On Penicillin-Binding Protein 1b Affinity-Labeling Reagents

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Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

The synthesis of some 3-aryl-3-(trifluoromethyl)3*H*-diazirine and benzophenone-based photoaffinity labels is reported. The photolabile group is bound to a scaffold that also accommodates functional groups to which an indicator unit (biotin) and the bioactive ligand can be attached orthogonally. To three of the labels, moenomycin was conjugated with the aim to provide tools for the identification of the moenomycin binding site within the transglycosylase domain of the enzyme PBP 1b. Some preliminary photoaffinity-labeling experiments were carried out.

Introduction. – Peptidoglycan is an essential cell-wall constituent of almost all eubacteria. It is a heteropolymer consisting of glycan strands cross-linked *via* short peptide chains. The final events of peptidoglycan biosynthesis at the outside of the cytoplasmic membrane are the formation of the macromolecular architecture by two major types of reaction, *i*) formation of the polysaccharide strands (transglycosylation) and *ii*) formation of peptide cross-linkages between the glycan chains (transpeptidation) (for review, see [1]). A representation of the transition state of the transglycosylation reaction is shown in *Fig. 1,a* [2]. Both the glycosyl donor (the growing peptidoglycan chain) and the glycosyl acceptor (the disaccharide-derived lipid II) (see [1]) are attached to the outer face of the cytoplasmic membrane *via* a C_{55} anchor. The transglycosylation 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 [3]. It comprises 844 amino acid residues and contains a short cytosolic N-terminus, a membrane span, the D198-G435 glycosyl-transferase module, and the Q447-N844 acyl-transferase module. Within the glycosyl-transferase module, Glu233 has been shown to be central to the transglycosylation, and, in the active site of the acyl transferase, Ser510 is essential, the acylation of which forms the basis of the antibiotic properties of the β-lactams. Recently, the soluble extracellular region of PBP 1b from *Streptococcus pneumoniae* has been expressed and characterized biochemically [4]. From the most-recent kinetic characterization of the *E. coli* PBP 1b, it was deduced that a doubly charged metal ion bound to the diphosphate group assists departure of the leaving group, whereas the essential Glu233 acts as a base, removing the proton from the 4-OH group of the glycosyl acceptor (see *Fig. 1,a*) [5].

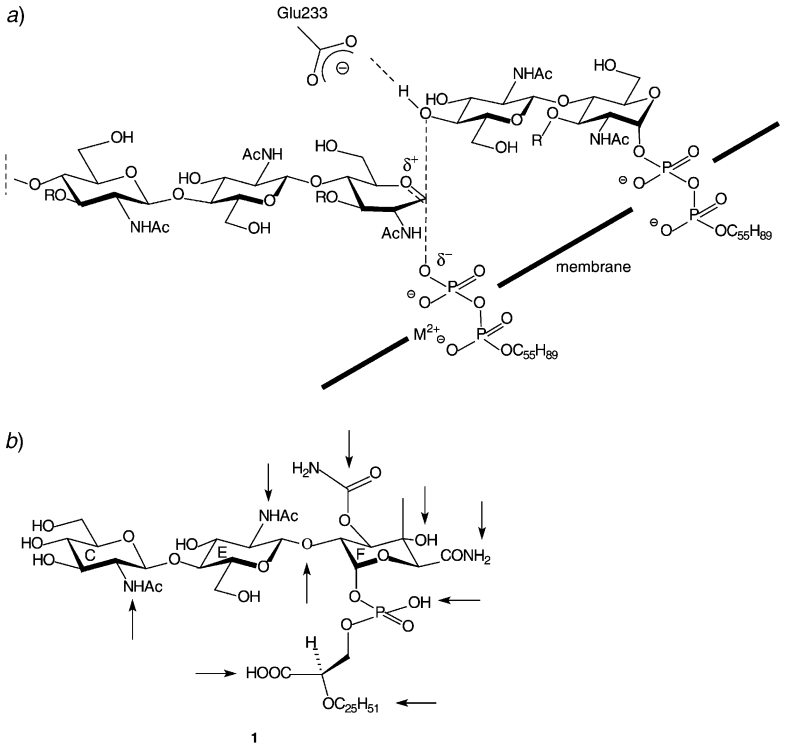
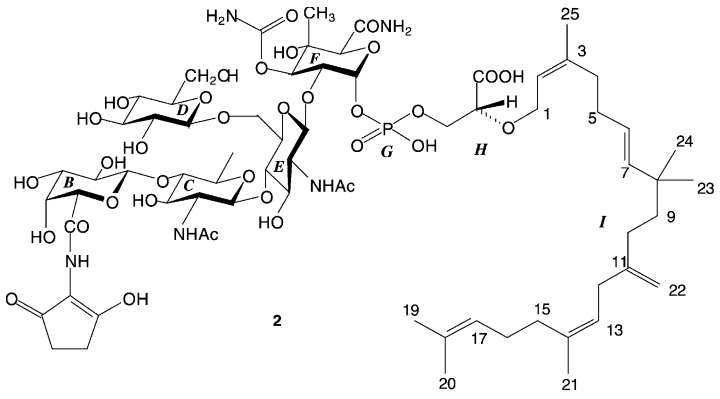


Fig. 1. a) *Putative mechanism of the transglycosylation reaction*; b) *SAR results of moenomycin A*. Arrows indicate indispensible pharmacophoric groups.

We are interested mainly in the interactions of the antibiotic moenomycin A with PBP 1b. The moenomycin antibiotics are known to interfere with the transglycosylation reaction [6] (for a recent review on transglycosylase inhibition, see [7]). They belong to the rare group of compounds known with certainty to inhibit the transglycosylase [8] and are, thus, important tools for localizing the transglycosylase domain on PBP 1b and for unraveling the mechanism of the transglycosylation reaction. Furthermore, they are very interesting lead compounds for the development of medicinally valuable new antibiotics [9]. Moenomycin A (**2**) has been demonstrated to bind reversibly to *E. coli* PBP 1b [1]. From structure-activity relationships [10][11], it has been concluded that the moenomycins, after unselective anchoring to the cytoplasmic membrane *via* their lipid moiety (for a discussion, see [12]), bind highly selectively *via* their carbohydrate part to the donor-binding subsites of the enzyme. The structural features that are known to be responsible for the antibiotic activity are indicated by arrows in **1** (see *Fig. 1,b*) [10][11]. It is assumed that units *C* and *E* of **1** and **2** compete with the second and the third sugar unit of the growing peptidoglycan chain for the binding subsites at the enzyme. The different binding of the first two sugar units ($1 \rightarrow 4$ in the growing peptidoglycan strand *vs.* $1 \rightarrow 2$ in **1** and **2**) has been suggested to be responsible for the inhibition of the enzyme (for a detailed discussion, see [2][10]). The conclusions from

structure-activity-relationship (SAR) studies are corroborated by NMR results, especially from saturation-transfer-difference (STD) NMR experiments [13][14] from which it has been inferred that the *N*-acetyl groups of units *C* and *E* are in contact with the enzyme in the ligand/protein complex [15] (see also [16])[1]). The generation of a soluble and functional extracellular form of PBP 1b [4] in *Vernet*'s laboratory was undertaken with the goal to determine eventually a crystal structure. Even the crystal structure of the complex with moenomycin or a suitable moenomycin derivative with this PBP 1b construct may become available. But some care should be taken. There are differences in the *in vivo* (*Staphylococcus aureus MIC* values) and the *in vitro* SAR results which have been taken as a hint that the access to the donor and acceptor binding sites of the transglycosylase differs in the enzyme preparations from those in the intact bacterium [1][11]. With this in mind, we started some time ago to develop moenomycin-derived tools that will allow us to perform affinity-labeling studies [18–20]. It is hoped that they will bring about independent information on the mode of binding of the antibiotic to the transglycosylase domain of PBP 1b (for some reviews on photoaffinity labeling, see [21]).



We describe here the synthesis of three moenomycin-derived photoaffinity labels as well as some preliminary photoaffinity-labeling studies.

Results[2]). – *Syntheses.* All new photoactivatable probes[3]) are based on our concept that allows the attachment of the photophore, the biotin tag, and the ligand orthogonally to a trifunctional carrier [18][23]. In **4d**, **8**, **10e**, and **11**, the trifunctional scaffold is isoserine (= 3-amino-2-hydroxypropanoic acid), and in **4e**, **9**, and **10f**, it is an amine N-atom. The probes, **4d**, **4e**, **4f**, **8**, **9** contain furthermore an alkyl 3-nitrophenyl ether unit, *i.e.*, an element with a cleavable bond, which is broken by a photo-substitution reaction to yield the corresponding 3-nitrophenol [24]. As suggested by *Nakanishi* and co-workers [25], the latter reaction can be used at the end of the

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[1]) The conclusions drawn from some recent kinetic studies need to be rediscussed in view of our previous results [17].

[2]) Some results have been published in a preliminary communication [20].

[3]) For a comparison of photoaffinity-labeling probes, see [22].

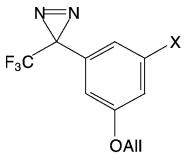
photoaffinity-labeling procedure to remove both the ligand and the biotin tag from the labeled peptide prior to MS sequencing to diminish complications in the MS analysis.

The synthesis of **3a** followed a route developed by *Nakanishi* and co-workers [25]. Amine **3a** has previously been oxidized to the corresponding nitro compound **3b** with dimethyldioxirane [25]. The reported yield was 66%. We found the reaction to be rather capricious. In our hands, the method of *Krohn* and co-workers [26], *i.e.*, $Zr(^tBuO)_4$-mediated oxidation with *tert*-butyl hydroperoxide, gave far more-reliable results. The yields of **3b** were uniformly in the range of 80%. On oxidative degradation (OsO_4, then $NaIO_4$, immobilized on silica gel [27]) **3b** provided aldehyde **4a** (yield 74%), and reduction of the latter with $NaBH_4$ furnished primary alcohol **4b** in 84% yield.

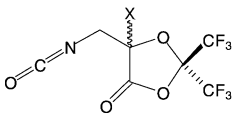
Isocyanates **5** (both enantiomers) were obtained from (*R*)- and (*S*)-malic acid, respectively, as described previously [28]. Coupling of **5a** with primary alcohol **4b** gave urethane **4c** in 88% yield. Reaction of **4c** with the biotin derivative **6b** (obtained from the corresponding commercial diamine and 1,1′-carbonylbis[1*H*-imidazole] (CDI)-activated biotin in pyridine) in 1,2-dimethoxyethane/H_2O 1:4 gave **4d** by amide formation and concomitant loss of the protecting group (65% yield after lyophilization and flash chromatography). The ^1H- and ^{13}C-NMR spectra of **4d** were fully assigned by using $^1H,^1H$-COSY and $^{13}C,^1H$-COSY experiments. The ^{19}F-NMR signal was found at δ 12.65 ($CDCl_3$ solution, δ (CF_3COOH) 0). The ESI mass spectrum showed the expected quasi-molecular-ion peaks. Isoserine derivative **4d** is a broadly applicable biotin-tagged photoaffinity label to which any suitably functionalized ligand can be attached.

We coupled **4d** to the moenomycin derivative **7b** (obtained from moenomycin A *via* the azo coupling/*Japp–Klingemann* route as described previously [29]). The bifunctional linker 4-thiocyanatobenzoyl chloride was used for the conjugation reaction. First **4d** was treated with the acid chloride in dry pyridine in the presence of *Steglich*'s base to provide the corresponding ester. Due to its hydrolytic sensitivity, the ester could not be isolated and was immediately treated with **7b**. Wasteful chromatographic separations were necessary to obtain pure **8**, which was, thus, isolated in a poor yield of 10%. Compound **8** could be detected on TLC by anisaldehyde and 4-(dimethylamino)benzaldehyde dipping reagents that suggested the presence of a sugar and the biotin part, respectively. The structure of **8** was confirmed by ^1H- and ^{13}C-NMR and mass spectrometry.

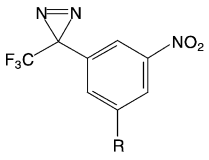
A second broadly applicable photoaffinity reagent based on a 3-nitro-alkoxyphenyl substituted 3-(trifluoromethyl)-3*H*-diazirine was obtained from aldehyde **4a** and amino compound **6a** by reductive amination to give secondary amine **4e** in 60% yield. Like **4d**, **4e** can be attached to any ligand armed with a suitable functional group. We coupled **4e** to the moenomycin-derived amine **7a** [29] *via* the squaric acid linker [30–32]. Thus, reaction of **4e** with diethyl squarate provided 'squaric acid ester amide' **4f** (a 3-amino-4-ethoxycyclobut-3-ene-1,2-dione) in 91% yield. As usual for 'squaric acid amides' [30], two sets of ^{13}C-NMR signals appeared for many C-atoms, indicating the presence of two conformers [29]. Accordingly, there were two ^{19}F-NMR signals. On reaction with the moenomycin derivative **7a** in H_2O/MeOH 1:1 at pH 9, **4f** furnished compound **9** (32% yield), which was characterized by NMR and mass spectrometry. The relevant photochemistry of both **8** and **9** has been discussed elsewhere [20].



