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Stereostructural Determination by a Synthetic and NMR-Based Approach of Three Oxazinins Isolated from Adriatic Mussels

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Two oxazinins, namely oxazinin-5 and -6, along with a related linear precursor (preoxazinin-7) were isolated from toxic mussels collected along the Northern Adriatic coasts in October 2005. Determination of the planar structure of these novel compounds was achieved through extensive NMR spectroscopic analysis, whereas a synthetic approach was crucial for their absolute stereochemical elucidation.

Introduction

Our ongoing research on toxic mussels from the Adriatic Sea carried out over the past two decades has allowed us to isolate and stereostructurally characterize not only several marine biotoxins, some of which have never been reported before,[1] but also new and intriguing classes of cytotoxic compounds, such as polychlorosulfolipids[2–3] and oxazinins[4–6] (Figure 1). Oxazinins represent a class of molecules featuring a typical oxazinine ring that resembles the oxazinone skeleton common to a number of bioactive natural products, such as bassiatin,[7] lateritin,[8] metacytofilin,[9] and javanicunin.[10] Preliminary toxicological studies performed on oxazinins suggest that their cytotoxicities are linked essentially to the –CN functionality.[4] As the occurrence of oxazinins has been repeatedly detected in the Adriatic shellfish over the past decade, a careful evaluation of their sanitary risk and environmental impact would definitely be in order.

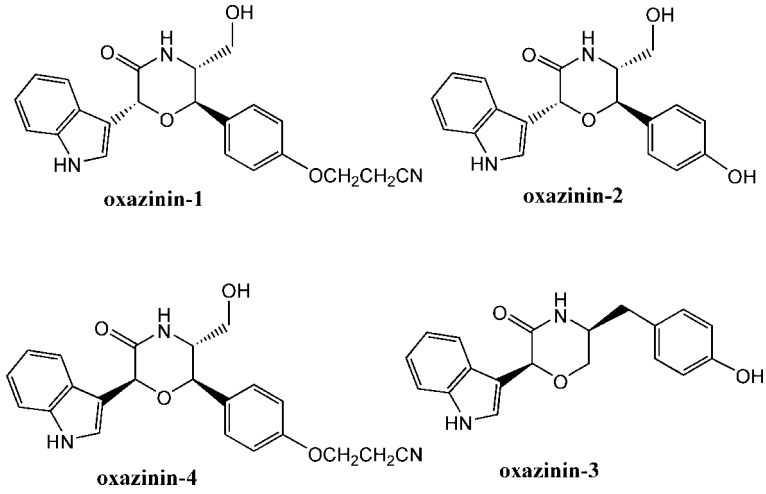

Figure 1. Stereostructures of oxazinin-1, -2, -3, and -4.

In this paper we report the structures and relative stereochemistries of two oxazinins (oxazinin-5 and -6) and of a related linear precursor (preoxazinin-7) achieved through sensitive NMR spectroscopic and MS techniques. In ad-

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dition, a synthetic study also led us to assess the absolute stereochemistry of these three novel compounds by comparison of the spectroscopic properties of the natural products with those of their corresponding model compounds.

Results and Discussion

In October 2005 by analyzing a batch of toxic mussels collected along the coast of the Northern Adriatic Sea, we isolated two oxazinins, namely oxazinin-5 (**1**) and -6 (**2**), along with a related linear precursor (preoxazinin-7; **3**). The chromatographic properties of these compounds as well as their ^{1}H and ^{13}C NMR spectra suggested that they belong to the oxazinin class.

