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Design and Synthesis of a Tag-Free Chemical Probe for Photoaffinity Labeling

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The novel aromatic diazirine-containing benzoic acid **22** was prepared via the diazirine **11** as the key intermediate. After formylation of the aryl ring and cleavage of the methyl ether function of aldehyde **12**, the phenolic hydroxy group was converted into the ether **21** terminating in an alkyne function. Oxidation of the aldehyde to the carboxylic acid provided the chemical probe **22** designed for tag-free photoaffinity labeling. In a proof-of-concept study it could be shown that irradiation of the simple ester **23** indeed yields the methanol insertion product **24**. A subsequent click reaction with benzyl azide **20** led to the triazole **25**. A more complicated example was realized with the esterification of bafilomycin A_1 (**27**) with acid **22**.

Introduction

A timeless challenge in biology and drug discovery involves finding a matching pair between one entity and a large number of possible partners. Thus, libraries of small molecules have been screened for binding to or inhibition of an enzyme.[1,2] Also the target of an active compound, frequently a natural product, needs to be found. Depending on the nature of the ligand, activity-based profiling[3] or affinity chromatography can be used. However, in some cases like low-abundance proteins, recourse to photoaffinity labeling (PAL) has to be made.[4] Besides target fishing, PAL can be used to elucidate the structural and functional properties of biological systems. In PAL a ligand is connected to a photoreactive group. Irradiation of the bound ligand–label conjugate generates a reactive intermediate that hooks onto the protein of interest by forming a covalent bond. Among various photophores, aryl(trifluoromethyl)diazirines[5] have found widespread applications. They are readily photolyzed to reactive carbenes at around 360 nm.[6] Furthermore, they can be kept small enough that the biological activity of the modified ligand does not suffer too much. In order to identify the PAL products after binding and photolysis, a reporter group should be contained within the photophore or somewhere else in the ligand.[7] Historically, radioisotopes have been employed in this context (cf. compounds **1**,[8,9] Figure 1). As an alternative to radioisotopes, photoaffinity biotinylation can be used (cf. compound **2**,[10–12] Figure 1). However, the polar and big biotin-anchored tag often renders the ligand-affinity conjugate substantially less active than the parent ligand. In order to circumvent these shortcomings compact bifunctional probes carrying a small functional group for selective modification after photoaffinity labeling (post-PAL modification, PPALM) have recently been described (cf. **3**, **4**, and **5**). These are formed by catalyzed azide–alkyne [3+2] cycloaddition[13–16] or Staudinger–Bertozzi ligation reactions.[17,18] In fact, Suzuki and co-workers showed that the azidoalkyl group of an ether derivative of diazirine **3** largely survives the conditions used for photoactivation of the 3-(trifluoromethyl)-3*H*-diazirin-3-yl group.[19] They also showed that the sequence of photoreaction followed by cycloaddition with benzyl azide worked in vitro on a derivative of **4**.[20]

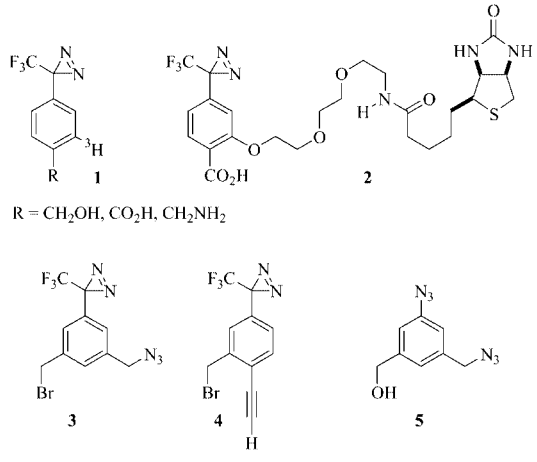

Figure 1. Some important examples of photoaffinity probes based on 3-(trifluoromethyl)-3*H*-diazirines and azides.

In connection with the task of localizing the binding partner of a natural product a tag-free 4-[3-(trifluoromethyl)-3*H*-diazirinyl]benzoic acid was sought. The idea was to

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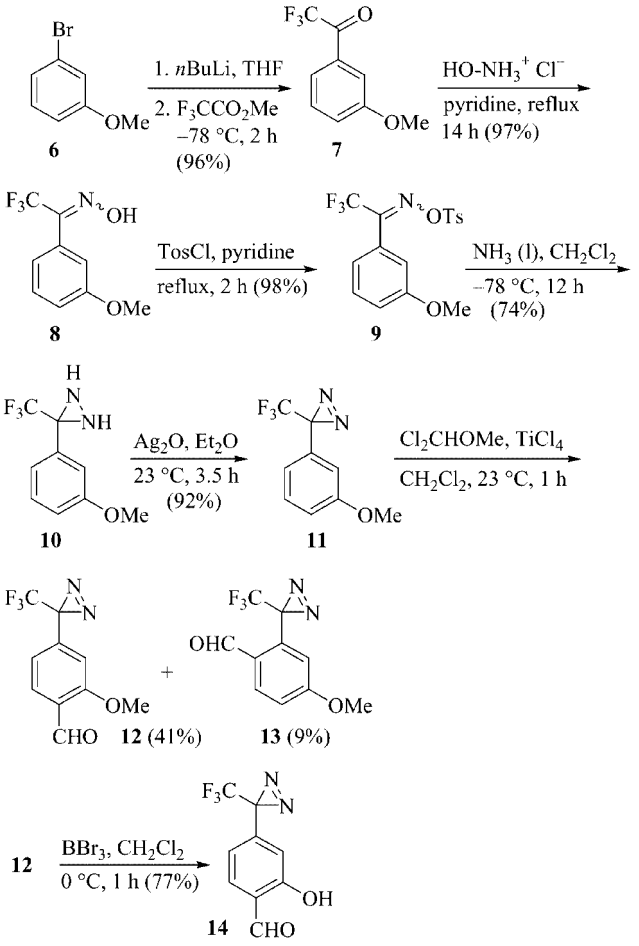
shorten the side-chain of benzoic acid **2** and terminate it with an azide or alkyne. This should allow selective fishing of a labeled protein by click chemistry. In this paper we detail the synthesis of the bifunctional benzoic acid **22** and illustrate the sequence of carbene trapping followed by click chemistry on a simple model system.

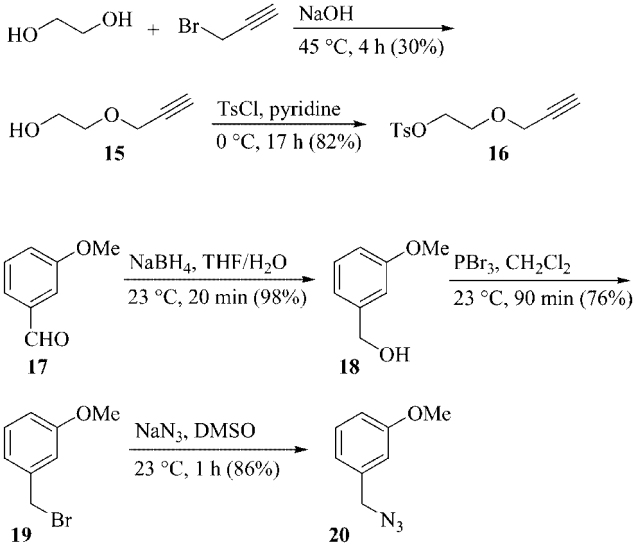
Results and Discussion

Starting from 3-bromoanisole (**6**) the key intermediate 3-(3-methoxyphenyl)diazirine **11** was prepared using the classical sequence[21] for introduction of the 3-(trifluoromethyl)-diazirin-3-yl group (Scheme 1). Compared with the literature methods some steps were slightly modified. Thus, metalation of bromide **6** with *n*-butyllithium followed by acylation of the aryllithium intermediate with methyl trifluoroacetate gave ketone **7** in excellent yield. Ketone **7** was converted into the oxime **8** using hydroxylamine hydrochloride in the presence of pyridine. Treatment of the oxime **8** (*E*/*Z* mixture) with tosyl chloride led to the *p*-tolylsulfonyloxime **9**. All these steps proceeded in almost quantitative yields. Formation of the diaziridine **10** using ammonia followed by oxidation of **10** with silver oxide[21a,21b] furnished diazirine **11**. The most convenient way to introduce the formyl group involves Friedel–Crafts alkylation of anisole **11** with dichloromethyl methyl ether in the presence of $TiCl_4$ followed by aqueous work-up.[22] These conditions gave the desired aldehyde **12** (41%) along with the regioisomer **13** (9%). The two aldehydes could be separated by flash chromatography. In order to prepare for the attachment of the side-chain, the aryl methyl ether was cleaved using boron tribromide in dichloromethane which led to hydroxybenzaldehyde **14**.



Scheme 1. Synthesis of the 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl] benzaldehyde **14**.

