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•Original article•

The biologically and ecologically important natural products from the Chinese sea hare *Bursatella leachii*: structures, stereochemistry and beyond

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[ABSTRACT] A novel amide alkaloid, bursatamide A (**1**), featuring an unprecedented propyl-hexahydronaphthalene carbon framework, was isolated from the infrequently studied sea hare *Bursatella leachi*, alongside a new 3-phenoxypropanenitrile alkaloid, bursatellin B (**2**), and twelve known compounds. The structures of **1** and **2** were elucidated through comprehensive spectroscopic data analyses, while their relative and absolute configurations (ACs) were established through total synthesis and a series of quantum chemical calculations, including calculated electronic circular dichroism (ECD) spectra, optical rotatory dispersion (ORD) methods, and DP4+ probability analyses. Bursatamide A (**1**) demonstrated inhibitory effects against the human pathogenic bacteria *Listeria monocytogenes* and *Vibrio cholerae*. *Erythro*-bursatellin B (**21**), a diastereoisomer of **2**, exhibited notable antibacterial activity against the fish pathogenic bacterium Streptococcus parauberis FP KSP28, with an MIC_{90} value of 0.0472 μg·mL^{-1}.

[KEY WORDS] *Bursatella leachii*; Marine natural product; Alkaloid; Sea hare; Antibacterial activity
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Introduction

Bursatella leachii, a distinct species within the *Aplysiidae* family, exhibits a remarkably wide distribution across tropical and subtropical marine environments [1]. Lacking protective hard shells, prey species such as sea hares have evolved diverse strategies to evade predation, developing unique chemical defense mechanisms [2]. One such method involves the release of purple ink and opaline when under attack. These secretions serve a dual purpose: directly deterring predator attacks and alerting nearby conspecifics to potential threats [3]. Notably, the purple ink expelled by sea hares as a defense mechanism contains a rich array of secondary metabolites with potential antibacterial [4] and cytotoxic effects [5]. These compounds include halogenated sesquiterpenes [6], diterpenes [7], polypropionates [8], and mycosporine-like amino acids [3]. Studies on *Bursatella leachii* have been relatively scarce, with only a few morpholine derivatives [9] and long-chain amides [10] isolated thus far. Consequently, further chemical investigation of this species may yield biologically and ecologically significant metabolites.

This investigation focused on *Bursatella leachii* specimens collected from Xiamen Island, Fujian, China. Chemical analyses were performed on the butanol-soluble and ether-soluble fractions of the acetone extract from this sea hare, leading to the isolation of two novel alkaloids: bursatamide A

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These authors have no conflict of interest to declare.

(**1**) and bursatellin B (**2**), along with twelve previously identified compounds. This paper presents the isolation process, structural elucidation (with particular emphasis on the complex stereochemistry determination), and biological assessment of these secondary metabolites.

Results and Discussion

Isolation and structure elucidation

The standard processing of the purple ink, epidermis, and residual tissue of the subject animal yielded pure compounds **1**–**14**. Chemical analysis of the purple ink resulted in the isolation of seven compounds (**2**, **3**, and **9**–**13**), while the ethyl ether-soluble portion of the epidermis produced four compounds (**3**–**6**). Additionally, the ethyl ether-soluble portion of the residual tissue yielded seven compounds (**1**, **4**–**8**, and **14**), respectively (Fig. 1).

The known compounds **3** to **14** were definitively identified as bursatellin (**3**) [11], cholesterol (**4**) [12], (3*β*,5*α*,8*α*)-5,8-Epidioxycholesta-6,9(11)-dien-3-ol (**5**) [13], 5*α*,8*α*-epidioxy, (3*β*,5*α*,8*α*)-5,8-epidioxycholest-6-en-3-ol (**6**) [14], *apo*-9′-fucoxanthinone (**7**) [15], (*S*)-5-hydroxy-3,4-dimethyl-5-pentyl-furan-2(5H)one (**8**) [16], thymidine (**9**) [17], deoxyguanosine (**10**) [18], deoxyadenosine (**11**) [19], adenosine (**12**) [20], cytidine(**13**) [21] and pyropheophorbide A (**14**) [22] through a comparative analysis of their nuclear magnetic resonance (NMR) data with previously reported values.

Bursatamide A, $[\alpha]_D^{20}$ +125 (*c* 0.25, CH_3OH), was isolated as a colorless oil. Its molecular formula, $C_{22}H_{27}NO_2$, was determined by a high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) ion peak at *m*/*z* 360.1934 $[M + Na]^+$, Calcd. 360.1934), indicating ten degrees of unsaturation. The ^1H NMR spectrum (Table 1) and ^1H–^1H correla-