The planar structure of compound **1** (*m/z* = 391.8 $[M + H]^+$) was mostly determined by comparison of its NMR spectroscopic data (Table 1) with those of the already reported oxazinins. In particular, in both COSY and TOCSY experiments the typical indole ring substituted at C-3′ and the *para* disubstituted phenyl ring linked to a $–OCH_2CH_2CN$ moiety were inferred on the basis of good overlap of their spectroscopic signals with those of the already-reported oxazinins[4] (Figure 2). The spin system 4-HN/C-5/(C-7)/C-6, where in comparison to oxazinin-3 the new structural modification lies, required more attentive analysis. Given its key position in the oxazinine ring, 5-H played a crucial role in the structure elucidation of the spin system it belonged to. Indeed, 5-H appeared to be coupled to 4-HN, 6-H_2, and 7-H (δ = 4.58 ppm). As this latter proton coupled to a carbon atom resonating at δ = 75.7 ppm and to an exchangeable proton at δ = 3.57 ppm (7-OH), we confidently located a hydroxy group at C-7, which is in full agreement with the molecular formula required by the HRMS (ESI) of **1**. At this point, diagnostic HMBC and ROE correlations (Figure 2) allowed us to connect all the isolated spin systems to unambiguously provide the planar structure of the molecule under investigation. Careful analysis of the $^3J_{H–H}$ values and ROE correlations (Figure 3a) was crucial to define the relative stereochemistry of the oxazine ring of **1**. In particular, a strong ROE between 2-H and 6a-H was conclusive for their *cis* orientation. Moreover, the coupling constants for 6a-H/5-H ($^3J_{H–H}$ = 7.5 Hz) and 6b-H/5-H ($^3J_{H–H}$ = 4.0 Hz) suggested a *trans* relationship between the 6a-H and 5-H protons, once a preferential chair-like conformation of the ring was assumed (Figure 3a). Unfortunately, the absence of a predominant conformation around the C-6/C-7 bond ($^3J_{H–H}$ =

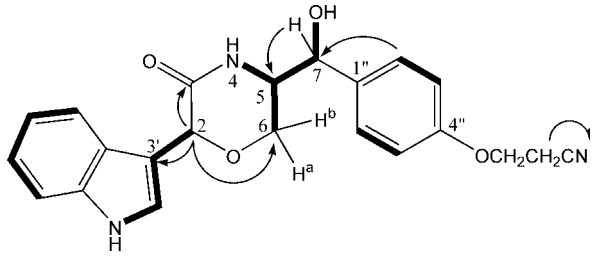


Figure 2. Planar structures of oxazinin-5 and -6: bold lines indicate the spin systems of the molecule as determined by COSY and TOCSY experiments; arrows represent selected HMBC correlations.

Table 1. ^{13}C (175 MHz) and ^{1}H (700 MHz) NMR spectroscopic data (in CD_3CN) of oxazinin-5 and (2*R*)-**6**.

Position	Oxazinin-5			(2*R*)-**6**		
	δ_H [ppm]	δ_C [ppm]	*J* [Hz] m	δ_H [ppm]	δ_C [ppm]	*J* [Hz] m
1						
2	5.29	74.3	s	5.28	75.0	s
3		170.2			170.5	
4-NH	6.76		br. s	6.74		br. s
5	3.81	58.5	4.0, 7.5, 8.3 m	3.79	57.5	4.0, 7.5, 8.3 m
6a	3.50	64.2	7.5, 12.0 dd	3.50	63.7	7.5, 12.0 dd
6b	3.59		4.0, 12.0 dd	3.59		4.0, 12.0 dd
7	4.58	75.7	8.3 d	4.52	76.2	8.3 d
1′	9.33		br. s	9.26		br. s
2′	7.27	125.3	s	7.26	124.9	s
3′		113.2			113.2	
3′a		127.8			127.4	
4′	7.58	120.5	8.2 d	7.59	120.8	8.2 d
5′	7.05	120.3	7.5, 8.2 dd	7.05	120.6	7.5, 8.2 dd
6′	7.14	122.5	7.5, 8.2 dd	7.13	123.2	7.5, 8.2 dd
7′	7.40	112.3	8.2 d	7.41	112.8	8.2 d
7′a		137.4			137.4	
1′′		135.5			137.6	
2′′-6′′	7.34	129.2	8.5 d	7.17	129.4	8.8 d
3′′-5′′	6.97	115.6	8.5 d	6.78	116.1	8.8 d
4′′		158.9			157.8	
7′′	4.19	64.0	6.0 t			
8′′	2.85	19.6	6.0 t			
CN		119.7				
7-OH	3.57		br. s		3.65	br. s

8.3 Hz) did not give us a chance to rely on Murata's method,[11] which would allow us to extend the relative stereochemistry to C-7 as well.

