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# Synthesis of Penicillenol $C_1$ and of a Bis-Azide Analogue for Photoaffinity Labeling

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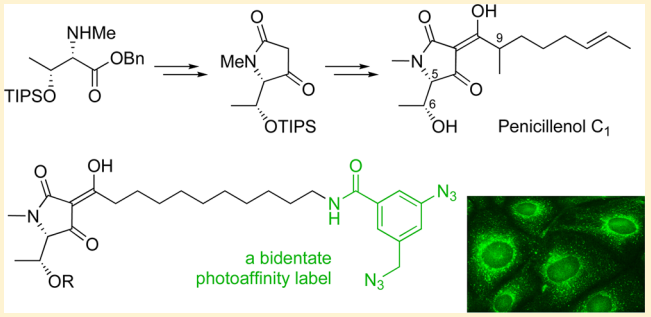
Supporting Information

**ABSTRACT:** Two diasteroisomers of the *Penicillium* metabolite penicillenol $C_1$ were synthesized for the first time by 3-acylation of an L-threonine-derived tetramic acid with enantiopure 2-methyloct-(6*E*)-enoic acids. The 5*S*,6*R*,9*S* isomer has NMR spectra and optical rotation identical with those of the natural compound. A bis-azide-tagged penicillenol analogue was also synthesized for photoaffinity labeling of target proteins. The photolysis of the bis-azide in the presence of methanol as a protein-mimicking nucleophile led to reaction only of the aryl azide, while leaving the benzyl azide available for pull-downs or the attachment of fluorescent tracers. As a proof of concept, the distribution of this bis-azide-tagged tetramic acid in living cells was visualized via a Staudinger ligation between the azide tag and a phosphane fluorophore.




## INTRODUCTION

The penicillenols **1** represent a small family of pyrrolidine-2,4-diones (aka tetramic acids)[1] that were isolated from *Penicillium* sp. GQ-7, an endophytic fungus associated with *Aegiceras corniculatum*.[2] They share the structural motif of an *N*-methylated tetramic acid derived from threonine and bearing an $\alpha$-methyl branched $C_8$-fatty acyl residue at C-3 (Figure 1). The penicillenols $A_1$ (**1a**) and $A_2$ (**1b**) were recently synthesized by Yoda et al. from L-threonine and D-allothreonine, respectively.[3] By comparison with the reported NMR and optical rotation data of the natural products, their configurations were inferred to be (5*S*,6*R*,9*S*)-**1a** and (5*R*,6*R*,9*S*)-**1b**. The penicillenols $B_1$ (**1c**) and $B_2$ (**1d**) also carry an $\alpha$-methyloctanoyl residue of hitherto unspecified configuration yet have lost the stereogenic centers C-5 and C-6 due to elimination of water. For the penicillenols $C_1$ (**1e**) and $C_2$ (**1f**) the discovering group assumed configurations at C-5 to be *S* for **1e** and *R* for **1f** on the grounds of CD and NMR spectra. So far, the penicillenols have been only cursorily tested for biological activity, which is somewhat surprising, given the high incidence of biological effects reported for the closely related melophlins, metabolites of bacteria dwelling on the marine sponge *Melophlus sarasinorum*.[4] Likewise, there are no data available for penicillenols concerning their uptake by and distribution in cells.

Herein we describe a total synthesis of penicillenol $C_1$ (**1e**) that differs from Yoda's approach to the penicillenols A by an early *N*-methylation of threonine and by the method of ring closure. We also report the synthesis of a bis-azide-tagged penicillenol analogue that should allow the identification of target proteins. Photolysis of one azide enables attachment to nucleophilic residues in the protein, while visualization of the resulting protein−tetramate conjugate becomes possible via a Staudinger reaction of the second azide with a fluorescent phosphane. We provide evidence for the feasibility of this approach.

## RESULTS AND DISCUSSION

**Syntheses of Penicillenol $C_1$ ((5*S*,6*R*)-1e).** We intended to synthesize **1e** by 3-acylation of the tetramic acid **12** (Scheme 1). Preliminary studies[5] had indicated that its benzyl ether **11** should be accessible by a Wittig-type cyclization reaction of the *O*-protected *N*-methylated L-threoninate **9** with the ylide $Ph_3PCCO$ (**10**).[6] This strategy differs from Yoda's synthesis of penicillenols A, which was based on the cyclization of *N*,*O*-protected threonines with Meldrum's acid and an *N*-methylation postponed until the very last step.[3] We now optimized the synthesis of the immediate cyclization precursor, benzyl (2*S*,3*R*)-2-methylamino-3-((triisopropylsilyl)oxy)-butanoate (**9**), so that only two intermediates, namely **4** and **6**, required purification. L-Threonine was *N*-protected with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (=FmocONSu) to give carboxylic acid **3**. This was converted to its Cs salt and treated with benzyl bromide to give the ester **4** in 91% yield after column chromatography. When it is prepared on a large scale, ester **4** can be purified conveniently by recrystallization from ethyl acetate. The $\beta$-hydroxy group of **4** was silylated with triisopropylsilyl triflate/$NEt_3$, affording the benzyl *N*-Fmoc-*O*-TIPS-threoninate **5** in 84% yield. Cleavage of the Fmoc group

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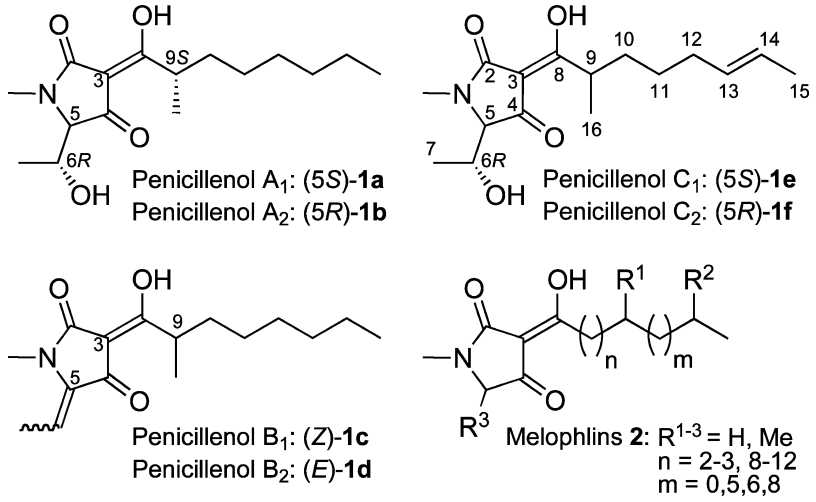
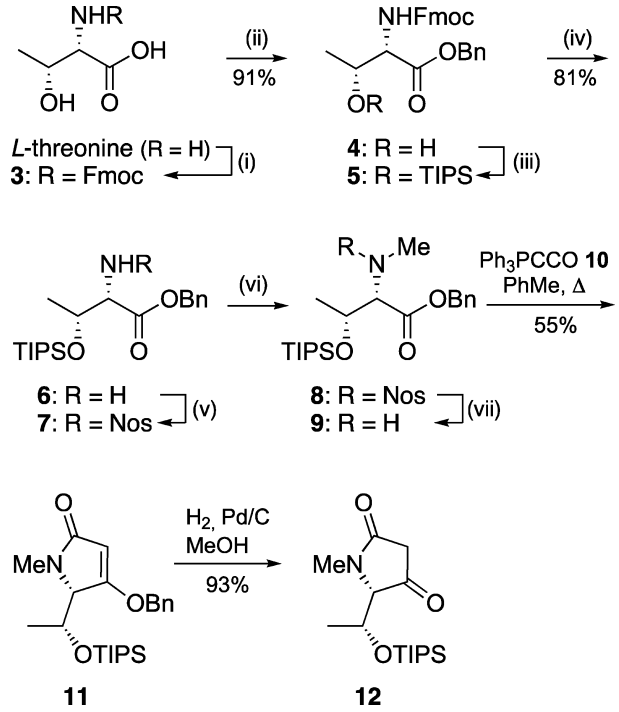



