquinoline alkaloid fragment of imbricatine (**1**). Compound **18** was independently prepared from the Raney nickel reduction product **6***a* (Scheme 1), providing synthetic verification of the constitution of the benzyltetrahydroisoquinoline fragment of imbricatine (**1**). Scheme 3 outlines the synthesis of both **13** and **18**.

The synthesis of **13** started with methyl 3,5-dihydroxybenzoate, a readily available precursor that contains the required arrangement of alkyl and hydroxyl substituents on the benzene ring. Protection of the phenols as the benzyl ether derivatives, followed by LAH reduction of the methyl ester and tosylation of the resulting benzyl alcohol, gave the alkylating agent **11**. Alkylation of diethyl acetamidomalonate with the tosylate **11** following literature procedures gave **12** in excellent yield (14). Hydrolysis and decarboxylation of **12** gave the protected amino acid, which was routinely converted into the amino acid methyl ester **13**. The Pictet–Spengler reaction of methyl

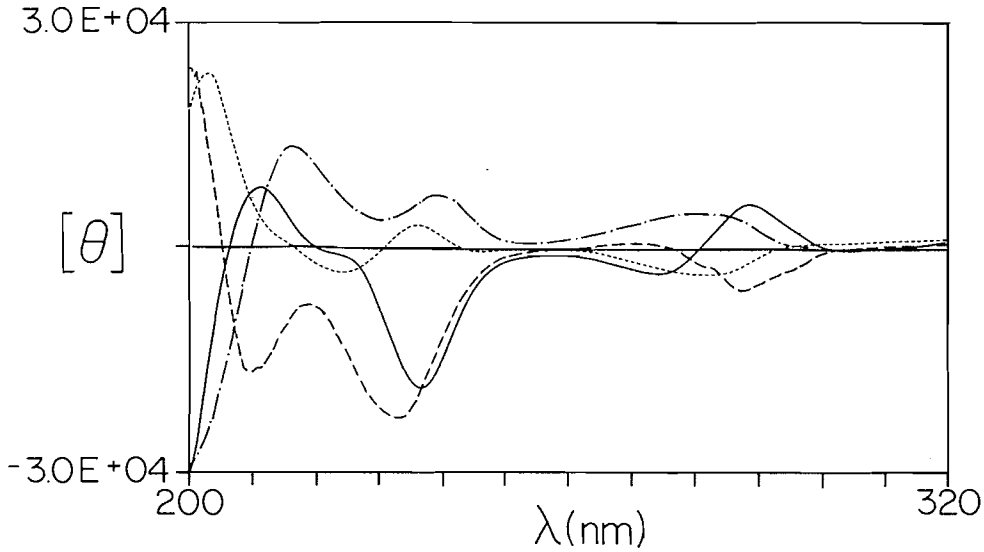
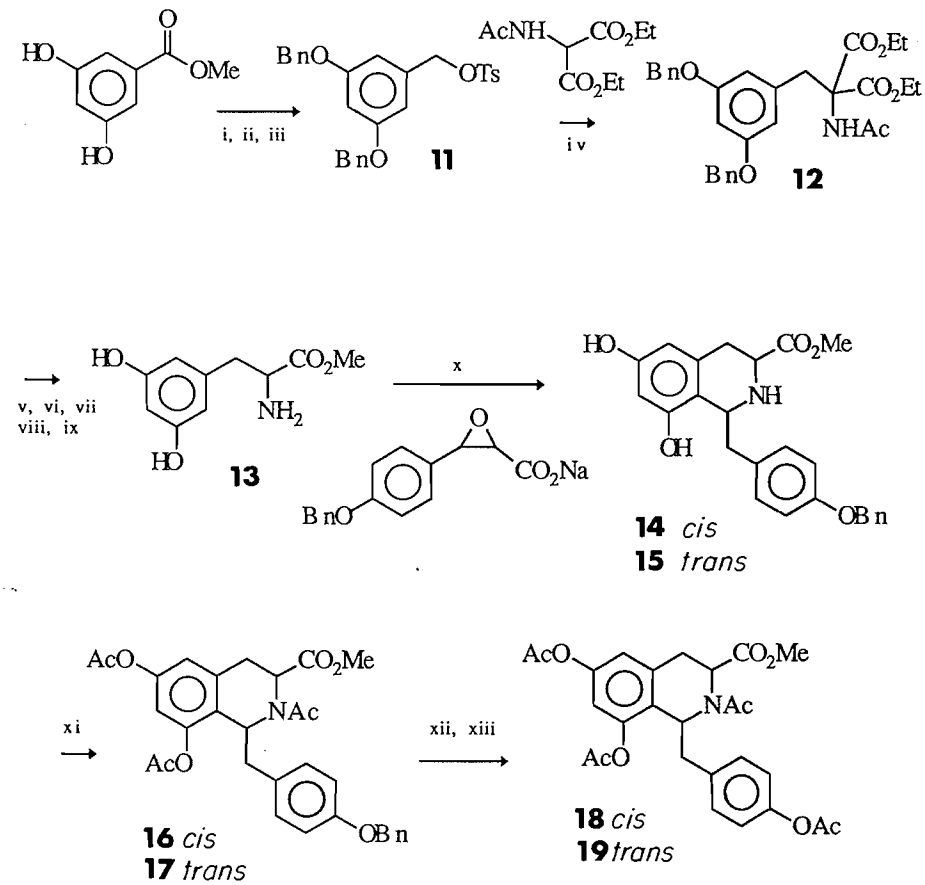


