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Moenomycin A: New Chemistry that Allows to Attach the Antibiotic to Reporter Groups, Solid Supports, and Proteins

Uwe Kempin,[a] Lothar Hennig,[a] Dietmar Knoll,[a] Peter Welzel,[a]*
Dietrich Müller,[b] Astrid Markus,[c] Jean van Heijenoort[d]

[a]Institut für Organische Chemie der Universität Leipzig, Talstr. 35,
D-04103 Leipzig (Germany)

[b]Fakultät für Chemie der Ruhr-Universität, D-44780 Bochum (Germany)

[c]Hoechst Marion Roussel, D-65926 Frankfurt (Germany)

[d]Biochimie Moléculaire et Cellulaire, Université Paris-Sud,
F-91405 Orsay Cedex (France)

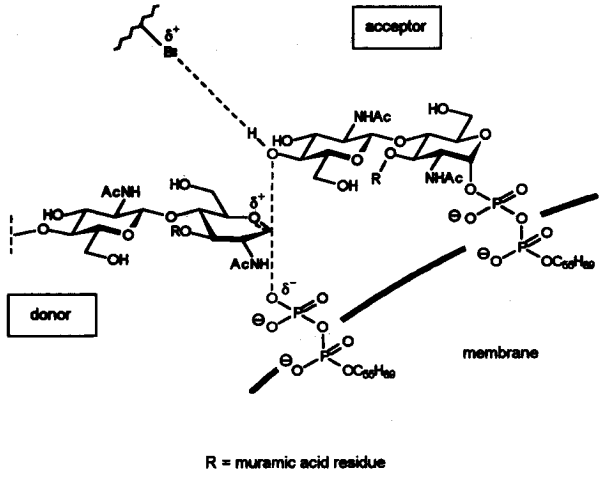
Abstract - Moenomycin A (**18**) on reaction with the diazonium salt derived from bifunctional (protected) **15** yields the coupling product **19** which on reduction is converted into the moenomycin thiol derivative **21**. Thiol **21** has been used to prepare selectively moenomycin dansyl and biotin adducts **26** and **28**, respectively. This work was performed with the aim to use moenomycin as a tool for studies of the transglycosylation step in peptidoglycan biosynthesis. © 1997 Elsevier Science Ltd.

Introduction

The biosynthesis of bacterial peptidoglycan is a two-stage process. First a disaccharide peptide monomer is formed in the cytoplasm and at the cytoplasmic face of the membrane, respectively. Then, at the outer face of the membrane two polymerization steps occur, a transglycosylation reaction, which leads to linear glycan strands and subsequently a transpeptidation reaction which cross-links the peptide units of different strands.

In *E. coli* both reactions are catalyzed by bifunctional enzymes, the high-molecular weight *penicillin-binding proteins* such as the PBPs 1a and 1b.[1] The transpeptidase active site is located in the C-terminal module and ist study has been greatly facilitated by the covalent binding of radiolabelled penicillin. The N-terminal module (at the outer surface of the cytoplasmic membrane) catalyzes the transglycosylation reaction. The

transglycosylation reaction is believed to proceed in such a way that the growing peptidoglycan chain linked to a C_{55} polyprenol (bacterioprenol) via a pyrophosphate bridge acts as the glycosyl donor whereas the



Scheme 1

disaccharide intermediate, the so-called lipid II, is the glycosyl acceptor (see Scheme 1). This mode of glycan chain elongation is not explicitly proven for *E. coli* but has been demonstrated for a poorly lytic mutant of *Bacillus licheniformis*.[2] The active site of the transglycosylase is still unknown and the mechanism of the transglycosylation reaction is poorly understood.

The moenomycin antibiotics have been shown to bind reversibly to PBP 1b and to be highly active inhibitors of the enzyme.[3] The close structural similarities between moenomycin A (**18**) and structural analogues related to it on the one hand and the donor and acceptor components of the transglycosylation reaction (see Scheme 1) on the other are striking and have been taken as a hint that the moenomycins are competitive inhibitors. However, until now this assumption is not substantiated by experiment. The transglycosylase may be discussed in the broader context of glycosyltransferases which catalyze the transfer of sugar residues from activated glycosyl phosphate derivatives to specific acceptors.[4] In a number of cases it has been demonstrated that glycosyltransferases recognize both the glycosyl donor and the glycosyl acceptor.[5] Accepting this view, moenomycin could in principle bind both at the donor and the acceptor site of the enzyme.[6]

Leaving the structural determinations aside, investigations on the moenomycins until now have concentrated on structure-activity relations.[7] This work may turn out to be useful for mapping the binding sites at the enzyme. However, the potential of the moenomycins as tools to identify and characterize the transglycosylase active site directly has not yet been exploited. A first step into this direction is the subject of the present publication. We were interested to find reactions that would allow to couple moenomycin A (**18**) to solid supports (for affinity

chromatography), to reactive or activatable groups (for affinity labelling), to proteins (for raising antibodies), and so forth.

Work on structure-activity relations has shown that units E, F, G, H, I of moenomycin A (**18**) are indispensable for transglycosylase inhibiting properties. Very few structural changes in this part of the molecule are tolerated without loss of activity. This means that additional groups have to be attached to either of units A to D.

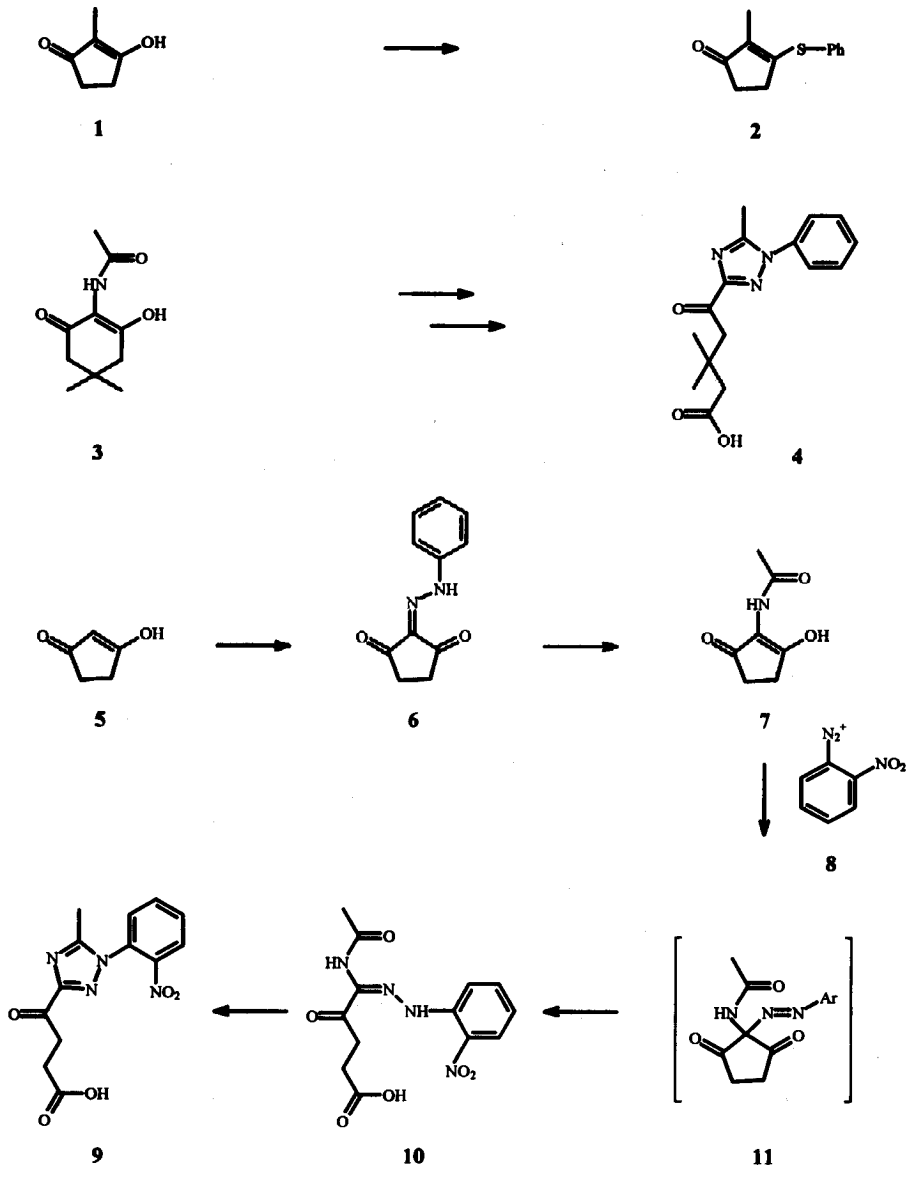
Moenomycin A (**18**) has a large array of functional groups with different reactivities. The reaction anticipated to combine the tools mentioned above with moenomycin had to be chemoselective and attack the antibiotic at a single position. Furthermore, we wanted to establish a modular system, i.e. to introduce a new functional group into the moenomycin molecule with an orthogonal reactivity to all genuine functional groups of the antibiotic. This new functional group would then have the duty to attach moenomycin to all labels and solid supports. We assumed that the enolized ß-diketone (unit A, the so-called moenomycin chromophore[8-11]) would serve the first purpose and could selectively be attacked by both soft nucleophiles and electrophiles. For the linking purposes a thiol function was anticipated although one could also think of groups reacting in thermal cycloaddition reactions.

Reaction of 1 with Thiophenol

Enolized ß-diketones are readily converted into the corresponding vinylsulfides by (i) tosylate, triflate or phosphate formation and (ii) reaction with a thiol.[12,13] Simple enolized 1,3-diketones can also be converted into the corresponding vinylsulfides on acid-catalyzed reaction with a thiol.[14] Thus, under these conditions **1** on reaction with thiophenol provided **2** in 76% yield (Scheme 2). However, the reaction failed with moenomycin.[15]

Reaction of moenomycin A (18) and model compounds with soft electrophiles.

We based our studies on selective reactions of moenomycin A (**18**) on work described some 30 years ago by Stetter,[16] Eistert and Regitz.[17] Thus, model compound **3** (prepared from dimedone by (i) reaction with tosyl azide[18] and (ii) hydrogenation in the presence of acetic anhydride[19]) on treatment with benzenediazonium chloride in aqueous solution in the presence of an excess of sodium acetate (0°C, 15 min) yielded triazole derivative **4**, which according to the results of Regitz and Eistert is formed by Japp-Klingemann reaction[20] of the initially formed azo diketone intermediate to give an amidrazone of type **10** and subsequent cyclization. For the synthesis of **7** the tosyl azide route failed. Therefore, **5** was treated with benzenediazonium chloride whereupon **6** was formed. On hydrogenation/acetylation **6** yielded **7**.[21] This compound did not react with benzenediazonium chloride, but with the more electrophilic *o*-nitro derivative **8** amidrazone **10** was obtained nicely. On prolonged standing under the reaction conditions **10** cyclized to provide **9**.



1 2 3 4 5 6 7 8 9 10 11

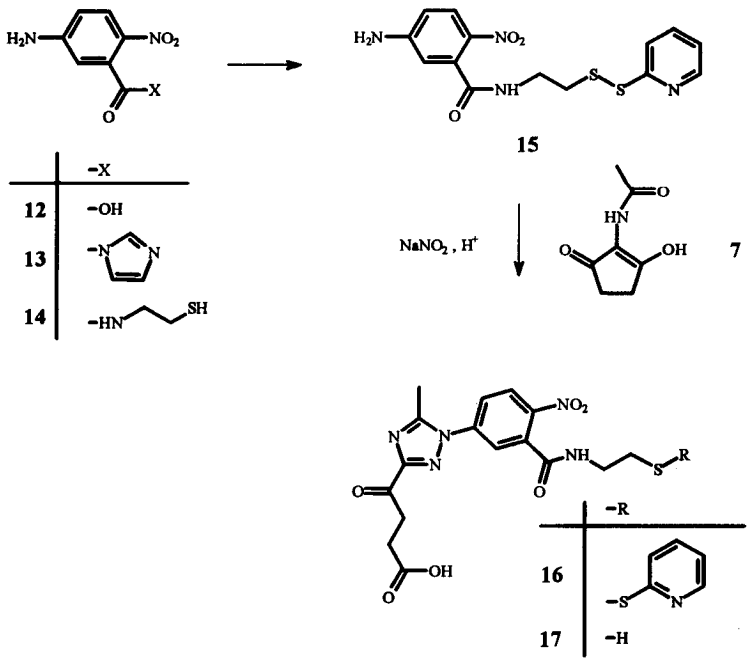
Scheme 2

When moenomycin A (**18**) under the same conditions was treated with *o*-nitrobenzenediazonium chloride (**8**), a clean reaction occurred, and the desired derivative **20** was isolated by reverse phase chromatography (HP 20,

water-methanol gradient) in about 56% yield (Scheme 4). Thus, the desired selective chemistry with moenomycin was at hand.