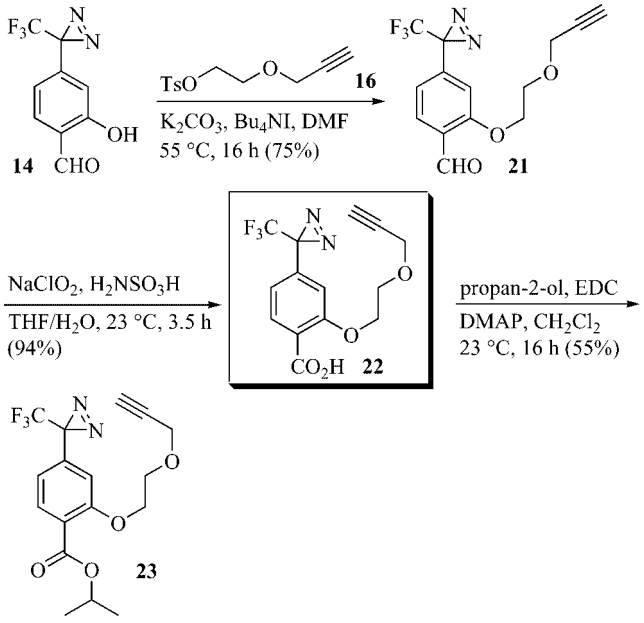
It was then planned to form an ether bond between phenol **14** and a suitable alkynol derivative. As shown in Scheme 2 the tosylate **16** was prepared in two steps by monoalkylation of ethanediol with propargyl bromide[23] followed by tosylation of the alkynol **15**. In order to demonstrate a click reaction of the alkyne functionality, we chose the simple benzyl azide **20**. This compound was easily accessible by routine steps from 3-methoxybenzaldehyde (**17**). Thus, reduction of the aldehyde to the benzyl alcohol **18** was followed by bromination using PBr_3 leading to benzyl bromide **19**. A final nucleophilic substitution of the bromide with sodium azide in DMSO provided the azide **20**. The methoxy group would later facilitate the identification of the click product by NMR spectroscopy.



Scheme 2. Preparation of the alkyne **16** and benzyl azide **20**.

Alkylation of the 2-hydroxybenzaldehyde **14** with the tosylate **16** was performed at a slightly elevated temperature in the presence of potassium carbonate and catalytic amounts of tetrabutylammonium iodide in DMF (Scheme 3). These conditions gave the ether **21** in 75% yield. The etherification was also possible under Mitsunobu conditions.[24] However, the yield was lower and separation of the byproducts turned out to be problematic. The final oxidation of the aldehyde **21** to the acid **22** was rather problematic. Typical reagents for related oxidation reactions such as permanganate did not work.[21] Finally, we found the combination of sodium chlorite with sulfamic acid to be suitable.[25] This way the benzoic acid **22** could be obtained in 94% yield. In large-scale reactions, simple extraction of the reaction mixture followed by concentration of the organic layer pro-

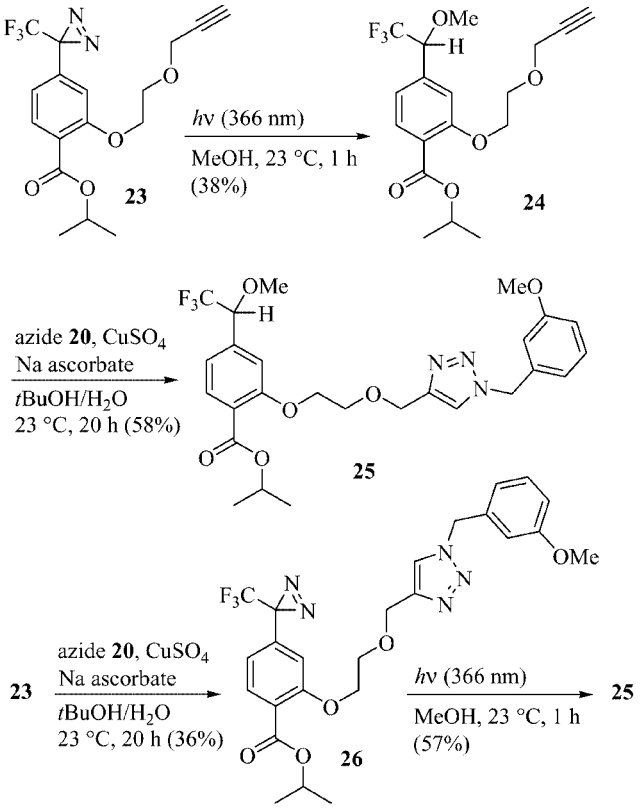
vided the pure acid **22** as an amorphous solid without any further purification. As a model the acid **22** was esterified with 2-propanol using the condensing agent 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC) providing ester **23** in reasonable yield.



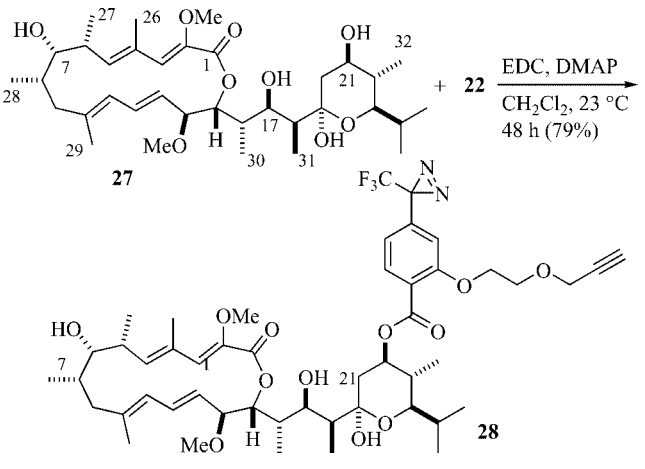
Scheme 3. Synthesis of the photoaffinity probe **22** by alkylation of phenol **14** and oxidation of the benzaldehyde **21**.

By irradiating a solution of diazirine **23** in methanol with a UV lamp (8 W, 366 nm), the corresponding carbene was generated, as evidenced by the isolation of the methanol adduct **24** (Scheme 4). This adduct is characterized by the methoxy signal (δ = 3.41 ppm) and the methine hydrogen (δ = 4.48 ppm). Owing to the CF_3 group the latter appears as a quartet (^2J = 6.6 Hz). Although the yield of adduct **24** was moderate, its formation shows that the side-chain does not react with the intermediate carbene. According to LC-MS analysis, irradiation on a larger scale produces the reduced derivative (2,2,2-trifluoroethyl substituent) as a side-product. In a subsequent click reaction between alkyne **24** and the azide **20** the triazole **25** was formed. Remarkably, the order of events could also be reversed. Thus, the diazirine function seems to be compatible with the conditions of the click reaction, as indicated by the successful formation of triazole **26**. This shows that the alkyne function might be used as a handle for conjugation with other small groups prior to irradiation. Subjection of diazirine **26** to irradiation in methanol gave compound **25** as well.

In order to illustrate the potential use of acid **22** we treated it with the natural product bafilomycin A_1 (**27**). This macrolactone, isolated from *Streptomyces griseus*, is a well-known inhibitor of V-ATPase.[26,27] In this regard it is closely related to concanamycin A.[28] As has been described before, the hydroxy group at C-21 can be selectively acylated, alkylated, and oxidized without affecting the V-ATPase activity too much.[29] On the other hand, modifications to 7-OH cause a significant reduction in activity. Accordingly, reaction of bafilomycin A_1 (**27**) with acid **22** (3 equiv.) in the presence of the condensing agent EDC and DMAP provided the ester **28** in good yield (Scheme 5). Even though an excess of acid **22** was used, besides ester **28** some unreacted **27** was recovered. In the NMR spectrum the signal of the methine 21-H resonates at δ = 5.11–5.20 ppm as opposed to 3.53 ppm in **27**. The carbon atom C-21 appears at δ = 75.6 ppm ([D_6]acetone) as opposed to 70.4 ppm.

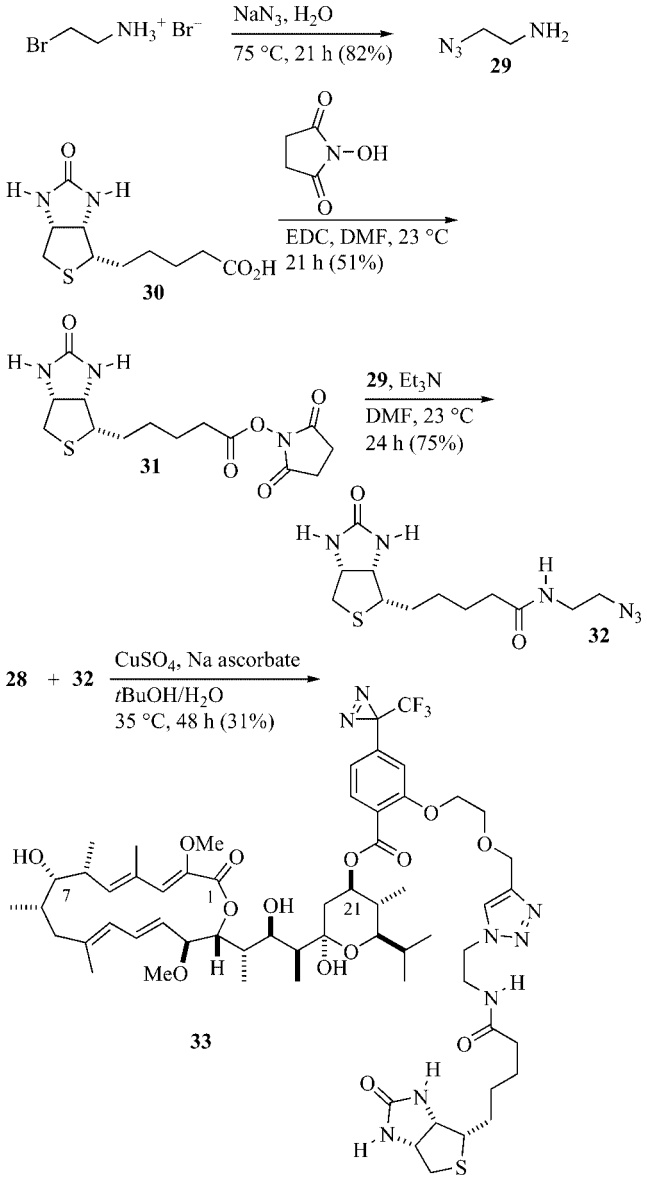


Scheme 4. Photoreaction of the alkynyl-functionalized phenyl(trifluoromethyl)diazirine **23** in methanol followed by a click reaction with benzyl azide **20**.