3a X = NH_2
b X = NO_2



5a X = α-H (D)
b X = β-H (L)

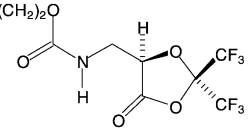


4a-e

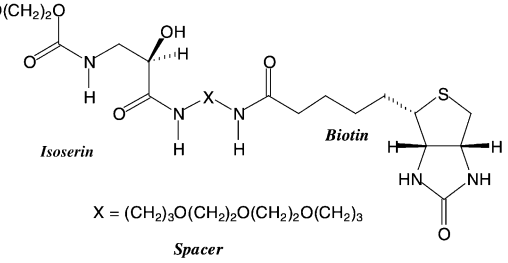
4a R = OCH_2CHO

4b R = OCH_2CH_2OH

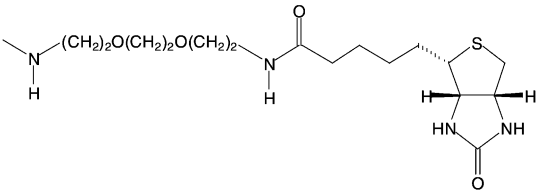
4c R = $O(CH_2)_2O$



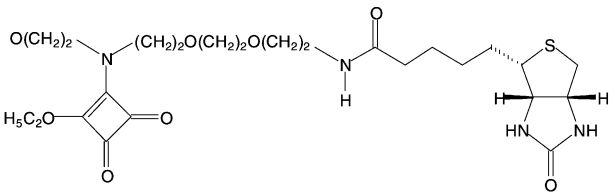
4d R = $O(CH_2)_2O$



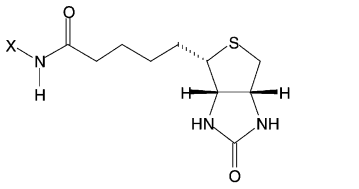
4e R = $O(CH_2)_2$ $(CH_2)_2O(CH_2)_2O(CH_2)_2$



4f R = $O(CH_2)_2$ $(CH_2)_2O(CH_2)_2O(CH_2)_2$

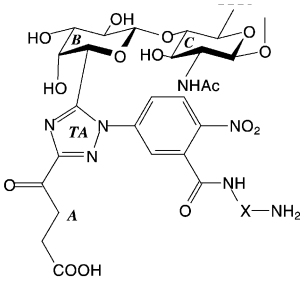


The synthesis of the benzophenone-based photoprobes **10e** and **10f** started with 4-benzoylbenzoic acid, which was converted to the allyl [33] and the 3-hydroxypropyl esters **10a** and **10c**, respectively, making use of *Staab*'s 1,1′-carbonylbis[[1*H*]-imidazole]



6a $X=H_2N(CH_2)_2O(CH_2)_2O(CH_2)_2$

b $X=H_2N(CH_2)_3O(CH_2)_2O(CH_2)_2O(CH_2)_3$



7a $X=CH_2CH_2$

b $X=CH_2CH_2O(CH_2)_2OCH_2CH_2$

c $X=CH_2(CH_2)_2O(CH_2)_2O(CH_2)_2O(CH_2)_2CH_2$

method [34]. Ozonolysis followed by reduction converted allyl ester **10a** to the 2-oxoethyl-ester **10b**.

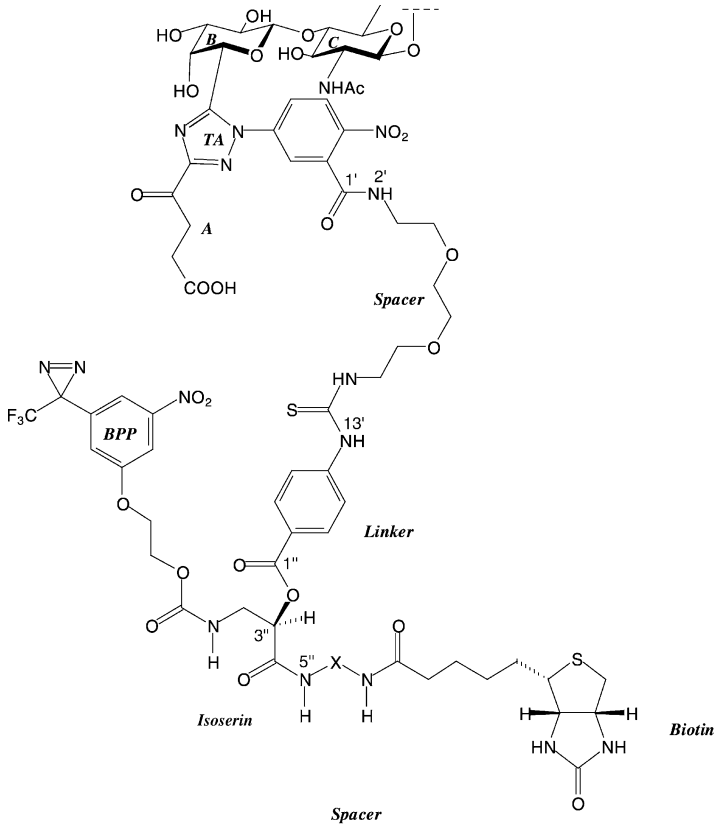
Reaction of **10a** with *Burger*'s isocyanate **5b** [28] furnished urethane **10d**, which on reaction with the biotin-derived amine **6a**, provided the isoserine derivative **10e** to which again any ligand can be attached by suitable methods. We prepared **11** from **10e** and the moenomycin-derived amine **7b** as described above for **8**. The structure of **11** is in accord with the ^{1}H- and ^{13}C-NMR and mass spectra. Finally, amine **10f** was prepared in 43% yield by reductive amination from aldehyde **10b** and the biotin-derived amine **6a**.

Antibiotic Properties of Selected Moenomycin Conjugates. The minimum inhibitory concentration (*MIC*) values against seven different *Staph. aureus* strains (ATCC 25923, ATCC 29213, MRSA 1309, SG 511, PEG 18, PEG 5, and KNS PEG 5) were determined by a serial twofold micro-dilution method on microtiter plates as described previously [12]. All compounds displayed strong antibiotic activity, albeit weaker than that of moenomycin A itself [35] (*Table 1*).

Table 1. *Minimum Inhibitory Concentration* (*MIC*) *of Moenomycin Derivatives against a Number of* Staph. aureus *Strains*

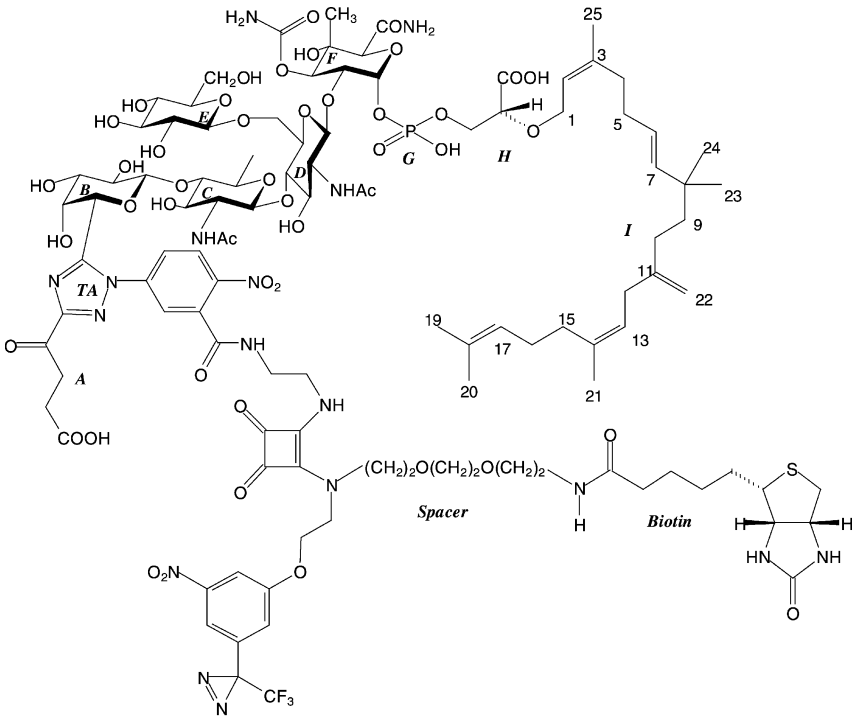
	MIC	
	[μmol/l]	[μg/ml]
2	0.0069	0.029
7a	0.15	0.27
7b	0.038	0.013
7c	0.053	0.11
8	0.32	0.81
9	0.48	0.14
11	0.058	0.16

Preliminary Photoaffinity-Labeling Experiments. PBP 1b was obtained and purified by ampicillin affinity chromatography as described previously [11]. Cross-linking with affinity label **9** was achieved by irradiation at 350 nm (*Rayonet* reactor). The *Coomassie*-Blue staining after SDS-PAGE (sodium dodecyl sulfate polyacrylamide



electrophoresis) revealed the expected pattern (not shown, see [36]). Evidence for a selective affinity labeling was obtained from the Western-blot analysis. Visualization of the transferred band by the streptavidine–alkaline-phosphatase-conjugate method clearly demonstrated a biotin-containing, *i.e.*, labeled component in the band (*Fig. 2*, *Lane 3*). Two control experiments were carried out. In the first, all experimental steps were performed as described above, with the exception that the enzyme was incubated both with **9** and an excess of moenomycin A (**2**). After SDS-PAGE and Western blotting, the visualization by the streptavidin–alkaline-phosphatese-conjugate method showed a band with greatly diminished intensity (*Fig. 2*, *Lane 1*) indicating the competition of **9** and **2** for the binding site at the enzyme. In the second control experiment, again all steps were executed as described for the labeling experiment, with the exception that irradiation was omitted. After blotting, the PBP 1b band gave no color reaction on applying the streptavidin–alkaline-phosphatase-conjugate method (*Fig. 2*, *Lane 2*).

We were interested to further localize the labeling site on the enzyme. An endoproteinase Lys-C digest seemed ideally suited since, as *Table 2* shows, theoretically



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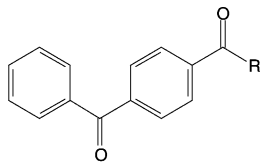
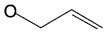
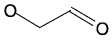
only two large fragments are expected from such a digest, one comprising a large part (109 amino acid residues) of the PBP 1b transglycosylase domain and a second one from the C-terminal part of the enzyme. After submitting PBP 1b to an endoproteinase Lys-C digest, the SDS-tricine-PAGE (*Coomassie*-Blue staining) displayed many bands (not shown). However, when the affinity-labeled specimen of PBP 1b was submitted to the endoproteinase Lys-C digest, only one major band in the expected molecular-mass region (*Fig. 3*) could be identified after SDS-tricine-PAGE followed by Western blotting and visualization with the streptavidin–alkaline-phosphatese conjugate method. We take this result as a hint that indeed the transglycosylase domain of PBP 1b was labeled. As *Fig. 3* indicates, there is still a large portion of uncleaved and only partially degraded PBP 1b is present. We did not attempt to optimize the digest since the amount of material in the desired mass-region band was obviously sufficient for mass-spectrometric sequencing. This work has yet to be performed.

We would like to express our gratitude to *Renate Herold* (this department) for determining the *MIC* values and to Dr. *J. A. Ayala* (Madrid) for providing us with the PBP 1b overproducing *E. coli* strain JM109/pJP13. Financial support by the *Deutsche Forschungsgemeinschaft*, the *Fonds der Chemischen Industrie*, and *Sandoz Industrial Products GmbH* (formerly *BC Biochemie GmbH*) is gratefully acknowledged.

