Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 2965–2981

BIOORGANIC & MEDICINAL CHEMISTRY

Studies on the Interaction of the Antibiotic Moenomycin A with the Enzyme *Penicillin-Binding Protein 1b*

Thomas Rühl,[a] Mohammed Daghish,[a] Andrij Buchynskyy,[a] Karen Barche,[a] Daniela Volke,[a] Katherina Stembera,[a] Uwe Kempin,[a] Dietmar Knoll,[a] Lothar Hennig,[a] Matthias Findeisen,[a] Ramona Oehme,[a] Sabine Giesa,[a] Juan Ayala[b] and Peter Welzel[a,*]

[a]*Universität Leipzig, Fakultät für Chemie und Mineralogie, Johannisallee 29, D-04103 Leipzig, Germany*
[b]*Centro de Biologica Molecular, Universidad Autonoma, Canto Blanco, E-28049 Madrid, Spain*

Received 25 October 2002; accepted 18 March 2003

Abstract—The interaction of a moenomycin derivative with the enzyme *penicillin binding protein 1b* (PBP 1b) has been studied by means of STD NMR. The results obtained initiated the synthesis of a number of moenomycin derivatives modified in unit A including a moenomycin–ampicillin conjugate and determination of their antibiotic activities. A protocol is described that allows studying the interaction of moenomycin analogues with PBP 1b by fluorescence correlation spectroscopy.

Introduction

Peptidoglycan is an essential cell wall constituent of almost all eubacteria. It is a heteropolymer consisting of glycan strands crosslinked by short peptide chains. A number of indispensible functions can be attributed to this macromolecule that is located at the outside of the cytoplasmic membrane. It preserves cell integrity by withstanding the internal osmotic pressure and gives the cell a defined shape. Furthermore, the biosynthesis of peptidoglycan is intimately associated with the cell division process. The final events of peptidoglycan biosynthesis are the formation of the macromolecular architecture from a disaccharide oligopeptide precursor by two major types of reaction, (i) formation of the polysaccharide strands (transglycosylation) and (ii) formation of peptide crosslinkages between the glycan chains (transpeptidation).

A representation of the transition state of the transglycosylation reaction is shown in Figure 1.[1]

Both the glycosyl donor (the growing peptidoglycan chain) and the glycosyl acceptor (the disaccharide-derived lipid II) are attached to the cytoplasmic membrane via a C_{55} anchor. The transglycoslyation reaction is catalyzed by a number of multimodular bifunctional polymerases (that catalyze also the transpeptidation reaction) designated as class A *high molecular mass penicillin-binding proteins* (PBPs). Of these PBP 1b from *Escherichia coli* has been studied in great detail.[2] In addition, a number of membrane-bound monofunctional glycosyltransferases are known. Recently, a monofunctional glycosyltransferase from *Staphylococcus aureus* that shares considerable homology with the transglycosylase domain of *bifunctional (class A) high molecular mass PBPs* has been expressed as a truncated protein lacking the membrane domain and purified to homogeneity.[3]

The moenomycin antibiotics are known to interfere with the transglycosylation reaction.[4,5] Moenomycin A (**1a**) has been demonstrated to bind reversibly to *E. coli* PBP 1b.[1] The structural similarities between the moenomycins and both glycosyl donor and glycosyl acceptor of the transglycosylation reaction are obvious. From structure–activity relationships[6,7] it has been concluded that the moenomycins first bind to the cytoplasmic membrane via their lipid moiety[8] and that membrane anchoring is an essential step preceding the highly selective binding of the sugar part to the donor binding site of the enzyme. The structural features that are known to be responsible for the antibiotic activity are indicated by arrows in **2**.[6,7,9] The structural similarity of units C and E of **1a** and **2**, respectively, with the second

*Corresponding author. Tel.: +49-341-97-36551; fax: +49-341-97-36599; e-mail: welzel@organik.chemie.uni-leipzig.de

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00187-1

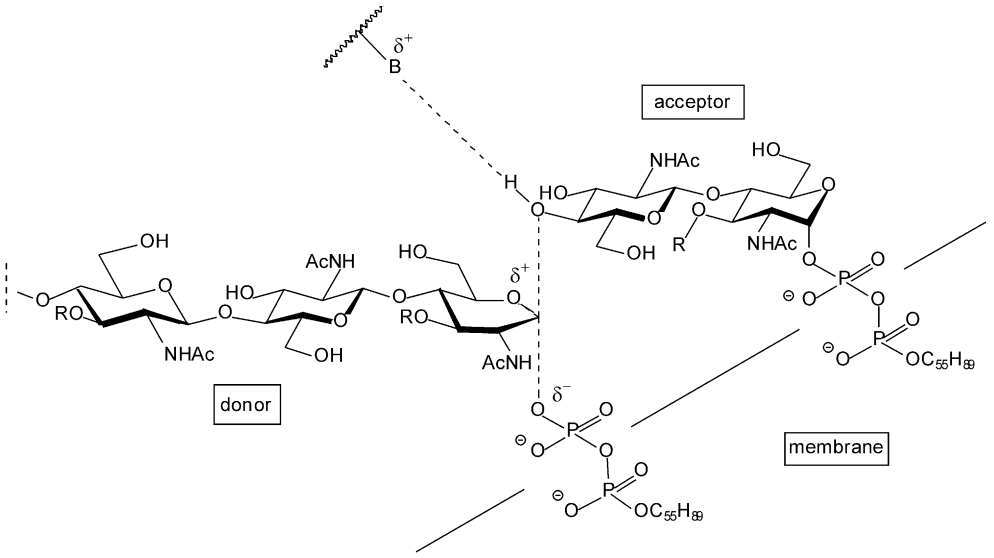


Figure 1.

and the third sugar unit of the growing peptidoglycan chain are obvious. The different binding of the first two sugar units (1→4 in the growing peptidoglycan strand versus 1→2 in **1a** and **2**, respectively) has been suggested to be responsible for the inhibition of the enzyme.[10]

The results of SAR studies are in agreement with NMR results. For a long time it was impossible to obtain good quality ^{1}H NMR spectra due to the formation of moenomycin aggregates in aqueous solution. We succeeded to overcome this obstacle when the ^{1}H NMR spectra were measured at concentrations below the cmc.[11] Well-resolved ^{1}H NMR spectra of the moenomycins in aqueous solution ($c < 5 \times 10^{-4}$ mol/L) were then obtained which could be fully assigned.[12] Based on NMR-derived distance constraints and molecular dynamics simulations a three-dimensional structure of moenomycin in aqueous solution was proposed by Kurz et al.[13] as illustrated in Figure 2. The arrows in this presentation indicate groups that have been shown to be essential for the antibiotic activity (vide supra). Since these substituents are exposed in close proximity at the surface of the molecule it has been speculated that they are part of a polar binding epitope.[13]

It is the purpose of the present publication to extend the knowledge on the interaction of moenomycin with the binding site at the enzyme.

Results and Discussion

STD experiments

Recently, saturation transfer difference (STD) NMR has been applied by the group of Meyer[14] and by others[15] for mapping carbohydrate epitopes in direct contact with lectins. STD NMR relies on the transfer of saturation from the protein to the ligand and is strongest for those segments of the ligand that are in closest contact with the protein. Saturation is transferred into solution through fast exchange of ligand molecules from the bound to the free state where the saturation transfer is detected. We repeated the published methyl β-D-galactopyranoside/*Ricinus communis* agglutinin experiment of Mayer and Meyer[14] and found the same transfer results that were reported but with some deviations in quantity.

On the other hand, experiments with moenomycin A (**1a**) and an *E. coli K12 (JM109/pJP13)* membrane extract in Tris–maleate buffer (in D_2O, apparent pH 6.8) which contained PBP 1b solubilized by Triton

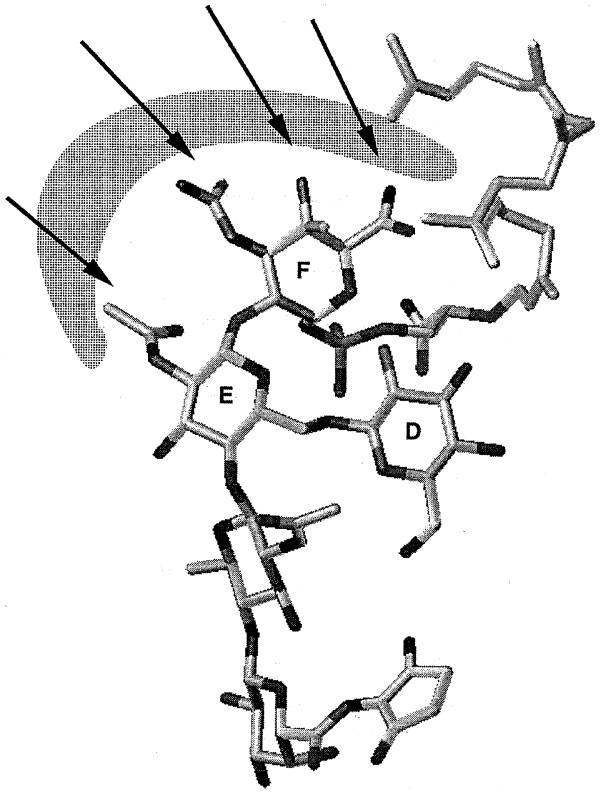


Figure 2. NMR solution structure (D_2O) of moenomycin A (from ref 13).

X-100 were inconclusive. The STD spectrum showed Triton X-100 and possibly moenomycin signals which, however, could not be separated from each other. Then membrane preparations in phosphate buffer (in D_2O, apparent pH 7.0) were used. The concentration of the membrane fragments was about 40 μM and moenomycin A was added until a final concentration of 4×10^{-4} mol/L (this is close to the cmc, vide supra) was reached. No STD could be observed. We attribute this failure to the fact that binding of moenomycin to PBP 1b is very strong[7] and possibly the rate of exchange between free and bound ligand is so slow that the concentration of free ligand labeled by saturation transfer is too low to be detected. We have shown previously that the lipid part contributes considerably to the binding between moenomycin A and PBP 1b.[7] Therefore, for further STD experiments the delipido derivative **3**, which was prepared as described previously,[4] was used. The 600 MHz ^{1}H NMR spectrum of **3** in aqueous solution is depicted in Figure 3 (top). Using APT, TOCSY, H,H COSY, ^{13}C HSQC/^{13}C HMQC and HMBC the ^{1}H and ^{13}C spectra could be fully assigned. The data are collected in Table 1 (Scheme 1).

For the new STD experiment, a *PBP 1b* solution (membrane fraction without detergents, about 40 μM in PBP 1b, phosphate buffer, D_2O, apparent pH 7.0, 20 °C) was used and delipidomoenomycin **3** was added (final concentration 4×10^{-4} mol/L). The experiment was performed essentially as described in ref 14 (see Experimental). In spite of the long acquisition time (22 h), the resulting STD spectrum was only of modest quality. Only the most intensive signals of the ligand could be observed [*N*-acetyl groups at δ = 1.91 and 1.95; 4-methyl group of unit F (δ = 1.10), methyl group of unit C (δ = 1.27) and the signal of the chromophore A part (at δ = 2.26)] demonstrating that these groups had been in contact with the protein. In comparison to the other signals, the intensity of the *N*-acetyl group signals was amplified to a much higher degree. On the other hand, no signal for the anomeric proton of unit F at δ = 5.66 could be detected. This feature indicates site specific differences in the saturation transfer processes. Because of the unfavourable quality of the spectrum not only the integrals but also the peaks heights have been compared carefully. Both modes indicated an intensity increase of the *N*-acetyl signals in comparison to the 4-methyl group signal of unit F and the four-proton singlet of the methylene groups of unit A. This result is in agreement with the known absolute requirement of the *N*-acetyl groups for the antibiotic activity of moenomycin analogues and also with the speculative model of Kurz[13] derived from their moenomycin NMR structural work. Interestingly, the STD NMR spectrum also shows the signal of the chromophore A part (at δ = 2.26) which has been shown to be of minor importance for the antibiotic activity. Probably, ring A has contact with the protein at positions different from the moenomycin binding site. The findings of Kurz et al.[13] suggest, that rings A and B display a high degree of conformational flexibility. In any case, the STD NMR result prompted us to look again at the role of the chromophore unit A.