Scheme 5. Esterification of bafilomycin A1 (**27**) at 21-OH with acid **22** yielding ester **28**.

We also demonstrated a click reaction with the ester **28**. It seemed that the azido-biotin derivative **32** would be a suitable reagent (Scheme 6).[30] In order to prepare this reagent, 2-bromoethylamine was converted into azidoethylamine (**29**) by treating it with sodium azide.[31] In a separate operation biotin (**30**) was activated as its *N*-hydroxysuccinimide derivative **31**[32] in a condensation reaction performed in the presence of the reagent EDC. Thereafter, treatment of *N*-hydroxysuccinimidobiotin (**31**) with azidoethylamine (**29**) in DMF in the presence of triethylamine furnished the azide[30] **32** in good yield. When the azido-biotin derivative **32** was treated with the bafilomycin A_1 derivative **28** under typical click conditions (copper sulfate, sodium ascorbate as reducing agent) in a mixture of *t*BuOH and H_2O the ligation product **33** could be isolated in a moderate yield as a colorless amorphous solid. The two bafilomycin A_1 derivatives **28** and **33** are currently being tested for inhibition of V-ATPase. Owing to the smaller appendage one would expect a higher activity for **28** over **33**.



Scheme 6. Synthesis of the biotin azide **32** and its click reaction with the alkyne **28**.

Conclusions

In this paper we have described the synthesis of the novel bifunctional probe **22** featuring the 3-(trifluoromethyl)-3*H*-diazirin-3-yl group as well as an alkynyl side-chain. In a model study we have shown that the carbene insertion product can be evidenced by a Cu^I-catalyzed Huisgen alkyne–azide 1,3-dipolar cycloaddition reaction. Esterification of bafilomycin A_1 (**27**) with the acid **22** gave the ester **28**. In a click reaction with the biotinyl azide **32** the triazole **33** was obtained. With the availability of biotinyl azides such as **32** or fluorescence dyes containing an azide group,[13] applications of **22** in chemical biology present itself. Studies along these lines are currently being pursued.

Experimental Section

General: ^1H and ^{13}C NMR: Bruker Avance 400 spectrometer, Bruker AMX 600 spectrometer; spectra were recorded at 295 K in $CDCl_3$, [D_6]acetone, or [D_6]DMSO; chemical shifts are calibrated to the residual proton and carbon resonances of the solvent: $CDCl_3$ (δ_H = 7.25, δ_C = 77.0 ppm), [D_6]acetone (δ_H = 2.04, δ_C = 29.8 ppm), [D_6]DMSO (δ_H = 2.49, δ_C = 39.5 ppm). Melting points: Büchi Melting Point B-540, uncorrected. HRMS (FT-ICR): Bruker Daltonic APEX 2 with electron-spray ionization (ESI). The minimal resolution of this machine is 1 ppm ($\Delta m/m \times 10^6$). Flash chromatography: J. T. Baker silica gel, 43–60 μm. Thin-layer chromatography: Macherey–Nagel Polygram Sil G/UV_{254} plates. Analytical LC-MS: HP 1100 Series connected with an ESI-MS Agilent G1946C detector, positive mode with a fragmentor voltage of 40 eV; column: Nucleosil 100–5, C-18 HD, 5 mm, 70 × 3 mm Macherey–Nagel; eluent: NaCl solution (5 mM)/acetonitrile; gradient: 0–10–15–17–20 min with 20–80–80–99–99% acetonitrile; flow: 0.5 mL min^{-1}. All solvents used in the reactions were distilled before use. Dry tetrahydrofuran was distilled from sodium and benzophenone, whereas dry CH_2Cl_2, dimethylformamide, pyridine, and DMSO were distilled from CaH_2. Petroleum ether with a boiling range of 40–60 °C was used. Reactions were generally run under argon. All commercially available compounds were used as received unless stated otherwise. Reactions with diazirine-containing compounds were protected from light. Bafilomycin A_1 was a gift from Dr. Carlo Farina, Brane Discovery Srl, via Zambeletti 25, 20021 Baranzate (MI), Italy.

2,2,2-Trifluoro-1-(3-methoxyphenyl)ethanone (7):[21b,33] *n*BuLi (2.5 M in hexane, 28 mL, 1.5 equiv.) was slowly added to a solution of 1-bromo-3-methoxybenzene (8.9 g, 6.0 mL, 47.6 mmol) in dry THF (100 mL) at –78 °C. The reaction mixture was stirred at this temperature for 2 h before a solution of methyl trifluoroacetate (8.1 g, 6.3 mL, 63.3 mmol, 1.3 equiv.) in dry THF (40 mL) was added over a period of 30 min. After the mixture had been stirred for an additional 2 h, a solution of conc. HCl (10.5 mL) in methanol (25 mL) was added. The resulting solution was warmed up to 0 °C, diluted with Et_2O (60 mL), washed with water (2 × 10 mL), dried with $MgSO_4$, and filtered. The solvent was evaporated in vacuo and the crude product purified by kugelrohr distillation, 60 °C/10^{-1} mbar (ref.[33] 84–85 °C/12 Torr) to give the title com-

pound (9.38 g, 46.0 mmol, 96%) as a colorless liquid. R_f = 0.21 (hexane/EtOAc, 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.86 (s, 3 H, CH_3), 7.24 (dd, J = 8.1, 2.0 Hz, 1 H, 4-H), 7.44 (t, J = 8.0 Hz, 1 H, 5-H), 7.55 (s, 1 H, 2-H), 7.65 (d, J = 7.6 Hz, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.5 (CH_3), 113.9 (d, J = 1.5 Hz, C-2), 116.6 (q, $^1J_{CF}$ = 291.3 Hz, CF_3), 122.3 (C-4), 122.7 (q, J = 3.0 Hz, C-6), 130.1 (C-5), 131.0 (C-1), 160.0 (C-3), 180.4 (q, $^2J_{CF}$ = 35.1 Hz, CCF_3) ppm.

2,2,2-Trifluoro-1-(3-methoxyphenyl)ethanone Oxime (8):[33] A solution of 2,2,2-trifluoro-1-(3-methoxyphenyl)ethanone (**7**) (9.38 g, 46.0 mmol) and hydroxylamine hydrochloride (4.2 g, 60.4 mmol, 1.3 equiv.) in pyridine (100 mL) and dry ethanol (50 mL) was refluxed at an oil-bath temperature of 85 °C for 14 h. The solvent was removed under reduced pressure and the resulting residue partitioned between Et_2O (80 mL) and water (60 mL). The organic layer was washed with 1 N HCl (3 × 10 mL) and water (1 × 10 mL), dried with $MgSO_4$, and filtered. Evaporation of the solvent afforded the pure oxime (9.8 g, 44.72 mmol, 97%) as a colorless liquid (*E*/*Z* mixture, 6:4 ratio). R_f = 0.63 ($CHCl_3$/EtOAc, 10:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.83 (s, 3 H, CH_3), 6.98–7.11 (m, 3 H, 2-H, 4-H, 6-H), 7.33 (t, J = 8.3 Hz, 0.4 H, 5-H), 7.40 (t, J = 7.8 Hz, 0.6 H, 5-H), 9.19 (s, 0.6 H, NOH), 9.34 (s, 0.4 H, NOH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.36 (CH_3), 55.38 (CH_3) 113.9 (C-2), 114.2 (C-2), 116.1 (C-4), 116.3 (C-4), 118.2 (q, $^1J_{CF}$ = 282.5 Hz, CF_3), 120.5 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 120.7 (C-6), 120.8 (C-6), 127.0 (C-1) 129.65 (C-5), 129.73 (C-5), 131.1 (C-1), 147.5 (q, $^2J_{CF}$ = 32.9 Hz, CCF_3), 147.7 (q, $^2J_{CF}$ = 30.7 Hz, CCF_3), 159.40 (C-3), 159.54 (C-3) ppm.

2,2,2-Trifluoro-1-(3-methoxyphenyl)-*N*-{[(4-methylphenyl)sulfonyl]-oxy}ethanimine (9): Toluenesulfonyl chloride (4.3 g, 22.5 mmol, 1.5 equiv.) was added to a solution of 2,2,2-trifluoro-1-(3-methoxyphenyl)ethanone oxime (**8**) (3.3 g, 15.0 mmol) in pyridine (50 mL) and the resulting solution was refluxed for 2 h at an oil-bath temperature of 125 °C. After cooling, the solvent was evaporated and the residue partitioned between Et_2O (250 mL) and water (60 mL). The organic layer was washed with 1 N HCl (3 × 15 mL) and water (1 × 15 mL), dried with $MgSO_4$, and filtered. Evaporation of the solvent afforded the pure title compound (5.5 g, 14.7 mmol, 98%) as a colorless solid, m.p. 111 °C. R_f = 0.17 (toluene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.47 (s, 3 H, CH_3), 3.81 (s, 3 H, OCH_3), 6.87 (s, 1 H, 2-H), 6.93 (d, J = 7.6 Hz, 1 H, 4-H), 7.04 (dd, J = 8.5, 1.9 Hz, 1 H, 6-H), 7.37 (t, J = 7.3 Hz, 3 H, 5-H, 3′-H, 5′-H), 7.87 (d, J = 8.3 Hz, 2 H, 2′-H, 6′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7 (CH_3), 55.4 (OCH_3), 113.9 (C-2), 117.1 (C-4), 119.5 (q, $^1J_{CF}$ = 277.4 Hz, CF_3), 120.4 (C-6), 125.6 (C-5), 129.2 (C-3′, C-5′), 129.9 (C-2′, C-6′), 130.0 (aryl C), 131.1 (aryl C), 146.1 (C-4′), 153.9 (q, $^2J_{CF}$ = 33.6 Hz, CCF_3), 159.5 (C-3) ppm.

