Supporting Information

(ja072225g)

Total Synthesis, Assignment of Absolute Stereochemistry and Structural Revision of Chlorofusin

Wen-Jian Qian, Wan-Guo Wei, Yong-Xia Zhang, and Zhu-Jun Yao*

*State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai
Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road,
Shanghai 200032, China*

E-mail: yaoz@mail.sioc.ac.cn

List of Content

1. Detailed experimental procedures.....(Page 2 ~ Page 12)
2. Data comparisons and HPLC analyses.....(Page 13 ~ Page 25)
3. Copies of ¹H NMR spectra for compounds **6-8**, (*S*)-**2**, **9a-b**, *rac*-**11a~rac-11d**, **12**, **13**, **15**, **1a** and the mixture of **1a** and **14**, and copies of ¹³C NMR spectra for compounds **6-8**, (*S*)-**2**, **9a-b**, *rac*-**11a~rac-11d**, **13** and **1a**.....(Page 26 ~ Page 53)

I. Detailed experimental procedures

General All non-aqueous reactions were conducted in oven-dried glassware under positive pressure of nitrogen, with magnetic stirring unless otherwise note. All melting points were uncorrected. Infrared (IR) spectra were obtained either as neat films, or as a thin film of a dichloromethane solution of the compound on sodium chloride discs, using a Fourier transform infrared spectrometer (FTIR). ^1H NMR spectra were recorded at 300 MHz at 300K unless otherwise stated. ^{13}C NMR spectra were recorded at 75.0 MHz at 300K unless otherwise stated. Chemical shifts are reported in parts per million relative to CDCl_3 (^1H , δ 7.27; ^{13}C , δ 77.0), $\text{DMSO}-d_6$ (^1H , δ 2.50; ^{13}C , δ 39.6) unless otherwise stated. Data for ^1H NMR are reported as follows: chemical shift, integration, multiplicity (app = apparent, par obsc = partially obscure, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants. All ^{13}C NMR spectra were recorded with complete proton decoupling. Low and high-resolution mass spectra were obtained in the Mass Spectrometry Laboratory of Shanghai Institute of Organic Chemistry. Optical rotations were recorded on a JASCO P-1030 Polarimeter at 589 nm, and are recorded as $[\alpha]_D$ (concentration in grams/100 mL solvent). Flash chromatography was performed on silica gel H (400 mesh). Yields refer to chromatographically and spectroscopically pure material, unless otherwise stated. HPLC analyses were performed on an Agilent 1100 series HPLC using a C18 reversed-phase analysis column (VYDAC 238DE54, 120 Å, 5 μm , 4.6 mm i.d. x 250 mm, flow rate 1 mL/min) and semi-preparation HPLC column (VYDAC 238DE1010, 120 Å, 10 μm , 10 mm i.d. x 250 mm, flow rate 3 mL/min).

Azaphilone 6. To a $\text{Cu}_2\text{L}_2\text{O}_2$ complex^[S1] freshly prepared from $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ (674 mg, 1.81 mmol), and (-)-sparteine (499 μL , 2.17 mmol) in 1.6 mL anhydrous CH_2Cl_2 under an oxygen

atmosphere at $-78\text{ }^\circ\text{C}$ was added alkynylbenzaldehyde **4** (200 mg, 0.72 mmol) and diisopropylethylamine (DIEA) (191 μL , 1.16 mmol) in CH_2Cl_2 (400 μL). After 5 min, 4-dimethylaminopyridine (DMAP) (221 mg, 1.81 mmol) was added and the mixture was warmed and stirring at $-10\text{ }^\circ\text{C}$ for 48 h under an oxygen atmosphere (monitored by TLC). The

^[S1] Zhu, J.; Grigoriadis, N. P.; Lee, J. P.; Porco, J. A., Jr., *J. Am. Chem. Soc.* **2005**, *127*, 9342-9343.

reaction was quenched at -10 °C with 10% sulfuric acid (2 mL) and brine (2 mL). The mixture was extracted three times with EtOAc, and the combined extracts were concentrated *in vacuo*. This crude residue was re-dissolved in CH₃CN (2.0 mL), and aqueous NH₄Cl (1.0 mL) was then added. The resulting heterogeneous mixture was stirring at rt for 1 h. The mixture was extracted three times with EtOAc. The organic extracts were dried over anhydrous Na₂SO₄, and concentrated. The residue was purified by silica gel column chromatography (CH₂Cl₂: MeOH = 100: 2 to 100: 4) to afford azaphilone **6** (116 mg, 55%, two steps) as a yellow oil. $[\alpha]_D^{24}$ -80.2 (c 0.25, CHCl₃). ¹H NMR (CDCl₃, 300 MHz): δ 7.88 (1H, s), 6.15 (1H, s), 5.54 (1H, s), 4.14 (2H, t, *J* = 6.3 Hz), 3.92 (1H, brs), 2.53 (2H, t, *J* = 7.5 Hz), 2.07 (3H, s), 2.05 – 1.93 (2H, m), 1.56 (3H, s). ¹³C NMR (CDCl₃, 75 MHz): δ 196.2, 195.6, 170.9, 161.4, 152.8, 143.6, 115.9, 106.7, 105.5, 83.5, 62.9, 30.0, 28.4, 25.6, 20.8. IR (KBr) ν_{max} 3363, 2930, 2858, 1723, 1656 cm⁻¹. HRMS (MALDI, *m/z*) calcd for C₁₅H₁₇O₆ (MH⁺): 293.1020; found, 293.1020. The e.e. value of **6** (91.4%) was determined by HPLC (250*4.6 mm Chiralpak OJ-H column, 40% *i*-PrOH in hexane, 1.0 mL/min, 308K, UV 214nm) with comparison of a racemic sample.

Azaphilone 7. To a solution of **6** (132 mg, 0.45 mmol) in anhydrous CH₂Cl₂ (10 mL) was successively added anhydrous pyridine (110 μL, 1.36 mmol), butyryl chloride (140 μL, 1.36 mmol) and 4-dimethylaminopyridine (DMAP) (5 mg, 0.041

mmol). Being stirred at room temperature, the reaction was monitored by TLC (this reaction usually completed after 1 h). The reaction mixture was quenched by addition of water and CH₂Cl₂. The aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. Purification on silica gel column (CH₂Cl₂: MeOH = 100: 1) provided azaphilone **7** (114 mg, 70%) as a yellow oil. $[\alpha]_D^{24}$ -64.2 (c 0.62, CHCl₃). ¹H NMR (CDCl₃, 300 MHz): δ 7.87 (1H, s), 6.13 (1H, s), 5.53 (1H, s), 4.13 (2H, t, *J* = 6.3 Hz), 2.50 (2H, t, *J* = 7.5 Hz), 2.43 (2H, t, *J* = 7.5 Hz), 2.07 (3H, s), 2.05 – 1.93 (2H, m), 1.71 – 1.62 (2H, m), 1.53 (3H, s), 0.97 (3H, t, *J* = 7.5 Hz). ¹³C NMR (CDCl₃, 125 MHz): δ 193.3, 192.9, 172.9, 170.9, 160.8, 153.8,

142.3, 115.2, 109.1, 107.3, 84.1, 62.9, 35.1, 29.9, 25.7, 22.2, 20.9, 18.2, 13.5; IR (KBr) $\nu_{\max}$ 2967, 2934, 1739, 1645 cm^{-1} . HRMS (ESI, m/z) calcd for $\text{C}_{19}\text{H}_{23}\text{O}_7$ (MH^+): 363.1444; found, 363.1438. The e.e. of **7** (93.8%) was determined by HPLC (250*4.6 mm Chiralpak OJ-H column, 40% *i*-PrOH in hexane, 1.0 mL/min, 308K, UV 214nm) with comparison of a racemic sample.