Synthesis of the heterobifunctional reagent 15

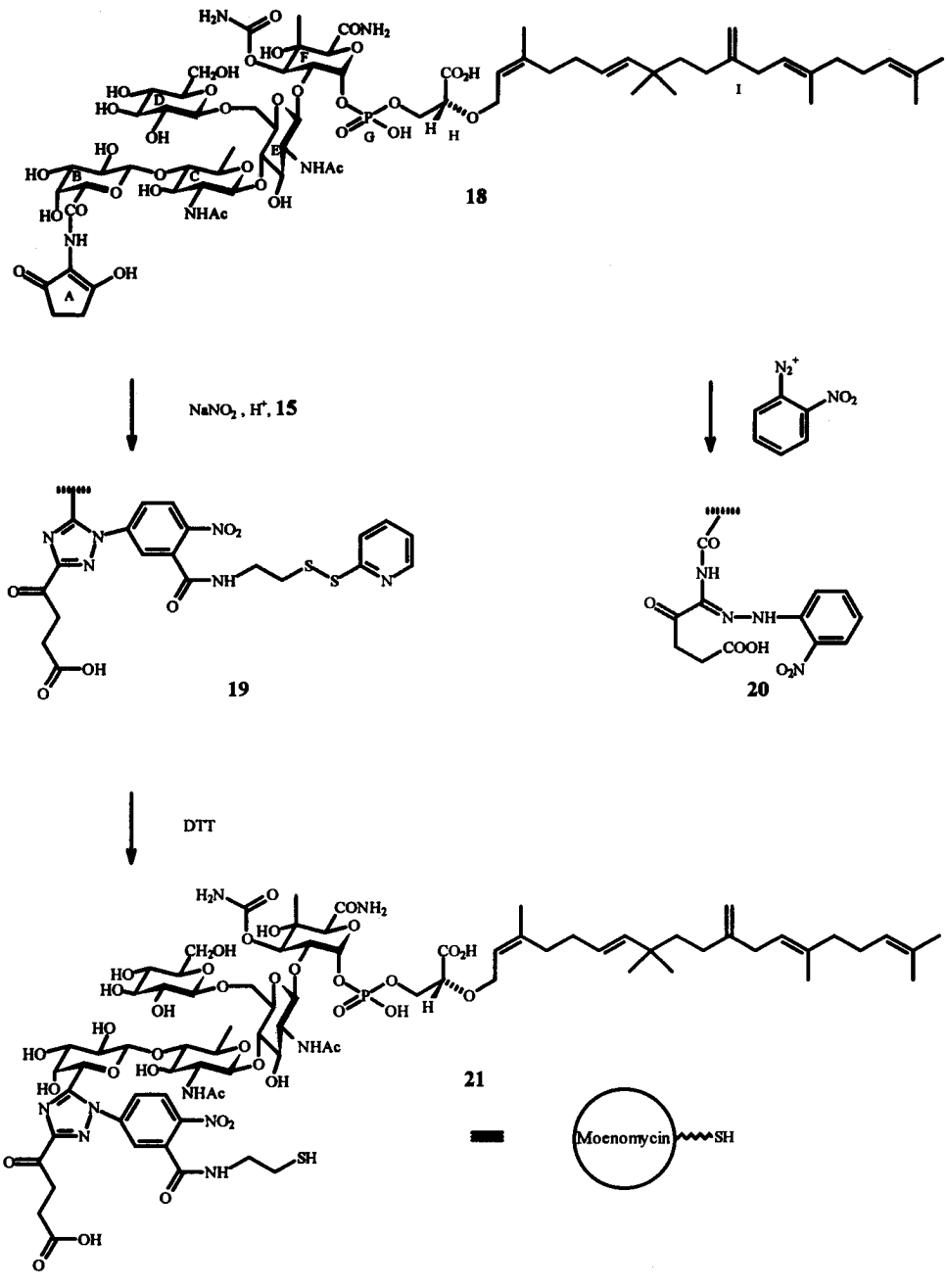
We started from 5-amino-2-nitrobenzoic acid (**12**). The amino and nitro functions were needed for the reactions described above, whereas the carboxylic acid group was chosen to carry the thiol function via a deliberately exchangeable spacer. For a first generation of tools we coupled cysteamine to **12** using Staab's procedure.[22] The thiol function was then protected with 2,2′-dipyridyl disulfide to give **15**. In a model experiment **15** was converted into the corresponding diazonium salt and this was then coupled with **7** to give **16** by the sequence of reactions discussed above. Finally, the disulfide **16** was reductively deprotected with dithiothreitol (DTT) to furnish thiol **17**.



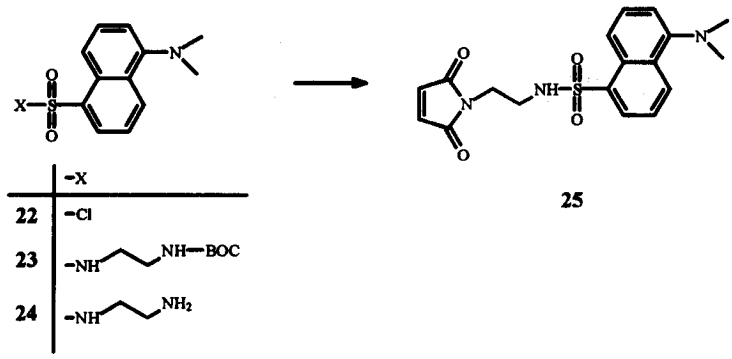
Scheme 3

Introduction of the orthogonal thiol function into Moenomycin A (18)

The diazonium salt obtained from **15** was coupled to **18**. HPLC indicated the formation of the amidrazone (identified by the UV absorption at 384 nm) which slowly (within 2 days) cyclized to furnish triazole **19**



Scheme 4



Scheme 5

(no UV absorption at > 300 nm) in 90% yield. **19** was characterized by ^{1}H and ^{13}C NMR spectroscopy and furnished the correct molecular ion (ESI MS). On reduction with DTT the desired moenomycin derived thiol **21** was obtained in 61% yield. When **18** was converted into **21** without isolation of **19**, the overall yield was 75%. Purification was performed by ultrafiltration (cutoff at 3.000 daltons).

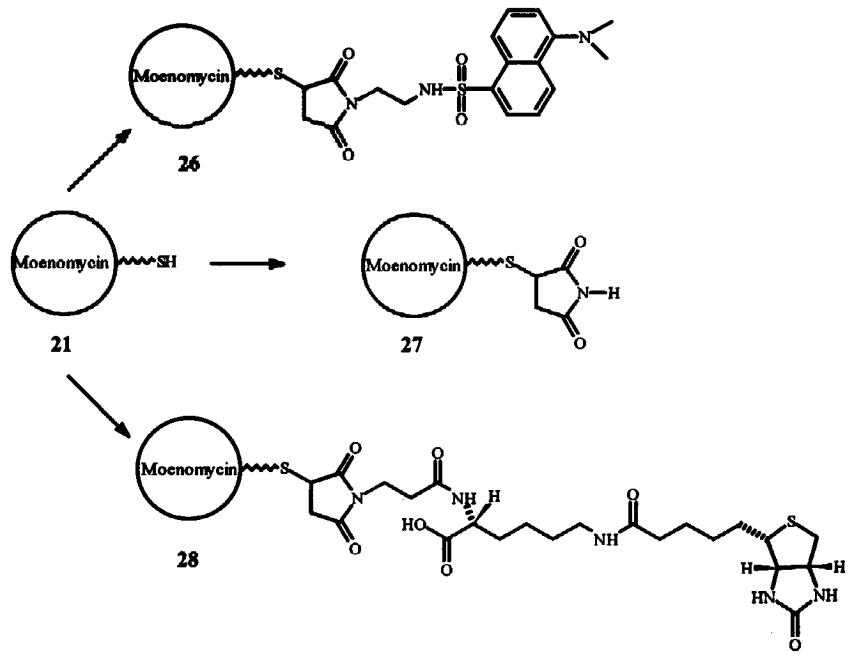
Coupling of 21 to suitably derivatized maleimides.

Thiols such as **21** are known to react with soft electrophilic reagents such as α-haloesters and maleimides, respectively. In our case the second type of reagents proved to be advantageous. Thus, in a model experiment, **21** on reaction with maleimide provided coupling product **27** in 25%. The low yield resulted from purification problems. In a second series of experiments **25** was prepared from dansyl chloride (**22**) using a known procedure.[23] **25** was then treated with **21** to furnish the moenomycin-dansyl construct **26**. Purification was performed by (i) ultrafiltration and (ii) gel filtration (Sephadex G-25M®). The yield was 41%. When this compound was excited with 340 nm light, the emission wavelength was at 517 nm in a water/Triton X-100 solution.

Finally the commercially available N-(3-maleinimidopropionyl)-biocytin was coupled to **21** to provide the biotin-labelled moenomycin derivative **28**. This compound was purified by preparative reverse phase HPLC and characterized by ^{13}C NMR and FAB MS.

Antibiotic and transglycosylase inhibiting properties of 20, 21, 26, 27, and 28

The biological activities of the new moenomycin derivatives were studied in the Izaki, Matsuhashi, and Strominger[24] test (slightly modified version[25]) which measures the inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan, and by the inhibitory effect directly on the transglycosylation reaction (determined by the



Scheme 6

in vitro assay developed earlier in one of our laboratories,[26] using a crude extract from an over-producer of polymerase PBP 1b (*E. coli JA200 plc19-19)* and as substrate lipid II which is the immediate precursor of uncross-linked peptidoglycan). Furthermore, the minimum inhibitory concentrations (MIC) against various microorganisms (serial two-fold agar dilution method, Müller Hinton Agar) have been determined.

The results (see Tables 1, 2, and 3) demonstrate that all compounds tested are active in the *in-vitro* and the *in-vivo* (gram-positive bacteria) test systems. Most importantly, coupling products **26**, **27** and **28** are active, although less than moenomycin. However, it should be noted that both **26** and **28** have considerably higher molecular masses than moenomycin A (**18**). Thus, on the basis of molar concentrations the activity differences are smaller. We assume that moenomycin at the active site is bound to the membrane via its lipid moiety and that the sugar part is in a polar surrounding. It may be that the newly introduced groups interact with the membrane leading to a change of the overall conformation and thus the in-vivo activity of moenomycin. This point is under study.

Table 1: **Effect of 20, 21, 26, 27, and 28 on the *in-vitro* formation of uncross-linked peptidoglycan by transglycosylation.**

	Final concentration (mg/L)	% inhibition
20	10.0	97
	1.0	50
26	10.0	74
	1.0	22
27	1.0	95
	0.1	78
28	1.0	89
	0.1	65

Table 2: Effect of compounds **20**, **26**, **27**, **28** and moenomycin A (**18**, for comparison) on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetyl-glucosamine into cross-linked high-molecular weight peptidoglycan.

	final concentration (mg/L)	% inhibition
18	10	96
	1	95
	0.1	55
20	10	94
	1	87
	0.1	9
21	10	87
	1	64
	0.1	17
26	10	69
	1	67
	0.1	6
27	10	95
	1	83
	0.1	14
28	10	95
	1	82
	0.1	9

Table 3: **Minimum inhibitory concentrations (in mg/L) of compounds 20, 21, 26, 27, 28, and of moenomycin A (18, for comparison) against various test organisms.**

	MIC (mg/L)			
	S. aureus SG 511	*S. aureus* 503	*S. pyogenes* 77A	*E. faecium* Md8B
18	0.049	0.049	< 0.02	> 100
20	0.015	0.008	< 0.001	> 64
21	4	2	2	> 64
26	0.781	0.781	< 0.002	> 100
27	0.391	0.391	0.049	> 50
28	1.563	1.563	0.049	> 50

In conclusion: It has been demonstrated that a thiol function can be selectively introduced into moenomycin and that this thiol can be used to couple moenomycin to differently substituted maleimides. This chemistry has been performed without protecting group chemistry (as far as moenomycin is involved). As a first application, moenomycin has been labeled with a dansyl and a biotin group, respectively. Further applications will be reported in due course.

Experimental

Methods and materials. For flash chromatography (FC), see ref.[27] The matrix for the FAB mass spectra was 3-nitrobenzylalcohol. For HPLC the following instrumentation was used: Analytical HPLC: Jasco PU-980 pump with Uniflows Degasys DG-1310 system, Sepsil column (C18, 5 µm, 250 mm x 2.1 mm), Sepsil precolumn (C18, 5 µm, 20 mm x 2.1 mm), flow rate 0.5 ml/min, sample volume 20 µl, eluent: a 63:37 mixture of buffer (0.6 g KH_2PO_4, 26.2 g $K_2HPO_4 \cdot 3\ H_2O$, water, final volume 1 l) and acetonitrile, adjusted to pH 8 with phosphoric acid[28], detection with the Jasco MD-910 diode array detector, data processing with the DP-L 910/V software. Preparative HPLC: Jasco PU-987 pump, Jasco 875-UV UV-VIS detector, Sepsil column (C18, 10 µm, 250 mm x 20 mm), 0.5 ml sample volume.- Either an Amicon gas-pressurized cell (model 8050) with an Amicon membrane (3000 daltons pore size) or reverse phase chromatography (HP-20 resin, swollen in methanol for 12 h, washed with acetone, water, 0.1 M NaOH, water, 0.1 M HCl, water, methanol, water) or gel filtration (PD-10 prepacked columns, Sephadex G-25M, 9.1 ml) were used for the removal of low molecular weight impurities and inorganic salts. The fluorescence spectra were recorded with the Fluoromax-2 (SPEX). For all other methods, see ref. [29] Special abbreviations: A′ for the modified unit A of moenomycin, TA for triazole, DAE for 1,2-diaminoethane, MI for maleimide, ßA for ß-alanine, SUC for the $COCH_2CH_2CO$ unit, BTR the ring part of biotin.