3-(3-Methoxyphenyl)-3-(trifluoromethyl)diaziridine (10):[21b,33] A solution of 2,2,2-trifluoro-1-(3-methoxyphenyl)-*N*-{[(4-methylphenyl)sulfonyl]oxy}ethanimine (**9**) (5.5 g, 14.73 mmol) in dry CH_2Cl_2 (65 mL) was added to liquid ammonia (50 mL) at –78 °C over a period of 1 h. The solution was stirred at –78 °C for 12 h and then warmed up to room temperature overnight which led to evaporation of the excess ammonia and some of the CH_2Cl_2. The colorless solid was removed by filtration and the filtrate was washed with water (3 × 5 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The resulting crude product was purified by flash chromatography ($CHCl_3$/EtOAc, 20:1) to give the diaziridine **10** (2.39 g, 11.0 mmol, 74%) as a slightly yellow liquid. R_f = 0.30 ($CHCl_3$/EtOAc, 20:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.24 (d, J = 8.6 Hz, 1 H, NH), 2.78 (d, J = 8.3 Hz, 1 H, NH), 3.81 (s, 3 H, CH_3), 6.97 (d, J = 8.3 Hz, 1 H, 4-H), 7.14 (s, 1 H, 2-H), 7.19 (d, J = 7.6 Hz, 1 H, 6-H), 7.32 (t, J = 8.0 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.3 (CH_3), 58.0 (q, $^2J_{CF}$ = 35.9 Hz, CCF_3), 113.6 (C-2), 115.8 (C-4), 120.3 (C-6), 123.5 (q, $^1J_{CF}$ = 278.8 Hz, CF_3), 129.9 (C-5), 133.0 (C-1), 159.7 (C-3) ppm.

3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine (11): A solution of sodium hydroxide (2.4 g, 60.0 mmol) in water (30 mL) was added slowly to a boiling solution of silver(I) nitrate (10.2 g, 60.0 mmol) in water (60 mL). The precipitate was filtered and washed with water (100 mL), acetone (100 mL), and Et_2O (100 mL) to give Ag_2O as a brown solid. The freshly prepared Ag_2O (9.5 g, 41.0 mmol, 3.8 equiv.) was added to a solution of diaziridine **10** (2.38 g, 10.9 mmol) in dry Et_2O (100 mL) and stirred at room temperature for 3.5 h. The dispersion was filtered, the filter cake rinsed with Et_2O, and the organic layer dried with $MgSO_4$. Filtration and evaporation of the solvent gave the pure diazirine **11** (2.17 g, 10.04 mmol, 92%) as a colorless liquid. R_f = 0.84 ($CHCl_3$/EtOAc, 20:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.80 (s, 3 H, CH_3), 6.69 (s, 1 H, 2-H), 6.77 (d, J = 7.6 Hz, 1 H, 4-H), 6.91–6.97 (m, 1 H, 6-H), 7.30 (t, J = 8.1 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (q, $^2J_{CF}$ = 40.3 Hz, CCF_3), 55.3 (CH_3), 112.2 (d, J = 1.5 Hz, C-2), 115.2 (C-4), 118.7 (C-6), 122.1 (q, $^1J_{CF}$ = 274.4 Hz, CF_3), 130.0 (C-5), 130.5 (C-1), 159.8 (C-3) ppm.

2-Methoxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (12) and 4-Methoxy-2-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (13):[22a] $TiCl_4$ (460 mg, 2.4 mmol, 1.5 equiv.) at 0 °C, followed by dichloromethyl methyl ether (276 mg, 2.4 mmol, 1.5 equiv.) was slowly added to a stirred solution of diazirine **11** (350 mg, 1.62 mmol) in dry CH_2Cl_2 (15 mL). The reaction mixture was warmed up to room temperature and stirred at this temperature for 1 h before it was cooled again to 0 °C and quenched with water (10 mL). The organic phase was diluted with CH_2Cl_2 (15 mL) and washed with water (2 × 5 mL), saturated $NaHCO_3$ solution (2 × 5 mL), and water (2 × 5 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The resulting crude product was purified by flash chromatography (hexane/EtOAc, 5:1) to afford aldehyde **12** (161 mg, 0.66 mmol, 41%) and the isomeric aldehyde **13** (34 mg, 0.14 mmol, 9%) as yellow oils.

Aldehyde 12: R_f = 0.40 (hexane/EtOAc, 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.92 (s, 3 H, CH_3), 6.68 (s, 1 H, 3-H), 6.83 (d, J = 8.1 Hz, 1 H, 5-H), 7.82 (d, J = 8.1 Hz, 1 H, 6-H), 10.42 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.6 (q, $^2J_{CF}$ = 40.3 Hz, CCF_3), 55.8 (CH_3), 109.5 (C-3), 118.6 (C-5), 121.8 (q, $^1J_{CF}$ = 274.4 Hz, CF_3), 125.4 (C-1), 129.0 (C-6), 136.6 (C-4), 161.5 (C-2), 188.7 (CHO) ppm.

Aldehyde 13: R_f = 0.28 (hexane/EtOAc, 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.88 (s, 3 H, CH_3), 7.05 (dd, J = 8.7, 2.2 Hz, 1 H, 5-H), 7.16 (s, 1 H, 3-H), 7.89 (d, J = 8.6 Hz, 1 H, 6-H), 10.38 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.2 (q, $^2J_{CF}$ = 42.5 Hz, CCF_3), 55.8 (CH_3), 116.2 (aryl C), 116.8 (aryl C), 121.6 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 129.1 (aryl C), 130.8 (aryl C), 133.8 (aryl C), 164.6 (C-4), 188.2 (CHO) ppm.

2-Hydroxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (14): Tribromoborane (1 M in CH_2Cl_2, 5.5 mL, 5.5 mmol, 1.1 equiv.) was added slowly to a solution of 2-methoxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (**12**) (1.2 g, 4.91 mmol) in dry CH_2Cl_2 (15 mL) at –20 °C. The reaction was warmed up to 0 °C, stirred at this temperature for 1 h, and then quenched with water (5 mL). The solution was diluted with CH_2Cl_2 (60 mL), the layers were separated, and the organic layer washed with saturated NaCl solution (3 × 10 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash

chromatography (hexane/CH_2Cl_2, 2:1) to give the hydroxy aldehyde **14** (870 mg, 3.78 mmol, 77%) as a colorless solid, m.p. 44 °C. R_f = 0.32 (hexane/CH_2Cl_2, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.75–6.80 (m, 2 H, 3-H, 5-H), 7.59 (d, J = 7.8 Hz, 1 H, 6-H), 9.91 (s, 1 H, CHO), 11.04 (s, 1 H, OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (q, $^2J_{CF}$ = 41.0 Hz, CCF_3), 115.8 (C-3), 117.3 (d, J = 1.5 Hz, C-5), 120.8 (C-1), 121.6 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 133.9 (C-6), 138.0 (C-4), 161.3 (C-2), 195.9 (CHO) ppm. HRMS (ESI): calcd. for $C_9H_5F_3N_2O_2$ $[M + H]^+$ 229.02304; found 229.02302.

2-(Prop-2-ynyloxy)ethanol (15): Finely pulverized NaOH (2.16 g, 54 mmol, 1.2 equiv.) was added to a vigorously stirred solution of 3-bromoprop-1-yne (5.34 g, 44.9 mmol) and ethylene glycol (5.57 g, 89.8 mmol, 2 equiv.) at 0 °C. The resulting suspension was refluxed at 45 °C for 4 h, filtered, and the filtrate extracted with $CHCl_3$ (3 × 25 mL). The combined organic layers were washed with water (3 × 10 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 2:1) to afford alcohol **15** (1.62 g, 16.18 mmol, 30%) as a colorless liquid. R_f = 0.18 ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (t, J = 2.3 Hz, 1 H, C≡CH), 2.71 (s, 1 H, OH), 3.56–3.61 (m, 2 H, CH_2), 3.68–3.72 (m, 2 H, CH_2), 4.15 (d, J = 2.3 Hz, 2 H, CH_2C≡C) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 58.2 (CH_2C≡C), 61.4 (CH_2OH), 71.1 (CH_2CH_2OH), 74.7 (C≡CH), 79.4 (C≡CH) ppm.

