## Appendix Supporting Information

# Synthesis and antiproliferative activity of marine bromotyrosine purpurealidin I and its derivatives

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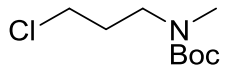
**4. References** **P9**

## 1. Synthesis

### General

All reactions were carried out using commercially available starting materials unless otherwise stated. The melting points were measured with Stuart SMP40 automated melting point apparatus and are uncorrected. $^{1}$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra in $CDCl_3$, $d_6$-DMSO, or $CD_3OD$ at room temperature were recorded on a Varian Mercury *Plus* 300 spectrometer or Bruker Avance 400 MHz NMR with smart probe. Chemical shifts ($\delta$) are given in parts per million (ppm) relative to the $^{1}$H and $^{13}$C NMR reference solvent signals ($CDCl_3$: 7.26 and 77.16 ppm; $CD_3OD$: 3.31 and 49.00 ppm; $d_6$-DMSO: 2.50 ppm and 39.52, $d_6$-acetone: 2.05 and 29.84 ppm). Multiplicities are indicated by s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet) and m (multiplet).The coupling constants *J* are quoted in Hertz (Hz). LC-MS and HRMS-spectra were recorded using Waters Acquity UPLC®-system (with Acquity UPLC® BEH C18 column, 1.7 µm, 50 × 2.1 mm, Waters) with Waters Synapt G2 HDMS with the ESI(+), high resolution mode. The mobile phase consisted of $H_2O$ (A) and acetonitrile (B) both containing 0.1% HCOOH. Microwave synthesis were performed in sealed tubes using Biotage Initiator+ instrument equipped with an external IR sensor. The flash chromatography was performed with Biotage SP1 flash chromatography purification system with 254 nm UV-detector or Biotage Isolera™ Spektra Systems with 200-800 nm UV-detector using SNAP 10, 25, 50 or 100 g cartridges. The TLC-plates were provided by Merck (Silica gel 60-F254) and visualization of the amine compounds was done using ninhydrin staining.

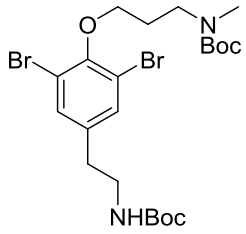
### *tert*-Butyl (3-chloropropyl) (methyl) carbamate (15) [1]




3-Chloro-*N*-methylpropylamine hydrochloride (0.50 g, 3.5 mmol) was dissolved to dry DCM (10 mL) under argon atmosphere. $Boc_2O$ (0.90 g, 4.16 mmol, 1.2 equiv) in DCM (2 mL) and $Et_3N$ (0.96 mL, 7.0 mmol, 2.0 equiv) were added dropwise at 0 °C. The reaction mixture was brought to room temperature in 1 h and stirred further 12 h. It was quenched with a 1 M solution of HCl in $H_2O$ (20 mL) and extracted with DCM (25 mL). The organic layer was further washed with a 1 M solution of HCl in $H_2O$ (2 × 15 mL) and a saturated solution of $NaHCO_3$ in $H_2O$ (3 × 15 mL). The aqueous layers were back-extracted with DCM (20 mL) and the combined organic layer was dried with anh. $Na_2SO_4$. The crude product was purified by column chromatography (silica gel, isocratic eluent, hexane/EtOAc, 8:2); TLC was stained with iodine. Compound **15** was obtained as a colorless oil (0.76 g, quant.).

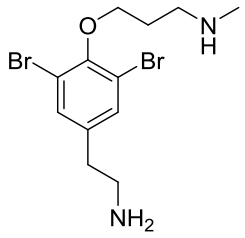
$^{1}$H NMR (300 MHz, $CDCl_3$) δ 3.53 (t, 2H, *J* = 6.6 Hz), 3.34 (t, 2H, *J* = 6.6 Hz), 2.85 (s, 3H), 2.02-1.93 (m, 2H), 1.44 (s, 9H).