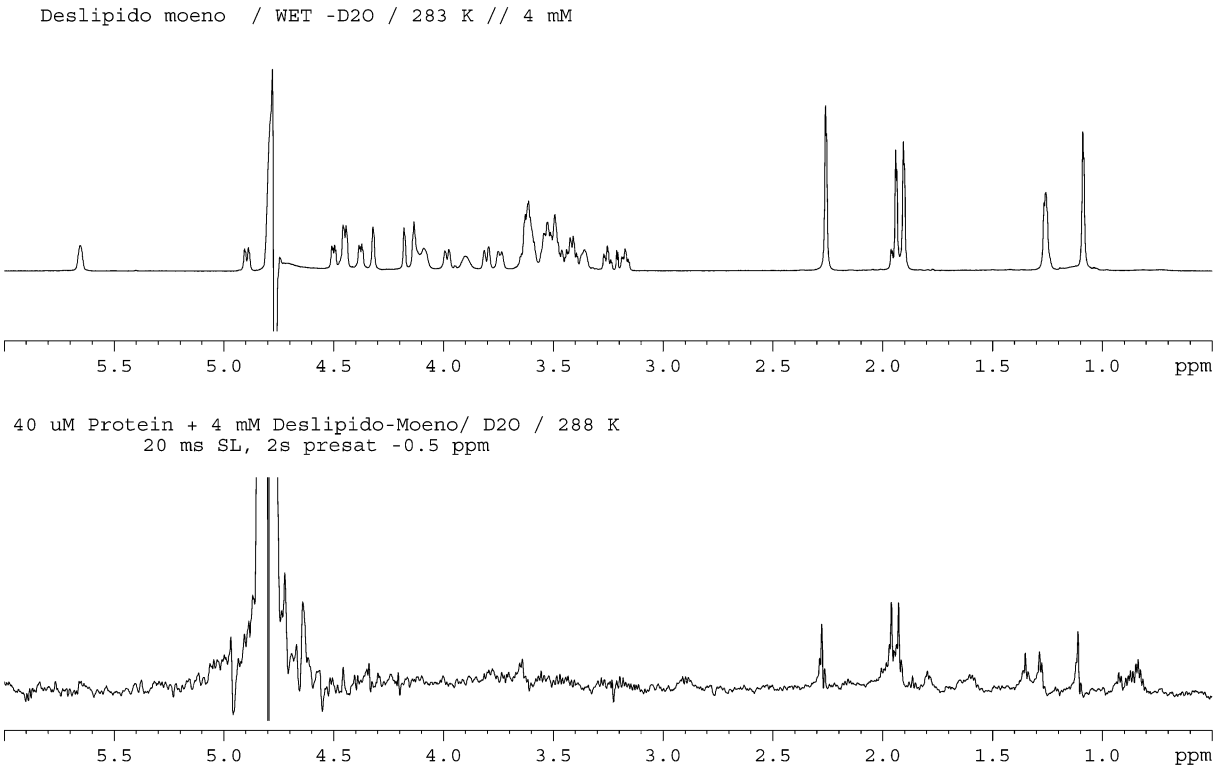


Figure 3. Autocorrelation G(t) of the interaction of PBP 1b with **11.** Each sample was measured in 25 mM Tris/HCl, pH 7.2, 200 mM NaCl, 1% Triton X-100 for 20 s. Data were analysed with ConforCor 2 Version 2.5 SP2 (two-components model for **11**) and three-components model for PBP 1b/**11**. Tetramethylrhodamine (TMR, 10 nM); **11** (M-TMR, 10 nM); **11** (10 nM) + PBP 1b (160 nM).

Table 1. ^{1}H, ^{13}C, and ^{31}P chemical shifts of **3** (in D_2O)

Position	^{1}H chemical shifts	^{13}C chemical shifts	^{31}P chemical shifts
1^{A}	—	110.08	—
2^{A}	—	201.45	—
3^{A}	2.26	30.56	—
4^{A}	2.26	30.56	—
5^{A}	—	201.45	—
1^{B}	4.50	103.10	—
2^{B}	ca. 3.47	70.84	—
3^{B}	ca. 3.63		—
4^{B}	4.14	69.10	—
5^{B}	4.18	74.87	—
$CONH^{B}$	—	169.95	—
1^{C}	4.46	101.49	—
2^{C}	3.64	55.82	—
CH_3CONH^{C}	—	174.79	—
CH_3CONH^{C}	1.91/1.95	22.58	—
3^{C}	*ca. 3.55*		—
4^{C}	3.42	83.38	—
5^{C}	*ca. 3.52*	71.34	—
6^{C}	1.27	16.85	—
1^{D}	4.38	103.44	—
2^{D}	3.18		—
3^{D}	3.42	75.93	—
4^{D}	3.26	69.91	—
5^{D}	3.36	76.19	—
6^{D}	ca. 3.61; 3.81	60.95	—
1^{E}	4.46	102.30	—
2^{E}		55.24	—
CH_3CONH^{E}	—	174.50	—
CH_3CONH^{E}	1.91/1.95	22.49	—
3^{E}			—
4^{E}	ca. 3.47	79.85	—
5^{E}			—
6^{E}	ca. 3.63; 3.98	68.94	—
1^{F}	5.66	94.5*1	—
2^{F}	3.74	76.4*1	—
3^{F}	4.90	75.15	—
$OCONH_2^{F}$	—	158.38	—
4^{F}	—	73.26	—
CH_3^{F}	1.10 (s)	14.88	—
5^{F}	4.33		—
$CONH_2^{F}$	—	172.96	—
P	—	—	−0.7
1^{H}	3.90/4.09	68.9*1	—
2^{H}	4.12	72.5*1	—
3^{H}	—	177.8*1	—

Moenomycin derivatives with modified units A

We have previously developed a method for removing the chromophore part selectively from moenomycine by $K_3[Fe(CN)_6]$ oxidation to give **1b**.[16] We have now determined the antibiotic activity of this compound and found a MIC of 6.0×10^{-8} mol L^{-1}. For moenomycin 7 $\times$ 10^{-9} mol L^{-1} have been determined in a parallel experiment (see Table 2). This result means that the interaction of the chromophore unit A with the enzyme contributes to some extent to the antibiotic activity of the moenomycins. Nevertheless, **1b** is still an antibiotically very active compound. We took this occasion to prepare a number of new moenomycin A derivatives modified in ring A in order to find out if different pendants at ring A would lead to changes in the antibiotic activity in a systematic way. First, we prepared the 4-nitrophenyl- and the 4-[(2-aminoethyl)carbamoyl]-phenyl-substituted triazoles **4** and **5a** and the two 4-nitro-aryl-substituted triazoles **6b** and **6c** with basic substituents of differing chain length in the 3-position of the aromatic ring by the usual Japp–Klingemann route (Scheme 2).[17]

Table 2. MIC values of moenomycin analogues with a modified chromophore unit (in mol L^{-1})

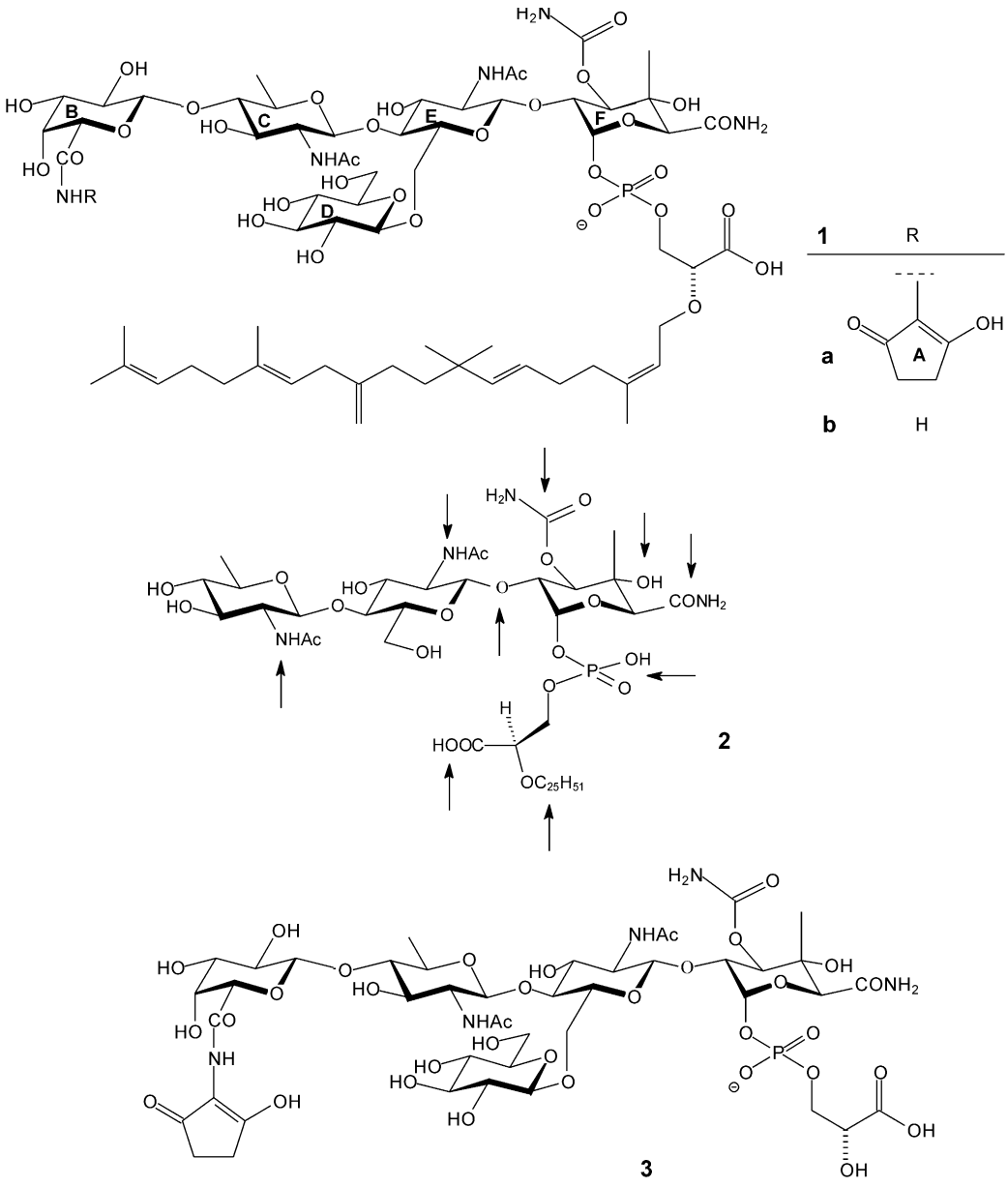
1a	7×10^{-9}
1b	6.0×10^{-8}
5a	1×10^{-7}
5b	6×10^{-8}
6b	5×10^{-8}
6c	4×10^{-8}
8a	4×10^{-7}
7a	1×10^{-7}
7b	2×10^{-7}
8b	2×10^{-7}
6a	2×10^{-7}

Furthermore amines **5a**, **6a**, and **6b** were treated with 4-isothiocyanatobenzoic acid to provide thioureas **5b**, **7a**, and **8a** with an extra free carboxyl group. From amines **6a** and **6b** on reaction with 4-isocyanatobenzenesulfonic acid thioureas **7b** and **8b** were obtained bearing sulfonic acid groups (Schemes 2 and 3).

In the ^{1}H NMR spectra of **5a** and of **7a** some anomalies were observed. The chemical shifts of 4^{C}-H of **5a** and of **7a** in methanol-d_4–DMSO-d_6 solution ($\delta = 2.42$ and $\delta = 2.46$, respectively) are smaller than that of 4^{C}-H of moenomycin A (**1a**, $\delta = 3.17$ in methanol-d_4 solution, $\delta = 3.09$ in DMSO-d_6 solution, referenced to $\delta = 5.69$ for 1^{F}-H). On the other hand, the 5^{B}-H of **5a** ($\delta = 5.02$) and **7a** ($\delta = 4.97$, both spectra in methanol-d_4–DMSO-d_6 solution) had a larger chemical shift than that of moenomycin A (**1a**, $\delta = 3.85$ in methanol-d_4 and $\delta = 3.88$ in DMSO-d_6 solution, referenced to $\delta = 5.69$ for 1^{F}-H). The reasons for these observations are at present not clear. All other ^{1}H chemical shifts of **5a** and of **7a** are in the same region as those of **1a**. The smaller chemical shift of 4^{C}-H of **5a** and of **7a** than in moenomycin A could be the result of a shielding effect of the aromatic ring.[18] However, in **5a** also the signal of 5^{C}-H ($\delta = 2.96$ in methanol-d_4–DMSO-d_6) appears at higher field than in moenomycin A (**1a**, $\delta = 3.39$ in methanol-d_4, $\delta = 3.42$ in DMSO-d_6). If it is assumed that this upfield shift also originates from a shielding effect of the aromatic ring this would indicate two conformations. We did not observe these anomalies in the spectra with a nitro-substituent in the 4-position. The ^{1}H NMR spectra of **7a** and **5a** revealed a rather fast H $\rightarrow$ D exchange in methanol-d_4–DMSO-d_6 solution at the α-position of the keto group (2^{A}-H, $\delta = 3.19$–3.13 in **5a** and $\delta = 3.14$ in **7a**). This caused the 3^{A}-H signal around $\delta = 2.50$ to be a doublet after exchange of one proton and as a singlet after exchange of both protons (see Experimental).

