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Oxazinins from toxic mussels: isolation of a novel oxazinin and reassignment of the C-2 configuration of oxazinin-1 and -2 on the basis of synthetic models

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Abstract—The analysis of a batch of toxic mussels (*Mytilus galloprovincialis*) from the Northern Adriatic Sea led to the isolation of a novel oxazinin, oxazinin-4. Its structure including the relative stereochemistry has been elucidated through extensive NMR analysis. A synthetic route to oxazinins has been crucial in establishing the absolute stereochemistry of oxazinin-4 and for reassigning the absolute C-2 configuration of oxazinin-1 and -2 previously isolated from toxic shellfish and stereostructurally characterized.

1. Introduction

During our continuous analysis on toxic shellfish from the Northern Adriatic Sea,[1] we have isolated and fully characterized several cytotoxic compounds[2–4] in addition to typical marine biotoxins. These cytotoxic compounds appear to be of great interest not only for their quite unique structural features, but also because they cause human seafood intoxication. In 2001, we reported the structure and the relative stereochemistry of three novel compounds oxazinin-1, -2, and -3 of which oxazinin-1 has emerged as a cytotoxic molecule.[5]

Subsequently, we have assigned the absolute stereochemistry of both oxazinin-1 and -2[6] by applying the Riguera's method (Fig. 1).[7] Recently, Couladouros et al. reported an effective synthesis of oxazinin-1, -2, and -3, which has allowed the absolute stereochemistry of these compounds to be determined (Fig. 2).[8]

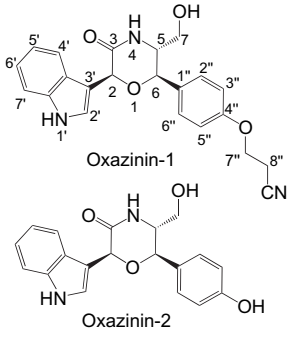

Figure 1. Previously reported stereostructures of oxazinin-1 and -2.[5]

In the present paper we report the structure of a novel diastereoisomer of oxazinin-1 (oxazinin-4) isolated from toxic mussels. The successive elucidation of the relative stereostructure of oxazinin-4 in combination with the isolation of a further amount of oxazinin-1 gave us the chance to

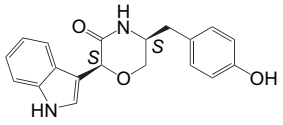

Figure 2. Stereostructure of oxazinin-3.

Keywords: Oxazinins; Adriatic shellfish; Natural products; Synthetic models; *Mytilus galloprovincialis*; NMR spectroscopy.
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reassess and correct the relative configuration of both oxazinin-1 and -2. A synthetic study allowed us to definitively confirm the C-2 configuration of oxazinin-1 and -2 and unambiguously assign the absolute stereochemistry of oxazinin-4.

2. Results and discussion

A toxic batch of mussels (*Mytilus galloprovincialis*) was collected along the Emilia Romagna coasts, Italy in March 2002 during a period of high toxicity. Oxazinin-4 was successfully isolated according to the previous reported procedure.[5] The chromatographic behavior, the preliminary NMR data, and the mass spectrum of this novel compound (Table 2 and Section 3) indicated a similar structure as oxazinin-1. Such a consideration eased us to individuate the presence of both an indole ring substituted at C-3 and a *para*-disubstituted phenyl ring linked to a $-OCH_2CH_2CN$ moiety. Running COSY, HOHAHA, and ROESY spectra were crucial to determine the spin system including C-5, C-6, C-7, and H-N-4. A strong IR absorption band at 1661 cm^{-1} along with a carbonyl carbon resonating at δ 169.4 ppm pointed out an amide functionality. Finally, correlations emerging from a HMBC experiment (Fig. 3) allowed to establish the planar structure of oxazinin-4.

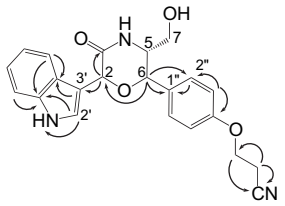


Figure 3. HMBC correlations for oxazinin-4.