### *tert*-Butyl [3-[2,6-dibromo-4-[2-[(*tert*-butoxycarbonyl)amino]ethyl]phenoxy]propyl](methyl) carbamate (14)



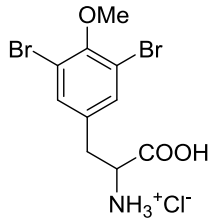

Compound **13** (0.76 g, 1.92 mmol), *t*-butyl (3-chloropropyl) methyl carbamate **15** (0.48 g, 2.31 mmol, 1.2 equiv) and $Cs_2CO_3$ (0.94 g, 2.88 mmol, 1.5 equiv) were dissolved in DMF (8 mL). The reaction mixture was stirred at 80 °C under argon atmosphere for 24 h, after which it was quenched with a 0.1 M solution of NaOH in $H_2O$ (20 mL). The reaction mixture was extracted with EtOAc (4 × 30 mL), the combined organic layers were dried over $Na_2SO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography, Biotage SNAP Cartridge KP-Sil 50 g, gradient elution: (hexane/EtOAc, 32→46%) to give **14** as a white amorphous solid (0.89 g, 83%). $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.32 (s, 2H), 4.01 (t, 2H, *J* = 12.9 Hz), 3.47 (t, 2H, *J* = 14.1 Hz), 3.32 (q, 2H, *J* = 6.9 Hz), 2.92 (s, 3H), 2.71 (t, 2H, *J* = 7.2 Hz), 2.13-2.03 (m, 2H), 1.45 (s, 9H), 1.43 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 155.9, 152.0, 137.8, 133.0, 118.3, 79.5, 71.4, 46.3, 41.6, 35.1, 34.7, 28.6, 28.5.

**3-[4-(2-Aminoethyl)-2,6-dibromophenoxy]-*N*-methylpropan-1-amine (6)**



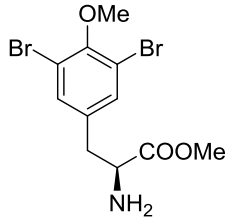
A solution of **14** (2.00 g, 3.62 mmol) in DCM (10 mL) was stirred with TFA (1.5 mL, 1.97 mmol, 5.5 equiv) under argon atmosphere for 48 h at rt. The reaction mixture was then concentrated under reduced pressure to remove excess TFA and DCM. The crude yellow oil obtained was treated with a saturated solution of $NaHCO_3$ in $H_2O$ (2 mL); the addition was monitored by using pH paper; addition of excess $NaHCO_3$ should be avoided as the product is water soluble. The resulting mixture was extracted with EtOAc (2 × 20 mL). The organic layer was dried over anh. $Na_2SO_4$ and concentrated under reduced pressure to give **6** as a yellow solid (1.52 g, quant.). $^1$H NMR (300 MHz, $CD_3OD$) *δ* 7.47 (s, 1H), 7.43 (s, 1H), 4.06–4.02 (m, 2H), 3.24–3.21 (m, 2H), 2.93–2.88 (m, 2H), 2.71–2.67 (m, 2H), 2.45 (s, 3H), 2.10–2.02 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) *δ* 153.0, 137.7, 134.4, 119.2, 71.4, 48.3, 41.3, 33.7, 33.1, 27.7.

**2-Amino-3-(3,5-dibromo-4-methoxyphenyl)propanoic acid hydrochloride (9)** [2]



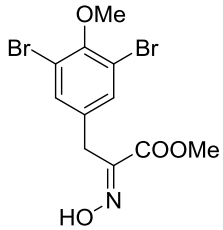
*O*-Methyl-L-tyrosine **7** (0.50 g, 2.6 mmol) in a 2 M solution of HCl in $H_2O$ (20 mL) was cooled to 0 °C. Bromine (1 mL) was added dropwise, and the reaction mixture was further stirred at rt overnight. It was concentrated to dryness by air, and the resulting solid was washed with *n*-hexane to obtain crude compound **9** as a brown solid (1.29 g, quant.).