FIG. 1. CD spectra recorded in 0.1 N HCl/MeOH. (———) *cis* model compound **7**(0.1 g/L); (– – – –) *trans* model compound **8**(0.2 g/L); (- - - - -) methyl ester **6*b*** of the *cis* Raney nickel reduction product (0.15 g/L); (— · —) methyl ester **20*b*** of the *trans* Raney nickel reduction product (0.15 g/L).



Reagents and reaction conditions: (i) K_2CO_3, acetone, benzylbromide, reflux 1 h; (ii) LAH, ether, reflux, 2.5 h; (iii) NaH, benzene, tosylchloride, r.t.; (iv) NaH, benzene, DMF, r.t., 6 h; (v) NaOH, dioxane, reflux, 3 h; (vi) HOAc, reflux, 1 h; (vii) hydrazine, 100°C, 24 h; (viii) MeOH, HCl, reflux, 1 h; (ix) H_2, Pt/C, MeOH, r.t., 10 h; (x) MeOH, HOAc, 35°C, 17 h; (xi) Ac_2O, pyridine, r.t., 24 h; (xii) H_2, Pt/C, MeOH, r.t., 3 h; (xiii) Ac_2O, pyridine, r.t., 24 h.

SCHEME 3

ester **13** with sodium 3-(4-benzyloxyphenyl)glycidate gave an inseparable mixture of the *cis* and *trans* benzyltetrahydroisoquinoline products **14** and **15** (12). Acetylation of the mixture of **14** and **15** gave the triacetoxy derivatives **16** and **17**, which could be separated by preparative thin-layer chromatography. The relative stereochemistries at C1 and C3 in **16** and **17** were established by comparison of their H1 and H3 coupling constants to those observed for the model compounds **9** and **10** where the relative stereochemistries had been rigorously established. The *trans* isomer was the major product (**17**:*trans*/**16**:*cis* 6.5:1) formed in the Pictet–Spengler reaction of methyl ester **13**, presumably due to an unfavorable steric interaction between the benzyl substituent at C1 and the C8 hydroxyl during the formation of the *cis* isomer. Removal of the benzyl protecting group in **16** by catalytic hydrogenation and acetylation of the resulting phenolic product gave the *cis* benzyltetrahydroisoquinoline derivative **18**, which was identical by tlc, ^{1}H nmr, and mass spectral comparison to compound **18** prepared by methylation (HCl/MeOH) and acetylation (Ac_2O/pyridine) of the Raney nickel reduction product **6***a*. The *trans* isomer **19** prepared from **17** by the same sequence of reactions had tlc and ^{1}H nmr characteristics different from compound **18** prepared from **6***a*.

It is well documented that many marine invertebrates utilize chemical cues to recognize their predators; however, relatively little information is available concerning the chemical structures of the messengers (16). Several studies examining the basis of escape and avoidance of marine invertebrates to seastars have found that saponins are the active substances (16*a*, 16*c*, 17). These results, in combination with the widespread occurrence of saponins in seastars (18), have fostered the common perception that most seastar initiated behavioral responses are elicited by saponins. Our results have shown that the metabolite of the seastar *D. imbricata* responsible for eliciting swimming in *Stomphia* sp. is not a saponin, but rather the very interesting new alkaloid imbricatine (**1**). This discovery suggests that a blanket assumption that other seastar initiated avoidance responses are caused by saponins is probably unjustified. Careful quantitative bioassay guided fractionation of seastar extracts is required to fully characterize the chemical mediator(s) of the responses.