2-(Prop-2-ynyloxy)ethyl Toluenesulfonate (16): Solid *p*-toluenesulfonyl chloride (1.14 g, 6 mmol, 1.1 equiv.) was added to a stirred solution of 2-(prop-2-ynyloxy)ethanol (**15**) (546 mg, 5.46 mmol) in pyridine (10 mL) at 0 °C. After 17 h saturated $NaHCO_3$ solution (20 mL) was added and the reaction mixture was stirred at the same temperature for another 10 min before it was poured into a saturated $NaHCO_3$ solution (20 mL) at room temperature. The resulting mixture was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with 1 N HCl (3 × 20 mL), water (1 × 20 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 3:1) to give the tosylate **16** (1.13 g, 4.45 mmol, 82%) as a colorless liquid. R_f = 0.62 (hexane/EtOAc, 3:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.41 (t, J = 2.4 Hz, 1 H, C≡CH), 2.42 (s, 3 H, CH_3), 3.68–3.72 (m, 2 H, pTsOCH$_2$CH_2), 4.09 (d, J = 2.3 Hz, 2 H, CH_2C≡C), 4.14–4.18 (m, 2 H, pTsOCH_2), 7.33 (d, J = 8.1 Hz, 2 H, 2-H, 6-H), 7.79 (d, J = 8.3 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6 (CH_3), 58.3 (CH_2C≡C), 67.1 (pTsOCH_2), 68.8 (pTsOCH$_2C$H$_2$), 75.0 (C≡CH), 78.9 (C≡CH), 128.0 (C-2, C-6), 129.8 (C-3, C-5), 132.9 (C-1), 144.9 (C-4) ppm.

(3-Methoxyphenyl)methanol (18): Sodium borohydride (777 mg, 20.6 mmol, 0.5 equiv.) was added to a solution of 3-methoxybenzaldehyde (**17**) (5.6 g, 41.1 mmol) in THF (120 mL) and H_2O (4 mL) and the mixture stirred at room temperature for 20 min. Then, water (120 mL) was added and the solution stirred for another 5 min. The mixture was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. This way the benzylic alcohol **18** (5.58 g, 40.39 mmol, 98%) was obtained as a colorless liquid, sufficiently pure for the subsequent step. R_f = 0.18 ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.96 (t, J = 5.2 Hz, 1 H, OH), 3.80 (s, 3 H, OCH_3), 4.65 (d, J = 5.5 Hz, 2 H, CH_2), 6.80–6.85 (m, 1 H, 4-H), 6.90–6.94 (m, 2 H, 2-H, 6-H), 7.26 (t, J = 8.1 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.2 (CH_3), 65.2 (CH_2), 112.2 (C-2), 113.2 (C-4), 119.1 (C-6), 129.5 (C-5), 142.5 (C-1), 159.8 (C-3) ppm.

1-(Bromomethyl)-3-methoxybenzene (19): Phosphorous tribromide (705 mg, 2.6 mmol, 0.36 equiv.) was added to a solution of (3-methoxyphenyl)methanol (**18**) (1.0 g, 7.24 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C. The mixture was stirred at room temperature for 90 min before it was treated with cold water (5 mL). The layers were separated and the water phase extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with water (2 × 10 mL), a saturated $NaHCO_3$ solution (1 × 10 mL), a saturated NaCl solution (1 × 10 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo to afford the pure bromide **19** (1.1 g, 5.47 mmol, 76%) as a colorless liquid. R_f = 0.25 (petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (s, 3 H, OCH_3), 4.46 (s, 2 H, CH_2), 6.84 (dd, J = 8.2, 2.2 Hz, 1 H, 4-H), 6.93 (s, 1 H, 2-H), 6.97 (d, J = 7.6 Hz, 1 H, 6-H), 7.25 (t, J = 7.8 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 33.5 (CH_2), 55.3 (CH_3), 114.2 (C-4), 114.4 (C-2), 121.3 (C-6), 129.8 (C-5), 139.1 (C-1), 159.7 (C-3) ppm.

1-(Azidomethyl)-3-methoxybenzene (20): A solution of sodium azide (0.5 M, 190.3 mg, 2.93 mmol, 1.1 equiv.) in DMSO (5.86 mL), which was prepared by stirring the sodium azide in DMSO at room temperature for 24 h, was added to 1-(bromomethyl)-3-methoxybenzene (**19**) (535 mg, 2.66 mmol). The mixture was stirred for 1 h at room temperature before it was quenched with water (10 mL). The mixture was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with water (2 × 10 mL) and a saturated NaCl solution (1 × 10 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The pure azide **20** (372 mg, 2.28 mmol, 86%) was obtained as a colorless liquid. R_f = 0.16 (petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.82 (s, 3 H, CH_3), 4.30 (s, 2 H, CH_2), 6.84–6.92 (m, 3 H, 2-H, 4-H, 6-H), 7.29 (t, J = 7.8 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 54.7 (CH_2), 55.2 (CH_3), 113.6 (aryl C), 113.8 (aryl C), 120.4 (C-6), 129.8 (C-5), 136.8 (C-1), 159.9 (C-3) ppm.

2-[2-(Prop-2-ynyloxy)ethoxy]-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (21): Potassium carbonate (710 mg, 5.14 mmol, 1.7 equiv.) was added to a cooled solution (0 °C) of 2-hydroxybenzaldehyde **14** (695 mg, 3.02 mmol) in dry DMF (60 mL) followed by stirring of the mixture for 30 min. The cooling bath was removed, then the tosylate **16** (870 mg, 3.42 mmol, 1.1 equiv.) dissolved in DMF (10 mL) was added, followed by Bu_4NI (120 mg, 0.3 mmol, 0.1 equiv.). The mixture was stirred at 55 °C for 16 h. It was then diluted with EtOAc (100 mL), washed with 1 N HCl (1 × 40 mL) and a saturated NaCl solution (1 × 40 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/Et_2O, 6:1) to give the slightly yellow aldehyde **21** (706 mg, 2.2 mmol, 75%), m.p. 40 °C. R_f = 0.45 (toluene/EtOAc, 25:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.47 (t, J = 2.2 Hz, 1 H, C≡CH), 3.94–3.96 (m, 2 H, ArOCH$_2$CH_2), 4.24–4.27 (m, 4 H, ArOCH_2, CH_2C≡C), 6.73 (s, 1 H, 3-H), 6.83 (d, J = 8.3 Hz, 1 H, 5-H), 7.83 (d, J = 8.1 Hz, 1 H, 6-H), 10.47 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.5 (q, $^2J_{CF}$ = 41.0 Hz, CCF_3), 58.6 (CH_2C≡C), 67.7 (ArOCH$_2C$H$_2$), 68.3 (ArOCH_2), 75.1 (C≡CH), 79.0 (C≡CH), 110.8 (C-3), 118.9 (C-5), 121.7 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 125.6 (C-1), 128.9 (C-6), 136.6 (C-4), 160.9 (C-2), 188.8 (CHO) ppm. HRMS (ESI): calcd. for $C_{14}H_{11}F_3N_2O_3$ $[M + Na]^+$ 335.0614; found 335.0612.

2-[2-(Prop-2-ynyloxy)ethoxy]-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid (22): Sulfamic acid (400 mg, 4.13 mmol, 1.85 equiv.) was added to a solution of aldehyde **21** (695 mg, 2.23 mmol) in THF (20 mL) and water (10 mL). The mixture was stirred for 5 min at room temperature before sodium chlorite (365 mg, 4.04 mmol, 1.81 equiv.) dissolved in water (4 mL) was added. The reaction mixture was stirred for 3.5 h at room temperature and then extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried

with $MgSO_4$, filtered, and concentrated in vacuo to afford the pure benzoic acid **22** (685 mg, 2.09 mmol, 94%) as a colorless solid, m.p. 70 °C. Purification by flash chromatography was accompanied by some loss of product. R_f = 0.16 ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.48 (t, J = 2.3 Hz, 1 H, C≡CH), 3.95–3.99 (m, 2 H, $ArOCH_2CH_2$), 4.26 (d, J = 2.3 Hz, 2 H, CH_2C≡C), 4.37–4.41 (m, 2 H, $ArOCH_2$), 6.78 (s, 1 H, 3-H), 6.92 (d, J = 8.3 Hz, 1 H, 5-H), 8.16 (d, J = 8.3 Hz, 1 H, 6-H), 10.58 (br. s, 1 H, CO_2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3 (q, $^2J_{CF}$ = 41.0 Hz, CCF_3), 58.5 (CH_2C≡C), 66.7 ($ArOCH_2CH_2$), 69.4 ($ArOCH_2$), 75.6 (C≡CH), 78.5 (C≡CH), 111.3 (C-3), 119.6 (C-1), 119.9 (d, J = 1.5 Hz, C-5), 121.7 (q, $^1J_{CF}$ = 275.2 Hz), 134.3 (C-6), 135.9 (C-4), 157.4 (C-2), 164.5 (CO_2H) ppm. HRMS (ESI): calcd. for $C_{14}H_{11}F_3N_2O_4$ [M + Na]$^+$ 351.0563; found 351.0560.