**Figure 1.** Structures of penicillenols **1** and melophlins **2**.

**Scheme 1. Synthesis of Tetramic Acid 12**[a]

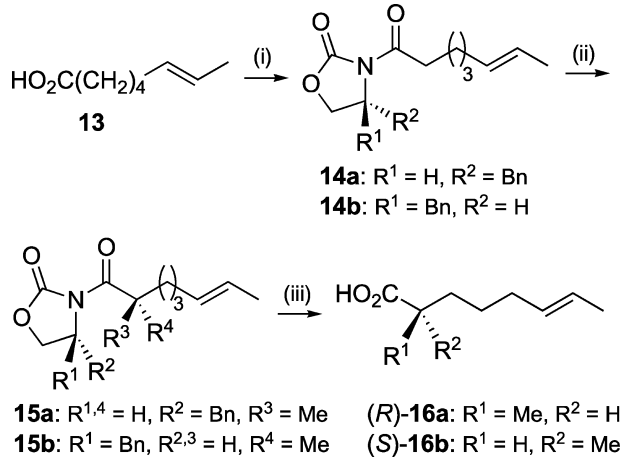



[a]Reagents and conditions: (i) FmocONSu, dioxane, room temperature, 15 h; (ii) (a) $Cs_2CO_3$, MeOH; (b) BnBr, DMF, room temperature, 91% (based on L-threonine); (iii) TIPSOTf, $NEt_3$, $CH_2Cl_2$, room temperature, 2 h, 84%; (iv) piperidine, $CH_2Cl_2$, 0 °C, 15 min, 81%; (v) $(o\text{-}NO_2)C_6H_4SO_2Cl$, $NEt_3$, $CH_2Cl_2$, room temperature, 99%; (vi) MeI, $K_2CO_3$, DMF, room temperature, 94% (crude); (vii) PhSH, $K_2CO_3$, DMF, room temperature, 70% (based on **6**).

with piperidine gave the α-amino ester **6** in 81% yield upon chromatographic purification. For the mono-methylation of the amino group of **6**, it was first nosylated to the sulfonamide **7** and then methylated with MeI/$K_2CO_3$ to afford the tertiary sulfonamide **8**. This was *N*-deprotected with PhSH/$K_2CO_3$ to furnish the benzyl *N*-methyl-*O*-TIPS-threoninate **9** in 70% yield (over three steps) after chromatographic purification. Refluxing of a mixture of amino ester **9** and ylide **10** in toluene gave benzyl tetramate **11** in 55% yield via a domino sequence comprised of amine addition to the C=C bond of **10** followed by an intramolecular Wittig olefination of the so-formed amide ylide. Hydrogenolysis of benzyl tetramate **11** eventually liberated tetramic acid **12**.

The (6*E*)-2-methyl-octenoic acid required for the 3-acylation of **12** was prepared by a stereoselective α-methylation of (6*E*)-octenoic acid **13**[7] (Scheme 2). Given the ambiguity of the configuration at C-9 in the natural **1e**, we synthesized both enantiomers by applying the appropriate Evans (*R*)- and (*S*)-4-benzyloxazolidin-2-one auxiliaries. Their attachment afforded the enantiomeric imides **14a,b**, which were diastereoselectively (95% de) methylated to the corresponding α-methylamides **15a,b**. These were hydrolyzed to the (6*E*)-2-methyloctenoic acids **16** in 67% overall yield based upon **13**.

**Scheme 2. Synthesis of (6*E*)-2-Methyloctenoic Acids 16**[a]




[a]Reagents and conditions: (i) (a) $Me_3CCOCl$, $NEt_3$, THF, −10 °C, (b) Evans auxiliary, $NEt_3$, LiCl, −10 °C (1 h) → 0 °C (2 h), 87%; (ii) NaHMDS, THF, −78 °C, then MeI, 3 h, 89% (95% de); (iii) LiOH, $H_2O_2$, THF, $H_2O$, 0 °C, 87%.

Tetramic acid **12** was then acylated with the carboxylic acids **16** under modified Yoshii[8]−Yoda[3] conditions (Scheme 3). Treatment of the acids **16** with ethyl chloroformate/$NEt_3$ to give the mixed anhydride followed by addition of tetramic acid **12** led to the tetramates **17**. The 4-O → C-3 acyl shift in the presence of $CaCl_2$ as recommended by Yoda proceeded readily,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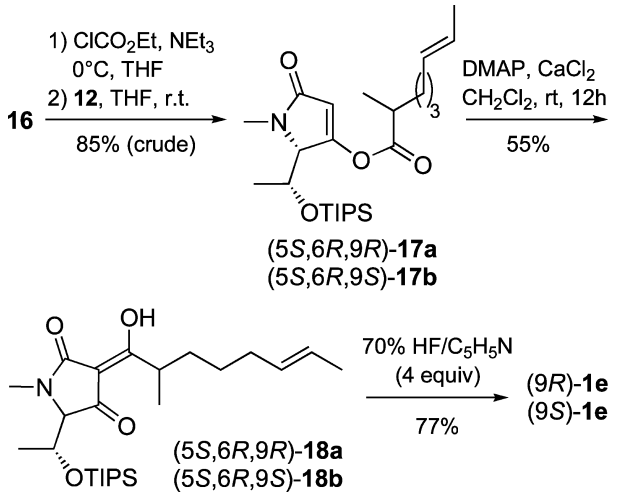
**Scheme 3. Synthesis of Penicillenols $C_1$ (9*R*)-1e and (9*S*)-1e**




affording the *O*-protected target compounds **18**. The cleavage of the silyl ether of **18** required exceptionally drastic conditions: namely, exposure to 4 equiv of 70% HF in pyridine for more than 12 h. Gratifyingly, deprotection went to completion without any signs of decomposition. The pure diasteroisomeric penicillenols $C_1$ (9*R*)-**1e** and (9*S*)-**1e** were obtained in almost 80% yield. This synthetic approach should be flexible enough to allow the preparation of structurally modified penicillenol analogues for an in-depth study of the biological properties of this family of 3-acyltetramic acids.

For the assignment of the configuration at C-9 of the natural penicillenol $C_1$ we compared the known chemical shifts in its $^{13}C$ NMR spectrum[2] with those of our synthetic diastereomers. Table 1 shows a perfect match of indicative carbon signals for the isomer (9*S*)-**1e**. A comparison of the optical rotations further corroborates this assignment. Natural **1e** and synthetic (9*S*)-**1e** both show an optical rotation of $[\alpha]^{24}_D = -47°$ ($c$ = 0.125, MeOH), while isomer (9*R*)-**1e** has $[\alpha]^{24}_D = -12°$ ($c$ = 0.125, MeOH).