Experimental

Imbricatine (1)

Imbricatine (**1**) was extracted and purified as described in the text. White amorphous solid; ir (KBr disc): 3700–2500 (b), 1630, 1595, 1515, 1447, 1397, 1244, 1175, 1080, 840 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; FABMS, *m/z* (M^+ + H): 515, $C_{24}H_{26}N_4O_7S$ + H.

Methylation and acetylation of imbricatine to give 2

Imbricatine (**1**) (9 mg) was dissolved in methanol saturated with gaseous HCl and the solution was refluxed for 2.5 h. The solvent was evaporated to dryness *in vacuo* and acetic anhydride (2 mL) and pyridine (2 mL) were added to the residue. After stirring 24 h at room temperature the acetylation reagents were removed *in vacuo* and the residue was purified by reverse phase hplc (CH_3CN/H_2O 3:7) to give derivative **2** (9 mg). **2**: ^{1}H nmr (400 MHz, $CDCl_3$) δ: 7.34,(s,1H), 7.29(d, *J* = 8 Hz,2H), 7.02(d, *J* = 8 Hz,2H), 6.89(bs,1H), 6.15(bd, *J* = 8 Hz,1H), 5.06(bt, *J* = 6 Hz,1H), 4.73(bdd, *J* = 6, 12 Hz,1H), 3.87(s), 3.75(s,1H), 3.61(s,3H), 3.36(dd, *J* = 6, 14 Hz,1H), 3.17(m,1H), 3.10(bd, *J* = 16 Hz,1H), 2.89 (dd, *J* = 6, 14 Hz,1H), 2.41(s,3H), 2.28(s,3H), 2.18(s,3H), 1.90(s,3H), 1.74(s,3H) ppm; FABMS, *m/z*: 753 ($C_{36}H_{40}N_4O_{12}S$ + H); EIHRMS, *m/z*: 603.1756 (M^+ − OAcbenzyl), $C_{27}H_{31}N_4O_{10}S$ (Δ*M* −0.5 mmu).

Raney nickel reduction of imbricatine (1)

Imbricatine (**1**) (16 mg) was refluxed with 0.5 mL of Ra-Ni suspension for 1 h under N_2. The reaction mixture was vacuum filtered and the Ra-Ni residue was washed with hot MeOH. The combined filtrate, after removal of MeOH under vacuum, was chromatographed on Sephadex LH20 using 9:1 MeOH/H_2O as the eluent to give crude compound **6***a*, which was further purified on reverse phase HPLC (20:80 MeOH/H_2O) to obtain pure **6***a* (8 mg). **6***a*: ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; CIMS, *m/z*: 316 (M^+ + H), 272, 242, 270, 208, 198, 164, 162, 152, 137, 124, 121, 109, 108, 107; EIHRMS, *m/z*: 208.1611 (M^+ − HObenzyl), $C_{10}H_{10}NO_4$ (ΔM +0.1 mmu). When imbricatine was refluxed with a large excess of Ra-Ni suspension for longer than 3 h, the reaction mixture turned a dark red and a mixture of the *cis* reduction product **6***a* and its C1 epimer **20***a* was formed. Methylation of the mixture as described below for **6***a* gave a mixture of **6***b* and **20***b* that was separated by preparative reverse phase tlc (6:4 MeOH/H_2O).

*Methylation of compound 6*a

The Raney nickel reduction product **6***a* was refluxed with HCl/MeOH for 1 h under nitrogen. After removal of the solvent, the reaction mixture was chromatographed on normal phase tlc(4:1 EtOAc/$CHCl_3$) to afford pure **6***b* as a yellow solid; ^{1}H nmr (400 MHz, acetone-d_6) δ: 7.02 (d, *J* = 8.6 Hz, H15, H17), 6.71 (d, *J* = 8.6 Hz, H14, H18), 6.33 (d, *J* = 1.9 Hz, H5), 6.12 (d, *J* = 1.9 Hz, H7), 4.41 (dd, *J* = 7.8, 3.0 Hz, H1), 3.68 (s, 3H, $COOCH_3$), 3.43 (dd, *J* = 13.5, 3.0 Hz, H12a), 3.42 (dd, *J* = 11.0, 3.0 Hz, H3), 2.71 (dd, *J* = 13.4, 7.8 Hz, H12b), 2.69 (dd, *J* = 15.2, 3.0 Hz, $H4_{eq}$), 2.47 (dd, *J* = 15.2, 11.0 Hz, $H4_{ax}$) ppm: EIMS, *m/z* (rel. int.): 236 (50, M^+ − HOphenyl), 222 (49, M^+ − HObenzyl), 191 (8), 176 (30), 162 (53).