Reaction of 2-methylcyclopentan-1,3-dione (1) with thiophenol

a) To a solution of **1** (50.7 mg, 45.2 mmol) and camphorsulfonic acid (5 mg, 2.0 mmol) in methanol (1 ml) thiophenol (54 mg, 49.0 mmol) was added and the reaction mixture was stirred at 50°C for 21 h and then the solvent was evaporated. The resulting residue was purified by FC (ethyl acetate-ethanol-triethylamine 400:80:1) to give **2** (79.5 mg, 76 %).

b) To a solution of **1** (45.1 mg, 40.2 mmol) and camphorsulfonic acid (0.7 mg, 0.3 mmol) in methanol (1 ml) thiophenol (107.3 mg, 97.3 mmol) was added and the reaction mixture was stirred at 20°C for 96 h. Solvent evaporation and FC (ethyl acetate-ethanol 10:1) furnished **2** (64.3 mg, 78 %) and 3-methoxy-2-methyl-cyclopent-2-enone (5.0 mg, 10 %).

2-Methyl-3-phenylsulfanyl-cyclopent-2-enone (2)[30]

R_f = 0.82 (ethyl acetate-ethanol 10:10).- UV (methanol): λ_{max} (ε) = 283.5 (23600), 205 nm (12900).- UV (methanol, + HCl): λ_{max} (ε) = 284.5 (23600), 205 nm (13300).- UV (methanol, + NaOH): λ_{max} (ε) = 283.5 (23400), 205 nm (26500).- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.75 (s, 3 H, CH_3-6), 2.28 (s, 4 H, CH_2-4, CH_2-5), 7.35 - 7.54 (m, 5 H, 2^{Ar}-H, 3^{Ar}-H, 4^{Ar}-H, 5^{Ar}-H, 6^{Ar}-H).- ^{1}H NMR (300 MHz, C_6D_6): δ = 1.75 (m, 2 H), 1.90 (m, 2 H, CH_2-4, CH_2-5), 1.81 (s, 3 H, CH_3-6), 7.00 - 7.23 (m, 5 H, 2^{Ar}-H, 3^{Ar}-H, 4^{Ar}-H, 5^{Ar}-H, 6^{Ar}-H).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 9.1 (C-6), 30.0 (C-4), 34.8 (C-5), 128.8 (C-2), 129.8 (C-2^{Ar}, C-6^{Ar}), 130.3 (C-4^{Ar}), 134.3 (C-1^{Ar}), 135.9 (C-3^{Ar}, C-5^{Ar}), 170.9 (C-3), 205.3 (C-1).

3-Methoxy-2-methyl-cyclopent-2-enone (formula not shown)[31]

R_f = 0.53 (ethyl acetate-ethanol 10:10).- UV (methanol): λ_{max} (ε) = 250 (17600).- UV (methanol, + HCl): λ_{max} (ε) = 250 nm (17000).- UV (methanol, + NaOH): λ_{max} (ε) = 250 (17700), 207 nm (12150).- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.59 (s, 3 H, CH_3-6), 2.40 (m, 2 H), 2.61 (m, 2 H, CH_2-4, CH_2-5), 3.92 (s, 3 H, CH_3-7).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 6.4 (C-6), 25.1 (C-4), 33.9 (C-5), 56.9 (C-7), 116.6 (C-2), 184.9 (C-3), 205.3 (C-1).

3,3-Dimethyl-5-(5-methyl-1-phenyl-1H-[1,2,4]triazol-3-yl)-5-oxopentanoic acid (4)

To a solution of freshly distilled aniline (46.6 mg, 0.50 mmol) in 9 per cent HCl (0.5 ml) a solution of sodium nitrite (34.5 mg, 0.50 mmol) in water (1 ml) was added dropwise at 0°C. After 15 min at 0°C this solution was added dropwise to a solution of **3** (100 mg, 0.50 mmol) and sodium acetate (400 mg) in water (10 ml). The reaction mixture was stirred for 15 min at 0°C and was then allowed to warm to 20°C (1 h). Solvents were removed by freeze-drying and the residue was stirred in acetone (30 ml). Filtration, solvent evaporation and FC ($CHCl_3$-ethyl acetate 10:10; then $CHCl_3$-ethyl acetate-acetic acid 10:10:0.1) furnished **4** (110.1 mg, 72%).- R_f = 0.75 ($CHCl_3$-methanol-acetic acid 10:10:0.1).- IR (KBr): $\tilde{\nu}$ = 1707, 1502, 1462, 1405, 1382, 1338, 1248, 1218, 1151, 1082 cm^{-1}.- UV (methanol): λ_{max} (ε) = 242 nm (11170).- ^{1}H NMR (200 MHz, C,H COSY, $CDCl_3$): δ = 1.18 (s, 6 H, CH_3-6, CH_3-7), 2.55 (s, 5 H, 5^{TA}-CH_3, CH_2-2), 3.22 (s, 2 H, CH_2-4), 7.43 - 7.54 (m, 5 H, 2-H^{Ar}, 3-H^{Ar}, 4-H^{Ar}, 5-H^{Ar}, 6-H^{Ar}).- ^{13}C NMR (50 MHz, gated decoupling, APT, C,H COSY, $CDCl_3$): δ = 13.6 (q, J_{CH} = 130.1 Hz, 5^{TA}-CH_3), 28.6 (q, J_{CH} = 126.6 Hz, C-7, C-6), 33.7 (bs, C-3), 45.8 (t, J_{CH} = 128.6 Hz, C-2), 48.8 (t, J_{CH} = 127.8 Hz, C-4), 125.3 (dt, J_{CH} = 136.8 Hz, J = 5.9 Hz, C-2^{Ar}, C-6^{Ar}), 130.0 (t, partly hidden, C-4^{Ar}), 130.1 (dd, J_{CH} = 164.8 Hz, J = 7.3 Hz, C-3^{Ar}, C-5^{Ar}), 137.2 (t, J = 7.5 Hz, C-1^{Ar}), 154.6 (q, J = 7.2 Hz, C-5^{TA}), 160.0 (s, C-3^{TA})[32], 177.3 (t, J = 6.6 Hz, C-5), 193.6 (t, J = 6.0 Hz, C-1).- $C_{16}H_{19}N_3O_3$ (301.4, 301.1), FAB MS: m/z = 302.0 $[M+H]^+$, 324.0 $[M+Na]^+$.

4-Oxo-5-(phenyl-hydrazono)-hexanoic acid (formula not shown)

1 (112.0 mg, 1.0 mmol) was treated with benzenediazonium chloride as described above. After freeze-drying the residue was partitioned between water (50 ml) and $CHCl_3$ (3x 50 ml). The organic layer was dried (Na_2SO_4), concentrated, and the resulting residue was purified by FC (ethyl acetate-petroleum ether 1:1) to give 4-oxo-5-(phenyl-hydrazono)-hexanoic acid (98.1 mg, 42%).- R_f = 0.23 (ethyl acetate-$CHCl_3$ 1:1).- IR (KBr): $\tilde{\nu}$ = 1713, 1660, 1640, 1602, 1562, 1497, 1372, 1233, 1203 cm^{-1}.- UV (methanol): λ_{max} (ε) = 335 (19000), 294 (6270), 233 nm (9400).- ^1H NMR (200 MHz, CD_3OD): δ = 1.99 (s, 3 H, CH_3-6), 2.62 (t, J = 6.8 Hz, 2 H, CH_2-2), 3.22 (t, J = 6.8 Hz, 2 H, CH_2-3), 6.94 (m, 1 H, 4^{Ar}-H), 7.26 - 7.36 (m, 4 H, 2^{Ar}-H, 3^{Ar}-H, 5^{Ar}-H, 6^{Ar}-H).- ^{13}C NMR (50 MHz, CD_3OD): δ = 8.8 (C-6), 29.9 (C-2), 32.5 (C-3), 115.5 (C-2^{Ar}, C-6^{Ar}), 123.1 (C-4^{Ar}), 130.5 (C-3^{Ar}, C-5^{Ar}), 141.1 (C-1^{Ar}), 145.9 (C-5), 177.4 (C-1), 199.6 (C-4).- $C_{12}H_{14}N_2O_3$ (234.3, 234.1), EI MS: m/z = 234 $[M]^{+\bullet}$.

2-(Phenyl-hydrazono)-cyclopentane-1,3-dione (6)

5 (97 mg, 0.98 mmol) was treated with benzenediazonium chloride as described above. Solvent was 6:1 ethanol-water (3.5 ml), after reaching 20°C the reaction mixture was stirred for 8 h. **6** precipitated from the solution and was obtained by filtration (181.3 mg, 88 %).- R_f = 0.81 ($CHCl_3$-ethyl acetate 1:1).- M.p. 184.6°C, decomp. ($CHCl_3$-petroleum ether).- IR (KBr): $\tilde{\nu}$ = 1703, 1654, 1531, 1467, 1437, 1417, 1301, 1127 cm^{-1}.- UV ($CHCl_3$): λ_{max} (ε) = 408 (12000), 247 nm (6030).- ^1H NMR (200 MHz, $CDCl_3$): δ = 2.69 (s, 4 H, CH_2-4, CH_2-5), 7.25 ($J_{4,5}$ ca. 7.0 Hz, 1 H, 4^{Ar}-H), 7.39 ($J_{2,3}$ ca. 7.0 Hz, 2 H, 3^{Ar}-H, 5^{Ar}-H), 7.51 ($J_{2,3}$ ca. 7.5 Hz, 2 H, 2^{Ar}-H, 6^{Ar}-H), 14.56 (bs, 1 H, NH).- ^1H NMR (200 MHz, [D_5]pyridine: δ = 2.66 (s, 4 H, CH_2-4, CH_2-5), 7.20 (m, 1 H, 4^{Ar}-H), 7.35 (m, 2 H, 3^{Ar}-H, 5^{Ar}-H), 7.57 (m, 2 H, 2^{Ar}-H, 6^{Ar}-H).- ^{13}C NMR (50 MHz, gated decoupling, $CDCl_3$): δ = 32.3 (t, $J_{C,H}$ = 133.5 Hz), 34.0 (t, $J_{C,H}$ = 133.9 Hz, C-4, C-5), 117.9 (d, $J_{C,H}$ = 164.0 Hz, C-2^{Ar}, C-6^{Ar}), 128.3 (d, $J_{C,H}$ = 163.3 Hz, C-4^{Ar}), 130.2 (d, $J_{C,H}$ = 164.0 Hz, C-3^{Ar}, C-5^{Ar}), 131.8 (s, C-2), 140.7 (s, C-1^{Ar}), 199.1 (s), 201.4 (s, C-1, C-3).- ^{13}C NMR (50 MHz, [D_5]pyridine): δ = 33.0 (C-4, C-5), 117.2 (C-2^{Ar}, C-6^{Ar}), 127.0 (C-4^{Ar}), 129.8 (C-3^{Ar}, C-5^{Ar}), 132.1 (C-2), 141.3 (C-1^{Ar}), 199.3 (C-1, C-3).- $C_{11}H_{10}N_2O_2$ (202.2, 202.1), FAB MS: m/z = 203 $[M+H]^+$.

N-(2-Hydroxy-5-oxo-cyclopent-1-enyl)-acetamide (7)

A mixture of **6** (179.0 mg, 0.88 mmol), 10 per cent Pd/C (3 mg), acetic acid (10 ml), and acetic anhydride (0.5 ml) was stirred under hydrogen (1 bar) at 20°C (until ≈ 50 ml hydrogen were consumed). Solvent evaporation and FC (tert-butyl methyl ether-toluene-acetic acid 60:40:0.1) furnished **7** (120.0 mg, 87 %).- R_f = 0.18 ($CHCl_3$-ethyl acetate-acetic acid 10:10:0.1).- M.p. 166 °C ($CHCl_3$-petroleum ether) (Lit.[33] 164-169°C).- IR (KBr): $\tilde{\nu}$ = 3269, 1620, 1544, 1363 cm^{-1}.- UV (methanol): λ_{max} (ε) = 257 nm (13200).- UV (methanol, + HCl): λ_{max} (ε) = 243 nm (10100).- UV (methanol, + NaOH): λ_{max} (ε) = 259 nm (20100).- ^1H NMR (200 MHz, $CDCl_3$)[11]: δ = 2.16 (s, 3 H, CH_3-7), 2.47 - 2.52 (m, 4 H, CH_2-3, CH_2-4), 8.38 (bs, 1 H, NH), 13.23 (bs, 1 H, OH).- ^1H NMR (200 MHz, [D_6]DMSO): δ = 2.06 (s, 3 H, CH_3-7), 2.40 (s, 4 H, CH_2-3, CH_2-4), 9.53 (bs, 1 H, NH).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.9 (C-7), 26.0 (C-3), 32.6 (C-4), 115.6 (C-1), 171.8 (C-6), 174.4 (C-2), 198.0 (C-5).- ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 22.1 (C-7), 29.1 (C-3, C-4), 114.6 (C-1), 171.5 (C-6), 187.1 (bs, C-2, C-5).- $C_7H_9NO_3$ (155.2, 155.1), EI MS: m/z = 155 $[M]^{+\bullet}$, 113 $[M - CH_2CO]^{+\bullet}$.