Antibiotic activities

Compound **1b** lacking the chromophore unit A has about 10% of the antibiotic activity of moenomycin A (vide supra), whereas in **6a** 4% of the antibiotic activity of moenomycin A are left. As described above, we have prepared new derivatives with neutral aryl substituents

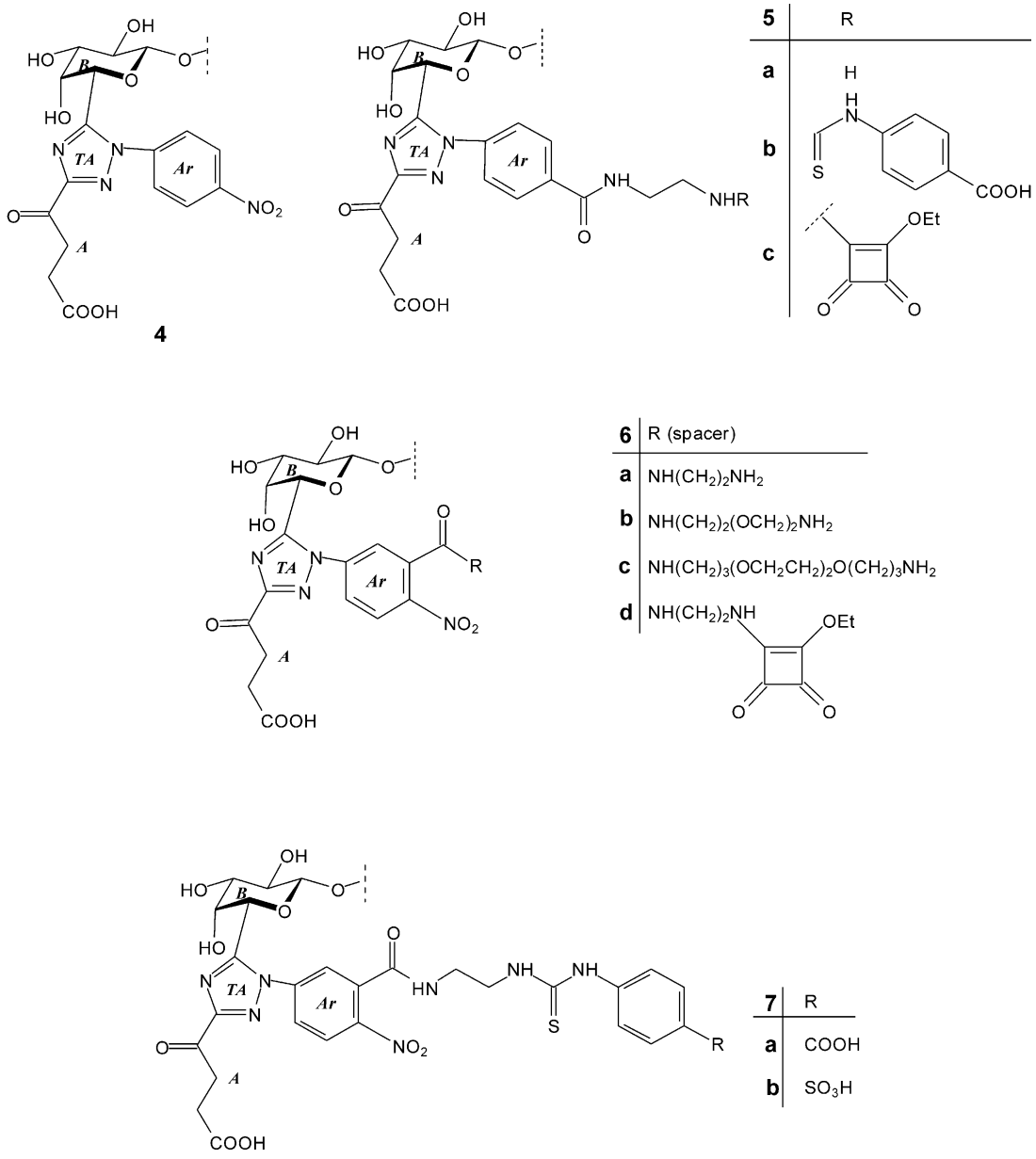


Scheme 1.

at the triazole ring (**4**), with basic substituents (**5a**, **6b**, **6c**), with weakly acidic substituents (**5b**, **7a**, **8a**) and with strongly acidic substituents (**7b**, **8b**). Their MIC values against seven *Staphylococcus aureus* strains were determined by a serial micro dilution method on microtiter plates as described previously.[8] As Table 2 shows, for most compounds the MIC values are in the 10^{-7} mol L^{-1} range. Compounds **5b**, **6b**, and **6c** have MIC values in the 10^{-8} mol L^{-1} range. Two of them have additional basic groups and one has an additional weakly acidic function. From these results, one may conclude that changes in the region of unit A of moenomycin cause some decrease in the antibiotic activity. The decrease of activity differs somewhat for different substituents but we could not find a systematic correlation between antibiotic properties and newly introduced functional groups. All these compounds are, however, still very active when compared to other classes of antibiotics.

The moenomycin-ampicillin conjugate 10

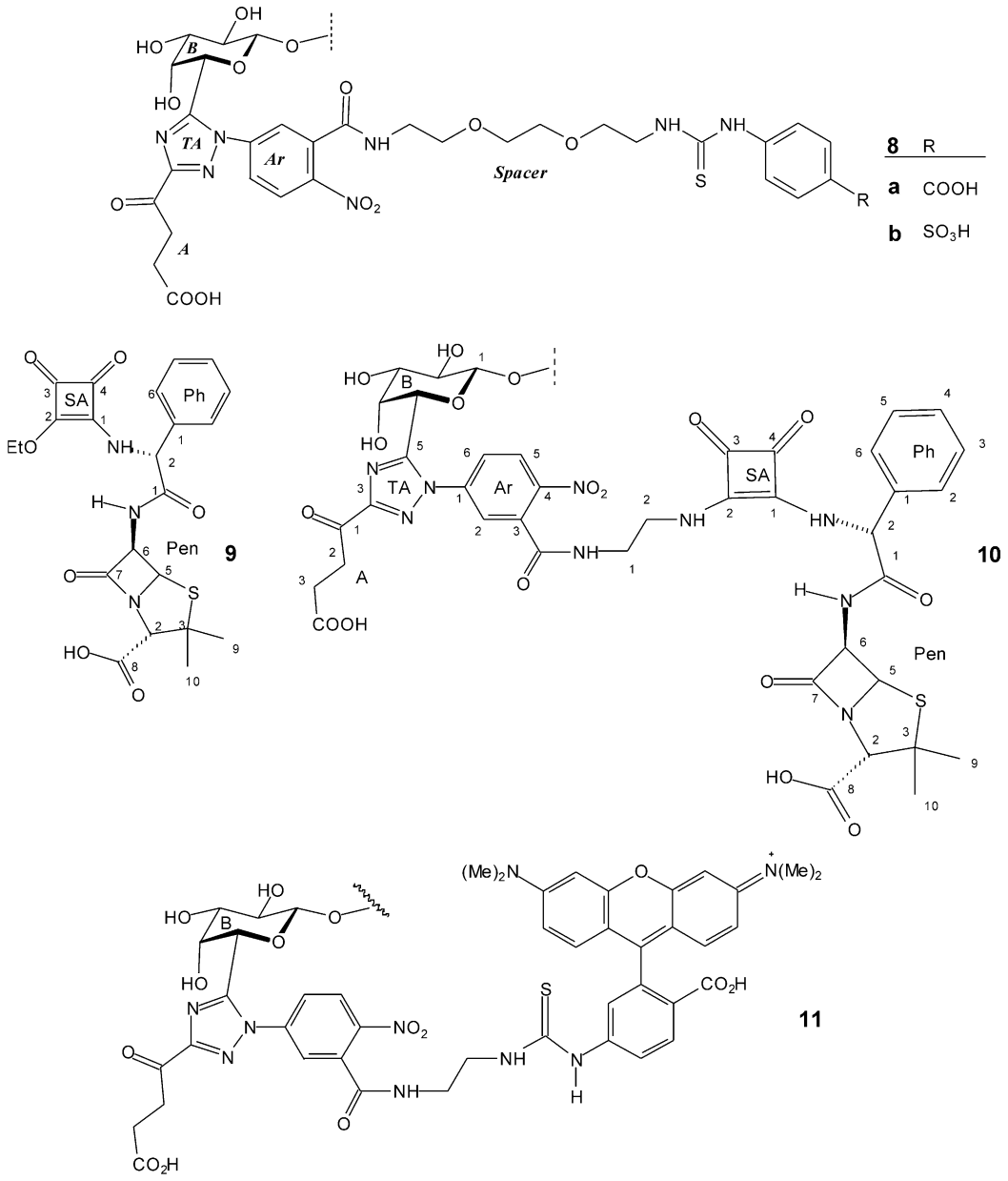
In view of the advantages which may be offered by drug synergism (for example impeding the development of resistance), we thought it would be interesting to develop chemistry that could be used to fuse moenomycin with antibiotics such as penicillin or vancomycin. All these compounds act at the outer face of the cytoplasmic membrane and inhibit the last two steps of the peptidoglycan biosynthesis. In a preliminary experiment without suitable spacers, ampicillin and **6a** were coupled via the squaric acid moiety under mild conditions. The low stability of ampicillin in aqueous solution at acidic and basic pH values demanded reaction conditions where the β-lactam ring of ampicillin is stable. Performing the reaction between **6a** and the ampicillin sodium salt in a buffered solution at pH 9.0 furnished the coupling product but with a hydrolized β-lactam



Scheme 2.

ring. Then the less nucleophilic polar solvent DMF with added Et_3N was used. First the moenomycin-squaric acid derivative **6d** was treated with ampicillin in DMF/Et_3N solution at 4 °C. The purification problems in this case (very similar R_f values of the product **10** and of **6d**, see Experimental) made this coupling sequence less advantageous than the reversed order of events. Here ampicillin squaric acid amide ester **9** was prepared from ampicillin sodium salt and diethyl squarate in phosphate buffer at pH 7.3.

The structure of **9** was proven by ^1H and ^{13}C NMR analysis based on comparison with the described ^1H and ^{13}C NMR spectra of ampicillin in the same solvent and by high resolution ESI ICR MS. Both in the ^1H and ^{13}C NMR spectra one signal was doubled (see Experimental). It is unclear whether this indicates an impurity or two conformations. The former possibility seems less likely since the ESI ICR MS spectrum gave no indication of an impurity. Then the crude **9** was attached to **6a** in DMF/Et_3N solution at 4 °C. The chimeric product **10** was isolated after careful purification in 19% yield (not optimized). The characteristic signals in the ^1H and ^{13}C NMR spectra of **10** were found with the help of 2D NMR experiments (^1H,^1H COSY, HMQC, HMBC) and by comparison with the NMR spectra of **9**. The IR spectrum of **10** showed a band at 1755 cm^{-1} which is characteristic for the β-lactam ring (for ampicillin at 1759 cm^{-1}). The high resolution ESI ICR MS of compound **10** showed besides the correct ion peaks at m/z = 1131.89462 ($[M+Na\text{-}3H]^{2-}$), 1120.90120 ($[M-2H]^{2-}$), 746.93268 ($[M-3H]^{3-}$), 559.94854 ($[M-4H]^{4}$) also peaks that differed from the expected ions by an additional mass unit. In conclusion, we have shown that the squaric acid method can be applied to the synthesis of moenomycin–ampicillin chimeric derivatives with the



Scheme 3.

intact β-lactam ring. The MIC value obtained for **10** was 1.8×10^{-7} mol/L^{-1}. Attempts to couple vancomycin to **6a** via the squaric acid linker were not successful until now.

Fluorescence correlation spectroscopy for studying moenomycin binding to PBP 1b

The interaction of moenomycin with PBP 1b has been studied previously by means of surface plasmon resonance.[19] Here, we report preliminary results that show that fluorescence correlation spectroscopy (FCS) can likewise be used for this purpose (Fig. 4). FCS measures statistical fluctuation of the fluorescence intensity in a small illuminated sample volume to obtain information about the binding properties of fluorescent molecules. Moenomycin was labeled with tetramethylrhodamine to give **11** as described previously.[20] We studied the interaction of **11** with the purified and solubilized membrane protein *PBP 1b*.[21] In the first experiments, the average diffusion time of **11** was determined. The measurements demonstrated that on addition of detergent the average diffusion time increased from 5.6×10^{-5} to 3.00×10^{-4} s due to the binding of **11** to Triton X-100 micelles (see Fig. 5). In principle in a solution of **11** and micelles, two diffusion times could be expected: (1) the ligand in solution and (2) the ligand incorporated into micelles.[22] But in our case the diffusion time **11** in solution could not be detected. Obviously, **11** was completely incorporated into the micelles in agreement with the previous observation that the lipid part of moenomycin binds very efficiently to membranes, vesicles and micelles.[8] For the analysis of the moenomycin/*PBP 1b* interaction constant concentrations of **11** were measured with increasing concentrations of *PBP 1b*. As soon as the protein was added to the solution a diffusion time of

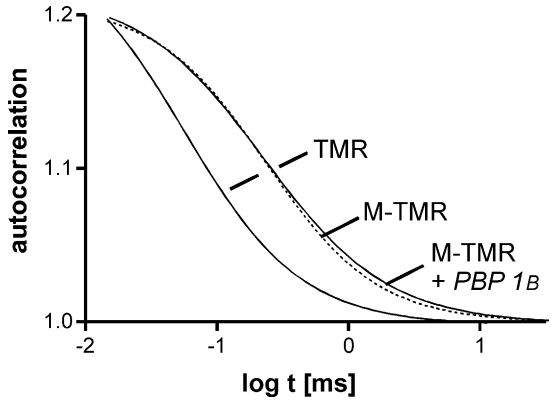


Figure 4. Autocorrelation G(t) of the interaction of PBP 1b with **11.** Each sample was measured in 25 mM Tris/HCl, pH 7.2, 200 mM NaCl, 1% Triton X-100 for 20 s. Data were analysed with ConforCor 2 Version 2.5 SP2 (two-components model for **11**) and three-components model for PBP 1b/**11**. Tetramethylrhodamine (TMR, 10 nM); **11** (M-TMR, 10 nM); **11** (10 nM) + PBP 1b (160 nM).