Since the structure of oxazinin-4 was coincident with that reported for oxazinin-1 only a diastereoisomeric relationship between the two compounds could explain the significant differences in their chromatographic and NMR properties (Tables 1 and 2). A ROESY spectrum was indispensable for assigning the relative configuration of oxazinin-4. Assuming that this compound presented a preferential chair-like conformation in analogy with oxazinin-1,[5–6] an intense

Table 2. ^{13}C and ^{1}H NMR spectroscopic data[a] of oxazinin-4 and **9b** (CD_3CN)

Position	Oxazinin-4			Compound **9b**		
	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)
2	75.3	5.48		75.6	5.43	
3	169.4			170.7		
4		6.61			6.66	
5	60.8	3.93		60.7	3.88	
6	77.6	4.79	9.7	78.1	4.69	9.9
7a	62.0	3.36		62.0	3.31	
7b		3.49			3.44	
1′		9.28			9.26	
2′	126.8	7.37		126.2	7.33	
3′	112.8			112.0		
3′a	126.8			126.9		
4′	120.0	7.42	8.2	120.3	7.41	8.2
5′	118.4	7.12	7.5, 8.2	120.5	7.08	7.7, 8.2
6′	120.1	7.20	7.5, 8.2	122.8	7.16	7.7, 8.2
7′	112.4	7.72	8.2	112.4	7.71	8.2
7′a	137.9			137.6		
1″	132.0			129.7		
2″–6″	130.4	7.41	8.4	129.8	7.25	8.2
3″–5″	116.0	6.98	8.4	116.1	6.79	8.2
4″	160.4			158.0		
7″	64.2	4.21	6.0			
8″	19.6	2.87	6.0			
CN	118.9					
7-OH		3.05			3.05	

[a] Assignments are based on HMQC and HMBC experiments.

Table 1. ^{13}C and ^{1}H NMR spectroscopic data of oxazinin-1, -2,[5] and **9a**[a] (CD_3CN)

Position	Oxazinin-1			Oxazinin-2			Compound **9a**		
	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)
2	72.9	5.59		72.8	5.57		72.8	5.57	
3	170.1			170.1			170.1		
4		6.70			6.64			6.64	
5	59.9	3.71		59.9	3.70		59.9	3.70	
6	71.1	4.61	9.2	71.2	4.56	9.2	71.2	4.56	9.2
7a	62.0	3.26		62.1	3.26		62.1	3.26	
7b		3.43			3.42			3.42	
1′		9.40			9.37			9.37	
2′	126.1	7.29		126.1	7.28		126.1	7.28	
3′	112.3			112.4			112.4		
3′a	127.5			127.5			127.5		
4′	120.1	7.58	8.2	120.1	7.58	8.2	120.1	7.58	8.2
5′	120.3	6.99	7.5, 8.2	120.3	7.00	7.7, 8.2	120.3	7.00	7.7, 8.2
6′	122.8	7.13	7.5, 8.0	122.8	7.14	7.7, 7.9	122.8	7.14	7.7, 7.9
7′	112.4	7.43	8.0	112.4	7.42	7.9	112.4	7.42	7.9
7′a	137.4			137.4			137.4		
1″	131.8			130.0			130.0		
2″–6″	130.0	7.20	8.4	129.9	7.08	8.4	129.9	7.08	8.4
3″–5″	115.5	6.88	8.4	116.0	6.73	8.4	116.0	6.73	8.4
4″	159.0			157.9			157.9		
7″	63.9	4.14	6.0						
8″	19.0	2.82	6.0						
CN	118.9								
7-OH		3.12			3.10			3.10	

[a] Assignments are based on HMQC and HMBC experiments.

ROE correlation between H-2 and H-6 was a clear clue of their cis-relationship, while the high coupling constant between H-5 and H-6 suggested their trans-orientation (Fig. 4).

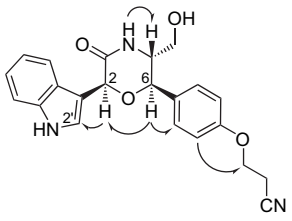
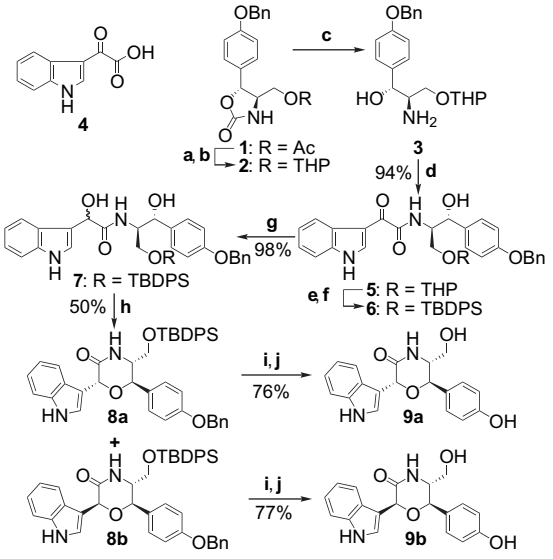


Figure 4. ROE correlations for oxazinin-4.