5-Acetylamino-5-[2-nitrophenylhydrazono]-4-oxo-pentanoic acid (10)

A solution of the diazonium salt obtained from 2-nitroaniline (47.1 mg, 0.30 mmol) as described above was added dropwise at 0°C to a solution of **7** (47.1 mg, 0.30 mmol) and sodium acetate (400 mg) in water (10 ml). After 1 h at 0°C the mixture was allowed to warm to 20°C. After acidification with dilute HCl (to pH = 3), usual work-up (CH_2Cl_2) and FC (ethyl acetate-$CHCl_3$-acetic acid 100:100:0.1) gave **10** (67.6 mg, 70%).- R_f =

0.65 ($CHCl_3$-methanol-acetic acid 10:10:0.1).- IR (KBr): $\tilde{\nu}$ = 3435, 3288, 1700, 1690, 1678, 1603, 1579, 1490, 1342, 1156 cm^{-1}.- UV (methanol): λ_{max} (ε) = 402 (8010), 321 (12080), 276 (7700), 220 nm (16550).- ^1H NMR (200 MHz, [D_6]DMSO): δ = 2.19 (s, 3 H, CH_3-7), 2.50 (m, partly hidden by the DMSO signal, 2 H, CH_2-2), 3.24 (t, partly hidden by the water signal, J = 6 Hz, 2 H, CH_2-3), 7.15 (dd, J = 8 Hz, J = 7 Hz, 1 H, 4-H^{Ar}), 7.82 (dd, J = 8 Hz, J = 7 Hz, 1 H, 5-H^{Ar}), 8.00 (d, J = 8 Hz, 1 H, 6-H^{Ar}), 8.20 (d, J = 8.0 Hz, 1 H, 3-H^{Ar}), 10.94 (bs, 1 H, =N-NH).- ^{13}C NMR (50 MHz, APT, [D_6]DMSO): δ = 22.9 (C-7), 28.8 (bs, C-2), 32.3 (C-3), 116.6 (C-6^{Ar}), 121.2 (C-4^{Ar}), 126.1 (C-3^{Ar}), 133.4, 135.2 (C-2^{Ar}, C-5), 136.9 (C-5^{Ar}), 139.6 (C-1^{Ar}), 169.8 (C-6), 174.2 (bs, C-1), 194.3 (C-4).- $C_{13}H_{14}N_4O_6$ (322.3, 322.1), FAB MS: m/z = 323.0 $[M+H]^+$, 345.0 $[M+Na]^+$.

4-[5-Methyl-1-(2-nitro-phenyl)-1H-[1,2,4]triazol-3-yl]-4-oxo-butanoic acid (9)

A solution of the diazonium salt obtained from 2-nitroaniline (44.8 mg, 0.32 mmol) as described above was added dropwise at 0°C to a solution of 7 (51.4 mg, 0.33 mmol) and KOH (400 mg) in water (3.5 ml). After 1 h at 0°C work-up as described for **10** and FC (ethyl acetate-$CHCl_3$-acetic acid 100:100:0.1) furnished **9** (80.7 mg, 78 %). 10.5 mg (20 %) of 7 were recovered.- R_f = 0.63 ($CHCl_3$-methanol-acetic acid 10:10:0.1).- UV (H_2O-methanol 1:1): no absorption > 250 nm.- ^1H NMR (200 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, 5^{TA}-CH_3), 2.55 (bs, 2 H, CH_2-2), 3.22 (bs, 2 H, CH_2-3), 7.63 - 7.87 (m, 3 H, H-4^{Ar}, H-5^{Ar}, H-6^{Ar}), 8.10 (d, J = 8.0 Hz, 1 H, H-3^{Ar}), 9.34 (bs, 1 H, COOH).- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 12.6 (5^{TA}-CH_3), 29.7 (bs, C-2), 35.4 (C-3), 130.4 (C-2^{Ar}), 126.3, 130.3, 132.2, 135.3 (C-3^{Ar}, C-4^{Ar}, C-5^{Ar}, C-6^{Ar}), 145.5 (C-1^{Ar}), 156.7 (C-5^{TA}), 159.7 (C-3^{TA})[34], 178.2 (bs, C-1), 193.6 (C-4).- ^{13}C NMR (50 MHz, APT, [D_6]DMSO): δ = 12.2 (5^{TA}-CH_3), 29.8 (C-2), 35.7 (C-3), 129.3 (C-2^{Ar}), 126.1, 129.8, 132.3, 135.3 (C-5^{Ar}, C-3^{Ar}, C-4^{Ar}, C-6^{Ar}), 145.1 (C-1^{Ar}), 155.8 (C-5^{TA}), 159.4 (C-3^{TA})[34], 175.7 (bs, C-1), 192.7 (C-4).- $C_{13}H_{12}N_4O_5$ (304.3, 304.1), FAB MS: m/z = 304.9 $[M+H]^+$, 326.9 $[M+Na]^+$, 348.9 $[M-H+2Na]^+$.

(5-Amino-2-nitro-phenyl)-imidazol-1-yl-methanone (13)

To a solution of **12** (100.0 mg, 0.54 mmol) in 1:1 pyridine-$CHCl_3$ (20 ml) N,N'-carbonyldiimidazole (130.0 mg, 0.80 mmol) was added and the reaction mixture was stirred at 20°C for 1 h. Solvent evaporation and FC ($CHCl_3$-ethyl acetate-acetone 10:5:5) furnished **13** (121.2 mg, 95%).- R_f = 0.68 ($CHCl_3$-methanol 1:1).- IR (KBr): $\tilde{\nu}$ = 2940, 1729, 1607, 1585, 1477, 1382, 1316, 1307, 1266, 1248, 1240, 1075, 1065, 1056 cm^{-1}.- UV (Aceton): λ_{max} (ε) = 374 nm (15300).- ^1H NMR (200 MHz, CD_3OD): δ = ca. 4.9 (bs, 2 H, NH_2), 6.74 (d, J = 2.5 Hz, 1 H, 6-H^{Ar}), 6.88 (dd, J = 9.1 Hz, J = 2.5 Hz, 1 H, 4-H^{Ar}), 7.14 (bs, 1 H, 5-H[#]), 7.53 (bs, 1 H, 4-H[#]), 8.06 (bs, 1 H, 2-H'), 8.14 (d, J = 9.1 Hz, 1 H, 3-H^{Ar}).- ^1H NMR (200 MHz, C,H COSY, [D_5]pyridine): δ = 6.95 (dd, J = 9.1 Hz, J = 2.5 Hz, 1 H, 4-H^{Ar}), 7.10 (d, J = 2.5 Hz, 1 H, 6-H^{Ar}), 7.27 (m, 1 H, 5-H[#]), 7.70 (m, 1 H, 4-H[#]), 7.90 (bs, 2 H, NH_2), 8.16 (d, J = 9.1 Hz, 1 H, 3-H^{Ar}), 8.46 (bs, 1 H, 2-H').- ^{13}C NMR (50 MHz, APT, [D_5]pyridine): δ = 113.0 (C-6^{Ar}), 115.1 (C-4^{Ar}), 117.7 (C-4[#]), 128.8 (C-3^{Ar}), 132.0 (C-5[#]), 133.5, 134.0 (C-1^{Ar}, C-2^{Ar}), 138.3 (C-2'), 156.6 (C-5^{Ar}), 165.3 (CONH^{Ar}).- $C_{10}H_8N_4O_3$ (232.2, 232.1), EI MS: m/z = 232 $[M]^{+\bullet}$.

5-Amino-N-[2-sulfanyl-ethyl]-2-nitro-benzamide (14)

To a solution of **13** (412.0 mg, 1.8 mmol) in pyridine (10 ml) cysteamine hydrochloride (201 mg, 1.8 mmol) was added and the solution was stirred at 20°C for 8 h. Solvent evaporation and FC ($CHCl_3$-methanol 10:1) furnished **14** (202.9 mg, 47%).- R_f = 0.37 ($CHCl_3$-methanol 1:1).- M.p. 184 °C ($CHCl_3$-methanol).- IR (KBr):

[#] Assignments may have to be exchanged.

$\tilde{\nu}$ = 1650, 1580, 1550, 1500, 1310, 1250 cm^{-1}.- UV (methanol): λ_{max} (ε) = 373 nm (5800).- ^1H NMR (200 MHz, homonuclear decoupling, [D_5]pyridine): δ = 2.31 (t, J = 8.4 Hz, 1 H, SH), 3.00 (dt, J = 8.4 Hz, J = 6.5 Hz, 2 H, CH_2-2^{Cys}), 3.85 (dt, J = 5.6 Hz, J = 6.4 Hz, 2 H, CH_2-1^{Cys}), 6.78 (dd, J = 9.0 Hz, J = 2.5 Hz, 1 H, 4-H^{Ar}), 7.12 (d, J = 2.5 Hz, 1 H, 6-H^{Ar}), 7.44 (bs, 2 H, NH_2), 8.04 (d, J = 9.0 Hz, 1 H, 3-H^{Ar}), 9.67 (bt, J = 5.6 Hz, 1 H, CONH).- ^1H NMR (200 MHz, homonuclear decoupling, [D_6]DMSO): δ = 2.43 (t, 1 H, SH), 2.63 (dt, 2 H, CH_2-2^{Cys}), ca. 3.3 (CH_2-1^{Cys}, partly hidden by the water signal), 6.49 (d, J = 2.5 Hz, 1 H, 6-H^{Ar}), 6.62 (dd, J = 9.0 Hz, J = 2.5 Hz, 1 H, 4-H^{Ar}), 6.77 (bs, 2 H, NH_2), 7.90 (d, J = 9.0 Hz, 1 H, 3-H^{Ar}), 8.51 (bt, J = 5.6 Hz, 1 H, CONH).- ^{13}C NMR (50 MHz, [D_5]pyridine): δ = 24.7 (C-2^{Cys}), 44.0 (C-1^{Cys}), 113.4, 113.5 (C-6^{Ar}, C-4^{Ar}), 128.4 (C-3^{Ar}), 135.2 (C-1^{Ar}), 138.5 (C-2^{Ar}), 155.9 (C-5^{Ar}), 169.0 (CONH^{Ar}).- ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 23.4 (C-2^{Cys}), 42.8 (C-1^{Cys}), 112.2, 112.7 (C-6^{Ar}, C-4^{Ar}), 127.7 (C-3^{Ar}), 133.2 (C-1^{Ar}), 137.2 (C-2^{Ar}), 155.0 (C-5^{Ar}), 167.4 (CONH^{Ar}).- $C_9H_{11}N_3O_3S$ (241.3, 241.1), EI MS : m/z = 241 $[M]^{+\bullet}$, 195 $[M-NO_2]^+$.- FAB MS: 242.2 $[M+H^+]$.