**Table 1. Chemical Shifts ($\delta$,[a] ppm) of Indicative Carbon Atoms of Natural Penicillenol $C_1$ and Deviations of the Corresponding Shifts of Synthetic Penicillenols (9*S*)-1e and (9*R*)-1e**

| no. of C atom[b] | atom type | $\delta$(natural **1e**) | $\delta$(natural **1e**) − $\delta$(9*S*-**1e**) | $\delta$(natural **1e**) − $\delta$(9*R*-**1e**) |
|---|---|---|---|---|
| 3 | $C^q$ | 101.1 | 0.0 | −0.2 |
| 6 | CH | 66.7 | 0.0 | 0.1 |
| 8 | $C^q$ | 192.7 | 0.0 | 0.1 |
| 9 | CH | 36.3 | 0.0 | 0.2 |
| 10 | $CH_2$ | 33.0 | 0.0 | −0.3 |
| 11 | $CH_2$ | 27.1 | 0.0 | 0.1 |
| 12 | $CH_2$ | 32.4 | 0.0 | 0.1 |
| 13 | CH | 130.8 | 0.0 | 0.1 |
| 16 | $CH_3$ | 17.2 | 0.0 | 0.2 |

[a] $^{13}C$ NMR spectra were recorded in $CDCl_3$ at 150 MHz (natural **1e**)[2] and 126 MHz (synthetic (9*S*)- and (9*R*)-**1e**). For a comparison of *all* signals cf. the Supporting Information. [b] Cf. Figure 1.

**Synthesis and Preliminary Tests of a Bis-Azide-Tagged Penicillenol Photolabel.** Little is known, yet, about the cellular targets of 3-acyltetramic acids. The Waldmann group showed by means of protein affinity pull-downs that melophlin A (**2**: $R^{1-3}$ = H, $n$ = 12, $m$ = 0) targets dynamins, GTPases crucial for endocytosis, cytokinesis, and signaling in eukaryotic cells.[9] While studies with melophlins are problematic due to their high cytotoxicities, penicillenol $C_1$ is far less cytotoxic and so lends itself ideally to protein target identification in living cells. In cytotoxicity (MTT) assays with cells of murine L929 fibrosarcoma, human KB-3-1 cervix carcinoma, and nonmalignant PTK2 rat kangaroo kidney cells both diastereomers of **1e** were antiproliferative only at very high concentrations, with $IC_{50}$ (72 h) > 50 $\mu$M (cf. the Supporting Information).

For a study of the protein targets of the penicillenols, we now prepared a derivative **23** that bears a known[10] bis-azide photoaffinity label, allowing a two-step target identification (Scheme 4). Incubation of **23** with cell lysates and subsequent irradiation at ca. 250 nm should cleave the aryl azide to a reactive intermediate that covalently links the tetramic acid to its target protein. The remaining benzyl azide may then be attached via click or Staudinger reactions to fluorescence labels or an affinity compound such as biotin. Scheme 4 depicts the synthesis of the bis-azide-tagged tetramic acid **23** by 3-acylation of tetramic acid **12** with the carboxylic acid **21** under Yoda conditions, followed by *O*-desilylation of the intermediate **22** with HF in pyridine. The acid **21** was prepared by amidation of the known 3-azido-5-(azidomethyl)benzoic acid **19**[11] with methyl 11-aminoundecanoate[12] in the presence of propylphosphonic acid cyclic anhydride ($T_3P$) and subsequent saponification of the ester **20**. Scheme 4 also shows a photochemical trapping reaction of the bis-azido carboxylic acid **21** with methanol as a nucleophilic protein surrogate to prove the validity of step 1 of the concept. Acid **21** was used in lieu of the tetramic acid **23**, since it is available in larger amounts, is easier to purify, and precludes the possibility of unwanted side reactions between the tetramate and an excess of the nucleophile. This reaction was complete after 28 h of irradiation with a 150 W mercury lamp. For real qualitative protein labeling, far shorter irradation times will suffice.[10] The primary product imidate, resulting from loss of $N_2$ and trapping of the intermediate seven-membered cyclic azaallene with methanol, could not be isolated but was readily hydrolyzed upon workup to leave the stable $\beta$-azidomethyllactam **24**, which was purified by HPLC and fully characterized. The bottom line is that the bis-azide label can be photolyzed to an intermediate that will link covalently to nucleophilic groups of proteins while retaining the benzyl azide for attachment of secondary fluorescence or affinity labels.

Finally, we tested whether the bis-azide-tagged tetramic acid **23** can be used to address particular subcellular components in living cells and if these can then be visualized by a Staudinger ligation between the azide(s) of **23** and a commercially available fluorescent dye−phosphane conjugate (DyLight488-phosphine, Thermo Scientific). PTK2 rat kangaroo kidney cells, which are flat and thus easy to observe, were incubated with **23** for 12 h and then fixed, permeabilized, and incubated with DyLight488-phosphine at 37 °C for 3 h. The images we obtained with a fluorescence microscope show an accumulation of green fluorescent dots, representing the tetramic acid **23**, in the vicinity of the cell nuclei (Figure 2). For a reliable identification of the subcellular target component, a colocalization with specific antibodies would be required. However, the important outcome of this test is that we can use bis-azide labels to trace the intracellular distribution of tetramic acids. In combination with the photolabeling of individual proteins we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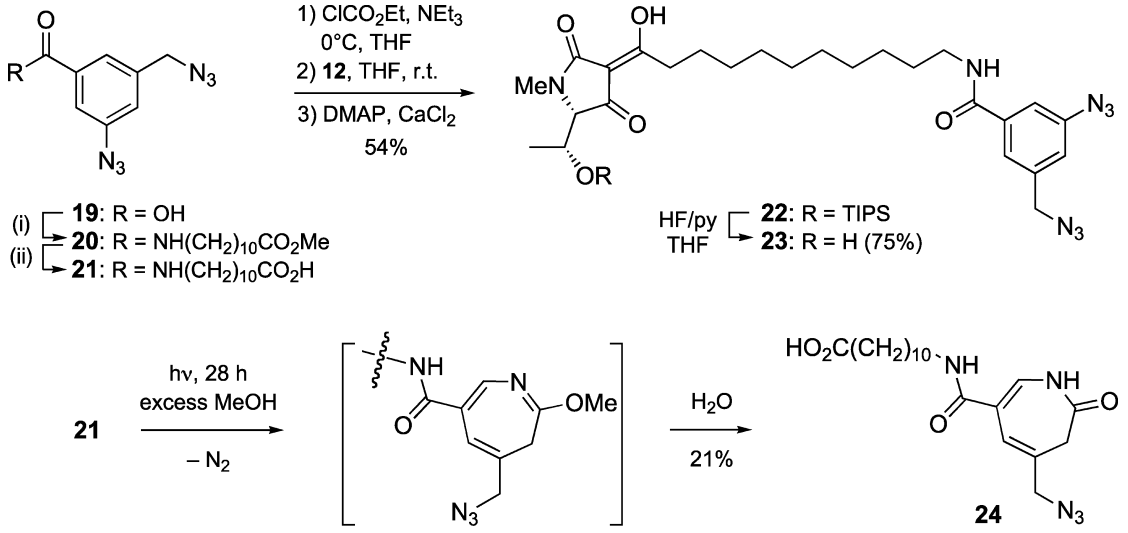
**Scheme 4. Synthesis of Bis-Azide-Tagged 3-Acyltetramic Acid 23 and Photoreaction of the Bis-Azide 21**[a]




[a]Reagents and conditions: (i) $T_3P$, $NEt_3$, $ClH_3N(CH_2)_{10}CO_2Me$, $CH_2Cl_2$, 0 °C, 1 h, 96%; (ii) 2 NaOH, MeOH, room temperature, 18 h, 85%.