These data surprisingly pointed to the same relative stereochemistry reported for oxazinin-1. In order to clarify such an ambiguity, we decided to re-examine the ROE correlations of oxazinin-1. This time we did not detect any correlation between H-2 and H-6 in oxazinin-1 indicating an actual trans-orientation of the two protons. Since this time we could employ a more powerful NMR instrument, such as a 700 MHz instead of the 500 MHz, and rely on a larger availability of pure oxazinin-1 isolated along with oxazinin-4, we can confidently conclude that the H-2/H-6 ROE correlation described for oxazinin-1 in our precedent paper was actually an artifact possibly due to the low signal–noise ratio of the former experiment.

In order to unambiguously prove the stereochemistry of oxazinin-1 and -4, we undertook the synthesis set up by Couladouros et al. (Scheme 1). Thus, the prerequisite amine **3** was obtained by protective group manipulation of the known[9] tyrosine derivative **1**. Subsequent coupling with 3-indoleglyoxylic acid **4**[10] afforded amide **5**. A further protecting group switched to the more stable *tert*-butyldiphenylsilyl ether **6**. Subsequent reduction of the keto functionality with $NaBH_4$ provided diol **7**, as a 1:1 mixture of diastereomers, thus setting the stage for the crucial morpholinone ring-forming step. Treatment of a solution of diol **7** in refluxing acetonitrile with PPTS afforded C-2 epimers **8a** and **8b**. Finally, debenzylation and silyl deprotection gave phenols **9a** and **9b**.



Scheme 1. Preparation of morpholinones **9a** and **9b**. Reagents and conditions: (a) 3 N $LiOH_{(aq)}$/THF/MeOH (1:10:10 v/v), 0 °C, 10 min; (b) DHP, PPTS, CH_2Cl_2, reflux, 1 h, 93% for two steps; (c) 3% $KOH_{(aq)}$/toluene (1:1 v/v), reflux; (d) **4**, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2/DMF (14:1 v/v), 0 °C→2 h, rt→22 h, 94% for two steps; (e) TsOH, MeOH, rt, 20 min, 98%; (f) TBDPSCl, 2,6-lutidine, 55 °C, 24 h, 95%; (g) $NaBH_4$, MeOH/THF (1:1 v/v), 0 °C→rt, 30 min, 98%; (h) PPTS, CH_3CN, reflux, 2 h, 50% combined yield; (i) H_2, $Pd(OH)_2$/C, EtOAc/EtOH (4:1 v/v), rt, 8 h; (j) 1.0 M TBAF, THF, rt, 30 min; **9a** 76% for two steps, **9b** 77% for two steps.

Once isolated, compounds **9a** and **9b** were fully investigated by NMR. Basically, a difference in their ROESY spectra was observed: a strong correlation peak between H-2 and H-6 was present in the spectrum of **9b**, while it was missing in the spectrum of **9a** (Fig. 5).

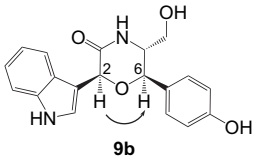


Figure 5. ROE correlation in compound **9b** indicating the cis-relationship of H-2 and H-6.

A comparison of NMR data and optical rotation collected on both the synthetic compounds with those recorded for the natural molecules (Tables 1 and 2) led us to unambiguously correlate **9a** to oxazinin-2. This was crucial for confirming the absolute stereochemistry previously assigned at C-5 and C-6, and for re-examining and properly reassigning the stereochemistry at C-2 as *R* (Fig. 6). Once established the overlapping of oxazinin-2 with **9a**, we could extend our analysis to oxazinin-1. In fact, the difference between oxazinin-1 and **9a**/oxazinin-2 is restricted to the presence of the $-OCH_2CH_2CN$ segment, which does not affect significantly the optical rotation. As a consequence, the absolute stereochemistry at C-5 and C-6 of oxazinin-1 was confirmed and the stereochemistry at C-2 was reassigned again as *R* (Fig. 6). Similarly, the absolute stereochemistry of oxazinin-4 was achieved on the basis of the parallelism of the

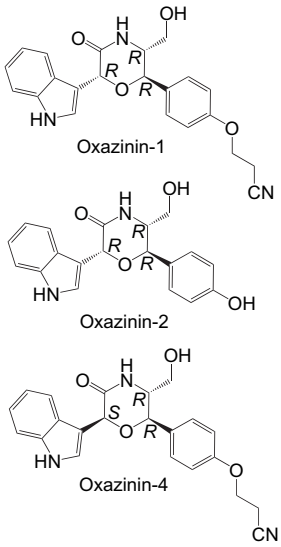


Figure 6. Absolute stereochemistry of oxazinin-1, -2, and -4.

optical rotation and NMR properties of oxazinin-4 with those of compound **9b** (Table 2; Fig. 6).