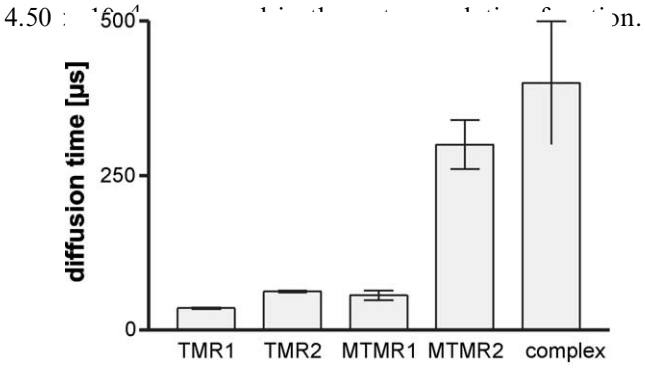


Figure 5. Increasing of the diffusion times during the interaction of *PBP 1b* with **11**. The diffusion times of tetramethylrhodamine (TMR) and **11** (MTMR) were influenced significantly by Triton X-100. TMR1 and MTMR1: tretramethylrhodamine or **11** in 25 mM Tris/HCl, pH 7.2, 200 mM NaCl without 1% Triton X-100. TMR2 and MTMR2: tetramethylrhodamine or **11** in 25 mM Tris/HCl, pH 7.2, 200 mM NaCl with 1% Triton X-100. Addition of PBP 1b in 25 mM Tris/HCl, pH 7.2, 200 mM NaCl, 1% Triton X-100 resulted in complex formation. Tetramethylrhodamine: 10 nM; **11**: 10 nM; complex: 10 nM **11** and 160 nM PBP 1b.

This diffusion time can be attributed to **11** bound to the protein. Thus, three different average diffusion times have been identified: tetramethylrhodamine, **11** incorporated into micelles and the complex of **11** with PBP 1b in Triton X-100 micelles. The autocorrelation functions for all three species are shown in Figure 4. A shift to higher relaxation times can be observed which is due to the increase of the molecular weight of the fluorescent species. The differences between the molar mass of **11** bound to micelles and the protein–ligand–micelles complex is very small. Competition experiments with moenomycin were performed to verify the experiments and showed an increase of the **11** fraction bound into micelles but not bound to the protein. The relative molecular mass of the solubilized PBP 1b could be estimated to be 124 kDa including a contribution of about 30–50 kD from micelles. Aggregations of the protein and micelles were observed in all experiments.

Experimental

All O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. Liquids and solutions were transferred by syringe. Small-scale reactions were performed in Wheaton serum bottles sealed with aluminum caps with open top and Teflon-faced septum (Aldrich). Usual workup means partitioning the reaction mixture between an aqueous phase and CH_2Cl_2, drying the combined organic solutions over Na_2SO_4, and removal of solvent by distillation using a rotatory evaporator (bath temperature 45 °C). Solvents were purified by standard techniques. The following materials and methods were used for chromatographic separations: flash chromatography (FC):[23] silica gel 32–63 µm (ICN Biomedicals); medium-pressure liquid chromatography (MPLC): silica gel 40–60 µm (Grace), Duramat pump (CfG); analytical TLC: Merck precoated silica gel 60 F_{254} plates (0.2 mm), spots were identified under a UV lamp ($\lambda = 254$ nm, Camag 29 200) and with a 2.22 mol/L H_2SO_4 solution which contained $Ce(SO_4)_2 \cdot 4H_2O$ (10 g/L) and $H_3[PO_4(Mo_3O_9)_4] \cdot H_2O$ (25 g/L)[24] and heating at 140 °C, or an anisaldehyde reagent for carbohydrates [2 mL of anisaldehyde, 8 mL of concd H_2SO_4 in ethanol (190 mL)]. Medium-pressure liquid chromatography (MPLC): LiChroprep RP-18 material 40–63 µm (Merck) was used. The samples were applied to a pre-column (~2 g of RP-18 material) and eluted at 1.5–2.5 bar using a Duramat (CFG) dosage pump. Analytical HPLC was performed using a HPLC system (Jasco) consisting of an intelligent HPLC pump PU-980, a three-line degasser DG-980-05, a ternary gradient unit LG-980-02 and a multi-wavelength detector MD-910. As eluent a mixture of buffer (prepared from 0.6 g KH_2PO_4, 26.2 g $K_2HPO_4 \times 3H_2O$, 3.0 g 1-heptanesulfonic acid sodium salt monohydrate and 1000 mL of water, pH 8.0) and acetonitrile 60:40 was used. A column 250 × 4.6 mm, nucleosil 300, C18, 5 µm (Jasco) with a pre-column was used. Ultrafiltration (UF) was performed using gas-pressurized (N_2, 3.5 bar) stirred ultrafiltration cells (Amicon, model 8050, 50 mL cell capacity and model 8400, 400 mL capacity) with membrane type YM3 (Amicon, molecular weight cut-off 3000 Da). NMR and MS equipment: NMR: DRX 400 (Bruker), DRX 600 (Bruker), Gemini 200 (Varian), Gemini 2000 (Varian); Mass spectrometry: FAB MS: VG Autospec (Fisons, matrix: 3-nitrobenzyl–alcohol), ESI MS: FT ICR MS APEX II (Bruker Daltonics, water–methanol). Following the molecular formula two masses are always communicated, the first was calculated using the International Atomic Masses, the second is the mono-isotopic mass. IR: Genesis FTIR (ATI Mattson). For the description of the NMR spectra the protons and carbons are indexed according to the indices in the formulae. The MIC (minimum inhibitory concentration) values against seven different *Staphylococcus aureus* strains (ATCC 25923, ATCC 29213, MRSA 1309, SG 511, PEG 18, PEG 5, KNS PEG 5) were determined by a serial twofold micro dilution method on microtiter plates (Iso-Sensitest medium, Oxoid). A series of decreasing concentrations of the compound under investigation was prepared in the

medium. For inoculations 1×10^5 cfu mL^{-1} were used. The MICs were determined (absence of visible turbidity) after 24 h at 37 °C. The MIC values were calculated as the average values from three measurements.

(*R*)-3-({(5*R*)-5-[3-(3-Carboxypropionyl)-1-(4-nitrophenyl)-1*H*-1,2,4-triazol-5-yl]-α-L-arabinopyranosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-2,6,13,17-nonadecatetraenyloxy)propionic acid (4). To a solution of 4-nitroaniline (62.5 mg, 0.45 mmol) in 9% HCl (1.2 mL) a solution of sodium nitrite (31.3 mg, 0.45 mmol) in water (1 mL) was added slowly at 0 °C. After 20 min at 0 °C, this solution was added slowly to a solution of **1a** (500 mg, 0.32 mmol) and sodium acetate (5 g) in water (50 mL), and the mixture was stirred at 20 °C for 48 h. The reaction mixture was desalted by RP18 chromatography eluting with water (200 mL) and then with acetonitrile–water 1:1 (UV monitoring at λ = 280 nm). Solvent evaporation followed by FC ($CHCl_3$–methanol–water 9:6.5:1.4), Sephadex LH 20 LC (methanol–water 3:1), and lyophilization furnished **4** (416 mg, 76%) as a pale orange solid. R_t = 14.8 min, RP HPLC (buffer–acetonitrile = 60:40, λ_{max} = 280 nm, flow 0.5 mL min^{-1}). UV (MeOH): λ_{max} (ε) = 272 nm (10,123). ^{1}H NMR (400 MHz, D_2O, H,H COSY): characteristic signals at δ = 8.52 (d, 2H, 3^{Ar}-H, 5^{Ar}-H), 7.85 (d, 2H, 2^{Ar}-H, 6^{Ar}-H, J = 8.5 Hz), 5.65 (bs, 1H, 1^F-H), 4.92 (d, 1H, 3^F-H, $J_{2F,\ 3F}$ = 10.2 Hz), 2.63 (d, 2H, CH_2-12^I, $J_{12,\ 13}$ = 7.1 Hz), 2.49 (t, 2H, CH_2-3^A, $J_{2A,\ 3A}$ = 6.2 Hz), 1.95, 1.93 (2×s, 6H, $NHCOCH_3^{C,\ E}$), 1.84–1.76 (m, 2H, CH_2-10^I), 1.62 (s, 3H, CH_3-25^I), 1.55 (s, 3H, CH_3-19^I), 1.51 (s, 3H, CH_3-21^I), 1.49 (s, 3H, CH_3-20^I), 1.29–1.21 (m, 2H, CH_2-9^I), 1.11 (s, 3H, CH_3-4^F), 1.08 (d, 3H, CH_3-6^C, J = 6.0 Hz), 0.83 (s, 6H, CH_3-23^I, CH_3-24^I). $C_{75}H_{111}N_8O_{36}P$ (1731.71, 1730.68), ESI ICR MS: m/z = 864.33338 (calcd 864.33465) $[M-2H]^{2-}$, m/z = 875.32560 (calcd 875.32563) $[M-3H+Na]^{2-}$.

(5^B*R*)-5^B-(1-{4-[(2-Aminoethyl)carbamoyl]phenyl}-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl)-5^B-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)carbamoyl]moenomycin A (5a). To a solution of 4-(2-amino-ethylcarbamoyl)-aniline (125 mg, 0.7 mmol) in 9% HCl (5 mL) at 0 °C a precooled (0 °C) solution of sodium nitrite (50 mg 0.7 mmol) in water (10 mL) was added. The mixture was stirred at 0 °C for 15 min. The solution of the diazonium salt was then added slowly to a solution of moenomycin complex (main component moenomycin A, 1 g) and sodium acetate (15 g) in water (500 mL). The reaction mixture was stirred for 144 h at ambient temperature. Progress of the reaction was monitored by TLC (*n*-propanol–water 7:3). After addition of water the solvent was removed by freeze-drying. Careful and repeated FC (*n*-propanol–2 mol/L ammonia 10:3 and RP-18 FC (water, acetonitrile–water 1:3 → 1:1 furnished a pure sample of **5a** (43 mg, 4%) as a pale yellow solid. $C_{78}H_{118}N_9O_{35}P$ (1772.81, 1771.746792), ESI MS: m/z 884.86774, calcd 884.86612 $[M-2H]^{-2}$. NMR spectra: Unit I (below):

Assignment		^{1}H NMR (600 MHz, CD_3OD/DMSO-d_6, H, H COSY, HMBC, HMQC)	^{13}C NMR (150 MHz, CD_3OD/DMSO-d_6)
Unit	Position		
I	1	4.03, m, 3.89, m	67.1
	2	5.26, m	124.0
	3	—	140.4
	4	1.93, m	33.7
	5	1.95, m	32.7
	6	5.10, dt, $J_{5,\ 6}$ = 6 Hz, $J_{6,\ 7}$ = 16 Hz	127.0
	7	5.19, d, $J_{6,\ 7}$ = 16 Hz	141.5
	8	—	36.6
	9	1.19, m	42.9
	10	1.72, m	32.4
	11	—	151.1
	12	2.52, d, $J_{12,\ 13}$ = 7 Hz	36.1
	13	4.97, t, J = 7 Hz	123.5
	14	—	137.5
	15	1.87, m	41.0
	16	1.92, m	27.8
	17	4.94, t, J = 7 Hz	125.6
	18	—	132.4
	19	1.51, s	26.4
	20	1.43, s	18.3
	21	1.44, s	16.5
	22	4.51, s, 4.50, s	109.7
	23	0.78, s	28.13, 28.18
	24	0.78, s	
	25	1.57, s	24.3
H	1	—	?
	2	3.80, m	81.6
	3	4.07, m, 3.90, m	69.0
A	1	—	194.0
	2	3.19–3.13 (exchangable)	36.1
	3	2.50, d, J = 6 Hz (probably caused by H→D exchange of one proton at C-2A)	29.9
	4	—	177.0
B	1	4.33, unter H_2O	103.8
	2	3.36, m	72.4
	3	3.46, m	74.1
	4	4.16, broad signal	71.1
	5	5.02, broad signal	71.2
C	1	4.24, m	102.5
	2	3.25, m	56.9
	3	3.23, m	?
	4	2.42, broad signal	84.3
	5	2.96, broad signal	72.1
	6	1.01, broad signal	18.6
	(CH_3*C*ONH)	—	173.4
	(*CH*$_3$CONH)	1.85 s	24.0

Assignment		^{1}H NMR (600 MHz, CD_3OD/DMSO-d_6, H, H COSY, HMBC, HMQC)	^{13}C NMR (150 MHz, CD_3OD/DMSO-d_6)
Unit	Position		
D	1	4.29, covered by the H_2O signal	104.7
	2	3.02, dd, $J_{1,2} = J_{2,3} = 9$ Hz	75.1
	3	3.26, m	78.2
	4	3.07, m	71.9
	5	3.19, m	78.3
	6	3.70, d, $J_{A,B} = 10$ Hz, 3.47, m	62.8
E	1	4.46, covered by the H_2O signal	103.2
	2	3.54, m	56.7
	3	3.46, m	73.9
	4	3.38, m	81.8
	5	3.49, m	?
	6	3.96, d, $J_{A,B} = 10$ Hz, 1H	69.6
	(CH_3***C***ONH)	—	173.4
	(***CH***$_3$CONH)	1.85, s	23.9
F	1	5.69, s	95.9
	2	3.50, m	78.6
	3	4.88, d, $J_{2,3} = 10$ Hz	75.8
	4	—	74.3
	5	4.26, s	73.7
	6	—	174.2
	(O***C***ONH$_2$)	—	158.8
	(***CH***$_3$)	1.05, s	17.0
TA	3	—	160.0
	5	—	155.3
Ar	1	—	141.7
	2, 6	8.04, d, $J = 9$ Hz	130.1
	3, 5	7.69, d, $J = 7$ Hz	126.8
	4	—	136.2
DAE	(***C***ONH)	—	168.4
	1	3.61, broad signal	?
	2	3.52, m	?