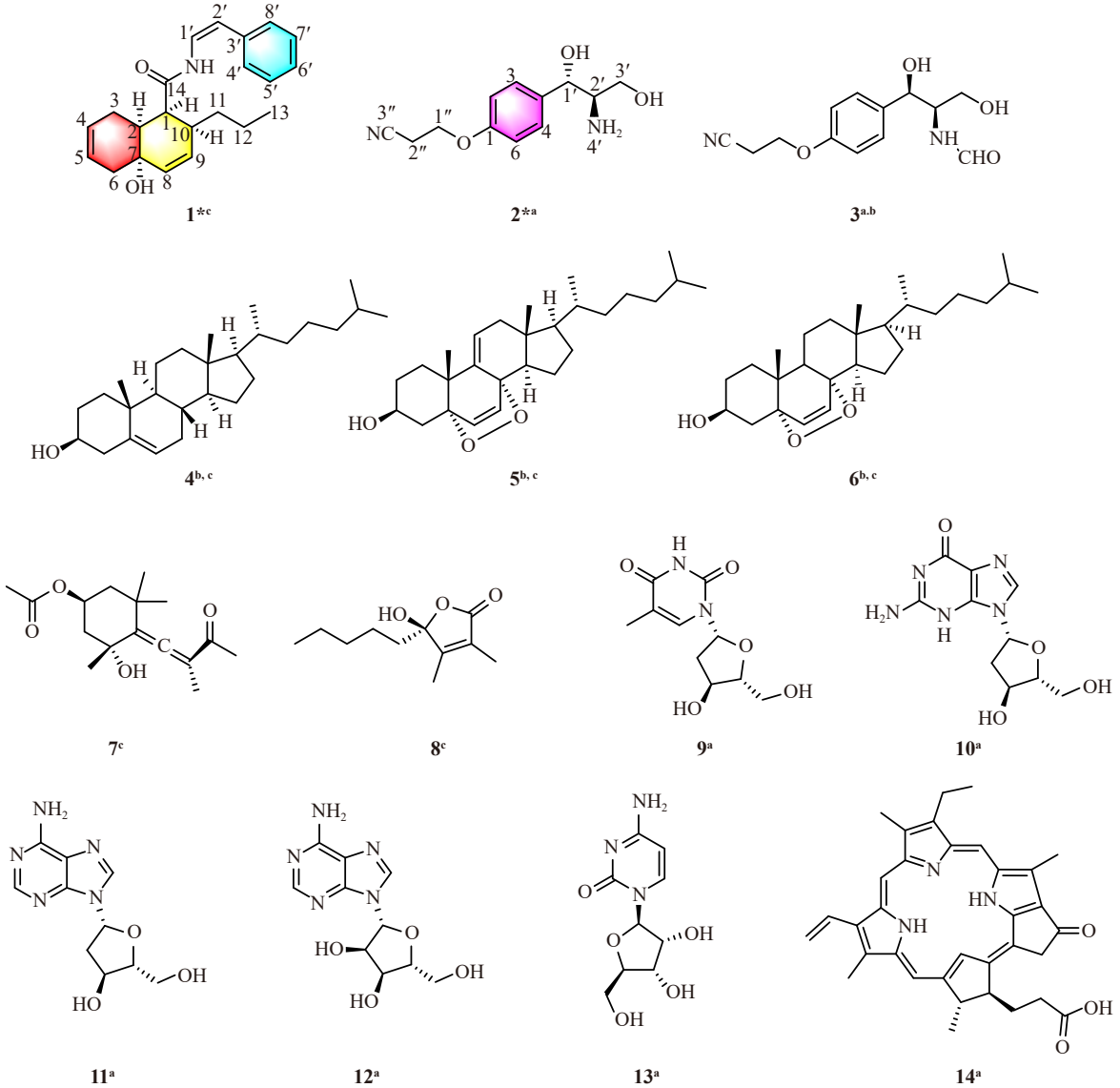


Fig. 1 Structures of compounds 1−14. (Compounds marked * were new. Compounds marked a, b and c were isolated from purple ink, epidermis, and the residual tissue, respectively.)

Table 1 ^{1}H NMR (δ_H) and ^{13}C NMR (δ_C) data of 1 in CD_3OD

No.	1	
	δ_H, mult (*J*, Hz)	δ_C
1	2.51 m	51.1 CH
2	2.12 dd (12.0, 6.3)	42.1 CH
3a	1.83 dd (18.4, 5.35)	25.6 CH_2
3b	2.51 m	
4	5.69 m	124.7 CH
5	5.59 m	124.3 CH
6a	2.16 m	37.4 CH_2
6b	2.16 m	
7		70.5 C
8	5.74 dd (10.1, 2.6)	136.9 CH
9	5.52 dd (10.1, 2.3)	129.3 CH
10	2.58 m	41.0 CH
11a	1.25 m	37.8 CH_2
11b	1.35 m	
12a	1.25 m	20.5 CH_2
12b	1.45 m	
13a	0.9 t (7.09)	
13b	0.9 t (7.09)	14.7 CH_3
13c	0.9 t (7.09)	
14		176.0 C
1′	6.81 d (9.6)	122.4 CH
2′	5.85 d (9.7)	114.3 CH
3′		136.9 C
4′	7.34 m	129.6 CH
5′	7.34 m	129.6 CH
6′	7.23 tt (5.6, 5.8)	128.0 CH
7′	7.34 m	129.6 CH
8′	7.34 m	129.6 CH

tion spectroscopy (COSY) analysis revealed one methyl signal at δ_H 0.9, five protons in an aromatic moiety at δ_H 7.34 (m, H-4′, H-5′, H-7′, H-8′, 4H), δ_H 7.23 (tq, *J* = 5.7, 2.6 Hz, H-6′, 1H) and three sets of olefinic protons at δ_H 5.74 (dd, *J* = 10.1, 2.6 Hz, H-8)/δ_H 5.52 (dd, *J* = 10.1, 2.3 Hz, H-9), δ_H 6.81 (d, *J* = 9.7, H-1′)/δ_H 5.85 (d, *J* = 9.7, H-2′), and δ_H 5.69 (m, H-4)/δ_H 5.59 (m, H-5).

The ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), and heteronuclear single quantum coherence (HSQC) spectra indicated 22 carbon signals in **1**, comprising one methyl (δ_C 14.7), four sp^3 methylenes (δ_C 20.5, 25.6, 37.4, and 37.8), three sp^3 methines (δ_C 41.0, 42.1, and 51.1), one oxygenated sp^3 quaternary carbon (δ_C 70.5), three disubstituted carbon-carbon double bonds (δ_C 114.3 and 122.4, δ_C 124.7 and 124.3, δ_C 129.3 and 136.9), and one ketone group (δ_C 176.0). The presence of one ketone, three double bonds, and a benzene group accounted for eight degrees of unsaturation. Consequently, the remaining structure consisted of a tricyclic carbon framework incorporating a benzene group.