Azaphilone 8. To a solution of **7** (96 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise SO_2Cl_2 (21 μL , 0.26 mmol) in CH_2Cl_2 at 0 $^\circ\text{C}$. The mixture was warmed to rt for 10 min, and then poured into ice-cold brine and extracted with CH_2Cl_2 (10 mL x 3). The combined

extracts were washed with H_2O and brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. Purification on silica gel column (CH_2Cl_2 : MeOH = 100: 1) provided azaphilone **8** (70 mg, 66%) as a yellow solid. mp: 99 – 100 $^\circ\text{C}$. $[\alpha]_{\text{D}}^{23}$ -173.96 (c 0.23, CHCl_3). ^1H NMR (CDCl_3 , 300 MHz): δ 7.92 (1H, s), 6.63 (1H, s), 4.16 (2H, t, J = 6.3 Hz), 2.61 (2H, t, J = 7.5 Hz), 2.43 (2H, t, J = 6.9 Hz), 2.08 (3H, s), 2.08–1.99 (2H, m), 1.70–1.63 (2H, m), 1.56 (3H, s), 0.97 (3H, t, J = 7.5 Hz). ^{13}C NMR (CDCl_3 , 100 MHz): δ 191.7, 186.3, 173.0, 170.9, 162.8, 152.9, 138.0, 114.9, 110.8, 106.3, 84.2, 62.8, 34.9, 30.2, 25.7, 22.2, 20.9, 18.2, 13.4. IR (KBr) $\nu_{\max}$ 1740, 1722, 1647, 1540, 1255, 1229, 1127, 1043, 835 cm^{-1} . HRMS (ESI, m/z) calcd for $\text{C}_{19}\text{H}_{21}\text{ClO}_7\text{Na}$ ($\text{M}^+ + \text{Na}$) 419.0868; found 419.0865.

Azaphilone (S)-2. To a solution of **8** (70 mg, 0.18 mmol) in MeOH (5 mL) was added K_2CO_3 (97 mg, 0.71 mmol). The reaction was stirred at room temperature for 0.5 h (monitored by TLC), and then quenched by addition of 10 N HCl (0.1 mL). The

mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Purification on silica gel column (CH_2Cl_2 : MeOH = 100: 1 to 100: 5) afforded azaphilone (S)-2 (50 mg, 80%) as a yellow solid. mp: 101-102 $^\circ\text{C}$. $[\alpha]_{\text{D}}^{24}$ -247.2 (c 0.09, CHCl_3). ^1H NMR (CDCl_3 , 500 MHz): δ 7.91 (1H, s),

6.63 (1H, s), 3.75 (2H, t, $J = 6.0$ Hz), 2.65 (2H, t, $J = 7.6$ Hz), 2.43 (2H, t, $J = 7.4$ Hz), 1.95-1.90 (2H, m), 1.69-1.63 (2H, m), 1.60 (1H, br s), 1.55 (3H, s), 0.97 (3H, t, $J = 7.4$ Hz). ^{13}C NMR (CDCl_3 , 125 MHz): δ 191.8, 186.4, 173.0, 163.8, 153.0, 138.3, 114.9, 110.6, 106.2, 84.4, 61.2, 35.0, 30.0, 29.4, 22.3, 18.2, 13.5. IR (KBr) ν_{max} 3551, 1719, 1645, 1580, 1535, 1183, 1125, 1086, 942, 835 cm^{-1} . HRMS (ESI, m/z) calcd for $\text{C}_{17}\text{H}_{19}\text{ClO}_6\text{Na}$ ($\text{M}^+ + \text{Na}$): 377.0763; found, 377.0762.

Compounds *rac*-11a-d: Compounds *rac*-11a-d were prepared from *rac*-2 following our previously reported procedure. Their ^1H NMR (CDCl_3), ^{13}C NMR (CDCl_3), IR and HRMS (ESI) were also published.^[S2]

In this paper, a new fast approach was used to obtain all the racemic compounds **11a-d** in one pot: To a solution of *rac*-2 (5 mg, 0.014 mmol) in MeCN (5 mL) was added NBS (4 mg, 0.0211 mmol). After this mixture was stirred at rt for 1 h, methylamine hydrochloride (2 mg, 0.021 mmol), aq. NaHCO_3 (2 mL), Ag_2O (5 mg, 0.021 mmol) were added successively. The reaction was stirred at rt for 3 days (monitored by TLC). The inorganic residues were removed by filtration over a pad of Celite. The solution was then extracted with the mixed solvent (CH_2Cl_2 -MeOH=10:1, 10 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Purification on silica gel column (CH_2Cl_2 :MeOH = 100:1 to 100:3) gave 4 mg (70%) of the mixture of diastereomers (**11a**: **11b**: **11c**: **11d** = 1: 1: 2.5: 4, by HPLC analysis). The HPLC conditions: $\lambda_{\text{max}} = 401$ nm, flow rate = 1.0 mL/min.

Gradient table (solvents: A = H_2O , B = MeCN)			
T/ min	0	20	25
B%	25	35	100

Retention times: $T_{\text{R}}(\text{11a}) = 12.8$ min; $T_{\text{R}}(\text{11b}) = 14.1$ min; $T_{\text{R}}(\text{11c}) = 16.0$ min; $T_{\text{R}}(\text{11d}) = 16.5$ min.

Compound *rac*-11a: ^1H NMR (($\text{DMSO}-d_6$, 300 MHz): δ 7.86 (1H,

^[S2] Wei, W.-G, Qian, W.-J., Zhang, Y.-X., Yao, Z.-J. *Tetrahedron Lett.* **2006**, 47, 4171-4174.

s), 6.10 (1H, s), 4.54 (1H, s), 4.23 – 4.09 (1H, m), 4.09 – 3.96 (1H, m), 3.21 (3H, s), 2.32 (2H, t, $J = 7.2$ Hz), 2.09 – 1.92 (2H, m), 1.91 – 1.72 (2H, m), 1.52 (2H, sextet, $J = 7.2$ Hz), 1.38 (3H, s), 0.90 (3H, t, $J = 7.2$ Hz). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 189.3, 188.7, 172.0, 150.9, 149.1, 113.8, 100.2, 97.5, 85.0, 70.8, 69.2, 37.5, 34.9, 34.4, 25.4, 23.4, 18.4, 13.7.

Compound *rac*-11b: ^1H NMR ((DMSO- d_6 , 300 MHz): δ 7.90 (1H, s), 6.11 (1H, d, $J = 5.7$ Hz), 4.48 (1H, d, $J = 5.7$ Hz), 4.21 – 4.10 (1H, m), 4.08 – 3.97 (1H, m), 3.21 (3H, s), 2.32 (2H, t, $J = 7.2$ Hz), 2.08 – 1.95 (2H, m), 1.95 – 1.85 (2H, m), 1.52 (2H, sextet, $J = 7.2$ Hz), 1.39 (3H, s), 0.90 (3H, t, $J = 7.2$ Hz) ppm. ^{13}C NMR

(DMSO- d_6 , 75 MHz): δ 189.3, 188.7, 172.1, 151.0, 149.1, 113.8, 100.2, 97.5, 85.1, 70.9, 69.2, 37.5, 34.9, 34.4, 25.4, 23.4, 18.4, 13.7.

Compound *rac*-11c: ^1H NMR ((DMSO- d_6 , 300 MHz): δ 7.83 (1H, s), 6.38 (1H, d, $J = 6.9$ Hz), 4.50 (1H, d, $J = 7.5$ Hz), 4.06 – 3.95 (1H, m), 3.84 – 3.71 (1H, m), 3.26 (3H, s), 2.47 – 2.38 (1H, m), 2.31 – 2.22 (1H, m), 2.33 (2H, t, $J = 7.2$ Hz), 2.12 – 1.97 (2H, m), 1.53 (2H,

sextet, $J = 7.5$ Hz), 1.36 (3H, s), 0.90 (3H, t, $J = 7.5$ Hz) ppm. ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 189.4, 188.9, 172.0, 151.8, 148.1, 115.3, 101.3, 96.7, 85.1, 69.6, 69.3, 38.9, 34.9, 31.0, 25.6, 23.4, 18.5, 13.7.