*Compound 20*b: obtained as a pale yellow solid; ^{1}H nmr (400 MHz, acetone-d_6) δ: 7.13 (d, *J* = 8.5 Hz), 6.80(d, *J* = 8.5 Hz), 6.31(d, *J* = 1.8 Hz), 6.17(d, *J* = 1.8 Hz), 4.22(dd, *J* = 10.0, 2.5 Hz), 4.01(dd, *J* = 10.5, 4.5 Hz), 3.70(s, 3H), 3.14(dd, *J* = 13.5, 2.5 Hz), 2.88(dd, *J* = 15.3, 4.5 Hz), 2.73(dd, *J* = 15.3, 10.5 Hz), 2.68(dd, *J* = 13.5, 10.0 Hz) ppm; EIMS, *m/z*(rel. int.): 270(5, M^+ − CO_2Me), 222(100, M^+ − HObenzyl); CIMS, *m/z*: 330(100).

Reductive hydrolysis of imbricatine (1)

Red phosphorus (150 mg) was added to a solution of imbricatine (**1**) (100 mg) in 57% aqueous HI and the reaction mixture was refluxed under N_2 for 48 h. At the end of this period, the reaction mixture was filtered to remove the solid phosphorus and the filtrate was evaporated under vacuum. The residue was taken up with 5 mL aqueous HCl (pH 2) and chromatographed on Sephadex LH20 using 1:1 MeOH/H_2O as an eluent to give the histidine thiol **4** (34 mg).

Oxidation of the thiol 4 to the disulfide 5

To a solution of histidine thiol **4** (17 mg) in 2 mL 0.1 N aqueous HCl was added a few crystals of I_2. The reaction mixture was stirred at room temperature for 26 h, diluted with 4 mL water, extracted with $CHCl_3$ to remove I_2, and then chromatographed on Sephadex LH20 using 1:1 MeOH/H_2O as an eluent. Disulfide **5** (15 mg) was obtained. **5**: ^{1}H nmr(300 MHz, D_2O) δ: 8.78(s, 1H), 4.20(t, *J* = 8 Hz, 1H), 3.85(s, 3H), 3.30(dd, *J* = 15, 8 Hz, 1H), 3.35(dd, *J* = 15, 8 Hz, 1H) ppm.

Measurement of the optical rotation of disulfide 5

Disulfide **5** (10 mg) was dissolved in 1.0 mL 0.1 N aqueous HCl and the optical rotation was measured using a 1-dm cell. The specific rotation $[\alpha]_D^{20}$ was found to be +67°.

Synthesis of the model compounds 7 and 8

Methyl-L-dopa (580 mg) in 10 mL MeOH was added to a solution of sodium 3-(4-methoxyphenyl)glycidate in 15 mL of water. The mixture was brought to pH 4 with AcOH and stirred at 35°C for 36 h. At the end of the reaction period, the mixture was concentrated under vacuum to yield an aqueous suspension which was partitioned between EtOAc (15 mL) and 10% HCl (15 mL). The aqueous layer was neutralized with K_2CO_3 and extracted with EtOAc (15 mL × 3). The EtOAc solution was washed with water, dried over Na_2SO_4, and concentrated under

vacuum to afford a brown residue that was chromatographed on normal phase hplc (3:2 EtOAc/$CHCl_3$) to give **7** (145 mg) and **8** (45 mg).