The planar structure of **1** was further elucidated through ^{1}H–^{1}H COSY and heteronuclear multiple bond correlation (HMBC) experiments. Two structural fragments **a**–**b** were identified by a careful analysis of the ^{1}H–^{1}H COSY spectrum of **1**, revealing clear correlations of H_2-11 (δ_H 1.35, 1.25) /H_2-12 (δ_H 1.45, 1.25) / H_3-13 (δ_H 0.88) (**a**), H-4 (δ_H 5.69) /H_2-3 (δ_H 1.83, 2.51) /H-2 (δ_H 2.12) /H-1 (δ_H 2.51) /H-10 (δ_H 2.58) / H-9 (δ_H 5.52) /H-5 (δ_H 5.59) /H_2-6 (δ_H 2.16) (**b**). The connection of these fragments was further determined through analysis of the HMBC spectrum. Key HMBCs of H_2-3 (δ_H 1.83, 2.51) and H-9 (δ_H 5.52) to C-7 (δ_C 70.5) established the connection of fragments **a** and **b**, forming a propyl-hexahydronaphthalene carbon framework A. The distinct HMBCs

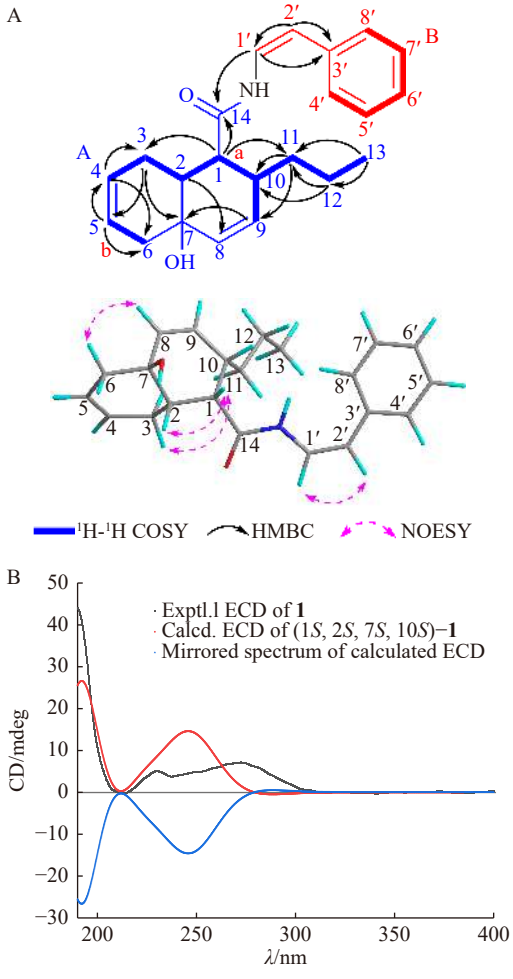


Fig. 2 A).^{1}H–^{1}H COSY, key HMBC, and NOESY correlations of 1. B). Experimental ECD spectrum (black), calculated spectrum (red), and mirrored spectrum of calculated ECD (blue) of 1.

from H-1′ (δ_H 6.81) /H-2′ (δ_H 5.85) to C-3′ (δ_C 136.9) indicated the presence of a styrene group as fragment B, considering the previously identified double bond at $\Delta^{1',2'}$ and a benzene group. The connection between **A** and **B** was determined to be an amide bond based on the HMBC correlations of H-1 (δ_H 6.81) to C-14 (δ_C 176.0), resulting in the construction of a novel propyl-hexahydronaphthalene amide carbon framework of **1** (Fig. 2).

Elucidating the relative configuration (RC) of **1** presented a significant challenge due to the arrangement of four consecutive chiral carbons (C-1, C-2, C-7, and C-10), which could only be partially characterized using the nuclear Overhauser effect spectroscopy (NOESY) spectrum. Furthermore, the oily nature of **1** impeded the formation of suitable crystals. To overcome this obstacle, we employed NMR calculations, a well-established and reliable method for characterizing structurally complex natural products [23]. Specifically, we utilized the time-dependent density functional theory (TDDFT) gauge-independent atomic orbital (GIAO) method, renowned for its efficacy in such analyses [24].

Compound **1** contains four chiral centers, resulting in eight possible RC arrangements (**1a**: 1*S**,2*R**,7*S**,10*S**, **1b**: 1*R**,2*R**,7*R**,10*S**, **1c**: 1*S**,2*R**,7*S**,10*R**, **1d**: 1*S**,2*S**,7*S**,10*S**, **1e**: 1*R**,2*R**,7*S**,10*S**, **1f**: 1*S**,2*R**,7*R**,10*S**, **1g**: 1*R**,2*S**,7*S**, 10*S**, **1h**: 1*R**,2*R**,7*S**,10*R**). To determine the correct configuration, conformational searches were conducted on all eight potential diastereoisomers. To optimize computational efficiency, an energy cutoff of 21 kJ·mol^{-1} was applied. Geometric optimization was subsequently performed at the DFT level using the B3LYP functional and the 6-311G (d,p) basis set. NMR calculations were then executed at the PCM/mPW1PW91/6-31G(d) level [25]. The GIAO approach was utilized to compute NMR shielding constants. The average of the shielding constants over the Boltzmann distribution for each stereoisomer was calculated and compared with the experimental data. This method revealed that the 1*S**, 2*S**, 7*S**, 10*S** isomer exhibited the best match (100%) with the experimentally observed NMR data for **1**, which aligns with the NOE cross-peak results for H-2 and H-10. (For more details, refer to the Supporting Information). The coupling constant of H-1′ (J = 9.6 Hz) and H-2′ (J = 9.7 Hz) indicated that the double bond at $\Delta^{1',2'}$ has a cis -relationship.

The absolute configuration (AC) of **1** was established through TDDFT electronic circular dichroism (TDDFT ECD) calculations [26]. As illustrated in Fig. 2B, the Boltzmann-averaged ECD spectrum of (1*S*, 2*S*, 7*S*, 10*S*)-**1** exhibited a close correspondence to the experimental ECD curve of **1**, facilitating the assignment of the AC as (1*S*, 2*S*, 7*S*, 10*S*)-**1**.

