

# **First Syntheses of Melophlins P, Q and R and Effects of Melophlins on the Growth of Microorganisms and Tumor Cells**

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**((Abstract))**

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The marine tetramic acids melophlin P, Q and R were synthesized for the first time in only four steps. Together with the congenerous melophlins A–C, and G they were also tested for antimicrobial and cytotoxic effects. Melophlins B, C, P, Q and R which share a 5-methyl residue showed some antibacterial activity, mainly in Gram-positive bacteria. Melophlins B, C and R which have methyl branched 3-acyl side chains in common, inhibited the growth of cells of human KB-3-1 cervix carcinoma, A-498 kidney carcinoma and U-937 leukemia at  $IC_{50} < 10 \mu\text{M}$ . They were similar in activity to cisplatin. Melophlin Q, also methyl branched, was astoundingly specific in inhibiting A-498 kidney cancer cells while melophlin P inhibited U-937 leukemia cells particularly well. The position of the methyl branch is decisive for the magnitude of the antiproliferative effect of the melophlin couples B/C and R/Q.

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## Introduction

The melophlins **1** are *N*-methyl-3-acyltetramic acids that differ only in the substituents at C(5) (H or Me) of the pyrrolidine-2,4-dione core and in the chain length (C<sub>12</sub> to C<sub>16</sub>) and branching of the 3-acyl residue (Fig. 1). The first two derivatives termed melophlin A and B were isolated by *Kobayashi et al.* from the Indonesian sponge *Melophlus sarasinorum* collected at Spermonde Islands [1]. Melophlin B was shown to be a mixture of two 5*S*-configured diastereomers. Three years later *Proksch et al.* obtained another thirteen congeners dubbed melophlins C–O from the same sponge collected near Makassar [2]. Again, a good deal of them was proved to be mixtures of diastereoisomers (e.g. melophlins C, J, L, M, N, O). Finally, in 2006 *Namikoshi et al.* reported the extraction of the melophlins P–S from a specimen of *Melophlus sarasinorum* harvested in Palau [3]. The melophlins P and Q turned out to be racemic mixtures of the 5*S* and 5*R*-enantiomers, while melophlin R was also racemic at C(5) with the stereogenic centre in its side chain remaining unspecified. Given the high incidence of bioactivity in 3-acyltetramic acids in general [4-7], reports on the biological properties of the melophlin family have been comparatively scarce. In early tests natural melophlins A and B displayed cytotoxic activity against HL-60 cells at 0.2 and 0.4 µg/mL, respectively, and also arrested NIH/3T3 cells in the G<sub>1</sub> phase of the cell cycle at 1 µg/mL [1]. Melophlins C, E, G, H, I, M, N, and O exerted no cytotoxicity in HL-60, HeLa or TF-1 cells, but melophlin C proved antibacterial in *Bacillus subtilis* and *Staphylococcus aureus* and antifungal in *Candida albicans* [2]. Another study by *Namikoshi et al.* of eleven melophlins including the set of the four isolated latest revealed that only melophlins H and O were modestly active in Chinese hamster lung fibroblasts V79 and none out

of eleven had any impact on the level of cytokine IL-8 in PMA-stimulated HL-60 cells [8]. Recently, the Waldmann group has embarked on systematic investigations of small libraries of synthetic acyltetramic acids, including assays for cytotoxic and antimicrobial activity, phenotypical and genetic changes as well as inhibition of phosphatases such as Cdc25A and VHR [9]. A study by *Lee et al.* of the antimicrobial properties of unnatural 3-acetyltetramic acids is currently being published [10].

This paper presents the first short syntheses of the melophlins P, Q and R as well as the results of biotests of structurally representative members of the melophlin family against further tumor cell lines, bacteria, yeasts and molds.

((Figure 1 here))

## Results and Discussion

### Chemistry

The melophlins P (**1p**), Q (**1q**) and R (**1r**) were prepared in four steps as previously described for the congeners B and C (Scheme 1) [11]. *N*-methylalanine *t*-butyl ester **2** was reacted with **3**, the cumulated ylide  $\text{Ph}_3\text{P}=\text{C}=\text{C}=\text{O}$ , immobilized on polystyrene [12] to give the tetramate **4** in 92% yield as product of a domino addition / intra-Wittig alkenation process. Since the natural products **1p–r** had been shown by oxidative degradation to be 1:1 mixtures of (5*R*)- and (5*S*)-stereoisomers [3], racemic **2** was employed. Immobilization of the phosphorus ylide greatly facilitated removal of by-product phosphine oxide. Quantitative cleavage of the ester **4** with trifluoroacetic acid (TFA) gave 1,5-dimethylpyrrolidine-2,4-dione **5**. This was 3-acylated under Jones' conditions [13] with  $\text{BF}_3$ -diethyl etherate and the respective acyl chloride **6**, which was racemic in the case of **6r**, to furnish the corresponding