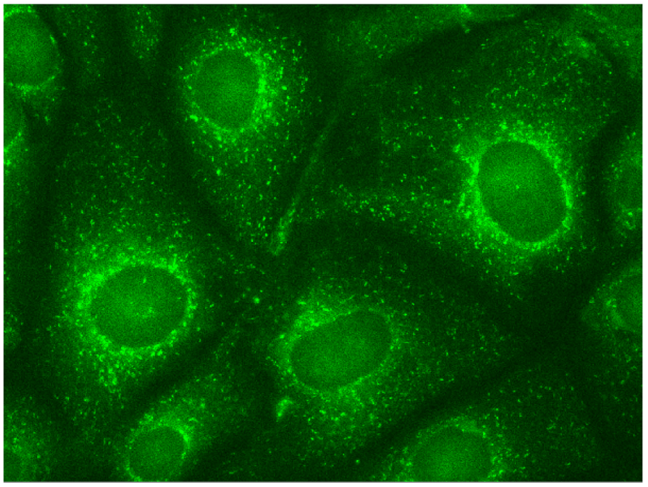


**Figure 2.** Distribution of bis-azide-tagged tetramic acid **23** (50 μg/mL) in PTK2 cells, visualized with a Zeiss Axioplan fluorescence microscope (63× magnification) after Staudinger ligation with fluorescent DyLight488-phosphine.

should now be in a position to visualize and identify the molecular targets of tetramic acids. It should be noted that the bis-azide **21** did not accumulate in specific compartments of PTK2 cells but rather gave a faint greenish background indicative of a negligible uptake and an unspecific distribution.

## CONCLUSIONS

The first synthesis of natural penicillenol $C_1$ (5*R*,6*S*,9*S*)-**1e** and its 9*R* diastereomer was based on the 3-acylation of a tetramic acid obtained by Wittig cyclization of a protected benzyl L-*N*-methylthreoninate with the phosphorus ylide $Ph_3PCCO$. The synthesis of the 3-acyl side chain used Evans auxiliaries for the introduction of the α-methyl branch. We also assigned the configuration at the carbon atom C-9 of the natural penicillenol $C_1$ to be *S* by comparison of NMR and optical rotation data. Since the cytotoxicity of penicillenol $C_1$ is low, it may be used as a probe to identify the proteins 3-acyltetramic acids interact with in living cells. We have demonstrated the feasibility of this strategy by test reactions with a bis-azide-tagged penicillenol C analogue. Its phenyl azido group was selectively cleaved by light irradiation, and the resulting intermediate was trapped with methanol, representing the nucleophilic moieties within every protein. The remaining benzyl azide may then be used for the attachment of fluorescent tracers or bioaffinity groups. As an example, we tracked the distribution of the bis-azide-tagged tetramic acid in PTK2 kidney cells by a Staudinger ligation with a fluorophore–phosphane conjugate under a fluorescence microscope. We are now preparing structural variants of bis-azide-tagged tetramic acids, and we are also running the first actual photolabeling experiments with whole cell lysates to find optimum irradiation conditions.

## EXPERIMENTAL SECTION

**General Remarks.** IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. Chemical shifts of NMR signals are given in parts per million (δ) downfield from tetramethylsilane for $^1H$ and $^{13}C$. Mass spectra were obtained under EI (70 eV) conditions. High-resolution mass spectra were obtained with a UPLC/Q-TOF MS system in ESI mode. For chromatography silica gel 60 (230–400 mesh) was used. HPLC was performed on Prontosil RP 200-5-C18, 5 μm, 250 × 4 mm (analytic) and 250 × 20 mm (preparative) columns.

**(5*S*,6*R*)-4-(Benzyloxy)-1-methyl-5-(1-((triisopropylsilyl)oxy)-ethyl)-1*H*-pyrrol-2(5*H*)-one (11).** A solution of benzyl (2*S*,3*R*)-2-methylamino-3-((triisopropylsilyl)oxy)butanoate (**9**;[5] 560 mg, 1.48 mmol) and $Ph_3PCCO$ (**10**; 530 mg, 1.65 mmol) in toluene (10 mL) was refluxed for 18 h and then concentrated under vacuum. The remainder was purified by column chromatography (cyclohexane/ethyl acetate 1/1, $R_f$ = 0.38) to leave **11** (330 mg, 55%) as a colorless oil. $[\alpha]^{20}_D$ = 46° (*c* = 1, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 2942, 2865, 1689, 1620, 1499, 1462, 1422, 1377, 1351, 1308, 1225, 1200, 1141, 1071, 989, 881, 846, 802, 755, 737, 716, 676 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 0.93–1.02 (m, 21 H), 1.09 (d, *J* = 6.3 Hz, 3 H), 2.87 (s, 3 H), 3.86 (d, *J* = 3.0 Hz, 1 H), 4.32 (qd, *J* = 6.3, 3.0 Hz, 1 H), 4.84 (d, *J* = 11.8 Hz, 1 H) 4.89 (d, *J* = 11.8 Hz, 1 H), 5.09 (s, 1 H), 7.25–7.33 (m, 5 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 12.1, 17.8, 18.3, 27.5, 66.4, 67.4, 72.5, 95.6, 127.6, 128.2, 128.4, 134.8, 171.9, 173.3. MS (EI) *m*/*z* 403 [$M^+$], 359, 268, 226, 203, 201, 157, 115, 91. HRMS: calcd for $C_{23}H_{38}NO_3Si^+$ 404.2615, found 404.2626 [M + H]$^+$.

**(5*S*,6*R*)-5-(1-((triisopropylsilyl)oxy)ethyl)-1-methylpyrrolidine-2,4-dione (12).** A mixture of tetramate **11** (430 mg, 1.1 mmol), dry MeOH (50 mL), and 5% Pd on charcoal (170 mg) was flushed and then pressurized (1 bar, balloon) with $H_2$ gas and stirred at room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temperature for 8 h. After filtration over Celite and evaporation of the volatiles the residue was taken up in ethyl acetate. The solution was filtered over a short pad of silica and concentrated under reduced pressure to leave the product as a colorless oil. Yield: 320 mg (93%), $R_f$ = 0.38 (cyclohexane/ethyl acetate 1/1). $[\alpha]^{28}_D$ = −20° (*c* = 1, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 2943, 2892, 2867, 1771, 1694, 1463, 1374, 1333, 1244, 1141, 1089, 1067, 998, 882, 811, 750, 717, 679 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.00−1.05 (m, 21 H), 1.40 (d, *J* = 6.7 Hz, 3 H), 2.93 (t, *J* = 0.8 Hz, 1 H), 2.95 (dd, *J* = 1.1, 0.8 Hz, 1 H), 3.11 (dd, *J* = 1.1, 0.8 Hz, 3 H), 3.68 (ddquin, *J* = 2.0, 1.1, 0.8 Hz, 1 H), 4.50 (qd, *J* = 6.7, 2.0 Hz, 1 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 12.6, 17.8, 21.7, 30.1, 41.2, 68.8, 74.6, 169.8, 205.6. MS (EI): *m*/*z* 270 (100) [M−$C_3H_7$]$^+$, 228 (11), 226 (9), 213 (7), 201 (45), 184 (15), 171 (10), 157 (34), 129 (6), 124 (11), 115 (26), 87 (13), 75 (13), 73 (14), 59 (19), 43 (9). HRMS: calcd for $C_{16}H_{32}NO_3Si^+$ 314.2151, found 314.2141 [M + H]$^+$.