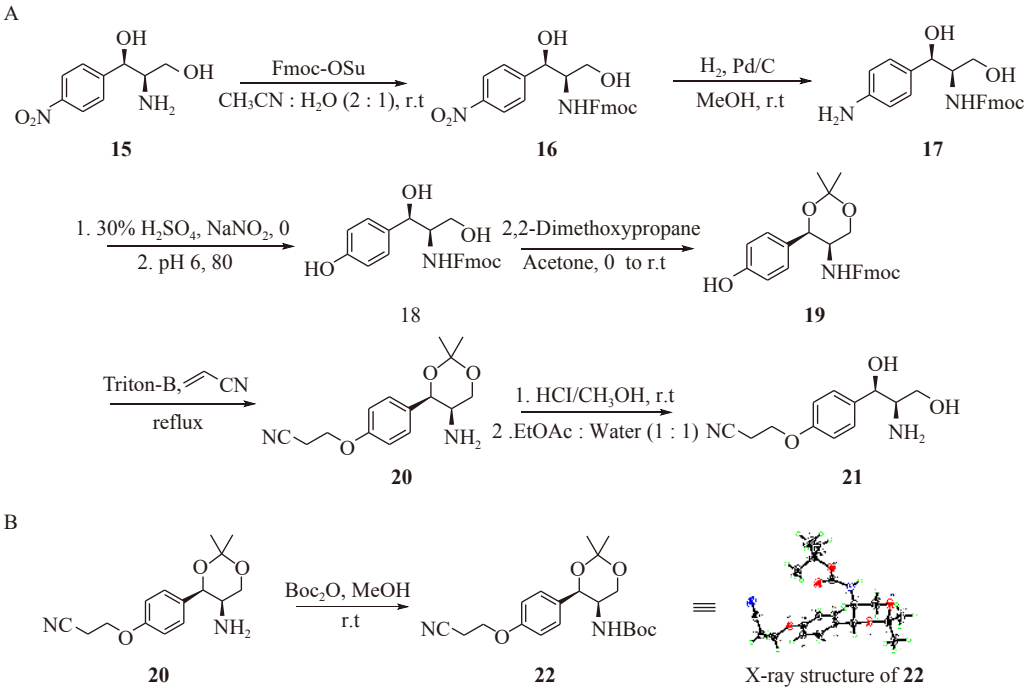
Bursatellin B (**2**), $[\alpha]_D^{20}$ +62.1 (*c* 1.0, CH_3OH), was isolated as a colorless oil. Its molecular formula, $C_{12}H_{16}N_2O_3$, was determined by an HR-ESI-MS ion peak at *m/z* 237.1231 $[M + H]^+$, Calcd. 237.1234), six degrees of unsaturation. The ^{1}H NMR spectrum of **2** revealed one benzene ring at (d, J = 8.7, H-2), δ_H 6.97 (d, J = 8.7, H-3), δ_H 6.97 (d, J = 8.7, H-5) and δ_H 7.33 (d, J = 8.7, H-6). The ^{13}C NMR indicated 12 carbon signals in **2**, including one disubstituted benzene ring (δ_C115.7, 115.7, 129.1, 129.1, 136.4 and 159.3), four sp^3 secondary carbons (δ_C 19.0, 60.0, 62,8, and 64.3 one sp^3 primary carbon (δ_C 74,2) and one nitrile group (δ_C 119.2). The signals observed in the ^{13}C, ^{1}H, and DEPT spectra of **2** demonstrated similarity to bursatellin (**3**) as described by Francis J *et al.* in 1987 [11]. The main differences were observed at C-2′ (δ_C 60.0 for **2**, δ_C 62.5 for **3**) and its neighboring carbon C-1′ (δ_C 74.2 for **2**, δ_C 72.0 for **3**), indicating a change in the substituent group of C-2′. Comparison of the molecular weight of **2** (HR-ESI-MS ion peak at *m/z* 237.1231 $[M + H]^+$ with that of **3** (HR-ESI-MS ion peak at *m/z* 266.9169 $[M + H]^+$ revealed that the aldehyde group at N-4′ in **2** was replaced by an H atom in **3**. Consequently, the planar structure of **2** was elucidated.

As bursatellin B is a colorless oil, making the cultivation of single crystals challenging, its AC was determined using a combination of chemical synthesis and optical rotatory dispersion (ORD). Our initial strategy involved the total synthesis of bursatellin B. However, since the AC of bursatellin B was unknown, we began by synthesizing the 1′*R*,2′*R* stereoisomer of bursatellin B.

The synthesis of amine **17** was accomplished in two steps from a commercially available chloramphenicol-free base (**15**). Initially, the amino group was protected using 9-fluorenylmethyl *N*-succinimidyl carbonate [27], followed by hydrogenation of the nitro group under Pd/C catalysis [28]. Subsequent diazotization of amine **17** and hydrolysis of the aryl diazonium salt yielded phenol **18** [29]. The diol group was then protected to form acetonide **19** by treatment with 2,2-dimethoxypropane and *p*-Toluenesulfonic acid at room temperature [30]. As reported by Dethe *et al.* [31], the oxa-Michael reaction presents challenges in the cyanoethylation of the phenolic hydroxyl group in compound **20** due to retro-Michael fragmentation. Consequently, attempts to achieve cyanoethylation using 3-bromopropionitrile were unsuccessful. After multiple trials, a dual transformation was achieved, involving Fmoc group deprotection and cyanoethylation of the phenolic hydroxyl group. This was accomplished using acrylonitrile with 10 mol% of triton-B under reflux conditions, yielding **20** at 39% [32]. Finally, **20** was deprotected to obtain **21** (MeOH; Hydrochloric acid; r.t.; 24 h) with a 90% yield (Scheme 1A).

During the synthesis of compound **18**, treatment with 30% H_2SO_4 posed a risk hydroxyl group configuration inversion under acidic conditions. To mitigate this risk, we trapped the intermediate amine with amino derivative **22** in methanol, achieving an 80% yield (Scheme 1B) [33]. Notably, product **22** was successfully crystallized in dichloromethane (CCDC: 2323123), confirming its RC as 1′*R*, 2′*R* and preventing any undesired inversion of the hydroxyl group during the reaction.

Comparison of the NMR data for final products **21** and **2** revealed distinct differences in the chemical shifts of C-1′ and C-3′ (Table 2). Given that compound **2** possesses two



Scheme 1 A). Synthetic route of 21. B). Synthetic route of 22 and perspective ORTEP drawings of the X-ray structures of 22 (displacement ellipsoids are drawn at the 50% probability level).