BF<sub>2</sub>-adducts **7** in a moderate 20-40% yield under classical thermal but in 30-65% yield under microwave irradiation conditions. The BF<sub>2</sub>-chelates **7** were finally converted to melophlins P, Q or R, respectively, by boiling in methanol. The required chlorides **6q** and **6r** were prepared from 13-methyltetradecanoic acid (13-MTA) [14] and 12-methyltetradecanoic acid (12-MTA), respectively. To assess the influence of metal chelation on the bioactivity we also prepared a stable Ca(II) complex of melophlin A, Ca(**1a**)<sub>2</sub>, by slowly adding an aqueous suspension of CaCO<sub>3</sub> to a methanolic solution of two equivalents of melophlin A and collecting the formed precipitate, similarly to a recently published method [15]. Earlier reports on the effect of metal chelation on the cytotoxic and antimicrobial activity of tetramic acids such as magnesidin [16] [17] and tenuazonic acid [18] were inconclusive.

((Scheme 1 here))

### **Biological evaluation**

Kinases and phosphatases play a pivotal role in the signal transduction of cancer cells. As 3-acyltetramates were frequently reported to inhibit these enzymes, we looked for antiproliferative effects of a representative, structurally diverse subset of melophlins (A–C, G, P–R) in human KB-3-1 cervix, human epithelial A-498 kidney carcinoma, and U-937 leukemia cells as well as L929 mouse fibroblasts (Table 1). In terms of stereochemistry, the compounds were prepared and tested as they had been isolated from natural sources, i.e. melophlins A and G achiral, melophlin B as mixture of two 5*S*-configured diastereomers, melophlins C and R as mixtures of four diastereomers, and melophlins P and Q as racemates of the 5*S*- and 5*R*-enantiomers. The melophlins B, C and R featuring branched 3-acyl side chains, as well as complex Ca(**1a**)<sub>2</sub> inhibited the growth of all four cancer cell types at one-digit micromolar

concentrations. They were nearly as active as the clinical standard drug cisplatin. It is worthy of note that melophlin C was previously reported inactive in HeLa cells, the parent cervix carcinoma cell line of KB-3-1 [2]. There were two melophlins with conspicuously good activity in only one of the four cell lines. Melophlin Q specifically inhibited A-498 cells at  $IC_{50} = 3.1 \mu\text{M}$  while the 3-unbranched melophlin P inhibited the growth of U-937 cells at  $IC_{50} = 2.1 \mu\text{M}$ . Of highest activity in both cell lines was melophlin R, though, with  $IC_{50}$  values of  $2.1 \mu\text{M}$  (A-498) and  $2.2 \mu\text{M}$  (U-937). This is actually better than activities for cisplatin in these cell lines as measured by us and reported in literature, i.e.  $IC_{50} = 3.08 \mu\text{M}$  (A-498; 120 h) [19] and  $6.41 \mu\text{M}$  (U-937; 48 h) [20]. The distinctly different cytotoxicities of the melophlin couples B/C and R/Q are remarkable, given their great structural similarity. *Ante-iso*-12-methyltetradecanoic acid (12-MTA) as well as *iso*-13-methyltetradecanoic acid (13-MTA), which constitute the side chains of melophlins R and Q, respectively, are known to interact with lipid membranes in different ways [21]. 13-MTA exhibits a greater ordering effect on the hydrophobic membrane core leading to a more rigid, impervious membrane structure. 12-MTA in contrast leads to a looser, more volatile membrane structure. 12-MTA was also shown to inhibit the proliferation of various lung and prostate carcinoma cell lines with  $IC_{50}$  concentrations ranging from 18 to 35  $\mu\text{g/mL}$  by inducing caspase-3 dependent apoptosis and by inhibiting the formation of 5-HETE [22]. Hence the different cytotoxicities of melophlins Q and R might be rationalized by assuming a less hindered membrane passage and a greater proapoptotic effect of the latter. The activities and cell line specificities of **1a** and its calcium complex  $\text{Ca}(\mathbf{1a})_2$  are too similar to decide whether the latter reaches its targets in an intact, undissociated

form. Since the cellular targets of the melophlins are as yet unidentified, any more detailed structure-activity correlations would be mere speculation at present.