**(*R*)-4-Benzyl-3-((6*E*)-octenoyl)oxazolidin-2-one (14a).** A solution of (6*E*)-octenoic acid (**13**;[7] 0.48 g, 3.4 mmol) in dry THF (20 mL) at −10 °C was treated with $NEt_3$ (1.18 mL, 8.5 mmol) followed by $Me_3CCOCl$ (0.42 mL, 3.4 mmol). After the mixture was stirred at −10 °C for 1 h, LiCl (0.22 g, 5.1 mmol) and (*R*)-4-benzyloxazolidin-2-one (0.60 g, 3.4 mmol) were added at this temperature. Stirring was continued for 1 h at −10 °C and then for a further 2 h at 0 °C. Saturated aqueous $NH_4Cl$ solution (20 mL) was added, and the mixture was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated. The crude product was purified by column chromatography (cyclohexane/diethyl ether 7/1, $R_f$ = 0.24). Yield: 0.89 g (87%) as a colorless oil. $[\alpha]^{28}_D$ = −65° (*c* = 1.0, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 3028, 2919, 2856, 1777, 1697, 1604, 1497, 1481, 1384, 1350, 1288, 1196, 1099, 1050, 1013, 965, 921, 843, 761 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.43−1.50 (m, 2 H), 1.58−1.83 (m, 5 H), 1.96−2.13 (m, 2 H), 2.77 (dd, *J* = 13.4, 9.3 Hz, 1 H), 2.82−3.05 (m, 2 H), 3.30 (dd, *J* = 13.4, 3.3 Hz, 1 H), 4.08−4.25 (m, 2 H), 4.67 (ddt, *J* = 9.3, 6.7, 3.3 Hz, 1 H), 5.34−5.55 (m, 2 H), 7.14−7.40 (m, 5 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 17.9, 23.7, 29.0, 32.3, 35.4, 37.9, 55.1, 66.1, 125.1, 127.3, 128.9, 129.4, 130.9, 135.3, 153.4, 173.3. MS (EI): *m*/*z* 301 (2) [M$^+$], 246 (5), 190 (8), 178 (27), 159 (8), 142 (23), 134 (43), 125 (26), 117 (35), 97 (43), 92 (22), 91 (33), 86 (20), 81 (13), 69 (20), 75 (74). HRMS: calcd for $C_{18}H_{24}NO_3^+$ 302.1751, found 302.1756 [M + H]$^+$.

**(*S*)-4-Benzyl-3-((6*E*)-octenoyl)oxazolidin-2-one (14b).** This compound was obtained from (*S*)-4-benzyloxazolidin-2-one as a colorless oil analogously to **14a**. Yield: 87%. $[\alpha]^{28}_D$ = 65° (*c* = 1.0, $CH_2Cl_2$).

**(4*R*,2′*R*,6′*E*)-4-Benzyl-3-(2′-methyloctenoyl)oxazolidin-2-one (15a).** A solution of amide **14a** (0.45 g, 1.5 mmol) in THF (15 mL) at −78 °C was treated dropwise with NaHMDS (1.5 M solution in THF, 1.5 mL, 2.3 mmol) and stirred for 30 min at this temperature. MeI (0.3 mL, 4.6 mmol) was added, and the reaction mixture was stirred for a further 3 h at −78 °C. Saturated aqueous $NH_4Cl$ solution (20 mL) was added, and the mixture was warmed to room temperature and then extracted twice with ethyl acetate. The organic extracts were washed with brine, dried, and concentrated. Column chromatography (cyclohexane/diethyl ether 10/1) left 0.44 g (89%) of **15a** as a colorless oil with $R_f$ = 0.55 (cyclohexane/ethyl acetate 3/1). $[\alpha]^{28}_D$ = −79° (*c* = 1.0, $CH_2Cl_2$). 95% de (GC). IR (ATR): $\nu_{max}$ 2927, 2855, 1776, 1695, 1604, 1497, 1454, 1383, 1348, 1289, 1238, 1208, 1195, 1100, 1075, 1015, 966, 921, 838, 761 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.26 (d, *J* = 6.9 Hz, 3 H), 1.34−1.46 (m, 3 H), 1.67 (dt, *J* = 4.9, 1.0 Hz, 3 H), 1.73−1.86 (m, 1 H), 1.95−2.10 (m, 2 H), 2.81 (dd, *J* = 13.3, 9.6 Hz, 1 H), 3.30 (dd, *J* = 13.3, 3.3 Hz, 1 H), 3.75 (qt, *J* = 6.9, 6.5 Hz, 1 H), 4.15−4.27 (m, 2 H), 4.71 (ddt, *J* = 9.6, 6.7, 3.3 Hz, 1 H), 5.34−5.54 (m, 2 H), 7.18−7.44 (m, 5 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 17.4, 17.9, 27.1, 32.5, 32.9, 37.6, 37.9, 55.3, 66.0, 125.1, 127.3, 128.9, 129.4, 130.9, 135.3, 153.0, 177.3. MS (EI): *m*/*z* 315 (10) [M$^+$], 233 (100), 218 (10), 190 (6), 178 (72), 159 (5), 139 (81), 117 (55), 111 (29), 91 (53), 82 (42). HRMS: calcd for $C_{19}H_{26}NO_3^+$ 316.1907, found 316.1920 [M + H]$^+$.

**(4*S*,2′*S*,6′*E*)-4-Benzyl-3-(2′-methyloctenoyl)oxazolidin-2-one (15b).** This compound was obtained from **14b** as a colorless oil analogously to **15a**. Yield: 89%. $[\alpha]^{28}_D$ = 79° (*c* = 1.0, $CH_2Cl_2$).

**(2*R*,6*E*)-2-Methyloctenoic Acid (16a).** A solution of amide **15a** (0.70 g, 2.2 mmol) in THF/$H_2O$ (88 mL, 10/1) at 0 °C was treated with LiOH (0.35 g, 8.4 mmol) and $H_2O_2$ (30% in $H_2O$, 1.2 mL, 11.3 mmol) and stirred for 2 h at room temperature. $Me_2S$ (0.9 mL, 11.3 mmol) was added, and the resulting mixture was stirred for another 10 min. It was acidified with 1 M $NaHSO_4$ solution and extracted three times with ethyl acetate. The organic extracts were washed with brine, dried, and concentrated. Column chromatography (cyclohexane/diethyl ether 7/1) left **16a** (0.30 g, 87%) as a colorless oil with $R_f$ = 0.42 (cyclohexane/ethyl acetate/acetic acid 100/33/1). $[\alpha]^{28}_D$ = −17° (*c* = 1.0, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 2933, 2858, 1702, 1464, 1416, 1378, 1289, 1237, 1076, 963 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.18 (d, *J* = 7.0 Hz, 3 H), 1.33−1.48 (m, 3 H), 1.64 (d, *J* = 4.7 Hz, 3 H), 1.66−1.75 (m, 1 H), 1.93−2.04 (m, 2 H), 2.45 (qt, *J* = 7.0, 6.3 Hz, 1 H), 5.29−5.53 (m, 2 H), 11.22 (br. s, 1 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 16.8, 17.9, 27.0, 32.4, 33.0, 39.3, 125.2, 130.8, 183.3. MS (EI): *m*/*z* 156 (15) [M + H]$^+$, 138 (21), 110 (9), 101 (6), 95 (5), 87 (22), 83 (75), 74 (100), 69 (27), 68 (32), 55 (88). Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 68.89; H, 10.35.

**(2*S*,6*E*)-2-Methyloctenoic Acid (16b).** This compound was obtained from **15b** as a colorless oil analogously to **16a**. Yield: 87%. $[\alpha]^{28}_D$ = 17° (*c* = 1.0, $CH_2Cl_2$). HRMS: calcd for $C_9H_{16}O_2^+$ 155.1078, found 155.1068.