Impurity signals (from RP-18): ^{1}H NMR: 0.74 (m); 0.80 (m); 1.13 (s); 1.14 (s); 1.24 (m) 1.48 (m); 1.74 (s); 2.75 (m); ^{13}C NMR: 14.4; 20.8; 21.3; 30.7; 45.0; 80.0.

(5^{B}*R*)-5^{B}-{1-[4-({2-[3-(4-Carboxyphenyl)thioureido]ethyl}-carbamoyl)phenyl]-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl}-5^{B}-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)carbamoyl]moenomycin A (5b). **5a** (43 mg, 0.025 mmol) was dissolved in 1:1 pyridine–water (1.5 mL) assisted by sonication. After addition of a solution of 4-isothiocyanatobenzoic acid (7 mg, 0.037 mmol) in 1:1 pyridine–water (1 mL) the mixture was stirred at 50 °C for 30 min and for 6 days at ambient temperature. Then a solution of 4-isothiocyanatobenzoic acid (3 mg, 0.017 mmol) in 1:1 pyridine–water (1 mL) was added and stirring continued for 24 h at 20 °C. Progress of the reaction was followed by TLC (*n*-propanol–water 7:3). Water was added and solvents were removed by freeze-drying furnishing **5b** (23 mg, 48%) as a pale yellow solid. ^{31}P NMR (81 MHz, CD_3OD/DMSO-d_6): δ = −1.28 (s). $C_{86}H_{123}N_{10}O_{37}PS$ (1952.01, 1950.750892) ESI MS: *m*/*z* 634.90768, calcd 634.90775 $[M-3H-CONH]^{-3}$; 649.24285, calcd 649.24302 $[M-3H]^{-3}$; 656.57016, calcd 656.57034 $[M+Na-4H]^{-3}$; 974.36913, calcd 974.36817) $[M-2H]^{-2}$; 985.35781, calcd 985.35914) $[M+Na-3H]^{-2}$; 996.35030, calcd 996.35011 $[M-2H+2Na-2H]^{2}$.

5-Amino-*N*-(8-amino-3,6-dioxaoctane)-2-nitrobenzamide. To a stirred solution of 5-amino-2-nitrobenzoic acid (700.0 g, 3.8 mmol) in dry pyridine (25 mL) CDI (800.0 g, 4.9 mmol) was added at 20 °C. After 10 min, the mixture was added to a solution of 1,8-diamino-3,6-dioxaoctane (2 mL) in 5 mL pyridine (5 mL). Heating to 80 °C for 24 h, solvent evaporation and FC (methanol-chloroform 3:7) gave the title compound (950.0 mg, 80%) as a yellow oil. UV/VIS (methanol, 1.2 × 10^{-4} mol L^{-1}): λ_{max} (ε_{max}) = 370 (2603), 372 (2605). IR (KBr): 3363, 1653, 1593, 1313. 1267 cm^{-1}. ^{1}H NMR (CD_3OD, 200 MHz): δ = 8.51 (1H, CONH, $W_{1/2}$ = 5.6 Hz), 7.97 (1H, d, 9.12 Hz, Ar–H), 6.70–6.57 (1H, m, Ar–H), 6.55 (1H, s, Ar–H), 3.75–3.567 (8H, m, C<u>H</u>$_2$O(C<u>H</u>$_2$)$_2$OC<u>H</u>$_2$), 3.57–3.52 (2H, m, CONHC<u>H</u>$_2$), 3.15–3.10 (2H, m, C<u>H</u>$_2$NH$_2$). ^{13}C NMR (DMSO-d_6, 50 MHz): δ = 167.19 (CO), 154.64 (CNH_2^{Ar}), 137.05 (CNO_2^{Ar}), 132.97 (<u>C</u>CO^{Ar}), 127.36, 112.32, 111.88 (3× CH^{Ar}), 73.02 (O<u>C</u>H$_2$CH$_2$NH$_2$), 69.64 (O<u>C</u>H$_2$<u>C</u>H$_2$O), 68.69 (NHCH$_2$CH$_2$O). $C_{13}H_{20}N_4O_5$ (312.33, 312.14337), FAB MS: *m*/*z* = 313.1 ($[M+H]^+$).

5-Amino-*N*-(13-amino-3,6,9-trioxadecane)-2-nitrobenzamide. The title compound was prepared as described for 5-amino-*N*-(8-amino-3,6-dioxaoctane)-2-nitrobenzamide. Yellow oil, yield: 61%. ^{1}H NMR (pyridine-d_5, 300 MHz): δ = 9.31 (1H, $W_{1/2}$ = 5.6 Hz, CONH), 7.43 (2H, bs, Ar–NH$_2$), 7.18–7.17 (1H, d, 8.5 Hz, Ar–H), 6.80–6.79 (1H, dd, *J* = 9.0, 2.4 Hz, Ar–H), 6.77–6.76 (1H, d, *J* = 2.4 Hz, Ar–H), 3.81–3.75 (2H, q, *J* = 6.3 Hz, CONHC<u>H</u>$_2$), 3.65–3.61 (2H, t, *J* = 6.3 Hz, OCH$_2$), 3.60–3.51 (10, m, 5× OCH$_2$), 3.14–3.09 (2H, t, *J* = 6.6 Hz, C<u>H</u>$_2$NH$_2$), 2.10–2.02 (2H, quintet, *J* = 6.6 Hz, CH$_2$), 1.94–1.88 (2H, quintet, *J* = 6.3 Hz, CH$_2$). ^{13}C NMR (D_2O, 50 Hz): δ = 172.93 (CO), 159.08 (CNH_2^{Ar}), 139.48 (C^{Ar}–NO_2), 137.85 (signal that could not be assigned), 132.75 (<u>C</u>CO^{Ar}), 121.68, 118.75, 117.15 (3× CH^{Ar}), 74.08, 73.94, 73.84, 73.74, 72.73 (5× OCH$_2$), 42.15 (CH$_2$NH$_2$), 41.31 (CONHCH$_2$), 32.37, 30.93 (2× CH$_2$). $C_{17}H_{28}N_4O_6$ (384.43, 384.201), FAB MS: *m*/*z* = 385.1 ($[M+H]^+$).

(5^{B}*R*)-5^{B}-{1-[3-(15-Amino-6,9,12-trioxa-2-azapentadecanoyl)-4-nitrophenyl]-3-(3-carboxypropionyl)-1H-1,2,4-triazol-5-yl}-5^{B}-de(2-hydroxy-5-oxocyclopent-1-enylcarbamoyl)moenomycin A (6c). To a stirred solution of 5-amino-*N*-(13-amino-3,6,9-trioxadecane)-2-nitrobenzamide (31.7 mg, 77 μmol) in 6% HCl (0.3 mL) at 3 °C a precooled (3 °C) solution of sodium nitrite (6.2 mg, 89.86 μmol) in water (0.25 mL) was added. After 15 min, the diazonium salt solution was dropped within 20 min into a solution of moenomycin A (118.0 mg, 74.6 μmol) sodium acetate (600.0 mg) in water (50 mL, cooled to 10 °C). The mixture was stirred at 20 °C for 24 h. The mixture was passed through a RP 18 column (elution with acetonitrile–water 3:7). Product fractions were combined and solvents were removed by evaporation. The residue was redissolved in water and freeze-dried. FC (H_2O–*n*-propanol 2:7), solvent evaporation and lyophilization provided **6c** (100.0 mg, 68%) as a pale yellow hygroscopic powder. ^{1}H NMR (CD_3OD/DMSO-d_6 0.5:0.4, 600 MHz): δ = 8.14–8.12 (1H, d, *J* = 8.4 Hz, Ar–H), 7.98 (1H, d, *J* = 8.4 Hz, Ar–H), 7.91 (1H, s, Ar–H), 5.69 (1H, s, $CH^{F\text{-}1}$), 4.37–3.67 (14H, m, carbohydrate signals, 3.44–3.26 (20H, m, CH_2^{I}, CH_2^{spacer}), 2.89–2.77 (2H, m, $CH^{D\text{-}2,3}$), 2.52–2.51 (2H, d, *J* = 7.08 Hz, $CH_2^{I\text{-}12}$), 2.44–2.42 (2H, m, CH_2^{A}), 2.00–1.82 (10H, m, $CH_2^{I\text{-}4,5,15,16}$ $CH_3CONH^{C,\,E}$), 1.78–1.71 (4H, m, $CH_2^{I\text{-}10}$, CH_2^{spacer}), 1.57 (3H, s, $CH_3^{I\text{-}25}$), 1.49 (3H, s, $CH_3^{I\text{-}19}$), 1.43 (3H, s, $CH_3^{I\text{-}20}$), 1.42 (3H, s, $CH_3^{I\text{-}21}$), 1.21–1.11 (7H, CH_2^{spacer}, $CH_2^{I\text{-}9}$, CH_3^{C-6}), 1.04 (3H, s, CH_3^{F}), 0.79 (6H, s, $CH_3^{I\text{-}23,\,24}$).- ^{13}C NMR (CD_3OD/DMSO-d_6 0.5:0.4, 100, 150 MHz): δ = 195.97 (CO^{A}), 181.53 ($COOH^{A}$), 174.85, 173.19 ($\underline{C}H_3\underline{C}ONH^{C,\,E}$), 167.65 (AR–$\underline{C}$ONH), 159.65 ($CONH_2^{F}$), 159.02 ($CO^{TA}$), 154.74 ($C^{TA}$), 150.10 ($CH^{I\text{-}11}$), 147.15 ($C^{Ar}$–$NO_2$), 142.00, 141.50, 141.10 ($CH^{I\text{-}7}$, $C^{I\text{-}3}$, $C^{I\text{-}14}$), 136.78 ($C^{I\text{-}18}$), 134.37 (C^{Ar}), 131.68 ($CH^{I\text{-}6}$), 126.69 (CH^{Ar}), 125.23 ($CH^{I\text{-}17}$), 123.10 ($CH^{I\text{-}2}$), 109.66 ($CH_2^{I\text{-}22}$), 104.29, 103.56 ($CH^{B\text{-}1,\,C\text{-}1,\,D\text{-}1,\,E\text{-}1}$), 77.12, 74.03 ($C^{pentasaccharide}$), 70.60, 69.35 (bs, 8× CH_2^{spacer}), 61.92 ($HOCH_2^{D}$), 56.03 ($CH^{E\text{-}2,C\text{-}2}$), 42.56 ($CH_2^{I\text{-}9}$), 40.56 ($CH_2^{I\text{-}15}$), 38.68, 38.10 ($CH_2NH_2$, $CONHCH_2^{spacer}$), 36.19, 35.71 ($CH_2^{I\text{-}12}$, $C^{I\text{-}8}$), 33.11, 32.32, 31.92 ($CH_2^{I\text{-}4,\,5,\,10}$), 29.28 ($CH_2^{A}$), 28.01, 27.67, 27.51 ($CH_3^{I\text{-}23,24}$, $CH_2^{I\text{-}16}$), 26.40 ($CH_3^{I\text{-}19}$), 24.24 ($CH_3^{I\text{-}25}$), 23.44 ($\underline{C}H_3CONH^{C,\,D}$), 18.33 ($CH_3^{I\text{-}21}$), 17.68 ($CH_3^{C}$), 16.62 ($CH_3^{I\text{-}20}$), 16.00 ($CH_3^{F}$). ^{31}P NMR ($CD_3OD$, 80 MHz): δ = −1.18. $C_{86}H_{133}N_{10}O_{40}P$ (1978.01, 1976.842), ESI MS: *m*/*z* = 987.41356 ($[M-2H]^{2-}$), calcd 987.41363, 657.94185 ($[M-3H]^{3-}$), calcd 657.9400.