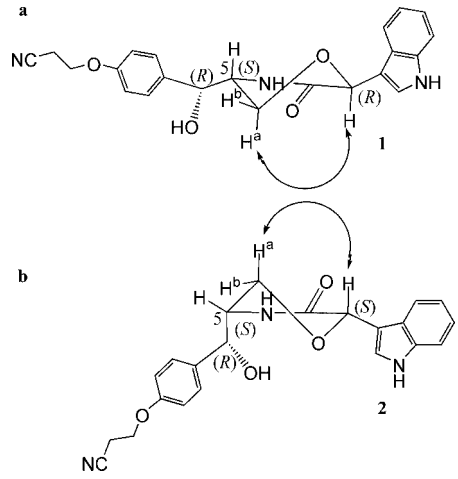
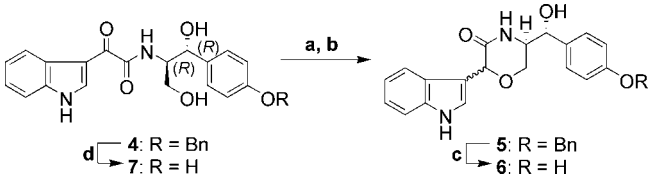


Figure 3. Preferential conformation of (a) **1** and (b) **2**. The ROE correlation that is crucial to assess the configuration of the oxazinine ring is indicated between 2-H and 6a-H, which are oriented in a diaxial relationship.

To establish not only the relative stereochemistry at C-7 but the absolute stereochemistry of the whole molecule as well, we resorted to the synthetic strategy developed by Couladouros et al. towards the morpholinone core of oxazinins.[12] Thus, reduction of the ketone functionality of the known[6] 3-indoleglyoxylic amide **4** (Scheme 1) by employing $NaBH_4$ afforded a triol that upon subsequent treatment with PPTS in refluxing acetonitrile furnished morpholinone **5** as a 1:1 mixture of C-2 diastereomers. Final hydrogenolysis of the benzyl protecting group provided an equimolar mixture of (2*R*)-**6** and (2*S*)-**6**. The two diastereomers were separated by employing a Chirex HPLC column with EtOAc as the eluent (see Experimental Section).



Scheme 1. Preparation of synthetic models **6** and **7**. Reagents and conditions: (a) $NaBH_4$, MeOH/THF (1:1), 0 °C→r.t., 30 min; (b) PPTS, CH_3CN, reflux, 3 h, 18%; (c) H_2, $Pd(OH)_2/C$, EtOAc/EtOH (4:1), r.t., 8 h, 75%; (d) H_2, $Pd(OH)_2/C$, EtOAc/EtOH (4:1), r.t., 8 h, 78%; (2*R*)-**6** and (2*S*)-**6** diastereoisomers were separated through a Chirex (*R*)-NGLY and DNB HPLC column with EtOAc as an eluent.

As the NMR spectroscopic properties of model (2*R*)-**6** were mostly superimposable with those of oxazinin-5, we could confirm the relative stereochemistry of the oxazine ring reported above. Moreover, as the circular dichroism (CD) spectrum of the synthetic model matched perfectly that of oxazinin-5 (Figure 4a,d), we were also able to determine the absolute stereochemistry of the whole molecule as reported in Figure 3a.