Compound *rac*-11d: ^1H NMR (DMSO- d_6 , 300 MHz): δ 7.83 (1H, s), 6.25 (1H, d, $J = 5.1$ Hz), 4.53 (1H, d, $J = 5.7$ Hz), 4.07 – 3.96 (1H, m), 3.82 – 3.71 (1H, m), 3.27 (3H, s), 2.46 – 2.38 (1H, m), 2.31 – 2.23 (1H, m), 2.33 (2H, t, $J = 7.2$ Hz), 2.11 – 1.97 (2H,

m), 1.52 (2H, sextet, $J = 7.5$ Hz), 1.40 (3H, s), 0.90 (3H, t, $J = 7.5$ Hz) ppm. ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 189.3, 188.9, 172.0, 151.8, 148.1, 115.3, 101.2, 96.7, 85.1, 69.6, 69.3, 38.9, 34.9, 31.0, 25.5, 23.4, 18.4, 13.7.

Compound (4*S*)-9a: To a solution of (4*S*)-2 (20 mg, 0.056 mmol) in MeCN (5 mL) was added

cyclopeptide **3b**^[S3] (90 mg, 0.060 mmol) and aq. NaHCO₃ (0.5 mL). The reaction was stirred at rt for 1.5 h, and then extracted with the mixed solvent (CH₂Cl₂:MeOH = 10:1, 10 mL x 3). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. Purification on silica gel column (CH₂Cl₂: MeOH = 100: 3 to 100: 7) afforded of (4*S*)-**9a** (82 mg, 80%) as an orange red solid. $[\alpha]_D^{23}$ -44.0 (*c* 0.41, CH₂Cl₂). ¹H NMR (DMSO-*d*₆, 500 MHz): δ 9.34 (2H, brs), 8.94 (1H, brs), 8.60 (1H, s), 8.11 (1H, s), 7.83 (1H, s), 7.76 (1H, d), 7.68 (1H, s), 7.40 - 7.00 (38H, m), 6.82 (1H, brs), 6.48 (1H, s), 6.31 (1H, brs), 5.41 (1H, brs), 5.15 (1H, m), 4.81 (2H, m), 4.53 (2H, m), 4.25 - 4.00 (3H, m), 4.00 - 3.75 (6H, m), 3.72 (1H, brs), 3.70 - 3.55 (1H, m), 2.42 - 2.10 (4H, m), 1.95-1.70 (6H, m), 1.70 - 1.50 (8H, m), 1.43 (3H, s), 1.38 - 1.12 (17H, m), 1.12 - 0.95 (6H, m), 0.95 - 0.65 (15H, m), 0.38 (1H, d, *J* = 6.3 Hz), 0.41 (1H, brs). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 192.4, 182.5, 173.8, 173.6, 173.4, 172.9, 172.7, 172.5, 171.5, 171.2, 168.2, 167.2, 151.9, 149.8, 151.9, 144.6, 144.5, 142.1, 128.6, 128.0, 127.7, 127.5, 127.4, 126.7, 126.4, 126.2, 126.1, 114.7, 112.0, 98.7, 84.8, 69.3, 64.9, 64.7, 64.3, 62.5, 59.2, 54.0, 52.6, 51.6, 51.4, 48.9, 48.4, 48.3, 40.6, 40.0, 39.8, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 38.3, 37.3, 35.5, 34.5, 31.2, 31.1, 29.9, 28.7, 28.5, 28.3, 27.3, 25.9, 23.9, 23.8, 23.7, 23.5, 23.2, 23.0, 22.9, 22.3, 22.1, 22.0, 20.6, 20.4, 20.1, 19.6, 18.0, 17.9, 16.3, 13.9, 13.4, 13.2. IR (KBr) ν_{max} 3309, 2938, 2929, 1654 cm⁻¹. HRMS (ESI, *m/z*) calcd for C₁₀₁H₁₂₇ClN₁₂O₁₈Na (*M*⁺+Na): 1853.8978; found, 1853.8972.

Compound (4*R*)-9b: To a solution of azaphilone *rac*-**2** (12 mg, 0.034 mmol) in MeCN (5 mL) was added cyclopeptide **3b** (52 mg, 0.035 mmol) and aq. NaHCO₃ (0.5 mL). The reaction was stirred at room temperature for 1.5 h, and then workup as that for compound (4*S*)-**9a**. Purification on silica gel

(CH₂Cl₂:MeOH = 100:3 to 100:7) afforded the mixture of (4*S*)-**9a** and (4*R*)-**9b** (50 mg, 80%) as orange red solids. This mixture was separated by a semi-preparation HPLC to yield

^[S3] Cyclopeptide **3b** was prepared by following a similar route in the reference: Desai, P.; Pfeiffer, S. S.; Boger, D. L. *Org. Lett.* **2003**, *5*, 5047-5050.

(4R)-**9b** (15 mg) and (4S)-**9a** (16 mg). HPLC semi-preparation conditions: $\lambda_{\text{max}} = 380 \text{ nm}$, flow rate = 3.0 mL/min.

Gradient table (A = H ₂ O, B = MeCN)					
T/min	0	5	10	15	20
B%	80	87	90	95	100

Retention times: $T_{R(9b)} = 13.6 \text{ min}$; $T_{R(9a)} = 14.3 \text{ min}$.

Data for (4R)-**9b**: $[\alpha]_D^{25} 76.9$ (c 0.065, CH₃CN). ¹H NMR (DMSO-*d*₆, 500 MHz): δ 9.32 (1H, brs), 9.24 (1H, brs), 8.92 (1H, s), 8.60 (1H, s), 8.08 (1H, s), 7.84 (1H, s), 7.74 (1H, d, $J = 7.2 \text{ Hz}$), 7.67 (1H, s), 7.50 (1H, d, $J = 7.4 \text{ Hz}$), 7.36 (1H, d, $J = 7.5 \text{ Hz}$), 7.30 - 7.00 (36H, m), 6.83 (1H, brs), 6.68 (1H, s), 6.34 (1H, brs), 5.41 (1H, s), 5.15 (1H, s), 4.95 (1H, m), 4.86 (1H, m), 4.54 (2H, m), 4.18 - 4.05 (3H, m), 4.0 - 3.78 (6H, m), 3.71 (1H, brs), 3.47 - 3.50 (1H, m), 2.32 (2H, t, $J = 6.9 \text{ Hz}$), 2.18 - 2.15 (1H, m), 2.1 - 2.06 (1H, m), 1.95 - 1.75 (4H, m), 1.75 - 1.50 (10H, m), 1.40 - 1.12 (17H, m), 1.29 (3H, s, judged by HMQC), 1.12 - 0.94 (6H, m), 0.91 (3H, t, $J = 7.3 \text{ Hz}$), 0.88 - 0.80 (6H, m), 0.70 - 0.78 (6H, brs), 0.49 (2H, d, $J = 6.2 \text{ Hz}$). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 192.5, 182.6, 173.7, 173.4, 172.8, 172.7, 171.68, 171.63, 171.2, 168.4, 167.3, 152.3, 144.7, 144.6, 144.5, 129.0, 128.6, 128.1, 127.9, 127.5, 126.6, 126.5, 126.2, 126.1, 114.5, 113.6, 99.0, 96.3, 84.5, 82.5, 71.5, 69.3, 67.2, 65.0, 64.8, 62.6, 59.4, 55.0, 54.8, 54.1, 52.8, 52.7, 52.1, 51.4, 50.7, 49.4, 48.4, 44.4, 37.4, 35.8, 34.6, 31.3, 29.9, 28.7, 28.6, 28.4, 27.5, 27.4, 27.0, 26.0, 24.0, 23.8, 23.5, 23.1, 22.7, 22.0, 20.7, 20.4, 20.1, 19.7, 19.5, 18.0, 17.2, 16.4, 13.9, 13.2. IR (KBr) ν_{max} 3300, 2960, 2927, 1654 cm⁻¹. HRMS (ESI, m/z) calcd for C₁₀₁H₁₂₇ClN₁₂O₁₈Na (M⁺+Na): 1853.8978; found, 1853.8972.