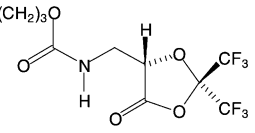


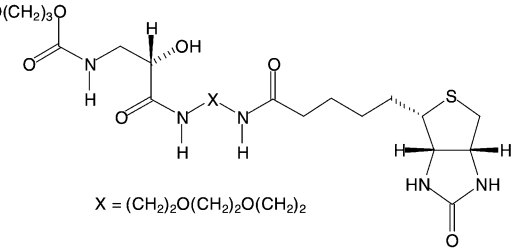
10a-f

10a R = 

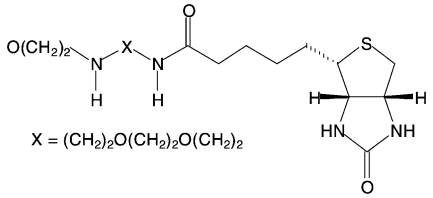
10b R = 

10c R = $O(CH_2)_3OH$

10d R = 

10e R = 

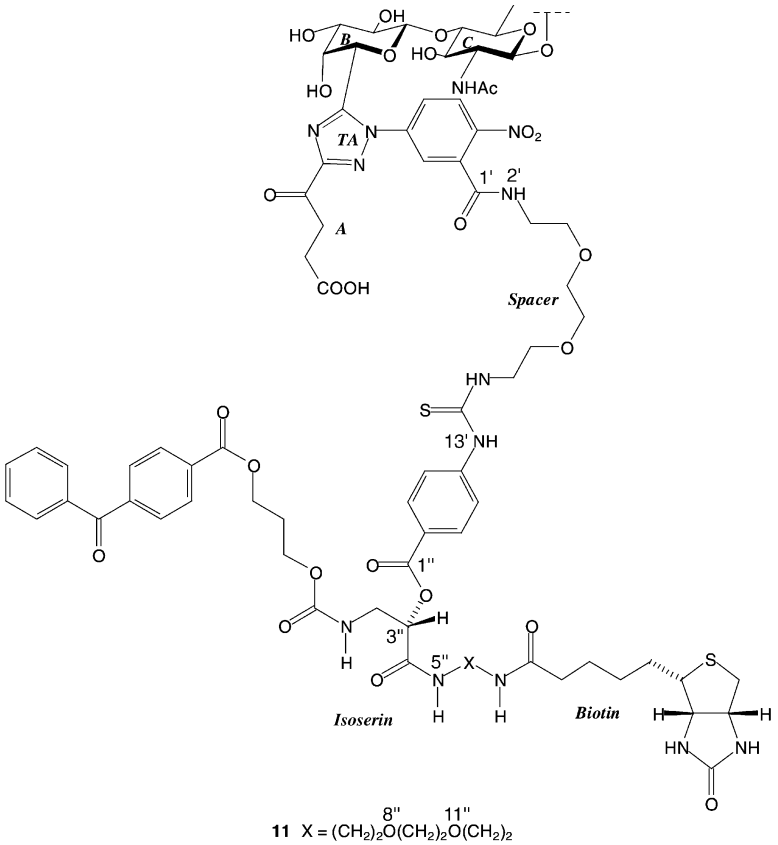
X = $(CH_2)_2O(CH_2)_2O(CH_2)_2$

10f R = 

X = $(CH_2)_2O(CH_2)_2O(CH_2)_2$

Experimental Part

General. All O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of Ar. Liquids and solns. were transferred by syringe. Small-scale reactions were performed in *Wheaton* serum bottles sealed with aluminium caps with open top and *Teflon*-faced septa (*Aldrich*). Usual workup means partitioning the reaction mixture between an aq. phase and CH_2Cl_2, drying the combined org. soln. (Na_2SO_4), and evaporation (bath temp. 45°). All light-sensitive compounds were handled under dimmed light and in brown glassware. Solvents were purified by standard techniques. TLC: pre-coated aluminium plates (silica gel *60* F_{254}, *Merck*); unless stated otherwise, visualization with phosphormolybdate/ceric sulfate reagent [37]. Flash column chromatography (FC): silica gel *60* (*Merck*; 40–63 μm). UV Spectra: λ_{max} (ε) in nm. IR Spectra: FT-IR spectrometer (*ATI Mattson*, *Genesis* series); in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian Gemini-200*, *Gemini-2000*, *Gemini-300*, *Bruker DRX-400*, and *DRX-600* spectrometers; $CDCl_3$ as a solvent unless otherwise stated; δ in ppm, with the signals of $CHCl_3$ (δ 7.26) and of $CDCl_3$ (δ 77.16) as internal references, *J* values in Hz; signal assignments by means of ^1H,^1H-COSY, HMQC, HMBC, and, in some complicated cases, partly by analogy with the fully analyzed moenomycin spectra [38]; H- and C-atoms are characterized according to *Formulae* **2** and **8**, bi = biotin, is = isoserine, sp = spacer, sa = squaric acid unit. MS: *VG ZAB-HSQ* (*VG Analytics*) with 3-nitrobenzyl alcohol as a matrix (FAB), *Finnigan MAT 212* (EI) and *FT ICR* (*MS 7 T APEX II*, *Bruker Daltonics*, H_2O/MeOH) in the pos. or neg. mode (ESI); in *m*/*z*; following the molecular formula, two calc.



masses are given in parentheses, the first one being calculated with the International Atomic Masses, the second one being the mono-isotopic mass.

*3-[3-Nitro-5-(prop-2-enyloxy)phenyl)-3-(trifluoromethyl)-3*H*-diazirine* (**3b**). To a soln. of 3-(prop-2-enyloxy)-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzenamine [25] (**3a**; 970.0 mg, 3.77 mmol) in dry CH_2Cl_2 (200 ml) containing freshly activated 3Å molecular sieves (5.0 g) at 20°, zirconium tetra(*tert*-butoxide) (0.15 ml, 0.4 mmol) was added, and the mixture was stirred for 2 h. Then, within 10 min *tert*-butyl hydroperoxide (2 ml, 11 mmol) was added. After 24 h (TLC monitoring (cyclohexane/AcOEt 8:2), visualization with tin(II) chloride, hydrochloric acid, 4-(dimethylamino)benzaldehyde), H_2O (5 ml) was added. Usual workup (CH_2Cl_2) and FC (MeOH/$CHCl_3$ 0.7:9.3, and AcOEt/cyclohexane 2:8) furnished **3b** (900.0 mg, 83%). Yellow highly viscous oil, which, according to the spectra, was slightly impure. ^{1}H-NMR (200 MHz, $CDCl_3$): 6.40 (*m*, 1 arom. H); 6.19 (*m*, 2 arom. H); 6.12–5.96 (*m*, CH_2=C*H*CH_2O); 5.49–5.42 (1 H, C*H*$_2$=CHCH_2O); 5.34–5.29 (1 H, C*H*$_2$=CHCH_2O); 4.60–4.56 (*m*, CH_2=CHCH_2O). ^{13}C-NMR (50 Hz, $CDCl_3$): 159.66 (C(5′)); 149.65 (C(3′)); 132.04 (C(1′)); 131.56 (CH_2=*C*HCH_2O); *ca.* 121.00 (CF_3); 119.93 (arom. CH); 119.29 (*C*H$_2$=CHCH_2O); 113.89, 110.45 (2 arom. CH); 69.92 (CH_2=CH*C*H$_2$O); 28.31 (*q*, ^2J(C,F) = 41.2 (C(3)). ^{19}F-NMR (188 Hz, $CDCl_3$): 11.35 (*s*, CF_3). EI-MS ($C_{11}H_8F_3N_3O_3$; 287.20, 287.052): 286.9 (10, $M^{+\cdot}$), 258.9 (25, $C_{11}H_8NO_3F_3^+$), 212.9 (35, $C_{11}H_8OF_3^+$), 40.8 (100, $C_3H_5^+$).

*2-{3-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}ethanol* (**4b**). To a soln. of 2-[3-nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetaldehyde [25] (**4a**; 419.0 mg, 1.50 mmol) in dry THF/MeOH 4:1 (50 ml) at –2°, $NaBH_4$ (55 mg, 0.89 mmol) was added, and the mixture was allowed to warm to 5°. After 1 h (TLC monitoring (AcOEt/petroleum ether/toluene 3:3:4)), the reaction was stopped by addition of AcOH (1 ml). Evaporation and FC (AcOEt/petroleum ether/toluene 0.4:1:1) provided **4b** (354 mg, 84%). Yellow

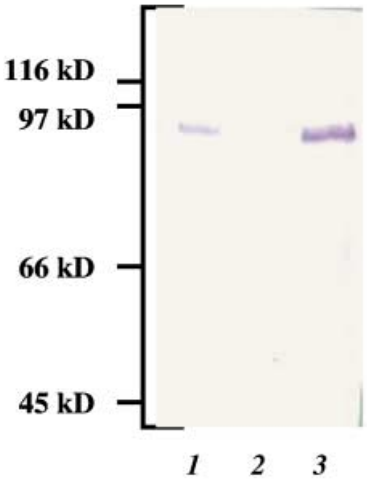


Fig. 2. *Western-blot analysis of the photoaffinity labeling of PBP 1b with* **9**. Photolabeling, SDS-PAGE, and Western blotting were performed as described in the *Exper. Part*. For staining, the streptavidine–alkaline-phosphatase-conjugate method was employed. *Lane 1:* control experiment, *i.e.*, PBP 1b was incubated with **9** and moenomycin A (1000-fold excess) prior to the photolabeling procedure; *Lane 2:* control experiment, *i.e.*, PBP 1b + **9**, not irradiated; *Lane 3:* photolabeling of PBP 1b with **9**.

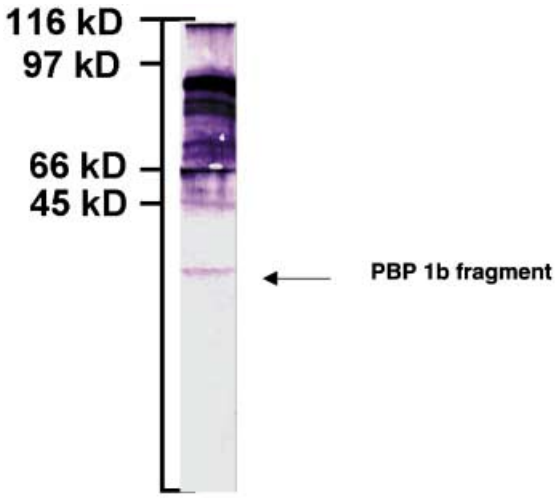


Fig. 3. *Western-blot analysis of the endoproteinase Lys-C digested photolabeled PBP 1b*. For staining, the streptavidine–alkaline-phosphatase-conjugate method was used. For exper. conditions and details, see text.

solid. UV ($CHCl_3$): 241.50 (5777), 263.50 (3599), 324.50 (1992). IR (KBr): 1622, 1587, 1547, 1444, 1349, 1294, 1263, 1164, 1082, 1061, 957, 905, 870, 750, 687. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.85–7.83 (*m*, 1 arom. H); 7.67–7.66 (*m*, 1 arom. H); 7.16–7.14 (*m*, 1 arom. H); 4.24–4.21 (*m*, OCH_2CH_2OH); 4.08–4.06 (*m*, OCH_2CH_2OH); 2.10 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.74 (C(1′)); 149.52 (C(3′)); 132.09 (C(5′)); 121.67 (*q*, = ^1J(C,F) = 274.8, CF_3); 119.76, 114.04, 110.02 (3 arom. CH); 70.49 (OCH_2CH_2OH); 61.10 (OCH_2CH_2OH); 28.32 (*q* ^2J(C,F) = 40.8, CF_3-CN_2). ^{19}F-NMR (282 MHz, $CDCl_3$): 12.63 (*s*, CF_3). EI-MS ($C_{10}H_8F_3N_3O_4$; 291.19, 291.047): 291.0 (10, $M^{+\bullet}$), 263.0 (25, $C_{10}H_8F_3NO_4^{+\bullet}$), 217.0 (25, $C_{10}H_8F_3O_2^+$), 172.9 (100, $C_8H_3F_3O^{+\bullet}$), 44.9 (75, $C_2H_5O^+$).

1,2-O-*(Hexafluoroisopropylidine)*-N-*{{2-{3-nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}ethoxy}carbonyl}*-D-*isoserine* (=*2-{3-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}ethyl {[(4*R*)-2,2-Bis(trifluoromethyl)-5-oxo-1,3-dioxolan-4-yl]methyl}carbamate*; **4c**). To a soln. of **4b** (220.0 mg, 0.76 mmol) in dry $CHCl_3$ (40 ml), **5a** [28] (316.0 mg, 1134 μmol) was added, and the mixture was stirred at 20° for 5 d (TLC control (petroleum ether/AcOEt 1:1)). Evaporation and removal of the excess isocyanate by bulb-to-bulb