**Methyl (*S*)-2-amino-3-(3, 5-dibromo-4-methoxyphenyl)propanoate (10)** [2]



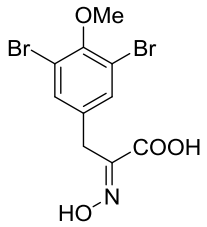
To a stirred solution of amino acid **9** (1.1 g, 2.8 mmol) in MeOH (15 mL) at 0 °C, was added $SOCl_2$ (0.5 mL, 5.60 mmol, 2.0 equiv). The mixture was stirred 30 min at 0 °C and then refluxed for 5 h. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ in $H_2O$ (15 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layer was dried over anh. $Na_2SO_4$ and concentrated under reduced pressure to give **10** as a pale yellow solid (1.04 g, 93%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 2H), 3.87 (s, 3H), 3.73 (s, 3H), 3.69 (br s, 2H), 3.02-2.96 (m, 1H), 2.79-2.77 (m, 1H).

**Methyl (*E*)-3-(3,5-dibromo-4-methoxyphenyl)-2-(hydroxyimino)propanoate (11)** [2]



To a well-stirred solution of **10** (0.90 g, 2.54 mmol) in EtOH (8 mL), was added $Na_2WO_4 \cdot 2\ H_2O$ (0.83 g, 2.54 mmol), 30% $H_2O_2$ (2.5 mL), and water (4.0 mL) at 0 °C. The reaction mixture was further stirred for 14 h at room temperature. It was then quenched with a saturated solution of $NH_4Cl$ in $H_2O$ (10 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over $Na_2SO_4$ and concentrated *in vacuo.* The crude product was purified by column chromatography (silica gel, isocratic elution, DCM/MeOH, 8:2) to give **11** as a white solid (0.38 g, 40%). $^1$H NMR (300 MHz, $CD_3OD$) *δ* 7.47 (s, 2H), 3.38 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H); $^{13}$C NMR (75 MHz, $CD_3OD$) *δ* 165.6, 154.0, 150.6, 137.0, 134.3, 118.6, 61.0, 52.9, 30.0.

**(*E*)-3-(3, 5-Dibromo-4-methoxyphenyl)-2-(hydroxyimino)propanoic acid (5)** [2]




To a solution of **11** (0.14 g, 0.37 mmol) in THF (12 mL) and $H_2O$ (1:1), LiOH (0.13 g, 5.43 mmol, 15.0 equiv) was added and the mixture was stirred for 14 h. The reaction mixture was then quenched with a 2 M solution of HCl in $H_2O$ (until neutral) and was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried over anh. $Na_2SO_4$ and concentrated *in vacuo*. The pale yellow crude solid was recrystallized from *n*-hexane to give **5** as a white solid (0.12 g, 90%).$^1$H NMR (300 MHz, $d_6$-DMSO) $\delta$ 12.8 (br s, 1H), 12.4 (br s, 1 H), 7.44 (s, 2H), 3.77 (s, 5H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 164.8, 151.8, 149.2, 136.1, 132.6, 117.1, 60.3, 28.6.