One-pot formation of 14

To a solution of **12** (150.0 mg, 0.8 mmol) in acetonitrile (10 ml) N,N'-carbonyldiimidazole (133.7 mg, 0.8 mmol) was added and the mixture was stirred at 20°C for 1 h. A solution of cysteamine (93.7 mg, 0.8 mmol) in pyridine (5 ml) was added and the mixture was refluxed for 2 h. Solvent evaporation and FC (methanol-$CHCl_3$ 1:20) furnished **14** (85.5 mg, 44%).[35]

5-Amino-2-nitro-N-[2-(pyridine-2-yldisulfanyl)-ethyl]-benzamide (15)

To a solution of 2,2'-dipyridyl disulfide (200 mg, 0.90 mmol) in methanol (10 ml) a solution of **14** (84 mg, 0.34 mmol) in methanol (5 ml) was added dropwise and the mixture was stirred at 20°C for 2 h.[36] Solvent evaporation and FC ($CHCl_3$-methanol 20:1) furnished **15** (114.2 mg, 92 %).- R_f = 0.38 ($CHCl_3$-methanol 10:1).- R_t = 4.11 min (RP-HPLC, methanol-H_2O 1:9).- IR (KBr): $\tilde{\nu}$ = 2922, 1639, 1603, 1580, 1317, 1260 cm^{-1}.- UV (methanol): λ_{max} (ε) = 370 (9850), 282 nm (4200).- ^1H NMR (200 MHz, [D_5]pyridine): δ = 3.32 (t, J = 6.5 Hz, 2 H, CH_2-2^{Cys}), 4.02 (dt, J = 6.5 Hz, J = 5.5 Hz, 2 H, CH_2-1^{Cys}), 6.77 (dd, J = 2.5 Hz, J = 9.0 Hz, 1 H, H-4^{Ar}), 7.01 (ddd, J = 1.0 Hz, J = 4.8 Hz, J = 7.3 Hz, 1 H, H-4^{Py}), 7.12 (d, J = 2.5 Hz, 1 H, H-6^{Ar}), 7.42 (bs, 2 H, NH_2), 7.57 (ddd, J = 1.8 Hz , J = 7.3 Hz, J = 7.8 Hz, 1 H, H-3^{Py}), 7.75 (d, J = 7.8 Hz, 1 H, H-2^{Py}), 8.03 (d, J = 9.0 Hz, 1 H, H-3^{Ar}), 8.44 (d, J = 4.8 Hz, 1 H, H-5^{Py}), 9.71 (t, J = 5.5 Hz, 1 H, CONH^{Ar}).- ^{13}C NMR (50 MHz, APT, [D_5]pyridine): δ = 38.7 (C-2^{Cys}), 39.5 (C-1^{Cys}), 113.3 (C-4^{Ar}), 113.5 (C-6^{Ar}), 120.3 (C-2^{Py}), 121.5 (C-4^{Py}), 128.3 (C-3^{Ar}), 135.0 (C-1^{Ar}), 137.8 (C-3^{Py}), 138.3 (C-2^{Ar}), 150.2 (C-5^{Py}), 155.8 (C-5^{Ar}), 160.5 (C-1^{Py}), 169.1 (CONH^{Ar}).- ^{13}C NMR (50 MHz, C,H COSY, CD_3OD): δ = 39.1 (C-2^{Cys}), 40.3 (C-1^{Cys}), 113.7 (C-4^{Ar}), 114.7 (C-6^{Ar}), 121.8 (C-2^{Py}), 122.9 (C-4^{Py}), 129.2 (C-3^{Ar}), 135.2 (C-1^{Ar}), 137.7 (C-2^{Ar}), 139.6 (C-3^{Py}), 150.7 (C-5^{Py}), 156.5 (C-5^{Ar}), 161.3 (C-1^{Py}), 171.4 (CONH^{Ar}).- $C_{14}H_{14}N_4O_3S_2$ (350.4, 350.1), FAB MS: m/z = 351.2 $[M+H]^+$.

4-(5-Methyl-1-{4-nitro-3-[2-(pyridine-2-yldisulfanyl)-ethylcarbamoyl]-phenyl}-1H-[1,2,4] triazol-3-yl)-4-oxobutanoic acid (16)

A solution of the diazonium salt obtained from **15** (150 mg, 0.43 mmol) as described above was added to a solution of **7** (66.4 mg, 0.43 mmol) and sodium acetate (1000 mg) in water (25 ml). The reaction mixture was stirred at 0°C for 30 min and at 20°C for 5 h. Usual work-up ($CHCl_3$) and FC ($CHCl_3$-methanol-acetic acid 6: 1: 0.1) furnished a mixture (212 mg) of **16** and the corresponding amidrazone.[37] After a second FC (ethyl acetate-$CHCl_3$-acetic acid 10:10:0.1) some pure **16** (50 mg) was obtained besides a fraction containing **16** and the amidrazone (103.5 mg). Since it was impossible to obtain the pure amidrazone because of its instability the 103.5 mg fraction was stirred at 20°C for 24 h in 1:20 acetic acid-methanol. Under these conditions the

amidrazone was completely converted into **16**. The overall yield was 132 mg (60 %).- R_f = 0.42 ($CHCl_3$-methanol, 10:1).- IR (KBr): $\tilde{\nu}$ = 2950, 1706, 1655, 1587, 1574, 1556, 1534, 1417, 1345 cm^{-1}.- UV (methanol): λ_{max} (ε) = 276 nm (11700).- ^1H NMR (200 MHz, homonuclear decoupling, C,H COSY, [D_6]DMSO): δ = 2.61 (t, 2 H, J = 6.5 Hz, CH_2-$2^{A'}$), 2.64 (s, 3 H, 5^{TA}-CH_3), 3.05 (t, 2 H, J = 6.5 Hz, CH_2-2^{Cys}), 3.29 (t, 2 H, J = 6.5 Hz, CH_2-$3^{A'}$), 3.57 (dt, J = 5.5 Hz, J = 6.5 Hz, 2 H, C-1^{Cys}), 7.26 (m, 1 H, H-4^{Py}), 7.82 (d, J = 1.3 Hz, 1 H, H-2^{Py}), 7.84 (m, 1 H, H-3^{Py}), 7.94 (d, 1 H, ^4J = 2.3 Hz, H-6^{Ar}), 8.04 (dd, 1 H, J = 8.7 Hz, J = 2.3 Hz, H-4^{Ar}), 8.31 (d, 1 H, J = 8.7 Hz, H-3^{Ar}), 8.46 (m, 1 H, H-5^{Py}), 9.14 (t, 1 H, J = 5.5 Hz, $CONH^{Ar}$).- ^{13}C NMR (50 MHz, APT, [D_6]DMSO): δ = 13.5 (5^{TA}-CH_3), 27.9 (C-$2^{A'}$), 34.7 (C-$3^{A'}$), 37.2 (C-2^{Cys}), 38.8 (C-1^{Cys}), 119.6 (C-2^{Py}), 121.5 (C-4^{Py}), 125.1 (C-6^{Ar}), 126.2 (C-3^{Ar}), 126.7 (C-4^{Ar}), 133.9 (C-1^{Ar}), 138.1 (C-3^{Py}), 140.3 (C-2^{Ar}), 146.6 (C-5^{Ar}), 149.9 (C-5^{Py}), 155.4 (C-5^{TA})^{34}, 158.9 (C-1^{Py}), 159.3 (C-3^{TA}), 164.6 ($CONH^{Ar}$), 174.0 (C-$1^{A'}$), 192.0 (C-$4^{A'}$).- $C_{21}H_{20}N_6O_6S_2$ (516.6, 516.1), FAB MS: m/z = 517.0 $[M+H]^+$, 538.9 $[M+Na]^+$.

4-{1-[3-(2-Mercapto-ethyl-carbamoyl)-4-nitro-phenyl]-5-methyl-1H-[1,2,4]triazol-3-yl}-4-oxobutanoic acid (17)

To a solution of dithiothreitol (420.0 mg, 2.72 mmol) in methanol (20 ml) a solution of **16** (82.0 mg, 0.15 mmol) in methanol (20 ml) was added dropwise and the mixture was stirred at 20°C for 3 h. Solvent evaporation and FC ($CHCl_3$-ethyl acetate-acetic acid 500: 500: 1) furnished **17** (55.4 mg, 86 %).- R_f = 0.29 ($CHCl_3$-methanol 10:1).- ^1H NMR (200 MHz, CD_3OD): δ = 2.68 - 2.80 (m, 7 H, CH_2-$2^{A'}$, 5^{TA}-CH_3, CH_2-2^{Cys}), 3.38 (t, J = 6.5 Hz, 2 H, CH_2-$3^{A'}$), 3.56 (t, J = 6.9 Hz, 2 H, CH_2-1^{Cys}), 7.94 (s, 1 H, H-6^{Ar}), 8.00 (d, 1 H, J = 8.6 Hz, H-4^{Ar}), 8.33 (d, 1 H, J = 8.6 Hz, H-3^{Ar}).- ^1H NMR (200 MHz, [D_6]DMSO): δ = 2.50 - 2.80 (m, 7 H, CH_2-$2^{A'}$, 5^{TA}-CH_3, CH_2-2^{Cys}), 3.25 (t, J = 6.5 Hz, 2 H, CH_2-$3^{A'}$), 3.39 (m, 2 H, CH_2-1^{Cys}), 7.92 (d, J = 2.2 Hz, 1 H, H-6^{Ar}), 8.00 (dd, J = 8.8 Hz, J = 2.2 Hz, 1 H, H-4^{Ar}), 8.27 (d, J = 8.8 Hz, 1 H, H-3^{Ar}), 9.01 (t, J = 5.4 Hz, 1 H, $CONH^{Ar}$).- ^{13}C NMR (50 MHz, CD_3OD): δ = 13.8 (5^{TA}-CH_3), 24.4 (C-2^{Cys}), 28.8 (C-$2^{A'}$), 35.6 (C-$3^{A'}$), 44.8 (C-1^{Cys}), 126.4 (C-6^{Ar}), 127.7 (C-3^{Ar}), 127.8 (C-4^{Ar}), 135.7 (C-1^{Ar}), 142.3 (C-2^{Ar}), 147.7 (C-5^{Ar}), 157.2 (C-5^{TA})^{34}, 160.3 (C-3^{TA}), 168.1 ($CONH^{Ar}$), 176.5 (C-$1^{A'}$), 193.4 (C-$4^{A'}$).- ^{13}C NMR (50 MHz, [D_6]DO): δ = 14.0 (5^{TA}-CH_3), 23.9 (C-2^{Cys}), 28.3 (C-$2^{A'}$), 35.2 (C-$3^{A'}$), 43.6 (C-1^{Cys}), 125.9 (C-6^{Ar}), 126.9 (C-3^{Ar}), 127.4 (C-4^{Ar}), 134.8 (C-1^{Ar}), 141.0 (C-2^{Ar}), 147.3 (C-5^{Ar}), 156.1 (C-5^{TA})^{34}, 159.6 (C-3^{TA}), 165.3 ($CONH^{Ar}$), 174.6 (C-$1^{A'}$), 192.8 (C-$4^{A'}$).- $C_{16}H_{17}N_5O_6S$ (407.4, 407.1), FAB MS: m/z = 408.1 $[M+H]^+$.

Conversion of Moenomycin A (18) into 19

A solution of the diazonium salt obtained from **15** (48.9 mg, 0.14 mmol) was added at 20°C to a solution of Moenomycin A (**18**) (200.1 mg, 0.13 mmol) and sodium acetate (400 mg) in water (50 ml) and the mixture was stirred at 20°C. Progress of the reaction was monitored by RP-HPLC-DAD (buffer-acetonitrile 63:37). After 2 d the intermediate amidrazone (formula not shown, R_t = 21.3 min (RP-HPLC, buffer-acetonitrile, 63:37); UV (buffer-acetonitrile 63:37): λ_{max} at 384 and 287 nm, ratio ≈ 4:1) was completely converted into triazole **19**. Ultrafiltration followed by FC ($CHCl_3$-methanol-H_2O 20:11:0.3) furnished **19** (234.2 mg, 90%).

2-*O*-{2-Acetamido-4-*O*-[2-acetamido-4-*O*-((5*R*)-5-{5-(3-carboxy-propionyl)-2-[4-nitro-3-(2-pyridine-2-yldithio-ethylcarbamoyl)-phenyl]-2*H*-[1,2,4]triazol-3-yl}-α-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl]-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyloxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyranuronamide (19)

R_t = 21.4 min (RP-HPLC, buffer-acetonitrile 63:37).- UV (buffer-acetonitrile 63:37): λ_{max} = 279 nm.- ^1H NMR (300 MHz, [D_6]DMSO): (low quality spectrum, characteristic signals) δ = 0.92 (s, CH_3-23^1, CH_3-24^1),