Table 2. *Fragments Expected from Endoproteinase Lys-C Digest of PBP 1b*

Cleavage site	Resulting peptide sequence	Peptide length [Å]	Peptide mass [Da]
12	MAGNDREPIGRK	12	1342.678
14	GK	2	203.127
20	PTRPVK	6	696.428
22	QK	2	274.164
49	VSRRRYEDDDDYDDYDDYEDEEPMPRK	27	3485.423
51	GK	2	203.127
53	GK	2	203.127
55	GK	2	203.127
58	GRK	3	359.228
62	PRGK	4	456.281
71	RGWLWLLLK	9	1183.723
90	LAIVFAVLIAIYGVYLDQK	19	2108.228
98	IRSRIDGK	8	943.556
121	VWQLPAAVYGRMVNLEPDMTISK	23	2617.340
126	NEMVK	5	619.300
138	LLEATQYRQVSK	12	1434.783
165	MTRPGEFTVQANSIEMIRRPFDFPDSK	27	3168.548
274	EGQVRARLTFDGDHLATIVNMENNRQFGFFRLDPRLITMISSPN-GEQRLFVPRSGFPDLLVDTLLATEDRHFYEHDGISLYSIGR-AVLANLTAGRTVQGASTLTQQLVK	109	12197.309
287	NLFLSSERSYWRK	13	1684.869
303	ANEAYMALIMDARYSK	16	1845.875
355	DRILELYMNEVYLGQSGDNEIRGFPLASLYYFGRPVEE-LSLDQQALLVGMVK	52	5948.017
367	GASIYNPWRNPK	12	1401.715
425	LALERRNLVLRLLQQQQIIDQELYDMLSARPLGVQPR-GGVISPQPAFMQLVRQELQAK	58	6663.674
429	LGDK	4	431.238
431	VK	2	245.174
437	DLSGVK	6	617.338
452	IFTTFDSVAQDAAEK	15	1641.789
462	AAVEGIPALK	10	967.570
463	K	1	146.106
466	QRK	3	430.265
513	LSDLETAIVVVDRFSGEVRAMVGGSEPQFAGYNRAMQARR-SIGSLAK	47	5038.587
525	PATYLTALSQPK	12	1288.703
595	IYRLNTWIADAPIALRQPNGQVWSPQNDDRRYSESGRVMLV-DALTRSMNVPTVNLGMALGLPAVTETWIK	70	7864.056
600	LGVPK	5	512.332
649	DQLHPVPAMLLGALNLTPIEVAQAFQTIASGGNRAPLSA-LRSVIAEDGK	49	5049.707
689	VLYQSFPQAERAVPAQAAYLTLWTMQQVVQRGTGRQLGAK	40	4460.354
698	YPNLHLAGK	9	1011.550
732	TGTTNNNVDTWFAGIDGSTVTITWVGRDNNQPTK	34	3679.750
828	LYGASGAMSIYQRYLANQTPTPLNLVPPEDIADMGVDYD-GNFVCSGGMRILPVWTSDPQSLCQQSEMQQQPSGNPFDQSS-QPQQQPQQQPAQQEQK	96	10609.909
838	DSDGVAGWIK	10	1046.503
844	DMFGSN	6	669.243

distillation at $2 \cdot 10^{-2}$ mbar followed by FC (cyclohexane/AcOEt 2:1) furnished **4c** (385.0 mg, 88%). Colorless oil. UV (MeOH): 233.5 (49959), 255.0 (sh, 26153). IR (KBr): 1840, 1707, 1541, 1325, 1271, 1240, 1200, 1136, 984. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.80–7.78 (*t*, *J* = 1.4, 1 arom. H); 7.65 (br. *s*, 1 arom. H); 7.09 (br. *s*, 1 arom. H); 5.15–5.11 (*m*, NH); 4.83–4.78 (*m*, C*H*CH_2NH); 4.52–4.48 (*t*, *J* = 4.8, COOCH_2); 4.29–4.24 (*t*, *J* = 4.4, ArOCH_2); 3.90–3.64 (*m*, CHCH_2NH). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 166.08 (CH(O)*C*(=O)O); 159.55 (arom. C(1′)); 155.87 (NHCOO); 149.64 (arom. C(3′)); 132.25 (arom. C(5′)); 121.73 (*q*, ^1J(C,F) = 274.8, *C*F_3–CN_2), 119.80 (arom. CH); 119.66 (*q*, *J* = 288.57, 1 CF_3); 118.85 (*q*, ^1J(C,F) = 286.9, 1 CF_3); 114.29 (arom. CH); 110.03 (arom. CH); 97.77 (*sept.* ^1J(C,F) = 36.6, $(CF_3)_2C$); 74.44 (*C*HCH$_2$NH); 67.32 (COO*C*H$_2$); 63.46 (ArO*C*H$_2$); 61.85 (not assigned); 41.30 (CH*C*H$_2$NH); 27.52 (*q*, ^2J(C,F) = 27.4, CF_3–*C*); 27.51 (not assigned). ^{19}F-NMR (282 MHz, $CDCl_3$): 12.45 (*s*, CF_3–CN_2); −2.98 to −3.09 (*m*, CF_3); −3.27 to −3.38 (*m*, CF_3). FAB-MS ($C_{17}H_{11}F_9N_4O_8$; 570.28, 570.043): 570.97 ([*M* + H]$^+$).

*(5^B*R*)-5^B-{1-[3-(10-Amino-1-oxo-5,8-dioxa-2-azadecyl)-4-nitrophenyl]-3-(3-carboxy-1-oxopropyl)-1*H*-1,2,4-triazol-5-yl}-5^B-de[(2-hydroxy-5-oxocyclopent-1-en-1-yl)carbamoyl]moenomycin A* (**7b**). To a soln. of 5-amino-N-(8-amino-3,6-dioxaoctyl)-2-nitrobenzamide (prepared as described for 5-amino-*N*-(2-aminoethyl)-2-nitrobenzamide [29]; 73.0 mg, 233.7 μmol) in 6% HCl soln. (0.7 ml) at 3°, a soln. of $NaNO_2$ (14.4 mg, 208.7 μmol) in H_2O (1 ml) was added, and the mixture was stirred at 3° for 15 min. The resulting soln. was added slowly to a soln. of **2** (300.0 mg, 189.6 μmol) and NaOAc (600 mg) in H_2O (50 ml) kept at 20°. The mixture was stirred at 20° for 24 h and then filtered through a column of *RP18* with MeCN/H_2O 3:7. The solvent of the fractions containing **7b** was removed by distillation, the residue taken up in H_2O (100 ml), and the soln. lyophilized. FC (H_2O/PrOH 2:7) followed by lyophilization provided pure **7b** (250.0 mg, 55%). Pale yellow hygroscopic powder. ^{1}H-NMR (600 MHz, CD_3OD; only signals that could be assigned): 8.47–8.45 (*d*, *J* = 8.4, 1 arom. H); 8.10 (*d*, *J* = 8.4, 1 arom. H); 8.05 (*s*, 1 arom. H); 5.85 (br. *s*, H–C(1^F)); 5.44 (*m*, H–C(2^I)); 5.36–5.33 (*d*, *J* = 15.2, H–C(7^I)); 5.29 (*m*, H–C(6^I)); 5.13–5.10 (*t*, *J* = 6.8, H–C(17^I)); 4.67–4.66 (2*s*, CH_2(22^I), overlapped by H_2O); 3.00–2.98 (*dd*, *J* ≈ 7.8, H–C(2^D)); 2.66–2.65 (*d*, *J* = 6.6, CH_2(12^I)); 2.06 (br. *s*, 6 H, *Me*CONH^{CE}); 1.88 (*m*, CH_2(10^I)); 1.68–1.65 (*s*, Me(25^I)); 1.58, 1.57 (2*s*, Me(20^I), Me(21^I)); 1.35 (Me(6^C)); 1.25 (*s*, Me(4^F)); 0.95 (br. *s*, Me(23^I), Me(24^I)). ^{13}C-NMR (150, 100 MHz; HMBC, HMQC; CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 95.11, 180.45, 176.80, 173.95, 173.19, 167.32, 159.21 (7 CO); 158.43, 154.32 (2 C^{TA}); 149.87 (C(11^I)); 146.69 (arom. C); 141.54 (arom. C); 140.63 (C(7^I); 136.36 (C(3^I)); 133.77 (arom. C); 131.41 (C(14^I)); 128.42 (arom. C); 127.01 (arom. C); 126.06 (C(6^I)); 125.59 (C(17^I)); 122.02 (C(13^I)); 121.42 (C(2^I)); 108.98 (C(22^I)); 104.61, 102.73, 101.61 (C(1^{B,C,D})); 94.94 (C(1^F)); 83.47 (C(4^C)); 80.87, 80.04 (C(2^H), C(4^E)); 77.30, 76.72, 76.59, 75.19, 73.82, 73.47, 73.16, 72.44, 72.25, 71.17, 70.21, 70.40, 69.83, 67.80, 69.16 (carbohydrate Cs, CH_2O^{sp}); 66.98 (C(1^I)); 66.25 (C(6^E)); 61.43 (C(6^D)); 55.53 (C(2^{E,C})); 42.00 (C(9^I)); 40.10 (C(15^I)); 39.67, 39.64 (*C*H$_2$NH$_2$, CONH*C*H$_2^{sp}$), 35.63 (C(12^I)); 35.21 (C(8^I)); 32.52, 31.84, 31.53, 31.42 (C(4^I), C(5^I), C(10^I), C(8^I)); 29.46 (CH_2^A); 27.34, 27.31 (C(23^I), C(24^I)); 26.86 (C(19^I)); 25.66 (C(25^I)); 23.53, 22.82 (*Me*CONH^{C,E}); 17.58 (C(20^I)); 17.15 (C(6^C)); 15.85 (C(21^I)); 15.48 (Me(4^F)). ESI-MS ($C_{82}H_{125}N_{10}O_{39}P$; 1905.91, 1904.7843): 951.38600 ([*M* − 2 H]$^{2-}$; calc. 951.38487), 633.91943 ([*M* − 3 H]$^{3-}$); calc. 633.92082).

*(5^B*R*)-5^B-{1-[3-(15-Amino-1-oxo-6,9,12-trioxa-2-azapentadecyl)-4-nitrophenyl]-3-(3-carboxy-1-oxopropyl)-1*H*-1,2,4-triazol-5-yl}-5^B-de[(2-hydroxy-5-oxocyclopent-1-en-1-yl)carbamoyl]moenomycin A* (**7c**). As described for **7b**, from 5-amino-*N*-(13-amino-3,6,9-trioxadecyl)-2-nitrobenzamide (prepared as described for 5-amino-*N*-(2-aminoethyl)-2-nitrobenzamide [29]; 31.7 mg, 77 μmol) and moenomycin A (118.0 mg, 74.6 μmol): **7c** (100.0 mg, 68%). Pale yellow hygroscopic powder. ^{1}H-NMR (600 MHz, CD_3OD/(D_6)DMSO 0.5:0.4; only signals that could be assigned): 8.14–8.12 (*d*, *J* = 8.4, 1 arom. H); 7.98 (*d*, *J* = 8.4, 1 arom. H); 7.91 (br. *s*, 1 arom. H); 5.69 (br. *s*, H–C(1^F)); 5.24 (br. *s*, H–C(2^I)); 5.21–5.18 (*d*, *J* = 15.8, H–C(7^I)); 5.13–5.10 (*d*, *J* = 15.8, H–C(6^I)); 4.97–4.95 (*t*, *J* = 7.8, H–C(17^I)); 4.88–4.86 (*d*, *J* = 10.5, H–C(3^F)); 2.52–2.51 (*d*, *J* = 7.0, CH_2(12^I)); 1.86–1.82 (br. *s*, 8 H, CH_2(15^I), MeCO^{C,E}); 1.57 (*s*, Me(25^I)); 1.49 (*s*, Me(19^I)); 1.43 (*s*, Me(21^I)); 1.42 (*s*, Me(20^I)); 1.21–1.11 (CH_2(9^I), Me(6^C)); 1.05 (*s*, Me(4^F)); 0.79 (*s*, Me(23^I), Me(24^I)). ^{13}C-NMR (100, 150 MHz, CD_3OD/(D_6)DMSO 0.5:0.4; only signals that could be assigned): 195.97, 181.53, 174.85, 173.19, 167.65, 159.65, 159.02 (7 CO); 154.74 (C^{TA}); 150.10 (C(11^I)); 147.15 (arom. CNO_2); 142.00, 141.50, 141.10, 136.78 (4C^I); 134.37 (arom. C); 131.68 (C^I); 126.69 (arom. C); 125.23 (C^I); 123.10 (C^I); 109.66 (C(22^I)); 104.29, 103.56 (C(1^{B,C,D,E})); 77.12, 74.03, 70.60, 69.35 (br., carbohydrate Cs, C^{sp}); 61.92 (CH_2); 56.03 (C(2^{E,C})); 42.56, 40.56 (2C^I); 38.68, 38.10 (2 C^{sp}); 36.19, 35.71 (C^I); 33.11, 32.32, 31.92 (3C^I); 29.28 (CH_2); 28.01, 27.67, 27.51 (C(23^I), C(24^I), C(16^I)); 26.40 (C(19^I)); 24.24 (C(25^I)); 23.44 (*Me*CONH^{C,E}); 18.33 (C(6^C)); 17.68 (Me(4^F)); 16.62 (C(20^I)); 16.00 (C(21^I)). ^{31}P-NMR (80 MHz, CD_3OD): −1.18. ESI-MS ($C_{86}H_{133}N_{10}O_{40}P$; 1978.02, 1976.8418): 987.41356 ([*M* − 2 H]$^{2-}$; calc. 987.41363), 657.94185 ([*M* − 3 H]$^{3-}$; calc. 657.9400).