Isopropyl 2-[2-(Prop-2-ynyloxy)ethoxy]-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoate (23): To a solution of benzoic acid **22** (100.0 mg, 0.3 mmol) in dry CH_2Cl_2 (4 mL) was added DMAP (73.3 mg, 0.6 mmol, 2 equiv.), followed by propan-2-ol (0.05 mL, 0.6 mmol, 2 equiv.), and EDC (0.06 mL, 51.2 mg, 0.33 mmol, 1.1 equiv.). The resulting solution was stirred at room temperature for 16 h and then diluted with CH_2Cl_2 (35 mL). The mixture was washed with water (3 × 5 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/Et_2O, 5:1) to give the ester **23** (61.0 mg, 0.17 mmol, 55%) as a colorless viscous oil. R_f = 0.32 (petroleum ether/Et_2O, 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34 [d, J = 6.3 Hz, 6 H, $CH(CH_3)_2$], 2.44 (t, J = 2.3 Hz, 1 H, C≡CH), 3.89–3.94 (m, 2 H, $ArOCH_2CH_2$), 4.16–4.21 (m, 2 H, $ArOCH_2$), 4.27 (d, J = 2.3 Hz, 2 H, CH_2C≡C), 5.16–5.28 [m, 1 H, $CH(CH_3)_2$], 6.70 (s, 1 H, 3-H), 6.80 (d, J = 8.1 Hz, 1 H, 5-H), 7.74 (d, J = 8.1 Hz, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 [$CH(CH_3)_2$], 28.4 (q, $^2J_{CF}$ = 41.0 Hz, CCF_3), 58.6 (CH_2C≡C), 67.9 ($ArOCH_2CH_2$), 68.72 [$CH(CH_3)_2$], 68.74 ($ArOCH_2$), 74.8 (C≡CH), 79.4 (C≡CH), 111.5 (C-3), 118.6 (C-5), 121.8 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 123.0 (C-1), 131.8 (C-6), 133.8 (C-4), 158.0 (C-2), 165.0 (CO_2iPr) ppm. HRMS (ESI): calcd. for $C_{17}H_{17}F_3N_2O_4$ [M + Na]$^+$ 393.1033; found 393.1034.

Isopropyl 2-[2-(Prop-2-ynyloxy)ethoxy]-4-(2,2,2-trifluoro-1-methoxyethyl)benzoate (24): A stirred 1 mM solution of diazirine **23** (5.5 mg, 14.9 mmol) in MeOH (15 mL) was irradiated at 366 nm with a UV lamp (8 W, Waldmann, type 600352) at a distance of 1 cm for 1 h in a Duran Schlenk tube under nitrogen. Thereafter, the reaction mixture was concentrated at reduced pressure and the resulting crude product was purified by flash chromatography (petroleum ether/EtOAc, 8:1) to afford the insertion product **24** (2.1 mg, 5.61 mmol, 38%) as a colorless oil. R_f = 0.16 (petroleum ether/EtOAc, 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35 [d, J = 6.3 Hz, 6 H, $CH(CH_3)_2$], 2.44 (t, J = 2.2 Hz, 1 H, C≡CH), 3.41 (s, 3 H, OCH_3), 3.91–3.95 (m, 2 H, $ArOCH_2CH_2$), 4.21–4.25 (m, 2 H, $ArOCH_2$), 4.28 (d, J = 2.3 Hz, 2 H, CH_2C≡C), 4.48 (q, J = 6.6 Hz, 1 H, $CHCF_3$), 5.19–5.30 [m, 1 H, $CH(CH_3)_2$], 7.02 (d, J = 8.1 Hz, 1 H, 5-H), 7.05 (s, 1 H, 3-H), 7.75 (d, J = 8.1 Hz, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 [$CH(CH_3)_2$], 58.4 (OCH_3), 58.7 (CH_2C≡C), 68.0 ($ArOCH_2CH_2$), 68.5 [$CH(CH_3)_2$], 68.6 ($ArOCH_2$), 74.7 (C≡CH), 79.5 (C≡CH), 81.0 ($^2J_{CF}$ = 31.5 Hz, CCF_3), 112.9 (C-3), 120.6 (C-5), 123.0 (C-1), 123.4 ($^1J_{CF}$ = 281.8 Hz, CF_3), 131.4 (C-6), 137.4 (C-4), 158.1 (C-2), 165.6 (CO_2iPr) ppm. HRMS (ESI): calcd. for $C_{18}H_{21}F_3O_5$ [M + Na]$^+$ 397.1233; found 397.1236.

Isopropyl 2-(2-{[1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl]-methoxy}ethoxy)-4-(2,2,2-trifluoro-1-methoxyethyl)benzoate (25): a) From alkyne **24**: The alkyne **24** (6.9 mg, 18.4 μmol) and the azide **20** (3.6 mg, 22.1 μmol, 1.2 equiv.) were suspended in *t*BuOH (1 mL) and H_2O (0.75 mL) at room temperature under nitrogen. Then, a 0.01 M aqueous solution of sodium ascorbate (0.37 mg, 0.19 mL, 1.84 μmol) was added followed by a 0.005 M aqueous solution of copper(II) sulfate pentahydrate (0.05 mg, 0.04 mL, 0.18 μmol). After being stirred for 20 h, water (2 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with water, dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to give the triazole **25** (5.7 mg, 10.6 μmol, 58%) as a colorless oil.

b) From diazirine **26**: A stirred 0.75 mM solution of diazirine **26** (2 mg, 3.7 μmol) in MeOH (5 mL) was irradiated at 366 nm with a UV lamp (8 W, Waldmann, type 600352) at a distance of 1 cm for 1 h in a Duran Schlenk tube under nitrogen. The reaction mixture was concentrated at reduced pressure and the resulting crude product was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to afford insertion product **25** (1.14 mg, 2.1 μmol, 57%) as a colorless oil. This material was identical in all respects (TLC, LC-MS and ^{1}H NMR) to the triazole prepared by the other route.

R_f = 0.25 (petroleum ether/EtOAc, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 [d, J = 6.1 Hz, 6 H, $CH(CH_3)_2$], 3.40 (s, 3 H, OCH_3), 3.76 (s, 3 H, $ArOCH_3$), 3.89–3.94 (m, 2 H, $ArOCH_2CH_2$), 4.17–4.23 (m, 2 H, $ArOCH_2$), 4.47 (q, J = 6.5 Hz, 1 H, $CHCF_3$), 4.75 (s, 2 H, OCH_2-triazole), 5.12–5.23 [m, 1 H, $CH(CH_3)_2$], 5.48 (s, 2 H, NCH_2), 6.80 (s, 1 H, 2′-H), 6.83–6.88 (m, 2 H, 4′-H, 6′-H), 7.01 (d, J = 8.1 Hz, 1 H, 5-H), 7.03 (s, 1 H, 3-H), 7.26 (t, J = 8.0 Hz, 1 H, 5′-H), 7.64 (s, 1 H, 5′′-H), 7.74 (d, J = 7.8 Hz, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 [$CH(CH_3)_2$], 54.1 (NCH_2), 55.3 ($ArOCH_3$), 58.4 (OCH_3), 64.9 (OCH_2-triazole), 68.4 [$CH(CH_3)_2$], 68.6 (OCH_2CH_2O), 81.0 (q, $^2J_{CF}$ = 31.0 Hz, CCF_3), 112.8 (C-3), 113.7 (C-2′), 114.1 (C-4′), 120.3 (C-6′), 120.5 (C-5), 122.7 (C-1, C-5′′), 123.4 (q, $^1J_{CF}$ = 282.0 Hz, CF_3), 130.1 (C-5′), 131.4 (C-6), 136.0 (C-1′), 137.5 (C-4), 145.5 (C-4′′), 158.2 (C-2), 160.1 (C-3′), 165.4 (CO_2iPr) ppm. HRMS (ESI): calcd. for $C_{26}H_{30}F_3N_3O_6$ [M + H]$^+$ 538.21593; found 538.21562; Δ(rel.) = 0.58 ppm.

Isopropyl 2-(2-{[1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methoxy}ethoxy)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoate (26): The alkyne **23** (24.0 mg, 64.8 μmol) and the azide **20** (12.7 mg, 77.8 μmol, 1.2 equiv.) were suspended in *t*BuOH (1 mL) and H_2O (0.2 mL) at room temperature under nitrogen. Then, a 0.01 M aqueous solution of sodium ascorbate (1.3 mg, 0.66 mL, 6.48 μmol) was added, followed by a 0.005 M aqueous solution of copper(II) sulfate pentahydrate (0.16 mg, 0.13 mL, 0.65 μmol). After being stirred for 20 h, water (3 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to give the triazole **26** (12.4 mg, 23.2 μmol, 36%) as a colorless oil. R_f = 0.34 (petroleum ether/EtOAc, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 [d, J = 6.1 Hz, 6 H, $CH(CH_3)_2$], 3.76 (s, 3 H, OCH_3), 3.88–3.93 (m, 2 H, $ArOCH_2CH_2$), 4.13–4.19 (m, 2 H, $ArOCH_2$), 4.74 (s, 2 H, OCH_2-triazole), 5.11–5.21 [m, 1 H, $CH(CH_3)_2$], 5.48 (s, 2 H, NCH_2), 6.66 (s, 1 H, 3-H), 6.79 (d, J = 7.1 Hz, 2 H, 5-H, 2′-H), 6.83–6.89 (m, 2 H, 4′-H, 6′-H), 7.26 (t, J = 7.8 Hz, 1 H, 5′-H), 7.61 (s, 1 H, 5′′-H), 7.73 (d, J = 8.1 Hz, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.8 [$CH(CH_3)_2$], 28.3 (q, $^2J_{CF}$ = 41.0 Hz, CCF_3), 54.1 (NCH_2), 55.3 ($ArOCH_3$), 64.9 (OCH_2-triazole), 68.5 ($ArOCH_2CH_2$), 68.6 [$CH(CH_3)_2$], 68.7 ($ArOCH_2$), 111.4 (C-3), 113.7 (C-2′), 114.1 (C-4′), 118.5 (C-5), 120.3 (C-6′), 121.8 (q, $^1J_{CF}$ = 275.2 Hz, CF_3), 122.7 (C-5′′), 122.8 (C-1), 130.1 (C-5′), 131.8 (C-

6), 133.8 (C-4), 136.0 (C-1′), 145.4 (C-4′′), 158.1 (C-2), 160.1 (C-3′), 164.9 (COO) ppm. HRMS (ESI): calcd. for $C_{25}H_{26}F_3N_5O_5$ [M + H]$^+$ 534.19585; found 534.19543; Δ(rel.) = 0.79 ppm.