1.54 (s), 1.55 (s), 1.62 (s), 1.67 (s, CH_3-20^I, CH_3-21^I, CH_3-19^I, CH_3-25^I), 2.64 (d, CH_2-12^I), 3.03 (bs, CH_2-2^{Cym}), 7.24 (m, H-4^{Py}), 7.68 (m, H-3^{Py}), 7.81 (m, H-2^{Py}), 7.95 (bs, 1 H, H-2^{Ar}), 8.14 (bd, 1 H, H-6^{Ar}), 8.24 (bd, 1 H, H-5^{Ar}), 8.44 (bd, 1 H, H-5^{Py}), 9.01 (bs, 1 H, CONH^{Ar}).- ^{13}C NMR (50 MHz, CD_3OD): δ = 15.1 (C-21^I), 15.5 (bs, 4^F-CH_3), 16.8 (bs, C-4^C, C-20^I), 22.4 (bs, NHCOC$H_3^{E, C}$), 22.9 (bs, C-25^I), 25.0 (C-19^I), 26.7 (C-16^I), 26.9 (C-23^I, C-24^I), 27.5 (bs, C-3$^{A'}$), 29.7 (bs, C-2$^{A'}$), 31.3 (C-10^I), 31.7 (C-5^I), 32.5 (C-4^I), 35.0 (C-12^I), 35.5 (C-8^I), 37.4 (bs, C-2^{Cym}), 39.4 (bs, C-1^{Cym}), 39.9 (C-15^I), 41.8 (C-9^I), 55.7 (non-resolved signals, C-2^E, C-2^C), 61.8 (bs, C-6^E), 66.6 (bs, C-3^H, C-1^I), 68.0 - 77.9 (not identified broad signals), 81.7 (bs, C-2^H), 84.5 (bs, C-4^C), 95.0 (bs, C-1^F), 102.0 (bs), 103.1 (bs), 103.8 (bs), 104.4 (bs) (C-1$^{C, E, B, D}$), 108.4 (C-22^I), 120.5 (C-2^{Py}), 121.7 (C-4^{Py}), 122.0 (bs, C-2^I), 122.6 (C-13^I), 124.5 (C-17^I), 125.7 (bs, C-2^{Ar}), 126.0 (C-6^I), 126.4 (bs, C-6^{Ar}), 128.1 (bs, C-5^{Ar}), 131.3 (C-18^I), 134.2 (C-3^{Ar}), 136.5 (C-14^I), 138.5 (C-3^{Py}), 140.7 (C-7^I), 140.9 (C-3^I), 141.6 (C-4^{Ar}), 146.8 (C-1^{Ar}), 149.7, 150.2 (C-5^{Py}, C-11^I), 154.8 (C-3TA)[34], 158.4 (OCONH$_2^F$), 159.2 (C-5TA), 160.2 (C-1^{Py}), 167.0 (CONH^{Ar}), 172.6 - 175.4 (complex of signals, NHCOCH$_3$ of units C and E, C-6^F, C-1^H, COOH$^{A'}$), 192.5 (bs, C-1$^{A'}$).- ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 16.5 (C-21^I), 17.1 (bs, 4^F-CH_3), 18.1 (bs, C-6^C), 18.4 (C-20^I), 23.8, 23.9 (bs, NHCOC$H_3^{E, C}$), 24.1 (bs, C-25^I), 26.3 (C-19^I), 26.9 (C-16^I), 28.0 (C-23^I, C-24^I), 29.9 (bs, C-3$^{A'}$), 31.6 (C-10^I), 31.8 (C-5^I), 32.8 (C-4^I), 35.3 (C-12^I), 36.1 (C-8^I), 37.6 (C-2^{Cym}), 37.8 (?), 55.8 (bs, C-2$^{E, C}$), 62.3 (bs, C-6^E), 65.7 (bs, C-3^H, C-1^I), 68.0 - 86.6 (not identified broad signals), 94.9 (bs, C-1^F), 102.1 - 105.0 broad signals (C-1$^{C, E, B, D}$), 109.7 (C-22^I), 120.3 (C-2^{Py}), 122.3 (C-4^{Py}), 122.7 (C-13^I), 124.1 (bs, C-2^I), 125.0 (C-17^I), 126.4 (C-6^I), 131.7 (C-18^I), 134.0 (C-3^{Ar}), 136.8 (C-14^I), 138.4 (bs, C-3^I), 138.9 (C-3^{Py}), 140.8 (C-7^I), 141.6 (bs, C-4^{Ar}), 147.6 (C-1^{Ar}), 150.1, 150.6 (C-11^I, C-5^{Py}), 155.5 (C-3TA)[34], 157.3 (OCONH$_2^F$), 159.7 (C-5TA), 160.1 (C-1^{Py}), 165.4 (CONH^{Ar}), 167.4 - 176.5 complex of signals (NHCOCH$_3^{C, E}$, C-6^F, C-1^H, COOH$^{A'}$), 193.5 (bs, C-1$^{A'}$).- $C_{83}H_{119}N_{10}O_{37}S_2P$ (1944.0, 1942.7), ESI MS: m/z = 1965 $[M+Na]^+$, 1943 $[M+H]^+$.

2-*O*-[2-Acetamido-4-*O*-(2-acetamido-4-*O*-{(5*S*)-5-[4-carboxy-1-(2-nitro-phenylhydrazono)-2-oxo-butyl-carbamoyl]-α-L-arabinopyranosyl}-2,6-dideoxy-β-D-glucopyranosyl)-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyloxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyran-uronamide (20)

A solution of the diazonium salt obtained from 2-nitroaniline (2.8 mg, 20 μmol) as described above was added dropwise to a solution of the moenomycin complex (Flavomycin®, 33.5 mg, 21 μmol, based on the molecular mass of moenomycin A) and sodium acetate (400 mg) in water (15 ml). After 1 h at 0°C the mixture was allowed to warm to 20°C and was then freeze-dried. The residue was purified by RP chromatography (HP-20, gradient water → methanol). The separation was monitored by RP-HPLC (methanol-acetonitrile-H_2O 6:3:1). Before HPLC the fractions were evaporated and lyophilized, the residues taken up in water (50 ml) and then analyzed. 21 mg (56%) of pure **20** were obtained.[38]- R_t = 10.4 min (RP-HPLC, acetonitrile-methanol-H_2O 6:3:1).- UV (methanol): λ_{max} (ε)= 403 (8820), 324 (12730), 272 nm (9120).- ^{1}H NMR (200 MHz, CD_3OD): characteristic signals δ = 0.97 (s, CH_3-23^I, CH_3-24^I), 1.60, 1.67, 1.77 (3s, CH_3-20^I, CH_3-21^I, CH_3-19^I, CH_3-25^I), 1.80 - 2.35 (m, CH_2-10^I, CH_2-15^I, NHCOCH_3^E, NHCOCH_3^C, CH_2-16^I, CH_2-5^I, CH_2-4^I), 2.69 (d, *J* = 7.0 Hz, CH_2-12^I), 5.95 (bs, 1-H^F), 7.11 (dd, *J* = 7.7 Hz, *J* = 8.4 Hz, 4-H^{Ar}), 7.73 (dd, *J* = 7.7 Hz, *J* = 8.1 Hz, 5-H^{Ar}), 8.06 (d, *J* = 8.1 Hz, 6-H^{Ar}), 8.22 (d, *J* = 8.4 Hz, 3-H^{Ar}).- ^{13}C NMR (50 MHz, CD_3OD):[39] δ = 16.4 (4^F-CH_3), 16.7 (C-21^I), 18.0 (C-6^C), 18.3 (C-20^I), 23.7, 23.9 (NHCOC$H_3^{E, C}$), 24.2 (C-25^I), 26.2 (C-19^I), 27.1,[39] 27.9, 28.1 (C-16^I, C-23^I, C-24^I), 29.3 (C-4$^{A'}$), 32.6, 32.9, 33.2, 33.6 (C-5^I, C-4^I, C-10^I, C-3$^{A'}$), 36.2 (C-12^I), 36.7 (C-8^I), 41.1 (C-15^I), 43.1 (C-9^I), 68.0 - 80.0 not identified broad signals, 85.2 (C-4^C), 96.4 (C-1^F), 103.0 (bs) - 105.0 (bs, C-1$^{B, C, D, E}$), 109.5 (C-22^I), 118.1 (C-6^{Ar}), 122.4, 122.8 (bs) (C-4^{Ar}, C-2^I), 123.7 (C-13^I), 125.6 (C-17^I), 127.1, 127.2 (C-6^I, C-3^{Ar}), 132.5 (C-18^I), 133.6 (C-2^{Ar}), 135.5, 137.6, 141.2, 141.9

(C-5^{Ar}, C-1$^{A'}$, C-1^{Ar}, C-14^{I}, C-7^{I}, C-3^{I}), 151.4 (C-11^{I}), 159.0 (bs, O<u>C</u>ONH$_2^{F}$), 170.1, 173.0 - 175.0 (complex of signals), 176.8 (C-1^{H}, C-6^{B}, COOH$^{A'}$, NH<u>C</u>OCH$_3^{C,E}$, C-6^{B,F}), 195.2 (C-2$^{A'}$).- The mass spectrum displayed two series of sgnals corresponding to the moenomycin A and C_3 derivatives, respectively. $C_{69}H_{103}N_8O_{31}P$ (1571.6 1570.7), moenomycin C_3 derivative, $C_{75}H_{113}N_8O_{37}P$ (1749.7, 1748.7), moenomycin A derivative, FAB: m/z = 1593.6 $[C_{69}H_{103}N_8O_{31}P+Na]^+$, 1609.6 $[C_{69}H_{103}N_8O_{31}P+K]^+$, 1749.4 $[C_{75}H_{113}N_8O_{37}P+H]^+$, 1771.4 $[C_{75}H_{113}N_8O_{37}P+Na]^+$, 1787.4 $[C_{75}H_{113}N_8O_{37}P+K]^+$.

2-*O*-{2-Acetamido-4-*O*-[2-acetamido-4-*O*-((5*R*)-5-{5-(3-carboxy-propionyl)-2-[3-(2-mercapto-ethyl-carbamoyl)-4-nitro-phenyl]-2*H*-[1,2,4]triazol-3-yl}-α-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl]-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyloxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyranuronamide (21)

To a solution of dithiothreitol (DTT) (175.0 mg, 1.13 mmol) in methanol (5 ml) a solution of **19** (220.3 mg, 0.11 mmol) in methanol (10 ml) was added dropwise and the reaction mixture was stirred at 20°C for 2 h.[40] Solvent evaporation, ultrafiltration followed by FC ($CHCl_3$-methanol-H_2O 20:10:2) and RP-HPLC (methanol-H_2O 63:37) furnished **21** (127.5 mg, 61%).- R_t = 12.5 min, RP-HPLC, (buffer-acetonitrile 63:37).- UV (buffer-acetonitrile 63:37): λ_{max} = 275 nm.- ^{1}H NMR (300 MHz): Unresolved signals in D_2O and [D$_6$]DMSO solution.- ^{13}C NMR (75 MHz, D_2O): δ = 15.2 (4^F-CH$_3$), 15.9 (C-21^I), 16.9 (C-6^C), 17.7 (C-20^I), 22.8 (bs, NHCO<u>C</u>H$_3^{E,C}$), 23.6 (C-25^I), 25.7 (C-19^I), 26.7 (C-16^I), 27.3 (C-23^I, C-24^I), 30.7 (bs), 31.6 (bs), 32.1 (bs, C-10^I, C-5^I, C-4^I), 34.9 (C-12^I), 35.4 (C-8^I), 39.8 (C-15^I), 41.7 (C-9^I), 55.0 (bs), (C-2^{E,C}), 60.6 (bs, C-6^E), 64.8 - 85.0 (not identified broad signals), 94.5 (bs, C-1^F), 101.0 - 103.3 broad signals (C-1^{C,E,B,D}), 108.9 (b, C-22^I), 121.5 (bs, C-2^I), 122.2 (C-13^I), 124.5 (C-17^I), 125.8 (bs, C-6^I), 131.0 (bs, C-18^I), 133.1 (bs, C-3^{Ar}), 136.1 (bs, C-14^I), 140.6 (bs, C-7^I, C-3^I), 141.2 (bs, C-4^{Ar}), 146.2 (bs, C-1^{Ar}), 149.5 (bs, C-11^I), 153.7 (C-3TA)^{34}, 158.1 (bs, O<u>C</u>ONH$_2^F$), 158.7 (C-5TA), 172.6 - 180.2 broad signals (NH<u>C</u>OCH$_3^{C,E}$, C-6^F, C-1^H, COOH$^{A'}$), 195.0 (bs, C-1$^{A'}$).- ^{13}C NMR (50 MHz, APT, [D$_6$]DMSO): δ = 15.7 (C-21^I), 16.0 (bs, 4^F-CH$_3$), 17.2 (bs, C-6^C), 17.5 (C-20^I), 22.9, 23.0 (bs, NHCO<u>C</u>H$_3^{E,C}$), 23.3 (C-25^I), 25.5 (C-19^I), 26.1 (C-16^I), 27.1 (C-23^I, C-24^I), 28.5 (bs, C-3$^{A'}$), 30.7 (C-10^I), 30.9 (C-5^I), 31.9 (C-4^I), 34.5 (C-12^I), 35.2 (C-8^I), 36.4 (bs, C-2$^{A'}$), 40.9 (C-9^I), 54.6 - 55.3 (C-2^{E,C}), 61.3 (bs, C-6^E), 64.8 (bs), 66.8 (bs, C-3^H, C-1^I), 68.0 - 77.6 (not identified broad signals), 80.0 (C-2^H, C-3^C), 84.3 (bs, C-6^C), 93.6 (bs, C-1^F), 101.0 (bs), 101.8 (bs), 102.8 (bs), 103.3 (bs, C-1^{C,E,B,D}), 108.7 (C-22^I), 121.7 (C-13^I), 123.1 (bs, C-2^I), 124.0 (C-17^I), 125.4 (C-6^I), 125.9 (bs, C-2^{Ar}), 127.7 (bs, C-6^{Ar}, C-5^{Ar}), 130.6 (C-18^I), 133.1 (bs, C-3^{Ar}), 135.7 (C-14^I), 137.3 (bs, C-3^I), 139.8 (C-7^I), 140.6 (bs, C-4^{Ar}), 146.6 (bs, C-1^{Ar}), 149.1 (C-11^I), 154.6 (C-3TA), 156.6 (O-<u>C</u>O-NH$_2^F$), 158.5 (C-5TA), 164.4 (CONH^{Ar}), 169.9, 171.6, 173.6, 174.4 complex of signals (NH<u>C</u>OCH$_3^{C,E}$, C-6^F, C-1^H, COOH$^{A'}$), 192.0 (bs, C-1$^{A'}$).- $C_{78}H_{116}N_9O_{37}SP$ (1834.9, 1833.7), FAB MS: m/z = 1856.7 $[M+Na]^+$, 1872.6 $[M+K]^+$.