**(5*S*)-3-((2′*R*,6′*E*)-1′-Hydroxy-2′-methylocten-1′-ylidene)-5-((*R*)-1″-((triisopropylsilyl)oxy)ethyl)-1-methylpyrrolidine-2,4-dione (18a).** A solution of acid **16a** (0.35 g, 2.2 mmol) and $NEt_3$ (0.70 mL, 5.1 mmol) in THF (20 mL) was cooled to 0 °C and treated dropwise with ethyl chloroformate (272 μL, 2.8 mmol). After 1 h tetramic acid **12** (0.63 g, 2.0 mmol) was added and the resulting mixture was stirred at room temperature for 12 h and then concentrated under vacuum. The residue was taken up in $CH_2Cl_2$ and washed with dilute $NaHSO_4$ solution. The water phase was re-extracted with $CH_2Cl_2$, and the combined organic layers were dried and concentrated under reduced pressure. The crude 4-*O*-acyltetramic acid **17a** was dissolved in $CH_2Cl_2$ (20 mL), cooled to 0 °C, and treated with DMAP (0.49 g, 4.0 mmol) and dry $CaCl_2$ (0.44 g, 4.0 mmol). The mixture was stirred at room temperature for 8 h and then washed with 0.05 M $Na_2$-EDTA solution. The water phase was re-extracted with $CH_2Cl_2$, and the combined organic layers were dried and concentrated. The crude product was dissolved in MeOH (17 mL), treated with water (3 mL) to give a milky emulsion, and centrifuged to leave **18a** as a red oil. Purification by HPLC (rinsing with 85% MeOH, then elution with 100% MeOH) afforded 468 mg (55%) of **18a** as a red oil consisting of a 5.6/1 mixture of the two tautomers A/B. $[\alpha]^{28}_D$ = −63° (*c* = 1, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 2939, 2866, 1710, 1651, 1614, 1461, 1376, 1329, 1264, 1213, 1139, 1096, 1069, 997, 965, 921, 881, 809, 778, 755, 704 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 0.96−1.04 (m, 21 H), 1.16 (d, *J* =6.9 Hz, 3 H), 1.27−1.39 (m, 3 H), 1.36$^B$/1.43$^A$ (d, *J* = 6.6 Hz, 3 H), 1.59−1.72 (m, 1 H), 1.62 (dt, *J* = 4.7, 1.2 Hz, 3 H), 1.90−2.02 (m, 2 H), 3.05$^B$/3.10$^A$ (s, 3 H), 3.47$^A$ (d, *J* = 1.9 Hz, 1 H), 3.55$^A$ (ddq, *J* = 7.4, 7.1, 6.9 Hz, 1 H), 3.70$^B$ (d, *J* = 2.5 Hz, 1 H), 3.77$^B$ (tq, *J* = 7.7, 6.9 Hz, 1 H), 4.56 (qd, *J* = 6.6, 1.9 Hz, 1 H), 5.30−5.49 (m, 2 H). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 12.5$^B$/12.6$^A$, 17.4, 17.9$^B$/18.0$^A$, 22.8, 27.2/27.3, 28.9, 32.56, 32.62, 35.6$^B$/36.1$^A$, 67.6$^B$/67.9$^A$, 69.4$^B$/72.4$^A$, 100.8, 125.0, 130.9, 174.4, 190.8, 193.0. MS (EI): *m*/*z* 451 (<1) [M]$^+$, 408 (100) [M − $C_3H_7$]$^+$, 394 (10), 364 (17), 251 (17), 201 (33), 157 (32), 149 (15), 115 (9). HRMS: calcd for $C_{25}H_{46}NO_4Si^+$ 452.3196, found 452.3192 [M + H]$^+$.

**(5*S*)-3-((2′*S*,6′*E*)-1′-Hydroxy-2′-methylocten-1′-ylidene)-5-((*R*)-1″-((triisopropylsilyl)oxy)ethyl)-1-methylpyrrolidine-2,4-dione (18b).** A 160 mg amount (55%) was obtained as a red oil, consisting of a 7/1 mixture of the two tautomers A/B, analogously to **18a**, from **12** (207 mg, 0.66 mmol) and **16b** (113 mg, 0.73 mmol). $[\alpha]^{28}_D$ = −104° (*c* = 1.0, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 2938, 2866, 1712, 1647, 1615, 1488, 1462, 1375, 1328, 1262, 1214, 1140, 1096, 1069, 967, 924, 882, 797, 753, 702, 679 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 0.97−1.02 (m, 21 H), 1.13$^A$/1.17$^B$ (d, *J* = 6.7 Hz, 3 H), 1.27−1.34


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(m, 2 H), 1.36$^{B}$/1.44$^{A}$ (d, $J$ = 6.6$^{B}$/6.9$^{A}$ Hz, 3 H), 1.39−1.49 (m, 1 H), 1.62 (dt, $J$ = 4.7, 1.1 Hz, 3 H), 1.64−1.74 (m, 1 H), 1.88−2.01 (m, 2 H), 3.05$^{B}$/3.11$^{A}$ (s, 3 H), 3.48$^{A}$ (d, $J$ = 1.6 Hz, 1 H), 3.59$^{A}$ (dqd, $J$ = 8.5, 6.7, 6.3 Hz, 1 H), 3.70$^{B}$ (d, $J$ = 2.5 Hz, 1 H), 3.76$^{B}$ (q, $J$ = 6.7 Hz, 1 H), 4.57 (qd, $J$ = 6.9, 1.6 Hz, 1 H), 5.27−5.48 (m, 2 H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 12.5$^{B}$/12.6$^{A}$, 16.7, 17.94$^{B}$/17.97$^{A}$, 23.0, 27.1, 29.0, 32.5, 33.5, 35.7, 67.5$^{B}$/68.1$^{A}$, 68.4$^{B}$/72.5$^{A}$, 101.2, 125.1, 130.9, 174.4, 190.5, 193.2. MS (EI): $m/z$ 451 (<1) $[M]^+$, 408 (100) $[M - C_3H_7]^+$, 394 (4), 364 (16), 251 (10), 201 (29), 157 (28), 115 (9). HRMS: calcd for $C_{25}H_{45}KNO_4Si^+$ 490.2749, found 490.2762 $[M + K]^+$.

**(9*R*)-Penicillenol C$_1$ ((5*S*,6*R*,9*R*)-1e).** A solution of **18a** (136 mg, 0.30 mmol) in THF (500 $\mu$L) in a plastic vial was treated with HF (70% in pyridine, 33 $\mu$L, 1.2 mmol, 4 equiv) and stirred overnight. The reaction was quenched by stirring with $Et_3SiH$ (191 $\mu$L, 1.2 mmol) for 30 min (*Caution*! the vessel must not be tightly sealed!) and subsequent pouring into MeOH/water (77/23, 8 mL). Centrifugation and purification by HPLC (MeOH/$H_2O$ 77/23 + 0.1% $HCO_2H$, flow rate 10 mL/min, $t_R$ of major isomer 29 min) left 69 mg (77%) of (5*S*,6*R*,9*R*)-**1e** as a red oil. $[\alpha]^{28}_D$ = −12° ($c$ = 0.125, MeOH). IR (ATR): $\nu_{max}$ 3448 br, 2972, 2934, 2157, 1698, 1602, 1453, 1408, 1377, 1339, 1259, 1212, 1121, 1087, 965, 851, 810, 797, 766, 710 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.11 (d, $J$ = 6.3 Hz, 3 H), 1.16 (d, $J$ = 6.9 Hz, 3 H), 1.27−1.38 (m, 2 H), 1.40−1.53 (m, 1 H), 1.61 (d, $J$ = 5.2 Hz, 3 H), 1.64−1.73 (m, 1 H), 1.90−1.99 (m, 2 H), 2.97 (s, 3 H), 3.57 (ddq, $J$ = 8.0, 6.9, 6.6 Hz, 1 H), 3.78 (d, $J$ = 4.4 Hz, 1 H), 4.17 (qd, $J$ = 6.9, 4.4 Hz, 1 H) 5.26−5.47 (m, 2 H). $^{13}$C NMR ($CDCl_3$, 126 MHz): $\delta$ 17.0, 17.5, 17.9, 27.0, 27.1, 32.3, 33.3, 36.1, 66.6, 68.5, 101.3, 125.2, 130.7, 174.1, 192.6, 194.9. MS (EI): $m/z$ 295 (58) $[M]^+$, 277 (10), 251 (100), 233 (11), 213 (53), 204 (17), 182 (13), 169 (40), 151 (9), 140 (78), 139 (55), 113 (34), 112 (11). HRMS: calcd for $C_{16}H_{26}NO_4^+$ 296.1862, found 296.1856 $[M + H]^+$.