NMR Spectroscopic Data for Bafilomycin A_1 (27): ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 0.76 (d, J = 6.8 Hz, 3 H, Me-33), 0.86 (d, J = 6.8 Hz, 3 H, Me-30), 0.91 (m, 9 H, Me-25, Me-28, Me-32), 0.98 (d, J = 7.1 Hz, 3 H, Me-31), 1.03 (d, J = 7.1 Hz, 3 H, Me-27), 1.08–1.13 (m, 2 H, 20-H), 1.22–1.30 (m, 1 H, 22-H), 1.77 (q, J = 7.0 Hz, 1 H, 18-H), 1.83–1.90 (m, 2 H, 8-H, 24-H), 1.92 (s, 3 H, Me-29), 1.97 (d, J = 0.8 Hz, 3 H, Me-26), 1.99–2.03 (m, 9-H), 2.11–2.20 (m, 2 H, 16-H, 20-H), 2.49–2.58 (m, 1 H, 6-H), 3.22 (s, 3 H, 14-OMe), 3.27–3.31 (m, 1 H, 7-H), 3.44 (dd, J = 10.4, 2.0 Hz, 1 H, 23-H), 3.53 (ddd, J = 15.4, 10.3, 5.4 Hz, 1 H, 21-H), 3.62 (s, 3 H, 2-OMe), 4.05 (t, J = 8.8 Hz, 1 H, 14-H), 4.11 (d, J = 5.6 Hz, 1 H, 7-OH), 4.17 (ddd, J = 10.7, 4.2, 1.5 Hz, 1 H, 17-H), 4.71 (d, J = 4.3 Hz, 1 H, 17-OH), 5.0 (dd, J = 8.3, 1.0 Hz, 1 H, 15-H), 5.13 (dd, J = 14.9, 9.1 Hz, 1 H, 13-H), 5.24 (d, J = 2.0 Hz, 1 H, 19-OH), 5.78 (d, J = 10.6 Hz, 1 H, 11-H), 5.95 (d, J = 8.8 Hz, 1 H, 5-H), 6.66 (dd, J = 10.9, 4.0 Hz, 1 H, 12-H), 6.70 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 7.3 (Me-31), 10.3 (Me-30), 12.6 (Me-32), 14.1 (Me-26), 14.7 (Me-33), 17.7 (Me-27), 20.3 (Me-29), 21.7 (Me-25), 22.2 (Me-28), 28.7 (C-24), 37.9 (C-6), 38.2 (C-16), 41.6 (C-8), 41.9 (C-22), 42.2 (C-9), 43.0 (C-18), 44.5 (C-20), 55.6 (14-OMe), 60.1 (2-OMe), 70.4 (C-21), 71.5 (C-17), 76.9 (C-23), 77.4 (C-15), 80.3 (C-7), 83.3 (C-14), 99.7 (C-19), 125.2 (C-11), 126.9 (C-13), 132.7 (C-4), 134.1 (C-3), 134.4 (C-12), 141.9 (C-2), 144.8 (C-10), 145.7 (C-5), 167.7 (C-1) ppm.

21-*O*-{2-[2-(Prop-2-ynyloxy)ethoxy]-4-[3-(trifluoromethyl)-3*H*-diaziren-3-yl]benzoyl}bafilomycin A_1 (28): Benzoic acid **22** (10.5 mg, 32.1 μmol, 2 equiv.) followed by DMAP (4.1 mg, 33.7 μmol, 2.1 equiv.), and EDC (6.0 μL, 33.7 μmol, 2.1 equiv.) were added to a stirred solution of bafilomycin A_1 (10.0 mg, 16.1 μmol) in CH_2Cl_2 (2 mL) . After stirring for 24 h at room temperature, additional acid **22** (5.3 mg, 16.1 μmol, 1 equiv.), DMAP (2.3 mg, 19.3 μmol, 1 equiv.), and EDC (3.4 μL, 19.3 μmol, 1.2 equiv.) were added and stirring was continued for a further 24 h. The solvent was evaporated in vacuo and the resulting crude product purified by flash chromatography (hexane/EtOAc, 3:2) to afford ester **28** (11.9 mg, 12.7 μmol, 79%) as a colorless solid as well as unreacted bafilomycin A_1 (2.0 mg, 3.2 μmol). R_f = 0.56 (hexane/EtOAc, 3:2). ^{1}H NMR (600 MHz, [D$_6$]acetone): δ = 0.81 (d, J = 6.9 Hz, 3 H, Me-33), 0.87 (d, J = 6.9 Hz, 3 H, Me-30), 0.91 (d, J = 6.5 Hz, 6 H, Me-25, Me-32), 0.96 (d, J = 6.9 Hz, 3 H, Me-28), 1.01 (d, J = 7.1 Hz, 3 H, Me-31), 1.04 (d, J = 6.9 Hz, 3 H, Me-27), 1.35–1.40 (m, 1 H, 20-H), 1.68 (ddq, J = 16.9, 10.5, 6.5 Hz, 1 H, 22-H), 1.84–1.89 (m, 2 H, 8-H, 18-H), 1.92 (s, 3 H, Me-29), 1.93–1.96 (m, 1 H, 24-H), 1.97 (d, J = 1.2 Hz, 3 H, Me-26), 2.01 (d, J = 10.8 Hz, 1 H, 9-H), 2.16 (ddq, J = 17.6, 6.9, 0.6 Hz, 1 H, 16-H), 2.40 (dd, J = 11.8, 4.9 Hz, 1 H, 20-H), 2.51–2.57 (m, 1 H, 6-H), 2.94 (t, J = 2.4 Hz, 1 H, C≡CH), 3.23 (s, 3 H, 14-OMe), 3.28–3.31 (m, 1 H, 7-H), 3.63 (s, 3 H, 2-OMe), 3.63–3.65 (m, 1 H, 23-H), 3.88–3.90 (m, 2 H, Ar-OCH$_2$CH_2), 4.05 (app. t, J = 8.9 Hz, 1 H, 14-H), 4.11 (d, J = 5.5 Hz, 1 H, 7-OH), 4.19 (ddd, J = 10.7, 4.3, 1.6 Hz, 1 H, 17-H), 4.25 (d, J = 2.4 Hz, 2 H, CH_2C≡CH), 4.27–4.30 (m, 2 H, Ar-OCH$_2$), 4.78 (d, J = 3.7 Hz, 1 H, 17-OH), 4.97 (dd, J = 8.6, 1.2 Hz, 1 H, 15-H), 5.11–5.20 (m, 2 H, 13-H, 21-H), 5.45 (d, J = 1.8 Hz, 1 H, 19-OH), 5.79 (d, J = 10.8 Hz, 1 H, 11-H), 5.95 (d, J = 8.8 Hz, 1 H, 5-H), 6.66 (dd, J = 14.9, 10.8 Hz, 1 H, 12-H), 6.70 (d, J = 0.6 Hz, 1 H, 3-H), 6.90 (d, J = 0.6 Hz, 1 H, 3-ArH), 7.01 (dd, J = 8.1, 0.8 Hz, 1 H, 5-ArH), 7.78 (d, J = 8.1 Hz, 1 H, 6-ArH) ppm. ^{13}C NMR (100 MHz, [D$_6$]acetone): δ = 7.4 (Me-31), 10.3 (Me-30), 12.8 (Me-32), 14.1 (Me-26), 14.6 (Me-33), 17.7 (Me-27), 20.3 (Me-29), 21.6 (Me-25), 22.2 (Me-28), 28.7 (C-24), 29.1 (q, $^2J_{CF}$ = 41.0 Hz, CCF$_3$), 37.9 (C-6), 38.3 (C-16), 39.0 (C-22), 40.6 (C-20), 41.6 (C-8), 42.2 (C-9), 42.9 (C-18), 55.6 (14-OMe), 58.8 (CH_2C≡C), 60.1 (2-OMe), 68.7 (ArOCH$_2$CH_2), 69.6 (ArOCH$_2$), 71.5 (C-17), 75.6 (C-21), 76.1 (C≡CH), 76.6 (C-23), 77.3 (C-15), 80.3 (C-7), 80.6 (C≡CH), 83.3 (C-14), 99.8 (C-19), 112.3 (Ar-3), 119.4 (Ar-5), 122.9 (q, $^1J_{CF}$ = 273.7 Hz, CF$_3$), 124.7 (Ar-1), 125.1 (C-11), 126.9 (C-13), 132.4 (Ar-6), 132.7 (C-4), 133.7 (Ar-4), 134.2 (C-3), 134.4 (C-12), 141.9 (C-2), 144.8 (C-10), 145.7 (C-5), 158.7 (Ar-2), 166.0 (ArCO$_2$), 167.7 (C-1) ppm. HRMS (ESI): calcd. for $C_{49}H_{67}F_3N_2O_{12}$ [M + Na]$^+$ 955.45383; found 955.45387; Δ(rel.) = 0.04 ppm.