One-pot formation of 21

A solution of the diazonium salt obtained from **15** (84.0 mg, 0.24 mmol) was added at 20°C to a solution of moenomycin A (**18**) (361.1 mg, 0.23 mmol) and sodium acetate (1000 mg) in water (30 ml) and the mixture was stirred at 20°C. Progress of the reaction was monitored by RP-HPLC-DAD (buffer-acetonitrile 63:37). After 65 h the intermediate amidrazone (formula not shown, R_t = 21.3 min (RP-HPLC, buffer-acetonitrile, 63:37); UV (buffer-acetonitrile 63:37): λ_{max} at 384 and 287 nm, ratio ≈ 4:1) was completely converted into triazole **19**. Then dithiothreitol (200 mg, 1.29 mmol) was added and the solution was stirred at 20°C for 4 h. Ultrafiltration followed by FC ($CHCl_3$-methanol-H_2O 18:11:2.7) furnished **21** (314.8 mg, 75%).- R_t = 13 min, RP-HPLC (buffer-acetonitrile 63:37), λ_{max} = 275 nm.

***tert*-Butyl [2-(5-dimethylamino-naphthalene-1-sulfonylamino)-ethyl]-carbamate (23)**

23 was prepared as described in ref.[23]- R_f = 0.48 (ethyl acetate-$CHCl_3$ 5:1).- IR (KBr): $\tilde{\nu}$ = 1690, 1162, 1145 cm^{-1}.- UV (methanol): λ_{max} (ε) = 335 (6975), 250 (21500), 221 nm (35450).- ^1H NMR (200 MHz, $CDCl_3$)[41]: δ = 1.35 (s, 9 H, $(CH_3)_3C^{BOC}$), 2.85 (s, 6 H, $N(CH_3)_2$), 3.00 (m, 2 H), 3.13 (m, 2 H, CH_2-2^{DAE}, CH_2-1^{DAE}), 5.00 (bt, 1 H, NH^{DAE}), 7.17 (d, J = 7.8 Hz, 1 H, H-6^{Dans}), 7.46 - 7.58 (2 H, H-3^{Dans}, H-7^{Dans}), 8.17 - 8.31 (2 H, H-2^{Dans}, H-8^{Dans}), 8.53 (d, J = 8.4 Hz, 1 H, H-4^{Dans}).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.7 ($(\underline{C}H_3)_3C^{BOC}$), 40.8, 44.0 (C-$1^{DAE}$, C-$2^{DAE}$), 45.8 ($N(CH_3)_2$), 80.1 (C-$1^{BOC}$), 115.7 (C-$6^{Dans}$), 119.2, 123.6, 128.9, 129.9, 130.0, 130.4, 130.9 (C-4^{Dans}, C-2^{Dans}, C-7^{Dans}, C-8^{Dans}, C-9^{Dans}, C-10^{Dans}, C-3^{Dans}), 135.1 (C-1^{Dans}), 152.5 (C-5^{Dans}), 156.8 (O-CO-NH).- $C_{19}H_{27}N_3O_4S$ (393.5, 393.2), FAB: m/z = 809.5 $[2M+Na]^+$, 787.5 $[2M+H]^+$, 416.2 $[M+Na]^+$, 393.2 $[M+H-H]^+$.

5-Dimethylamino-naphthalene-1-sulfonic acid (2-amino-ethyl)-amide (24)

24 was prepared as described in ref.[23]- R_f = 0.20 ($CHCl_3$-methanol 1:1).- IR (KBr): $\tilde{\nu}$ = 2927, 2855, 1631, 1615, 1589, 1459, 1315, 1142 cm^{-1}.- UV (methanol): λ_{max} (ε) = 336 (3750), 250 (11700), 217 nm (23670).- ^1H NMR (200 MHz, $CDCl_3$): δ = 2.88 (s, 6 H, $N(CH_3)_2$), 2.70 (t, 2 H), 2.92 (t, 2 H, CH_2-1^{DAE}, CH_2-2^{DAE}), 7.17 (d, J = 7.8 Hz, 1 H, H-6^{Dans}), 7.46 - 7.58 (m, 2 H, H-3^{Dans}, H-7^{Dans}), 8.24 (m, 2 H, H-2^{Dans}, H-8^{Dans}), 8.53 (d, J = 8.4 Hz, 1 H, H-4^{Dans}).- $C_{14}H_{19}N_3O_2S$ (293.4, 293.1), FAB MS: m/z = 294.2 $[M+H]^+$.

5-Dimethylamino-naphthalene-1-sulfonic acid [2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl]-amide (25)

25 was prepared as described in ref.[23]- R_f = 0.31 (ethyl acetate-$CHCl_3$ 1:1).- IR (KBr): $\tilde{\nu}$ = 1710, 1409, 1319, 1162, 1143, 792 cm^{-1}.- UV (methanol): λ_{max} (ε) = 334 (4000), 250 nm (12300).- ^1H NMR (200 MHz, $CDCl_3$): δ = 2.84 (s, 6 H, $N(CH_3)_2$), 3.15 (dt, 2 H, CH_2-1^{DAE}), 3.53 (t, 2 H, CH_2-2^{DAE}), 5.51 (bt, 1 H, NH^{DAE}), 6.33 (s, 2 H, H-3^{MI}, H-4^{MI}), 7.12 (d, J = 7.8 Hz, 1 H, H-6^{Dans}), 7.43 - 7.53 (2 H, H-3^{Dans}, H-7^{Dans}), 8.18 (m, 2 H, H-2^{Dans}, H-8^{Dans}), 8.48 (d, J = 8.4 Hz, 1 H, H-4^{Dans}).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 37.6, 42.0 (C-1^{DAE}, C-2^{DAE}), 45.9 ($N(CH_3)_2$), 115.6, 119.5, 123.7, 128.9, 129.8, 130.3, 130.9 (C-6^{Dans}, C-4^{Dans}, C-2^{Dans}, C-7^{Dans}, C-8^{Dans}, C-9^{Dans}, C-10^{Dans}, C-3^{Dans}), 133.9 (C-3^{MI}, C-4^{MI}), 134.8 (C-1^{Dans}), 152.5 (C-5^{Dans}), 171.0 (C-2^{MI}, C-5^{MI}).- $C_{18}H_{19}N_3O_4S$ (373.4, 373.1), FAB: m/z = 374.2 $[M+H]^+$, 373.2 $[M+H-H]^+$, 371.2 $[M+H-H_2]^+$.

5-Amino-N-(2-{1-[2-(5-dimethylamino-naphthalin-1-sulfonylamino)-ethyl]-2,5-dioxo-pyrrolidin-3-ylsulfanyl}-ethyl)-2-nitrobenzamide (formula not shown)

To a solution of **25** (18.6 mg, 0.05 mmol) in ethanol (1 ml) a solution of **14** (12.1 mg, 0.05 mmol) in ethanol (1 ml) was added and the mixture was stirred at 20°C for 20 min. Solvent evaporation and FC ($CHCl_3$-ethyl acetate-methanol 25:25:8) furnished 26.4 mg of the desired product (86%).- R_f = 0.70 (methanol-$CHCl_3$ 1:1).- IR (KBr): $\tilde{\nu}$ = 2927, 2855, 1703, 1645, 1602, 1584, 1501, 1401, 1319, 1259 cm^{-1}.- UV (methanol): λ_{max} (ε) = 367 (9970), 250 (11870), 221 nm (18500).- ^1H NMR (200 MHz, [D_6]DMSO): δ = ca. 2.4 (1 H, H_A-4^{Suc}), 2.84 (s, 6 H, $N(CH_3)_2$), 2.60 - 3.50 (m, CH_2-2^{Cys}, CH_2-1^{DAE}, CH_2-1^{Cys}, H-3^{Suc}, CH_2-2^{DAE}), 3.86 (B von ABX, 1 H, H_B-4^{Suc}), 6.48 (d, J = 2.5 Hz, 1 H, H-3^{Ar}), 6.60 (dd, J = 9.0 Hz, J = 2.5 Hz, 1 H, H-5^{Ar}), 6.77 (bs, 2 H, NH_2^{Ar}), 7.27 (d, J = 7.8 Hz, 1 H, H-6^{Dans}), 7.57 - 7.68 (m, 2 H, H-3^{Dans}, H-7^{Dans}), 7.91 (d, J = 9.0 Hz, 1 H, H-6^{Ar}), 8.07 - 8.26 (m, 3 H, H-2^{Dans}, H-8^{Dans}, NH), 8.45 - 8.52 (m, 2 H, NH, H-4^{Dans}).- ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 30.5 (C-4^{Suc}), 36.2 (C-3^{Suc}, C-2^{Cys}), 45.3 ($N(CH_3)_2$), 112.1 (C-4^{Ar}), 112.7 (C-6^{Ar}), 127.7 (C-3^{Ar}), 115.5, 119.2, 123.9, 128.2, 128.6, 129.2, 129.4, 129.8 (C-6^{Dans}, C-4^{Dans}, C-2^{Dans}, C-7^{Dans}, C-8^{Dans}, C-9^{Dans}, C-10^{Dans}, C-3^{Dans}), 133.2 (C-1^{Ar}), 135.9 (C-1^{Dans}), 137.2 (C-2^{Ar}), 151.7 (C-5^{Dans}), 155.0 (C-5^{Ar}), 167.4 ($CONH^{Ar}$), 175.0, 176.9 (C-2^{Suc}, C-5^{Suc}).- $C_{27}H_{30}N_6O_7S_2$ (614.7, 614.2), FAB MS: m/z = 615.2 $[M+H]^+$, 614.2 $[M+H-H]^+$.

2-*O*-{2-Acetamido-4-*O*-[2-acetamido-4-*O*-((5*R*)-5-{5-(3-carboxy-propionyl)-2-[3-(2-{(*RS*)-1-[2-(5-dimethylamino-naphtalene-1-sulfonamido)-ethyl]-2,5-dioxo-pyrrolidin-3-ylthio}-ethylcarbamoyl)-4-nitro-phenyl]-2*H*-[1,2,4]triazol-3-yl}-α-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl]-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyloxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyranuronamide (26)