**(9*S*)-Penicillenol C$_1$ ((5*S*,6*R*,9*S*)-1e).** A 38 mg amount (77%) was obtained analogously to (5*S*,6*R*,9*R*)-**1e** from **18b** (51 mg, 0.17 mmol). $[\alpha]^{28}_D$ = −47.0° ($c$ = 0.125, MeOH). IR (ATR): $\nu_{max}$ 3454 br, 2971, 2932, 2858, 1702, 1602, 1453, 1407, 1376, 1338, 1258, 1212, 1121, 1087, 965, 851, 811, 796, 768, 710 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.13 (d, $J$ = 6.6 Hz, 3 H), 1.17 (d, $J$ = 6.9 Hz, 3 H), 1.27−1.40 (m, 2 H), 1.41−1.52 (m, 1 H), 1.61 (d, $J$ = 4.7 Hz, 3 H), 1.63−1.74 (m, 1 H), 1.90−2.00 (m, 2 H), 2.98 (s, 3 H), 3.55 (ddq, $J$ = 8.0, 6.9, 6.6 Hz, 3 H), 3.78 (d, $J$ = 4.5 Hz, 1 H), 4.18 (qd, $J$ = 6.6, 4.5 Hz, 2 H), 5.24−5.50 (m, 2 H). $^{13}$C NMR ($CDCl_3$, 126 MHz): $\delta$ 17.2, 17.7, 17.9, 27.1, 27.2, 32.4, 33.0, 36.3, 66.7, 68.6, 101.1, 125.2, 130.8, 174.1, 192.7, 194.8. MS (EI): $m/z$ 295 (20) $[M]^+$, 277 (8), 251 (63), 233 (9), 213 (37), 204 (19), 182 (16), 169 (36), 151 (13), 140 (100), 139 (62), 113 (32), 112 (14). HRMS: calcd for $C_{16}H_{26}NO_4^+$ 296.1862, found 296.1863 $[M + H]^+$.

**Methyl 11-(3-Azido-5-(azidomethyl)benzamido)-undecanoate (20).** An ice-cold solution of 3-azido-5-(azidomethyl)-benzoic acid (**19**;[11] 1.07 g, 4.9 mmol), methyl 11-aminoundecanoate hydrochloride[12] (1.85 g, 7.36 mmol), and $NEt_3$ (2.73 mL, 19.6 mmol) in $CH_2Cl_2$ (60 mL) was treated dropwise with propanephosphonic acid anhydride ($T_3P$; 50% in THF, 3.38 mL, 5.6 mmol) and stirred for 1 h. Water was added, the phases were separated, the aqueous layer was re-extracted twice with $CH_2Cl_2$, and the combined organic phases were dried and concentrated. Column chromatography (cyclohexane/ethyl acetate 3/1, $R_f$ = 0.24) left **20** (1.95 g, 96%) as a light brown solid with mp 52 °C. IR (ATR): $\nu_{max}$ 3305, 2926, 2854, 2100, 1736, 1640, 1592, 1538, 1437, 1344, 1312, 1278, 1209, 1170, 1110, 998, 859, 764, 698 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.19−1.38 (m, 12 H), 1.58 (m, 4 H), 2.27 (t, $J$ = 7.5 Hz, 2 H), 3.40 (q, $J$ = 7.0 Hz, 2 H), 3.63 (s, 3 H), 4.36 (s, 2 H), 6.47 (t, $J$ = 6.3 Hz, 1 H), 7.05 (dd, $J$ = 2.1, 1.5 Hz, 1 H), 7.38 (t, $J$ = 1.9 Hz, 1 H), 7.43 (t, $J$ = 1.5 Hz, 1 H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 24.5, 24.7, 26.9, 28.7, 29.1, 29.2, 29.3, 29.5, 34.0, 40.2, 53.9, 63.9, 118.8, 121.4, 124.0, 131.7, 140.7, 143.3, 166.2, 174.3. MS (EI): $m/z$ 415 (21) $[M]^+$, 384 (19), 345 (44), 317 (29), 313 (25), 285 (10), 231 (20), 201 (27), 175 (42), 161 (24), 145 (43), 118 (33), 83 (30), 69 (51), 56 (100). HRMS: calcd for $C_{20}H_{29}N_7NaO_3^+$ 438.2230, found 438.2238 $[M + Na]^+$.

**11-(3-Azido-5-(azidomethyl)benzamido)undecanoic Acid (21).** A mixture of ester **20** (0.75 mg, 1.8 mmol), MeOH (40 mL), water (10 mL), and NaOH (0.14 g, 3.6 mmol) was stirred at room temperature for 18 h and then concentrated under reduced pressure. The remainder was extracted three times with $CH_2Cl_2$, and the extracts were dried, concentrated, and purified by column chromatography (cyclohexane/ethyl acetate/HOAc 1/1/0.01, $R_f$ = 0.32). Yield: 0.61 g (85%) as a white solid with mp 78 °C. IR (ATR): $\nu_{max}$ 3292, 2918, 2851, 2107, 2081, 1694, 1631, 1592, 1534, 1468, 1439, 1410, 1330, 1317, 1299, 1278, 1267, 1241, 1215, 1190, 1118, 1078, 1019, 920, 879, 795, 766 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.23−1.40 (m, 12 H), 1.53−1.66 (m, 4 H), 2.32 (t, $J$ = 7.4 Hz, 2 H), 3.41 (td, $J$ = 7.1, 5.9 Hz, 2 H), 4.37 (s, 2 H), 6.43 (t, $J$ = 5.9 Hz, 1 H), 7.06 (t, $J$ = 1.8 Hz, 1 H), 7.38 (t, $J$ = 1.8 Hz, 1 H), 7.41−7.47 (m, 1 H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 24.6, 26.9, 28.9, 29.05, 29.13, 29.2, 29.3, 29.4, 34.0, 40.3, 53.9, 117.5, 120.9, 122.6, 137.1, 138.0, 141.3, 166.2, 179.2. MS (EI): $m/z$ 401 (24) $[M^+]$, 375 (9), 345 (5), 342 (32), 331 (27), 313 (45), 303 (35), 289 (10), 275 (9), 261 (8), 231 (16), 201 (22), 190 (19), 184 (20), 175 (28), 164 (50), 162 (48), 147 (40), 145 (48), 136 (35), 122 (40), 106 (20), 90 (33), 83 (26), 69 (48), 63 (42), 56 (100). HRMS: calcd for $C_{19}H_{27}N_7NaO_3^+$ 424.2068, found 424.2067 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{27}N_7O_3$: C, 56.84; H, 6.78; N, 24.42. Found: C, 56.96; H, 6.80; N, 24.13.