Chlorofusin (synthetic, 1a): To a solution of (S)-**2** (5 mg, 0.014 mmol) in MeCN (5 mL) was added NBS (4 mg, 0.0211 mmol) at rt. After being stirred at room temperature 1 h, cyclopeptide **3a** (17 mg, 0.016 mmol), aq. NaHCO₃ (2 mL) and Ag₂O (5 mg, 0.021 mmol)

was added successively. The reaction was stirred at rt for 3 days (monitored by TLC). The

inorganic solid was removed by filtration over a pad of Celite. The solution was then extracted with the mixed solvent (CH₂Cl₂:MeOH = 10:1, 10 mL x 3). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. Purification on silica gel chromatography (CH₂Cl₂:MeOH = 100:3 to 100:7) provided a mixture of diastereomers (10 mg, 50%). This mixture was separated by a semi-preparation HPLC to afford the major product, chlorofusin **1a** (5 mg). Conditions of semi-preparation HPLC: λ_{max} = 410 nm, flow rate = 3.0 mL/min.

Gradient table (A = H ₂ O, B = MeCN)				
T/min	0	5	15	20
B%	60	65	67	100

Retention time of chlorofusin **1a**: $T_{R(1a)} = 9.29$ min. $[\alpha]_D^{24} -16.1$ (c 0.09, CH₃OH). ¹H NMR (DMSO-*d*₆, 500 MHz): δ 8.97 (1H, brs), 8.61 (1H, brs), 8.57 (1H, s), 7.82 (1H, brs), 7.74(s, 1H), 7.70 (1H, d, $J = 6.6$ Hz), 7.50 (1H, d, $J = 8.9$ Hz), 7.22 (1H, s), 7.07 (1H, s), 6.96 (2H, brs), 6.90 (1H, brs), 6.88 (1H, brs), 6.83 (1H, brs), 6.19 (1H, d, $J = 5.6$ Hz), 5.26 (1H, d, $J = 4.8$ Hz), 4.98 (1H, d, $J = 5.0$ Hz), 4.72 (1H, brs), 4.54 (1H, m), 4.50 (1H, d, $J = 5.44$ Hz), 4.44 (1H, m), 4.39 (1H, m), 4.05-3.86 (8H, m), 3.76 (1H, q, $J = 7.5$ Hz), 3.67 (1H, brs), 3.50 (2H, m), 2.40 (2H, m), 2.90 (1H, d, $J = 15.0$ Hz), 2.72 (1H, dd, $J_1 = 12.0$ Hz, $J_2 = 9.0$ Hz), 2.55 (1H, m), 2.38 (2H, m), 2.32 (2H, d, $J = 7.0$ Hz), 2.03 (2H, m), 1.84-1.63 (7H, m), 1.62-1.45 (7H, m), 1.40 (3H, s), 1.45-1.33 (3H, m), 1.24 (20H, m), 1.14 (3H, d, $J = 6.4$ Hz), 0.8 (3H, d, $J = 6.5$ Hz), 0.78 (3H, d, $J = 6.1$ Hz), 0.77 (3H, t, $J = 6.0$ Hz). ¹³C NMR (DMSO-*d*₆, 150 MHz): δ 189.1, 188.6, 174.5 (HMBC), 173.4 (2C), 172.8, 172.75, 172.69, 172.2, 172.1, 171.73, 171.68, 171.3, 170.7, 150.5, 147.9, 115.5, 101.6, 97.0, 85.0, 68.69, 68.65, 65.5, 65.3, 63.1 (HMQC), 62.4, 54.2, 53.0, 52.3, 51.4, 51.0, 50.9, 49.4 (2C), 39.3 (2C), 37.6, 36.4, 34.7, 31.6, 30.5, 30.3, 29.3, 28.9, 28.8, 28.7, 27.4, 26.2, 25.4, 24.4, 24.2, 23.6, 23.4, 23.2, 22.3, 20.9, 20.5 (2C), 20.4, 18.2, 16.8, 14.2, 13.5. IR (KBr) ν_{max} 3857, 3631, 1655 cm⁻¹. HRMS (ESI, m/z) calcd for C₆₃H₉₉ClN₁₂O₁₉Na (M⁺+Na): 1385.67357; found, 1385.67302.

Compounds 13 and 15: To a solution of compound (4*S*)-**9a** (72 mg, 0.039 mmol) in MeCN (4 mL) was added I₂ (30 mg, 0.118 mmol), aq. NaHCO₃ (2 mL) successively. The reaction

was stirred at room temperature for 2 days. The reaction mixture was quenched by addition of aq. $\text{Na}_2\text{S}_2\text{O}_3$. The aqueous layer was extracted with the mixed solvent ($\text{CH}_2\text{Cl}_2:\text{MeOH} = 10:1$, 10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. The above residue was treated with a solution of TFA- H_2O (10:1, 1 mL) containing *i*- Pr_3SiH (16 μL , 0.078 mmol) at rt for 1 h. The solvent was evaporated. The resulting residue was triturated with Et_2O (2 mL x 2).

Purification on silica gel chromatography ($\text{CH}_2\text{Cl}_2:\text{MeOH} = 100:3$ to $100:7$) provided of the mixture of diastereomers (16 mg, 30%). This mixture was separated by a semi-preparation HPLC to afford the major product **13** (8 mg) as a yellow solid, and **15** (3 mg) as a yellow solid. Conditions of semi-preparation HPLC: $\lambda_{\text{max}} = 410 \text{ nm}$, flow rate = 3.0 mL/min.

Gradient table (A = H_2O , B = MeCN)				
T/min	0	5	15	20
B%	60	65	67	100

Retention times: $T_{\text{R(13)}} = 9.7 \text{ min}$; $T_{\text{R(15)}} = 10.6 \text{ min}$.

Data for compound **13**: $[\alpha]_{\text{D}}^{22} 19.8$ (*c* 0.07, CH_3OH). ^1H NMR ($\text{DMSO}-d_6$, 500 MHz): δ 9.01 (1H, brs), 8.66 (1H, brs), 8.58 (1H, brs), 7.79 (1H, s), 7.75 (1H, brs), 7.74 (1H, d, $J = 8.4 \text{ Hz}$), 7.47 (1H, d, $J = 8.9 \text{ Hz}$), 7.21 (1H, s), 7.09 (1H, s), 6.95 (2H, brs), 6.91 (1H, brs), 6.84 (1H, brs), 6.76 (1H, brs), 5.98 (1H, d, $J = 3.5 \text{ Hz}$), 5.28 (1H, d, $J = 4.6 \text{ Hz}$), 4.98 (1H, d, $J = 4.4 \text{ Hz}$), 4.77 (1H, m), 4.59 (1H, m), 4.54 (1H, d, $J = 3.5 \text{ Hz}$), 4.46 (1H, m), 4.38 (1H, m), 4.22 (1H, m), 4.00 (1H, m), 4.00-3.80 (6H, m), 3.62 (1H, brs), 3.50 (2H, m), 2.93 (1H, d, $J = 11.2 \text{ Hz}$), 2.74 (1H, dd, $J_1 = 10.6 \text{ Hz}$, $J_2 = 15.8 \text{ Hz}$), 2.47 (1H, m), 2.32 (2H, t, $J = 7.1 \text{ Hz}$), 2.11-1.87 (4H, m), 1.87-1.66 (8H, m), 1.66-1.45 (4H, m), 1.38 (1H, m), 1.37 (3H, s), 1.23 (15H, brm), 1.14 (3H, d, $J = 6.2 \text{ Hz}$), 1.13 (1H, m), 1.08 (3H, d, $J = 5.8 \text{ Hz}$), 0.90 (3H, d, $J = 6.8 \text{ Hz}$), 0.89 (3H, t, $J = 7.8 \text{ Hz}$), 0.85 (3H, d, $J = 4.6 \text{ Hz}$), 0.84 (3H, t, $J = 6.1 \text{ Hz}$), 0.76 (6H, d,