## Unsuccessful coupling attempts towards purpurealidin I 1

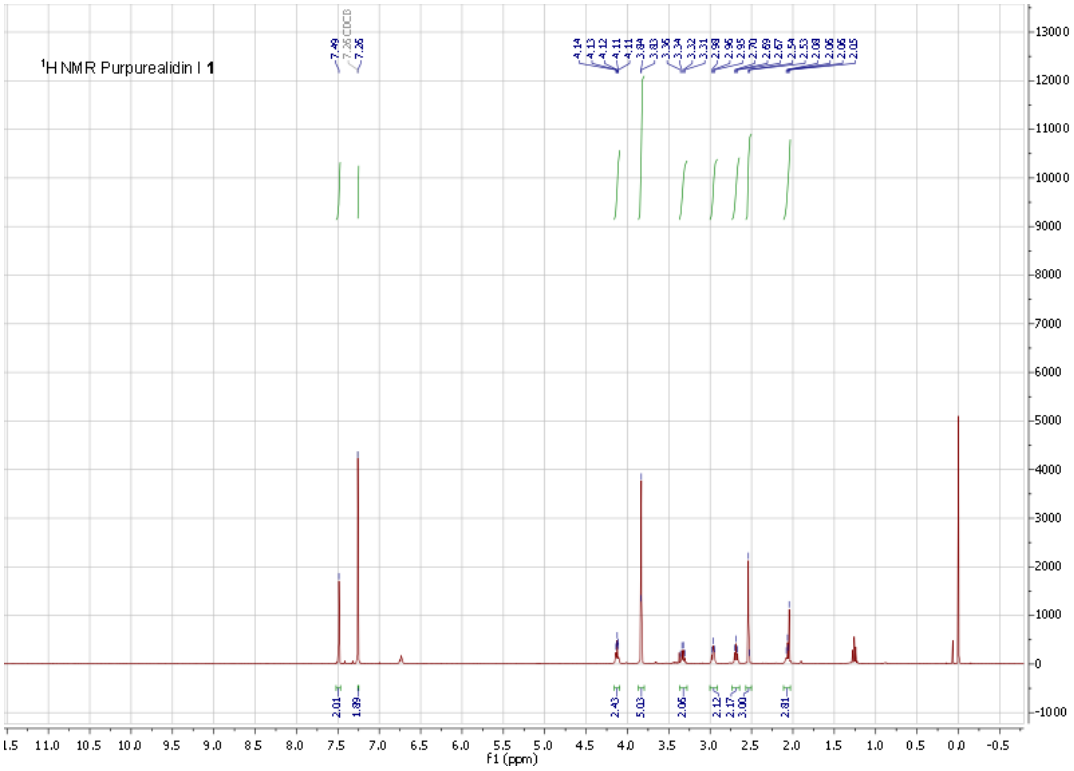
Initially, the direct condensation of hydroxylamine ester **11** was tried using amine **6** without success. The general experimental procedure employed was as follows: Carboxylic acid **5** (0.10 g, 0.26 mmol), **6** (0.29 g, 0.78 mmol, 3.0 equiv) and $K_2CO_3$ (0.11 g, 0.78 mmol, 3.0 equiv.) were charged to a 5-mL Biotage tube pre-flushed with argon. MeOH (3 mL) was then added and the tube was sealed. The reaction mixture was heated in an oil bath at 60 °C. Different work-up procedures were tried after running different batches of reaction (*e.g.* washing with water, dil. HCl, saturated $NaHCO_3$, and 1 M NaOH). In every trial, the product **1** was characterized by $^1$H NMR, but never obtained as a pure substance.