**(5*S*)-3-(1′-Hydroxy-11′-(3-azido-5-(azidomethyl)-benzamido)undecan-1′-ylidene)-5-((*R*)-1″-((triisopropylsilyl)-oxy)ethyl)-1-methylpyrrolidine-2,4-dione (22).** Analogously to **18**, 3-acyltetramic acid **22** (244 mg, 54%) was obtained as a red oil from bis-azido acid **21** (0.26 g, 0.65 mmol) and tetramic acid **12** (0.24 g, 0.77 mmol). $[\alpha]^{25}_D$ = −59° ($c$ = 0.8, $CH_2Cl_2$). IR (ATR): $\nu_{max}$ 3330, 2926, 2864, 2105, 1712, 1607, 1540, 1461, 1374, 1324, 1281, 1214, 1139, 1096, 1068, 975, 921, 882, 780, 754 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 0.95−1.03 (m, 21 H), 1.23−1.38 (m, 12 H), 1.42 (d, $J$ = 6.8 Hz, 3 H), 1.53−1.69 (m, 4 H), 2.77 (m, 2 H), 3.09 (s, 3 H), 3.43 (q, $J$ = 6.3 Hz, 2 H), 3.47 (d, $J$ = 1.6 Hz, 1 H), 4.39 (s, 2 H), 4.54 (qd, $J$ = 6.8, 1.6 Hz, 1 H), 6.20−6.38 (m, 1 H), 7.06 (s, 1 H) 7.38 (s, 1 H) 7.45 (s, 1 H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 12.5, 17.9, 22.8, 25.7, 26.9, 29.0, 29.16, 29.18, 29.21, 29.25, 29.3, 29.5, 32.5, 40.3, 53.9, 68.1, 72.4, 101.6, 117.5, 120.9, 122.6, 137.3, 138.0, 141.3, 166.1, 174.1, 186.7, 193.3. MS (EI): $m/z$ 653 (100) $[M - C_3H_7]^+$, 582 (4), 553 (6), 496 (72), 479 (25), 453 (29), 435 (12), 392 (3), 322 (10), 270 (22), 201 (54), 157 (62), 147 (12), 131 (56), 115 (29), 103 (43), 87 (15), 75 (40), 73 (22), 61 (22), 55 (12), 43 (60). HRMS: calcd for $C_{35}H_{56}N_8NaO_5^+$ 719.4041, found 719.4023 $[M + Na]^+$.

**(5*S*)-3-(1′-Hydroxy-11′-(3-azido-5-(azidomethyl)-benzamido)undecan-1′-ylidene)-5-((*R*)-1″-hydroxyethyl)-1-methylpyrrolidine-2,4-dione (23).** Analogously to **1e**, 3-acyltetramic acid **23** (65 mg, 75%) was obtained from **22** (111 mg, 0.16 mmol) as a red oil. $[\alpha]^{25}_D$ = −31° ($c$ = 1.0, MeOH). IR (ATR): $\nu_{max}$ 3305 br, 2926, 2854, 2105, 1706, 1637, 1606, 1541, 1455, 1373, 1313, 1260, 1213, 1088, 1025, 945, 860, 801, 763 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.14 (d, $J$ = 6.6 Hz, 3 H), 1.24−1.40 (m, 12 H), 1.56−1.71 (m, 4 H), 2.82 (td, $J$ = 7.5, 1.6 Hz, 2 H), 2.98 (s, 3 H), 3.44 (td, $J$ = 7.0, 6.0 Hz, 2 H), 3.79 (d, $J$ = 4.7 Hz, 1 H), 4.19 (qd, $J$ = 6.6, 4.7 Hz, 1 H), 4.40 (s, 2 H), 6.15−6.22 (m, 1 H), 7.06−7.11 (m, 1 H), 7.40 (t, $J$ = 1.6 Hz, 1 H), 7.42−7.48 (m, 1 H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 17.8, 26.9, 27.2, 29.01, 29.04, 29.18, 29.21, 29.3, 29.6, 32.8, 40.3, 54.0, 66.7, 68.6, 101.8, 117.5, 121.0, 122.6, 127.9, 137.3, 141.4, 166.0, 173.9, 188.7, 194.9; HRMS: calcd for $C_{26}H_{37}N_8O_5^+$ 541.2887, found 541.2920 $[M + H]^+$.

**11-((2*E*,4*E*)-5-Azidomethyl-7-oxo-1*H*-azepine-3-carboxamido)undecanoic Acid (24).** A solution of bisa-zide **21** (148 mg, 0.37 mmol) in MeOH (3 mL) was irradiated for 28 h with a 150 W Mercury UV lamp in a quartz cuvette from a distance of 10 cm. Purification by HPLC (MeOH/$H_2O$ 65/35 + 0.1% HCOOH) gave three main fractions ($t_R$ = 22, 26.5, 39 min, flow rate 10 mL/min). The third fraction contained the main product imidate, which hydrolyzed upon evaporation to leave amide **24**. The latter was also found in the first fraction. The second fraction contained a different unspecified azide. The first and third fractions had identical IR and $^1$H NMR spectra as well as identical retention times in analytical HPLC. They


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were pooled to afford 30 mg (21%) of **24** as a white solid with mp 76 °C. IR (ATR): $\nu_{max}$ 2926, 2853, 2103, 1703, 1657, 1627, 1585, 1541, 1436, 1367, 1295, 1263, 1181, 1110, 1015, 885, 799, 759 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.26−1.38 (m, 12 H), 1.52−1.66 (m, 4 H), 2.34 (t, $J$ = 7.4 Hz, 2 H), 2.94 (s, 2 H), 3.35 (dd, $J$ = 7.1, 6.0 Hz, 2 H), 4.03 (s, 2 H), 5.65−5.79 (m, 1 H), 6.33−6.54 (m, 1 H), 7.33 (d, $J$ = 5.2 Hz, 1 H), 8.21−8.37 (m, 1 H). $^{13}C$ NMR ($CDCl_3$, 126 MHz): $\delta$ 24.6, 26.7, 28.8, 28.90, 28.95, 29.0, 29.1, 29.4, 33.7, 39.4, 40.1, 56.1, 119.6, 121.5, 129.3, 131.5, 165.9, 166.6, 177.7. MS (EI): $m/z$ 392 (80) $[M + H]^+$, 349 (10), 331 (10). HRMS: calcd for $C_{19}H_{30}N_5O_4^+$ 392.2292, found 392.2304 $[M + H]^+$.

**Distribution of Bis-Azide-Tagged Tetramic Acid 23 in PTK2 Cells.** PTK2 cells were incubated overnight with compound **23** (50 μg/mL) and then fixed and permeabilized with formaldehyde (4%) and Triton X-100. The cells were then treated with DyLight488-phosphine (Thermo Scientific), incubated at 37 °C for 3 h, irradiated at 245 nm for 15 min, and finally photographed through a Zeiss Axioplan fluorescence microscope at 63× magnification.

## ■ ASSOCIATED CONTENT

### Supporting Information

Tables and figures giving $^1H$ and $^{13}C$ NMR spectra of **11**−**16**, **18**, **20**−**24**, (9*R*)-**1e**, and (9*S*)-**1e**, details of the cytotoxicity of (9*S*)-**1e**, (9*R*)-**1e**, and **21**, and chemical $^{13}C$ shifts of natural and synthetic **1e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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