Compound 7: obtained as a white solid; ^{1}H nmr (270 MHz, DMSO-d_6) δ: 7.17(d, J = 8 Hz, 2H), 6.84(d, J = 8 Hz, 2H), 6.70(s, 1H), 6.43(s, 1H), 4.00(J = 8, 4 Hz, 1H), 3.70(s, 3H), 3.58(s, 3H), 3.48(dd, J = 11, 5 Hz, 1H), 3.21(dd, J = 14, 4Hz, 1H), 2.71(dd, J = 16, 5 Hz, 1H), 2.56(dd, J = 14, 8 Hz, 1H) ppm; CIMS, m/z(rel. int): 344 (M^+ + H, 16), 284(1), 222(100), 162(11), 134(1), 121(13); EIHRMS, m/z: 343.1403 (M^+), $C_{17}H_{21}NO_5$ (ΔM −1.7 mmu).

Compound 8: obtained as a pale yellow oil; ^{1}H nmr (400 MHz, DMSO-d_6) δ: 7.18 (d, J = 8 Hz, 2H), 6.88 (d, J = 8 Hz, 2H), 6.60 (s, 1H), 6.47 (s, 1H), 4.00(dd, J = 10, 4 Hz, 1H), 3.87(dd, J = 9, 5 Hz, 1H), 3.74(s, 3H), 3.63(s, 3H), 2.86(dd, J = 14, 4 Hz, 1H), 2.79(dd, J = 16, 5 Hz, 1H), 2.74(dd, J = 14, 4 Hz, 1H), 2.64(dd, J = 16, 8 Hz, 1H) ppm; EIHRMS, m/z: 343.1412 (M^+), $C_{17}H_{21}NO_5$ (ΔM −0.8 mmu).

Acetylation of model compounds 7 and 8

Compounds **7** (25 mg) and **8** (25 mg) were reacted independently with acetic anhydride (2 mL) and pyridine (2 mL) at room temperature for 24 h to give the triacetyl derivatives **9** and **10** respectively.

Compound 9: white solid; ^{1}H nmr see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) δ: 172.7(s), 170.6(s), 168.1(s), 168.0(s), 158.5(s), 141.2(s), 140.4(s), 135.5(s), 130.6(d), 129.3(s), 122.7(d), 121.7(d), 114.0(d), 61.2(d), 55.4(d), 55.1(q), 52.3(q), 42.5(t), 29.7(t), 21.2(q), 20.4(q), 20.5(q) ppm; CIMS, m/z: 470(M^+ + H), 428, 348, 306, 264, 222, 162, 121; EIHRMS, m/z: 348.1093 (M^+ − MeObenzyl), $C_{17}H_{18}NO_7$ (ΔM +1.0 mmu).

Compound 10: clear oil; ^{1}H nmr (400 MHz, $CDCl_3$: exists as a 1: 1.4 mixture of conformers) δ: 6.98 (s), 6.89(s), 6.87(d, J = 8 Hz), 6.79(d, J = 8 Hz), 6.70(s), 6.69(s), 6.53(s), 5.36(t, J = 5 Hz), 4.92(t, J = 7 Hz), 4.86(dd, J = 8, 4 Hz), 4.63(dd, J = 6, 3 Hz), 3.78(s), 3.75(s), 3.58(s), 3.51(s), 3.3(m), 3.1–2.85(m), 2.43(dd, J = 16, 6 Hz), 2.26(s), 2.25(s), 2.24(s), 2.23(s), 2.13(s), 2.06(s) ppm; CIMS, m/z: 470 (M^+ + H); EIHRMS, m/z: 348.1090 (M^+ − MeObenzyl), $C_{17}H_{18}NO_7$ (ΔM +0.7 mmu).

Preparation of methyl 3,5-dibenzyloxybenzoate

Methyl 3,5-dihydroxybenzoate (5.0 g, 29 mmol) and K_2CO_3 (20.0 g) were stirred in 100 mL of anhydrous acetone for 30 min. Benzyl bromide (7.5 mL) was added slowly over a period of 5 min and the mixture was refluxed for 1 h. Filtration and evaporation to dryness *in vacuo* yielded a crude product that was purified by flash chromatography (400 mL 9:1 Hex/EtOAc, 400 mL, 8:2 Hex/EtOAc, 400 mL 7:3 Hex/EtOAc) to yield methyl 3,5-dibenzyloxybenzoate (9.4 g, 27 mmol: 92%), mp 66.0–67.5°C; ^{1}H nmr (400 MHz, $CDCl_3$) δ: 7.32(m, 10H), 7.28(d, J = 2.3 Hz, 2H), 6.76(t, J = 2.3 Hz, 1H), 4.97(s, 4H), 3.82(s, 3H) ppm; EIHRMS, m/z: 348.1364 (M^+), $C_{22}H_{20}O_4$ (ΔM +0.2 mmu).