(5^{B}*R*)-5^{B}-{1-[3-(10-Amino-5,8-dioxa-2-azadecanoyl)-4-nitrophenyl]-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl}-5^{B}-de(2-hydroxy-5-oxocyclopent-1-enylcarbamoyl)-moenomycin A (6b). **6b** was prepared from moenomycin A and 5-amino-*N*-(8-amino-3,6-dioxaoctane)-2-nitrobenzamide as described for **6c**. Yield: 55%. Pale yellow hygroscopic powder. ^{1}H NMR (CD_3OD, 600 MHz): δ = 8.47–8.45 (1H, d, *J* = 8.4 Hz, Ar–H), 8.10 (1H, d, *J* = 8.4 Hz, Ar–H), 8.05 (1H, s, Ar–H), 5.85 (1H, s, $CH^{F\text{-}1}$), 5.44 (1H, bs, $CH^{I\text{-}7}$), 5.36–5.06 (8H, m, $CH^{I\text{-}2,6,17,13}$, $CH^{B\text{-}5}$, $CH^{F\text{-}3}$), 4.67–4.66 (2H, d, *J* = 7.8 Hz, $CH_2^{I\text{-}22}$), 4.61–3.32 (38H, m, carbohydrate signals), OCH_2^{spacer}, CH_2^{I}-signals), 3.27–3.20 (3H, m, $CH^{D\text{-}2}$, CH_2^{A}), 3.00–2.98 (2H, t, *J* = 7.5 Hz, CH_2^{A}), 2.66–2.65 (2H, d, *J* = 6.8 CH_2^{I-12}), 2.60 (2H, s, CH_2^{A}), 2.14–1.99 (14H, m, $CH_2^{I-4,5,15,16}$, CH_2 NH_2^{spacer}, $CH_3CONH^{C,E}$), 1.88 (2H, m, $CH_2^{I\text{-}10}$), 1.75 (2H, s, $CH_2^{I\text{-}25}$), 1.68–1.65 (5H, m, $NHCH_2^{spacer}$, $CH_3^{I\text{-}19}$), 1.58 und 1.57 (6H, je s, $CH_3^{I\text{-}20,21}$), 1.44–1.40 (2H, m, impurity from RP-18), 1.35 (4H, m, $H_2NCH_2^{spacer}$, $CH_2^{I\text{-}9}$), 1.25 (3H, s, CH_3^{F}), 0.98–0.97 (2H, t, impurity from RP-18), 0.95 (6H, s, $CH_3^{I\text{-}23,24}$). ^{13}C NMR (HMBC, HMQC, CD_3OD/DMSO-d_6 4:1, 150, 100 MHz): δ = 195.11 (CO^{A}), 180.45 ($COOH^{A}$), 176.80 ($COOH^{H}$), 173.95, 173.19 ($CH_3\underline{C}ONH^{C,\,E}$), 167.32 (AR$\underline{C}$ONH), 159.21 ($CONH^{F}$), 158.43 ($C^{TA}$), 154.32 ($C^{TA}$), 149.87 ($CH^{I\text{-}11}$), 146.69 ($C^{Ar}$), 141.54 ($C^{Ar}$), 140.63 ($CH^{I\text{-}7}$), 136.36 ($CH^{I\text{-}3}$), 133.77 ($C^{Ar}$), 131.41 ($CH^{I\text{-}14}$), 128.42 ($C^{Ar}$), 127.01 ($C^{Ar}$), 126.06 ($CH^{I\text{-}6}$), 125.59 ($CH^{I\text{-}17}$), 122.02 ($CH^{I\text{-}13}$), 121.42 ($CH^{I\text{-}2}$), 108.98 ($CH^{I\text{-}22}$), 104.61, 102.73, 101.61 ($CH^{B\text{-}1,C\text{-}1,D\text{-}1}$), 94.94 ($CH^{F\text{-}1}$), 83.47 ($CH^{C\text{-}4}$), 80.87, 80.04 ($CH^{H\text{-}2}$, $CH^{E\text{-}4}$), 77.30, 76.72, 76.59, 75.19, 73.82, 73.47, 73.16, 72.44, 72.25, 71.17, 69.83, 67.80 (carbohydrate signals), 70.40, 70.21 (OCH_2^{spacer}), 66.98 ($CH_2^{I\text{-}1}$), 66.25 (OCH_2^{E}), 61.43 ($HOCH_2^{D}$), 55.53 ($CH^{E\text{-}2,C\text{-}2}$), 42.00 ($CH_2^{I\text{-}9}$), 40.10 ($CH_2^{I\text{-}15}$), 39.67, 39.64 ($\underline{C}H_2NH_2$, $CONHCH_2^{spacer}$), 35.63 ($CH_2^{I\text{-}12}$), 35.21 ($CH_2^{I\text{-}8}$), 32.52, 31.84, 31.53, 31.42 ($CH_2^{I\text{-}4,5,10}$, $C^{I\text{-}8}$), 29.46 (CH_2^{A}), 27.34, 27.31 ($CH_3^{I\text{-}23,24}$), 26.86 ($CH_3^{I\text{-}19}$), 25.66 ($CH_3^{I\text{-}25}$), 23.53, 22.82 ($CH_3CONH^{C,E}$), 17.58 ($CH_3^{I\text{-}20}$), 17.15 (CH_3^{C}), 15.85 ($CH_3^{I\text{-}21}$), 15.48 (CH_3^{F}). $C_{82}H_{125}N_{10}O_{39}P$, 1905.91, 1904.78), ESI MS: *m*/*z* = 951.38600 ($[M-H]^{2-}$), calcd 951.38487, 633.91943 ($[M-3]^{3-}$), calcd 633.92082.

(5^{B}*R*)-5^{B}-[1-(3-{[2-(2-{2-[3-(4-Carboxyphenyl)thioureido]-ethoxy}ethoxy)ethyl]-carbamoyl}-4-nitrophenyl)-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl]-5^{B}-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)carbamoyl]moenomycin A (8a). To a solution of **6b** (60 mg (0.031 mmol) in 1:1 pyridine–water (1 mL, sonication) a solution of 4-isothiocyanatobenzoic acid (14 mg 0.081 mmol) in 1:1 pyridine–water (2 mL, sonication) was added in two portions. The mixture was stirred at 50 °C for 1 h and at 20 °C for 12 h. Progress of the reaction was monitored by TLC (*n*-propanol–water 7:3). Addition of water, lyophilization and RP-18 chromatography (water, acetonitril–water 1:4) provided **8a** (28 mg, 43%) as a pale yellow solid. ^{31}P NMR (122 MHz, CD_3OD/DMSO-d_6): δ = 0.04 (s). $C_{90}H_{130}N_{11}O_{41}PS$ (2085.11, 2083.788400) ESI MS: *m*/*z* 1040.88804, calcd 1040.88692 $[M-2H]^{-2}$.

(5^{B}*R*)-5^{B}-{1-[3-({2-[3-(4-Carboxyphenyl)thioureido]ethyl}-carbamoyl)-4-nitrophenyl]-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl}-5^{B}-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)carbamoyl]moenomycin A (7a). **6a** (45 mg (0.025 mmol) was converted to **7a** by reaction with 4-isothiocyanatobenzoic acid (13.7 mg, 0.074 mmol) as described for **8a**. RP18 chromatography (acetonitrile–water 3:7 gave **7a** (32 mg, 65%) as a colourless solid. ^{31}P NMR (81 MHz, CD_3OD/DMSO-d_6): δ = −0.06 (s). $C_{86}H_{122}N_{11}O_{39}PS$ (1997.00, 1995.73597), ESI MS: *m*/*z* 664.23662, calcd 664.23805 $[M-3H]^{-3}$, 996.86145, calcd 996.86071 $[M-2H]^{-2}$. NMR spectra (below):

Assignment		^{1}H NMR (600 MHz, CD_3OD/DMSO-d_6, H,H COSY)	^{13}C (150 MHz, CD_3OD/DMSO-d_6, HMBC/HMQC)
Unit	Position		
I	1	3.99, m, 3.87, m	67.1
	2	5.21, m	123.6
	3	—	140.8
	4	1.90, m	33.6
	5	1.87, m	32.7
	6	5.08, dt, $J_{5,6}=6$ Hz, $J_{6,7}=16$ Hz	127.1
	7	5.16, d, $J_{6,7}=16$ Hz	141.5
	8	—	36.5
	9	1.16, m	42.9
	10	1.69, m	32.4
	11	—	151.1
	12	2.48, d, $J_{12,13}=7$ Hz	36.0
	13	4.93, t, $J=8$ Hz	123.6
	14	—	137.4
	15	1.81, m	41.0
	16	1.89, m	27.8
	17	4.89 t, $J=7$ Hz	125.5
	18	—	132.3
	19	1.46, s	26.2
	20	1.39, s	18.0
	21	1.40, s	16.4
	22	4.46, covered by the H_2O signal	109.4
	23	0.75, s	28.1
	24	0.75, s	28.1
	25	1.53, s	24.2
H	1	—	?
	2	3.76, broad signal	80.9
	3	4.03, m, 3.89, m	69.0
A	1	—	193.9
	2	3.14, m (mainly exchanged)	35.6
	3	2.46, s, (through H→D exchange of both protons at C-2^A)	29.9
	4	—	177.4
B	1	4.35, d, $J=7$ Hz	105.4
	2	3.42, m	72.1
	3	3.47, m	73.9
	4	4.05, broad signal	71.3
	5	4.97, broad signal	71.1
C	1	4.22, m	102.9
	2	3.38, m	56.7
	3	3.15, m	73.5
	4	2.71, m	85.2
	5	3.30, m	72.4
	6	1.16, m (not separated from CH_2-9^I)	17.9
	(CH_3***C***ONH)	—	173.6
	(***CH***$_3$CONH)	1.81 s	23.7
D	1	4.24, m	104.8
	2	3.00, m	75.1
	3	3.25, m	78.0
	4	3.07, m, covered by the methanol signal	71.8
	5	3.15, m	78.2
	6	3.66, m, 3.46, m	62.7
E	1	4.22, m	104.3
	2	3.64, m	55.8
	3	3.38, m	74.4
	4	3.17, m	82.6
	5	3.26, m	74.9
	6	3.85, m 1H	70.0
	(CH_3***C***ONH)	—	174.1
	(***CH***$_3$CONH)	1.80, s	23.7
F	1	5.69, s	95.9
	2	3.41, m	79.1
	3	4.83, d, $J=10$ Hz	76.2
	4	—	74.3
	5	4.25, s	73.9
	6	—	174.1
	(O***C***ONH$_2$)	—	159.1
	(***CH***$_3$)	1.01, s	16.6
TA	3	—	?
	5	—	155.7
Ar	1	—	147.8
	2	7.95, s	126.7
	3	—	135.3
	4	—	142.5
	5	8.14, d, $J_{5,6}=9$ Hz	127.3
	6	7.90, d	128.8
DAE	(***C***ONH)	—	167.8
	1	3.51, m	? (40.7)
	2	3.44, m	? (41.0)
	(NH***C***SNH)	—	?
BL	1	—	143.6
	2, 6	7.71, d, $J=8$ Hz	131.6
	3, 5	7.34, d, $J=8$ Hz	123.4

Impurity signals (from RP-18): ^{1}H NMR: 0.74 (m); 0.80 (m); 1.13 (s); 1.14 (s); 1.24 (m) 1.48 (m); 1.74 (s); 2.75 (m); ^{13}C NMR: 14.4; 20.8; 21.3; 30.7; 45.0; 80.0.

(5^B*R*)-5^B-{3-(3-Carboxypropionyl)-1-[4-nitro-3-({2-[3-(4-sulfophenyl)thioureido]ethyl}carbamoyl)phenyl]-1*H*-1,2,4-triazol-5-yl}-5^B-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)-carbamoyl]moenomycin A (7b). **6a** (48 mg, 0.026 mmol) was dissolved in 1:1 pyridine–water (1 mL) assisted by sonication. 4-Isothiocyanatobenzenesulfonic acid (15 mg, 65 mmol) dissolved (sonication) in 1:1 pyridine–water (2 mL) was added in two portions. The mixture was stirred at 50 °C for 1 h and at 20 °C for 19 h. Progress of the reaction was followed by TLC (*n*-propanol–water 7:3). Water addition, lyophilization and RP 18 chromatography (water, acetonitrile–water 1:4) gave **7b** (40 mg, 75% as a pale yellow solid. ^{31}P NMR (243 MHz, CD_3OD/DMSO-d_6): $\delta=-1.45$ (s). $C_{85}H_{122}N_{11}O_{40}PS_2$ (2033.05, 2031.702957) ESI MS: *m*/*z* 676.22747, calcd 676.22704 $[M-3H]^{-3}$; 1014.84557, calcd 1014.84420 $[M-2H]^{-2}$. NMR spectra (below):