In conclusion, a novel oxazinin has been isolated and stereostructurally characterized; and a synthetic route to oxazinin-2 has given the opportunity to correct the absolute stereochemistry of oxazinin-1 and -2.

3. Experimental

3.1. General

NMR spectra were measured on a Varian Unity Inova700 spectrometer and the solvent was used as an internal standard (CD_3CN: δ_H 1.94; δ_C 1.3 and 118.2). ESI positive ion mode spectra were obtained on a API-2000 triple quadrupole mass spectrometer equipped with a turbo ion spray source (Applied biosystem, Thornhill, ON, Canada). Optical rotations were measured on a Perkin–Elmer 192 polarimeter in methanol solution, using a sodium lamp at 589 nm. 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 on a Buchi 861 apparatus equipped with Develosil ODS and Toyopearl HW-40 SF columns. HPLC separations were performed on 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) obtained homogeneous materials. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography.

3.2. Collection and extraction

Toxic mussels *M. galloprovincialis* were collected along the coasts of Cesenatico (Adriatic Sea) in March 2002 at 3 m depth, 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, which 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 using a solvent gradient system from 60% to 100% of methanol. The fraction eluted with 90% of methanol was successively separated on a Toyopearl HW-40 SF column with 100% methanol as an eluent. The fraction containing oxazinins was first purified on reverse phase HPLC eluted with $CH_3CN/H_2O/CH_3OH$ 15:50:35 and then on a silica gel HPLC column using EtOAc/CH_3OH 95:5 as an eluent to afford 1.2 mg of pure oxazinin-4.

3.2.1. Oxazinin-4. $[\alpha]_D^{25}$ +60.0 (*c* 0.2, MeOH). ν_{max} (KBr) 3478, 3342, 3186, 2930, 2262, 1661, 1623 cm^{-1}. ESI positive ion mode MS: *m/z* 391.9 $[M+H]^+$ and *m/z* 413.7 $[M+Na]^+$. HRMS (ESI positive ion mode): $[M+H]^+$, found 392.1627. $C_{22}H_{22}N_3O_4$ requires 392.1610. ^{1}H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 1. ^{1}H–^{1}H COSY correlations: H-5/H-6; H-5/H_2-7; H-5/7-OH; H-1′/H-2′; H-4′/H-5′; H-5′/H-6′; H-6′/H-7′; H-2″(6″)/H-3″(5″); $H_2$7″/$H_2$8″. HMBC correlations: H-2/C-3,C-2′, C-3′,C-3′a,C-6; H-6/C-2,C-1″,C-2″(6″); H-2′/C-3′,C-3′a, C-7′a; H-4′/C-3′,C-6′,C-7′a; H-5′/C-3′a,C-7′; H-6′/C-5′,C-7′a; H-7′/C-5′,C-3′a; H-2″(6″)/C-6,C-4″,C-6″(2″); H-3″(5″)/C-1″,C-4″,C-5″(3″); H-7″/CN; H-8″/C-7″,CN.