Preparation of 3,5-dibenzyloxybenzyl alcohol

$LiAlH_4$ (0.012 g) was added slowly to methyl 3,5-dibenzyloxybenzoate (0.1 g, 0.29 mmol) in 10 mL of diethyl ether. The mixture was refluxed for 2.5 h and quenched with 10 mL of water. 20% NaOH (10 mL) was added and the mixture was extracted three times with diethyl ether (3 × 15 mL) and the ether layers were dried over magnesium sulfate. Evaporation *in vacuo* gave 3,5-dibenzyloxybenzyl alcohol (0.087 g, 0.27 mmol, 95% yield), mp 78.5–79.0°C; ^{1}H nmr (400 MHz, $CDCl_3$) δ: 7.31(m, 10H), 6.56(bs, 2H), 6.50(bs, 1H), 4.94(s, 4H), 4.50(s, 2H), 2.29(bs, 1H) ppm.

Preparation of 3,5-dibenzyloxybenzyl tosylate (11)

Sodium hydride (0.019 g 80% dispersion in oil) was added to a stirred solution of 3,5-dibenzyloxybenzyl alcohol (0.20 g, 0.63 mmol) in 10 mL of benzene. Tosyl chloride (0.12 g) was added slowly over a period of 10 min and the mixture was stirred for 9 h at room temperature. The resulting solution was centrifuged and the supernatant withdrawn. Evaporation of the benzene *in vacuo* yielded a mixture of the product 3,5-dibenzyloxybenzyl tosylate and starting material. Purification using flash chromatography (7:3 Hex/EtOAc) yielded the desired tosylate **11** (0.11 g, 0.23 mmol); ^{1}H nmr (400 MHz, $CDCl_3$) δ: 7.76(d, J = 8.2 Hz, 2H), 7.34(m, 10H), 7.28(d, J = 8.2 Hz, 2H), 6.54(t, J = 2.2 Hz, 1H), 6.46(d, J = 2.2 Hz, 2H), 4.96(s, 2H), 4.94(s, 4H), 2.39(s, 3H) ppm.

Preparation of benzylacetamidomalonate 12

Diethyl acetamidomalonate (0.13 g, 0.61 mmol) and sodium hydride (0.018 g 80% dispersion in oil) were stirred in benzene (5 mL) and DMF (5 mL) for 15 min. 3,5-Dibenzyloxybenzyl tosylate **11** (0.068 g, 0.14 mmol) was added slowly and the mixture was stirred at room temperature for 6 h. Evaporation *in vacuo* followed by flash chromatography (3:2 EtOAc/Hex) gave compound **12** (0.069 g, 0.13 mmol, 92% yield), mp 138.5–140.0°C; ^{1}H nmr (400 MHz, $CDCl_3$) δ: 7.34(m, 10H), 6.56(s, 1H), 6.51(t, J = 2.2 Hz, 1H), 6.26(d, J = 2.2 Hz, 2H), 4.98(s, 4H), 4.23(m, 4H), 3.58(s, 2H), 1.95(s, 3H), 1.27(t, J = 7.1 Hz, 6H) ppm; EIHRMS, m/z: 519.2254 (M^+), $C_{30}H_{33}NO_7$ (ΔM −0.3 mmu).

Hydrolysis and decarboxylation of 12

Compound **12** (0.055 g, 0.11 mmol) was dissolved in 10 mL of dioxane and 20% NaOH (2 mL) was added. The mixture was stirred under reflux for 3 h. After cooling, glacial acetic acid (2 mL) was added and the reaction was refluxed for an additional hour. After cooling, the mixture was extracted three times with 10-mL portions of EtOAc. Evaporation of the EtOAc *in vacuo* gave the crude 3,5-dibenzyloxy-*N*-acetamidophenylalanine (0.041 g, 0.099 mmol, 93% yield), which was used directly in the next step; ^{1}H nmr (400 MHz, CD_3OD) δ: 7.35(m, 10H), 6.53(bs, 2H), 6.45(bs, 1H), 4.80(s, 4H), 4.51(bs, 1H), 3.15(dd, J = 4.1, 13.6 Hz, 1H), 2.90(dd, J = 7.5, 13.6 Hz, 1H), 1.88 (s, 3H) ppm.