Through extensive NMR spectroscopic investigation (data reported in Table 2 and in the Experimental Section), compound **2** (*m/z* = 391.9 $[M + H]^+$) afforded the same planar structure as **1**, which suggests a diastereoisomeric relationship between them. The stereochemical elucidation of **2** was compounded by partial overlap of 6-H_2, which kept us from a straightforward evaluation of their *J* values. To overcome such a drawback, we recorded the ^1H NMR spectrum of **2** in deuterated solvents other than CD_3CN and eventually found that C_6D_6 afforded good separation of the 6a-H and 6b-H peaks (Table 2). At this point, the ROESY spectrum (in C_6D_6) proved once again crucial (Figure 3b). A strong ROE between 2-H and 6a-H was also diagnostic in this case for assessing their *cis* relationship, whereas the coupling constants for the 6a-H/5-H ($^3J_{H-H}$ = 3.9 Hz) and 6b-H/5-H ($^3J_{H-H}$ = 7.7 Hz) protons were this time indicative of a *cis* relationship between 6a-H and 5-H in a chair-like conformation of the oxazinine ring (Figure 3b). In analogy to compound **1**, the absolute stereochemistry of **2** was inferred by comparison (Figure 4b,e) of its NMR spectroscopic data and CD absorption with those of synthetic model (2*S*)-**6** (Scheme 1).

In-depth analysis of the COSY and TOCSY spectra of **3** allowed us to identify the following spin systems highlighted in bold lines in Figure 5a: (1) an indole ring, (2) a *para* disubstituted phenyl ring linked to a $–OCH_2CH_2CN$ moiety, and (3) the spin system 3-HN/C-4/(C-6)/C-5.

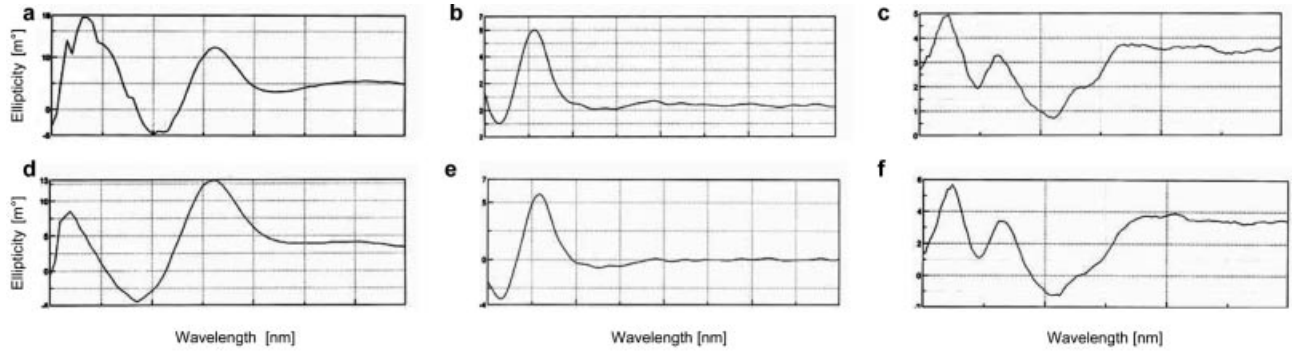


Figure 4. CD spectra of (a) oxazinin-5; (b) oxazinin-6; (c) preoxazinin-7; (d) (2*R*)-**6**; (e) (2*S*)-**6**; and (f) **7**.

Table 2. ^{13}C (175 MHz) and ^{1}H (700 MHz) NMR spectroscopic data of oxazinin-5 and (2*S*)-**6**.

Position	Oxazinin-6[a]		(2*S*)-**6**[a]			Oxazinin-6[b]	
	δ_H [ppm]	δ_C [ppm]	δ_H [ppm]	δ_C [ppm]	*J* [Hz][c] m	δ_H [ppm]	*J* [Hz][c] m
1							
2	5.38	73.7	5.38	74.5	s	5.72	s
3		170.4		170.1	br. s		br. s
4-NH	6.76		6.74			6.38	
5	3.62	56.8	3.59	57.0	3.9, 7.7, 9.2 m	3.24	3.9, 7.7, 9.2 m
6a	3.55	62.5	3.55	62.2	3.9, 11.8 dd	3.14	3.9, 11.8 dd
6b	3.57		3.57		7.7, 11.8 dd	3.43	7.7, 11.8 dd
7	4.72	75.8	4.66	76.2	9.2 d	3.97	9.2 d
1′	9.31		9.32			6.73	
2′	7.24	125.8	7.25	126.1		7.27	
3′		113.5		113.0			
3′a		127.6		127.9			
4′	7.63	119.5	7.64	120.4	8.2 d	8.08	8.2 d
5′	7.07	119.6	7.07	120.6	7.5, 8.2 dd	7.22	7.5, 8.2 dd
6′	7.16	121.8	7.17	122.3	7.5, 8.2 dd	7.21	7.5, 8.2 dd
7′	7.43	112.7	7.43	112.8	8.2 d	7.05	8.2 d
7′a		138.2		137.6			
1′′		136.2		133.6			
2′′-6′′	7.29	128.6	7.17	129.4	8.4 d	6.71	8.8 d
3′′-5′′	6.93	114.9	6.78	116.1	8.4 d	6.43	8.8 d
4′′		159.5		157.8			
7′′	4.18	64.0				3.11	6.0 t
8′′	2.84	19.1				1.63	6.0 t
CN		119.6					
7-OH	3.81			3.60	br. s	3.50	br. s