Table 2 ^{1}H NMR (δ_H) and ^{13}C NMR (δ_C) data of 2 and 21 in CD_3OD

No.	2		21	
	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C
1		159.3 C		159.8 C
2	7.33 (8.7)	129.1 CH	7.38 d (8.7)	129.3 CH
3	6.97 (8.7)	115.7 CH	7.01 d (8.6)	115.9 CH
4		136.4 C		134.9 C
5	6.97 (8.7)	115.7 CH	7.01 d (8.6)	115.9 CH
6	7.33 (8.7)	129.1 CH	7.38 d (8.7)	129.3 CH
1′	4.56 d (7.6)	74.2 CH	4.71 d (8.9)	71.7 CH
2′	3.00 m	60.0 CH	3.28 m	60.3 CH
3′a	3.34 m	62.8 CH_2	3.41 dd (11.7, 6.1)	59.7 CH_2
3′b	3.46 dd (11.1, 4.1)		3.54 dd (11.7, 3.6)	
1″	4.20 t (6.0)	64.3 CH_2	4.21 t (5.9)	64.3 CH_2
2″	2.93 t (6.0)	19.0 CH_2	2.94 t (5.9)	19.0 CH_2
3″		119.2 C		119.1 C

chiral centers, we deduced its RC of **2** as 1′*S**, 2′*R** by comparing the NMR data with that of (1′*R*, 2′*R*)-**21**.

Determining the AC of **2** presents significant challenges, as CD spectroscopy measurements of compound **2** are not viable due to insufficient absorption of the chromophore. However, ORD emerges as an effective method for AC determination by comparing experimental measurements with calculated spectra [34]. At the Na–D line (589 nm), **2** displays a substantial specific rotation, $[\alpha]_D^{20}$ +62.1, enabling a feasible comparison between experimental and calculated optical rotations for AC assignment. The configuration assignment for compound **2** was derived from ORD data for 13 conformers of **1** in the gas phase at three wavelengths (589, 546, and 365 nm) using the 6-311 + G (d) level of theory (for detailed information, please refer to the supplementary information). The general trends between the experimental and computed

data for (1*S*,2*R*)-**2** demonstrate acceptable consistency. Based on this evidence, **2** was identified, as shown in Fig. 3, namely, bursatellin B.

Bioactivity

The antimicrobial efficacy of all compounds was evaluated against several pathogenic bacteria. The results indicate that *erythro*-bursatellin B (**21**) demonstrated significant inhibition against the fish pathogenic bacterium Streptococcus parauberis FP KSP28, with a minimum inhibitory concentration for 90% (MIC_{90}) of 0.0472 μg·mL^{-1}(Table 3). This finding suggests that the RC at carbons C-1′ and C-2′ of **2** contributes to the observed differences in antibacterial activity.

Furthermore, compound **1** exhibited inhibitory activity against the human pathogenic bacteria *Listeria monocytogenes* and *Vibrio cholerae*, with MIC_{90} values of 33.7 and 12.5 μg·mL^{-1}, respectively.

Chemoecology analysis

The chemical analysis of *Bursatella leachii* in this study was motivated by the distinctive physiological effects of its secondary metabolites. As illustrated in Fig. 4, Bursatellin (**3**), present in the epidermal extract, was absent from visceral extracts. This observation suggests that bursatellin may function as a potential sunscreen agent in the organism [35].

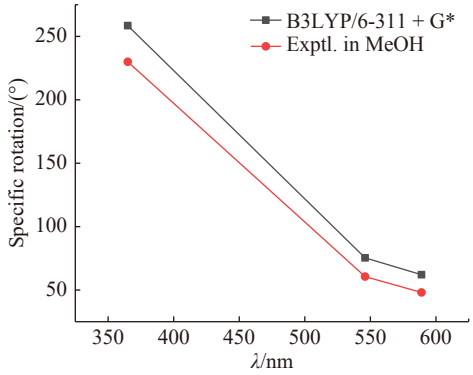


Fig. 3 Assignment of the AC of 2 based on specific rotations. Experimental (red) and computed of (1*S*, 2*R*)-2 (black) specific rotations at different wavelengths.

Kicklighter *et al.* proposed that uracil and the nucleosides uridine and cytidine function as alarm signals in the purple ink and opaline secreted by sea hares during predatory attacks [2]. These signals can elicit avoidance behavior in neighboring species. In the present study, in addition to isolating cytidine (**13**) from the purple ink and opaline, other nucleoside compounds were identified, including thymidine (**9**), deoxyguanosine (**10**), deoxyadenosine (**11**), and aden-

Table 3 Antibacterial activity assay results of 2 and 21

Compounds	MIC_{90} (μg·mL^{-1})		
	Streptococcus parauberis FP KSP28	*Pseudomonas fulva* ZXM181	*Phoyobacterium damselae* FP2244
Ampicillin sodium	4.64	37.14	0.02
natural bursatellin B (**2**)	23.6	23.6	23.6
erythro-bursatellin B (**21**)	0.0472	23.6	11.8