$J = 5.8$ Hz). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 189.3, 188.6, 174.8 (2C), 173.6, 173.4 (2C), 172.9, 172.2, 172.1 (2C), 171.9, 171.6, 170.8, 149.9, 148.8, 114.0, 100.7, 98.2, 85.0, 71.3, 69.5 (2C), 65.3 (2C), 63.2 (HMQC), 62.8, 54.3, 53.0, 52.7, 51.8, 51.3, 50.2, 49.5 (2C), 39.0 (2C), 37.5, 36.0, 34.9, 31.7, 30.3, 29.4, 29.1, 28.8, 28.6, 27.3, 26.4, 24.9, 24.7, 24.5, 23.8, 23.6, 23.4, 22.5, 21.0, 20.8, 20.7 (2C), 18.4, 16.8, 14.4, 13.7. IR (KBr) ν_{max} 3321, 2930, 1653 cm^{-1} . HRMS (ESI, m/z) calcd for $\text{C}_{63}\text{H}_{99}\text{ClN}_{12}\text{O}_{19}\text{Na}$ ($\text{M}^+ + \text{Na}$): 1385.67357; found, 1385.67301. Data for compound **15**: $[\alpha]_D^{22} -16.5$ (c 0.019, CH_3OH). ^1H NMR (DMSO- d_6 , 500 MHz): δ 9.09 (1H, brs), 8.67 (1H, brs), 8.46 (1H, brs), 7.86 (1H, s), 7.80 (1H, brs), 7.69 (1H, d, $J = 6.2$ Hz), 7.46 (1H, d, $J = 8.2$ Hz), 7.21 (1H, brs), 7.05 (1H, brs), 6.97 (1H, brs), 6.89 (1H, brs), 6.86 (1H, brs), 6.71 (1H, brs), 6.67 (1H, brs), 5.88 (1H, d, $J = 6.0$ Hz), 5.28 (1H, d, $J = 4.4$ Hz), 5.04 (1H, brs), 4.72 (1H, m), 4.58 (1H, m), 4.46 (1H, d, $J = 5.8$ Hz), 4.45 (1H, m), 4.39 (1H, m), 4.21 (1H, m), 4.05 (1H, m), 4.03-3.80 (6H, m), 3.62 (1H, brs), 3.50 (2H, m), 2.91 (1H, m), 2.73 (1H, m), 2.60 (1H, m), 2.33 (1H, t, $J = 6.9$ Hz), 2.11-1.87 (4H, m), 1.87-1.43 (12H, m), 1.39 (3H, s), 1.38 (1H, m), 1.36 (1H, m), 1.23 (15H, brm), 1.14 (3H, d, $J = 6.4$ Hz), 1.08 (3H, m), 0.90 (2H, d, $J = 7.0$ Hz), 0.89 (2H, t, $J = 4.9$ Hz), 0.85 (6H, brm), 0.78 (3H, d, $J = 6.6$ Hz), 0.76 (3H, d, $J = 6.3$ Hz). IR (KBr) ν_{max} 3316, 2932, 1656 cm^{-1} . HRMS (ESI, m/z) calcd for $\text{C}_{63}\text{H}_{99}\text{ClN}_{12}\text{O}_{19}\text{Na}$ ($\text{M}^+ + \text{Na}$): 1385.67357; found, 1385.67302.

Compound 12: To a solution of (*S*)-**2** (5 mg, 0.014 mmol) in MeCN (5 mL) was added NBS (4 mg, 0.021 mmol). This mixture was stirred at room temperature for 1 h, and then cyclopeptide **3b** (25 mg, 0.0169 mmol), aq. NaHCO_3 (2 mL), and Ag_2O (5 mg, 0.021 mmol) were added successively. The reaction mixture was stirred at

room temperature for 3 days. The inorganic residues were removed by filtration over a pad of Celite. The solution was extracted with the mixed solvent (CH_2Cl_2 :MeOH = 10:1, 10 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Purification on silica gel (CH_2Cl_2 : MeOH = 100:2 to 100:5) provided a mixture of

diastereomers (12 mg, 46%). This mixture was separated by a semi-preparation HPLC to afford a major product **12** (6 mg, $T_{R(12)} = 15.4$ min). Conditions of semi-preparation HPLC: $\lambda_{\max} = 402$ nm, flow rate = 3.0 mL/min.

Gradient table (A = H ₂ O, B = MeCN)			
T/min	0	20	25
B%	80	90	100

$[\alpha]_D^{25}$ 31.4 (*c* 0.085, CH₂Cl₂). ¹H NMR (DMSO-*d*₆, 500 MHz): δ 9.29 (1H, brs), 8.99 (1H, s), 8.60 (1H, s), 8.17 (1H, s), 7.81 (1H, d, *J* = 7.5 Hz), 7.75 (1H, s), 7.55 (1H, s), 7.38-7.09 (30H, m), 7.04 (6H, d, *J* = 7.7 Hz), 6.89 (1H, brs), 6.36 (1H, brs), 6.17 (1H, s), 5.40 (1H, brs), 5.14 (1H, m), 4.82 (1H, t, *J* = 10.0 Hz), 4.53 (1H, t, *J* = 7.2 Hz), 4.39 (1H, s), 4.15 (3H, m), 3.90 (4H, m), 3.71 (2H, brs), 3.58 (1H, q, *J* = 7.1 Hz), 3.07 (2H, t, *J* = 6.7 Hz), 2.42-2.32 (1H, m), 2.34 (2H, t, *J* = 7.0 Hz), 2.10-1.96 (3H, m), 1.94-1.77 (4H, m), 1.73-1.59 (4H, m), 1.59-1.40 (6H, m), 1.54 (2H, m), 1.43 (3H, s), 1.40-1.11 (36H, m), 0.98 (3H, d, *J* = 5.0 Hz), 0.90 (3H, t, *J* = 7.3 Hz), 0.85 (3H, t, *J* = 6.9 Hz), 0.82 (3H, d, *J* = 6.3 Hz), 0.76 (3H, t, *J* = 6.9 Hz), 0.36 (2H, d, *J* = 6.2 Hz). IR (KBr) $\nu_{\max}$ 2925, 2867, 1660 cm⁻¹. HRMS (ESI, *m/z*) calcd for C₁₀₁H₁₂₇ClN₁₂O₁₉Na (M⁺+Na): 1869.89267; found, 1869.89212.

II. Data comparisons and HPLC analyses

II-1a. Model chromophores 11a-11d and their corresponding X-ray structures.

Figure S-1: OREPT drawing of compound **11a**, **11b**, **11c'**, **11d'**^[S4]

^[S4] Crystallographic data for compounds **11a**, **11b**, **11c'**, and **11d'** (CIF files, in a separate file).

II-1b. HPLC analysis of model chromophores 11a-11d.

HPLC conditions: $\lambda_{\text{max}} = 401 \text{ nm}$, flow rate = 1.0 ml/ min. A = H₂O, B = CH₃CN

Gradient table (A = H₂O, B = CH₃CN):

T/ min	0	20	25
B%	25	35	100

The order of retention times: $t_{11a} < t_{11b} < t_{11c} < t_{11d}$.

Figure S-2: HPLC comparison of **11a-11d**.

II-2. HPLC analysis of product ratio for one-pot reaction of *rac*-2 and methylamine.