Assignment		^{1}H NMR (600 MHz, CD_3OD/DMSO-d_6, H, H COSY, HMBC, HMQC)	^{13}C NMR (150 MHz, CD_3OD/DMSO-d_6)
Unit	Position		
I	1	4.12, broad signal, 3.98, broad signal	66.9
	2	5.32, m	123.9
	3	—	140.5
	4	2.02, m	33.7
	5	2.01, m	32.7
	6	5.20, dt, $J_{5,6}=6$ Hz, $J_{6,7}=16$ Hz	127.0
	7	5.31, d, $J_{6,7}=16$ Hz	141.1
	8	—	36.6
	9	1.27, m (not separated from CH_3-6^C)	42.9
	10	1.81, m	32.4
	11	—	151.1
	12	2.60, d, $J_{12,13}=8$ Hz	36.0
	13	5.05, t, $J=7$ Hz	123.5
	14	—	137.4
	15	1.93, t, $J=8$ Hz	40.9
	16	2.00, m	27.8
	17	5.01, t, $J=7$ Hz	125.5
	18	—	132.3
	19	1.57, s	26.3
	20	1.51, s	18.2
	21	1.52, s	16.5
	22	4.59, s, 4.58, s	109.6
	23	0.87, s	28.1
	24	0.87, s	28.1
	25	1.65, s	24.3
H	1	—	177.0
	2	3.84, broad signal	81.2
	3	4.12, broad signal, 3.98, broad signal	69.1
A	1	—	194.1
	2	3.21–3.25, (exchangeable)	36.0
	3	2.52, covered by the DMSO signal	30.8
	4	—	177.9
B	1	4.48, covered by the H_2O signal	105.4
	2	3.49, m	72.1
	3	3.55, m	74.0
	4	4.16, s	71.3
	5	5.12, s	71.3
C	1	4.37, covered by the H_2O signal	102.9
	2	3.48, m	56.7
	3	3.24, m	73.3
	4	2.83, m	85.5
	5	3.39, m	72.3
	6	1.26, partial signal overlap	18.0
	(CH_3***C***ONH)	—	173.2
	(***CH***$_3$CONH)	1.909, s	23.9
D	1	4.30, m	104.6
	2	3.12, dd, $J_{1,2}=J_{2,3}=8$ Hz	75.1
	3	3.31, m	78.2
	4	3.15, m, covered by the methanol signal	71.7
	5	3.23, m	78.2
	6	3.75, d, $J_{A,B}=11$ Hz, 3.55, m	62.7
E	1	4.25, d, $J_{1,2}=8$ Hz	104.3
	2	3.66, m	55.9
	3	3.45, m	?
	4	3.32, m	82.4
	5	3.52, m	?
	6	3.94, d, $J_{A,B}=10$ Hz, 1H	69.4
	(CH_3***C***ONH)	—	173.4
	(***CH***$_3$CONH)	1.906, s	23.8
F	1	5.69, s	95.9
	2	3.51, m	78.8
	3	4.90, d, $J_{2,3}=10$ Hz	75.9
	4	—	74.2
	5	4.29, broad signal	73.8
	6	—	174.2
	(O***C***ONH$_2$)	—	158.8
	(***CH***$_3$)	1.13, s	16.9
TA	3	—	160.3
	5	—	155.7
Ar	1	—	147.1
	2	8.05, s	126.8
	3	—	135.4
	4	—	142.6
	5	8.27, d, $J_{5,6}=9$ Hz	127.2
	6	8.04, d (overlapping with 2-H^{Ar})	128.5
DAE	(***C***ONH)	—	167.5
	1	3.63, m, 1H	? (40.5)
	2	3.54, m	? (40.5)
	(NH***C***SNH)	—	182.4
BL	1	—	141.6
	2, 6	7.66, d, $J=9$ Hz	128.0

Impurity signals (from RP-18 material) as described above.

(5^{B}*R*)-5^{B}-{3-(3-Carboxypropionyl)-1-[4-nitro-3-({2-[3-(4-sulfophenyl)thioureido]ethyl}carbamoyl)phenyl]-1*H*-1,2,4-triazol-5-yl}-5^{B}-de[(2-hydroxy-5-oxo-1-cyclopenten-1-yl)-carbamoyl]moenomycin A (8b). **6b** (58 mg, 0.030 mmol) was converted into **8b** as described for **7b**. RP-18 chromatography (water, acetonitrile–water 1:4) gave **8b** 34 mg, (53%) as a pale yellow solid. ^{31}P NMR (243 MHz, CD_3OD/DMSO-d_6): $\delta=-0.97$ (s). $C_{89}H_{130}N_{11}O_{42}PS_2$ (2121.16, 2119.755386) ESI MS: *m*/*z* 528.93100, calcd 528.93157 $[M-4H]^{-4}$; 705.57685, calcd 705.57785 $[M-3H]^{-3}$; 1058.87021, calcd 1058.87042 $[M-2H]^{-2}$. NMR spectra: (below).

Assignment		^{1}H NMR (600 MHz, CD_3OD/DMSO-d_6, H, H-COSY, HMBC HMQC)	^{13}C NMR (150 MHz, CD_3OD/DMSO-d_6)
Unit	Position		
I	1	4.07, broad signal, 3.92, broad signal	66.9
	2	5.29, broad signal	124.2
	3	—	140.2
	4	1.99, m	33.7
	5	1.98, m	32.8
	6	5.17, dt, $J_{6,7}=15$ Hz	127.1
	7	5.26, d, $J_{6,7}=16$ Hz	141.6
	8	—	36.7
	9	1.27, m (overlapping with other signals)	42.9
	10	1.78, m	32.5
	11	—	151.2
	12	2.57, d, $J_{12,13}=7$ Hz	36.1
	13	5.02, broad signal	123.6
	14	—	137.6
	15	1.90, t, $J=7$ Hz	41.0
	16	1.97, m	27.9
	17	4.98, t, $J=6$ Hz	125.7
	18	—	132.4
	19	1.55, s	26.6
	20	1.48, s	18.5
	21	1.49, s	16.7
	22	4.56, s, 4.55, s	109.8
	23	0.84, s	28.3
	24	0.84, s	28.3
	25	1.62, s	24.5
H	1	—	176.9
	2	3.77, broad signal	81.6
	3	4.07, broad signal, 3.92, broad signal	?
A	1	—	194.2
	2	3.19–3.16 (covered by the methanol signal)	?
	3	2.45, covered by the DMSO signal	31.3
	4	—	178.0
B	1	4.40, m	105.5
	2	3.44, m	72.0
	3	3.47, m	74.1
	4	3.99, broad signal	71.5
	5	5.02, broad signal	71.4
C	1	4.40, m	103.0
	2	3.45, m	56.9
	3	3.27, m	73.4
	4	2.88, dd, $J_{3,4}=J_{4,5}=8$ Hz	86.0
	5	?	72.4
	6	1.21, d (partial signal overlap)	18.3
	(CH_3***C***ONH)	—	172.8
	(***CH***$_3$CONH)	1.85, s	24.1
D	1	4.30, covered by the H_2O signal	104.8
	2	3.04, dd, $J_{1,2}=J_{2,3}=7$ Hz	75.2
	3	3.28, m	78.2
	4	3.10, covered by the methanol signal	71.9
	5	3.19, broad signal	78.4
	6	3.70, d, $J_{A,B}=11$ Hz, 3.50, m	62.9
E	1	4.33, covered by the H_2O signal	104.2
	2	3.59, m	56.1
	3	3.40, m	74.3
	4	3.33, m	82.4
	5	3.38, m	75.2
	6	3.95, d, $J_{A,B}=10$ Hz, 1H	69.8
	(CH_3***C***ONH)	—	173.1
	(***CH***$_3$CONH)	1.85, s	24.1
F	1	5.69, s	95.9
	2	3.49, m	78.8
	3	4.87, d, $J_{2,3}=10$ Hz	76.0
	4	—	74.3
	5	4.25, covered by the H_2O signal	73.8
	6	—	174.1
	(O***C***ONH_2)	—	158.7
	(***CH***$_3$)	1.08, s	17.2
TA	3	—	160.5
	5	—	156.0
Ar	1	—	148.1
	2	7.89, s	127.2
	3	—	135.4
	4	—	142.4
	5	8.18, d, $J_{5,6}=9$ Hz	127.2
	6	8.01, d	129.1
DAO	(***C***ONH)	—	167.1
	1	3.42, m	41.2
	2	3.54, broad signal	70.4
	3	3.54, broad signal	71.5
	4	3.54, broad signal	71.5
	5	3.54, broad signal	71.5
	6	3.42, m	41.2
	(NH***C***SNH)	—	182.3
BL	1	—	142.1
	2, 6	7.57, d, $J=8$ Hz	127.8
	3, 5	7.41, d, $J=8$ Hz	123.5
	4	—	143.5

Impurity signals (from RP-18) as described above.

6β-[(*R*)-2-(2-Ethoxy-3,4-dioxo-1-cyclobuten-1-ylamino)-2-phenylacetamido]-penicillanic acid (9). The mixture of ampicillin sodium salt (Sigma, 0.2 g, 0.53 mmol) and diethyl squarate (0.105 g, 0.62 mmol) in phosphate buffer (10 mL, pH 7.28) was stirred at rt. After 6 h, when ampicillin sodium salt could be not observed anymore by TLC (MeCN/H_2O = 4:1); the solvent was removed by lyophilization. From the crude product (1.032 g, mixture of compound **9** and inorganic salts) the salts could not be removed by UF. The sample for NMR analysis was prepared as follows: the mixture was poured in DMSO-d_6, filtered, and the filtrate was used for NMR measurements. ^{1}H NMR (200 MHz, DMSO-d_6, ^{1}H ^{1}H COSY): δ = 9.44, 9.10 (s, broad s, 1H, 1H, 2-NH, 6^{Pen}-NH), 7.49–7.25 (m, 5H, 2,3,4,5,6^{Ph}-H), 6.01, 5.55 (2 broad s, 0.4H and 0.3H), 5.41–5.25 (m, 2H, 5^{Pen}-H, 6^{Pen}-H), 4.63 (m, 2H, $CH_3C\underline{H}_2O$), 3.83 (s, 1H, 2^{Pen}-H), 1.47, 1.39 (s, s, 3H, 3H, CH_3-9^{Pen}, CH_3-10^{Pen}), 1.32 (m, 3H, $C\underline{H}_3CH_2O$). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 177.49, 171.61, 169.45 (C-1,2,3,4^{SA}), 172.34 (C-1), 169.07 (C-7^{Pen}), 128.32 (C-2,6^{Ph}), 128.01 (C-4^{Ph}), 127.43 (C-3,5^{Ph}), 74.07 (C-2^{Pen}), 68.80 ($CH_3\underline{C}H_2O$), 66.65

(C-5^{Pen}), 64.28 (C-3^{Pen}), 60.30, 57.60 (C-6^{Pen}, C-2), 31.72/31.28 (C-9^{Pen}), 27.43 (C-10^{Pen}), 15.63 ($\underline{C}H_3CH_2O$), the signal of C-8^{Pen} was not observed. $C_{22}H_{23}N_3O_7S$ (473.60, 473.12567), ESI ICR MS (neg. mode, MeCN/H_2O): m/z = 963.25480 (calcd 963.25404) $[2M+H_2O-H]^-$, 945.24287 (calcd 945.24407) $[2M-H]^-$, 472.11825 (calcd 472.11839) $[M-H]^-$.