((Table 1 here))

3-Acyltetronic and -tetramic acids are also frequently distinguished by high antimicrobial activities. Table 2 summarizes the results of agar diffusion assays with selected melophlins and microorganisms in the form of the diameters of observed growth inhibition zones. None of the compounds had any effect on the growth of the yeasts, molds and Gram-negative bacteria quoted in the legend to table 2. Melophlin A, its Ca complex and melophlin G were at that inactive in all tested bacteria. However, melophlins B, C, P, Q and R which have a 5-methyl residue at the pyrrolidine-2,4-dione ring in common showed distinct activity in Gram-positive bacteria, melophlins B and C also in *E. coli tolC*. This is in line with Lee's findings for his 3-acetyltetramates [10]. Structurewise, the presence or absence of a methyl branching in the side chain seems to be more decisive for the specificity than its position or the overall length of the alkyl chain.

((Table 2 here))

### **Conclusion**

Despite their structural prima facie similarity, the melophlines display a varied spectrum of activities in bacteria and tumor cells which is dependent on structural parameters such as the C(5) substitution and the length and branching of the 3-acyl side chain. Antibacterial activities were found in the case of the 5-methyl substituted derivatives B, C, P, Q and R. Antiproliferative activities in a panel of four cell lines were on average most pronounced for melophlins B, C and R featuring branched 3-acyl side chains. Their activities lay in the range of that of cisplatin. Melophlin Q

was astoundingly specific in inhibiting A-498 kidney cancer cells whereas melophlin P inhibited U-937 leukemia cells particularly well. With protocols at hand for the parallel synthesis of 3-acyltetramic acids it will be worthwhile to optimize the pharmacological profile of semisynthetic melophlins by systematically varying the structural key parameters.

**Abbreviations:** Cdc25, cell division cycle dual-specificity phosphatase 25; ERK, extracellular signal-regulated kinase; 5-HETE, 5-hydroxyeicosatetraenoic acid; MTT, 3(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PMA, phorbol 12-myristate 13-acetate; TNF, tumor necrosis factor; VHR, VH1 related dual-specific phosphatase.

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## **Experimental Part**

*General.* Microwave irradiations were carried out in sealed vials in an MLS Microchemist™ system. Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR-spectra were recorded on a Perkin-Elmer One FT-IR spectrophotometer. Magnetic resonance (NMR) spectra were recorded under conditions as indicated on a Bruker Avance 300 spectrometer. Chemical shifts ( $\delta$ ) are given in parts per million downfield from TMS as internal standard. Mass spectra were recorded using a Varian MAT 311A (EI). Elemental analyses were carried out with a Perkin-Elmer 2400 CHN elemental analyser. For column chromatography Merck silica gel 60 (230-400 mesh) was used. Solvents were dried and distilled (THF, diethyl ether and dioxane over Na/Ph<sub>2</sub>CO, CH<sub>3</sub>OH over CaO, acetone over



K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub> and CHCl<sub>3</sub> over P<sub>2</sub>O<sub>5</sub>) and stored under argon. Starting compounds were purchased from the usual sources and were used without further purification.

### *Chemistry*

*13-Methyltetradecanoyl chloride 6q*: Under an atmosphere of dry argon, a mixture of 13-methyltetradecanoic acid [14] (1.05 g, 4.33 mmol), freshly distilled SOCl<sub>2</sub> (0.82 g, 6.87 mmol) and two drops of dry DMF was stirred at room temperature overnight. All volatiles were removed under reduced pressure to leave **6q** as a faintly yellow liquid (2.90 mmol, 67%); IR (ATR): 2924, 1794, 1466, 952, 720. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): 0.84 (*d*, *J* = 6.6, 6 H); 1.00-1.40 (*m*, 18 H); 1.40-1.59 (*m*, 1 H); 1.62-1.75 (*m*, 2 H); 2.85 (*t*, *J* = 7.3, 2 H). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>): 22.6, 25.1, 27.4, 28.0, 28.4, 29.1, 29.3, 29.5, 29.6, 29.7, 29.9, 39.0, 47.1, 173.8.

*12-Methyltetradecanoyl chloride 6r*: 680 mg (2.6 mmol, 46%) from 12-methyltetradecanoic acid (1.38 g, 5.70 mmol), analogously to the synthesis of **6q**. Yellowish oil; IR (ATR): 2924, 1798, 1687, 1462, 1130, 951. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): 0.82 (*d*, *J* = 6.4, 3 H), 0.83 (*t*, *J* = 7.1, 3 H), 1.03-1.35 (*m*, 19 H), 1.66 (*qui*, *J* = 7.3, 2 H), 2.86 (*t*, *J* = 7.3, 2 H). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>): 11.4, 19.2, 25.0, 27.1, 28.4, 29.0, 29.3, 29.5, 29.55, 29.6, 30.0, 47.1, 34.4, 36.6, 173.8.