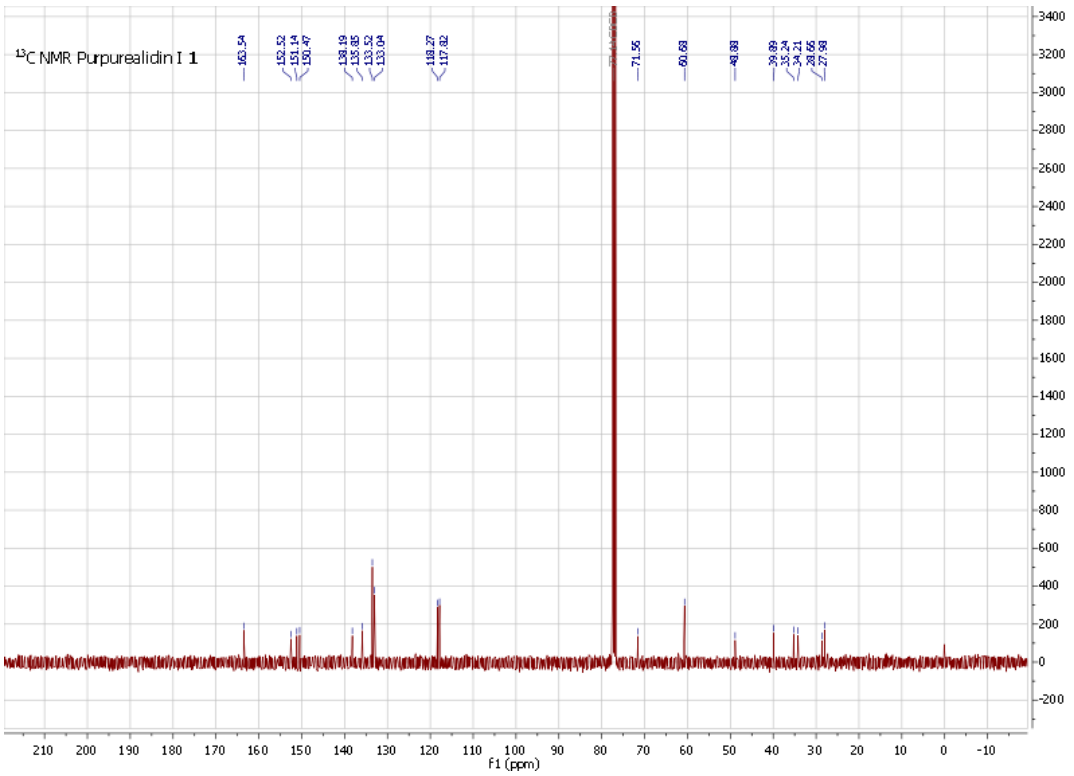
**Table S1.** Coupling attemps towards purpurealidin I **1**

| **Carboxylic acid 5 (equiv.)** | **Amine 6 (equiv.)** | **Conditions (carried out in a sealed tube)** | **Result** |
|---|---|---|---|
| 1.0 | 3 | MeOH, $K_2CO_3$, 80 °C, 48 h | Mixture |
| 1.0 | 0.5 | MeOH, $K_2CO_3$, 80 °C, 48 h | Mixture |
| 1.0 | 3.0 | DMF, 80 °C, 48 h | Mixture |
| 1.0 | 3.0 | MeOH, $K_2CO_3$, 65 °C, 2 h, MW | Mixture |
| 1.0 | 3.0 | MeOH, $K_2CO_3$, 80 °C, 48 h | 48% crude yield. Product **1** seen in $^1$H NMR, the purification failed |
| 1.0 | 0.5 | MeOH, *t*-BuOK, 80 °C, 48 h | Mixture |