(*R*)-3-({(5*R*)-5-[1-(3-{[2-(2-{[(*R*)-((2*S*,5*R*,6*R*)-2-Carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-ylcarbamoyl)phenylmethyl]amino}-3,4-dioxo-1-cyclobuten-1-ylamino)ethyl]carbamoyl}-4-nitrophenyl)-3-(3-carboxypropionyl)-1*H*-1,2,4-triazol-5-yl]-α-L-arabinopyranosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamid-osyloxy}hydroxyphosphoryloxy)-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-2,6,13,17-nonadecatetraenyloxy)propionic acid (10). (a) **6a** (53 mg, 0.029 mmol) and the crude compound **9** (0.52 g, ca. 90 mg of **10**, 0.182 mmol) in a mixture of DMF (10 mL) and Et_3N (1 mL) were stirred at 4 °C for 24 h. Progress of the reaction was monitored by TLC (MeCN/H_2O = 4:1, R_f (**33**) = 0.27, R_f (**5**) = 0.1). Then the solvents were removed under vacuum (10^{-2} bar) at rt. To the residue water was added and lyophilized. Inorganic salts and an excess of **32** were removed by UF (YM3, 6 × 40 mL) in the refrigerator. Water was lyophilized. The crude product was dissolved in a small amount of water and kieselguhr was added. After lyophilization the kieselguhr with the adsorbed compounds was transferred onto the top of a FC column. A water-cooled column (4 °C, water-ice) was used. After solvent evaporation and lyophilization 12.6 mg (19%) of pure product **10** were obtained. (b) The mixture of compound **6d** (20 mg, 0.010 mmol) and ampicillin sodium salt **31** (4.2 mg, 0.011 mmol) in DMF (3 mL) and Et_3N (0.5 mL) was stirred at 4 °C for 26 h. Progress of the reaction could not be efficiently monitored by TLC (MeCN/H_2O = 4:1) because of the very close R_f values of **9** (R_f = 0.26) and **6d** (R_f = 0.28). In another solvent system (*n*-propanol/water = 7:2) the separation was not better. After workup procedure as described above and lyophilization the product was analyzed by ESI ICR MS. IR (KBr): 3345, 1755, 1674, 1602, 1508, 1408, 1327, 759, 698 cm^{-1}. IR (KBr) ampicillin: 3355, 1759, 1675, 1604, 1508, 1404, 1325 cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6, ^{1}H,^{1}H COSY, HMBC, HMQC): Only characteristic signals could be assigned, since most of the signals were very broad. δ = 7.50–7.15 (broad m, 5H, 2,3,4,5,6^{Ph}-H), 5.34 (d, 7^I-H, J = 15.1 Hz), 5.25 (broad s, 6^I-H), 5.10 (broad s, 1H, 13^I-H), 5.06 (broad s, 1H, 17^I-H), 4.89 (broad s, 1H), 4.65 (s, 2H, CH_2-22^I), 2.65 (broad d, 2H, CH_2-12^I, J = 5.8 Hz), 2.10–1.95 (broad s, 8H, CH_2-4,5,15,16^I), 1.84 (broad s, 8H, CH_2-10^I, $CH_3CONH^{C,E}$), 1.67 (s, 3H, CH_3-25^I), 1.63 (s, 3H, CH_3-19^I), 1.56, 1.55 (s, s, 6H, CH_3-20,21^I), 1.47 (broad s, CH_3-10^{Pen}), 1.32 (broad s, CH_2-9^I), 1.23 (broad s, 3H), 1.07 (broad s, 3H, CH_3^F), 0.93 (s, 6H, CH_3-23,24^I). ^{13}C NMR (150 MHz, DMSO-d_6, HMBC, HMQC): δ = 192.71 (C-1^A), 183.07, 181.99 (C-3,4^{SA}, COOH^A), 173.94 (COOH^H), 172.23 (C-1), 171.70 ($CONH_2^F$), 169.86, 169.54 ($CH_3\underline{C}ONH^{C,E}$), 168.84 (C-7^{Pen}), 168.30 ($\underline{C}ONH^{Ar}$), 166.71, 164.65 (C-1,2^{SA}), 158.63 (C-3TA), 156.60 ($O\underline{C}ONH_2^F$), 154.51 (C-5TA), 149.25 (C-11^I), 146.70 (C-1^{Ar}), 139.87 (C-7^I), 137.40 (C-3^{Ar}), 135.87 (C-14^I), 130.77 (C-18^I), 128.42, 127.46, 126.72 (broad signals, C-2,6^{Ar}, C-2,3,4,5,6^{Ph}), 125.47 (C-6^I), 124.09 (C-17^I), 123.22 (C-2^I), 121.79 (C-13^I), 108.77 (C-22^I), 102.93, 102.28, 101.15 (broad s, C-1^{B,C,D,E}), 93.77 (C-1^F), 81.00–67.00 (many broad overlapping signals, mainly of sugar carbons), 64.87 (C-3^{Pen}), 61.37 (C-6^D), 59.30, 59.06 (C-6^{Pen}, C-2), 55.33, 54.50 (broad s, C-2^{E,C}), 42.86 (C-2^A), 40.99 (C-9^I), 39.50 (C-15^I, hidden by solvent signals, HMQC), 36.91 (C-2^{AE}), 35.80 (C-1^{AE}), 35.25 (C-8^I), 35.11 (C-3^A), 34.53 (C-22^I), 31.96 (C-4^I), 30.98 (C-5^I), 30.75 (C-10^I), 29.04 (C-9^{Pen}), 28.29, 27.92 (C-10^{Pen}), 27.16 (C-23,24^I), 26.14 (C-16^I), 25.55 (C-19^I), 23.37 (C-25^I), 23.09, 22.99 ($\underline{C}H_3CONH^{C,E}$), 17.59 (C-20^I), 17.24 (CH_3^C), 16.16 (CH_3^F), 15.76 (C-21^I). $C_{98}H_{134}N_{13}O_{43}PS$ 2245.23, 2243.81568, ESI ICR MS (neg. mode, MeCN/H_2O): m/z 1154.42339 (calcd 1153.87345) $[M+3Na-5H]^{2-}$, 1143.42962 (calcd 1142.88194) $[M+2Na-4H]^{2-}$, 1131.89462 (calcd 1131.89099) $[M+Na-3H]^{2-}$, 1120.90120 (calcd 1120.90004) $[M-2H]^{2-}$, 769.28044 (calcd 768.91266) $[M+3Na-6H]^{3-}$, 761.95228 (calcd 761.59154) $[M+2Na-5H]^{3-}$, 746.93268 (calcd 746.93076) $[M-3H]^{3-}$, 571.21341 (calcd 570.93707) $[M+2Na-6H]^{4-}$, 559.94854 (calcd 559.94612) $[M-4H]^{4-}$.

Preparation of PBP 1b-containing membrane fractions

Cells of *Escherichia coli* strain JM109 [K-12 *recA*1, Δ(*lac-pro*AB), *endA*1, *gyrA*96, *thi*1, *hsdR*17, *supE*44, *relA*1, F′(*tra*-D36, *proAB*$^+$, *lacI9*, *lacZ*ΔM15)] carrying the plasmid pJP13 (this strain expresses the structural gene of PBP 1b) were grown in modified Lennox broth (10 g/L Bacto-Trypton, 5 g/L yeast extract, 5 g/L NaCl, 100 μg/mL ampicillin) to an $OD_{550\,nm}$ of 0.25 and the temperature was increased from 30 to 42 °C to induce transcription and overexpression of PBP 1b.[21] After 4 h, cells were harvested by centrifugation (5000*g*) and washed. The cells were resuspended in 0.01 M Tris–maleate buffer (pH 6.8) containing 0.1 mM $MgCl_2$ and 150 mM NaCl and sonicated (18 μm, 3 × 30 s). The lysed cell suspension was centrifuged at 100,000*g* for 90 min to obtain the membrane fraction. The supernatant was discarded. The pellet was resuspended in the same buffer and mixed with an equal volume of buffer containing 2% (w/v) detergent (Triton X-100) and phenylmethanesulfonyl fluoride (final concentration 2 mM) at 30 °C for 30 min to extract PBP 1b. Insoluble residues were removed by centrifugation. Protein concentrations were quantified by the bicinchoninic acid (BCA) method. The membrane extracts were stored at −70 °C.

For STD NMR measurements, the buffer of the membrane extract was exchanged against the same buffer [in D_2O, 0.01 M Tris–maleate (apparent pH 6.8), 0.1 mM $MgCl_2$, 150 mM NaCl]. For the exchange we used 30 K filtron units (PALL, 3 × 12,000*g*, 1 h, 4 °C). The NMR results were unconclusive as discussed in the general part. Further experiments were carried out with membrane particles. Thus, the harvested *E. coli* cells (see

Table 3. STD experiments that were performed

Entry	Protein preparation	Ligand	Buffer in D_2O	Detergent	Temperature	Result
1	Membrane extract	Moenomycin A	Tris–maleate, pH 6.8	1% Triton X-100	rt	STD signals could not be assigned
2	Membrane fraction	Moenomycin A	Sodium phosphate, pH 7.0	—	rt	No STD signals
3	Membrane fraction	Delipidomoenomycin A	Sodium phosphate, pH 7.0	—	rt	No STD signals
4	Membrane fraction	Delipidomoenomycin A	Sodium phosphate pH 7.0	—	20 °C	See Figure 3

below) were washed with 0.2 M sodium phosphate buffer (in D_2O, apparent pH 7.0) and were resuspended in the same buffer. After sonication (18 μm, 3×30 s) the lysed cell suspension was centrifuged at 15,000*g* for 20 min and 4 °C to get the membrane particles. The membrane particles were stored at −20 °C, but not longer than 24 h to avoid degradation.

STD experiments

1D STD NMR experiments were performed according to ref 14 on a Bruker Avance DRX 600-MHz spectrometer equipped with a 5-mm inverse triple-resonance probe head. A Gauss shape pulse of 50 ms at −0.6 ppm (on-resonance irradiation) and +40 ppm (off-resonance irradiation) with a repetition duration of 2 s was used. A trim pulse of 20 ms was applied to eliminate the protein signals. The total experimental time was about 22 h (16 k scans) (Table 3).

FCS measurements

FCS measurements were performed with the ConfoCor® 2 (Evotec Biosystems, Hamburg and Carl Zeiss Jena, Jena). Excitation of tetramethylrhodamine (TMR) was performed at a wavelength of 543 nm (HeNe-laser) with an OD-filter of 1.0 mm. For calibration 5 nM rhodamine 6G was used. For the measurements a droplet of 50 μL of the sample solution was placed on a glass cover slide (Nunc® chamber) on the microscope table. Each sample was measured for 30 s (bleach time 2 s) and repeated 10 times. For every experiment, three independent measurements were performed. The solubilized PBP 1b was prepared as described previously.[21] A purified and characterized solution of PBP 1b was carefully defrosted in ice, centrifuged to remove undissolved particles. For the binding studies a series of different protein concentrations (20 nM bis 2 μM) in 10 mM Tris–maleate (pH 6.8) was preincubated with **11** (5–15 nM) in the dark. The sample solutions were prepared 20 min before the measurements to ensure that equilibrium had been reached. Stock solutions of all further additives (Triton X-100 and NaCl) were diluted in binding buffer. Control experiments were done with binding buffer, tetramethylrhodamine in binding buffer, *PBP 1b* in binding buffer and *PBP 1b* with tetramethylrhodamine. For the competition experiments preincubated samples of PBP 1b/**11** were mixed with moenomycin A in different concentrations (100–1000 μM). Data analysis was performed with the software FCS Access® 2.5 SP2 (Evotec Biosystems, Hamburg, Germany). Depending on the number of fluorescent labelled species in the sample an one- or two-component model was used.

Acknowledgements

We thank Prof. B. Meyer for the STD NMR pulse sequence and Prof. S. Berger for the implementation of the pulse sequence and verification of the experimental conditions. Financial support by the Deutsche Forschungsgemeinschaft, BC Biochemie GmbH (Frankfurt), and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

1. Review: van Heijenoort, J. *Glycobiology* **2001**, *13*, 25 R-36R.
2. Terrak, M.; Ghosh, T. K.; van Heijenoort, J.; van Beemen, J.; Lampilas, M.; Aszodi, J.; Ayala, J. A.; Ghuysen, J.-M.; Nguyen-Destèche, M. *Mol. Microbiol.* **1999**, *34*, 350, and references therein.
3. Wang, Q. M.; Peery, R. B.; Johnson, R. B.; Alborn, W. E.; Yeh, W.-K.; Skatrud, P. L. *J. Bacteriol.* **2001**, *183*, 4779.
4. Vogel, S.; Buchynskyy, A.; Stembera, K.; Richter, K.; Hennig, L.; Müller, D.; Welzel, P.; Maquin, F.; Bonhomme, C.; Lampilas, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1963, and references therein.
5. For a recent review on transglycosylase inhibition, see: Goldman, R. C.; Gange, D. *Curr. Med. Chem.* **2000**, *7*, 801.
6. El-Abadla, N.; Lampilas, M.; Hennig, L.; Findeisen, M.; Welzel, P.; Müller, D.; Markus, A.; van Heijenoort, J. *Tetrahedron* **1999**, *55*, 699, and references therein.
7. Stembera, K.; Vogel, S.; Buchynskyy, A.; Ayala, J. A.; Welzel, P. *Chembiochemistry* **2002**, *3*, 559.
8. For a discussion, see: Anikin, A.; Buchynskyy, A.; Kempin, U.; Stembera, K.; Welzel, P.; Lantzsch, G. *Angew. Chem.* **1999**, *111*, 3931. Anikin, A.; Buchynskyy, A.; Kempin, U.; Stembera, K.; Welzel, P.; Lantzsch, G. *Angew. Chem., Int. Ed.* **1999**, *38*, 3703.
9. Donnerstag, A.; Marzian, S.; Müller, D.; Welzel, P.; Böttger, D.; Stärk, A.; Fehlhaber, H.-W.; Markus, A. *Tetrahedron* **1995**, *51*, 1931.
10. (a) For a detailed discussion, see: Ritzeler, O.; Hennig, L.; Findeisen, M.; Welzel, P.; Müller, D.; Markus, A.; Lemoine, G.; Lampilas, M.; van Heijenoort, J. *Tetrahedron* **1997**, *53*, 1675. (b) Kosmol, R.; Hennig, L.; Welzel, P.; Findeisen, M.; Müller, D.; Markus, A.; van Heijenoort, J. *J. Prakt. Chem.* **1997**, *339*, 340.
11. For a discussion of the aggregation behaviour of moenomycin A, see: Lantzsch, G.; Binder, H.; Heerklotz, H.; Welzel, P.; Klose, G. *Langmuir* **1998**, *14*, 4095.
12. Hennig, A.; Findeisen, M.; Weelzel, P.; Haessner, R. *Magn. Reson. Chem.* **1998**, *36*, 615, and references therein.
13. Kurz, M.; Guba, W.; Vertesy, L. *Eur. J. Biochem.* **1998**, *252*, 500.
14. Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **2001**, *123*, 6108 and references therein.
15. Vogtherr, M.; Peters, T. *J. Am. Chem. Soc.* **2000**, *122*, 6093.

16. Marzian, S.; Happel, M.; Wagner, U.; Müller, D.; Welzel, P. *Tetrahedron* **1994**, *50*, 5299, and references therein.
17. Kempin, U.; Hennig, L.; Knoll, D.; Welzel, P.; Müller, D.; Markus, A.; van Heijenoort, J. *Tetrahedron* **1997**, *53*, 17669.
18. For leading references, see: Martin, N. H.; Allen, N. W.; Moore, J. C. *J. Mol. Graph. Model.* **2000**, *18*, 242.
19. Stembera, K.; Vogel, S.; Buchynskyy, A.; Ayala, J.; Welzel, P. *Chem. Biochem.* **2002**, *3*, 559.
20. Buchynskyy, A.; Kempin, U.; Vogel, S.; Hennig, L.; Findeisen, M.; Müller, D.; Giesa, S.; Knoll, H.; Welzel, P. *Eur. J. Org. Chem.* **2002**, 1149.
21. Stembera, K.; Buchynskyy, A.; Vogel, S.; Knoll, D.; Osman, A. A.; Ayala, J. A.; Welzel, P. *Chembiochemistry* **2002**, *3*, 332.
22. Wohland, T.; Friedrich, K.; Hovius, R.; Vogel, H. *Biochemistry* **1999**, 8671.
23. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
24. Kritchevsky, D.; Kirk, M. R. *Arch. Biochem. Biophys.* **1952**, *35*, 346.