*3-[1'-(Difluoroboryloxy)-palmitoylidene]-1,5-dimethyl-pyrrolidine-2,4-dione 7p*: To a shaken solution of **5** [11] (115 mg, 1.0 mmol) in 5 mL ethereal boron trifluoride-diethyl ether, palmitoyl chloride **6p** (550 mg, 2.0 mmol) was added. This mixture was then heated in a sealed tube under microwave irradiation (1 kW max) for 45 min at 100°C. The cooled reaction mixture was treated with sat. aq. NH<sub>4</sub>Cl (11 mL) and immediately extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to yield an orange oil. Purification by column chromatography (silica gel, CHCl<sub>3</sub>; *R<sub>f</sub>* = 0.40) left **7p** (228 mg, 55%) as a pale yellow

solid; m.p. 82-85°C; IR (ATR): 2849, 1722, 1653, 1577, 1529, 1024. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): 0.85 (*t*, *J* = 6.5, 3 H), 1.13-1.28 (*m*, 24 H), 1.43 (*d*, *J* = 7.1, 3 H), 1.54-1.73 (*m*, 2 H), 2.75-2.88 (*m*, 2 H), 3.14 (*s*, 3 H), 3.89 (*q*, *J* = 7.1, 1 H). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>): 14.0, 14.1, 22.7, 26.6, 27.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 33.9, 64.9, 98.5, 170.9, 189.5, 190.5. EI-MS: 413 (73, *M*<sup>+</sup>), 393 (15), 347 (3), 230 (41), 217 (100), 202 (17), 175 (9), 106 (11); HR-EI-MS: 413.29100 (C<sub>22</sub>H<sub>38</sub>BF<sub>2</sub>NO<sub>3</sub>); calc. 413.29126.

*3-[1'-(Difluoroboryloxy)-13'-methyltetradecylidene]-1,5-dimethyl-pyrrolidine-2,4-dione 7q*: 366 mg (0.92 mmol, 65%) from **5** (180 mg, 1.4 mmol) and **6q** (730 mg, 2.8 mmol), analogously to the synthesis of **7p**. Brown solid, m.p. 68°C; *R*<sub>f</sub> = 0.35 (CHCl<sub>3</sub>); IR (ATR): 2851, 1721, 1648, 1579, 1529, 1181, 1021. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): 0.83 (*d*, *J* = 6.6, 6 H), 0.96-1.56 (*m*, 19 H), 1.43 (*d*, *J* = 7.2, 3 H), 1.57-1.73 (*m*, 2 H), 2.83 (*t*, *J* = 7.6, 2 H), 3.13 (*s*, 3 H), 3.88 (*q*, *J* = 7.2, 1 H). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>): 14.0, 22.6, 25.6, 27.4, 27.9, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 29.9, 33.9, 39.0, 64.9, 98.5, 170.9, 189.5, 190.5. EI-MS: 399 (31, *M*<sup>+</sup>), 379 (20), 364 (8), 333 (5), 230 (57), 217 (100), 216 (27), 210 (16), 203 (20), 197 (14), 175 (16). Anal. calc. for C<sub>21</sub>H<sub>36</sub>BF<sub>2</sub>NO<sub>3</sub>: C 63.2, H 9.1, N 3.8; found: C 62.9, H 8.9, N 3.6.

*3-[1'-(Difluoroboryloxy)-12'-methyltetradecylidene]-1,5-dimethyl-pyrrolidine-2,4-dione 7r*: 160 mg (0.41 mmol, 30%) from **5** (165 mg, 1.3 mmol) and **6r** (680 mg, 2.6 mmol), analogously to the synthesis of **7p**. Colorless solid, m.p. 81-86°C; *R*<sub>f</sub> = 0.22 (CHCl<sub>3</sub>); IR (ATR): 2854, 1722, 1644, 1570, 1533, 1456, 1180, 1059, 1030. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): 0.78 (*d*, *J* = 6.4, 3 H), 0.80 (*t*, *J* = 7.2, 3 H), 0.94-1.37 (*m*, 19 H), 1.41 (*d*, *J* = 7.1, 3 H), 1.64 (*qui*, *J* = 7.5, 2 H), 2.80 (*t*, *J* = 7.5, 2 H), 3.11 (*s*, 3 H), 3.88 (*m*, 1 H). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>): 11.4, 14.0, 19.2, 25.6, 27.1, 27.9, 29.1, 29.2, 29.4, 29.5, 29.6, 29.6, 30.0, 33.9, 34.4, 36.6, 65.0, 98.6, 170.8, 189.2,