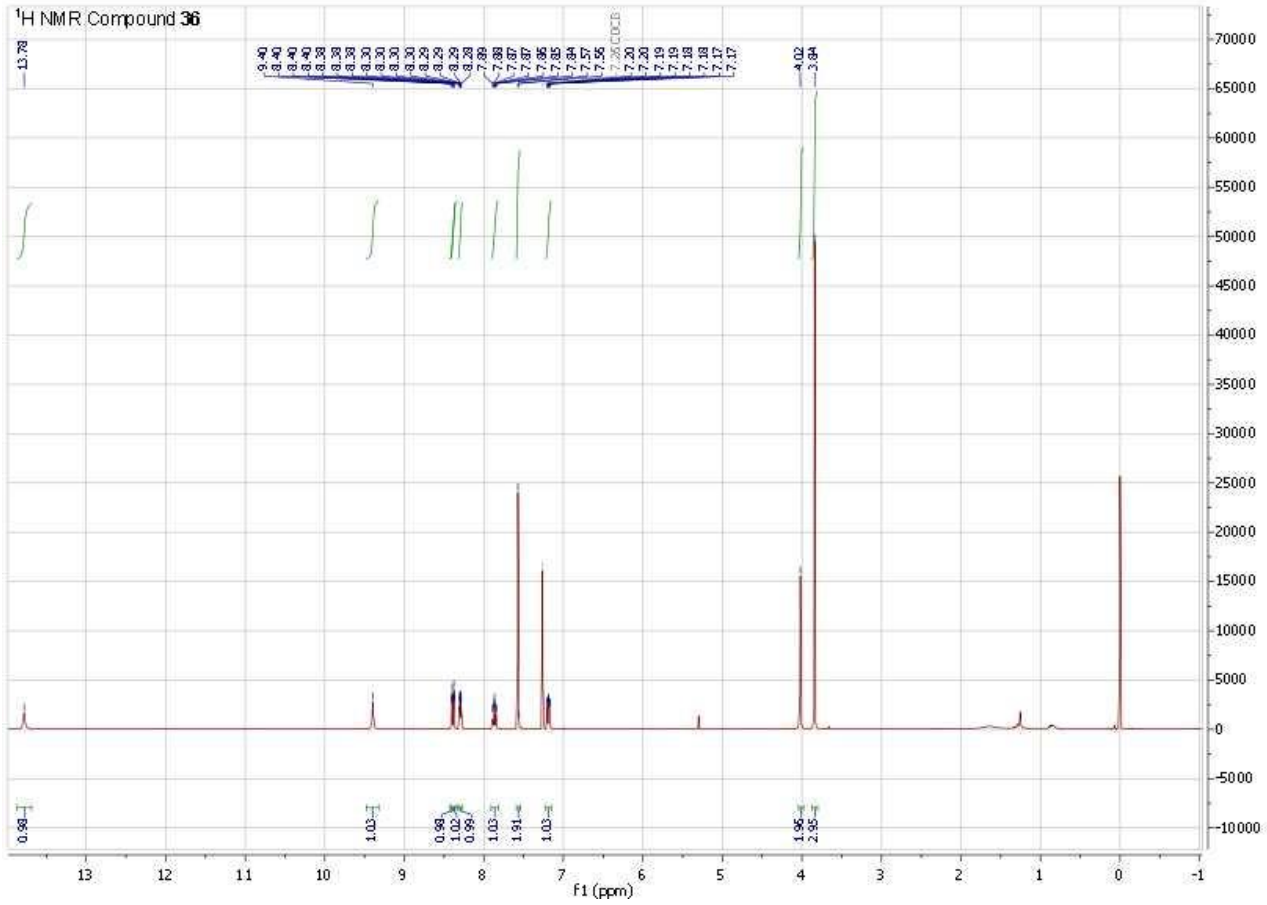
## 2. NMR spectra

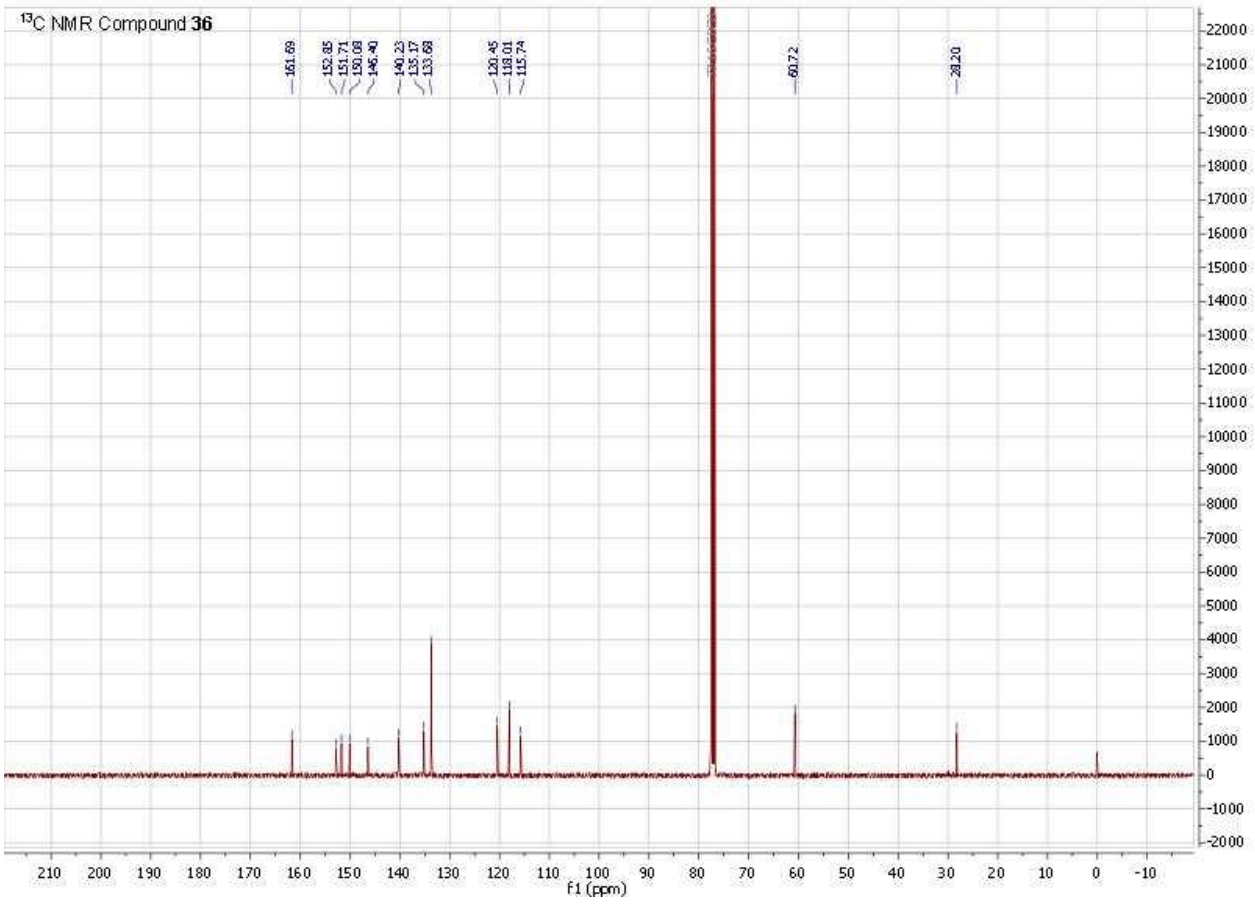
### 2.1 $^{1}$H and $^{13}$C NMR spectra of purpurealidin I **1**



**2.2** $^{1}$H and $^{13}$C NMR spectra of compound **36**



## 3. Single crystal x-ray analysis

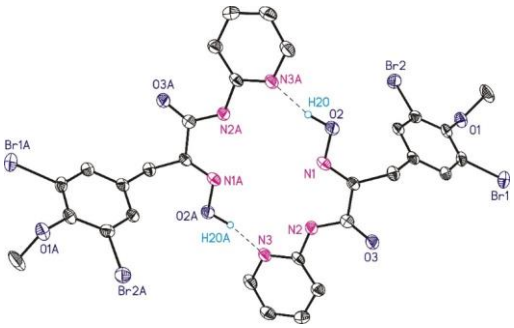



**Figure S1**. ORTEP (50% probability ellipsoids) of the dimer formed by **36** in the solid state due to formation of intermolecular hydrogen bonds, indicated with dotted lines. All carbon- and nitrogen bonded hydrogen atoms and packing solvent molecules have been omitted for clarity. Symmetry code: "A" = –1 – *x*, 1 – *y*, 1 – *z*.