[a] Chemical shifts recorded in CD_3CN. [b] Chemical shifts recorded in C_6D_6. [c] *J* values were evaluated in C_6D_6.

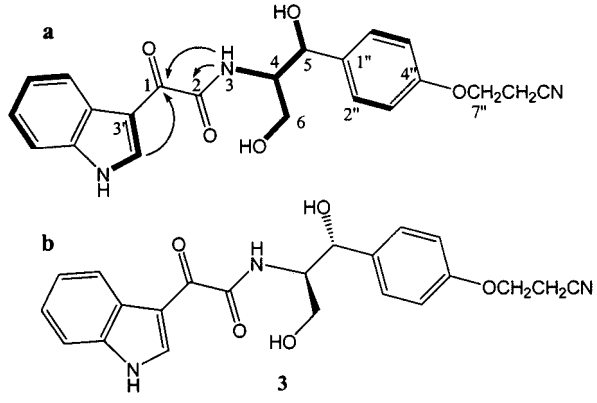


Figure 5. Structure of preoxazinin-7: (a) Bold lines indicate the spin systems of the molecule determined by COSY and TOCSY experiments; arrows represent selected HMBC correlations; (b) absolute stereochemistry afforded by comparison with the synthetic model.

An HMQC experiment was conclusive for linking each proton to its corresponding carbon atom (Table 3). Eventually, by taking into account the molecular formula ($C_{21}H_{21}O_5N_3$) determined by HRMS (ESI+), we had to locate one carbon and one oxygen atom in addition to those already included in the spin systems of the molecule described above. In this regard, a ^{13}C resonance value at δ = 183.3 ppm was helpful to identify a ketone functionality, which was located at position C-1 thanks to the HMBC correlations between the amidic proton and C-1, and between 2′-H and C-1. The HMBC correlation between 5-H and C-2′′ allowed the whole structure of **3** to be defined.

The last step of this study was to elucidate the stereostructure of this new oxazinin member. Also, in this case the synthetic approach was definitive. In fact, the overlapping NMR properties and the coincidence (Figure 4c,f) of the CD spectrum of **3** with that of synthetic model **7** (Scheme 1) afforded the absolute stereochemistry at C-4 and C-5 (Figure 5b).

The co-occurrence of **1**, **2**, and **3** enabled us to hypothesize a possible biogenetic pathway leading to this interesting class of cytotoxic compounds. In particular, **3** − whose biogenesis is most likely derived from tryptophan and tyrosine − might be considered a hypothetical precursor of oxazinins by generating the typical oxazinine ring through an intramolecular nucleophilic addition of the hydroxy group at C-6 to the carbonyl at C-1, followed by a reduction of the resulting semiacetal functionality.

Our study on contaminated shellfish has all along contributed to delineate the complex toxin profile of the Adriatic Sea to reveal a peculiarity unmatched anywhere else across the world. Unfortunately − as it happens for most of the natural biotoxins − scarce availability of the pure toxic compound limits the evaluation of the real hazards posed to human health. To obtain a quantity of the pure compounds that is sufficient for in-depth toxicological studies, a synthetic approach could be taken into account. Indeed, synthetic studies towards the introduction of the pendant –OCH_2CH_2CN moiety of oxazinins are underway; upon their successful completion, the synthetic strategy toward oxazinins previously described and employed in this paper for the preparation of models **1**, **2** and **3** would represent a valid way to overcome the lack of pure samples.