*(5^B*R*)-5^B-{1-{3-{13-{4-{(3*R*)-18-Biotinamido-3-{{{{2-{3-nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}ethoxy}carbonyl}amino}methyl}-1,4-dioxo-2,9,12,15-tetraoxa-5-azaoctadecyl}phenyl}-1-oxo-12-thioxo-5,8-*

*dioxa-2,11,13-triazatridecyl}-4-nitrophenyl}-3-(3-carboxy-1-oxopropyl)-1*H*-1,2,4-triazol-5-yl}-5^{B}-de[(2-hydroxy-5-oxocyclopent-1-en-1-yl)carbamoyl]moenomycin A* (**8**). A soln. of **4d** (80.0 mg, 94 μmol) and *N*,*N*-dimethylpyridin-4-amine (DMAP; 1.0 mg, 8.2 μmol) in dry pyridine (5 ml) was stirred at 0° for 10 min (Ar, dimmed light). Upon addition of 4-isothiocyanatobenzoyl chloride (18.5 mg, 94 μmol), the soln. turned brown. The mixture was stirred at 0° for 2 h, then allowed to warm to 5° and stirred at this temp. for 13 min (TLC control (MeOH/$CHCl_3$ 2:8), 4-(dimethylamino)cinnamaldehyde dipping reagent). A suspension of **7b** (150.0 mg, 79 μmol) in H_2O (5 ml) was added. The mixture was stirred at 5° for 30 h. H_2O (30 ml) was added, and the mixture was evaporated. The residue was taken up in H_2O (30 ml) and lyophilized. Chromatography (*RP18* MeCN/H_2O 1:1, R_f 0.9), solvent evaporation, lyophilization, repeated FC (MeOH/$CHCl_3$ 2:8, R_f 0.1; PrOH/H_2O 3:7, R_f 0.7), and chromatography (*RP18*, MeCN/H_2O 3:7) provided **8** (30.0 mg, 10%). Pale yellow hygroscopic powder. ^{1}H-NMR (600 MHz, CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 8.20–8.19 (*d*, *J* = 8.8, 1 arom. H); 8.07–8.06 (*d*, *J* = 8.8, 1 arom. H); 7.91–7.90 (*m*, 3 arom. H); 7.78 (br. *s*, 2 arom. H); 7.79–7.67 (*d*, *J* = 8.8, 2 arom. H); 7.04 (br. *s*, 1 arom. H); 5.66 (br. *s*, H–C(1^F)); 5.32 (*m*, H–C(2^I)); 5.31–5.28 (*d*, *J* = 15.9, H–C(7^I)); 5.25–5.18 (*m*, H–C(6^I)); 5.12–5.10 (*t*, *J* = 4.7, H–C(13^I)); 5.09–5.03 (*t*, *J* = 7.6, H–C(17^I)); 4.95–4.93 (*d*, *J* = 10.6, H–C(3^F)); 4.58, 4.57 (2*s*, CH_2(22^I)); 4.17–4.15 (*m*, SCHC*H*^{bi}); 2.82–2.79 (*dd*, $^2J_{AB}$ = 12.9, $^3J_{BX}$ = 4.7, 1 H, SC*H*$_2$CH^{bi}), 2.60–2.58 ($^2J_{AB}$ = 12.9, 1 H, SC*H*$_2$CH^{bi}); 2.47 (*s*, CH_2^A); 2.07–1.98 (*m*, CH_2(4^I), CH_2(5^I), CH_2(15^I)); 1.94–1.91 (*m*, CH_2(16^I)); 1.88 (*s*, 6 H, *Me*CONH^{C,D}), 1.82–1.79 (CH_2(10^I)); 1.66 (*s*, Me(25^I)); 1.63–1.60 (*m*, 4 H, CH_2^{bi}); 1.57 (*s*, Me(19^I)); 1.51 (*s*, Me(21^I)); 1.50 (*s*, Me(20^I)); 1.40–1.26 (*m*, Me(6^C), CH_2(9^I), 2 CH_2^{sp}); 1.11 (*s*, Me(4^F)); 0.87 (*s*, Me(23^I), Me(24^I)). ^{13}C-NMR (150, 100 MHz; HMBC, HMQC; CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 174.27, 173.46, 171.88, 168.96, 166.28, 165.64, 164.52, 160.26 (9 CO); 159.50 (arom. OC); 157.38 (C^{TA}); 157.23 (CO); 154.75 (CO); 150.08 (C(11^I)); 146.88 (arom. CNO_2); 145.41 (arom. CNO_2); 141.55 (arom. C); 140.55 (C(3^I), C(7^I)); 136.45 (C(14^I)); 134.25 (arom. C); 131.31 (C(18^I)); 130.82 (arom. C); 127.97 (arom. C); 126.02 (arom. C); 124.51 (C(17^I)); 122.47 (C(13^I)); 121.19 (br. *s*, arom. C, CF_3); 119.69 (arom. C); 113.97 (arom. C); 111.01 (arom. C); 108.61 (C(22^I)); 104.27, 103.72, 102.53, 101.91 (C(1^{B,C,D,E})); 94.83 (C(1^F)); 77.20, 74.09, 68.11 (carbohydrate Cs, CH_2O^{sp}); 62.97, 60.39 (C(6^D), biotin signals); 56.07 (S*C*HCH$_2^{bi}$); 55.81, 55.97 (C(2^E), C(2^C)); 44.02 (CH_2^{is}); 42.51 (C(9^I)); 41.82 (C(15^I)); 36.86, 36.70 (CH^{sp}); 35.87, 35.60 (CH_2^A, CH_2^{bi}); 34.99 (C(8^I)); 32.54 (CH_2^A); 31.72 (CH_2^{sp}); 31.40 (C(10^I)); 29.64, 29.44, 28.84, 28.59 (2 CH_2^{bi}, C(5^I), C(16^I), CH_2^A); 27.13 (C(23^I), C(24^I)); 26.76 (C(25^I)); 25.92 (CH_2^{bi}); 25.32 (*Me*CONH^{C,D}); 23.32, 22.80 (C(6^C)); 17.20 (C(19^I), C(22^I)); 16.13 (C(21^I)); 15.48 (Me(4^F)). ^{19}F-NMR (188 MHz, $CDCl_3$): −67.08 (*s*, CF_3). ESI-MS ($C_{124}H_{178}F_3N_{19}O_{25}PS_2$; 2918.96, 2917.100): 971.02420 ([*M* − 3 H]$^{3-}$; calc. 971.02343).

N-*{11-{3-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}-3,6-dioxa-9-azaundecyl}biotinamide* (**4e**). Commercially available *N*-[2-(2-ethoxyethoxy)ethyl]biotinamide (199.0 mg, 0.53 mmol), which we prepared using the method of *Braun et al.* [39]; and **4a** (99.0 mg, 0.34 mmol) were dissolved in MeOH (10 ml). At 20°, the pH was adjusted to 6.5 by addition of 0.25M CF_3COOH in MeOH. Sodium cyanoborohydride (23.0 mg, 0.37 mmol) was added, and the mixture was stirred for 30 h at 20° (TLC control ($CHCl_3$/MeOH 8:2), 4-(dimethylamino)cinnamaldehyde dipping reagent). AcOH (0.2 ml) was added, and solvents were evaporated. FC ($CHCl_3$/MeOH 9:1) provided **4e** (133.0 mg, 60%). Colorless oil. UV (CH_2Cl_2): 229 (13484), 263 (4924), 325 (2463). ^{1}H-NMR (200 MHz, CH_3OD; ^{1}H,^{1}H-COSY): 7.95–7.93 (*m*, 1 arom. H); 7.72 (br. *s*, 1 arom. H); 7.21 (br. *s*, 1 arom. H); 4.53–4.48 (*m*, SCH$_2$C*H*^{bi}); 4.34–4.24 (*m*, SCHC*H*, C*H*$_2$OAr); 3.69–3.65 (*m*, 3 CH_2O); 3.58–3.52 (*m*, CH_2O); 3.39–3.33 (*m*, C*H*$_2$CONH, overlapped by MeOH); 3.25–3.18 (*m*, SC*H*CH); 3.16–3.10 (*m*, C*H*$_2$NH); 2.98–2.93 (*dd*, $^2J_{AB}$ = 12.5, 1 H, SC*H*$_2$CH); 2.95–2.89 (*m*, C*H*$_2$NH); 2.74–2.67 (*d*, $^2J_{AB}$ = 12.5, 1 H, SC*H*$_2$CH^{bi}); 2.25–2.18 (*t*, *J* = 7.0, C*H*$_2$CONH^{bi}); 1.83–1.26 (*m*, 3 CH_2^{bi}). ^{13}C-NMR (75 MHz, $CDCl_3$): 176.09 (CONH^{bi}); 165.96 (HNCONH^{bi}); 161.41 (*C*H$_2$OAr); 151.10 (arom. CNO_2); 132.67 (arom. *C*–CN_2); 120.48, 114.80, 111.83 (arom. CH); 71.39, 71.28 (2 CH_2O); 70.64, 70.56 (O*C*H$_2$CH$_2$NHCH$_2$*C*H$_2$OAr); 69.20 (CONHCH$_2$*C*H$_2$O); 63.39 (*C*HCHS^{bi}); 61.67 (SCH$_2$*C*H^{bi}); 56.93 (S*C*HCH^{bi}); 41.01 (HN*C*H$_2$CH$_2$OAr); 40.30 (SCH$_2$*C*H^{bi}); 36.75 (*C*H$_2$CONH^{bi}); 29.71, 29.49, 26.80 (3 CH_2^{bi}). ^{19}F-NMR (282 MHz, $CDCl_3$): 2.65 (*s*, CF_3); 2.03 (*s*, CF_3COO). ESI-MS ($C_{26}H_{36}F_3N_7O_7S$; 647.67, 647.24): 648.24211 ([*M* + H]$^+$; calc. 648.2418).

N-*{9-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)-11-{3-nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}-3,6-dioxa-9-azaundecyl}biotinamide* (**4f**). To a soln. of **4e** (120.0 mg, 0.185 mmol) in EtOH (7 ml), 3,4-diethoxycyclobut-3-ene-1,2-dione (75 μl, 0.51 mmol) was added, and the mixture was stirred at 20° for 19 h (TLC control ($CHCl_3$/MeOH 8:2), 4-(dimethylamino)cinnamaldehyde dipping reagent). Evaporation and FC ($CHCl_3$/MeOH 10:1) provided **4f** (130.0 mg, 91%). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$; ^{1}H,^{1}H-COSY): 7.79–7.77 (*m*, 1 arom. H); 7.67 (*m*, 1 arom. H); 7.01 (*m*, 1 arom. H); 6.62 ($w_{1/2}$ = 3.92, 0.5 H, N*H*CH$_2$); 6.46 ($w_{1/2}$ = 5.0, 0.5 H, N*H*CH$_2$); 6.37 ($w_{1/2}$ = 5.74, 1 H, HNCONH); 5.47 ($w_{1/2}$ = 5.62, 1 H, HNCONH); 4.84–4.72

(*m*, CH_3CH_2O); 4.53–4.46 (*m*, SCHC*H*^{bi}); 4.39–4.20 (*m*, SCH_2CH^{bi}, $ArOCH_2$, CH_2N); 3.97–3.95 (CH_2N); 3.75–3.71 (*m*, CH_2O); 3.60–3.50 (*m*, 3 CH_2O); 3.43–3.38 (*m*, $CONHCH_2$); 3.18–3.05 ($w_{1/2}$ = 5.56, $SCHCH_2^{bi}$); 2.94–2.85 (*dd*, $^2J_{AB}$ = 13.2, $^3J_{BX}$ = 4.8, 1 H, SCH_2); 2.74–2.68 (*d*, $^2J_{AB}$ = 13.2, 1 H, SCH_2); 2.29–2.18 (*m*, CH_2CONH^{bi}); 1.71–1.61 (2 CH_2^{bi}); 1.49–1.24 (*m*, *Me*CH_2O, CH_2^{bi}). ^{13}C-NMR (50 MHz, $CDCl_3$): 189.06, 188.76 (CO^{sa}); 183.13, 183.03 (CO^{sa}); 177.58, 177.18 and 173.16, 172.84 ($C{=}C^{sa}$); 173.71, 173.63 (CH_2CONH); 164.14 (CO^{bi}); 159.49, 159.41 (arom. OC); 149.78 (arom. CNO_2); 132.52, 132.38 (arom. CCN_2); 130.08, 121.85 (2*q*, each with ^1J(C,F) = 273, CF_3); 119.91, 119.60 (arom. CH); 114.69 (arom. CH); 110.44, 109.96 (arom. CH); 70.93–69.77 (MeCH_2O^{sa}, CH_2O); 68.22 (CH_2O); 67.17 (CH_2O); 62.03 ($CHCHS^{bi}$); 60.44 ($CHCH_2S^{bi}$); 55.89 ($SCHCH_2^{bi}$); 50.99 (2 CH_2N); 40.86 (SCH_2CH^{bi}); 39.40 (CH_2NCO); 36.21 (CH_2CONH); 28.43 (^2J(C,F) = 42.0, CCF_3); 28.44, 28.39 (2 CH_2^{bi}); 25.90 (CH_2^{bi}); 16.25 (*Me*CH_2O). ^{19}F-NMR (188 MHz, $CDCl_3$): 11.36, 11.34 (CF_3). ESI-MS ($C_{32}H_{40}F_3N_7O_{10}S$; 771.77, 771.251): 772.25759 ($[M+H]^+$; calc. 772.25095), 794.23993 ($[M+Na]^+$, calc. 794.24017), 1543.51046 ($[2M+H]^+$; calc. 1543.50972).