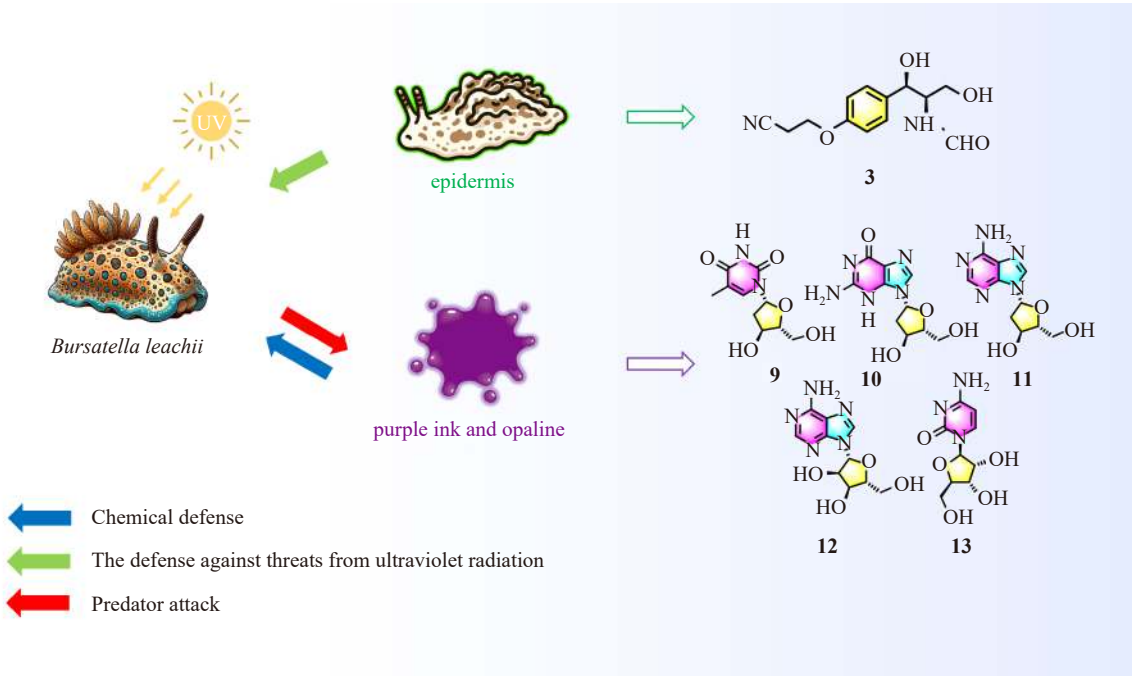


Fig. 4 Physiological effects of the secondary metabolites in *Bursatella leachii*.

osine (**12**). These compounds may serve as distinctive intraspecific chemical signals in *Bursatella leachii*.

Conclusions

In conclusion, the chemical analysis of *Bursatella leachii* has resulted in the isolation and comprehensive characterization of two novel compounds: bursatamide A (**1**), a unique amide, and bursatellin B (**2**), a new phenoxypropanenitrile alkaloid. While amides are a well-established class of natural products in sea hares, bursatamide A features a previously undescribed disubstituted-hexahydronaphthalene scaffold, making it the first example of this carbon framework. This discovery not only expands the structural diversity of marine amides but also adds to the complexity of marine natural products.

The stereochemistry of compounds **1** and **2** was elucidated through total synthesis, DP4+ probability, ORD, and TDDFT-ECD calculations, offering diverse approaches to address the challenges in determining the stereochemistry of flexible carbon chain skeleton molecules without single crystals. The novel amide bursatamide A (**1**) exhibited inhibitory activity against the human pathogenic bacteria *Listeria monocytogenes* and *Vibrio cholerae*. Analysis of sea hare purple ink components revealed that bursatellin B (**2**) demonstrates antibacterial activity against fish pathogenic bacteria. The *erythro*-bursatellin B (**21**) displayed more potent antimicrobial activity against Streptococcus parauberis FP KSP28 and *Phoyobacterium damselae* FP2244, providing valuable insights for the identification of potential antibiotic lead compounds targeting fish pathogenic bacteria.

This study also addresses the ecological roles of these compounds in the sea hare lifecycle. However, further investigation is necessary to elucidate these functions fully. Such research may facilitate more precise and targeted pharmacological investigations.

Experimental

General experimental procedures.

IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. UV spectra were obtained using a Varian Cary 300 Biospectrophotometer. Optical rotations were measured with a PerkinElmer 241MC polarimeter. Circular dichroism spectroscopy was performed on a Chirascan-plus V100 spectrometer. ESI-MS and HR-ESI-MS spectra were acquired using a Q-TOF Micro LCMS-MS mass spectrometer. EIMS and HR-EI-MS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. NMR spectra were measured on either a Bruker DRX-500 or a Bruker DRX-400 spectrometer with the residual $CHCl_3$ (δ_H 7.26 ppm, δ_C 77.16 ppm) and CH_3OH (δ_H 3.31 ppm, δ_C 49.00 ppm) as internal standards. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. Structural assignments were made with additional information from ^1H–^1H COSY, HSQC, NOESY, and HMBC experiments. All solvents used for CC were of analytical grade, and solvents used for HPLC were of HPLC grade. Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 10 mm (i.d.) × 25 cm] column) was also employed. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 300−400 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Extraction and isolation

Purple ink and opaline collected from *Bursatella leachii* were first filtered to remove particulate matter, concentrated under reduced pressure, and dissolved in methanol. The resulting solution was further purified by filtration through a 0.22 μm microporous membrane. Subsequent high-performance liquid chromatography (HPLC) of the methanol extract led to the isolation of a novel compound **2** and previously identified compounds (**3, 9**–**13**).

Additionally, the epidermis and residual tissue from the sea hare specimens were sectioned and subjected to comprehensive extraction using acetone. The ethyl ether fraction of the acetone extract was then purified through multiple chromatographic steps, including MCI gel and silica gel column chromatography, leading to the isolation of compounds (**1**, **4**–**8**, and **14**).

Antibacterial activity assays

The marine strains *Streptococcus parauberis* FP KSP28, *Pseudomonas fulva* ZXM181, and Phoyobacterium damselae FP2244 were provided by the National Fisheries Research & Development Institute, Korea. The human pathogenic bacteria *Vibrio cholerae* and *Listeria monocytogenes* were supplied by Jiangsu Marine Fisheries Research Institute. The MIC_{90} values for all antimicrobial agents were determined using the 96-well micro-dilution method. Mueller–Hinton II broth (cation-adjusted, BD 212322) was utilized for determining MIC_{90} values. Compounds were initially prepared as stock solutions at a concentration of 20 mmol·L^{-1} in DMSO. All samples were subsequently diluted with culture broth to achieve an initial concentration of 500 μmol·L^{-1}. Serial 1 : 2 dilutions were then performed by adding culture broth to obtain concentrations ranging from 500 μmol·L^{-1} to 0.24 μmol·L^{-1}. 100 μL of each dilution was dispensed into 96-well plates, including sterile controls, growth controls (containing culture broth plus DMSO, without compounds), and positive controls (containing culture broth plus control antibiotics such as tetracycline). Each test and control well received 5 μL of an exponential-phase bacterial suspension (approximately 10^5 CFU/well). The 96-well plates were incubated at 37 °C for 24 h. MIC_{90} values for these compounds were defined as the lowest concentration that completely inhibited bacterial growth. All MIC_{90} values were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Bursatamide A (1) (2.2 mg): colorless oil; $[\alpha]_D^{20}$ +125 (*c*

0.25, CH_3OH); IR (KBr) ν_{max} 3423, 2958, 2924, 1679, 1648, 1502, 1477, 1201 cm^{-1}; CD (CH_3CN) λ ($\Delta\varepsilon$) 230.1 (+5.02), 272.6 (+7.12); for ^1H and ^{13}C NMR data (Table 1); HR-ESI-MS (ESI) *m/z* 360.1930 $[M + Na]^+$, Calcd. for $C_{22}H_{27}NNaO_2$, 360.1934).