Preparation of methyl-3-(3,5-dihydroxyphenyl)alanine (13)

N-Acetamido-3-(3,5-dibenzyloxyphenyl)alanine was stirred in hydrazine (2 mL) at 100°C for 24 h. The mixture was evaporated to dryness *in vacuo*, taken up in methanol saturated with HCl, and refluxed for 1 h. The mixture was then evaporated to dryness *in vacuo* and taken up in methanol. Stirring under H_2 in the presence of Pt/C at room temperature for 10 h gave, after reverse phase flash chromatography (100% H_2O, 80% H_2O/acetone, 60% H_2O/acetone), the amino acid methyl ester **13**: ^{1}H nmr (400 MHz, CD_3OD) δ: 6.25(t, J = 1.8 Hz, 1H), 6.22(d, J = 1.8 Hz, 2H), 4.26(t, J = 6.6 Hz, 1H), 3.83(s, 3H, 3.36(s, 1H), 3.10(dd, J = 6.6, 13.8 Hz, 1H), 3.05(dd, J = 6.6, 13.8 Hz, 1H), 2.62 (s, 1H) ppm; EIHRMS, m/z: 211.0851 (M^+), $C_{10}H_{13}NO_4$ (ΔM +0.7 mmu).

Preparation of the benzyltetrahydroisoquinolines 14 and 15

Sodium 3-(4-benzyloxyphenyl)glycidate (15) (0.15 g, 0.53 mmol) in 10 mL of water was added to a solution of compound **13** (0.11 g, 0.53 mmol) in 15 mL of methanol. Acetic acid (2 mL) was added and the mixture was stirred at 35°C for 17 h under N_2. The solvent was evaporated *in vacuo* and the crude material was taken up in EtOAc. The organic layer was washed twice with 1 N HCl and once with H_2O and dried over sodium sulfate. Evaporation of the EtOAc to dryness gave 0.20 g of the crude mixture. Purification was accomplished using silica gel (1:1 Hex/EtOAc) to yield an inseparable mixture (0.079 g, 0.19 mmol, 36% yield) of the tetrahydroisoquinolines **14** and **15**. Data for the mixture of **14** and **15**: EIHRMS, m/z: 419.1728 (M^+), $C_{25}H_{25}NO_5$ (ΔM −0.4 mmu); Compound **15**: ^{1}H nmr (400 MHz, CD_3OD) δ: 7.37(m, 5H), 7.17(d, J = 8.1 Hz, 2H), 6.97(d, J = 8.1 Hz, 2H), 6.22(s, 1H), 6.13(s, 1H), 5.06(s, 2H), 4.31(d, J = 10.0 Hz, 1H), 4.01(d, J = 11.3 Hz, 1H), 3.74(s, 3H), 3.29(m, 2H), 2.85(m, 2H) ppm; Compound **14**: ^{1}H nmr (400 MHz, CD_3OD) δ: 7.37(m, 5H), 7.06(d, J = 8.1 Hz, 2H), 6.88(d, J = 8.1 Hz, 2H), 6.26(s, 1H), 6.06(s, 1H), 5.03(s, 2H), 4.46(bs, 1H), 3.44(m, 1H), 2.78(m, 3H) ppm.

Acetylation of the mixture of 14 and 15:

The tetrahydroisoquinoline mixture (**14** and **15**) (0.030 g, 0.072 mmol) was stirred in pyridine (2 mL) and acetic anhydride (2 mL) for 19 h. Evaporation of the reagents *in vacuo* yielded 0.048 g of the crude mixture. Purification was accomplished on preparative tlc (1:1