3.3. Synthetic studies

3.3.1. THP ether 2. Acetate **1** (1.5 g, 4.39 mmol) was dissolved in a mixture of THF/MeOH (40:40 mL), the solution was cooled to 0 °C, and aqueous LiOH (3 N, 4 mL) was added slowly. After 10 min the solution was neutralized with a 1 N aqueous HCl and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with brine (2×30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. To a stirred solution of the above crude product (1.3 g) in dichloromethane (80 mL), 3,4-dihydro-2*H*-pyran (8 mL) was added at ambient temperature, followed by a catalytic amount of pyridinium *p*-toluenesulfonate (20 mg) and the mixture was refluxed at 80 °C. After 1 h the reaction mixture was poured into water (50 mL) and extracted with dichloromethane (2×50 mL). The combined organic extracts were washed with brine (2×50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (30% EtOAc in hexane) affording 1.56 g (4.07 mmol) of the tetrahydropyranyl ether **2** (93% combined yield for two steps) as colorless amorphous solid. R_f=0.38 (60% EtOAc in hexane); $[\alpha]_D^{25}$ +62 (*c* 0.30, Acetone); ν_{max} (KBr) 3284, 1758, 1614, 1514 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44–7.24 (m, 14H, Ar*H*), 7.00 (d, *J*=8.7 Hz, 4H, Ar*H*), 6.37 (d, *J*=3.7 Hz, 1H, N*H*CO), 6.30 (d, *J*=5.7 Hz, 1H, N*H*CO), 5.21 (m, 2H, ArC*H*OCO), 5.08 (s, 4H, OCH_2Ph), 4.65 (br s, 2H, OC*H*O), 3.97–3.80 (m, 6H, CH_2OTHP+ CH_2C*H*HO), 3.63–3.50 (m, 4H, C*H*NH+CH_2CH*H*O), 1.85–1.54 (m, 12H, CH_2CH_2CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.2, 159.1, 158.9, 136.6, 130.6, 130.5, 128.5, 128.5, 128.4, 128.2, 128.0, 127.4, 127.3, 127.2, 115.1, 99.4, 99.1, 80.1, 80.0, 70.0, 69.0, 68.8, 62.4, 62.3, 60.2, 59.9, 30.3, 30.2, 25.2, 25.1, 19.2, 19.1. HRMS (ESI positive ion mode): $[M+H]^+$, found 384.1828. $C_{22}H_{26}NO_5$ requires 384.1811.

3.3.2. Amide 5. An aqueous solution of 3% KOH (40 mL) was added to a stirred solution of cyclic carbamate **2** (1.5 g, 3.91 mmol) in toluene (40 mL) and the mixture was

warmed to reflux for 24 h. After cooling to ambient temperature the reaction mixture was poured into a saturated aqueous ammonium chloride (30 mL) neutralized with a 1 N aqueous HCl (pH=8) and extracted with EtOAc (3× 100 mL). The combined organic extracts were washed with brine (2×50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. To a stirred solution of the crude amine **3** (1.4 g, 3.91 mmol) and 3-indoleglyoxylic acid **4** (874 mg, 4.62 mmol) in a mixture of dichloromethane (500 mL) and DMF (36 mL) were added at 0 °C *N*,*N*-diisopropylethylamine (867 μL, 4.98 mmol), 1-hydroxy-benzotriazole (720 mg, 5.33 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (954 mg, 4.98 mmol). The temperature was maintained at 0 °C for 2 h, and then the mixture was warmed gradually to ambient temperature. After 22 h the reaction mixture was poured into water (200 mL) and extracted with dichloromethane (2× 200 mL). The combined organic extracts were washed with brine (2×200 mL), dried over Na_2SO_4, and concentrated under reduced pressure; the residue was purified by flash column chromatography (50% EtOAc in hexane) affording 1.95 g (3.68 mmol) of the amide **5** (94% yield) as colorless oil. R_f=0.30 (50% EtOAc in hexanes); $[\alpha]_D^{25}$ −80 (*c* 0.80, $CHCl_3$); ν_{max} (NaCl) 3393, 3284, 1743, 1683, 1633, 1500, 1455 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 9.08 (br s, 2H, ArN*H*), 8.92 (s, 2H, Ar*H*), 8.43 (d, *J*=7.6 Hz, 2H, Ar*H*), 8.29 (d, *J*=8.8 Hz, 1H, Ar*H*), 8.14 (d, *J*=8.8 Hz, 1H, Ar*H*), 7.44–7.24 (m, 20H, Ar*H*+N*H*CO), 6.94 (d, *J*=7.2 Hz, 4H, Ar*H*), 5.10 (m, 2H, C*H*OH), 5.02 (s, 4H, OCH_2Ph), 4.64 (br s, 1H, OC*H*O), 4.57 (br s, 1H, OC*H*O), 4.31 (m, 2H, C*H*NH), 3.98–3.79+3.69–3.53 (m, 4H+m, 6H, CH_2OTHP+ CH_2CH_2O+OH), 1.78–1.50 (m, 12H, CH_2CH_2CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.7, 180.3, 163.0, 162.8, 158.4, 138.2, 138.2, 135.7, 133.3, 128.5, 128.3, 127.9, 127.5, 126.6, 124.1, 124.1, 123.4, 123.3, 122.4, 114.8, 111.6, 99.8, 99.5, 74.3, 73.7, 70.0, 68.4, 68.0, 63.2, 62.5, 55.2, 54.7, 30.7, 30.3, 29.7, 25.2, 25.2, 19.9, 19.4; HRMS (ESI positive ion mode): $[M+H]^+$, found 529.2329. $C_{31}H_{33}N_2O_6$ requires 529.2338.