Bursatellin B (2) (3.5mg): colorless oil; $[\alpha]_D^{20}$ +62.0 (*c* 0.50, CH_3OH); IR (KBr) ν_{max} 3346, 2913, 2252, 2028, 1611, 1587, 1513, 1474, 1435, 1405, 1312, 1179, 1021, 953, 896 cm^{-1}; for ^1H and ^{13}C NMR data (Table 2); HR-ESI-MS (ESI) *m/z* 237.1231 $[M + H]^+$, Calcd. for $C_{12}H_{17}N_2O_3$, 237.1234).

(9*H*-fluoren-9-yl)methyl ((1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)carbamate (16): A solution of **15** (2.12 g, 10 mmol) and $NaHCO_3$ (168 mg, 2 mmol) in acetonitrile (100 mL) and water (50mL) was prepared, to which Fmoc-Osu (3.37 g, 10 mmol) was added. The reaction mixture was stirred at room temperature for 10 h. Subsequently, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with a saturated NaCl solution. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). Purification *via* column chromatography, using MeOH/CH_2Cl_2 (4 : 96) as the eluent, yielded **16** (3.96 g, 91%). ^1H NMR (400 MHz, Methanol-d_4) δ 8.11 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.67–7.49 (m, 4H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.35–7.16 (m, 2H), 5.07 (d, *J* = 2.9 Hz, 1H), 4.29–4.19 (m, 1H), 4.18– 4.12 (m, 1H), 4.08 (d, *J* = 6.7 Hz, 1H), 3.87 (d, *J* = 2.9 Hz, 1H), 3.79–3.72 (m, 1H), 3.60–3.51 (m, 1H), two signal due to proton (OH) was not observed; ^{13}C NMR (101 MHz, MeOD) δ 158.57, 152.01, 148.45, 145.24, 142.55, 128.73, 128.37, 128.07, 126.08, 124.07, 120.89, 71.88, 67.66, 62.68, 59.48, 48.31. HR-ESI-MS (ESI) *m/z* 435.1551 $[M + H]^+$, Calcd. for $C_{24}H_{23}N_2O_6$, 435.1551).

(9*H*-fluoren-9-yl)methyl ((1*R*,2*R*)-1-(4-aminophenyl)-1,3-dihydroxypropan-2-yl)carbamate (17): TA stirred mixture of **16** (3.8 g, 9.3 mmol) in MeOH (120 mL) was combined with Pd/C (380 mg) in a hydrogenation chamber equipped with a Parr hydrogenation apparatus. The chamber was thoroughly purged. The reaction mixture was stirred at ambient temperature for 10 h. Hydrogen was released cautiously. The solvent was removed under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). The resulting material was purified *via* column chromatography, using MeOH/CH_2Cl_2 (3 : 97) as the eluent, to yield amine **17** (3.17 g, 90%). ^1H NMR (600 MHz, Methanol-d_4) δ 7.77 (dd, *J* = 7.6, 2.1 Hz, 2H), 7.62 (dd, *J* = 13.9, 7.5 Hz, 2H), 7.37 (td, *J* = 7.5, 4.3 Hz, 2H), 7.30 (dt, *J* = 10.2, 7.5 Hz, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.2 Hz, 2H), 4.75 (d, *J* = 4.8 Hz, 1H), 4.33 (dd, *J* = 10.2, 6.6 Hz, 1H), 4.22–4.12 (m, 2H), 3.80 (q, *J* = 5.7 Hz, 1H), 3.65 (dd, *J* = 11.0, 5.8 Hz, 1H), 3.45 (dd, *J* = 11.0, 6.2 Hz, 1H); ^{13}C NMR (151 MHz, MeOD) δ 158.82, 147.94, 145.39, 145.25, 142.51, 133.04, 128.72, 128.35, 128.16, 128.12, 126.31, 126.21, 120.86, 116.39, 73.14, 67.82, 62.85, 59.95, 48.38. HR-ESI-MS (ESI) *m/z* 427.1630 $[M + Na]^+$, Calcd. for $C_{24}H_{24}N_2NaO_4$, 427.1630).

(9*H*-fluoren-9-yl)methyl ((1*R*,2*R*)-1,3-dihydroxy-1-(4-hydroxyphenyl)propan-2-yl)carbamate (18): A stirred mixture of amine **17** (3.10 g, 7.7 mmol) in 30% aqueous H_2SO_4 solution was treated with $NaNO_2$ (106.26 mg, 1.54 mmol) and magnetically stirred at 0 °C for 30 min. The solution was then alkalized to pH 6 with $NaHCO_3$. The resulting mixture was heated at 80 °C for 30 min. The residue was subsequently dissolved in EtOAc and washed with a saturated NaCl solution. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). Purification *via* column chromatography, using MeOH/CH_2Cl_2 (3 : 97) as the eluent, yielded phenol **18** (1.34 g, 43%). ^1H NMR (600 MHz, Methanol-d_4) δ 7.75 (dd, *J* = 7.7, 2.3 Hz, 2H), 7.59 (dd, *J* = 16.3, 7.5 Hz, 2H), 7.36 (td, *J* = 7.4, 5.0 Hz, 2H), 7.28 (dt, *J* = 12.1, 7.4 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 3H), 6.75 (d, *J* = 8.3 Hz, 2H), 4.82 (d, *J* = 4.6 Hz, 1H), 4.32 (dd, *J* = 10.3, 6.7 Hz, 1H), 4.20–4.08 (m, 2H), 3.82 (q, *J* = 5.7 Hz, 1H), 3.68 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.48 (dd, *J* = 11.0, 6.1 Hz, 1H); ^{13}C NMR (151 MHz, MeOD) δ 158.78, 157.75, 145.30, 145.20, 142.47, 134.39, 128.69, 128.62, 128.12, 128.08, 126.25, 126.15, 120.84, 115.94, 72.88, 67.80, 62.84, 59.90, 48.32; HR-ESI-MS (ESI) *m/z* 428.1469 $[M + Na]^+$, Calcd. for $C_{24}H_{23}NNaO_5$, 428.1468).