190.6;  $^{19}\text{F}$ -NMR (376 MHz,  $\text{CDCl}_3$ ): -143.9 (*s*,  $\text{O}^{10}\text{BF}_2\text{O}$ ), -144.0 (*s*,  $\text{O}^{11}\text{BF}_2\text{O}$ );  $^{11}\text{B}$ -NMR (128 MHz,  $\text{CDCl}_3$ ): 0.717 (*s*); EI-MS: 399 (35,  $M^+$ ), 379 (21), 350 (14), 230 (47), 217 (100), 202 (23), 175 (10). Anal. calc. for  $\text{C}_{21}\text{H}_{36}\text{BF}_2\text{NO}_3$ : C 63.2, H 9.1, N 3.8; found: C 62.8, H 8.9, N 3.5.

*Melophlin P 1p*: A stirred solution of **7p** (565 mg, 1.39 mmol) in methanol (50 mL) was heated under reflux for 2 h. After this time the reaction mixture was allowed to cool to room temperature. The cooled solution was diluted with ethyl acetate (50 mL) and evaporated under reduced pressure. The residue was taken up in ethyl acetate (50 mL), washed twice with water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated to yield **1p** (460 mg, 91%) as a pale yellow oil.  $R_f = 0.30$  (diethyl ether); IR (ATR): 2915, 2849, 1694, 1653, 1628, 1490, 1063.  $^1\text{H}$ -NMR (300 MHz,  $\text{CD}_3\text{OD}$ ): 0.85-0.94 (*m*, 3 H), 1.25-1.36 (*m*, 24 H), 1.33 (*d*,  $J = 7.0$ , 3 H), 1.58-1.73 (*m*, 2 H), 2.78-2.87 (*m*, 2 H), 2.95 (*s*, 3 H), 3.75-3.88 (*m*, 1 H).  $^{13}\text{C}$ -NMR (75.5 MHz,  $\text{CDCl}_3$ ): 14.1, 14.8, 22.7, 24.9, 26.0, 26.2, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.6, 32.8, 62.7, 100.5, 172.8, 187.9, 194.7. EI-MS: 365 (17,  $M^+$ ), 347 (17), 220 (4), 182 (36), 169 (100), 154 (54), 127 (17), 97 (3), 69 (4), 58 (11). HR-EI-MS: 365.29240 ( $\text{C}_{22}\text{H}_{39}\text{NO}_3$ ); calc. 365.29297.

*Melophlin Q 1q*: 220 mg (0.63 mmol, 90%) from **7q** (280 mg, 0.7 mmol), analogously to the synthesis of **1p**. Yellow oil; IR (ATR): 2924, 2854, 1714, 1653, 1617, 1366, 1236.  $^1\text{H}$ -NMR (300 MHz,  $\text{CD}_3\text{OD}$ ): 0.89 (*d*,  $J = 6.6$ , 6 H), 1.13-1.46 (*m*, 18 H), 1.34 (*d*,  $J = 6.8$ , 3 H), 1.47-1.60 (*m*, 1 H), 1.60-1.72 (*m*, 2 H), 2.83 (*t*,  $J = 7.5$ , 2 H), 2.97 (*s*, 3 H), 3.83 (*q*,  $J = 6.8$ , 1 H).  $^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ ): 14.8, 22.6, 25.9, 26.2, 27.4, 27.9, 29.2, 29.3, 29.4, 29.5, 29.6, 29.9, 32.5, 39.0, 62.7, 100.4, 172.8, 187.8, 194.6. EI-MS: 351 (16,  $M^+$ ), 333 (10), 323 (1), 307 (1), 280 (1), 266 (1), 252 (1), 238 (1), 220 (2), 206 (1), 182 (26), 169 (100), 154 (34), 140 (3), 127

(11).

*Melophlin R 1r*: 127 mg (0.36 mmol, 90%) from **7r** (160 mg, 0.4 mmol), analogously to the synthesis of **1p**. Orange oil; IR (ATR): 2960, 2923, 2853, 1714, 1645, 1617, 1534, 1488, 1373, 1308, 1238. <sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): 0.87 (*d*, *J* = 6.7, 3 H), 0.88 (*t*, *J* = 6.7, 3 H), 1.05-1.45 (*m*, 22 H), 1.66 (*qui*, *J* = 7.5, 2 H), 2.82 (*t*, *J* = 7.1, 2 H), 2.97 (*s*, 3 H), 3.83 (*q*, *J* = 6.8, 1 H). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): 12.0, 15.2, 19.9, 26.9, 27.2, 28.4, 30.4, 30.5, 30.6, 30.7, 30.9, 30.9, 31.3, 33.6, 37.9, 35.8, 63.6, 102.3, 172.9, 189.2, 197.7. EI-MS: 351 (25, *M*<sup>+</sup>), 333 (13), 323 (3), 294 (3), 277 (3), 200 (3), 207 (4), 193 (7), 182 (30), 169 (90), 154 (100), 127 (26).