Hex/EtOAc) to yield **16** (0.0035 g, 0.0064 mmol) and **17** (0.023 g, 0.042 mmol). Compound **16**: ir (NaCl window): 3031, 2925, 2853, 1769, 1652, 1615, 1510 cm^{-1}; ^1H nmr(400 MHz, $CDCl_3$) δ: 7.40(m, 5H), 7.22(d, J = 8.7 Hz, 2H), 6.92(d, J = 8.7 Hz, 2H), 6.93(d, J = 2.1 Hz, 1H), 6.88(d, J = 2.1 Hz, 1H), 5.06(s, 2H), 5.03(dd, J = 5.3, 8.0 Hz, 1H), 4.46(t, J = 9.6 Hz, 1H), 3.85(s, 3H), 3.25(m, 3H), 2.81(dd, J = 5.3, 13.7 Hz, 1H), 2.29(s, 3H), 2.20(s, 3H), 1.71(s, 3H) ppm; EIHRMS, m/z: 545.2043 (M^+), $C_{31}H_{31}NO_8$ (ΔM −0.7 mmu). Compound **17** existed as a mixture of conformers. *Major conformer*: ^1H nmr (400 MHz, $CDCl_3$) δ: 7.33(m, 5H), 6.89(d, J = 2.2 Hz, 1H), 6.75(d, J = 8.6 Hz, 2H), 6.67(d, J = 2.2 Hz, 1H), 6.66(d, J = 8.6 Hz, 2H), 5.64(dd, J = 2.8, 6.7 Hz, 1H), 5.01(s, 2H), 4.54(dd, J = 2.4, 5.4 Hz, 1H), 3.49(s, 3H), 3.24(dd, J = 6.7, 13.2 Hz, 1H), 2.86(m, 2H), 2.25(s, 3H), 2.16(s, 3H), 2.12(s, 3H), 2.10(m, 1H) ppm. *Minor conformer*: ^1H nmr (400 MHz, $CDCl_3$) δ: 7.33(m, 5H), 6.93(d, J = 2.2 Hz, 1H), 6.86(s, 4H), 6.79(d, J = 2.2 Hz, 1H), 5.18(t, J = 6.3 Hz, 1H), 5.04(s, 2H), 4.83(dd, J = 3.5, 6.4 Hz, 1H), 3.57(s, 3H), 3.02(dd, J = 3.5, 16.3 Hz, 1H), 2.95 (second order d, J = 6.3 Hz, 1H), 2.93(second order d, J = 6.3 Hz, 1H), 2.86(m, 3H), 2.26(s, 3H), 2.22(s, 3H), 1.99(s, 3H) ppm; EIHRMS, m/z: 545.2043 (M^+), $C_{31}H_{31}NO_8$ (ΔM −0.7 mmu).

Preparation of compound 18

The *cis* benzyltetrahydroisoquinoline **16** (0.0039 g, 0.0071 mmol) was stirred in methanol (2 mL) under H_2 in the presence of Pt/C catalyst for 3 h. The mixture was filtered and evaporated to dryness *in vacuo*. Purification was accomplished using preparative tlc (9:1 EtOAc/Hex) to yield the phenol, which was stirred in pyridine (1 mL) and acetic anhydride (1 mL) at room temperature for 15 h. Evaporation of the reagents *in vacuo* followed by purification using preparative tlc (9:1 EtOAc/Hex) yielded compound **18**: ^1H nmr (400 MHz, $CDCl_3$) see Table 1; EIHRMS, m/z: 497.1691 (M^+), $C_{26}H_{27}NO_9$ (ΔM +0.5 mmu).

Preparation of compound 19

An identical procedure was employed to prepare the *trans* isomer **19** starting from **17**. The *trans* isomer **19** existed as a mixture of conformers. EIHRMS, m/z: 438.1556 ($M^+ - C_2O_2H_3$), $C_{24}H_{24}NO_7$ (ΔM +0.3 mmu). *Major conformer*: ^1H nmr(400 MHz, $CDCl_3$) δ: 6.88(d, J = 2.1 Hz, 1H), 6.86(d, J = 8.6 Hz, 2H), 6.79(d, J = 8.6 Hz, 2H), 6.71(d, J = 2.1 Hz,1H), 5.65(dd, J = 2.8, 7.2 Hz, 1H), 4.59(dd, J = 2.5, 5.3 Hz, 1H), 3.49(s, 3H), 3.21(dd, J = 7.2, 13.0 Hz, 1H), 2.97(m, 3H), 2.26(s, 3H), 2.25(s, 3H), 2.15(s, 3H), 2.13(s, 3H) ppm. *Minor conformer*: ^1H nmr (400 MHz, $CDCl_3$) δ: 6.99(m, 4H), 6.94(d, J = 2.6 Hz, 1H), 6.82(d, J = 2.6 Hz, 1H), 5.21(t, J = 6.2 Hz, 1H), 4.86(dd, J = 3.5, 6.3 Hz, 1H), 3.57(s, 3H), 3.08(dd, J = 3.5, 16.3 Hz, 1H), 2.95(m, 3H), 2.28(s, 3H), 2.27(s, 3H), 2.22(s, 3H), 2.06(s, 3H) ppm.

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