Table 3. ^{13}C (175 MHz) and ^{1}H (700 MHz) NMR spectroscopic data of preoxazinin-7 and **7** (CD_3CN).

Position	Preoxazinin-7			**7**		
	δ_H [ppm]	δ_C [ppm]	*J* [Hz] m	δ_H [ppm]	δ_C [ppm]	*J* [Hz] m
1		183.3			182.0	
2		162.3			163.6	
3-NH	7.79		br. s	7.78		br. s
4	4.06	57.4	m	4.03	57.8	m
5	5.00	72.0	3.2 d	4.92	72.6	3.2 d
6a	3.63	62.8	11.4, 5.6 dd	3.61	63.2	11.4, 5.6 dd
6b	3.70	62.8	11.4, 5.6 dd	3.69		11.4, 5.6 dd
1′	10.10		br. s		10.13	br. s
2′	8.81	139.4	s	8.83	139.8	s
3′		113.4			113.2	
3′a		127.6			127.6	
4′	8.31	122.3	9.1 d	8.31	122.7	9.1 d
5′	7.29	124.5	7.3, 9.1 dd	7.29	124.8	7.3, 9.1 dd
6′	7.30	124.5	7.3, 9.1 dd	7.30	124.8	7.3, 9.1 dd
7′	7.54	113.0	9.1 d	7.55	113.0	9.1 d
7′a		137.2			139.8	
1′′		136.5			134.6	
2′′-6′′	7.32	128.1	8.9 d	7.21	128.3	9.0 d
3′′-5′′	6.88	114.8	8.9 d	6.74	115.8	9.0 d
4′′		158.4			157.2	
7′′	4.14	63.6	6.2 t			
8′′	2.81	19.0	6.2 t			
CN		119.4				
5-OH	3.89		br. s	4.10		br. s
6-OH	3.56		br. s	3.48		br. s

Moreover, it would be interesting to trace the origin of oxazinins. At the moment, there are no reports about this, even though it is reasonable to hypothesize that their occurrence in shellfish might be derived from an exogenous source − most likely a microorganism such as a microalga. Discovery of the producing organism(s) would allow: (1) the isolation of a large quantity of pure compounds required for toxicological studies and (2) for the assumed oxazinin biogenetic pathway to be proved.

Experimental Section

General: NMR spectra were measured with a Varian Unity Inova700 spectrometer and the solvent was used as an internal standard (CD_3CN: δ_H = 1.94 ppm; δ_C = 1.3 and 118.2 ppm). MS (ESI+) was recorded with an API-2000 triple quadrupole mass spectrometer equipped with a turbo ion-spray source (Applied Biosystem; Thornhill, ON, Canada). CD spectra were recorded with a J-710 spectropolarimeter (Jasco, Tokyo, Japan) equipped with a J-710 for Windows software (Jasco). All spectra were measured in MeOH at room temperature. NMR and MS experiments were performed at "Centro di Servizi Interdipartimentale di Analisi Strumentale", Università degli Studi di Napoli Federico II. Medium-pressure liquid chromatography (MPLC) was performed with a Buchi 861 apparatus equipped with Develosil ODS and Toyopearl HW-40 SF columns. HPLC separations were performed with a Varian apparatus, equipped with Waters 490 MS UV and RI-3 index detectors and Luna 5u C18 and Luna 5u Silica columns. UV detector was set at 230 nm; TLC was performed on silica gel 60 plates (Merck, precoated), with EtOAc/MeOH (95:5) as a mobile phase; the oxazinins were detected by heating the plates after spraying with 50% sulfuric acid. All reactions were carried out under a dry argon atmosphere with anhydrous, freshly distilled solvents under anhydrous conditions unless otherwise noted. All reactions were magnetically stirred with Teflon stir bars, and temperatures were measured externally. Reactions requiring anhydrous conditions were carried out in oven-dried (120 °C, 24 h) or flame-dried (vacuum < 0.5 Torr) glassware. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography.