*Ca(1a)<sub>2</sub>*: A suspension of CaCO<sub>3</sub> (5.7 mg, 0.057 mmol) in water (2 mL) was slowly syringed to a solution of (5*S*)-**1a** [11] (40 mg, 0.114 mmol) in methanol (5 mL). The reaction mixture was stirred at room temperature for 30 min and the formed colorless precipitate was collected, washed with water and dried. Yield: 40 mg (0.054 mmol, 95%); colorless solid of m.p. 155°C (decomp.); IR (ATR): 2919, 2851, 1657, 1597, 1488, 1467, 1239, 1024, 994, 889, 779, 720. <sup>1</sup>H-NMR (300 MHz, acetone-*d*<sub>6</sub>/DMSO-*d*<sub>6</sub>): 0.8-0.9 (*m*, 3 H), 1.2-1.3 (*m*, 24 H), 1.4-1.6 (*m*, 2 H), 2.73 (*t*, *J* = 6.2, 2 H), 2.83 (*s*, 3 H), 3.48 (*s*, 2 H); EI-MS: 399 (16), 216 (43), 203 (100). Anal. calc. for C<sub>42</sub>H<sub>72</sub>CaN<sub>2</sub>O<sub>6</sub>: C 68.1, H 9.8, N 2.0; found: C 67.4, H 9.5, N 1.7.

#### *Cell lines and culture conditions.*

Human A-498 kidney cancer, KB-3-1 cervix carcinoma and U-937 leukemia cells as well as murine L929 fibroblasts were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and cultivated in the media recommended by the supplier at 37°C and 10% CO<sub>2</sub>.

#### *MTT cytotoxicity assays.*

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) was used to identify the metabolic activity of cells which are capable of reducing it by dehydrogenases to a violet formazan product. 60  $\mu$ L of serial dilutions of the test compounds were added to 120  $\mu$ L aliquots of a cell suspension (50.000/mL) in 96-well microplates. Blank and solvent controls were incubated under identical conditions. After 5 days 20  $\mu$ L MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg/mL. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100  $\mu$ L PBS and dissolved in 100  $\mu$ L isopropanol containing 0.4% hydrochloric acid. The microplates were gently shaken for 20 min to ensure a complete dissolution of the formazan and finally measured at 595 nm using an ELISA plate reader. All experiments were carried out in two parallel experiments, the percentage of viable cells was calculated as the mean with respect to the controls set to 100%. With U-937 the cytotoxicity was measured using a ViaLight Plus Sample Kit from Lonza. This kit is based upon the bioluminescent measurement of ATP that is present in all metabolically active cells.

*Agar diffusion assays.*

Agar plates containing 15 mL of medium were inoculated with bacterial or yeast suspensions in liquid broth to give a final OD of 0.01 (bacteria) or 0.1 (yeasts). The microorganisms were from the HZI collection and grown on standard medium. In the case of molds, spores were collected from well-grown Petri dishes which were rinsed with 10 mL sterile aqua dest. 1 mL of the spore suspension was added to 100 mL of molten agar medium. 20  $\mu$ L of test samples in methanol (1 mg/mL) were applied onto 6 mm cellulose discs. The methanol was allowed to evaporate and the discs

were placed upon the inoculated agar plate. The diameters in mm of the resulting growth zones were determined after 24 h of incubation at 30°C.

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**Table 1.** Inhibitory concentrations<sup>a</sup> IC<sub>50</sub> in  $\mu\text{M}$  of various melophlins **1** and cisplatin when applied to cells of human KB-3-1 cervix carcinoma, A-498 kidney carcinoma, U-937 leukemia and to L929 mouse fibroblasts.