*(5^B*R*)-5^B-{1-{3-{{{2-{{2-{(8-Biotinamido-3,6-dioxaoctyl)}{2-{3-nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy}ethyl}amino}-3,4-dioxocyclobut-1-en-1-yl}amino}ethyl}amino}carbonyl}-4-nitrophenyl}-3-(3-carboxy-1-oxopropyl)-1*H*-1,2,4-triazol-5-yl}-5^B-de[(2-hydroxy-5-oxocyclopent-1-en-1-yl)carbamoyl]moenomycin A* (**9**). To a suspension of **7a** (67.0 mg, 36 μmol) in MeOH/H_2O 1:1 (8 ml), 0.2M Et_3N in MeOH was added until a pH value of 9 was reached (dimmed light). At 20°, a soln. of squaric acid ester amide **4f** (28.0 mg, 36 μmol) in MeOH (5 ml) was added. The mixture was stirred at 20° (TLC control PrOH/H_2O 7:3), anisaldehyde and 4-(dimethylamino)cinnamaldehye dipping reagents). After 72 h, the mixture was evaporated, the residue taken up in H_2O (20 ml) and separated by chromatography (*RP18*, MeCN/H_2O 3:7). The product fractions were evaporated. The residue was redissolved in H_2O (30 ml) and the soln. adsorbed onto 'Kieselguhr' (1.0 g) by lyophilization. FC (PrOH/H_2O 7.5:2.5) provided **9** (30.0 mg, 33%). Hygroscopic pale yellow powder. ^{1}H-NMR (600 MHz; ^{1}H,^{1}H-COSY, HMBC; CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 8.17 (*d*, *J* = 8.4, 1 arom. H); 8.00–7.97 (*m*, 2 arom. H); 7.81 (br. *s*, 1 arom. H^{BPP}), 7.60 (*s*, 1 arom. H^{BPP}); 7.03 (br. *s*, 1 arom. H^{BPP}); 5.69 (br. *s*, H–C(1^F)); 5.31 (*m*, H–C(2^I)); 5.27–5.25 (*d*, *J* = 15.2, H–C(7^I)); 5.21–5.17 (*m*, H–C(6^I)); 5.03–5.01 (*t*, *J* = 7.0, H–C(17^I)); 4.99–4.97 (*m*, CH_2OAr); 4.94–4.93 (br., carbohydrate Hs); 4.46 (*m*, H–C(1^C)); 4.35–4.33 (*m*, SCH_2CH^{bi}); 4.12 (*s*, $O(CH_2)_2O^{sp}$); 4.17–4.15 (*m*, $SCHCH^{bi}$); 4.10–3.73 (br., carbohydrate Hs); 3.60 (*s*, $HN(CH_2)_2CONH$); 3.53–3.41 (br., carbohydrate Hs); 3.38–3.35 (*m*, $N(CH_2CH_2O)_2^{sa}$); 3.07–3.04 (*m*, $SCHCH^{bi}$); 2.90 (*m*, H–C(4^C)); 2.80–2.77 (*dd*, $^2J_{AB}$ = 12.5, $^3J_{BX}$ = 4.7, 1 H, SCH_2CH^{bi}); 2.58–2.56 (*d*, $^3J_{BX}$ = 12.5, 3 H, SCH_2CH^{bi}, $COCH_2^A$); 2.44 (*s*, 2 H, $NCH_2CH_2O^{sa}$); 2.07–2.05 (*t*, *J* = 7.3, 1 CH_2); 2.01–1.96 (*m*, $CH_2(4^I)$, $CH_2(15^I)$, $CH_2(16^I)$); 1.94–1.80 (*m*, $COCH_2CH_2COOH^A$, *Me*$CONH^E$, CH_2^{bi}); 1.86 (*s*, *Me*-$CONH^C$); 1.79–1.74 (*m*, $CH_2(10^I)$); 1.63 (*s*, Me(25^I)); 1.55 (*s*, Me(19^I)); 1.49 (*s*, Me(21^I)); 1.48 (*s*, Me(20^I)); 1.49–1.39 (*m*, 6 H, 2 CH_2^{bi}, partly overlapped); 1.30–1.23 (*m*, 7 H, CH_2^{bi}, $CH_2(9^I)$, $NCH_2CH_2O^{sa}$); 1.10 (Me(4^F)); 0.85 (Me(23^I), Me(24^I)). ^{13}C-NMR (150, 100 MHz; HMBC, HMQC; CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 194.35, 183.79, 183.47, 179.63, 175.41, 174.03, 172.88, 170.21, 167.35, 165.38 (8 CO, $C{=}C^{sa}$); 160.80 (C^{TA}); 160.07 (arom. CO); 158.41, 155.29 (2 CO); 150.66 (C(11^I)); 147.52 (arom. C); 147.25 (arom. C^{BPP}–NO_2); 142.14 (arom. C); 141.21 (C(7^I)); 137.08 (C(3^I)); 134.87 (arom. C); 132.16 (CCN_2CF_3); 131.94 (C(17^I)); 128.50 (arom. C); 126.83 (arom. C); 126.66 (C(6^I)); 126.23 (arom. C); 125.13 (C(13^I)); 124.20 (arom. C); 123.16 (C(2^I)); *ca.* 121.5 (CF_3); 120.28 (C^{BPP}); 114.75 (C^{BPP}); 111.87 (C^{BPP}); 109.15 (C(22^I)); 105.01, 104.45, 103.76, 102.56 (C(1^{B,C,D,E})); 85.23 (C(4^C)); 81.78 (C(4^E)); 77.82, 75.73, 74.73, 73.87, 73.58, 72.18 (carbohydrate Cs, OCH_2); 62.89, 62.48, 61.16 (C(6^D), SCH_2CH^{bi}); 56.71 ($CHCHS^{bi}$); 56.39, 55.59 ($C(2^{E,C})$); 36.43 (CH_2N); 36.22 (C(8^I)); 35.64 (C(12^I)); 33.23 (CH_2^{bi}); 32.41, 32.14 (C(4^I), C(4^I)); 32.05 (C(10^I)); 30.39 (CH_2^A); 29.43, 29.22 (CH_2^{bi}); 27.71, 27.42 (C(23^I), C(24^I)); 26.55 (CH_2^{bi}); 25.86 (C(25^I)); 23.90, 23.36 (*Me*CO^C, $MeCO^E$); 17.72 (C(20^I), C(6^c)); 16.50 (C(21^I)); 16.02 (Me(4^F)). ^{19}F-NMR (282 MHz, $CDCl_3$): 11.79 (*s*, CF_3). ^{31}P-NMR (282 MHz, $CDCl_3$): −1.84. ESI-MS ($C_{108}H_{151}F_3N_{17}O_{46}PS$; 2543.50, 2541.940): 1269.96424 ($[M+H]^{2-}$; calc. 1269.96320), 864.30686 ($[M-3H]^{3-}$; calc. 864.30637).

Prop-2-enyl 4-Benzoylbenzoate (**10a**). A mixture of 4-benzoylbenzoic acid (2.5 g, 11 mmol), *N,N'*-diisopropylcarbodiimide (2.5 ml, 16 mmol), DMAP (0.1 g, 0.8 mmol), and dry CH_2Cl_2 (25 ml) was stirred for 2 h at 20° (dimmed light). Allyl alcohol (10 ml) was added, and the mixture stirred at 20° for 24 h (TLC control (petroleum ether/AcOEt 1:1), potassium permanganate dipping reagent). The mixture was evaporated, the residue taken up in CH_2Cl_2 (*ca.* 300 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. (2 × 300 ml) and H_2O (600 ml), dried, and evaporated, and the residue purified by FC (petroleum ether/AcOEt 9:2): **10a** (2.5 g, 85%). Colorless oil. UV (MeOH): 256.50 (27952). IR (KBr): 1722, 1662, 1267, 1107, 930, 710. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.20–8.14 (*m*, 2 arom. H); 7.87–7.78 (*m*, 4 arom. H); 7.66–7.57 (*m*, 1 arom. H); 7.54–7.45 (*m*, 2 arom. H); 6.16–5.96 (*m*, $CH_2{=}CHCH_2$); 5.49–5.38 (*ddd*, *J* = 17.2, 2.9, 1.0, 1 H, $CH_2{=}CHCH_2$); 5.35–5.28 (*dd*, *J* = 10.1, 2.2, 1.0, 1 H, $CH_2{=}CHCH_2$); 4.89–4.84 (*dt*, ^3J = 5.3, ^4J = 1.0, $CH_2{=}CHCH_2$). ^{13}C-NMR

(50 Hz, $CDCl_3$): 196.08 (CO_2); 165.60 (C=O); 141.55 (C(4)); 137.12 (C(1′)); 133.42 (C(4′)); 133.11 (CH_2=*C*HCH$_2$); 133.09 (C(1)); 130.28 (C(2)); 129.94, 129.73 (C(3), C(2′)); 128.63 (C(3′)); 118.38 (*C*H$_2$=CHCH$_2$); 66.19 (CH$_2$=CH*C*H$_2$). FAB-MS ($C_{17}H_{14}O_3$; 266.30, 266.0943): 267.1 ($[M+H]^+$).

2-Oxoethyl 4-Benzoylbenzoate (**10b**). Into a soln. of **10a** (2 g, 7.51 mmol) in CH_2Cl_2/MeOH 4:1 (50 ml) at −73° (stirring), ozone (50 l h^{-1}, 2.5 g h^{-1}) was introduced for 10 min (TLC control (every 2 min; petroleum ether/AcOEt 8:2), fuchsin dipping reagent). Purging with N_2, addition of Me_2S (2 ml, 44 mmol), stirring at 20° for 3 h, evaporation, and FC (petroleum ether/AcOEt 10:4) provided **10b** (1.80 g, 90%). Colorless oil. UV (MeOH): 256.00 (33929). IR (KBr): 3431, 1718, 1649, 1273. ^{1}H-NMR ($CDCl_3$, 200 MHz): 9.74 (*s*, CHO); 8.24–8.18 (*m*, 2 arom. H); 7.89–7.78 (*m*, 4 arom. H); 7.67–7.58 (*m*, 1 arom. H); 7.54–7.46 (*m*, 2 arom. H); 4.96 (*s*, CH_2). ^{13}C-NMR (75 MHz, $CDCl_3$, APT): 195.93 (+, CO); 195.25 (−, HCO); 165.22 (+, COOR); 142.02 (+, C(4)); 136.87 (+, C(1′)); 133.16 (−, C(4′)); 131.97 (+, C(1)); 130.19 (−, C(2)); 129.94, 129.79 (−, C(3), C(2′)); 128.59 (−, C(3′)); 69.38 (+, CH_2). FAB-MS ($C_{16}H_{12}O_4$; 268.27, 268.073559): 269.0 ($[M+H]^+$).

3-Hydroxypropyl 4-Benzoylbenzoate (**10c**). A mixture of 4-benzoylbenzoic acid (2.1 g, 9.3 mmol), dry pyridine (70 ml), 1,1′-carbonylbis[1*H*-imidazole] (2.3 g, 14 mmol) was stirred at 20° for 2 h. Propane-1,3-diol (5 ml, 66 mmol) was added, and the mixture was stirred at 20° for 24 h. Pyridine was evaporated and CH_2Cl_2 (200 ml) was added. Usual workup followed by FC (CH_2Cl_2) furnished **10c** (2.6 g, 91%). Colorless oil. UV (MeOH): 256.0 (25209). IR (KBr): 3282, 1716, 1650, 1317, 1280, 1111, 1059, 708. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.16–8.12 (*m*, 2 arom. H); 7.85–8.78 (*m*, 4 arom. H); 7.62–7.46 (*m*, 3 arom. H); 4.56 (*t*, *J* = 6.2, CH_2CO_2); 3.80 (*t*, *J* = 6.2, CH_2OH); 2.27 (br. *s*, OH); 2.07–2.01 (*quint.*, *J* = 6.2, $CH_2CH_2CH_2$). ^{13}C-NMR (50 Hz, $CDCl_3$, APT): 196.10 (+, CO); 166.20 (+, COO); 141.48 (+, C(4)); 136.98 (+, C(1′)); 133.30 (C(1)); 133.06 (C(4′)); 130.18 (−, C(2)); 129.85, 129.59 (−, C(3), C(2′)); 128.55 (−, C(3′)); 62.42 (+, CH_2CO_2); 59.21 (+, CH_2OH); 31.93 (+, $CH_2CH_2CH_2$). ESI-MS ($C_{17}H_{16}O_4$; 284.3, 284.105): 285.0 ($[M+H]^+$), 306.9 ($[M+Na]^+$).