Figure S-3. HPLC analysis of the one pot protocol product

II-3. ¹H NMR data comparison of model chromophores and chlorofusin (reported)

Table S-1. ¹H NMR data comparison

Position	Chlorofusin ^a	11a	11b	11c	11d
1	7.75 (s)	7.86 (s)	7.90 (s)	7.83 (s)	7.83 (s)
8	4.51 (d)	4.54 (s)	4.48 (d)	4.50 (s)	4.53 (d)
10	2.36 (brm)	1.91-1.72 (m)	1.95-1.85 (m)	2.47-2.38 (m) 2.31-2.22 (m)	2.46-2.38 (m) 2.31-2.23 (m)
11	1.98 - 2.18 (m)	2.09-1.92 (m)	2.08-1.95 (m)	2.12-1.97 (m)	2.11-1.97 (m)
12	3.76 (q) and 4.00 (m)	4.09-3.96 (m) 4.23-4.09 (m)	4.08-3.97 (m) 4.21-4.10 (m)	3.84-3.71(m) 4.06-3.95(m)	3.82-3.71 (m) 4.07-3.96 (m)
13	1.41 (s)	1.38 (s)	1.39 (s)	1.36 (s)	1.40 (s)
14	6.24 (d)	6.10 (s)	6.11 (d)	6.38 (s)	6.25 (d)
16	2.32 (t)	2.32 (t)	2.32 (t)	2.32 (t)	2.33 (t)
17	1.53 (sextet)	1.52 (sextet)	1.52 (sextet)	1.53 (sextet)	1.52 (sextet)
18	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)

^aAll NMR data were collected in DMSO-d₆. Chemical shift calibration of residual ¹H-DMSO signal was 2.52, and ¹³C-DMSO signal at 39.6 for the natural product (see JACS **2001**, *123*, 554-560). Data for our sample is 2.50 and 39.6 unless otherwise note.

Table S-2. ¹³C NMR data comparison

Position	11a ^a	11b	11c	11d	Chlorofusin
5	188.9	188.9	189.0	188.9	188.7
3	188.3	188.3	188.5	188.5	188.1
15	171.6	171.7	171.6	171.6	171.4
1	150.5	150.6	151.4	151.4	150.0
7	148.7	148.7	147.7	147.7	147.5
2	113.4	113.4	114.9	114.9	115.2
6	99.8	99.8	100.9	100.8	101.3
9	97.1	97.1	96.3	96.3	96.7
4	84.6	84.7	84.7	84.7	84.7
12	70.4	70.5	69.2	69.2	68.4

8	68.8	68.8	69.0	68.9	68.4
16	34.5	34.6	34.5	34.5	34.4
10	34.0	34.0	30.6	30.6	30.3
11	25.0	25.0	25.2	25.1	25.1
13	23.0	23.0	23.0	23.0	22.9
17	18.0	18.0	18.1	18.0	17.9
18	13.3	13.3	13.3	13.3	13.2

^a **11a-d** Chemical shift calibration of residual ¹³C-DMSO signal at 40.0.

II-4. Coupling of **2** with peptide **3b**.

II-4a. HPLC separation of **(4S)-9a** and **(4R)-9b**.

HPLC conditions: λ_{max} = 380 nm, flow rate = 1.0 mL/ min.

Gradient table (A = H ₂ O, B = CH ₃ CN)					
T/ min	0	10	20	25	30
B%	80	85	87	100	100

Figure S-4: HPLC analysis of **9a** and **9b**

II-4b. ^1H NMR comparison of **9a**, **9b** and chlorofusin (reported).

Table S-3. ^1H NMR data comparison

Position	Chlorofusin	(4S)- 9a	(4R)- 9b
1	7.75(s)	7.83(s)	7.84(s)
13	1.41(s)	1.43(s)	1.29(s)
16	2.32(t)	2.33(t)	2.32(t)
17	1.53(sextet)	1.54(m)	1.55(m)
18	0.90(t)	0.91(t)	0.91(t)

II-5. ^1H , ^{13}C NMR comparison of 13 and model chromophores.

Table S-4. ^1H NMR data comparison

Position	11a	11b	11c	11d	13	chlorofusins
1	7.86 (s)	7.90 (s)	7.83 (s)	7.83 (s)	7.79 (s)	7.75 (s)
8	4.54 (s)	4.48 (d)	4.50 (s)	4.53 (d)	4.54 (d)	4.51 (d)
10	1.91-1.72 (m)	1.95-1.85 (m)	2.47-2.38 (m)	2.46-2.38 (m)	2.00-1.80 (m)	2.36 (brm)
11	2.09-1.92 (m)	2.08-1.95 (m)	2.12-1.97 (m)	2.11-1.97 (m)	2.05-2.00 (m)	1.98-2.18 (m)
12	4.09-3.96 (m)	4.08-3.97 (m)	3.84-3.71 (m)	3.82-3.71 (m)	4.06 (m)	3.76 (q) and
	4.23-4.09 (m)	4.21-4.10 (m)	4.06-3.95 (m)	4.07-3.96 (m)	4.22 (m)	4.00 (m)
13	1.38 (s)	1.39 (s)	1.36 (s)	1.40 (s)	1.36 (s)	1.41 (s)
14	6.10 (s)	6.11 (d)	6.38 (s)	6.25 (d)	5.98 (d)	6.24 (d)
16	2.32 (t)	2.32 (t)	2.32 (t)	2.33 (t)	2.31 (t)	2.32 (t)
17	1.52 (sextet)	1.52 (sextet)	1.53 (sextet)	1.52 (sextet)	1.52 (sextet)	1.53 (sextet)
18	0.90 (t)	0.9 (t)	0.90 (t)	0.90 (t)	0.89 (t)	0.90 (t)

Table S-5. ^{13}C NMR data comparison

Position	11a	11b	11c	11d	Chlorofusins	13 ^a
5	188.9	188.9	189.0	188.9	188.7	188.9
3	188.3	188.3	188.5	188.5	188.1	188.2
15	171.6	171.7	171.6	171.6	171.4	171.7
1	150.5	150.6	151.4	151.4	150.0	149.5
7	148.7	148.7	147.7	147.7	147.5	148.4
2	113.4	113.4	114.9	114.9	115.2	113.6
6	99.8	99.8	100.9	100.8	101.3	100.3
9	97.1	97.1	96.3	96.3	96.7	97.8
4	84.6	84.7	84.7	84.7	84.7	84.6
12	70.4	70.5	69.2	69.2	68.4	71.0
8	68.8	68.8	69.0	68.9	68.4	69.0
16	34.5	34.6	34.5	34.5	34.4	34.5
10	34.0	34.0	30.6	30.6	30.3	29.1
11	25.0	25.0	25.2	25.1	25.1	24.5
13	23.0	23.0	23.0	23.0	22.9	23.0
17	18.0	18.0	18.1	18.0	17.9	18.0
18	13.3	13.3	13.3	13.3	13.2	13.3

^aChemical shift calibration of residual DMSO signal at 40.0 ppm.

II-5. HPLC analysis for conversion of **11d** to **11a** under acidic conditions.

Conditions of semi-preparation HPLC: $\lambda_{\text{max}} = 401 \text{ nm}$, flow rate = 1.0 ml/ min.

Gradient table (A = H ₂ O, B = CH ₃ CN)			
T/ min	0	20	25
B%	25	35	100

Figure S-5. HPLC analysis for the conversion of **11d** to **11a**.

II-6. ¹H, ¹³C NMR comparison of synthetic chlorofusin **1a**, natural chlorofusin (reported) and model compounds **11a**-**11d**.

Table S-6. ¹H NMR data comparison

Position	11a	11b	11c	11d	1a	chlorofusin
1	7.86 (s)	7.90 (s)	7.83 (s)	7.83 (s)	7.74 (s)	7.75 (s)
8	4.54 (s)	4.48 (d)	4.50 (s)	4.53 (d)	4.50 (d)	4.51 (d)
10	1.91-1.72 (m)	1.95-1.85 (m)	2.47-2.38 (m) 2.31-2.22 (m)	2.46-2.38 (m) 2.31-2.23 (m)	2.37 (m)	2.36 (brm)
11	2.09-1.92 (m)	2.08-1.95 (m)	2.12-1.97 (m)	2.11-1.97 (m)	2.08-2.00 (m)	1.98 - 2.18 (m)
12	4.09-3.96 (m) 4.23-4.09 (m)	4.08-3.97 (m) 4.21-4.10 (m)	3.84-3.71(m) 4.06-3.95(m)	3.82-3.71 (m) 4.07-3.96 (m)	3.76 (q) and 4.05-4.00 (m)	3.76 (q) and 4.00 (m)
13	1.38 (s)	1.39 (s)	1.36 (s)	1.40 (s)	1.40 (s)	1.41 (s)
14	6.10 (s)	6.11 (d)	6.38 (s)	6.25 (d)	6.19 (d)	6.24 (d)
16	2.32 (t)	2.32 (t)	2.32 (t)	2.33 (t)	2.32 (t)	2.32 (t)
17	1.52 (sextet)	1.52 (sextet)	1.53 (sextet)	1.52 (sextet)	1.53 (sextet)	1.53 (sextet)
18	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)