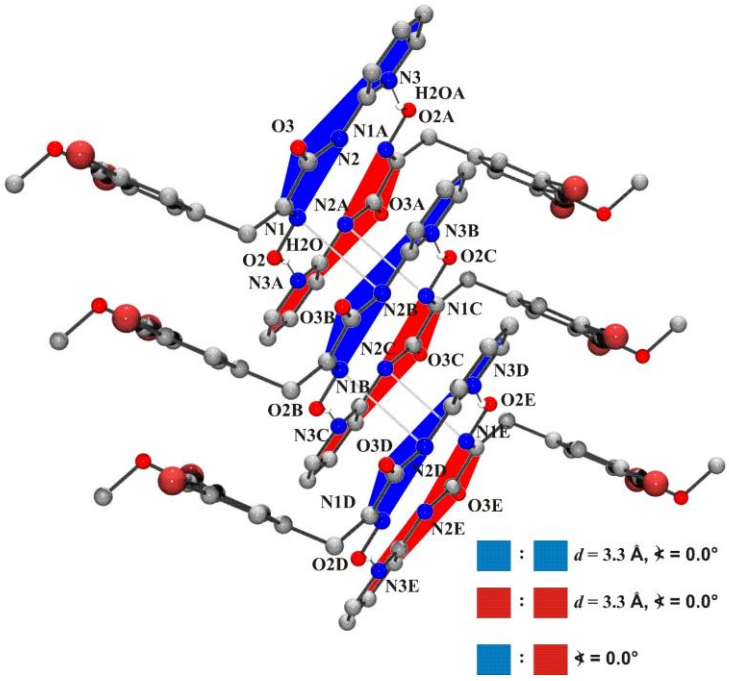



**Figure S2**. Ball-and-stick model of a representative part of one of the bands formed by **36** in the solid state due to formation of intermolecular hydrogen bonds indicated by dotted lines and dispersion interactions. The sign *d* refers to the averaged distance of mean planes of atoms belonging to blue or red shaded areas, while the sign ∢ refer to the interplanar angles. Grey lines indicate the shortest intermolecular distances of 3.272 Å due to dispersion interactions between nitrogen atoms N1 and N2B as well as symmetry related atoms. Symmetry codes: "A" = –1 – *x*, 1 – *y*, 1 – *z*. "B" = –*x* + 1, *y*, *z*. "C" = –*x*, 1 – *y*, 1 – *z*. "D" = –*x* + 2, *y*, *z*. "E" = –*x* + 1, 1 – *y*, 1 – *z*.

| D–H···A | D–H | H···A | D···A | D–H···A |
|---|---|---|---|---|
| Intra N2–H2···N1 | 0.86 | 2.22 | 2.637(4) | 109 |
| Inter O2–H2O···N3 | 0.97(3) | 1.74(3) | 2.687(4) | 164(4) |

CCDC 1873696 contains the detailed crystallographic data of this report. This data are available free of charge via the Cambridge Crystallographic Data Centre:
www.ccdc.cam.ac.uk/data_request/cif .

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Summary of Data CCDC 1873696

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Compound Name:
Formula: C15 H13 Br2 N3 O3,C1 H1 Cl3
Unit Cell Parameters: a 4.4118(3) b 24.7517(14) c 18.6721(11) P21/n
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## 4. References

[1] Buchholz, M.; Heiser, U.; Schilling, S.; Niestroj, A.J.; Zunkel, K. Demuth, H.-U. The First Potent Inhibitors for Human Glutaminyl Cyclase: Synthesis and Structure-Activity Relationship. *J. Med. Chem.* **2006**, *49*, 664-677. DOI**:** 10.1021/jm050756e

[2] Hillgren, J.M.; Öberg, C.T.; Elofsson, M. Syntheses of pseudoceramines A–D and a new synthesis of spermatinamine, bromotyrosine natural products from marine sponges. *Org. Biomol. Chem*. **2012**, *10*, 1246-1254. DOI: 10.1039/C1OB06722B

[3] García, J.; Pereira, R.; de Lera, A.R. Total synthesis of the natural isoprenylcysteine carboxyl methyltransferase inhibitor spermatinamine. *Tetrahedron Lett.* **2009**, *50*, 5028-5030. https://doi.org/10.1016/j.tetlet.2009.06.087

[4] Kottakota, S.K.; Evangelopoulos, D.; Alnimr, A; Bhakta, S; McHugh, T.D.; Gray, M.; Groundwater, P.W.; Marrs E.L.; Perry J.D.; Spilling C.D., Harburn J.J. Synthesis and biological evaluation of purpurealidin E-derived marine sponge metabolites: Aplysamine-2, aplyzanzine A, and suberedamines A and B. *J Nat Prod.* 2012, 75, 1090-1101. DOI: 10.1021/np300102z