Collection and Extraction: Toxic mussels *M. galloprovincialis* were collected along the coasts of Cesenatico (Adriatic Sea) in October 2005 at a depth of 3 m, which corresponds to the upper levels of mussel farm in this area. Reference specimens were deposited at the Dipartimento di Chimica delle Sostanze Naturali, Napoli (Italy). After collection, the mussels were stored at –20 °C until extraction. The digestive glands (5000 g of dry weight after extraction) were removed, homogenized with a Waring blender, and extracted with CH_3CN/H_2O (8:2 + 0.1% HCOOH) twice at room temperature. The combined extracts, after filtration, were concentrated in vacuo to give a residue that was dissolved in CH_3CN/H_2O (2:1) and partitioned with CH_2Cl_2. The dichloromethane layer was concentrated and then chromatographed by MPLC on a Develosil ODS column ($MeOH/H_2O$, 3:2 to 1:0). The fraction eluted with $MeOH/H_2O$ (9:1) was successively separated on a Toyopearl HW-40 SF column with MeOH as the eluent. The fraction containing oxazinins was first purified on reverse-phase HPLC ($CH_3CN/H_2O/CH_3OH$, 15:50:35) and then on a silica gel HPLC column ($AcOEt/CH_3OH$, 95:5) 2.2, 2.8, and 2.4 mg of pure oxazinin-**5**, -**6**, and -**7**, respectively.

Oxazinin-5: ^{1}H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 1. IR (KBr): $\tilde{\nu}$ = 3476, 3342, 3187, 2932, 2259, 1661, 1622 cm^{-1}. MS (ESI+): *m/z* = 391.9 $[M + H]^+$, 414.1 $[M + Na]^+$. HRMS (ESI+): calcd. for $C_{22}H_{22}N_3O_4$ $[M + H]^+$ 392.1610; found 392.1621.

Oxazinin-6: ^{1}H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 2. IR (KBr): $\tilde{\nu}$ = 3478, 3344, 3186, 2931, 2263, 1660, 1623 cm^{-1}. MS (ESI+): *m*/*z* = 391.9 [M + H]$^+$, 414.1 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{22}H_{22}N_3O_4$ [M + H]$^+$ 392.1610; found 392.1602.

Preoxazinin-7: ^{1}H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 3. IR (KBr): $\tilde{\nu}$ = 3483, 3337, 3192, 2936, 2270, 1658, 1622 cm^{-1}. MS (ESI+): *m*/*z* = 408.2 [M + H]$^+$, 430.1 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{22}H_{22}N_3O_5$ [M + H]$^+$ 408.1559; found 408.1568.

Synthetic Studies

Reduction of Amide 4: Sodium borohydride (51 mg, 1.35 mmol) was added in small portions to a stirred solution of amide **4** (300 mg, 0.67 mmol) in a mixture of MeOH (5 mL) and THF (5 mL) at 0 °C. The reaction was warmed to ambient temperature and after 30 min saturated aqueous ammonium chloride (10 mL) was carefully added. The mixture was extracted with EtOAc (4 × 50 mL); the combined organic extracts were washed with brine (20 mL), dried with Na_2SO_4, and concentrated under reduced pressure to afford the product as an amorphous white solid. TLC analysis (R_f = 0.14, 0.24; acetone/CH_2Cl_2, 2:3) and ^{1}H NMR spectroscopic analysis (data not shown) revealed it to be an equimolar mixture of the corresponding C-1 diastereomeric triols. HRMS (ESI+): calcd. for $C_{26}H_{27}N_2O_5$ [M + H]$^+$ 447.1920; found 447.1939. On the basis of the previous related synthetic studies,[6,12] both diastereomers were expected to lead to the same mixture of diastereomeric morpholinones; thus, no attempt was made to separate them, and the mixture was used in the next step without further purification.