To a solution of **21** (100.0 mg, 0.055 mmol) in ethanol-water (1:1, 10 ml) a solution of **25** (21.0 mg, 0.056 mmol) in ethanol (2 ml) was added and the mixture was stirred at 20°C for 30 min. The pH was adjusted to 8 by addition of triethylamine and the mixture was stirred at 20°C for 2 h. After solvent evaporation the residue was purified by FC ($CHCl_3$-methanol-H_2O 18:11:2) and gel filtration (PD-10 column, Sephadex G-25M®, equilibrated with water) furnished **26** (50.1 mg, 41%).- R_f = 0.28 ($CHCl_3$-methanol-buffer 18:11:2.7).- Fluorescence spectra: Exitation at 340 nm, emission at 517 nm, in water/Triton X-100 solution, the Triton X-100 concentration was 4 times the cmc.- ^{1}H NMR (300 MHz, [D$_6$]DMSO): characteristic signals δ = 0.96 (s, CH_3-23^I, CH_3-24^I), 1.57 (s), 1.65 (s), 1.70 (s, CH_3-20^I, CH_3-21^I, CH_3-19^I, CH_3-25^I), 2.67 (d, J = 7.0 Hz, CH_2-12^I), 2.85 (s, N(CH_3)$_2$), 5.73 (bs, 1-H^F), 7.28 (d, 6-H^{Ar}), 7.58 - 7.72 (m) and 7.98 - 8.30 (m, aromatic H's), 8.48 (d, 6-H^{Dans}).- ^{13}C NMR (50 MHz, D_2O): δ = 15.2 (4^F-CH_3), 15.9 (C-21^I), 17.0 (bs, C-6^C), 17.6 (C-20^I), 22.8 (bs, NHCO$\underline{C}H_3^{E,C}$), 23.5 (C-25^I), 25.7 (C-19^I), 26.7 (C-16^I), 27.2 (C-23^I, C-24^I), 29.9 (bs), 31.6 (bs), 32.2 (bs, C-3$^{A'}$, C-10^I, C-5^I, C-4^I), 34.8 (C-12^I), 35.3 (C-8^I), 39.8 (C-15^I), 41.7 (C-9^I), 45.3 (N(CH_3)$_2$), 55.0 (bs), 55.3 (bs, C-2^{E,C}), 60.9 (bs, C-6^E), 64.0 - 88.0 (not identified broad signals), 94.5 (bs, C-1^F), 101.0 - 103.8 (bs, C-1^{C,E,B,D}), 108.9 (C-22^I), 121.2 (C-2^I), 122.3 (C-13^I), 124.5 (C-17^I), 125.9 (bs, C-6^I, C-2^{Ar}), 115.8, 119.5, 123.6, 126.0 - 130.5 broad signals (C-6^{Dans}, C-4^{Dans}, C-2^{Dans}, C-7^{Dans}, C-8^{Dans}, C-9^{Dans}, C-10^{Dans}, C-3^{Dans}, C-6^{Ar}, C-5^{Ar}), 130.8 (C-18^I), 133.3 (bs, C-3^{Ar}), 135.0 (C-1^{Dans}), 136.0 (C-14^I), 140.2, 141.0 (C-7^I, C-4^{Ar}), 146.4 (C-1^{Ar}), 149.3 (C-11^I), 151.4 (C-5^{Dans}), 153.8 (C-3TA)^{34}, 158.1 (O-$\underline{C}$O-NH$_2^F$), 158.6 (C-5TA), 166.5 (CONH^{Ar}), 172.8 - 179.6 broad signals (NH$\underline{C}$OCH$_3^{C,E}$, C-6^F, C-1^H, C-4$^{A'}$, C-2^{Suc}, C-5^{Suc}), 194.7 (bs, C-1$^{A'}$).- ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 16.0 (C-21^I), 16.4 (bs, 4^F-CH_3), 17.5 (bs, C-6^C), 17.8 (C-20^I), 23.4 (bs, NHCO$\underline{C}H_3^{E,C}$), 23.7 (C-25^I), 25.8 (C-19^I), 26.4 (C-16^I), 27.4 (C-23^I, C-24^I), 28.6 - 32.0 (bs, C-3$^{A'}$, C-10^I, C-5^I, C-4^I), 34.8 (C-12^I), 35.5 (C-8^I), 36.2 - 38.0 (bs, C-2$^{A'}$, C-2^{Cys}, C-3^{Suc}), 45.3 (N(CH_3)$_2$), 55.0 (bs), 55.5 (bs, C-2^{E,C}), 61.9 - 79.0 (not identified broad signals), 80.4 (bs, C-2^H, C-3^C), 85.6 (bs, C-4^C), 94.3 (bs, C-1^F), 101.3 - 103.8 (bs, C-1^{C,E,B,D}), 109.0 (C-22^I), 122.1 (C-13^I), 124.4 (C-17^I), 125.8 (bs, C-6^I, C-2^{Ar}), 115.5, 119.3, 123.9, 128.3, 128.6, 129.0, 129.3, 129.4, 129.9, 131.9 (C-6^{Dans}, C-4^{Dans}, C-2^{Dans}, C-7^{Dans}, C-8^{Dans}, C-9^{Dans}, C-10^{Dans}, C-3^{Dans}, C-5^{Ar}, C-6^{Ar}), 131.0 (C-18^I), 133.4 (bs, C-3^{Ar}), 136.0 (C-1^{Dans}), 136.1 (C-14^I), 140.2 (C-7^I), 140.8 (bs, C-4^{Ar}), 147.0 (bs, C-1^{Ar}), 149.5 (C-11^I), 151.7 (C-5^{Dans}), 154.9 (C-3TA)^{34}, 156.9 (O-CO-NH$_2^F$), 158.9 (C-5TA), 164.6 (CONH^{Ar}), 167.3 - 175.0 (NH$\underline{C}$OCH$_3^{C,E}$, C-6^F, C-1^H, C-4$^{A'}$), 175.1, 176.9 (C-2^{Suc}, C-5^{Suc}), 192.5 (bs, C-1$^{A'}$).- $C_{96}H_{135}N_{12}O_{41}S_2P$ (2208.3, 2206.8), FAB MS: m/z = 2229.9 [M+Na]$^+$, 2251.8 [M+2Na-H]$^+$.

2-*O*-(2-Acetamido-4-*O*-{2-acetamido-4-*O*-[(5*R*)-5-(5-(3-carboxy-propionyl)-2-{3-[2-((*RS*)-2,5-dioxo-pyrrolidin-3-ylthio)-ethylcarbamoyl]-4-nitro-phenyl}-2*H*-[1,2,4]triazol-3-yl)-α-L-arabinopyranosyl]-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyl-oxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyranuronamide (27)

To a solution of **21** (20.0 mg, 10.9 μmol, R_t = 14.1 min, RP-HPLC, (buffer-acetonitrile 63:37), λ_{max} = 275 nm) in buffer (10 ml) maleimide (2 mg, 20.6 μmol) was added and the mixture was stirred at 20°C for 2 h. Ultrafiltration followed by RP-HPLC (methanol-H_2O 63:37), UV-detection at λ = 208 nm) furnished **27** (5.2 mg,

25 %).- R_t = 13.6 min, RP-HPLC (buffer-acetonitrile 63:37), λ_{max} = 275 nm.- $C_{82}H_{119}N_{10}O_{39}SP$ (1931.9, 1930.7), FAB: m/z = 1953.6 $[M+Na]^+$, 1969.7 $[M+K]^+$, 1991.6 $[M+Na+K-H]^+$, 2007.6 $[M+2\ K-H]^+$.

2-*O*-[2-Acetamido-4-*O*-(2-acetamido-4-*O*-{(5*R*)-5-[2-(3-{2-[(*RS*)-1-(2-{(*R*)-1-carboxy-5-[5-((3a*S*)-2-oxo-(3a*r*,6a*c*)-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4*t*-yl)-pentanoylamino]-pentylcarbamoyl}-ethyl)-2,5-dioxo-pyrrolidin-3-ylthio]-ethylcarbamoyl}-4-nitro-phenyl)-5-(3-carboxy-propionyl)-2*H*-[1,2,4]triazol-3-yl]-α-L-arabinopyranosyl}-2,6-dideoxy-β-D-glucopyranosyl)-2-deoxy-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonodeca-2,6,13,17-tetraenyloxy)-ethoxy]-hydroxy-phosphoryl}-4-C-methyl-α-D-glucopyranuronamide (28)

To a solution of **21** (80.0 mg. 43.6 μmol) (R_t = 13.6 min, RP-HPLC (buffer-acetonitrile 63:37), λ_{max} = 275 nm) in water (5 ml) N-(3-maleimidopropionyl)-biocytin (20.4 mg, 38.9 μmol) was added and the mixture was stirred at 20°C for 1 h. Lyophilization followed by RP-HPLC (methanol-H_2O 37:63, UV detection at λ = 208 nm) furnished **28** (60 mg, 65 %).- R_t = 8.9 min, RP-HPLC, (buffer-acetonitrile 63:37), λ_{max} = 275 nm.- ^{1}H NMR (300 MHz): unresolved signals in D_2O and [D_6]DMSO solution.- ^{13}C NMR (75 MHz, [D_6]DMSO, broad signals)[42]: δ = 15.7 (C-21^I), 16.0 (bs, 4^F-CH_3), 17.2 (bs, C-6^C), 17.5 (C-20^I), 22.8, 22.9 (bs, $NHCOCH_3^{E,C}$), 23.2 (C-25^I), 25.4 (C-19^I), 26.0 (C-16^I), 27.1 (C-23^I, C-24^I), 25.2, 27.9, 28.1, 29.0, 29.5, 29.9, 32.2, 33.1 and 35.8 (not identified broad signals from the cysteine, succinimide, ß-alanine, biocytine moieties and the succinyl part derived from unit A), 30.6 (C-10^I), 30.8 (C-5^I), 31.8 (C-4^I), 34.4 (C-12^I), 35.1 (C-8^I), 40.9 (C-9^I), 53.9, 54.3 (bs), 55.0 (bs, C-2^{E,C}), 55.3, 59.2, 61.0 (C-4^{BTR},C-3a^{BTR}, C-6a^{BTR}), 60.2 (?), 62.9 - 78.0 (not identified broad signals), 80.0 (C-2^H, C-3^C), 93.6 (bs, C-1^F), 101.1 - 102.8 (broad signals) (C-1^{C,E,B,D}), 108.7 (C-22^I), 121.7 (C-13^I), 123.1 (bs, C-2^I), 124.0 (C-17^I), 125.4 (C-6^I), 125.9 (C-2^{Ar}), 127.6 (bs, C-6^{Ar}, C-5^{Ar}), 130.7 (C-18^I), 133.0 (bs, C-3^{Ar}), 135.8 (C-14^I), 137.4 (bs, C-3^I), 139.8 (C-7^I), 140.5 (bs, C-4^{Ar}), 146.5 (bs, C-1^{Ar}), 149.1 (C-11^I), 154.6 (C-3TA)[34], 156.7 (O-CO-NH_2^F), 158.6 (C-5TA), 162.8 (C-2^{BTR}), 164.3 ($CONH^{Ar}$), 168.3, 169.7, 170.2, 171.5, 171.9, 173.6, 174.7, 176.3 ($NHCOCH_3^{C,E}$, C-6^F, C-2^{Suc}, C-5^{Suc}, $COOH^{\beta A}$, $COOH^{Lys}$, C-1^H, $COOH^{A'}$), 192 (bs, C-1$^{A'}$).- $C_{101}H_{149}N_{14}O_{44}S_2P$ (2358.5, 2356.9), FAB MS: m/z = 2379 $[M+Na]^+$, 2397 $[M+K]^+$, 2417 $[M-H+K+Na]^+$.

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[34] Assignments in analogy to **4**.

[35] Under the basic conditions the thiol is easily oxidized to give the corresponding disulfide.- ^1H-NMR (200 MHz, [D_5]pyridine): $\delta = 3.26$ (t, $J = 6.7$ Hz, $J = 8.4$ Hz ,$CH_2\text{-}2^{Cys}$), 4.04 (dt, $J = 6.7$ Hz, $J = 5.6$ Hz, $CH_2\text{-}1^{Cys}$), 6.80 (dd, $J = 2.5$ Hz, $J = 9.0$ Hz, H-4^{Ar}), 7.20 (d, $J = 2.5$ Hz, H-6^{Ar}), 7.42 (bs, Ar-NH_2), 8.05 (d, $J = 9.0$ Hz, H-3^{Ar}), 9.74 (bt, $J = 5.6$ Hz, $CONH^{Ar}$). - ^{13}C-NMR: (50 MHz, [D_5]pyridine): $\delta = 38.5$ ($C\text{-}2^{Cys}$), 39.8 ($C\text{-}1^{Cys}$), 113.5 ($C\text{-}4^{Ar}$, $C\text{-}6^{Ar}$), 128.3 ($C\text{-}3^{Ar}$), 135.0 ($C\text{-}1^{Ar}$), 138.3 ($C\text{-}2^{Ar}$), 155.8 ($C\text{-}5^{Ar}$), 169.2 ($CONH^{Ar}$).

[36] The procedure has to be followed exactly, otherwise the disulfide is formed.[35]

[37] ^{13}C-NMR (50 MHz, CD_3OD, taken from the spectrum of the mixture): 22.1 ($C\text{-}7^{A'}$), 28.0 ($C\text{-}2^{A'}$), 32.0 ($C\text{-}3^{A'}$) 37.5 ($C\text{-}2^{Cys}$), 39.1 ($C\text{-}1^{Cys}$), 113.5, 114.5 ($C\text{-}6^{Ar}$, $C\text{-}4^{Ar}$), 120.6 ($C\text{-}2^{Py}$), 121.7 ($C\text{-}4^{Py}$), 127.3 ($C\text{-}3^{Ar}$), 133.2 ($C\text{-}1^{Ar}$), 134.4 ($C\text{-}2^{Ar}$), 138.4 ($C\text{-}3^{Py}$), 139.2 ($C\text{-}1^{Ar}$), 146.5 ($C\text{-}5^{Ar}$), 149.6 ($C\text{-}5^{Py}$), 160.1 ($C\text{-}1^{Py}$), 169.1 ($C\text{-}6^{Ar}$), 175.7 ($C\text{-}1^{A'}$), 192.2 ($C\text{-}4^{A'}$).

[38] UV-control of the conversion of Moenomycin A (**18**) into **20**. A solution of the diazonium salt obtained from 2-nitroaniline (22.4 mg, 0.33 mmol) was prepared as described above. The UV maximum of this solution was at $\lambda_{max} = 225$ nm. Moenomycin A (**18**) (0.3 mg, 0.2 µmol) was dissolved in buffer (10 ml, pH = 7.75). The UV maximum of this solution was at $\lambda_{max} = 259$ nm. Addition of a small amount of the diazonium salt solution (≈ 0.02 ml) caused the absorption at $\lambda = 259$ nm to increase (formation of the azo compound). Within 5 min the absorption at 259 nm decreased and a slow increase of the amidrazone band at $\lambda \approx 320$ nm was observed.

[39] Signals at 14.1, 14.7, 20.8, 20.9, 21.2, 27.1, 30.5, 30.7, 31.0, 71.6 could not be assigned.

[40] Release of thiopyridone was indicated by the increase of the UV absorptions at $\lambda = 271$ nm und $\lambda = 350$ nm.

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