Table S-7. ¹³C NMR data comparison

Position	11a	11b	11c	11d	1a ^a	Chlorofusin
5	188.9	188.9	189.0	188.9	188.8	188.7
3	188.3	188.3	188.5	188.5	188.3	188.1
15	171.6	171.7	171.6	171.6	171.4	171.4
1	150.5	150.6	151.4	151.4	150.2	150.0
7	148.7	148.7	147.7	147.7	147.6	147.5
2	113.4	113.4	114.9	114.9	115.2	115.2
6	99.8	99.8	100.9	100.8	101.3	101.3
9	97.1	97.1	96.3	96.3	96.7	96.7
4	84.6	84.7	84.7	84.7	84.7	84.7
12	70.4	70.5	69.2	69.2	68.4	68.4
8	68.8	68.8	69.0	68.9	68.4	68.4
16	34.5	34.6	34.5	34.5	34.4	34.4
10	34.0	34.0	30.6	30.6	30.2	30.3
11	25.0	25.0	25.2	25.1	25.1	25.1
13	23.0	23.0	23.0	23.0	22.9	22.9
17	18.0	18.0	18.1	18.0	17.9	17.9
18	13.3	13.3	13.3	13.3	13.2	13.2

^aChemical shift calibration of residual DMSO signal at 39.9 ppm.**II-7. ¹H NMR comparison of synthetic chlorofusin (1a) at different temperatures.**

In contrast to its chromophore part, the peptide part of chlorofusin (**1a**) is very dynamic in the solution. ¹H NMR of its peptide region was found very sensitive to the temperatures, especially for those active amide protons.

Figure S-6. ^1H NMR spectra of **1a** between 300 K and 340 K.

II-8. Preparation of chlorofusin **1a** and **14**, and their NMR comparison.

II-8a. HPLC analysis of the crude products from reactions of (a) *rac*-**2** and (b) (*S*)-**2** with **3a**.

HPLC conditions: $\lambda_{\text{max}} = 410 \text{ nm}$, flow rate = 1.0 mL/min.

Gradient table (A = H_2O , B = CH_3CN)					
T/ min	0	10	20	25	30
B%	50	60	65	100	100

Figure S-7. HPLC analysis of crude products from reactions of *rac*-**2** (a) and (*S*)-**2** (b) with cyclopeptide **3a**.

II-8b. ¹H NMR comparisons after HPLC purification.

Figure S-8. Spectral comparisons of ¹H NMR of pure **1a** and that of mixture of **1a** & **14**.

II-9. Preparation of **15** and **13**, and their ¹H NMR comparison

Compound **15** (fully *cis* for oxygenated functionalities in the chromophore region) does not show in the manuscript. Such a structure was suggested as the natural product in the previous JACS paper (see *JACS* **2001**, *123*, 554). We prepared **15** through the following steps from (*S*)-**2**. After HPLC purification, its ¹H NMR clearly showed that it is not the real structure of natural chlorofusin.

Table S-8. ¹H NMR data comparison of natural chlorofusin (reported), chlorofusin isomers **13** and **15**, and the model chromophores **11a-11d**

Position	11a	11b	11c	11d	15	Chlorofusin	13
1	7.86 (s)	7.90 (s)	7.83 (s)	7.83 (s)	7.86 (s)	7.75 (s)	7.79 (s)
8	4.54 (s)	4.48 (d)	4.50 (s)	4.53 (d)	4.46 (d)	4.51 (d)	4.54 (d)
10	1.91-1.72 (m)	1.95-1.85 (m)	2.47-2.38 (m)	2.46-2.38 (m)	1.90 – 1.70 (m)	2.36 (brm)	2.00–1.80 (m)

			2.31-2.22 (m)	2.31-2.23 (m)			
11	2.09-1.92 (m)	2.08-1.95 (m)	2.12-1.97 (m)	2.11-1.97 (m)	2.05 – 1.90 (m)	1.98 - 2.18 (m)	2.05-2.00 (m)
12	4.09-3.96 (m)	4.08-3.97 (m)	3.84-3.71(m)	3.82-3.71 (m)	4.04 (m)	3.76 (q) and	4.06 (m)
	4.23-4.09 (m)	4.21-4.10 (m)	4.06-3.95(m)	4.07-3.96 (m)	4.20 (m)	4.00 (m)	4.22 (m)
13	1.38 (s)	1.39 (s)	1.36 (s)	1.40 (s)	1.39 (s)	1.41 (s)	1.36 (s)
14	6.10 (s)	6.11 (d)	6.38 (s)	6.25 (d)	5.88 (s)	6.24 (d)	5.98 (d)
16	2.32 (t)	2.32 (t)	2.32 (t)	2.33 (t)	2.33 (t)	2.32 (t)	2.31 (t)
17	1.52 (sextet)	1.52 (sextet)	1.53 (sextet)	1.52 (sextet)	1.54 (sextet)	1.53 (sextet)	1.52 (sextet)
18	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)	0.89 (t)

II-10. Full NMR assignment of synthetic chlorofusin 1a and data comparison

¹ H NMR (500MHz, DMSO-d ₆)			¹³ C NMR (500 MHz, DMSO-d ₆)		
	natural	1a ^a		natural	1a ^a
Chromophoric Unit			Chromophoric Unit		
Positon			Position		
1	7.75	7.74	1	150.0	150.2
8	4.51	4.50	2	115.2	115.2
10	2.36	2.38	3	188.1	188.3
11	2.08	2.03	4	84.7	84.7
12	3.76	3.76	5	188.7	188.8
12	4.00	4.00	6	101.3	101.3
13	1.41	1.40	7	147.5	147.6
14	6.24	6.19	8	68.4	68.4
16	2.32	2.32	9	96.7	96.7
17	1.53	1.53	10	30.3	30.2
18	0.90	0.90	11	25.1	25.1
Thr-1			12	68.4	68.3
NH	8.71	8.61	13	22.9	22.9
α-CH	3.64	3.67	15	171.4	171.4
β-CH	4.00	4.00	16	34.4	34.4
γ-CH ₃	1.14	1.14	17	17.9	17.9
OH	5.26	5.26	18	13.2	13.2
Ala-2			Thr-1		
NH	8.59	8.57	α-CH	63.1	62.8 ^b
α-CH	3.93	3.90	β-CH	65.0	65.0
β-CH ₃	1.24	1.24	γ-CH ₃	20.3	20.2
Asn-3			α-carbonyl	173.0	
NH	6.91	6.95	Ala-2		
α-CH	4.73	4.72	α-CH	50.8	50.6
β-CH ₂	2.60	2.55	β-CH ₃	16.5	16.5
β-CH ₂	2.91	2.90	α-carbonyl	171.6	
γ-NH ₂	6.80	6.83	Asn-3		
γ-NH ₂	6.88	6.88	α-CH	49.0	49.1
Asn-4			β-CH ₂	37.3	37.3
NH	7.82	7.82	γ-carbonyl	170-174	
α-CH	4.39	4.39	α-carbonyl	170.9	