Morpholinones 5: A catalytic amount of pyridinium *p*-toluenesulfonate (10 mg) was added to a stirred solution of the above triols (290 mg, 0.65 mmol) in acetonitrile (100 mL) at ambient temperature, and the mixture was warmed to 80 °C. After completion of the reaction (3 h) half of the volume of the solvent was removed under reduced pressure and the rest was poured into water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (30 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane, 3:7) to afford an equimolar mixture of C-2 diastereomeric morpholinones **5** (50 mg, 0.12 mmol, 18% yield over 2 steps) as a colorless oil. R_f = 0.38 (acetone/CH_2Cl_2, 2:3). ^{1}H NMR (500 MHz, CD_3CN): δ = 9.31 (br. s, 1 H, ArN*H*), 9.27 (br. s, 1 H, ArN*H*), 7.63 (d, *J* = 8.0 Hz, 1 H, Ar*H*), 7.59 (d, *J* = 8.0 Hz, 1 H, Ar*H*), 7.48–6.96 (m, 26 H, Ar*H*), 6.80 (br. s, 1 H, N*H*CO), 6.76 (br. s, 1 H, N*H*CO), 5.38 (s, 1 H, COC*H*O), 5.29 (s, 1 H, COC*H*O), 5.10 (s, 2 H, OCH_2Ph), 5.08 (s, 2 H, OCH_2Ph), 4.70 (d, *J* = 7.9 Hz, 1 H, C*H*OH), 4.54 (d, *J* = 7.9 Hz, 1 H, C*H*OH), 3.83–3.46 (m, 8 H, C*H*NHCO + CHCH_2O + CHO*H*) ppm. ^{13}C NMR (125 MHz, CD_3CN): δ = 134.6, 132.2, 129.7, 129.5, 129.4, 129.1, 129.0, 128.9, 128.9, 128.7, 128.6, 126.3, 126.2, 122.8, 122.8, 120.4, 120.4, 120.3, 115.8, 115.7, 112.5, 112.4, 75.4, 75.0, 74.7, 74.5, 70.7, 70.6, 64.1, 62.8, 58.6, 58.5 ppm. HRMS (ESI+): calcd. for $C_{26}H_{25}N_2O_4$ [M + H]$^+$ 429.1814; found 429.1797.

Morpholinones 6: To a solution of **5** (30 mg, 0.07 mmol) in a mixture of EtOAc/EtOH (4:1; 20 mL) at ambient temperature was added a catalytic amount of $Pd(OH)_2$/C (5 mg), and the mixture was stirred under a hydrogen atmosphere for 8 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give the corresponding free phenols as a white amorphous solid. The residue was purified by flash column chromatography (acetone/CH_2Cl_2, 2:3) to afford 17.8 mg of morpholinones **6** (0.053 mmol, 75%) as an amorphous white solid. The two diastereomers [(2*R*)-**6** and (2*S*)-**6**] were separated by employing a Chirex (*R*)-NGLY and DNB HPLC column (50 × 4.60 mm, 5 μm) with EtOAc as the eluent. Their ^{1}H and ^{13}C-NMR resonances are reported in Tables 1 and 2, respectively. Data for (2*R*)-**6**: HRMS (ESI+): calcd. for $C_{19}H_{19}N_2O_4$ [M + H]$^+$ 339.1345; found 339.1360. Data for (2*S*)-**6**: HRMS (ESI+): calcd. for $C_{19}H_{19}N_2O_4$ [M + H]$^+$ 339.1345; found 339.1329.

Synthetic Model 7: To a solution of **4** (30 mg, 0.07 mmol) in a mixture of EtOAc/EtOH (4:1; 20 mL) at ambient temperature was added a catalytic amount of $Pd(OH)_2$/C (5 mg), and the mixture was stirred under a hydrogen atmosphere for 8 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give the corresponding free phenol as a white amorphous solid. The residue was purified by flash column chromatography (acetone/CH_2Cl_2, 2:3) to afford 18.5 mg of **7** (0.052 mmol, 78%) as an amorphous white solid. Its ^{1}H and ^{13}C-NMR resonances are reported in Table 3. HRMS (ESI+): calcd. for $C_{19}H_{19}N_2O_5$ [M + H]$^+$ 355.1339; found 355.1355.

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