N-*{3-[(4-Benzoylbenzoyl)oxy]propoxy}carbonyl}-1,2*-O-*(hexafluoroisopropylidene)*-L-*isoserine* (= *3-{{{{[(4*S*)-2,2-Bis(trifluoromethyl)-5-oxo-1,3-dioxolan-4-yl]methyl}amino}carbonyl}oxy}propyl 4-Benzoylbenzoate*; **10d**). As described for **4c** from **10a** and **5b**. UV (MeOH): 256 (2414). IR (KBr): 3350, 1734, 1659, 1527, 1319, 1281, 1268, 1228, 1130, 982, 721. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.16–8.12 (*m*, 2 arom. H); 7.86–7.78 (*m*, 4 arom. H); 7.67–7.58 (*m*, 1 arom. H); 7.54–7.45 (*m*, 2 arom. H); 5.22–5.16 (*m*, CH_2NHCOO); 4.83–4.78 (*t*, *J* = 4.9, $CHCH_2NH$); 4.48–4.41 (*t*, *J* = 6.2, CO_2CH_2); 4.32–4.26 (*t*, *J* = 6.2, CO_2CH_2); 3.91–3.59 (*m*, CH_2NH); 2.20–1.95 (*quint.*, $CH_2CH_2CH_2$). ^{13}C-NMR (50 and 75 MHz, $CDCl_3$): 196.42 (Ph*C*O); 166.25 (CO); 166.00 (CO); 156.41 (HNCO); 141.58 (C(4)); 136.94 (C(1)); 133.25 (C(1′)); 133.18 (C(4′)); 130.30 (C(2)); 129.98 (C(2), C(3′)); 129.66 (C(3)); 128.63 (C(2′)); 119.62 (*q*, ^1J(C,F) = 289.1, CF_3); 118.84 (*d*, ^1J(C,F) = 286.9, CF_3); 74.40 (O*C*HCH$_2^{is}$); 62.48 (NHCO$_2$*C*H$_2$CH$_2$); 62.00 (ArCO$_2$*C*H$_2$); 41.23 (CH*C*H$_2$NH); 28.34 ($CH_2CH_2CH_2$). ^{19}F-NMR (282.3 MHz, $CDCl_3$): −2.81 to −2.87 (*m*, CF_3); −3.10 to −3.16 (*m*, CF_3); −4.80 (*s*, unassigned). ESI-MS ($C_{24}H_{19}F_6NO_8$; 563.41, 563.101); 586.2 ($[M+Na]^+$).

*(8*S*)-18-Biotinamido-8-hydroxy-5,9-dioxo-4,13,16-trioxa-6,10-diazaoctadecyl 4-Benzoylbenzoate* (**10e**). To a soln. of **10d** (700.0 mg, 1.24 mmol) in 1,2-dimethoxyethane (15 ml), a soln. of **6a** (600.0 mg, 1.60 mmol) in 1,2-dimethoxyethane (10 ml) was added dropwise at 0° (dimmed light). The mixture was stirred at 0° for 2.5 h and then allowed to warm to r.t. H_2O (10 ml) was added, the mixture evaporated, and the residue taken up in H_2O (30 ml) and freeze-dried. FC (MeOH/$CHCl_3$ 2.5:7.5) provided **10e** (475.0 mg, 62%). Colorless oil. UV (MeOH): 256 (22063). IR (KBr): 3290, 3282, 1695, 1645, 1547, 1319, 1275, 1111, 721. ^{1}H-NMR (600 MHz, $CDCl_3$; ^{1}H,^{1}H-COSY): 8.08–8.06 (*d*, *J* = 8.4, 2 arom. H); 7.85–7.76 (*d*, *J* = 8.4, 2 arom. H); 7.74–7.73 (*d*, *J* = 6.8, 2 arom. H); 7.59–7.55 (*m*, 1 arom. H); 7.55 ($w_{1/2}$ = 11, CONH); 7.45–7.43 (*m*, 2 arom. H); 6.10 (*m*, $w_{1/2}$ = 19, 0.5 H, CONH); 4.52 (*m*, SCH$_2$C*H*^{bi}); 4.38–4.36 (*m*, SCHC*H*^{bi}, 1 COOCH$_2$); 4.19–4.18 (*m*, 1 COOCH$_2$, C*H*OH^{is}); 3.56–3.52 (*m*, 10 H, 3 CH$_2$O, C*H*$_2$CHOH, CONHC*H*$_2$); 3.42–3.40 (*m*, 5 H, CH$_2$O, CONHC*H*$_2$, OH); 3.11 (*m*, SC*H*CH^{bi}); 2.87–2.85 (*dd*, $^2J_{AB}$ = 13.1, $^3J_{BX} \approx$ 4, 1 H, SC*H*$_2$CH^{bi}); 2.76–2.74 (*d*, $^2J_{AB}$ = 13.1, 1 H, SC*H*$_2$CH^{bi}); 2.26–2.24 (*m* (= '*t*'), *J* = 6.3, C*H*$_2$CONH^{bi}); 2.07–2.05 (*m* (= '*t*'), *J* = 5.8, ArCOOCH$_2$C*H*$_2$); 1.70–1.40 (*m*, 3 CH$_2^{bi}$). ^{13}C-NMR (50 Hz, $CDCl_3$; HETCOR): 198.00 (Ph*C*O); 175.70 (*C*ONHCH$_2$); 174.61 (*C*ONHCH$_2$); 167.75 (Ar*C*OOCH$_2$); 166.21 (HN*C*OOCH$_2$); 160.17 (HN*C*ONH); 143.42, 138.86, 135.13 (arom. C(1), C(1′), and C(4)); 135.02 (arom. C(4′)); 132.11, 131.79, 131.50, 130.49 (6 arom. CH); 74.22 (*C*HOH^{is}); 72.16 (2 CH$_2$O); 71.88, 71.51 (2 CH$_2$O); 64.08, 64.00 (O*C*H$_2$CH$_2$CH$_2$O); 63.80 (SCH*C*H); 62.35 (SCH$_2$*C*H); 57.75 (S*C*HCH); 46.87 (HOCH*C*H$_2$NH^{is}); 42.50 (S*C*H$_2$CH^{bi}); 41.32, 40.85 (2 CONH*C*H$_2^{sp}$); 37.84 (*C*H$_2$CONH^{bi}); 30.48 (ArCOOCH$_2$*C*H$_2$); 30.30, 30.09, 27.65 (3 CH$_2^{bi}$). ESI-MS ($C_{37}H_{49}N_5O_{11}S$; 771.88, 771.315): 772.32286 ($[M+H]^+$; calc. 772.32220), 794.30583 ($[M+Na]^+$; calc. 794.30470), 810.27953 ($[M+K]^+$; calc. 810.27864).

*(5^B*R*)-5^B-{{3-{13-{4-{(3*S*)-3-{{{{3-[(4-Benzoylbenzoyl)oxy]propoxy}carbonyl}amino}methyl}-13-biotinamido-1,4-dioxo-2,8,11-trioxa-5-azatridecyl]phenyl}-1-oxo-12-thioxo-5,8-dioxa-2,11,13-triazatridecyl}-4-nitro-*

*phenyl}-3-(3-carboxy-1-oxopropyl)-1*H*-1,2,4-triazol-5-yl}-5*^{B}*-de[(2-hydroxy-5-oxocyclopent-1-en-1-yl)carbamoyl]moenomycin A* (**11**). As described for **8**, from **10e** (170.0 mg, 221 μmol), 4-isothiocyanatobenzoyl chloride (45.0 mg, 227 μmol), and **7a** (170.0 mg, 94 μmol): **11** (33.0 mg, 13%). Pale yellow hygroscopic powder. ^{1}H-NMR (600 MHz, CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 8.22–7.35 (*m*, 14 arom. H); 5.68 (br. *s*, H–C(1^F)); 5.33 (*m*, H–C(2^I)); 5.32–5.29 (*d*, *J* = 16.2, H–C(7^I)); 5.16–5.13 (*m*, H–C(6^I)); 5.10–5.00 (*t*, *J* = 6.8, H–C(17^I)); 5.59, 5.58 (2*s*, CH_2(22^I)); 1.94–1.90 (*m*, 6 H, *Me*CONH^{C,E}); 1.67 (*s*, Me(25^I)); 1.58 (*s*, Me(19^I)); 1.52 (*s*, Me(21^I)); 1.51 (*s*, Me(20^I)); 1.31–1.17 (*m*, 10 H, CH_2^{bi}, Me(6^C), CH_2(9^I)); 1.11 (*s*, Me(4^F)); 0.88 (Me(23^I), Me(24^I)). ^{13}C-NMR (150, 100 MHz; HMBC, HMQC; CD_3OD/(D_6)DMSO 4:1; only signals that could be assigned): 172.46, 159.42 (2 CO); 157.64, 154.8 (2 C^{TA}); 150.19 (C(10^I)); 146.96 (arom. CNO_2); 141.84, 140.67 (arom. C); 137.32, 136.53, 136.29, 135.95 (C(3^I), arom. C); 133.50 (C(14^I)); 131.38, 130.42, 130.41, 130.32, 130.12, 130.08, 129.78, 129.77, 128.99, 128.43, 128.32 (arom. C); 126.15 (C(6^I)); 124.56 (C(13^I)); 122.57 (C(2^I)); 108.60 (C(22^I)); 103.73, 102.61, 101.98 (C(1^{B,C,D})); 95.02 (C(1^F)); 81.43 (C(4^H)); 77.31–69.74 (carbohydrate Cs, CH_2O^{sp}); 62.63, 62.28, 61.91, 60.53 (C(6^D), CO_2CH_2, biotin Cs); 56.15, 55.26, 55.03, 54.32 (C(2^C), C(2^E), CH^{bi}); 41.92, 35.67, 35.05 (C(15^I), 2 CH_2^{sp}); 32.54 (C(12^I)); 32.12, 31.48 (CH_2); 31.83 (C(10^I)); 29.82, 29.43, 28.65, 27.13, 26.82 (2 C^I, 3 CH_2^{bi}); 25.96 (CH_2^{bi}); 25.31 (C(25^I)); 23.36 (*Me*CON^{C,E}); 20.39 (C(20^I)); 19.97 (C(6^C)); 16.07 (C(21^I)); 16.00 (Me(4^F)). ESI-MS ($C_{123}H_{169}N_{16}O_{49}PS_2$; 2750.87, 2749.040): 1373.50848 ([*M* – 2 H]$^{2-}$; calc. 1373.51289), 915.33825 ([*M* – 3 H]$^{3-}$; calc. 915.33950).

2-{[2-[2-(2-Biotinamidoethoxy)ethoxy]ethyl]amino}ethyl 4-Benzoylbenzoate (**10f**). A soln. of **6a** (600.0 mg, 1.6 mmol) and aldehyde **10b** (100.0 mg, 0.37 mmol) in dry MeOH (40 ml; stirring under dimmed light) was adjusted to pH 6.5 with AcOH. At 20°, sodium cyanoborohydride (23 mg, 0.84 mmol) was added, and the mixture was stirred at 20° for 48 h (TLC control (MeOH/$CHCl_3$/^{i}PrNH$_2$ 2:10:0.1), 4-(dimethylamino)cinnamaldehyde dipping reagent). Evaporation and FC (MeOH/$CHCl_3$/^{i}PrOH 0.75:10:0.1) furnished **10f** (100.0 mg, 43%). Colorless oil. UV (MeOH): 206.0 (20783), 257 (16030). IR (KBr): 3388, 3327, 2931, 1691, 1652, 1275, 756. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.85–7.76 (*m*, 4 arom. H); 7.70–7.57 (*m*, 3 arom. H); 7.54–7.40 (*m*, 2 arom. H); 7.04 (br. *s*, 0.5 H, CONH); 6.20 (br. *s*, 0.5 H, HNCONH); 5.65 (br. *s*, 0.5 H, HNCONH); 4.55–4.42 (*m*, SCH_2CH^{bi}); 4.29–4.23 (*m*, $SCHCH^{bi}$); 4.15–3.90 (*m*, $ArCOOCH_2$, $CONHCH_2$); 3.74–3.30 (*m*, 12 H, 4 CH_2O, 2 CH_2NH); 3.15–3.06 (*m*, SCH^{bi}); 2.90–2.80 (*dd*, $^2J_{AB}$ = 12.8, $^3J_{BX}$ = 4.4, 1 H, SCH_2CH^{bi}); 2.72–2.65 (*d*, $^2J_{AB}$ = 12.8, 1 H, SCH_2CH^{bi}); 2.21–2.13 (*t*, *J* = 7.2, CH_2CONH^{bi}); 1.68–1.33 (*m*, 4 H, 3 CH_2^{bi}, overlapped by ^{i}PrNH$_2$). ^{13}C-NMR (50 MHz, $CDCl_3$): 196.21 (Ph*C*O); 173.62 (CONH); 172.14 (Ar*C*OO); 163.90 ($HNCONH^{bi}$); 140.34, 138.48, 137.13, 132.96, 130.17, 129.85, 128.56, 127.51, 127.36, 127.20 (10 C, arom. CH, arom. C); 70.56, 70.47 ($OCH_2CH_2O^{sp}$); 69.80 (CH_2CO_2Ar); 68.82 (2 CH_2O^{sp}); 61.97, 60.28 (SCH_2CH^{bi}, $SCHCH^{bi}$); 59.76 (SCH^{bi}); 55.62 (SCH_2^{bi}); 53.61, 50.68 (CH_2NHCH_2); 47.20 ($CONHCH_2^{sp}$); 43.85 (SCH_2CH^{bi}); 39.27 (^{i}PrNH$_2$); 37.82 ($CONHCH_2^{sp}$); 35.85 (CH_2CONH^{bi}); 28.36, 28.18, 25.74 (3 CH_2^{bi}). ESI-MS ($C_{32}H_{42}N_4O_7S$; 626.77, 626.277): 627.28528 ([*M* + H]$^+$, calc. 627.28470).