3.3.3. Amide 6-deprotection. To a stirred solution of amide **5** (1.95 g, 3.68 mmol) in MeOH (100 mL) was added a catalytic amount of *p*-toluenesulfonic acid monohydrate (30 mg) at ambient temperature. After 20 min the acid was quenched with a saturated aqueous $NaHCO_3$ (2 mL), the reaction mixture was poured into water (100 mL) and extracted with EtOAc (4×100 mL). The combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4, and concentrated under reduced pressure; the residue was purified by flash column chromatography (50–80% EtOAc in hexane) affording 1.60 g (3.60 mmol) of the corresponding diol (98% yield) as amorphous white solid. R_f=0.33 (80% EtOAc in hexane); $[\alpha]_D^{25}$ −112 (*c* 0.98, Acetone); ν_{max} (KBr) 3395, 3286, 1736, 1684, 1618, 1509, 1431 cm^{-1}. ^1H NMR (500 MHz, Acetone-d_6): δ 11.3 (br s, 1H, ArN*H*), 8.99 (d, *J*=3.1 Hz, 1H, Ar*H*), 8.38 (m, 1H, Ar*H*), 8.03 (m, 1H, Ar*H*), 7.56 (m, 1H, Ar*H*), 7.46–7.26 (m, 8H, Ar*H*), 6.96 (d, *J*=8.7 Hz, 2H, Ar*H*), 5.17 (m, 1H, C*H*OH), 5.05 (s, 2H, OCH_2Ph), 4.87 (m, 1H, N*H*CO), 4.27 (m, 1H, OH), 4.17 (m, 1H, C*H*NH), 3.84 (m, 1H, CH*H*OH), 3.74 (m, 1H, C*H*HOH), 2.99 (s, 1H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 181.6, 163.3, 159.0, 139.7, 138.4, 137.2, 136.1, 129.2, 128.5, 128.4, 128.1, 127.7, 124.4, 124.4, 123.5, 122.7, 115.2, 113.1, 72.0, 70.3, 62.8, 57.9; HRMS (ESI positive ion mode): $[M+H]^+$, found 445.1777. $C_{26}H_{25}N_2O_5$ requires 445.1763.

3.3.4. Amide 6-protection. The above diol (1.1 g, 2.47 mmol) was dissolved in 2,6-lutidine (4 mL) and TPSCl (1.3 mL, 4.95 mmol) was added at ambient temperature. The mixture was warmed to 55 °C and stirred for 24 h. The reaction mixture was poured into saturated aqueous ammonium chloride (50 mL) and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with saturated aqueous copper sulfate (2×30 mL) brine (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (40% EtOAc in hexane) affording 1.60 g (2.35 mmol) of the amide **6** (95% yield) as colorless oil. R_f=0.60 (50% EtOAc in hexane); $[\alpha]_D^{25}$ −29.0 (*c* 0.48, Acetone); ν_{max} (NaCl) 3398, 1739, 1681, 1641, 1635, 1510, 1430 cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ 10.2 (br s, 1H, ArN*H*), 8.83 (d, *J*=3.2 Hz, 1H, Ar*H*), 8.33 (m, 1H, Ar*H*), 7.86 (d, *J*=9.3 Hz, 1H, Ar*H*), 7.75–7.62 (m, 5H, Ar*H*), 7.55 (m, 1H, Ar*H*), 7.46–7.26 (m, 14H, Ar*H*+CON*H*), 6.92 (d, *J*=8.6 Hz, 2H, Ar*H*), 5.05 (br s, 3H, OCH_2Ph+C*H*OH), 4.23 (br s, 1H, OH), 4.18 (m, 1H, C*H*NH), 3.80 (m, 1H, CH*H*OTBDPS), 3.67 (m, 1H, C*H*HOTBDPS), 1.01 (s, 9H, (CH_3)$_3$C); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.8, 136.4, 136.4, 135.6, 130.9, 130.4, 129.4, 129.3, 128.8, 128.6, 128.5, 124.8, 123.9, 122.7, 115.4, 113.3, 72.2, 70.5, 64.4, 57.7, 27.2, 27.0; HRMS (ESI positive ion mode): $[M+H]^+$, found 683.2920. $C_{42}H_{43}N_2O_5Si$ requires 683.2941.