2-Azidoethylamine (29): 2-Bromoethylamine hydrobromide (500 mg, 2.44 mmol) was added to a solution of sodium azide (475.9 mg, 7.32 mmol, 3 equiv.) in H_2O (2 mL). The stirred solution was heated to 75 °C for 21 h before it was cooled to 0 °C. Et_2O (2 mL) was added followed by solid KOH (800 mg). The organic phase was separated and the aqueous layer extracted with Et_2O (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, filtered, and the solvent removed carefully by rotary evaporation (35 °C, 750 mbar) to afford the azide **29** (171 mg, 1.99 mmol, 82%) as a colorless liquid. R_f = 0.39 (EtOAc/MeOH, 3:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (s, 2 H, NH$_2$), 2.80–2.84 (m, 2 H, CH$_2$N$_3$), 3.30 (t, J = 5.7 Hz, 2 H, CH_2NH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.2 (CH$_2$NH$_2$), 54.6 (CH$_2$N$_3$) ppm.

1-{[5-(2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanoyl]oxy}pyrrolidine-2,5-dione (31): *N*-Hydroxysuccinimide (56.5 mg, 0.49 mmol, 1.2 equiv.) followed by EDC (86.9 μL, 0.49 mmol, 1.2 equiv.) was added to a solution of D-biotin (100.0 mg, 0.41 mmol) in DMF (10 mL). The solution was stirred for 21 h at room temperature. The solvent was evaporated in vacuo and the resulting residue dissolved in CH_2Cl_2 (200 mL). The organic phase was washed with $NaHCO_3$ solution (2 × 10 mL) and a saturated NaCl solution (1 × 10 mL), dried with $MgSO_4$, and filtered. The solvent was removed under vacuo to give biotin-NHS **31** (71.0 mg, 0.21 mmol, 51%) as a colorless solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.35–1.54 (m, 3 H, 4′-H, 5′-H), 1.58–1.68 (m, 3 H, 3′-H, 5′-H), 2.57 (d, J = 11.4 Hz, 1 H, SCH$_2$), 2.66 (t, J = 7.3 Hz, 2 H, 2′-H), 2.78–2.85 [m, 5 H, CH$_2$CH$_2$ (succinyl), SCH$_2$], 3.06–3.12 (m, 1 H, SCH), 4.11–4.16 (m, 1 H, 3a-H), 4.27–4.32 (m, 1 H, 6a-H), 6.36 (s, 1 H, 1-NH), 6.42 (s, 1 H, 3-NH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 24.3 (C-3′), 25.4 [CH$_2$CH$_2$ (succinyl)], 27.6 (C-5′), 27.8 (C-4′), 30.0 (C-2′), 39.9 (SCH$_2$), 55.2 (SCH), 59.2 (C-6a), 61.0 (C-3a), 162.7 [(HN)$_2$CO], 168.9 (CO$_2$), 170.3 [N(CO)$_2$] ppm.

***N*-(2-Azidoethyl)-5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanamide (32):** Et$_3$N (0.03 mL, 0.20 mmol, 1 equiv.) was added to a solution of 2-azidoethylamine (**29**) (24.1 mg, 0.28 mmol, 1.4 equiv.) in DMF (3 mL), followed by the addition of biotin-NHS **31** (70.0 mg, 0.20 mmol) in DMF (2 mL). The resulting solution was stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the crude product purified by flash chromatography (acetone/MeOH, 10:1) to give the desired azido-biotin **32** (47.7 mg, 0.15 mmol, 75%) as a colorless solid. R_f = 0.22 (acetone/MeOH, 10:1). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.20–1.38 (m, 2 H, 4′-H), 1.39–1.55 (m, 3 H, 3′-H, 5′-H), 1.55–1.65 (m, 1 H, 5′-H), 2.06 (t, J = 7.3 Hz, 2 H, 2′-H), 2.56 (d, J = 12.9 Hz, 1 H, SCH$_2$), 2.80 (dd, J = 12.4, 5.1 Hz, 1 H, SCH$_2$), 3.05–3.11 (m, 1 H, SCH), 3.19–3.24 (m, 2 H, CH_2CH$_2$N$_3$), 3.31 (d, J = 7.6 Hz, 2 H, CH$_2$N$_3$), 4.08–4.14 (m, 1 H, 3a-H), 4.26–4.32 (m, 1 H, 6a-H), 6.35 (s, 1 H, 1-NH), 6.42 (s, 1 H, 3-NH), 8.03 (t, J = 5.3 Hz, 1 H, CONH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 25.2 (C-3′), 28.0 (C-5′), 28.2 (C-4′), 35.1 (C-2′), 38.1 (CH_2CH$_2$N$_3$), 39.9

(SCH_2), 50.0 (CH_2N_3), 55.4 (SCH), 59.2 (C-6a), 61.0 (C-3a), 162.7 [$(HN)_2CO$], 172.4 (CONH) ppm. HRMS (ESI): calcd. for $C_{12}H_{20}N_6O_2S$ $[M + H]^+$ 313.14412; found 313.14419; Δ(rel.) = 0.22 ppm.

21-*O*-{2-(2-{[1-(2-{[5-(2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoyl]amino}ethyl)-1*H*-1,2,3-triazol-4-yl]methoxy}ethoxy)-4-[3-(trifluoromethyl)-3*H*-diaziren-3-yl]benzoyl}bafilomycin A_1 (33): Azido-biotin **32** (2.0 mg, 6.4 μmol, 1.2 equiv.) was added to a stirred solution of bafilomycin A_1 derivative **28** (5.0 mg, 5.1 μmol) in H_2O (1 mL) and *t*BuOH (1 mL) at room temperature. An aqueous sodium ascorbate solution (0.01 M, 55 μL, 0.5 μmol) was added followed by an aqueous solution of copper(II) sulfate pentahydrate (0.005 M, 10 μL, 0.05 μmol). The reaction mixture was stirred for 24 h at 35 °C before further sodium ascorbate solution (55 μL, 0.5 μmol) and copper(II) sulfate pentahydrate solution (10 μL, 0.05 μmol) were added. After another 24 h of stirring the solvent was removed under vacuum and the crude product purified by flash chromatography (CH_2Cl_2/MeOH, 10:1) to afford the ligation product **33** (2.0 mg, 1.6 μmol, 31%) as a colorless solid. Owing to the small amount a ^{13}C spectrum could not be acquired. According to the ^1H NMR spectrum, the purified **33** contains a small amount of unreacted azide **32**. R_f = 0.18 (CH_2Cl_2/MeOH, 10:1). ^1H NMR (600 MHz, [D_6]acetone): δ = 0.81 (d, J = 7.0 Hz, 3 H, Me-33), 0.87 (d, J = 6.6 Hz, 3 H, Me-30), 0.91 (m, 6 H, Me-25, Me-32), 0.96 (d, J = 6.7 Hz, 3 H, Me-28), 0.99 (d, J = 7.0 Hz, 3 H, Me-31), 1.04 (d, J = 7.0 Hz, 3 H, Me-27), 1.35–1.48 (m, 3 H, 20-H, 4′-H), 1.54–1.72 (m, 4 H, 22-H, 3′-H, 5′-H), 1.72–1.80 (m, 1 H, 5′-H), 1.84–1.89 (m, 2 H, 8-H, 18-H), 1.92 (s, 3 H, Me-29), 1.93–1.96 (m, 1 H, 24-H), 1.98 (m, 3 H, Me-26), 2.00–2.02 (m, 1 H, 9-H), 2.13–2.16 (m, 1 H, 16-H), 2.19 (t, J = 7.2 Hz, 2 H, $NHCOCH_2$), 2.40 (dd, J = 11.8, 4.4 Hz, 1 H, 20-H), 2.51–2.57 (m, 1 H, 6-H), 2.67–2.71 (m, 2 H, SCH_2), 2.90–2.94 (m, 3 H), 3.17–3.22 (m, 1 H, SCH), 3.23 (s, 3 H, 14-OMe), 3.28–3.31 (m, 1 H, 7-H), 3.35–3.42 (m, 4 H, CH_2CH_2triazole), 3.63 (s, 3 H, 2-OMe), 3.65–3.68 (m, 1 H, 23-H), 3.88–3.91 (m, 2 H, $ArOCH_2CH_2$), 4.03–4.11 (m, 2 H, 7-OH, 14-H), 4.16–4.20 (m, 1 H, 17-H), 4.26–4.29 (m, 2 H, $ArOCH_2$), 4.30–4.34 (m, 1 H, 3a-H), 4.48–4.52 (m, 1 H, 6a-H), 4.68 (s, 2 H, OCH_2-triazole), 4.77 (d, J = 4.2 Hz, 1 H, 17-OH), 4.98 (m, 1 H, 15-H), 5.17 (m, 2 H, 13-H, 21-H), 5.50 (s, 1 H, 19-OH), 5.72 (s, 1 H, NH), 5.80 (d, J = 10.4 Hz, 1 H, 11-H), 5.92 (s, 1 H, NH), 5.94–5.97 (m, 1 H, 5-H), 6.66 (dd, J = 14.9, 11.0 Hz, 1 H, 12-H), 6.71 (s, 1 H, 3-H), 6.89 (s, 1 H, 3-ArH), 7.02 (d, J = 8.9 Hz, 1 H, 5-ArH), 7.29–7.37 (m, 1 H, triazole), 7.81 (d, J = 8.1 Hz, 1 H, 6-ArH), 7.96 (s, 1 H, CONH) ppm. HRMS (ESI): calcd. for $C_{61}H_{87}F_3N_8O_{14}S$ $[M + Na]^+$ 1267.59068; found 1267.58951; Δ(rel.) = 0.90 ppm.

Supporting Information (see also the footnote on the first page of this article): Copies of the ^1H and ^{13}C NMR spectra of the described compounds.

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