MIC *Values.* The *MIC* values against seven different *Staph. aureus* strains (ATCC 25923, ATCC 29213, MRSA 1309, SG 511, PEG 18, PEG 5, and 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, $1 \cdot 10^5$ cfu ml^{-1} were used. The *MIC*s were determined (absence of visible turbidity) after 24 h at 37°. The *MIC* values were calculated as the average values from three measurements.

Photoaffinity Labeling of PBP 1b with Affinity Label **9**. PBP 1b was obtained and purified by ampicillin affinity chromatography as described previously [36]. PBP 1b (74.25 μg) from a dialyzed ampicillin-affinity chromatography fraction (150 μg/ml in 50 mM *Tris*·HCl (pH 7.6) containing 0.1 mM $MgCl_2$ and 1 mM 2-mercaptoethanol) was mixed in the dark with photoaffinity label X (5 μg) in an *Eppendorf* tube. The mixture was gently swirled for 90 min in the dark to facilitate complex formation. The soln. was then transferred into a glass tube (5-mm diameter) and irradiated at 350 nm for 20 min in a *Rayonet* reactor which was cooled. The SDS PAGE on a 8.5% gel was performed by the *Laemmli* method as described previously (*BioRad Mini-Protean 3*, 0.75 mm). Proteins were detected after separation by staining with *Coomassie* Brilliant Blue *G250* (*Serva*) and by Western blotting. For Western-blot analysis, the semi-dry method was used in a discontinuous buffer system. The proteins were transferred within 1 h at 25 V onto a *Millipore* PVDF membrane (pore size 0.45 μm) using the *Trans-Blot* unit of *BioRad*. For the detection of labeled protein, the streptavidine–alkaline-phosphatase conjugate was employed. In a control experiment, the Western blotting and visualization was also performed with botinylated bovine serum albumin. Two control experiments were carried out: *i*) Two *Eppendorf* tubes were charged with PBP 1b from a dialyzed ampicillin-affinity-chromatography fraction, each with 74.25 μg. *ii*) Two *Eppendorf* tubes were charged with PBP 1b (each 74.25 μg from a dialyzed ampicillin-affinity chromatography fraction), photoaffinity label **9** (5 μg), and moenomycin A (5 mg). The samples were treated

as described above, with the exception that one sample of each series was not irradiated. All samples were analyzed by SDS PAGE and Western blot as described. For the endoproteinase digest, a sample containing labeled PBP 1b was precipitated with 80% acetone. The pellet was solubilized in 50 µl of digest buffer (25 mM *Tris*·HCl (pH 8.5), 1 mM EDTA, 0.1% (*w*/*v*) SDS) in *Eppendorf* tubes and mixed with endoproteinase Lys-C (100 µg/ml in digest buffer) at a ratio of 1:20. The digest was incubated for 18 h at 37°. For control, an aliquot of the endoproteinase-Lys-C-containing soln. was incubated with melittin, whereupon the typical four cleavage products were formed. After the endoproteinase digest, the sample was analyzed by SDS-tricine PAGE, which was performed in a vertical apparatus (*BioRad Mini-Protean 3*, 0.75 mm) as described by *Schägger* and *von Jagow* [40]. Routinely, two gels were prepared, one for *Coomassie* Brilliant Blue *G250* (*Serva*) staining and one for the Western-blot analysis. For Western blotting, the semi-dry method was used in a discontinuous buffer system. The peptides were transferred onto a PVDF membrane (*Millipore*, pore size 0.2 µm) by using the *BioRad-Trans-Blot* unit (1 h at 25 V). For the detection, again the streptavidine–alkaline-phosphatase-conjugate method was used (control with biotinylated bovine serum albumine).

REFERENCES

[1] J. van Heijenoort, *Glycobiology* **2001**, *11*, 25R.
[2] O. Ritzeler, L. Hennig, M. Findeisen, P. Welzel, D. Müller, A. Markus, G. Lemoine, M. Lampilas, J. van Heijenoort, *Tetrahedron* **1997**, *53*, 1675; R. Kosmol, L. Hennig, P. Welzel, M. Findeisen, D. Müller, A. Markus, J. Van Heijenoort, *J. Prakt. Chem*. **1997**, *339*, 340.
[3] M. Terrak, T. K. Ghosh, J. van Heijenoort, J. van Beemen, M. Lampilas, J. Aszodi, J. A. Ayala, J.-M. Ghuysen, M. Nguyen-Destèche, *Mol. Microbiol*. **1999**, *34*, 350, and ref. cit. therein.
[4] A. M. Di Guilmi, A. Dessen, O. Dideberg, T. Vernet, *J. Bacteriol*. **2003**, *185*, 1650.
[5] B. Schwartz, J. A. Markwalder, S. P. Seitz, Y. Wang, R. L. Stein, *Biochemistry* **2002**, *41*, 12552.
[6] S. Vogel, A. Buchynskyy, K. Stembera, K. Richter, L. Hennig, D. Müller, P. Welzel, F. Maquin, C. Bonhomme, M. Lampilas, *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1963, and ref. cit. therein.
[7] R. C. Goldman, D. Gange, *Curr. Med. Chem*. **2000**, *7*, 801.
[8] M. Ge, Z. Chen, H. R. Onishi, J. Kohler, L. L. Silver, R. Kerns, S. Fukuzawa, C. Thompson, D. Kahne, *Science (Washington, D.C.)* **1999**, *284*, 507.
[9] C.-H. Wong, M. C. Bryan, P. T. Nyffeler, H. Liu, E. Chapman, *Pure Appl. Chem*. **2003**, *75*, 179; T. K. Ritter, C.-H. Wong, *Angew. Chem*. **2001**, *113*, 3616; *Angew. Chem., Int. Ed*. **2001**, *40*, 3508.
[10] N. El-Abadla, M. Lampilas, L. Hennig, M. Findeisen, P. Welzel, D. Müller, A. Markus, J. van Heijenoort, *Tetrahedron* **1999**, *55*, 699, and ref. cit. therein.
[11] K. Stembera, S. Vogel, A. Buchynskyy, J. A. Ayala, P. Welzel, *ChemBioChem* **2002**, *3*, 559.
[12] A. Anikin, A. Buchynskyy, U. Kempin, K. Stembera, P. Welzel, G. Lantzsch, *Angew. Chem*. **1999**, *111*, 3931; *Angew. Chem., Int. Ed*. **1999**, *38*, 3703.
[13] M. Mayer, B. Meyer, *J. Am. Chem. Soc*. **2001**, *123*, 6108, and ref. cit. therein.
[14] M. Vogtherr, T. Peters, *J. Am. Chem. Soc*. **2000**, *122*, 6093.
[15] T. Rühl, M. Daghish, A. Buchynskyy, K. Barche, D. Volke, K. Stembera, U. Kempin, D. Knoll, L. Hennig, M. Findeisen, R. Oehme, S. Giesa, J. Ayala, P. Welzel, *Bioorg. Med. Chem*. **2003**, *11*, 2965.
[16] A. Hennig, M. Findeisen, P. Welzel, R. Haessner, *Magn. Reson. Chem*. **1998**, *36*, 615, and ref. cit. therein; M. Kurz, W. Guba, L. Vertesy, *Eur. J. Biochem*. **1998**, *252*, 500.
[17] L. Chen, D. Walker, B. Sun, S. Walker, D. Kahne, *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 5658.
[18] T. Rühl, L. Hennig, Y. Hatanaka, K. Burger, P. Welzel, *Tetrahedron Lett*. **2000**, *41*, 4555; *Tetrahedron Lett*. **2001**, *42*, 9297.
[19] T. Rühl, D. Volke, K. Stembera, Y. Hatanaka, H. Hennig, F. Schumer, P. Welzel, *Chem. Commun.* **2002**, 1630.
[20] M. Daghish, L. Hennig, M. Findeisen, S. Giesa, F. Schumer, H. Hennig, A. G. Beck-Sickinger, P. Welzel, *Angew. Chem*. **2002**, *114*, 2404; *Angew. Chem., Int. Ed*. **2002**, *41*, 2293.
[21] F. Kotzyba-Hilbert, I. Kapfer, M. Goeldner, *Angew. Chem*. **1995**, *107*, 1391; *Angew. Chem., Int. Ed.* **1995**, *34*, 1296; S. A. Fleming, *Tetrahedron* **1995**, *51*, 12479; G. Dormán, *Top. Curr. Chem*. **2001**, *211*, 169; G. Dormán, G. D. Prestwich, *Trends Biotechnol*. **2000**, *18*, 64; Y. Hatanaka, Y. Sadakane, *Curr. Top. Med. Chem*. **2002**, *2*, 271.
[22] P. A. Weber, A. G. Beck-Sickinger, *J. Peptide Res.* **1997**, *49*, 375.
[23] B. A. Gilbert, R. R. Rando, *J. Am. Chem. Soc*. **1995**, *117*, 8061.

[24] E. Havinga, M. E. Kronenberg, *Pure Appl. Chem*. **1968**, *16*, 137.
[25] K. Fang, M. Hashimoto, S. Jockusch, N. J. Turro, K. Nakanishi, *J. Am. Chem. Soc*. **1998**, *120*, 8543; M. Hashimoto, Y. Liu, K. Fang, H. Li, G. Campiani, K. Nakanishi, *Bioorg. Med. Chem*. **1999**, *7*, 1181.
[26] K. Krohn, J. Küpke, H. Rieger, *J. Prakt. Chem*. **1997**, *339*, 335.
[27] M. Daumas, Y. Vo-Quang, L. Vo-Quang, F. Le Goffic, *Synthesis* **1989**, 64.
[28] K. Burger, E. Windeisen, R. Pires, *J. Org. Chem*. **1995**, *60*, 7641; R. Pires, K. Burger, *Synthesis* **1996**, 1277, and ref. cit. therein.
[29] A. Buchynskyy, U. Kempin, S. Vogel, L. Hennig, M. Findeisen, D. Müller, S. Giesa, H. Knoll, P. Welzel, *Eur. J. Org. Chem.* **2002**, 1149, and ref. cit. therein.
[30] L. F. Tietze, M. Arlt, M. Beller, K.-H. Glüsenkamp, E. Jähde, M. F. Rajewsky, *Chem. Ber.* **1991**, *124*, 1215.
[31] A. Schmidt, *Synthesis* **1980**, 961; L. F. Tietze, C. Schröter, S. Gabius, U. Brinck, A. Goerlach-Graw, H.-J. Gabius, *Bioconjugate Chem.* **1991**, *2*, 148; V. P. Kamath, P. Diedrich, O. Hindsgaul, *Glycoconjugate J.* **1996**, *13*, 315; V. Pozsgay, E. Dubois, L. J. Pannell, *J. Org. Chem*. **1997**, *62*, 2832; J. Zhang, A. Yergey, J. Kowalak, P. Kovác, *Carbohydr. Res.* **1998**, *313*, 15.
[32] A. Buchynskyy, U. Kempin, S. Vogel, L. Hennig, M. Findeisen, D. Müller, S. Giesa, H. Knoll, P. Welzel, *Eur. J. Org. Chem*., submitted.
[33] M. A. Winnik, C.-K. Hsiao, *Chem. Phys. Lett*. **1975**, *33*, 518.
[34] H. A. Staab, *Angew. Chem*. **1962**, *74*, 407; *Angew. Chem., Int. Ed.* **1962**, *1*, 351.
[35] S. Vogel, A. Buchynskyy, K. Stembera, K. Richter, L. Hennig, D. Müller, P. Welzel, F. Maquin, C. Bonhomme, M. Lampilas, *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1963, and ref. cit. therein.
[36] K. Stembera, A. Buchynskyy, S. Vogel, D. Knoll, A. A. Osman, J. A. Ayala, P. Welzel, *ChemBioChem* **2002**, *3*, 332.
[37] D. Kritchevsky, M. R. Kirk, *Arch. Biochem. Biophys.* **1952**, *35*, 346.
[38] L. Hennig, M. Findeisen, P. Welzel, R. Haessner, *Magn. Reson. Chem*. **1998**, *36*, 615, and ref. cit. therein; M. Kurz, W. Guba, L. Vertesy, *Eur. J. Biochem*. **1998**, *252*, 500.
[39] M. Braun, X. Camps, O. Vostrowsky, A. Hirsch, E. Endress, T. M. Bayerl, O. Birkert, G. Gauglitz, *Eur. J. Org. Chem*. **2000**, *7*, 1173.
[40] H. Schägger, G. von Jagow, *Anal. Biochem.* **1987**, *166*, 368.

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