3.3.5. Diol 7. Sodium borohydride (56 mg, 1.5 mmol) was added in small portions to a stirred solution of amide **6** (500 mg, 0.73 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 (3×30 mL); the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (80% EtOAc in hexane) affording 490 mg (0.72 mmol) of the diol **7** (98% yield) as amorphous white solid. R_f=0.15, 0.22 (50% EtOAc in hexane); ν_{max} (KBr) 3394, 1729, 1664, 1516, 1462 cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ 9.25 (br s, 1H, ArN*H*), 9.21 (br s, 1H, ArN*H*), 7.67–7.58 (m, 6H, Ar*H*), 7.54–7.29 (m, 30H, Ar*H*), 7.27–7.18 (m, 3H, Ar*H*), 7.15–6.86 (m, 8H, Ar*H*), 6.75 (d, *J*=8.5 Hz, 2H, Ar*H*), 6.63 (br s, 1H, N*H*CO), 5.22 (br s, 1H, COC*H*OH), 5.20 (s, 1H, COC*H*OH), 5.09 (br s, 1H, CHC*H*OH), 5.05 (s, 2H, OCH_2Ph), 5.03 (s, 2H, OCH_2Ph), 4.97 (br s, 1H, CHC*H*OH), 4.09 (br m, 4H, C*H*NH+O*H*), 3.90–3.80 (m, 2H, O*H*), 3.78–3.57 (m, 4H, CH_2OTBDPS), 1.05 (s, 18H, (CH_3)$_3$C); HRMS (ESI positive ion mode): $[M+H]^+$, found 685.3117. $C_{42}H_{45}N_2O_5Si$ requires 685.3097.

3.3.6. Morpholinones 8a and 8b. A catalytic amount of pyridinium *p*-toluenesulfonate (10 mg) was added to a stirred solution of diol **7** (490 mg, 0.72 mmol) in acetonitrile (100 mL) at ambient temperature and the mixture was warmed to 80 °C. After completion of the reaction (2 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 (3×30 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (30% EtOAc in hexane) affording, in order of elution, morpholinones **8a** (122 mg, 0.183 mmol) and **8b** (120 mg, 0.180 mmol) (50% combined yield) as colorless oils.

3.3.6.1. Compound 8a. R_f=0.46 (50% EtOAc in hexane); $[\alpha]_D^{25}$ +36.9 (*c* 0.80, MeOH); ν_{max} (NaCl) 3398, 3289, 1666, 1612, 1513, 1454 cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ 9.35 (br s, 1H, ArN*H*), 7.58 (m, 3H, Ar*H*), 7.52 (d, *J*=6.8 Hz, 2H, Ar*H*), 7.48–7.22 (m, 13H, Ar*H*), 7.17 (t, *J*=7.4 Hz, 1H, Ar*H*), 7.06 (d, *J*=8.6 Hz, 2H, Ar*H*), 6.97 (t, *J*=7.4 Hz, 1H, Ar*H*), 6.82 (d, *J*=8.6 Hz, 2H, Ar*H*), 6.64 (br s, 1H, N*H*CO), 5.59 (s, 1H, COC*H*O), 5.03 (s, 2H, OCH_2Ph), 4.73 (d, *J*=9.2 Hz, 1H, CHC*H*O), 3.83 (m, 1H, C*H*NHCO), 3.56 (dd, *J*=11.0, 2.9 Hz, 1H, CH*H*OTPS), 3.38 (dd, *J*=11.0, 4.7 Hz, 1H, C*H*HOTPS), 1.03 (s, 9H, (CH_3)$_3$C); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 159.8, 136.5, 136.4, 130.9, 130.9, 130.1, 129.5, 129.3, 128.9, 128.8, 128.8, 128.6, 127.5, 126.1, 122.9, 120.4, 120.3, 115.6, 112.5, 112.2, 73.4, 70.9, 70.6, 64.1, 59.6, 27.3, 19.8; HRMS (ESI positive ion mode): $[M+H]^+$, found 667.3018. $C_{42}H_{43}N_2O_4Si$ requires 667.2992.