| Compd./cell line             | KB-3-1        | A-498                      | U-937            | L929          |
|------------------------------|---------------|----------------------------|------------------|---------------|
| <b>1a</b>                    | 26 $\pm$ 0.4  | 21 $\pm$ 1.6               | 8.8 $\pm$ 0.4    | 24 $\pm$ 2.0  |
| <b>1b</b>                    | 9.3 $\pm$ 0.4 | 6.8 $\pm$ 0.4              | 3.3 $\pm$ 0.1    | 11 $\pm$ 2.0  |
| <b>1c</b>                    | 3.6 $\pm$ 0.2 | 3.7 $\pm$ 0.0              | 2.6 $\pm$ 0.9    | 3.4 $\pm$ 0.1 |
| <b>1g</b>                    | 22 $\pm$ 0.2  | 14 $\pm$ 2.2               | 6.7 $\pm$ 0.2    | 22 $\pm$ 1.1  |
| <b>1p</b>                    | 17 $\pm$ 2.1  | 15 $\pm$ 1.8               | 2.1 $\pm$ 0.2    | 13 $\pm$ 0.4  |
| <b>1q</b>                    | 16 $\pm$ 1.8  | 3.1 $\pm$ 0.4              | 3.6 $\pm$ 1.1    | 19 $\pm$ 0.1  |
| <b>1r</b>                    | 5.9 $\pm$ 0.2 | 2.1 $\pm$ 1.0              | 2.2 $\pm$ 0.6    | 6.1 $\pm$ 0.1 |
| Ca( <b>1a</b> ) <sub>2</sub> | 10 $\pm$ 1.0  | 9.0 $\pm$ 0.6              | 6.1 $\pm$ 0.9    | 8.3 $\pm$ 0.4 |
| cisplatin                    | 1.1 $\pm$ 0.1 | 3.1 $\pm$ 0.4 <sup>b</sup> | n/m <sup>c</sup> | 2.5 $\pm$ 0.4 |

<sup>a</sup> Values in  $\mu\text{M}$  are derived from concentration-response curves obtained by measuring the percentage of grown cells relative to untreated controls after 5 days of incubation using an MTT assay in case of KB-3-1, A-498 and L929 cells or an ATP assay in case of the U-937 cells. Values represent means of two experiments in parallel. <sup>b</sup> Also see ref [19]. <sup>c</sup> Not measured; ref [20] quotes an IC<sub>50</sub> = 6.41  $\mu\text{M}$  for an MTT assay (48 h).

**Table 2.** <sup>a</sup> Antibiotic activity of various melophlins **1**, streptomycin sulfate (strep) and penicillin-G, potassium salt (pen) in selected bacteria<sup>b</sup>

|  | <b>1b</b> | <b>1c</b> | <b>1p</b> | <b>1q</b> | <b>1r</b> | <b>strep</b> | <b>pen</b> |
|--|-----------|-----------|-----------|-----------|-----------|--------------|------------|
| <i>E. coli tolC</i> [Gram(-)]          | 8         | 9         | 0         | 0         | 0         | -            | -          |
| <i>Staphylococcus aureus</i> [Gram(+)] | 9         | 9         | 8         | 13        | 13        | 16           | 33         |
| <i>Micrococcus luteus</i> [Gram(+)]    | 0         | 0         | 8         | 9         | 9         | 20           | 15         |
| <i>Mycobacterium phlei</i> [Gram(+)]   | 10        | 10        | 0         | 0         | 0         | 22           | 44         |

<sup>a</sup> Agar plates inoculated with the respective microorganisms were incubated with 6 mm cellulose discs containing 20  $\mu\text{L}$  of a methanolic solution ( $1 \text{ mg mL}^{-1}$ ) of the compounds tested. The diameters (in mm) of the resulting growth-inhibition zones were determined after 24 h of incubation at 30 °C and are cited here. <sup>b</sup> None of the compounds inhibited the growth of the yeasts *Hansenula anomala* and *Saccharomyces cerevisiae*, or of the molds *Aspergillus fumigatus*, *Botrytis cinerea* and *Pythium debaryanum*, or of the Gram-negative bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Compounds **1a**,  $\text{Ca}(\mathbf{1a})_2$  and **1g** were inactive in all tested bacteria.

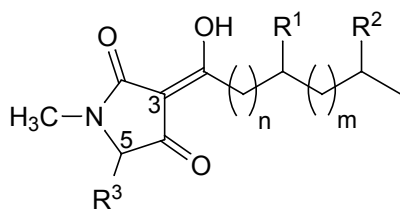
((Caption to Fig. 1))

Fig. 1. Structures of natural melophlins (**1**) from the sponge *Melophlus sarasinorum*, with those tested in bold typeface

((Caption to Scheme 1))