(9*H*-fluoren-9-yl)methyl ((4*R*,5*R*)-4-(4-hydroxyphenyl)-2,2-dimethyl-1,3-dioxan-5-yl)carbamate (19): A stirred mixture of 2, 2-dimethoxypropane (1.0 g, 9.63 mmol) in acetone (20 mL) was combined with phenol **18** (1.3 g, 3.21 mmol) and *p*-Toluenesulfonic acid (55.2 mg) at 0 °C. The mixture was subsequently stirred for an additional 8 h at 25 °C. The solvent was then evaporated under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). The resulting material was purified *via* column chromatography, using ether/EtOAc (2 : 1) as the eluent, to yield **19** (621 mg, 43%). ^1H NMR (600 MHz, Methanol-d_4) δ 7.77 (d, *J* = 7.2 Hz, 2H), 7.50 (ddd, *J* = 7.7, 5.2, 1.2 Hz, 2H), 7.37 (q, *J* = 7.1 Hz, 2H), 7.28 (dtd, *J* = 14.9, 7.5, 1.1 Hz, 2H), 7.23–7.19 (m, 2H), 6.73–6.69 (m, 2H), 5.21 (d, *J* = 2.2 Hz, 1H), 4.34 (dd, *J* = 11.7, 1.9 Hz, 1H), 4.25 (dd, *J* = 10.4, 6.8 Hz, 1H), 4.07 (t, *J* = 7.2 Hz, 1H), 4.00 (dd, *J* = 10.4, 7.5 Hz, 1H), 3.79–3.74 (m, 2H), 1.58 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (151 MHz, MeOD) δ 158.22, 157.74, 145.37, 145.10, 142.50, 142.44, 131.19, 128.73, 128.71, 128.29, 128.21, 128.15, 126.39, 126.13, 120.85, 120.83, 115.80, 100.79, 73.43, 67.95, 65.70, 50.89, 48.30, 29.83, 19.11; HR-ESI-MS (ESI) *m/z* 446.1966 $[M + H]^+$, Calcd. for $C_{27}H_{29}NO_5$, 446.1962).

3-(4-((4*R*,5*R*)-5-amino-2,2-dimethyl-1,3-dioxan-4-yl)phenoxy)propanenitrile (20): A stirred mixture containing a catalytic amount of Triton B in acrylonitrile (10.0 mL) was combined with a solution of **19** (621 mg, 1.39 mmol) and refluxed for 10 h. Subsequently, the solvent was evaporated under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). The resulting material was purified *via* column chromatography, using MeOH/CH_2Cl_2

(5 : 95) as the eluent, yielding **20** (148 mg, 39%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.34–7.28 (m, 2H), 7.02–6.94 (m, 2H), 5.17 (d, J = 1.9 Hz, 1H), 4.35 (dd, J = 11.9, 2.2 Hz, 1H), 4.20 (t, J = 6.0 Hz, 2H), 3.79 (dd, J = 11.9, 1.8 Hz, 1H), 2.92 (t, J = 6.0 Hz, 2H), 2.73 (q, J = 1.9 Hz, 1H), 1.55 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 158.90, 133.73, 128.13, 119.17, 115.70, 100.59, 74.00, 66.16, 64.27, 50.48, 30.00, 19.02, 18.97; HR-ESI-MS (ESI) *m/z* 277.1549, Calcd. for $C_{15}H_{22}N_2O_3$, 277.1547).

3-(4-((1*R*,2*R*)-2-amino-1,3-dihydroxypropyl) phenoxy)propanenitrile (21): A stirred mixture of **20** (100 mg, 361.88 μmol) in methanol (10 mL) and 6 mol·L^{-1} HCl solution (5 mL) was maintained at room temperature for 2 h. The solvent was subsequently evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with a saturated NaCl solution. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting colorless oil, **21**, was obtained (77 mg, 90% yield). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.38 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 4.71 (d, J = 8.9 Hz, 1H), 4.21 (t, J = 5.9 Hz, 2H), 3.54 (dd, J = 11.7, 3.6 Hz, 1H), 3.41 (dd, J = 11.7, 6.1 Hz, 1H), 3.33–3.26 (m, 2H), 2.94 (t, J = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, MeOD) δ 159.74, 134.88, 129.28, 119.16, 115.93, 71.68, 64.30, 60.33, 59.73, 19.03; HR-ESI-MS (ESI) *m/z* 237.1231 [M + H]$^+$, Calcd. for $C_{12}H_{18}N_2O_3$, 237.1234).

***Tert*-butyl ((4*R*,5*R*)-4-(4-(2-cyanoethoxy)phenyl)-2,2-dimethyl-1,3-dioxan-5-yl)carbamate (22):** To a stirred mixture of **20** (50 mg, 180.9 μmol) in MeOH (5 mL), $(Boc)_2O$ (47.3 mg, 217.1 μmol) was added at room temperature. The solvent was evaporated under reduced pressure. The residue was loaded onto a silica gel column (200−300 mesh). The resulting material was purified *via* column chromatography, using ether/EtOAc (4 : 1) as the eluent, to yield **22** (58.4 mg, 85%). Recrystallization using a MeOH and CH_2Cl_2 mixture was performed for X-ray analysis. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.38 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 4.71 (d, J = 8.9 Hz, 1H), 4.21 (t, J = 5.9 Hz, 2H), 3.54 (dd, J = 11.7, 3.6 Hz, 1H), 3.41 (dd, J = 11.7, 6.1 Hz, 1H), 3.33–3.26 (m, 2H), 2.94 (t, J = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.02, 155.32, 132.08, 127.22, 117.13, 114.42, 99.50, 79.25, 72.26, 65.06, 62.78, 48.19, 29.74, 28.22, 18.59, 18.56; HR-ESI-MS (ESI) *m/z* 399.1891 [M + Na]$^+$, Calcd. for $C_{20}H_{28}N_2NaO_5$, 399.1896).

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