3.3.6.2. Compound 8b. R_f=0.20 (50% EtOAc in hexane); $[\alpha]_D^{25}$ +71.7 (*c* 1.69, MeOH); ν_{max} (NaCl) 3396, 3283, 1676, 1613, 1514, 1460 cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ 9.28 (br s, 1H, ArN*H*), 7.68 (d, *J*=7.8 Hz, 1H, Ar*H*), 7.61 (m, 3H, Ar*H*), 7.56 (t, *J*=6.4 Hz, 1H, Ar*H*), 7.47–7.28 (m, 13H, Ar*H*), 7.21 (d, *J*=8.5 Hz, 2H, Ar*H*), 7.16 (t, *J*=7.4 Hz, 1H, Ar*H*), 7.09 (t, *J*=7.4 Hz, 1H, Ar*H*), 6.87 (d, *J*=8.5 Hz, 2H, Ar*H*), 6.72 (br s, 1H, N*H*CO), 5.46 (s, 1H, COC*H*O), 5.04 (s, 2H, OCH_2Ph), 4.78 (d, *J*=9.7 Hz, 1H, CHC*H*O), 3.96 (m, 1H, C*H*NHCO), 3.53 (d, *J*=4.3 Hz, 2H, CH_2OTPS), 1.06 (s, 9H, (CH_3)$_3$C); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 160.5, 137.1, 137.0, 131.6, 131.6, 130.7, 130.4, 130.1, 129.5, 129.2, 127.7, 126.9, 123.4, 121.2, 121.0, 116.4, 113.1, 78.5, 76.3, 71.3, 64.8, 61.0, 27.9, 20.4; HRMS (ESI positive ion mode): $[M+H]^+$, found 667.2971. $C_{42}H_{43}N_2O_4Si$ requires 667.2992.

3.3.7. General procedure for the preparation of 9a and 9b. To a solution of **8a** or **8b** (30 mg, 0.045 mmol) in a mixture of EtOAc/EtOH (25 mL, 4:1 v/v) at ambient temperature was added a catalytic amount of $Pd(OH)_2$/C (10 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. A solution of the above phenol in THF (1 mL) was treated for 30 min at ambient temperature with 1.0 M solution of TBAF in THF (50 μL). The reaction mixture was poured into saturated aqueous ammonium chloride (5 mL) and extracted with EtOAc (3×5 mL). The combined organic phases were washed with water (3 mL), brine (3 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (40% acetone in dichloromethane) affording 11.6 mg of morpholinone **9a** (0.034 mmol, 76% yield for two steps) or 11.7 mg of morpholinone **9b** (0.035 mmol, 77% yield for two steps) as colorless oils.

3.3.7.1. Compound 9a. R_f=0.27 (50% acetone in dichloromethane); $[\alpha]_D^{25}$ +132.5 (*c* 0.2, MeOH); ν_{max} (NaCl) 3274, 1663, 1618, 1520, 1460 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 1. HRMS (ESI positive ion mode): $[M+H]^+$, found 339.1361. $C_{19}H_{19}N_2O_4$ requires 339.1345.

3.3.7.2. Compound 9b. R_f=0.43 (50% acetone in dichloromethane); $[\alpha]_D^{25}$ +73.2 (*c* 0.6, MeOH); ν_{max} (NaCl) 3314, 1656, 1616, 1518, 1453 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 2. HRMS (ESI positive ion mode): $[M+H]^+$, found 339.1350. $C_{19}H_{19}N_2O_4$ requires 339.1345.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.05.070.

References and notes

1. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S. *Pure Appl. Chem.* **2003**, *75*, 325–336 and literature cited there.
2. Ciminiello, P.; Fattorusso, E.; Forino, M.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Org. Chem.* **2001**, *66*, 578–582.
3. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Am. Chem. Soc.* **2002**, *124*, 13114–13120.
4. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Meglio, P.; Ianaro, A.; Poletti, R. *Tetrahedron* **2004**, *60*, 7093–7098.
5. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A. *Eur. J. Org. Chem.* **2001**, 49–53.
6. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, C.; Fattorusso, E.; Forino, M.; Magno, S. *Tetrahedron* **2001**, *57*, 8189–8192.
7. Latypov, S. K.; Ferreiro, M. J.; Quinoa, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 4741–4751.
8. Couladouros, E. A.; Moutsos, V. I.; Pitsinos, E. N. *Tetrahedron Lett.* **2004**, *45*, 7779–7781.
9. Kawamine, K.; Takeuchi, R.; Miyashita, M.; Irie, H.; Shimamoto, K.; Ohfune, Y. *Chem. Pharm. Bull.* **1991**, *39*, 3170–3171.
10. Shaw, K. N. F.; McMillan, A.; Gudmundson, A. G.; Armstrong, M. D. *J. Org. Chem.* **1958**, *23*, 1171–1178.