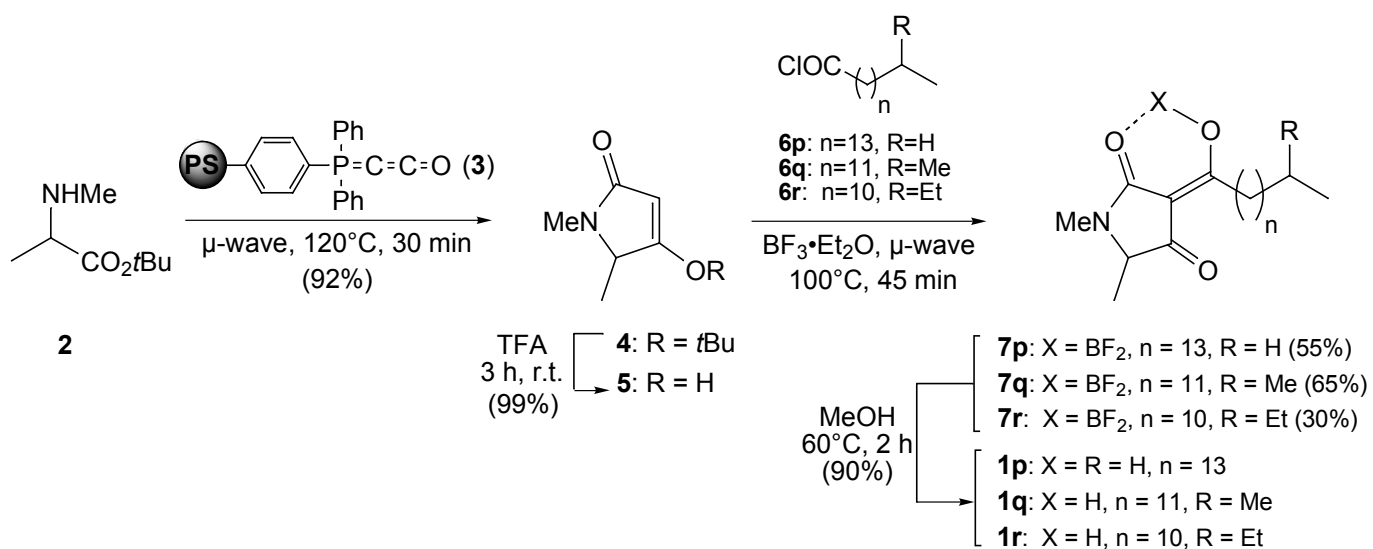
Scheme 1. Syntheses of melophlins P (**1p**), Q (**1q**) and R (**1r**)

((Figure 1 to be reduced to 70%))



| melophlin     | R <sup>3</sup> | R <sup>1</sup> | R <sup>2</sup> | n         | m        |
|---------------|----------------|----------------|----------------|-----------|----------|
| <b>A (1a)</b> | <b>H</b>       | <b>H</b>       | <b>H</b>       | <b>12</b> | <b>0</b> |
| <b>B (1b)</b> | <b>(5S)-Me</b> | <b>Me</b>      | <b>H</b>       | <b>2</b>  | <b>6</b> |
| <b>C (1c)</b> | <b>Me</b>      | <b>Me</b>      | <b>H</b>       | <b>3</b>  | <b>5</b> |
| D (1d)        | H              | H              | H              | 11        | 0        |
| E (1e)        | H              | H              | Me             | 11        | 0        |
| F (1f)        | H              | Me             | H              | 11        | 0        |
| <b>G (1g)</b> | <b>H</b>       | <b>H</b>       | <b>H</b>       | <b>10</b> | <b>0</b> |
| H (1h)        | H              | H              | Me             | 10        | 0        |
| I (1i)        | H              | Me             | H              | 10        | 0        |
| J (1j)        | Me             | H              | H              | 10        | 0        |
| K (1k)        | H              | H              | Me             | 9         | 0        |
| L (1l)        | Me             | H              | H              | 9         | 0        |
| M (1m)        | Me             | H              | H              | 8         | 0        |
| N (1n)        | Me             | H              | Me             | 8         | 0        |
| O (1o)        | Me             | Me             | H              | 8         | 0        |
| <b>P (1p)</b> | <b>Me</b>      | <b>H</b>       | <b>H</b>       | <b>12</b> | <b>0</b> |
| <b>Q (1q)</b> | <b>Me</b>      | <b>H</b>       | <b>Me</b>      | <b>10</b> | <b>0</b> |
| <b>R (1r)</b> | <b>Me</b>      | <b>Me</b>      | <b>H</b>       | <b>10</b> | <b>0</b> |
| S (1s)        | Me             | Me             | H              | 3         | 8        |

((Scheme 1; to be reduced to 70%))



((picture for graphical abstract /TOC))