β -CH ₂	2.46	2.50	Asn-4		
β -CH ₂	2.73	2.72	α -CH	52.0	52.0
γ -NH ₂	6.98	6.96	β -CH ₂	36.2	36.1
γ -NH ₂	7.22	7.22	γ -carbonyl	170.3	
Leu-5			α -carbonyl	170-174	
NH	7.49	7.50	Leu-5		
α -CH	4.46	4.44	α -CH	49.2	49.1
β -CH ₂	1.11	1.11	β -CH ₂	38.7	39.0
β -CH ₂	1.58	1.58	γ -CH	24.0	23.9
γ -CH	1.39	1.39	δ -CH ₃	20.6	20.6
δ -CH ₃	0.75	0.77	δ -CH ₃	23.2	23.3
δ -CH ₃	0.76	0.78	α -carbonyl	173.1	
Thr-6			Thr-6		
NH	7.05	7.07	α -CH	62.1	62.1
α -CH	3.90	3.90	β -CH	64.9	65.0
β -CH	3.90	3.90	γ -CH ₃	20.2	20.2
γ -CH ₃	1.08	1.08	α -carbonyl	170-174	
OH	5.03	4.98	Leu-7		
Leu-7			α -CH	52.7	52.7
NH	9.06	8.97	β -CH ₂	38.7	39.0
α -CH	3.93	3.93	γ -CH	24.1	24.1
β -CH ₂	1.58	1.58	δ -CH ₃	20.1	20.1
γ -CH	1.73	1.73	δ -CH ₃	23.1	23.1
δ -CH ₃	0.80	0.80	α -carbonyl	172.4	
δ -CH ₃	0.90	0.90	Ada-8		
Ada-8			α -CH	53.9	53.9
NH	7.68	7.70	β -CH ₂	30.0	30.0
α -CH	4.00	4.00	γ -CH ₂	25.9	25.9
β -CH ₂	1.73	1.73	δ -CH ₂	28.6	29.0
γ -CH ₂	1.36	1.36	ϵ -CH ₂	28.5	28.6
γ -CH ₂	1.24	1.24	ζ -CH ₂	28.4	28.5
δ -CH ₂	1.24	1.24	η -CH ₂	31.2	31.3
ϵ -CH ₂	1.24	1.24	θ -CH ₂	22.0	22.0
ζ -CH ₂	1.24	1.24	CH ₃	13.9	13.9
η -CH ₂	1.24	1.24	α -carbonyl	171.9	
θ -CH ₂	1.24	1.24	Orn-9		
CH ₃	0.85	0.85	α -CH	51.2	51.1
Orn-9			β -CH ₂	28.3	28.4
NH	6.67	6.70	γ -CH ₂	27.0	27.1
α -CH	4.57	4.54	δ -CH ₂	50.5	50.6
β -CH ₂	1.73	1.73	α -carbonyl	170-174	
γ -CH ₂	1.53	1.53			
γ -CH ₂	1.73	1.73			
δ -CH ₂	3.40	3.42 and 3.50 ^b			

^a300K; ^b assignment assisted by HMQC experiments.

Carbonyls Observed (500MHz, DMSO-d⁶)

Chlorofusin (Cyclopeptide part)	1a
173.1	173.1
173.0	173.1
172.4	172.4
171.9	171.9
171.6	171.8
170.9	171.0
170.3	170.4
170-174	174.2 ^c
170-174	172.5
170-174	172.3
170-174	171.3

^c assignment assisted by HMBC experiments.

III. Selected NMR spectra copies (^1H NMR spectra for compounds **6-8**, (*S*)-**2**, **9a-b**, *rac*-**11a~rac-11d**, **12**, **13**, **15**, **1a** and the mixture of **1a** and **14**, and ^{13}C NMR spectra for compounds **6-8**, (*S*)-**2**, **9a-b**, *rac*-**11a~rac-11d**, **13** and **1a**).

Archive directory: /export/home/shi-m/vnmrsys/data
 Sample directory:
 File: CARBON

Pulse Sequence: s2pu1

Current Data Parameters

NAME txo
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters

Date_ 20060828
Time 17.02
INSTRUM av500
PROBHD 5 mm TXO 19F/1
PULPROG zg
TD 65536
SOLVENT DMSO
NS 103
DS 0
SMH 15015.015 Hz
FIDRES 0.229111 Hz
AQ 2.1824322 sec
RG 256
DW 33.300 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec

===== CHANNEL f1 =====

NUC1 1H
P1 4.00 usec
PL1 0.00 dB
SF01 500.1320806 MHz

F2 - Processing parameters

SI 65536
SF 500.1300059 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters

CX 20.00 cm
CY 0.00 cm
F1P 10.000 ppm
F1 5001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.50000 ppm/cm
HZCM 250.06500 Hz/cm

7.828

6.380
6.367

4.508
4.484

4.000

3.788
3.762

3.318
3.283

2.501
2.357
2.333
2.310

2.087
2.044

1.563
1.540
1.515
1.480
1.382

0.928
0.901
0.877

-0.001

Current Data Parameters
NAME tso
EXPNO 8
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081211
Time 16.45
INSTRUM av500
PROBHD 5 mm TXO 197/1
PULPROG zg
TD 65536
SOLVENT DMSO
NS 68
DS 0
SWH 15015.015 Hz
FIDRES 0.229111 Hz
AQ 2.1824322 sec
RG 512
OW 33.300 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 4.00 usec
PL1 0.00 dB
SF01 500.1320806 MHz

F2 - Processing parameters
SI 65536
SF 500.1300050 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
CY 0.00 cm
F1P 10.000 ppm
F1 5001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PRACH 0.50000 ppm/cm
HZCM 250.06500 Hz/cm

Current Data Parameters
NAME bbi
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20061231
Time 9.52
INSTRUM av500
PROBHD 5 mm BBI 1H-13
PULPROG zg
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 15015.015 Hz
FIDRES 0.229111 Hz
AQ 2.1824322 sec
RG 256
DW 33.300 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 3.00 usec
PL1 -4.00 dB
SFO1 500.1318915 MHz

F2 - Processing parameters
SI 65536
SF 500.1300054 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

10 NMR plot parameters
CX 20.00 cm
CY 0.00 cm
F1P 10.000 ppm
F1 5001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.50000 ppm/cm
HZCM 250.06500 Hz/cm

Pulse Sequence: s2pu1

Current Data Parameters
NAME h1
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20061228
Time 10.47
INSTRUM drx400
PROBHD 5 mm QNP 13C
PULPRDG zg
TD 65536
SOLVENT D2O
NS 64
DS 0
SWH 12019.230 Hz
FIDRES 0.183399 Hz
AQ 2.7263477 sec
RG 256
DW 41.600 usec
DE 20.00 usec
TE 300.0 K
D1 2.00000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 5.00 usec
PL1 0.00 dB
SF01 400.1316015 MHz

F2 - Processing parameters
SI 65536
SF 400.1300030 MHz
WOW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 10.000 ppm
F1 4001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.50000 ppm/cm
HZCM 200.06500 Hz/cm

Current Data Parameters
NAME q-b-35-3
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20061118
Time 3.39
INSTRUM drx400
PROBHD 5 mm QNP 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 16
DS 0
SWH 12019.230
FIDRES 0.183399
AQ 2.7263477
RG 64
OW 41.600
DE 20.00
TE 300.0
D1 2.00000000

===== CHANNEL f1
NUC1 1H
P1 5.00
PL1 0.00
SF01 400.1317262

F2 - Processing parameters
SI 65536
SF 400.1300029
WDW no
SSB 0
LB 0.00
GB 0
PC 2.00

1D NMR plot parameters
CX 20.00
F1P 10.000
F1 4001.30
F2P 0.000
F2 0.00
PPMCM 0.50000
HZCM 200.06500

Current Data Parameters
NAME bbi
EXPNO 9
PROCNO 1

F2 - Acquisition Parameters
Date_ 20070313
Time 11.10
INSTRUM av500
PROBHD 5 mm BBI 1H-13
PULPROG zg
TD 65536
SOLVENT DMSO
NS 400
DS 0
SMH 15015.015 Hz
FIDRES 0.229111 Hz
AQ 2.1824322 sec
RG 1024
DW 33.300 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec

----- CHANNEL f1 -----
NUC1 1H
P1 3.00 usec
PL1 -4.00 dB
SF01 500.1325085 MHz

F2 - Processing parameters
SI 65536
SF 500.1300058 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
CY 0.00 cm
F1P 10.000 ppm
F1 5001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCH 0.50000 ppm/cm
HZCM 250.06500 Hz/cm